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Research Article

Comparison of Different Media for the Pigment Production of Pathogenic and Non Pathogenic *Cryptococcus neoformans* Isolates

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Abstract: Melanin production by species of *Cryptococcus* is widely used to characterize *Cryptococcus neoformans*. An agar medium which contains a precursor of melanin is used to test the pigment production by *Cryptococcus neoformans*. This study aimed to compare the pigment production of pathogenic and non pathogenic *Cryptococcus neoformans* on various media. *Cryptococcus* isolates which were obtained from pigeon droppings and HIV samples were inoculated on different media such as mustard seed agar, henna agar, cabbage agar, and tobacco agar and observed for the rate of growth and pigment production. 10 isolates of *C. neoformans* showed growth on the first day on Tobacco agar medium, whereas 5 isolates showed pigment production on same medium. 8 isolates of C. neoformans showed growth on the first day on Henna medium and 5 isolates showed growth on the same medium.Whereas in mustard seed agar and cabbage agar, there was no *Cryptococcus* growth on first day.
Keywords: *Cryptococcus neoformans*, melanin, virulence, pathogenicity.

INTRODUCTION

Cryptococcosis is an illness that affects a wide variety of mammals including humans, with occasional cases also reported in birds, reptiles, and amphibians. *Cryptococcus neoformans* is an opportunistic human pathogen and primarily affects people who are immunosuppressed; however, this does not seem to be the case for *Cryptococcus gattii* in humans, or for either organisms in animals. Colonies of melanin producing *Cryptococcus* species show a display of colours varying from brown to black when grown in agar media such as sunflower seed agar (Helianthus anus), Niger seed agar (*Phalaris canariensis*), bird seed agar (*Guizotia abyssinica*), Potato-carrot agar, and other chemically defined media as L-dopa and caffeic acid agar[1]. Some recent studies have shown the production of pigment in mustard seed and chilli pepper agar, Pinus halepensis seed and black berry agar and in media containing substrates methyl dopa, epinephrine, and nor-epinephrine [2-6]. Another useful medium is Pal's medium based on similar principle [7]. These media are useful in selecting colonies of *Cryptococcus neoformans* from mixed cultures expected from environmental samples and clinical samples such as respiratory specimans and urine. The property of producing brown colonies on these media provides a definitive identification of *Cryptococcus neoformans* and is due to production of black pigment from various substrates which accumulates in the fungal cell due to an enzyme laccase [8].

The laboratory identification of medically important *Cryptococcus* species takes into account the particular characteristics of this genus. The majority are yeasts that produce capsules, are able to grow at 37 ° C, and produce enzyme urease and laccase. When cultured in media containing phenolic or polyphenolic substrates, they form a pigment called melanin [9]. Enzyme laccase present in yeast act on these phenolic substrates generating quinones, which undergo a process of autopolymerization and turn into melanin. The dark pigment retained in the cell wall of the fungus is responsible for the color shown by the colonies [10-11]. Other species of *Cryptococcus* may also produce melanin in the media, but also intensely as *Cryptococcus neoformans* and *Cryptococcus gatti* [1, 12]. The non pathogenic Cryptococcus isolated from pigeon droppings lack virulent factors like melanin synthesis, as virulent factors go, those of *C. neoformans* would be considered low-grade. Hence, This study was undertaken to differentiate pathogenic Cryptococcus neoformans from non pathogenic Cryptococcus neoformans for the pigment production of on various media.

MATERIALS AND METHODS

This study was carried out in the Microbiology division of Botany department, St.Joseph's college, Tiruchirappalli. A total of 21 *C. neoformans* isolates which were obtained from pigeon droppings. Total of thirty three samples of pigeon droppings were collected from 10 different sites in Tiruchirappalli district. Similarly pathogenic *Cryptococcus neoformans* isolates were also taken from sputum samples of HIV/AIDS patients of Namakkal district. The isolates were inoculated on the following media: 1.Mustard seed agar, 2.Henna agar,3.Cabbage agar,4.Tobacco agar for the pigment production. All the media were prepared in

the media section of the microbiology laboratory as per the techniques which are described by other workers [4, 7, 13, 14, and 15]. They were incubated at 37°C for a period of 2 weeks. The plates were observed daily for growth and pigment production. *Candida albicans* was used as a negative control.

RESULTS AND DISCUSSION

A comparative study of pathogenic and non pathogenic *Cryptococcus neoformans* isolates were tested for the production of pigments. A total of 10 pathogenic *Cryptococcus* isolates were collected from the HIV/AIDS positive patients from Namakkal district and 21 non pathogenic isolates were collected from pigeon droppings in Tiruchirappalli district. The growth of pathogenic *C. neoformans was* observed on all the media. Ten isolates of *C. neoformans* showed growth on the first day on Tobacco agar medium, whereas five isolates showed pigment production on same medium. Tobacco agar is the suitable medium for the appreciation of the brown pigment of *C. neoformans*. A maximum number of isolates showed pigments on this media within 72 hours [16]. Tendolkar *et al* also reported the same finding, where all the isolates produced a brown pigment within 48-72 hours of their incubation. In the present study, 8 isolates of *C. neoformans* showed growth on the first day on Henna medium and five isolates showed pigment production on the same medium. Our results were contradictory with the results drawn by Nandhakumar *et al* in 2006; he reported that all isolates of *C. neoformans* produced a brown pigment on Henna medium at 24 hours post inoculation, whereas in mustard seed agar and cabbage agar, there was no growth on first day. But, on the second day of inoculation growth was observed and on 4th day pigment production was observed in mustard seed agar medium. In cabbage agar medium the growth was observed on second day and pigment production was observed on 3rd and 4th day. In various media, non pathogenic isolates did not produce any pigment(Table-1; Fig.1) But *Cryptococcus* growth was observed on the first day on henna agar and tobacco agar, whereas in mustard seed agar and cabbage agar, the growth was observed within 3 days(Table -2,3; Fig.2) Pigment (Melanin) production, is one of the virulence factors which play an important role in pathogenicity. One characteristic that differentiates pathogenic isolates of *C. neoformans* from nonpathogenic isolates and other *Cryptococcus* species is the organism's ability to form a brown to black pigment on a medium [17]. Hence, non pathogenic *C.neofromans* did not produce pigment because of the lacking of virulence factor. As virulence factors go, those of *C. neoformans* would be considered low-grade. Virulence factors increase the degree of pathogenicity of a microbe. *C. neoformans* has a number of virulence factors; generally, the virulence of an isolate cannot be attributed to any single factor, but rather it is attributed to many working in unison to cause progressive disease [18].

Table -1 Mean day of growth and pigment production of non pathogenic *C. neoformans*

Media	Day of growth	Day of pigment
Mustard seed agar	2.4	Nil
Cabbage agar	3.25	Nil
Henna agar	1	Nil
Tobacco agar	1.2	Nil

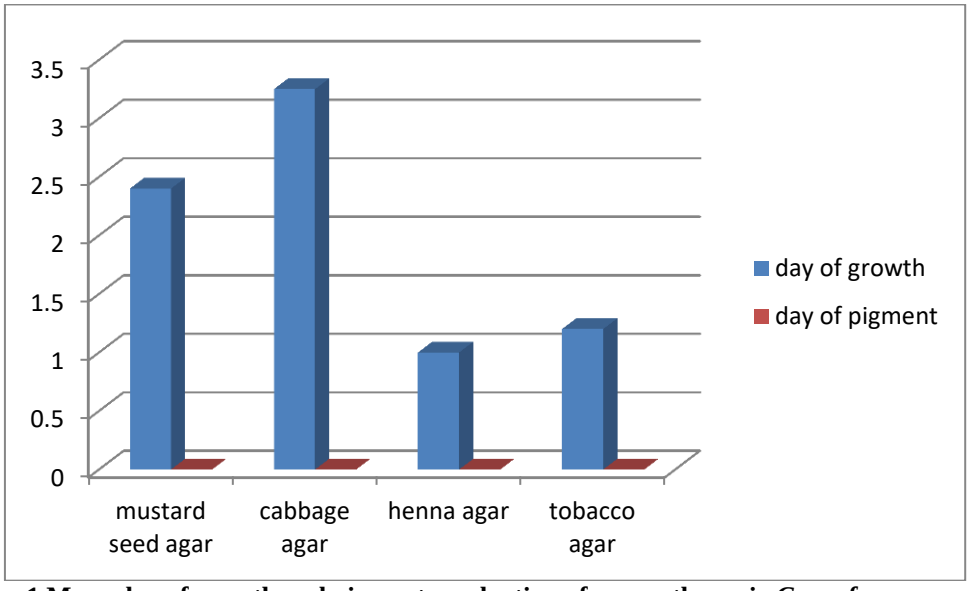

Figure -1 Mean day of growth and pigment production of non pathogenic *C. neoformans*

Table -2 Mean day of growth and pigment production of pathogenic *C. neoformans*

Media	Day of growth	Day of pigment
Mustard seed agar	2.2	3.0
Cabbage agar	3	3.5
Henna agar	1	2.0
Tobacco agar	1.5	2.3

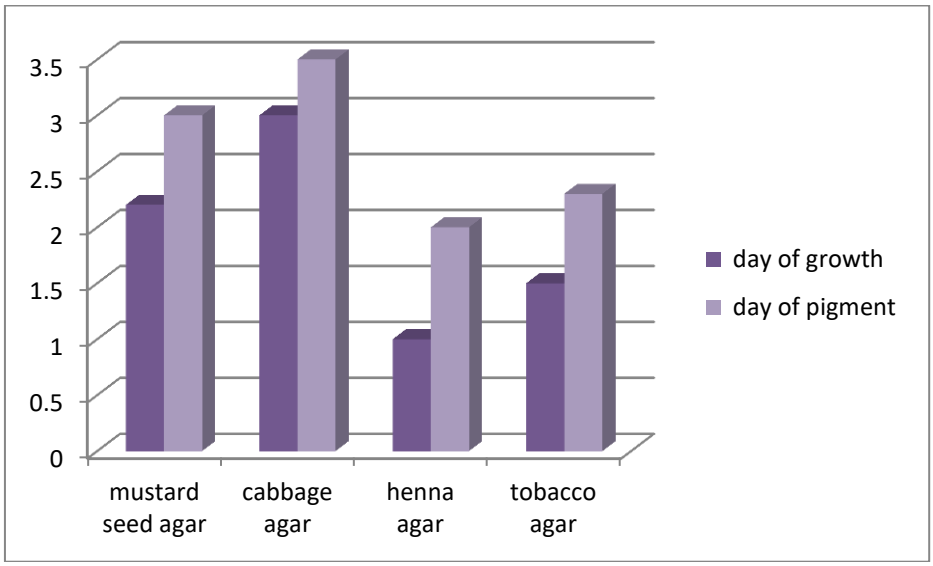

Figure -2 Mean day of growth and pigment production of pathogenic *C. neoformans*

Table – 3 Number wise distribution of pathogenic *Cryptococcus neoformans* on different growth media

G/P	Mustard seed agar	Cabbage agar	Henna agar	Tobacco agar
1/2	-	-	5	-
1/3	-	-	3	5
2/4	7	3	2	5
3/4	2	4	-	-
4/5	-	3	-	-
5/6	1	-	-	-

CONCLUSION

It can be concluded from the present investigation that the various media tested for the pigment production was the most valuable tool for the presumptive differentiation of pathogenic and non pathogenic *Cryptococcus neoformans* isolates

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