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RESEARCH PAPER

Mycelial biomass production of medicinal mushroom *Ganoderma* P. Karst.

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Abstract : The fruiting body, mycelia and spores of *Ganoderma* contain approximately 400 different bioactive compounds. At present, the demand for natural products with pharmacological activity and medicinal uses is markedly increasing and the submerged culture offer faster production of mycelia biomass with high nutrients and exopolysaccharide of medicinal importance in shorter period of time within reduced space and gets lesser chances of contamination with consistent quality. Mycelial biomass production of *Ganoderma* was carried out using five different liquid media *viz*., Glucose aspargine media (GLM), Hwang liquid media (HLM), Potato dextrose broth (PDB), Yeast wine media (YWM) and Glucose peptone liquid media (GPM). The highest dry weight was observed in yeast wine media (1.08g/300ml).

Key Words : Bioactive compound, Dry weight, Exopolysaccharides, Liquid media, Mycelial mat

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INTRODUCTION

Traditionally the edible or medicinal mushrooms were cultivated in solid cultures which take several months to produce fruiting bodies and the bioactive metabolites produced from these fruiting bodies not retain consistent quality. At present, the demand for natural products with pharmacological activity and medicinal uses is markedly increasing and this is the case with mushrooms from the genus *Ganoderma* (Gonzalez *et al.,* 2002). In addition, attempts are being made to obtain useful mycelial products or to produce effective substances into a medium by means of liquid culture of the mycelia (Nasreen *et al.,* 2005). *Ganoderma* is one of the oldest mushroom known to be used medicinally and a famous traditional Chinese medicine which has been used as tonic and invigorating medicine (Yan-Qun and Zhi-Cong, 2012). It has a worldwide distribution in both tropical and temperate geographical regions, including South and North America, Africa, Europe and Asia, growing as a parasite or a saprotroph on a wide variety of trees. Nutritionally, *G. lucidum* contains mainly protein, fat, carbohydrate and fibre.

Medicinally, over 300 reports have been published concerning the constituents of *Ganoderema* species. The fruiting body, mycelia and spores of *Ganoderma* contain approximately 400 different bioactive compounds, which mainly include triterpenoids, polysaccharides, nucleotides, steroids, fatty acids, protein/peptides and trace elements (Wasser, 2005). Bioactive polysaccharides in mushroom

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can be extracted from the mycelia of the mushroom species for medicinal and pharmacological activity. Increasing cell biomass production and active ingredient concentrations can enhance the medicinal efficiency of *Ganoderma* (Yan-Qun and Zhi-Cong, 2012). The submerged culture offer potential advantages of faster production of mycelia biomass with high nutrients and exopolysaccharide of medicinal importance in shorter period of time within reduced space (Kim *et al*., 2009) and gets lesser chances of contamination with consistent quality (Kwon *et al*., 2009). In this paper we studied the mycelial biomass production of *Ganodrma* in different liquid media to determine the effect of different liquid media on biomass yield and their mycelial mat characteristics.

MATERIAL AND METHODS

This experiment was carried out in the laboratory of Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University. Survey for the collection of naturally growing *Ganoderma* was conducted at the different regions of Navsari *viz.,* Navsari Agricultural University campus, Dandi and Gandevi. The sites of collection were soil, open lands, farm lands, roadside, research field etc. The data was analysed using CRD and the difference among mean value was tested by using critical differences (CD) values at 5 per cent level of probability.

Sources of materials:

The fruiting bodies of *Ganoderma* collected from the dead stump of unknown trees and stem of Pink shower, *Cassia grandia* in different location of Navsari, Gujarat during June 2018 to September 2018. Identification was done on the basis of macroscopic and microscopic traits of basidiocarp (Suansia and John, 2020).

Isolation:

Several tissue cultures were prepared from the freshly collected fruiting bodies on sterilized Potato dextrose agar (PDA) and Malt extract agar (MEA) media in the petri plates. Cultures were maintained on sterilized PDA slants at room temperature and regular sub-culturing was done at 20 days interval.

Preparation of different liquid media:

Mycelial biomass production of *Ganoderma* was carried out using five different liquid media *viz*., Glucose aspargine media (GLM), Hwang liquid media (HLM), Potato dsextrose broth (PDB), Yeast wine media (YWM) and Glucose peptone liquid media (GPM) (Table A).

Table A: Five different liquid media and their ingredients for preparation of 1L media

Sr. No.	Liquid media	ingredients for 1L media
1.	Glucose aspargine media	Glucose - 10g
		Asparagine - 2g
		KH_2PO_4 - 1g
		$MgSO_4.7H_2O$ - 0.5g
		$ZnSO_4.7H_2O$ - 0.2g
		$MnSO_4.4H_2O$ - 0.1g
		Thiamine - 0.1g
		Biotin - 5µg
2.	Hwang liquid media	Glucose - 20g
		Peptone - 2g
		KH_2PO_4 - 0.46g
		K_2HPO_4 - 1g
		$MgSO_4.7H_2O$ - 0.5g
3.	Potato dextrose broth	Potato starch - 4g
		Dextrose - 20g
4.	Yeast wine liquid media	Wine - 15ml
		Soybean peptone - 7g
		Yeast extract - 3g
5.	Glucose peptone liquid media	Glucose - 16g
		Peptone - 3g
		Corn flour - 20.93g
		Soybean powder - 6.44g

Inoculation:

Three pieces of 5mm diameter mycelial discs of actively growing five-days-old culture from petriplate of previously isolated *Ganoderma* were transferred into each 1000 ml conical flask containing 300 ml of the liquid media. These flasks were incubated for 7 days in complete darkness.

Harvesting, drying and weighing of biomass:

The culture media containing the mycelia was decanted and each medium was separately filtered using Whatman filter paper no. 01 until a clear filtrate was obtained. The mycelial mat was washed with sterile distilled water to remove any trace of medium and then oven dried at 60 ± 1ºC until a constant dry weight was obtained (Feng *et al.,* 2010). The dry mycelia were weighed in terms of grams per 300ml of media. The mycelial dry weight was obtained by the following

formula (Karthikeyan *et al.,* 2007):

Biomass = Weight of (filter paper + biomass) -Weight of filter paper

Dry mycelium was ground and the air-dried powder was stored in an air-tight container at room temperature for further use.

RESULTS AND DISCUSSION

In the case of Glucose aspargine media white coloured small colonies were scattered on the surface of the media. In the case of Hwang liquid media, Potato dextrose broth and Yeast wine media light, cream thick complete mycelial mat was formed where as in the case of Glucose peptone liquid media white thin incomplete mycelial mat was formed (Table 1 and Fig. 1). Yeast wine media (1.08g/300ml) exhibited significantly more mycelial dry weight as compared to the rest liquid.

The difference in mycelial growth on different media may occur due to availability of different carbon sources, nitrogen sources and other required nutrients. The medium containing glucose and yeast extract was significant in yielding the highest mycelial growth as compared to other carbon and nitrogen sources. Moreover, yeast extracts being a vitamin B complex source, supports effective cell development (Shah and Modi, 2018). Other authors working on basidiomycetes like *Lentinus tuberregium*, *Grifola frondosa*, *Agaricus blazei* and *Psathyerella atroumbonata* also found yeast extract to be the best nitrogen source for the growth of mycelial biomass (Manjunathan and Kaviyarasan, 2011; Lee *et al.,* 2004; Hamedi *et al.,* 2007 and Jonathan and Fasidi, 2001).

The results corroborate the earlier findings of Nithya *et al*. (2014) that mycelial mat of *Ganoderma* was white to light cream, scattered, more or less extensive. Bhattacharyya (2015) studied the growth of mycelia of *Ganoderma* in glucose aspargine medium (Lilly and Burnett, 1951) and reported that the faster production of mycelia biomass with high nutrients and exopolysaccharide was observed in shorter period of time within reduced space.

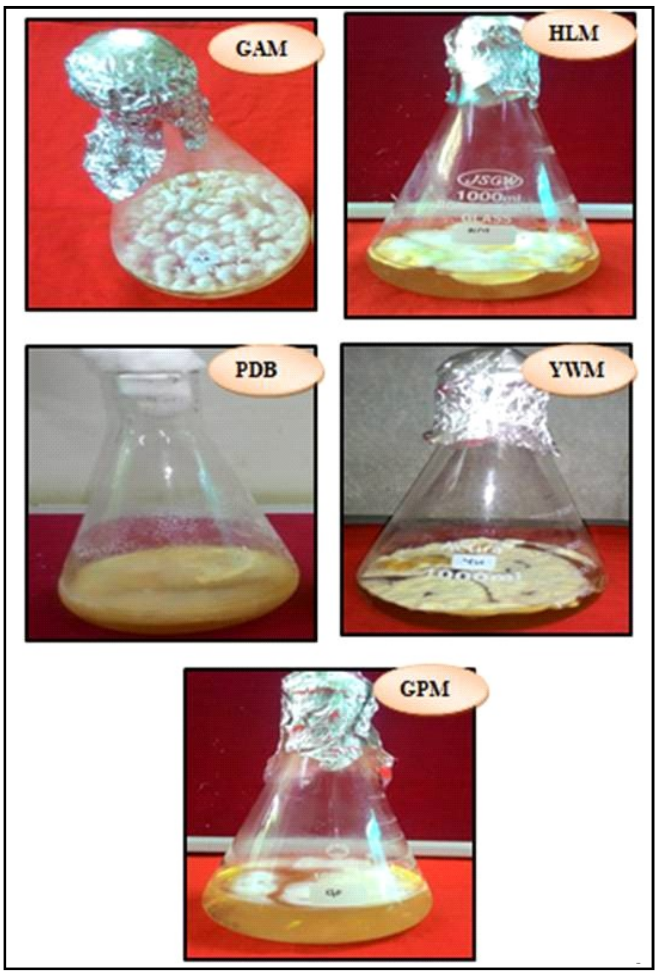

Fig. 1 : Mycelial biomass production of *Ganoderma* isolate on different liquid media

Conclusion:

It can be concluded from this study that Yeast wine

Table 1: Mycelial biomass of *Ganoderma* isolate

Sr. No.	Liquid media	Dry weight (g/300ml)	Characteristic of mycelial mat
1.	Glucose aspargine media	1.16* (0.84)	White coloured small colonies, scattered on the surface of media
2.	Hwang liquid media	1.24 (1.04)	Light cream thick mycelial mat
3.	Potato dextrose broth	1.17 (0.88)	Light cream thick mycelial mat
4.	Yeast wine media	1.25 (1.08)	Light cream thick mycelial mat
5.	Glucose peptone liquid media	1.13 (0.78)	White thin mycelial mat
	S.E.±	0.01	-
	C.D. (P=0.05)	0.02	-

*Figures are square root (X + 0.5) transformed values Figures in parenthesis are original value

media was the best for the mycelial biomass production. This might be due to presence of yeast extract and other required nutrients. Scientifically yeast extract is vitamin B complex sources required for the cell development and growth of *Ganoderma*.

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