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Utilization of Garlic (*Allium sativum* L.), Jasmine (*Jasminum officinale* L.), Thyme (*Thymus basilicum*) and Wheat Bran (*Triticum aestivum* L.) Wastes for Fungal Growth and Removal of Textile Dyes

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ABSTRACT

There are great environmental concerns to develop new and efficient ways to remove azo-dyes from wastewater. Among these ways abiotic and biotic agents were tested for removal of dyes. Cultivate of *Aspergillus niger* growth and the bioremoval of four textile dyes using several medicinal plants wastes has been studied. Three wastes of distillation medicinal aromatic plants namely; garlic (*Allium sativum* L.), jasmine (*Jasminum officinale* L.) and thyme (*Thymus basilicum*) in addition to wheat bran (*Triticum aestivum* L.) were used as growth media for *A. niger.* This fungus is used for textile dye bioremoval. The plant waste supported good growth of fungi in rather short incubation time (7 days). The aim of this study is to adopt low-cost technology for removal of some textile dyes by biotic or abiotic agents. Four commercial dyestuffs; direct violet, direct green, reactive red and acid red were included in this study. It was found that color bioremoval of the various dyes within 72 h of incubation using *A. niger* biomass varied from 40.2 to 99.6% of the original dye color. This finding was dye-dependent. In absence of fungi, the tested abiotic sorbents (wheat bran, jasmine, garlic and thyme) showed comparatively low removal capacity amounting < 60% in the majority of treatments. The bioremoval efficiency by fungi obviously rose up to > 90%. These findings confirm the role of fungi in decolorization of textile dyes.

Keywords: biotic and abiotic agents, fungi, medicinal plants waste, textile dyes removal

INTRODUCTION

The treatments of wastewaters contained dyestuffs are difficult and they require special advanced treatment technologies. It is known that adsorption is one of the methods commonly and efficiently used for treatment of textile wastewaters (Şayan 2006). Azo dyes are frequently used in dyeing and textile industries worldwide. Thus, the industrial effluents often contain residual dye, which deteriorates the water quality, and may become a threat to human health since some azo dyes or their metabolites may be mutagenic or carcinogenic (Heiss *et al.* 1992). There is no general method for the removal of color from dye wastewater. Methods of primary clarification, including sedimentation and flotation, are not effective for the removal of color without simultaneous chemical treatment. Processes such as membrane separation, coagulation and ion exchange are also used for the removal of color from dye wastewaters, but the cost of these processes is the main drawback of these techniques (Mishra and Tripathy 1993). Numerous approaches have been studied for the development of cheaper and effective adsorbents. Many non-conventional low-cost adsorbents, including natural materials, biosorbents, and waste materials from industry and agriculture, have been proposed by several workers. These materials could be used as sorbents for the removal of dyes from solution. Some of the reported sorbents include clay materials, siliceous material, agricultural wastes (bagasse pith, maize cob, rice husk, coconut shell), industrial waste products (waste carbon slurries, metal hydroxide sludge), biosorbents (chitosan, peat, biomass) and others (starch, cyclodextrin, cotton) (Crini 2006). Fungal strains have been used to develop bioprocesses for mineralization of azo dyes (Yang and Yu 1996; Palleria and Chambers 1997; Zhang *et al.* 1999). However, the long growth cycle and moderate decolorization rate limit the performance of the fungal decolorization system (Banat *et al.* 1996; Jang *et al*. 2007). Wafaa (2006) investigated that the removal of dyes and their derivatives from aqueous solutions uses sugarcane bagasse, sawdust, rice straw, charcoal and fungal biomass as dye removing agents. Seven fungal strains known to have high capacity in removing textile dyes were used also. Results of this study indicated that *Penicillium commune*, *P. freii*, and *P. allii* removed 96, 64 and 65%, respectively, of direct violet dye after 2 h of incubation. In addition, the use of rice straw was shown to be more efficient in dye removal, than was bagasse or sawdust. Rice straw was effective in removing 72% of direct violet dye within 24 h. The distillation wastes of various aromatic plants and the non-utilized parts of medicinal and other plants are of limited economic use these waste are usually obtained as a by-product in hydro-distillation of fresh herbage is a potential source of nitrogen and other nutrients. Some agricultural by- products and wastes have been used for mass culturing of a variety of microorganisms in general and fungi in particular. In this paper, experiments have been carried out to estimate the removing capacity of four textile dyes from aqueous solution using the medicinal plants wastes.

MATERIALS AND METHODS

Plant materials

Distillation wastes of three medicinal aromatic plants, garlic (*Al-*

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lium sativum L.), jasmine (*Jasminum officinale* L.) and thyme (*Thymus basilicum*) beside wheat bran (*Triticum aestivum* L.) were evaluated as fungal growth media adopting the procedure described by Singh *et al*. (2002). Moistened shade dried distillation wastes were filled in 500 g portions in polyethylene bags. The bags were plugged with cotton and autoclaved at 121°C for 1 hr.

Preparation of fungal inocula

Fungal strain *Aspergillus niger* characterized by high textile dye removing capacity (Wafaa *et al.* 2003) was selected. Fungi were cultivated on potato dextrose agar medium (PDA; Merck, Germany) at 28°C for 7 days, then kept at 4°C (Oshoma and Ikenebomeh 2005). For inocula preparation, a spore suspension of fungi was obtained by growing the microorganism in 250 ml Erlenmeyer flasks containing 50 ml of molt extract broth medium for 2 weeks at 28°C. There after, spores were harvested in 0.1% aqueous Tween 80 (v/v) and adjusted to *ca*. 3×10^3 spores ml^{-1} as colony forming units on PDA plates (Gombert *et al*. 1999).

Preparation of fungal biomass inoculum

Fungal strains were cultivated, separately, in 250 ml Erlenmeyer flasks containing 150 ml of basal mineral salts medium containing (g/l) 0.5 KH_2PO_4, 0.2 $MgSO_4 \cdot 7H_2O$, 0.1 NaCl, and supplemented with 10 g/l sucrose (Sisco Research Laboratories, India), and 1 g/l yeast extract (Sigma-Aldrich, St. Louis, USA). All other chemicals used were of analytical grade. Flasks were incubated on a rotary incubator shaker at 150 rpm at 28°C for 3–4 days. Fungal pellet were obtained by centrifugation at 8,000 rpm for 20 min at 20°C. A suspension of fungal biomass containing 30 mg fungal biomass (dry weight) was used as the inoculum. Uninoculated flasks containing media and the dyes (250 mg/l) were included as controls for comparison. The fungal mycelium was collected by filtration after 48 h, and dried at 105°C to determine dry weight.

Survival of fungi on distillation wastes

The polyethylene-cotton plugged bags containing the autoclaved plant wastes with 60% w/v sterile water were inoculated by injecting 5 ml spore suspension of *A. niger* by hypodermic syringes. Bags were then incubated in well- illuminated incubator at 28°C for 30 days. Representative samples were taken for serial dilution preparations. The PDA plates were inoculated with the proper dilutions and incubated at 28°C for 7 days. Incubation experiments were designed to monitor the multiplication patterns of these representative fungi on distillation wastes. Developed fungal colonies were enumerated at 0, 7, 14 and 30-day intervals.

Table 1 Periodical fluctuation in *A. niger* after 1, 2 and 4 weeks incubation of the various distillation wastes incubation.

Waste	*Aspergillus niger* 10^6				Doubling
	0	1	2	4	time (hr)
Wheat bran	0.011	7000	8800	11000	8.21
Garlic	0.004	10500	3400	2000	7.95
Jasmine	0.007	9400	7300	11000	8.30
Thyme	0.007	3400	7200	10000	9.41

Textile dye removed by *A. niger* and distillation

All the distillation wastes were dried and milled. The artificial effluent was prepared by dissolving the various dyes separately in distilled deionized water to produce solutions containing 300 mg L^{-1} of either. A portion of (2.0, 1.5, 1.2, 1.2 g) of garlic, jasmine, thyme and wheat bran wastes were added to 180 ml of the artificial effluent with 300 mg L^{-1} dye concentration.

RESULTS

A 30-day incubation experiment was executed to monitor the survival patterns of *A. niger* on the various distillation wastes. Those were compared with the corresponding on wheat bran. A dense population of fungal member was encountered on wheat bran as early as 7 days incubation (**Table 1**). Survival of *A. niger* (**Fig. 1**) on wastes was found to substrate-dependent. After 7 days of cultivation, a storm of microbial multiplication was scored particularly with jasmine (8.3) and thyme. Garlic waste showed a unique pattern of fungal growth. This was authenticated by comparatively the lowest calculated doubling time of 7.95 h. Longer periods were required for the fungal member proliferation on the other growth substrates, doubling times of 8.21-9.30 h were recorded for the latter media. Irrespective of fungal member and incubation interval, jasmine waste seemed the most favorable for fungal cultivation followed by wheat bran. Garlic and thyme wastes showed relatively similar influence on microbial growth. As to fungal candidate, *A. niger* successfully grew on the various distillation wastes.

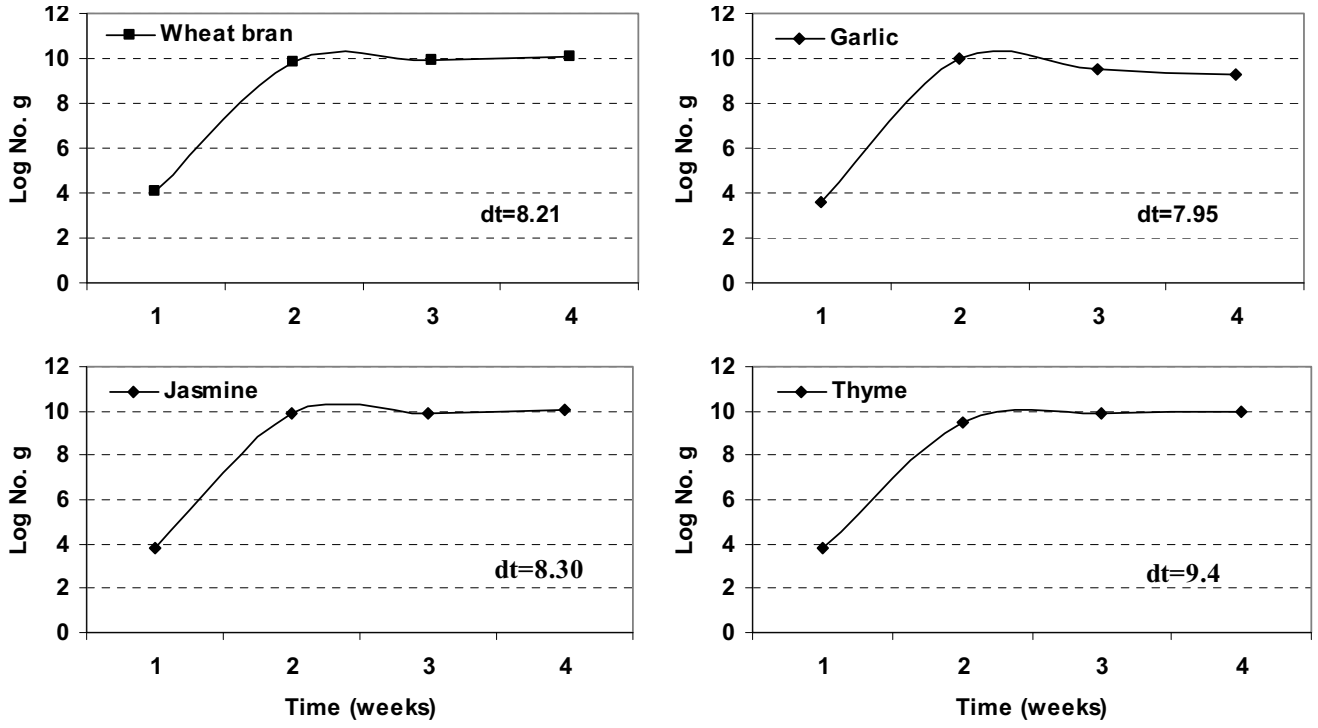

Fig. 1 Periodical fluctuations in *Aspergillus niger* numbers after 1, 2 and 4 weeks of distillation wastes incubation.

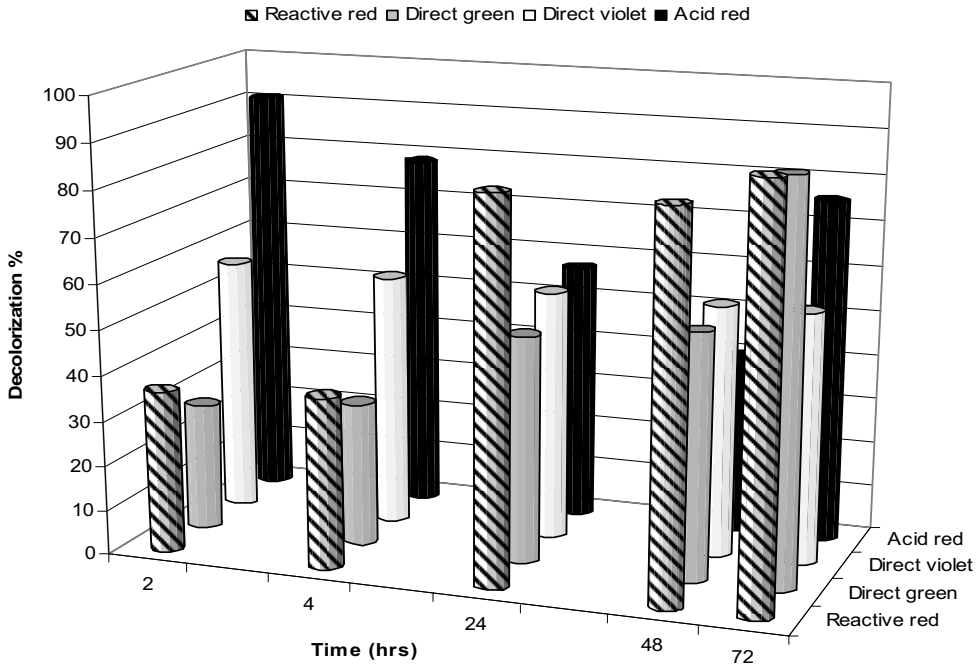

Fig. 2 Removal of textile dyes using garlic wastes.

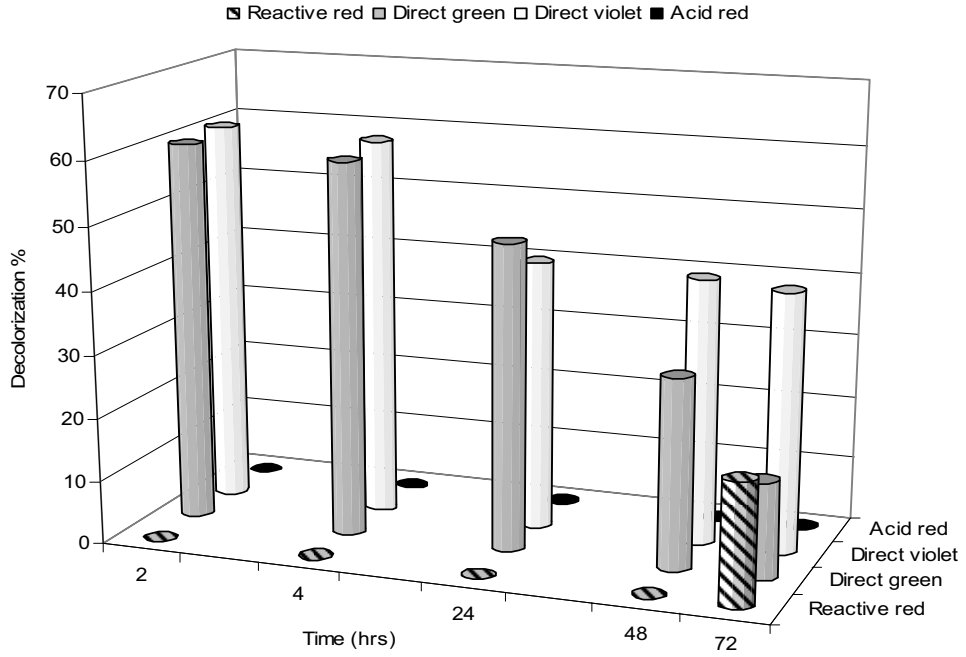

Fig. 3 Removal of textile dyes using thyme wastes.

Remediation of textile dyes using garlic, jasmine and thyme

The removal rate of the various textile dyes by *A. niger* and/or distillation wastes scored at the different experimental interval is present in **Figs. 2-7**. As to the direct violet textile dye (**Figs. 6, 7**), *A. niger* showed extraordinary removing ability with decolorization percentage of 96.8 within 2 h of incubation. The dye removal rate rose to 99.3% with prolonged incubation to 4 h. This was followed by slight reduction in the removal capacity among 6-48 h where the decolorization percentage decreased to 88.5-96.8%. The microorganisms did recover its activity at the 72 h period recording a dye removal estimate of 99.6%.

When abiotic sorbents represented by garlic, jasmine and thyme wastes as well as wheat bran were used, significant reductions in the removal rate of the textile dye was scored. Regardless of sampling date, the highest removal percentage of 55.7 was recorded for garlic waste (**Fig. 2**) while the lowest (35.2) was attributed to jasmine (**Fig. 3**). Respective removal percentages of 49.2 and 36.1 were obtained by thyme and wheat bran. Variations among the abiotic sorbents were almost the same when used in combination with *A. niger*. The percentages of direct violet textile dye removal of the fungus-abiotic sorbent mixtures were falling in the range 30.6-49.9. The activity of *A. niger* in decolorization of the reactive red textile dye steady increased with incubation period being 80.5% at 2 h interval and 85.5% at the end of the experiment with an average of 84.2% (**Fig. 6**). As previously recorded with direct violet, the disappearance of reactive red dye markedly decreased due to abiotic sorbents, whatever they are. This was clear in case of thyme waste where the substrate failed to show any dye removing capacity during an incubation period extended to 48 h. A very low removing rate of 19.3% was recorded thereafter. An exception was observed with garlic waste where an average dye decolorization percentage of 68.4% was estimated. Wheat bran showed somewhat high removal activity (71.1%) after 2 h of incubation, this was followed by gradual reductions in the activity up to the 24-h interval.

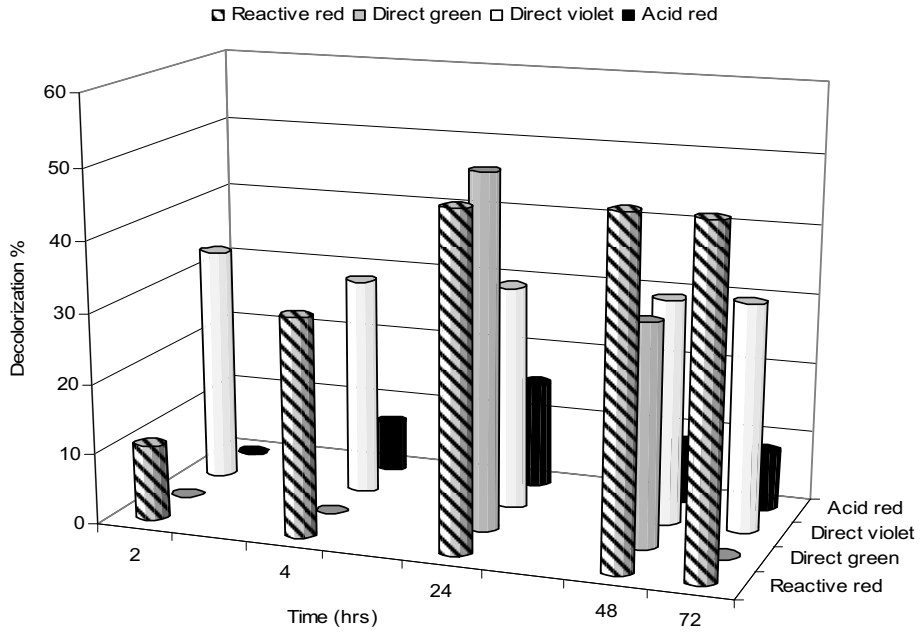

Fig. 4 Removal efficiency (%) of textile dyes by jasmine wastes.

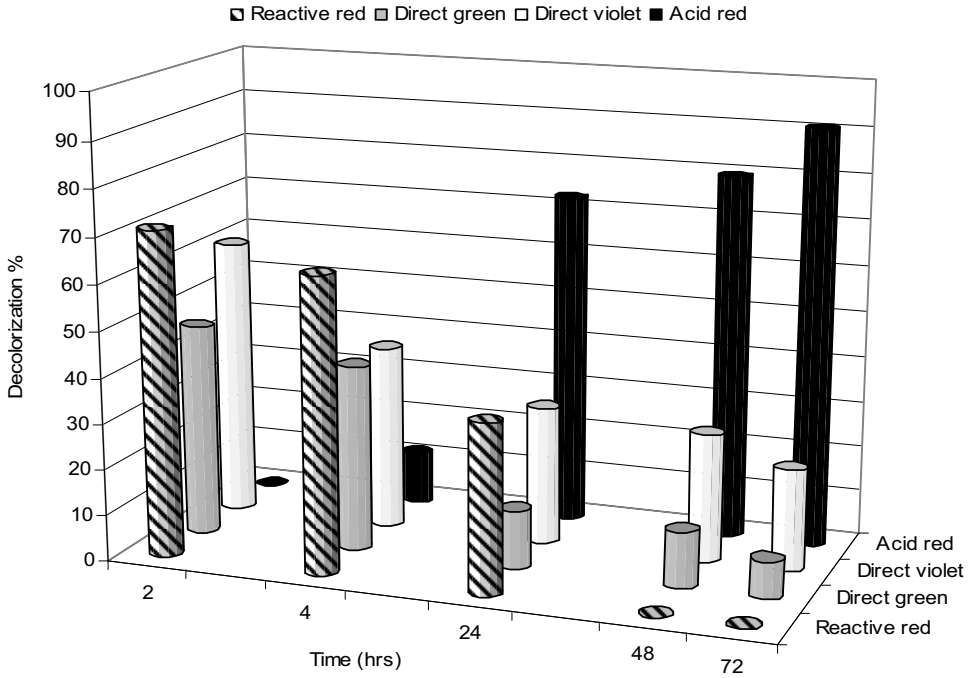

Fig. 5 Removal of textile dyes using wheat bran wastes.

Thereafter, the substrate lost its capacity to decolonize the dye. The reactive red textile dye kept its color throughout the experimental period when treated with *A. niger* and wheat bran mixture. Other fungus- abiotic sorbent mixtures showed very low removal efficiency of 13.8-33.8% with the highest decolorization percentage in case of *A. niger*-jasmine waste. Relatively low removal rates (55.0-58.7%) were recorded for the direct green dye due to *A. niger* during 2-6 h of incubation at 28°C (**Fig. 6**). This was followed by a reduction in the fungal dye decolorization activity approximated 50 and 47% at the 24- and 48-h intervals, respectively. Slight increase of 16.9% was estimated at the end of the incubation period. Abiotic sorbents showed variable decolorizing levels within 2 h, records differed from 0.0 to 60.0% depending upon the substrate. Thereafter, variations among wastes were less pronounced. In general, garlic distillation waste supported the highest dye removal percentage (49.3) followed by thyme (43.9%) and wheat bran (26.4). Jasmine exhibited no dye removing activity except at the 48-h period being 31.5% (**Figs. 2, 4, 5**).

For the biotic and abiotic sorbent mixtures, the fungus-thyme system had no apparent decolorizing activity during 72 h, while very low activity (14.7%) was exhibited by the fungus- garlic mixture at the end of the experiment (**Fig. 6**). The direct green textile dye removal rate of *A. niger*-wheat bran mixture exceeded that of the fungus-jasmine where average decolorizing percentages were 39.9 and 21.8%, respectively (**Fig. 6**).

The acid red textile dye removal percentages considerably varied among the biotic and abiotic sorbents (**Fig. 6**). As previously reported with the majority of other dyes, the decolorizing efficiency of *A. niger* steady increased with time up to 4 h then gradually decreased. A maximal removing activity of *ca*. 46% was scored as early as 2 h of incubation while a minimal of 20.0% was recorded for the 72-h old culture. Thyme alone failed to show any removing activity. On the contrary, garlic deemed the pioneer among the tested abiotic sorbents with decolorization percentages of 39.8-91.2 with an average of 66.8. Other substrates were arranged in the descending order: wheat bran (48.0%) >

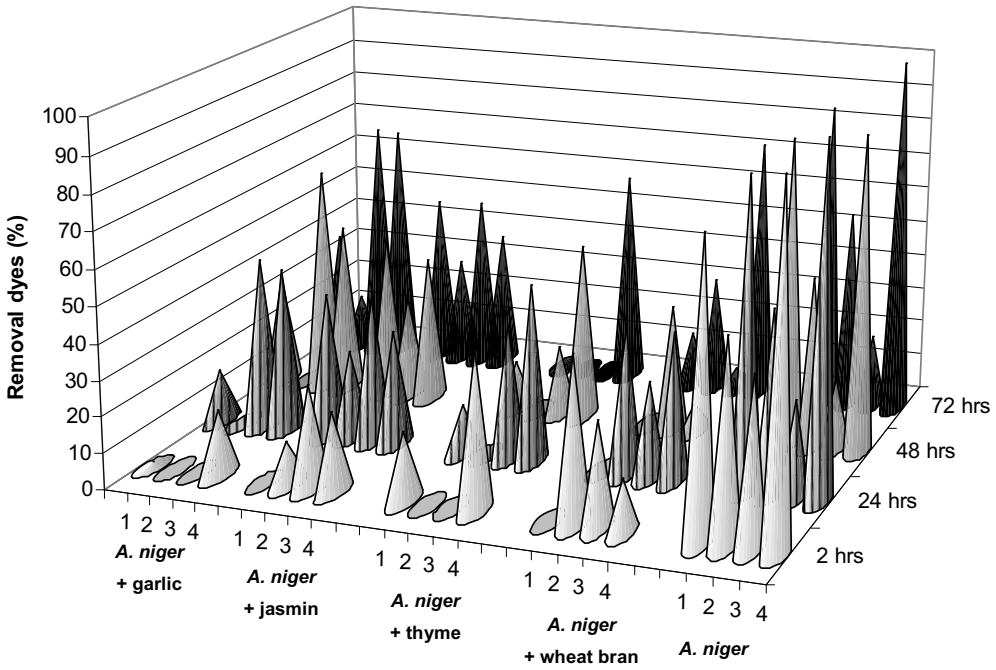

Fig. 6 Bioremoval of four textile dyes (1, reactive red, 2, direct green, 3, direct violet, 4, acid red) using biotic and abiotic mixures.

jasmine (8.7%). When *A. niger* was introduced to the distillation wastes, low dye removal activities of < 50% were estimated depending upon the fungus-substrate mixture. In such particular treatments, fungus-garlic system was found the most active resulting in a removing capacity of 40.0%. The microorganisms-jasmine mixture showed almost similar decolonizing level of *ca*. 37%. The fungus-thyme complex was the inferior with an average activity of 13.5%. *A. niger*-wheat barn mixture occupied a medium rank where the dye removal rate approximated 39%. Regardless of the experimental treatments, the direct violet textile dye seemed the most susceptible to the action of biotic and/or abiotic agents used, an average removal rate of 47.9% was scored. Respective estimates of 32.4, 27.5 and 31.3% were obtained for reactive red, direct green and acid red textile dyes, respectively. In respect to abiotic sorbents, those, in general, were more active on direct violet dye compared to other dyes. A decolorization percentage of 44.1% was recorded for the former and of 30.9-36.6% for the latter. In comparison with other dyes, the reactive red strongly resisted the effect of biotic-abiotic sorbent mixtures where the general score of removal rate was 15.4%. On the contrary, such mixtures exerted relatively higher decolorizating ability towards direct violet dye with removal percentage of 39.7%. The direct green and acid red dyes showed 16.1 and 29.8% color removal due to biotic-abiotic agent mixtures (**Fig. 6**). The biomass yield of 72-h mono-fungal- and waste-microbe cultures as affected by the textile dye treatment (data not show). In substrate-free systems, the direct-violet did support higher biomass accumulation where 16.7% increase was scored. A higher increase of 53.3% was attributed to the reactive red dye. Other dyes had no effect on the dry matter production. In mixed cultures of *A. niger* and distillation wastes, the biomass yield was found to be dye-dependent. As to fungus-garlic system, the reactive red was the most supportive dye and resulted in *ca*. 60% increase in the culture dry weight while direct green was the least (40.5% increase). On the other hand, the latter dye resulted in higher cultural dry weight, compared to other dyes, in case of *A. niger*-thyme mixture. Extraordinary stimulation for dry matter production was noticed in the microorganism- jasmine system in presence of the textile dye acid red where estimated increase approximated 122%. As low as 6.7-15.0% increases in growth yield were recorded in the various fungus- wheat bran-textile dye treatments.

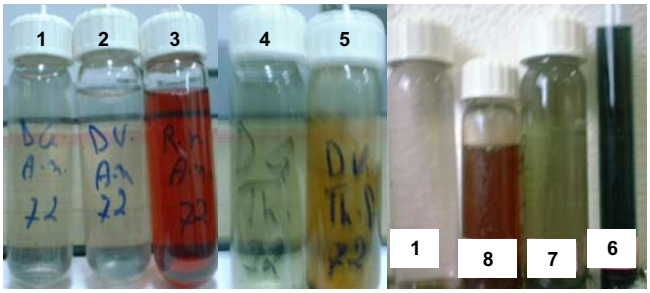

Fig. 7 Removal of three textile dyes by *A. niger* biomass and medicinal plants wastes: (1), Removal of direct green by *A. niger* biomass, (2), Removal of direct violet by *A. niger* biomass, (3) Reactive red treated with fungi, (4), Removal of direct green by Thyme waste, (5), Removal of direct violet by *A. niger* + Thyme waste, (6), Control 250 ppm direct green dye, (7), Removal of direct green dye by garlic waste, (8), Removal of direct green dye by thyme waste.

DISCUSSION

Though some agricultural by- products and wastes have been used for mass culturing of a variety of microorganisms and fungi in particular (Koysalya and Jeyarajan 1990; Reeta *et al*. 2009), no information so far exists on the possibility of using distillation wastes as growth media for microorganisms. This was experimented in the present work for one unique fungal member. The microbial candidate is *A. niger* as a textile dye bio-removing fungus (Wafaa *et al.* 2003; Wafaa 2006; Wafaa *et al*. 2008a). Besides, the latter particular fungus belongs to microorganisms of extreme biotechnological importance since it is used for production of various primary metabolites (organic acids) and enzymes (Jernejc and Legisa 2004). In fact, citric acid production by this fungus is among the most efficient bioprocesses in terms of productivity, since *A. niger* can convert up to 80% of the substrate to the final product. A series of short-term incubation experiments was designed to monitor the multiplication patterns of these representative fungi on distillation wastes of garlic, jasmine and thyme in addition to wheat bran. All tested by-products did obviously support the growth of fungal member in rates varied depending upon the substrate and microbial strain. During 7 days of culture age, growth of *Aspergillus* strain was lass. Fungal strain oppositely behaved with prolonged incubation but

grew nicely. Many other investigators mentioned that several agro-industrial processes can generate wastes which could be used as substrates for microbial growth (Balis *et al.* 1996; Zervakis *et al.* 1996; Del Re *et al.* 2003). Microorganisms are capable of utilizing various components of the organic matter in such wastes as a carbon source for growth and for synthesis of cellular biomass as well. Numerous reports described the utilization of waste products including apple pamace (Barba *et al.* 2001), banana waste (Barba *et al.* 2002), kiwifruit peels (Sparringa *et al.* 2002) and potato waste (Bergmeyer and Bernt 1974). A number of adsorbents from agricultural byproducts such as date pith, sawdust, corn cob, barley husk, rice hull, rice straw, and bagasse pith were tested for removal of several dyes and proved to be promising in this respect (Low *et al*. 2000; Robinson *et al*. 2002; Banat *et al*. 2003; Garg *et al*. 2003; Wafaa 2006; Mahejabeen and Hajira 2007). The textile industries usually use mixture of different types of dyes for different applications. Hence, there is a need to identify sorbents capable of removing wide range of different types of dyes. This has been stated in previous work (Janos *et al*. 2003; Ong *et al*. 2007). In previous work (Wafaa 2006) assessed the removal of direct and reactive dyes using biotic and abiotic agents. Removal of these dyes from aqueous solutions was investigated using sugarcane bagasse, sawdust, rice straw, charcoal and fungal biomass as dye removing agents. The results indicated that *Penicillium commune*, *P. freii*, and *P. allii* removed 96, 64 and 65%, respectively, of direct violet dye after 2h of incubation. In addition, the use of rice straw was shown to be more efficient in dye removal, than was bagasse or sawdust. Also the results of this study indicate that low-cost, renewable, bioadsorption agents are relatively effective in removing textile dyes from solution. A vast array of bibliography has dealt with removing dyes from wastewater (Liversidge *et al.* 1997; Choy *et al.* 1999; Kattri and Singh 2000; Low *et al.* 2000; reviewed by Viswanath *et al*. 2008). Here, it should be mentioned that release of wastewaters by various industries poses serious environmental problems due to various dyes persistent and recalcitrant nature. Textile industries are responsible for the discharge of large quantities of dyes into natural waterways due to inefficiencies in dyeing techniques. The presence of dyes in water ways is easily detectable even when released in small concentrations (Nigam *et al*. 2000). This is not only unsightly, but the coloration of the water by the dyes may have an inhibitory effect on photosynthesis affecting aquatic ecosystems. Dyes may also be problematic if they are broken down anaerobically in the sediment, as toxic amines are often produced due to incomplete degradation by microorganisms (Weber and Wolfe 1987). Hence, colour removal from textile wastewater is of major environmental concern (Juang *et al.* 1996). Conventional physical and chemical treatments for dye removal (Robinson *et al.* 2001) have been shown to be either expensive, e.g., activated carbon and membrane filtration (also incapable of treating large volumes) or produce a concentrated sludge, e.g., Fentans Reagent. Other techniques are extensively adopted; among those is the absorption which deemed an effective procedure for treating dye-containing effluents (Keith *et al.* 1999; Robinson *et al.* 2001). Robinson *et al*. (2002) succeeded to remove dyes from an artificial textile dye effluent by the agricultural waste residues corncob and barley husk. Biological treatment for dye removal has many advantages over the chemical and physical ones. This is possibly attributed to degradation of dye molecules to carbon dioxide and water with the formation of less sludge besides maintaining the environment clean to some extent (Tatarko and Bumpus 1998). Many authors studied the removal of textile dye methylene blue by adsorption technique using biosorbent *Ulva lactuca* and *Sargassum* as a natural, renewable bioabsorbents About 96% removal was obtained by using these biosorbents. (Turner *et al*. 2007; El-Sikaily *et al*. 2007; Hajira *et al*. 2007; *Sari et al*. 2008). Several fungal species were tested for the decolorizing various dyes. Those include *Trametes versicolor* (Young and Yu 1997), *Coriolus versicolor* (Knapp and Newby 1999), *Funalia trogii* (Yesilada *et al.* 1995), *Aspergillus niger* (Fu and Viraraghavan 2000; Wafaa *et al*. 2008b), *Rhizopus arrhizus* (Zhou and Banks 1993), *Rhizopus oryzae* (Gallagher *et al*. 1997) and *Penicillium* spp. (Wafaa and Moawad 2003). Among the goals of this work, is the adoption of low-cost technology for removal of some textile dyes by biotic or abiotic agents. It was found that bioremoval of the various dyes within 72 h of incubation using *A. niger* varied from 40.2 to 99.6% of the original dye color, a finding was dye-dependent. In absence of fungal member, the tested abiotic sorbents (wheat bran, jasmine, garlic and thyme) showed comparatively low removal capacity of <60% in the majority of treatments. For fungi-received wastes, the bioremoval efficiency obviously raised up to >90%. These findings confirm those of other investigators that fungi play a role in decolorization of textile dyes. Zhou and zimmermann (1993) mentioned that absorption of anthraxquinone, phthalocyanine and azo dyes to the cells of some microbial strains result in the decolonization of the effluents. Here, the biotic removal of dyes seems to depend on interaction between the fungal mycelium and the dye in media. This interaction is based on a biosorption of such chemical on the intact fungal biomass. It was reported that the primary mechanism for dye removal in biological systems might occur by absorption into the cell walls of the microorganisms. Wallace (2001) studied the dye absorption by the cell wall and concluded the following; 1) dye absorption follows Freundlich absorption isotherms at low dye loads per weight of biomass, 2) depending upon the dye, the dye may remain in the cell wall and 3) absorption does not inhibit the reduction rate of microbes that exhibit the ability to reduce azo days. In general, fungi have shown the ability to remove dyes from industrial effluents by various mechanisms. Dyes might be removed through biosorption (Payman *et al*. 1998; Zheng *et al*. 1999; Fu and Viraraghavan 2000), biodegradation (Nigam *et al*. 1995; Conneel *et al*. 1999) and/or enzymatic mineralization (Wong and Yu 1999; Pointing and Vrijmoed 2000; Wesenberg *et al.* 2003). However, one or more of these mechanisms could be involved in color removal depending on the fungus used. Prolonged incubation, in some cases, resulted in the release of the dye once again to the culture media. This was reported by Wafaa and Moawad (2003) as well, they found that the decolorization percentage of direct dye by *A. flavus* and *A. terres* were 71.8 and 71.6 after 5 and 6 days of incubation. The percentages decreased to 13 and 32 respectively after 8 days. In this respect, Pointing *et al.* (2000) using *Pycnoporus sanguineus* fungi found that the strain lost the ability to decolorize some azo dyes after repeated sub-culturing.

CONCLUSION

This paper suggests that the tested medicinal plant wastes produced during distillation process could successfully be used as a proper growth medium for *A. niger* strain without costly pretreatment or nutrient supplementation. In addition, such abiotic agents unexpectedly showed ability for dye removal which indicates their possible contribution in the environmental protection against these chemical pollutants.

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