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Alternative culture media for fungal growth using different formulation of protein sources

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ABSTRACT

The exorbitant costs of culture media have deprived the use of readymade culture media such as Potato Dextrose Agar (PDA) in schools and laboratories with financial limitations. Generally legume seeds are found to be a good protein source for nutritional purposes. This study was carried out to find the feasibility of using legume seeds as an alternative nutrient source to grow fungi. Cowpea, green gram, black gram and soya meat (processed soya bean) were used in this study. The test organisms used were Aspergillus, Trichoderma, Fusarium, Sclerotium and Pencillium sp. On average Fusarium sp shows significantly ($p<0.05$) higher growth (5.85±0.18) in blackgram and Aspergillus shows significantly ($p<0.05$) less growth (1.58±0.31) in PDA. In comparison with the performance on conventional potato dextrose agar (PDA) media, the prepared protein formulations were found to be relatively cheap and good alternative culture media for mycological studies.

Keywords: Culture media, fungi, protein formulation, PDA

INTRODUCTION

Microorganisms need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce. Culture media used in the laboratory for the cultivation of microorganisms supply the nutrients required for growth and maintenance. PDA is a common medium used to grow fungi in laboratories. This is a basic medium composed of dextrose, potato extract and agar. As the readily available culture media are expensive, there is a need to find alternative media or reduce the amount of agar added during the preparation of culture media in order to reduce the overall cost involved. Few of the studies described below have focused on addressing this issue. Arnan-Parh *et.al* has worked on cowpea as a cost effective alternative culture media for the growth of bacteria [1]. Though the authors have used cooked (boiled) cowpea to increase the shelf life up to three months in their study, we presume that it is not essential to boil as cowpea is readily available. There are reports that used starch sources such as sago, palmyrah tuber flour, tubers of sweet potato and cassava as alternative growth media for fungi [2, 3]. Further there are also reports using vegetables as an alternative source for preparing culture media for the growth of fungi and bacteria [4]. The present study is aimed at replacing the nutrient source by a solid form of protein formulation. This is because most of the other studies cited here do not use substrate in dried form and they require an additional step of drying except for sago before processing further. Legumes such as cowpea, green gram, black gram and soya meat serve as a good protein source and they are locally available cheap materials. These protein sources were selected to formulate the media for the growth of some selected fungi.

MATERIALS AND METHODS

Collection of samples

Edible legumes such as green gram, black gram, soya meat and cowpea were purchased from supermarkets in Jaffna and identity was confirmed using a taxonomist in the University.

Solid media formulation

The samples were cleaned and finely powdered using electric blender and sieved as fine powder. The powder was stored separately in sterile containers until its use. Four different solid media were prepared as follows. 3 g from each protein source was taken and mixed with 0.5-3.0 g agar (HIMEDIA) and dissolved in 100 ml distilled water. The solidification times of each media preparation was recorded in triplicates. Finally 2 g of agar and 3 g of each protein formulation of pH of 5.6±0.2 were used in all successive experiments.

Microbial Inoculation

The standardized culture of each test fungi namely *Aspergillus*, *Trichoderma*, *Penicillium*, *Fusarium* and *Sclerotium* sp were prepared by dissolving 3.9 g of PDA in 100ml distilled water, autoclaving, pouring and inoculating into plates. The inoculated plates were incubated at 28±3 °C for 2 days. Then a fungal disc was cut by using sterile cork borer and placed on the surface of the relevant culture media in six replicates. The organisms introduced on PDA media served as control.

After incubation, the plates and respective controls were observed for the degree of growth in terms of the diameter (cm) in two different directions in perpendicular to which fungal hyphae have grown.

Statistical data analysis

All data were statistically analyzed by STATISTICA software (version 6; Statsoft Inc., Tulsa, USA). The diameter of the fungal growth obtained for each protein formulation as well as the specific fungi growing on different protein formulation were first analyzed by one-way analysis of variance (ANOVA) when the results of the one way ANOVA show the mean values of the samples are significantly different, the ANOVA was followed by Post hoc comparison of means and Duncan's Multiple Range Test (DMRT), using STATISTICA 6.0 software. The level of statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

The results showed that when the different protein sources were used with varying proportions of agar have different solidification times as expected. However similar solidification time for PDA was obtained when 1.5-2.0 g of agar was added to different protein sources which is consistent with the results obtained for bacterial growth media produced by Deivanayaki and Iruthayaraj for vegetable sources and Annan Prah et al. for Cowpea alone [4, 1]. However if the amount of agar used is increased, approximately there is a reduction of time duration taken for solidification (Table 1.1).

Table 1: Mean solidification times of various protein sources with different proportions of agar

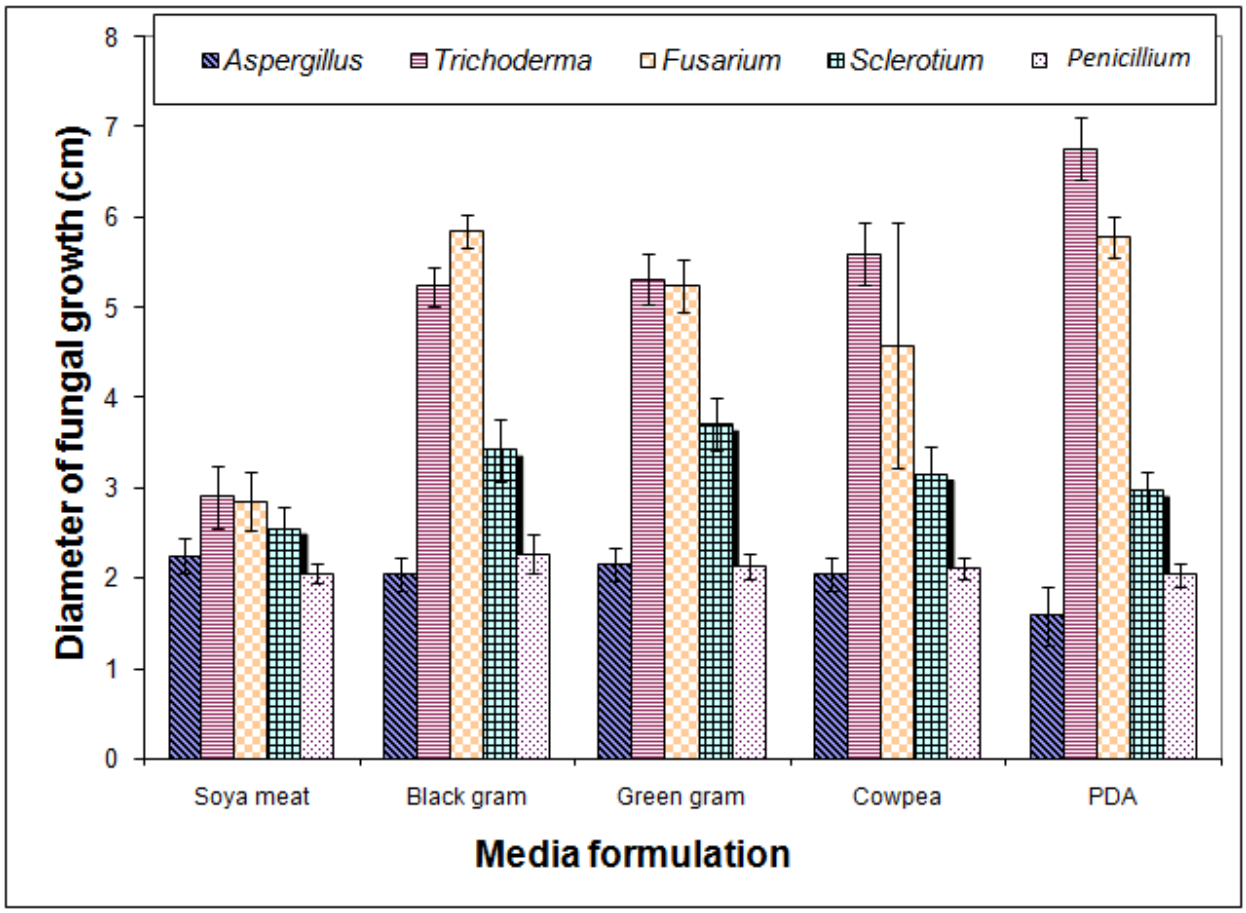
Weight of protein sources(x)g	Weight of agar(Y)g	SOY MEAT (mean setting time) (min)	BLACK GRAM (mean setting time) (min)	GREEN GRAM (mean setting time) (min)	COWPEA (mean setting time) (min)
3	1	>43	42	43	42
3	1.5	37	41	33	30
3	2	33	34	30	24
3	2.5	31	33	28	22
3	3	22	18	22	20
3	3.5	16	14	18	17
3	4	12	12	13	12

The mean value was obtained from the experiment carried out in triplicates. PDA - mean solidification time 15 min. X –weight of cowpea, soy meat, black gram and green gram. Y -Weight of agar powder.

Even though the pH values were kept constant at 7 throughout the experiment, it was our view to check whether these protein formulations can be used without adjusting the pH as many of the schools in the developing countries

lack a pH meter. The pH values of different formulations ranged from 6.12- 6.89. It was interesting to note that there was no significant change (data not shown) in the diameter of growth whether we adjusted the pH to 7.0 or went on with the experiments as such.

Figure 1: Fungal growth in different protein formulations



Graph drawn using Microsoft Excel (2003). Error bars show standard deviation.

The formulated media consisting of protein source and agar supported the growth of all test organisms (Fig. 1). *Trichoderma sp* recorded significantly ($p<0.05$) higher growth (6.76±0.34) in PDA which is followed by cowpea (5.60±0.34), greengram (5.31±0.27) and blackgram (5.23±0.21). *Fusarium sp* showed significantly ($p<0.05$) higher growth in blackgram (5.85±0.18) and PDA (5.78±0.23) which is followed by greengram (5.25±0.28) and cowpea (4.58±1.35) Moderate growth was observed for *Sclerotium sp* which showed 3.70±0.28 in greengram, 3.41±0.34 in blackgram, 3.15±0.30 in cowpea and 2.96±0.21 in PDA. *Aspergillus* and *Penicillium* did not show significant ($p<0.05$) growth among the four alternative protein sources tested and the least growth in PDA (1.58±0.31 and 2.03±0.13 respectively)

A number of studies have been carried out to find alternative source of culture media to replace nutrient agar and PDA. In a study Sago was effectively used to replace nutrient source as well as agar for the growth of selected bacteria [3]. It has been shown that coconut (*Coccus nucifera*) liquid endosperm will alone support the growth of bacteria (unpublished data). In another study vegetable was successfully used as a nutrient source with agar for microbial growth [4]. However caution should be exercised while extrapolating the results because the requirements for vegetative growth are generally less stringent when compared with that of sporulation (5).

PDA is used as a common culture medium to grow various fungi. The cost of 1kg of PDA (Biochmika) is approximately $ 100 (12,750LKR). It costs around 400 LKR to prepare 1 litre of PDA medium whereas it costs approximately 300 LKR to prepare same volume of different protein formulations. Thus the use of different protein formulations as culture media in laboratories with basic facilities is very much feasible and cheaper when compared to commercially prepared PDA. Although these protein formulations can be prepared instantly, they can even be stored in air tight containers for about three months at room temperature in tropical climate.

CONCLUSION

Based on the findings of this study, it is concluded that, *Fusarium sp* showed significantly ($p<0.05$) higher growth in blackgram and *Sclerotium sp* showed the highest growth in greengram among the tested protein sources. *Penicillium sp* exhibited significantly ($p<0.05$) higher growth (2.26 ± 0.22) in blackgram. *Aspergillus sp* shows significantly less growth in PDA when compared with that of all the protein formulations. All the tested fungi except *Trichoderma sp* did not show higher growth in PDA.

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