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ANTIMICROBIAL EFFECT OF PENCILLIUM *SP.* OF YELLOW PIGMENTS AGAINST SOME BACTERIAL AND FUNGAL STRAINS

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ABSTRACT
The aim of this study is to evaluate the antimicrobial activity of pigments produced from *Pencillium sp*. strain. There is at present a growing interest in evaluating pigments for use as a natural dye and preservative. These pigments are looked upon for their safe use as a natural food dye in replacement of synthetic ones because of undesirable toxic effects including mutagenicity and potential carcinogenicity. The fungus was grown over PDA in tubes at 30ºC and the spore suspension was used as inoculums for the production of pigments. The pigment obtained by growing the Pencillium sp. had a antifungal action against some species of *Trichoderma sp., Aspergillus niger, Aspergillus terreus and Fusarium sp.* . The yellow pigments produced from *Pencillium sp.* also inhibit bacteria of genera of *Bacillus subtilis, klebsiella pneumonia and Staphylococcus aureus*. It can be concluded that the microbial pigment produced can find application in the areas of textile, pharmaceuticals and food industries because of its antibacterial and antifungal properties.

KEY WORDS: *Pencillium sp.* Antibiotic, Antifungal and antibacterial activities.

INTRODUCTION
Among pigments produced by fermentation, pigments had potential use in meats, beverages, sauces and soups[1,2]. Manufacturing procedures of red rice were described in addition to the therapeutic activities,including the bettering of digestion and revitalizing the blood*(3)*. The Antimicrobial activity of *M.purpureus* was first reported in 1977[4,5]. Several wild and mutant strains of *Monascus purpureus* were shown to produce antibiotics active against *Bacillus, Streptococcus and Pseudomonas,* three genera of bacteria generally found in spoiling food. Ethyl acetate extract of fermentation cultures of these fungi were tested for antimicrobial activities against human pathogenic gram-positive and gram negative bacteria and fungi. From these antimicrobial activities use as a preservatives which increase the shelf life of product[6].

MATERIALS & METHODS
Fungal strain
Strain was cultivated in tubes on patato-dextrose-agar at 30ºC for a week. *Pencillium sp.* had higher pigment productivity was used for the study of antimicrobial properties[7].

Pigment biosynthesis
The cultivation of *Pencillium sp.* for the production of pigments was carried out in solid-state fermentation on soaked broken rice and broken wheat. The flasks were sterilized at 121°C for 20 min by autoclaving. The flasks were allowed to cool and inoculated with 10 % of the inoculum and incubated for 10-12 days at 28^0C. After incubation period the flasks were sterilized at 121^0 C for 30 minutes by autoclaving. The sterilized fermented substrates were dried at 50°C for 24 hours. Dried substrates were pulverized and stored for further analysis.

Bioassay of Antibiotic
Antibacterial activity
Antibacterial activity of the compound was tested against human pathogenic test organisms by agar cup diffusion method. The test organisms include against 3 strains of *Bacillus subtilis, Klebsiella pneumonia and Staphylococcus aureus*. The test bacterial pathogens were maintained on freshly prepared nutrient agar slants. Nutrient agar plates were inoculated with 0.2 ml of overnight grown culture. The culture was evenly spread out with a help of a sterile cotton swab. Then agar cups were prepared in the plates with a cork borer (8mm in diameter). Each cup was then loaded with 100µl of the crude extracts. The control cup was filled with distilled water and tetracyline. The plates were incubated at 37°C for 24 h and the zone of inhibition was measured and compared with the control. Three replicates were maintained in each case. The magnitude of antimicrobial action was assessed by the diameter (mm) of inhibition zones and compared with co-assayed antibiotic Tetracycline as antibacterial[6].

Antifungal avtivity
The test organisms include four pathogenic fungi *Trichoderma sp., Aspergillus niger, Aspergillus terreus and Fusarium sp.*. The test fungi were maintained on freshly prepared PDA slants. PDA plates were inoculated with 0.2 ml of cultured test fungi. The culture was evenly spread out with a help of a sterile cotton swab. Then agar cups were prepared in the plates with a cork borer (8mm in diameter). Each cup was then loaded with 100 µl of the crude extracts. The control cups were filled with distilled water and tetracycline. The plates were incubated at 37°C for 24 h and the zone of inhibition was measured and compared with the control. Three replicates were maintained in each case. The magnitude of antimicrobial

action was assessed by the diameter (mm) of inhibition zones and compared with co-assayed antibiotic Tetracycline as antifungal agent.

RESULTS & DISCUSSION

The mixture of pigments synthesized by Pencillium sp. grown on broke rice and broken wheat has been extracted on 80% ethanol and presents for absorbance maxima were expressed as the concentration of pigment produced. The absorbance maxima of pigment extract was found by measuring the absorbance from 375nm-755nm. The maximum absorbance was observed at 375nm. The dominating wavelength of 375nm corresponds to yellow pigments.

Antibacterial activity

The study of antibacterial activity of *Pencillium sp.* was performed by using 2 types of extracts, (extracts of broken wheat and broken rice) obtained in ethanol (extract ratio 8:2). The values of diameters for the inhibition growth zones against 3 strains of *Bacillus subtilis, klebsiella pneumonia and Staphylococcus aureus* are presented in Table 3.1.

In *Bacillus subtilis* strain, inhibition zone in case of broken wheat was 1.4 cm and in case of broken rice was 1.8 cm, while in case of *Klebsiella pneumonia* the inhibition zone in broken wheat was 1.9 cm and in broken rice was 2.3 cm and for *Staphylococcus aureus* the inhibiton zone was in broken wheat was 1.6 cm and in broken rice was 1.3 cm. Highest inhibition zone diameter was observed against *Klebsiella pneumoniae* that was almost equal to the reference antibacterial agent followed by *Staphylococcus aureus.*

TABLE 1: The values of diameters of the inhibition zones for bacterial strains

Bacterial Strain	Inhibition Zone diameter, (cm)					
	Broken wheat			Broken rice		
	PC	BW(I)	BW(II)	PC	BR(I)	BR(II)
Bacillus subtilis	2.6	1.4	1.4	2.7	1.4	1.8
Klebsiella pneumonia	2.3	1.9	2	2.4	1.5	2.3
Staphlococcus aureus	1.9	1.6	1.7	1.7	1.3	1.8

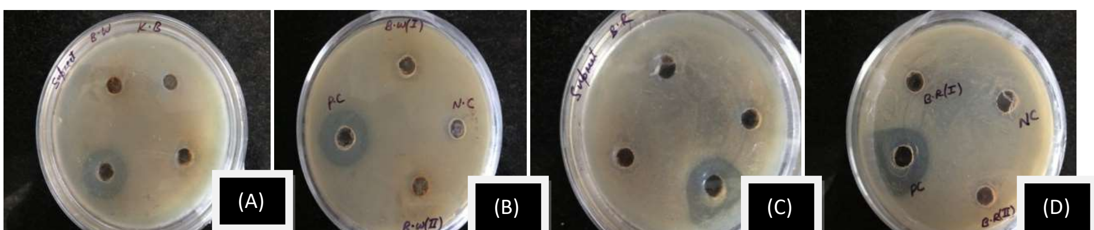

FIGURE 1: Antibacterial activity of the Pigment against *Klebsiella pneumonia* (A) Front view of broken wheat (B) Back view of broken wheat (C) Front view of broken rice (D) Back view of broken rice

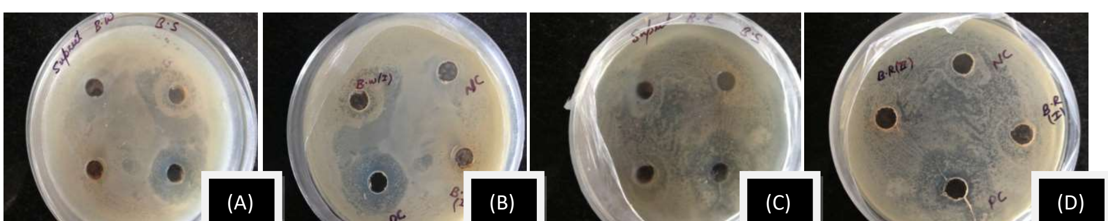

FIGURE 2: Antibacterial activity of the Pigment against *Bacillus subtilis* (A) Front view of broken wheat (B) Back view of broken wheat (C) Front view of broken rice (D) Back view of broken rice

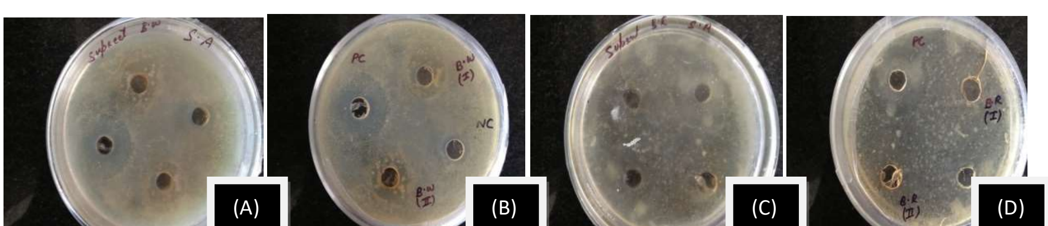

FIGURE 3: Antibacterial activity of the Pigment against *Staphylococcus aureus* (A) Front view of broken wheat (B) Back view of broken wheat (C) Front view of broken rice (D) Back view of broken rice

Antifungal activity

Antifungal activity was estimated using 4 fungal strains as indicator microorganisms, cultivated on potato-dextrose-agar (control plates). The assay of antifungal activity by using the culture demonstrated that, except the *Aspergillus niger* strain, all fungal strains were more or less effective against the antibiotic. The values obtained for the inhibition zone diameters has demonstrated that the extracts obtained on fermented broken wheat and broken rice presented as antifungal to positive control activity which is almost similar but different as function of tested strain type. In case of *Trichoderma sp.* inhibition zone in case of broken wheat was 2.1 cm and in case of broken rice was 2.2 cm while in case of *Aspergillus terreus* inhibition zone only in broken wheat 2.2cm and for *Fusarium sp.* inhibition zone in broken wheat 2.2cm and in broken rice 2.1 cm.

TABLE 2: The values of diameters of the inhibition zones for fungal strains

Bacterial Strain	Inhibition Zone diameter, (cm)					
	Broken wheat			Broken rice		
	PC	BW(I)	BW(II)	PC	BR(I)	BR(II)
Trichoderma sp.	2.3	2.1	2.0	2.1	2.0	2.2
Aspergillus terreus	2.4	2.2	2.2	-	-	-
Fusarium sp.	2.2	1.9	2.2	2.0	2.1	1.8

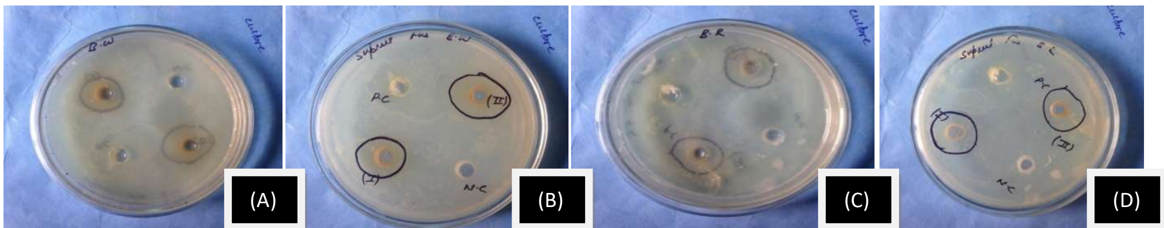

FIGURE 4: Antifungal activity of the Pigment against *Fusarium sp.* (A) Front view of broken wheat (B) Back view of broken wheat (C) Front view of broken rice (D) Back view of broken rice.

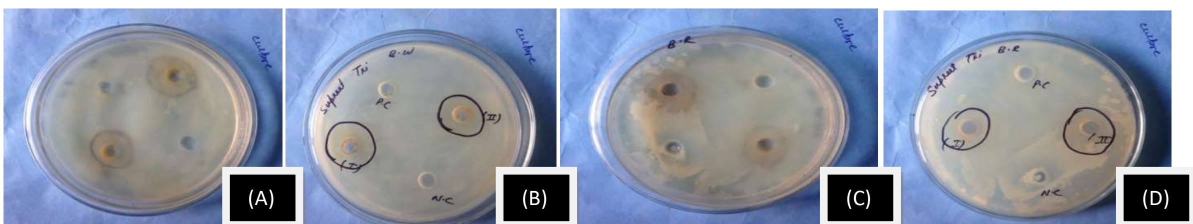

FIGURE 5: Antifungal activity of the Pigment against *Trichoderma sp.* (A) Front view of broken wheat (B) Back view of broken wheat (C) Front view of broken rice (D) Back view of broken rice.

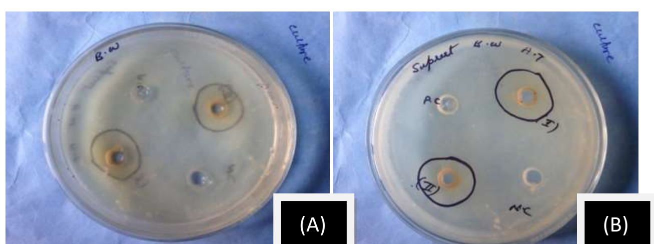

FIGURE 6: Antifungal activity of the Pigment against *Aspergillus terreus* (A) Front view of broken wheat (B) Back view of broken wheat.

CONCLUSION

Pigments produced from natural sources are of worldwide interest and is gaining importance. These pigments are looked upon for their safe use as a natural food dye in replacement of synthetic ones because of undesirable toxic effects including mutagenicity and potential carcinogenicity. By antibacterial and antifungal studies, it revealed that pigment produced by *Pencillium sp.* showed activity against

some microbial strains. Highest inhibition zone diameter (2 cm) was observed against *Klebsiella pneumoniae* that was almost equal to the reference antibacterial agent (tetracycline, 2.3cm). Similarly, the compound showed strong antifungal activity against *Fusarium sp.* and moderate activity against *Trichoderma sp.*

It can be concluded that the microbial pigment produced can find application in the areas of textile, pharmaceuticals and food industries because of its antibacterial and antifungal properties.

According to the American Heart Association, due to elevated level of cholesterol more than one million die each year because of cardiovascular disease. Medications available are very expensive posing side effects ,where Red yeast rice is a natural colourant, containing naturally produced monacolins which has the ability to reduce LDL-cholesterol and increase HDL- cholesterol thus improving heart health, besides a food colourant.

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