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STUDIES AND ANALYSIS OF GROWTH OF *ASPERGILLUS NIGER* FUNGI ON DEPROTEINIZED LEAF JUICE (DPJ)

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ABSTRACT: Current study provides the growth of Aspergillus niger fungi on Deproteinized Leaf Juice (DPJ). The process of green crop fractionation results into three major fractions i.e. pressed crop residue (PCR), leaf protein concentrate (LPC) and deproteinized juice (DPJ). The PCR has been recommended in animal nutrition (Raymond and Harris, 1957). The leaf juice expressed during fractionating of green foliage is employed for the preparation of food grade leaf protein concentrate (LPC). The fungus Aspergillus niger grew well on DPJ samples. The growth of the fungus was evaluated on the basis of the amount of mycelia dry weight(MDW) per 25 ml of DPJ obtained after filtration. The Deproteinized juice (DPJ) released during fractionation of green foliage is considered as a by-product. It is rich in soluble plant nutrients i.e. sugars, free amino acids, amides, vitamins, minerals and other nutrients of plant origin. Random disposal of DPJ may cause environmental bio-pollution and therefore its use as a source of fertilizer, in animal nutrition or as a medium for growing useful microorganism has been advocated to avoid pollution as well as to make the process of GCF more efficient and economical.

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1.INTRODUCTION

The process of green crop fractionation results into three major fractions i.e. pressed crop residue (PCR), leaf protein concentrate (LPC) and deproteinized juice (DPJ). The PCR has been recommended in animal nutrition (Raymond and Harris, 1957). The leaf juice expressed during fractionating of green foliage is employed for the preparation of food grade leaf protein concentrate (LPC). For this purpose, it is heated for the coagulation of proteins in it, which results into a curd

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referred as LPC. The LPC is a food grade product and is used as a source of protein and vitamin A in human and poultry diet. The LPC is separated from the remaining portion of the juice i.e. deproteinized juice (DPJ) by filtration. The DPJ is a by-product of GCF system, which is produced in large volume. About 50% of fresh weight from green foliage contributes the DPJ, which is rich in water soluble nutrients like carbohydrates, free amino acids, minerals, lipids, and vitamins. It also contains a small fraction of protein (Barnes, 1976; Shahane and Mungikar, 1985). This product, with 4 to 5% solids, is generally rich in nitrogen and phosphorus (Ream et al., 1983). In addition, Festenstein (1961) observed that this fraction may contain from 11 to 47 g dry matter (DM), 0.25 to 1.25 g nitrogen (N) and 2.5 to 22.0 g carbohydrates per litre. At Rothamsted 1experimental Station in UK the DM content in this fraction was found to be between 1.2 to 4.0%. The data on the chemical composition of DPJ obtained from 10 crops has been given by (Ajaykumar, 1989). It is evident from the data that the percent DM and nutrient composition of this fraction varies from species to species. The DPJ from Lucerne contained as high as 8.2% DM. Pirire (1971) stated that, on an average, the N and carbohydrate content in the DM of DPJ are 3 and 40% respectively. Ajay kumar (1989) showed that the N content in the DM of DPJ fluctuated between 1.67 and 4.33% while the amount of carbohydrate between 25 to 54%, depending on the species from which it was derived. The dominant monosaccharides in DPJ are glucose and fructose, however, the content of these reducing sugars in DPJ is subject to great variation, depending upon the species and the maturity of the plants used. It varies from 0.5 to 6.0% (Bakeris et al., 1983). The contents of nitrogenous substances also vary widely. It has been emphasized by Pirie (1942) that this product should be disposed properly to avoid local environmental bio-economical and efficient, proper use of the nutrients in DPJ has to be made. It is well known that the DPJ contained biologically active substance like sugars, amino acids and vitamins. These are essential components of the nutrient media which may be useful for cultivation of microorganisms. The presence of carbohydrate in this product makes it suitable for microbial biomass production. The research on the suitability of DPJ as a culture medium started in Uppsala during 1955. Jonsson (1962) cultivated seven types of microorganisms on DPJ from pea vines and other leafy materials. He observed that the DPJ supports growth of Rhizobium meliloti, Penicillium chrysogenum and Aspergillus niger. Worgan and Wilkins (1977) studied growth of the species of Fusarium, Trichoderma and Aspergillus on DPJ of Lucerne. Pirie (1971) also stressed that uncoagulable material in DPJ is suitable for the cultivation of microorganism. Ream et al., (1983) recommended the use of DPJ for the production of single cell protein (SCP) in a fermentation process. Butt et al., (1972) used DPJ for propagation of yeast. Pardez-Lopez and Gagagro (1973) used Lucerne DPJ for single cell (SCP) production. They observed that DPJ supported growth of yeast. Kummerlin (1984) obtained good yields of Candida utilis on DPJ from 10 plant species. However, Pirie (1987) is of opinion that the filamentous fungi are better than yeasts for biomass production on the DPJ as the former can be collected by filtering, rather than centrifuging as is done for SCP

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production. Furthermore, mycelia tend to contain less nucleic acids than yeast, and therefore, use of DPJ for the cultivation of some edible fungi is most suitable. At the Indian Statistical Institute, Calcutta, Chanda et al., (1984) made attempts to grow commercially important microorganism on DPJ samples obtained from various crops. With few exceptions, DPJs were found to support the growth of bacteria, fungi and actinomycetes. It was observed that the samples of DPJ from cruciferous and leguminous plants could be used successfully for the production of various types of microbial metabolites such as lipase, citric acid, penicillin and yeast SCP. Chanda (1983, 1985) specially recommended its use for the production of antibiotics. At the Institute of Science, Aurangabad, Baukhandi et al., (1984) used lucerne DPJ for SCP production from Candida tropicalis and C. lypolytica. They observed rapid growth of these yeast strains on DPJ and pointed out that about 1.5 to 4.0 g of dry cell mass could be obtained from 100 ml of DPJ. These results also indicated the nutritive sufficiency in the DPJ for microbial growth. At the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad Deshpande and Joshi (1971) made attempts to grow eight different fungi on DPJ obtained after LP extraction. It was observed that this by-product was a good substrate for the growth of fungi. Five fungal species of Aspergillus, Fusarium, Helminthosporium, Penicillium and Phoma were cultivated by Ghewande and Deshpande (1975) on the DPJ expressed during fractionation of hybrid Napier grass. They also reported the possibility of utilizing this by-product for the production of microbial biomass. On the contrary. Mukadam et al., (1976) observed that the DPJ from some plants may inhabit germination of pathogenic fungal spores. Umalkar et al., (1976) found that the DPJ supports growth of fungi. They attributed this to the presence of certain nutrients like sugar, amino acids and salts in DPJ. Gangawane and Nehemiah (1980), using the DPJ from lucerne, successfully grown different rhizobial strains.In the present investigation attempts have been made to study the microbiological aspects of DPJ in detail. For this purpose, the samples of DPJ obtained from several plant species were employed for cultivation of Aspergillus niger. Experiments were also undertaken to explore the possibility of growing these fungi efficiently by correcting nutrient status of the deproteinized juice.

2.MATERIALS AND METHODS

Preparation of DPJ

Green foliages from 100 plants were employed for green crop fractionation (GCF) as listed and explained in Chapter III. The deproteinized juice (DPJ) released during the fractionation was collected and dried in hot air oven at 65^{o}C till constant weight. The samples of one hundred dry DPJ were stored in sealed glass bottles until used. While storing adequate care was taken to reduce absorption of moisture by the DPJ samples.

Preparation of culture media

The fungi (*Aspergillus niger*) was cultivated on synthetic glucose nitrate (GN) medium as well as on aqueous solution of the DPJ. The GN medium was prepared by dissolving glucose 10 gm, KNO_3

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2.5 gm, KH_2PO_4 1 gm and $MgSO_4$ 0.5 gm in one litre of distilled water (Shinde, 1982). Oven dry DPJ alone was dissolved in distilled water at various concentrations and was used for the growth of fungi.

Sterilization

Twenty five ml of either the GN medium or the solution of DPJ was poured into 100 ml conical flask. The flasks were the plugged with non-absorbent cotton and autoclaved at 15 lbs for 30 minutes.

Inoculation

The autoclaved flasks were transferred into the inoculation room for inoculating with Aspergillus niger. The stock fungal culture was collected from the Departmental culture collection, wherein the fungi were maintained on Potato Dextrose Agar (PDA) medium. The inoculation was always done under the hood of laminar air flow. The inoculums in the from of spore suspension was prepared by adding 10 ml sterile distilled water to a six day old slop culture of the fungus. The medium, either GN or DPJ, was inoculated with 5 drops of the spore suspension which contained 5 x 102 spores per microscopic field. The inoculated flasks were incubated for seven days at room temperature.

Collection of microbial biomass

After each incubation period (7 to 10 days) the fungal biomass was harvested by filtration through pre-weighed Whatman No. 1 filter paper. The mycelia biomass was dried along with the filter paper in an oven at 65 ≠ 5oC till constant weight. The yield of mycelia dry weight (MDW) was then recorded by substracting the weight of filter paper. In all experiments, each treatment was replicated for 3 times. At each experiment a control flask was also kept simultaneously wherein the flask remained un-inoculated. The MDW was corrected each time by substracting the dry weight obtained from control flasks. The data obtained on the yield of MDW were statistically analysed for the calculation of standard deviation (CD) and coefficient of variation (c.v.), and value of "t", following Panse and Sukhatme (1978) and Mungikar (1997, 2003).

3.RESULTS AND DISCUSSION

The fungus Aspergillus niger grew well on DPJ samples. The growth of the fungus was evaluated on the basis of the amount of mycelia dry weight(MDW) per 25 ml of DPJ obtained after filtration. When the yield of MDW on 1% DPJ was compared to that obtained on GN medium, it was observed that on the DPJ of almost all plants the weight of MDW was more than that obtained on GN medium except eight DPJ samples where the weight was less than that on GN medium. When the fungus was grown on 2% DPJ the yield of MDW was higher in all hundred DPJ samples than that obtained on GN medium (Table 1). The weight of MDW on GN medium was 0.054 g per 25 ml, it ranged from 0.020 to 0.171 g on 1% DPJ while 0.076 to 0.266 on 2% DPJ. On an average 1% DPJ yielded 0.076 g MDW while 2% yielded 0.151 g MDW per 25 ml of DPJ. Thus when DPJ was used at 2% concentration the average yield MDW was at par to that obtained on GN medium. The variation in

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the fungal growth among 100 plants under investigation was almost similar (30%) at both concentrations of DPJ studied. On the basis of "t" test there was significant difference in yield of fugal biomass when the DPJ was used at higher concentrations. The results obtained thus indicate that the DPJ at concentration of 2% from most of the species was suitable for cultivation of fungi. Figure 1 and 2 illustrates the distribution of microbial biomass produced on the 100 samples of DPJ in the form of frequency curve. Both the frequency curves were normal showing normal distribution of fungal biomass production on the DPJ from various plants with maximum frequency for 74.5 mg for 1% DPJ and 178.5 mg for 2% DPJ (Fig.1). Apart from microbial biomass production in the form of MDW the sporulated by fungi was also observed. The fungus sporulation favourably on all DPJ samples from all of the plants. The amount of sporulation was higher when 2% DPJ was used.

The overall results obtained during present investigations supports the findings reported by the earlier workers in this laboratory. The overall results indicated suitability of DPJ as medium for growth Aspergillus niger, a commercially important fungus employed for the production of organic acid and enzymes.

4.CONCLUSION

The Deproteinized juice (DPJ) released during fractionation of green foliage is considered as a by-product. It is rich in soluble plant nutrients i.e. sugars, free amino acids, amides, vitamins, minerals and other nutrients of plant origin. Random disposal of DPJ may cause environmental bio-pollution and therefore its use as a source of fertilizer, in animal nutrition or as a medium for growing useful microorganism has been advocated to avoid pollution as well as to make the process of GCF more efficient and economical. Earlier research workers from this laboratory have shown that DPJ is most suitable for growing fungi, bacteria and Actinomycetes (Baviskar et al., 1999). However the studies undertaken by them were restricted to the DPJ of Lucerne and few other plants. during present investigation 100 plants, wild as well as cultivated were investigated by preparing DPJ from them for the growth of fungi. It was confirmed that the fungi like A. niger can grow on DPJ from majority of the plants under investigations. Furthermore the 2% concentration of DPJ was found to be suitable for fungal biomass production. The failure in growth of fungus on some of the plants like Goniogyna hirta, Millettia ovalifolia, vigna unguiculata, Alternanthera sessilis, Amaranthus hybridus, A. viridis, Annona squamosa, Carrisa congesta, Jasminum multiflorum, Malvostrum coromandelianum and Peristrophe paniculata etc. might be due to the presence of toxic compounds in the DPJ, which has to be confirmed. Thus it can be concluded that the DPJ can be used for growing useful microorganisms and exploited in the fungal biotechnology for the production of various metabolites like enzymes, antibiotics, alcohol, single cell protein (SCP), toxins etc.

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