ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 09, pp.56887-56893, September, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

COST EFFECTIVE ALTERNATIVE FUNGAL CULTURE MEDIA FORMULATION USING FRUIT AND VEGETABLES WASTE

Arati Kadam, Suraj Patil, Mrunalini Sonne, Kirti Dahigaonkar, Jaspal Kaur Oberoi and *Pratibha Jadhav

Abeda Inamdar Senior College, 2390, KB Hidayatullah Road, Azam Campus, Pune - 411001

ARTICLE INFO

Article History:
Received 15th June, 2017
Received in revised form
07th July, 2017
Accepted 23rd August, 2017
Published online 29th September, 2017

Key words:

Culture media,
Cost effective alternative media.

ABSTRACT

Potato Dextrose Agar is general purpose medium used for the cultivation of broad range of fungi. Use of commercially available culture media for research purpose is costly so cheap culture media needs to be formulated. The feasibility of developing alternative culture media for PDA was assessed using locally available cheap materials such as Vegetables and fruits wastes, as they contain considerable amount of carbohydrate, protein and macro elements. These wastes are easily available in local shops, vegetable markets and kitchen. Now a day's waste disposal is also a major problem, so use of these nutrient rich waste materials for cultivation of fungi could be Good Avenue to look for. Waste such as Drumstick peel, seed; Cauliflower stalk, Potato peel, Fenugreek stem and orange peel was used to formulate media. Ability of media to support growth of *Aspergillus* and *Trichoderma* was tested. Elemental components of the media were determined. The medium had considerable protein and carbohydrate content. The pH of the media was adjusted to 4.0 ± 0.2 before sterilization. Growth on formulated media was comparable to commercially available media.

Citation: Arati Kadam, Suraj Patil, Mrunalini Sonne, Kirti Dahigaonkar, Jaspal Kaur Oberoi and Pratibha Jadhav, 2017. "Cost effective alternative fungal culture media formulation using fruit and vegetables waste", *International Journal of Current Research*, 9, (09), 56887-56893.

INTRODUCTION

They were selected as a natural nutrient source to prepare the alternative culture media. Culture Media is widely used to enrich, isolate and cultivate microorganisms of interest from the environment. Potato Dextrose Agar medium is commonly used as general purpose medium for the cultivation of broad range of fungi. Fungi are a group of eukaryotic spore bearing microorganisms. They generally reproduce asexually and sexually. Some are agents of diseases in plant and animals (parasitic) and some are saprophytic. They play major role in nutrient recycling. PDA contains potato infusion, dextrose and agar. Readymade media such as PDA are expensive. In today's world waste disposal is also a major problem. Lot of research is carried out so as to use domestic waste for production of cheap media. Higher cost of cultivation media is a matter of concern (Adesemoye and Adedire *et al.,* 2005). To reduce the cost of media, various substitutes are being tried out for commercial media and agar. Different media for the growth and isolation of organisms have been reported from different substrates. Some vegetables and fruits have been used to cultivate fungi, such as Carrot, Tomato, Cabbage, Pumpkin (Deivanayaki and Antony Iruthayaraj, 2012) etc. with easily available low cost material as substitutes for PDA. Some others have used cow pea, green gram and black gram as starch and protein substitutes to reduce the cost of microbial media (Basu *et al*., 2015). Microorganisms are almost omnipresent and very diverse. Microorganisms thrive in different conditions and have variety of growth requirements; like nutrients, pH, osmotic conditions and temperature (Bhattacharya *et al*., 2002). The current limitations of cultivation of microbes in lab need to be addressed by formulation of newer media.

******Corresponding author:* **Pratibha Jadhav,**
Abeda Inamdar Senior College, 2390, KB Hidayatullah Road, Azam Campus, Pune – 411001

MATERIALS AND METHODS

Collection of samples

Vegetables and fruits like Drum stick (seeds and peels), Orange peel, Potato peel, Cauliflower stalk, and Fenugreek stem were collected from local shops, vegetable market and kitchen. The collected samples were transported to the laboratory and processed immediately.

Treatment of samples

Peels, stalks and seeds were sun dried for 2-3 days. Dried material was grinded to powder using electronic blender. The powdered samples were kept in air tight containers until its use.

Table 1. Mediaformulation by mixture of various vegetable wastes

Sr. No.	Name of Formulation	Cauliflower Stalk (gm/100ml)	Potato Peel (gm/100ml)	Fenugreek stem (gm/100ml)	Orange peel (gm/100ml)	Agar (gm/100ml)
1	CPFO-A	0.30	0.25	0.25	0.20	2.50
2	CPFO-B	0.20	0.25	0.25	0.30	2.50
3	CPFO-C	0.25	0.30	0.20	0.25	2.50
4	CPFO-D	0.25	0.20	0.30	0.25	2.50
5	CPFO-E	0.25	0.25	0.30	0.20	2.50
6	CPFO-F	0.25	0.25	0.20	0.30	2.50

Table 2. Media formulation using Drum stick

Sr. no	Drum stick peel powder gm/100ml	Drum stick seed powder gm/100ml	Agar gm/100ml
1	1	0	2.5
2	0	1	2.5
3	1	1	2.5

Table 3. Diameter of fungal growth on different media

Media → / Fungus↓	DP	DS	CPFO-A	CPFO-B	CPFO-C	CPFO-D	CPFO-E	CPFO-F	PDA
Trichoderma spp	4.0	2.5	2.0	4.0	2.5	3.5	3.0	3.5	4.0
Aspergillus spp	4.5	2.0	2.5	4.0	3.0	4.0	3.0	2.0	4.5

Table 4. Concentration of sugar and protein in alternative media

Media name	DNSA (Reducing sugar)mg/1000ml	Folin Lowry (Protein) mg/1000ml
CPFO-A	9.5	9.2
CPFO-B	10	8.7
CPFO-C	9.8	9.9
CPFO-D	9.9	8.9
CPFO-E	7.9	8.8
CPFO-F	7.2	6.9
DS	1.9	7.5
DP	0.7	6.3

Table 5. Nutrients present in CPFO-B formulation powder by chemical analysis

Spectrum: Objects 1673

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
O	8	K-series	50.26	50.26	45.42	6.93
C	6	K-series	43.53	43.53	52.40	5.88
K	19	K-series	3.62	3.62	1.34	0.20
Mo	42	L-series	1.45	1.45	0.22	0.12
Mg	12	K-series	0.44	0.44	0.26	0.07
Na	11	K-series	0.37	0.37	0.23	0.07
Ni	28	L-series	0.19	0.19	0.05	0.15
Si	14	K-series	0.11	0.11	0.05	0.04
Al	13	K-series	0.04	0.04	0.02	0.03
Total:			100.00	100.00	100.00	

Table 6. Nutrients present in CPFO-D formulation powder by chemical analysis

Spectrum: Objects 4686

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
O	8	K-series	43.72	46.32	42.54	4.91
C	6	K series	39.94	42.31	51.77	4.48
K	19	K-series	3.08	3.26	1.23	0.12
Cl	17	K-series	2.91	3.08	1.28	0.12
Na	11	K-series	1.92	2.03	1.30	0.15
Ca	20	K-series	1.46	1.55	0.57	0.07
N	7	K-series	0.96	1.02	1.07	0.25
Mg	12	K series	0.34	0.36	0.22	0.04
Si	14	K-series	0.03	0.03	0.02	0.03
Al	13	K-series	0.03	0.03	0.01	0.03
Total:			94.40	100.00	100.00	

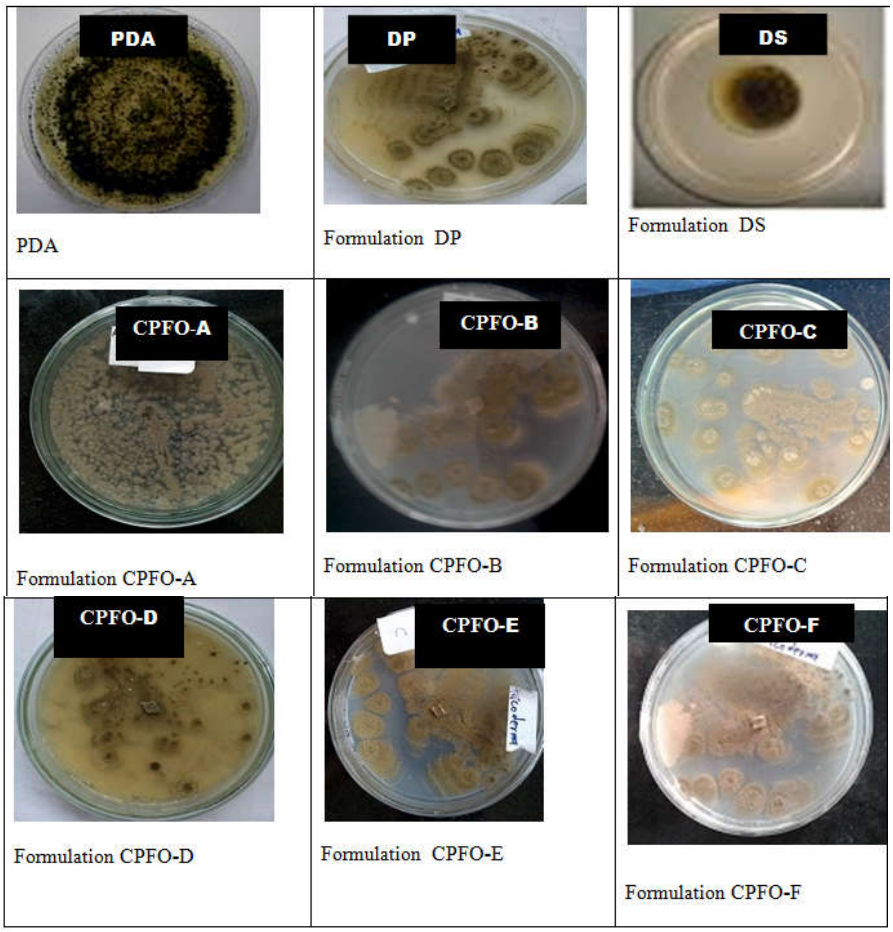


Fig. 1. Growth of *Trichoderma sp* on different media

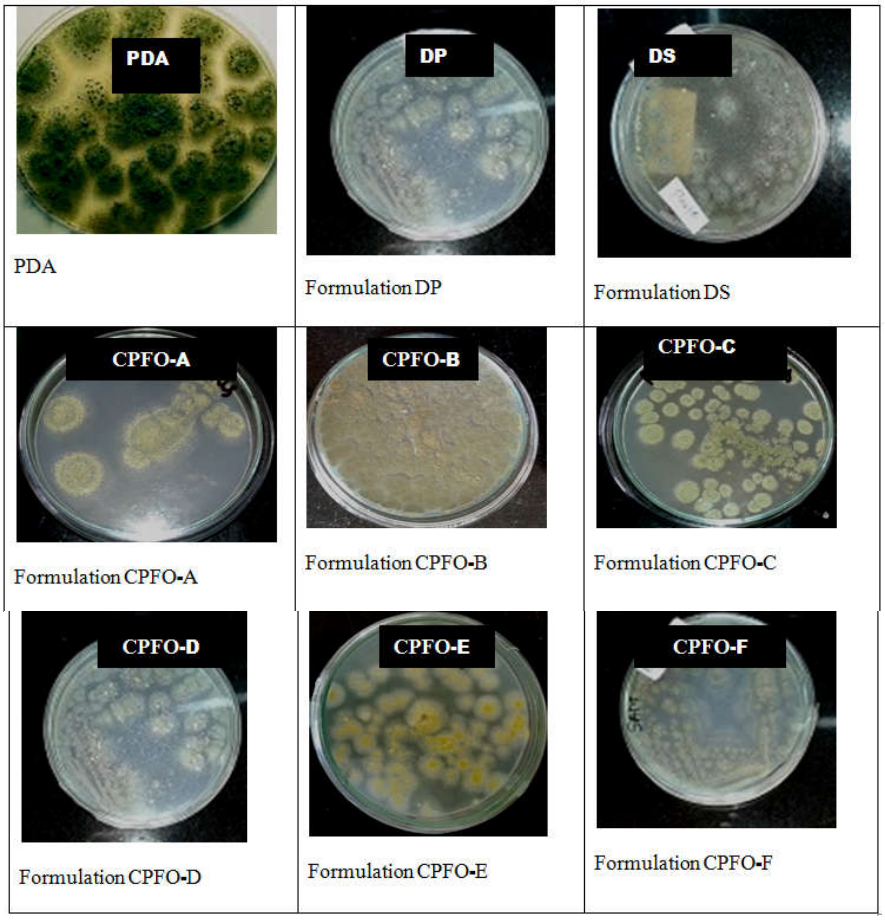
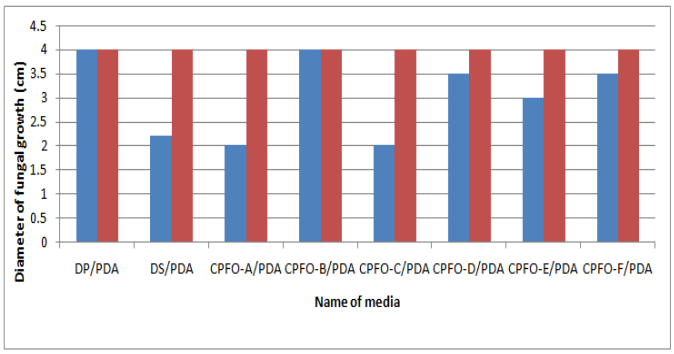
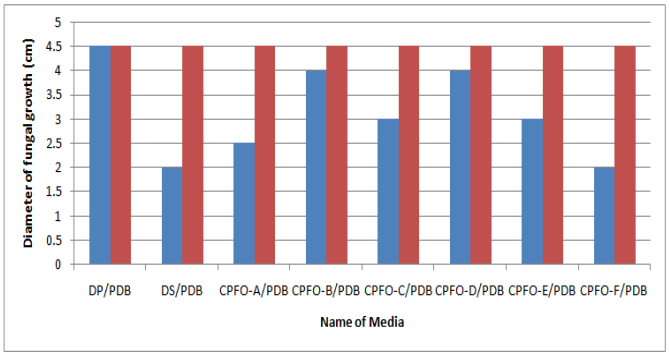


Fig. 2. Growth of *Aspergillus sp* on different media



Graph 1. Growth of *Trichoderma sp.* on different media



Graph 2. Growth of *Aspergillus sp.* on different media

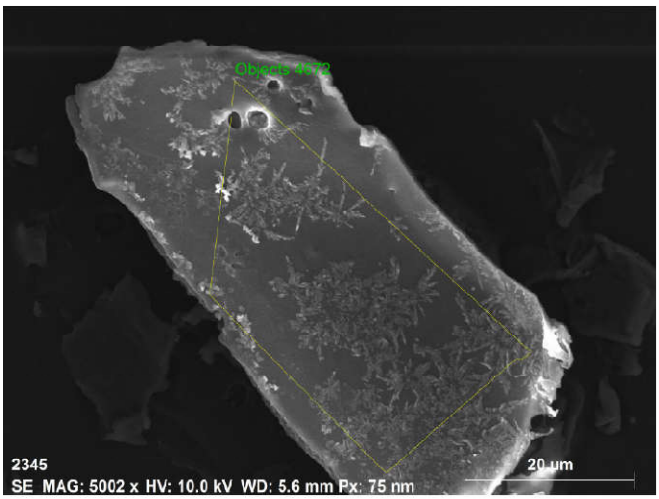
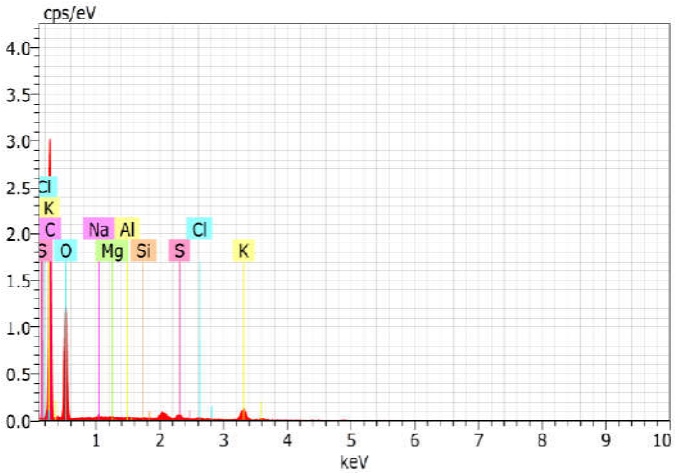


Fig. 3. B Formulation powder analysis (unpublished data)



Graph 3. Elemental analysis of CPFO-B formulation powder

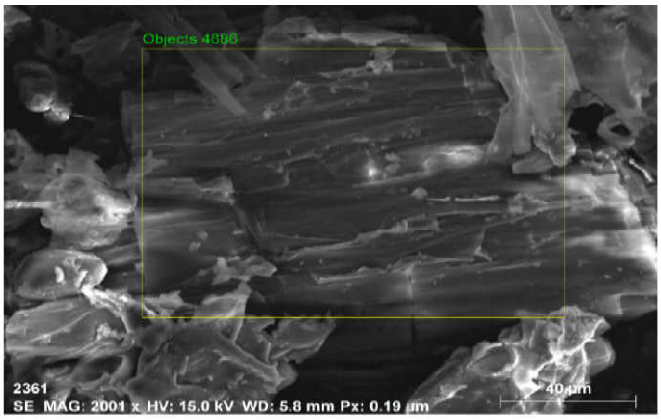
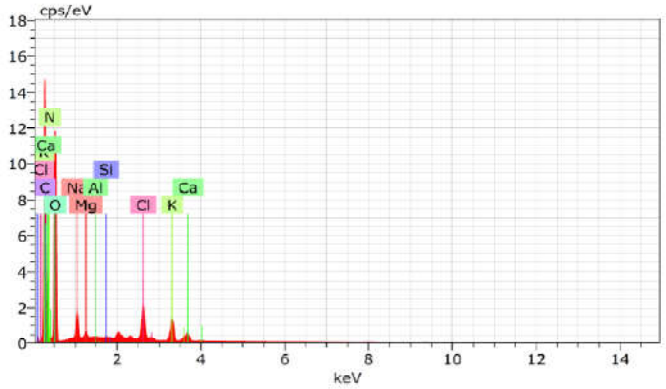


Fig. 4. CPFO-D formulation powder analysis (unpublished data)



Graph 4. Elemental analysis of CPFO-D formulation powder

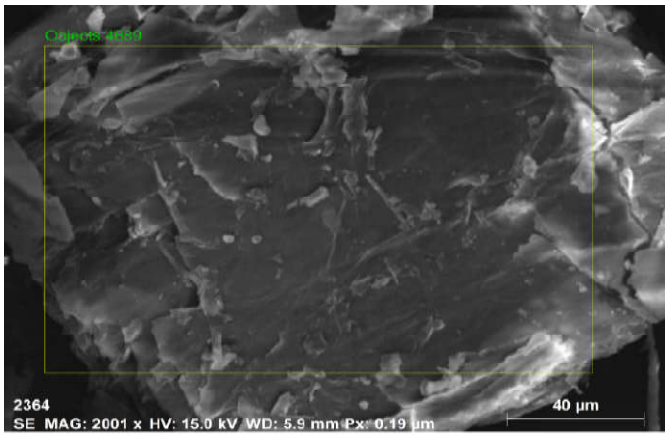
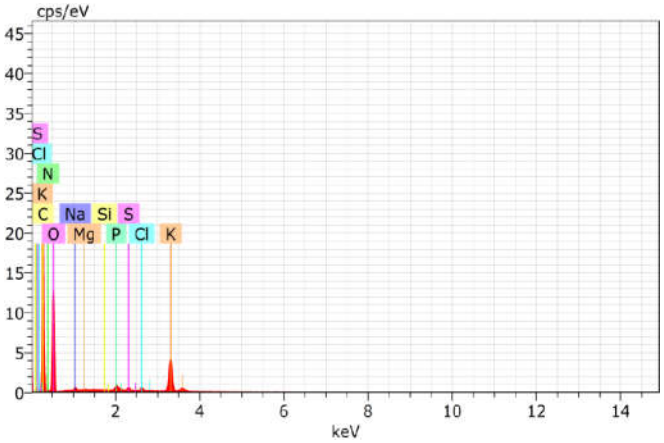


Fig. 5. DP Formulation powder analysis (unpublished data)



Graph 5. Elemental analysis of DP Formulation powder

Table 7. Nutrients present in DP formulation powder by chemical analysis

Spectrum: Objects 4689

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
O	8	K-series	44.86	44.86	41.10	5.40
C	6	K-series	42.89	42.89	52.34	5.03
K	19	K-series	7.50	7.50	2.81	0.26
N	7	K-series	2.55	2.55	2.66	0.64
P	15	K-series	0.74	0.74	0.35	0.06
Cl	17	K-series	0.54	0.54	0.22	0.05
S	16	K series	0.46	0.46	0.21	0.05
Na	11	K-series	0.39	0.39	0.25	0.05
Mg	12	K-series	0.06	0.06	0.04	0.03
Si	14	K-series	0.02	0.02	0.01	0.03
		Total:	100.00	100.00	100.00	

Table 8. Comparative cost study of media

Sr. No.	Liquid Media	Total Prize(Rs)/100L
1.	PDB	17980
2.	DP	1460
3.	DS	1150
4.	CPFO-A	1607
5.	CPFO-B	1417
6.	CPFO-C	1331
7.	CPFO-D	1336
8.	CPFO-E	1255
9.	CPFO-F	1327

Test organisms used

Trichoderma sp, Aspergillus sp.

Media Formulation

The dry powder was kept in warm water for 2-3 hours. Then filtered with the help of filter paper and the filtrate were used to prepare nine different solid formulated media with varying proportion of components. Then agar, which is solidifying agent was added in 100 ml distilled water (Famurewa *et al.,* 2007) In all experiments the pH of the media was adjusted to 4 ±0.2. The dissolved media was sterilized in autoclave at 121°C for 20 minutes under 15 psi pressures and were poured into sterile Petri dishes separately.

Preparation of fresh culture

In this study two different fungi namely *Trichoderma sp., Aspergillus sp.*, were obtained from ATCC. For study, fungal cultures were grown on PDA medium. The cultures were incubated at room temperature for 2-3 days.

Inoculation of fungus into alternative media

Actively growing pure culture of test fungi such as *Trichoderma sp, Aspergillus sp*, were taken. Then a fungal culture was placed on the surface of each alternative nutrient culture media in triplicates. The tested fungi were also inoculated on PDA media which served as control. Then all the plates were incubated at room temperature for 2-3 days.

Fungal growth was observed in formulated media

Fungal growth was measured in terms of fungal diameter at room temperature for total duration of 48 hrs. Fresh culture was used for determining growth in terms of diameter. Diameter of fungal growth was measured in cm (Sathiya Vimal *et al.,* 2013).

Estimation of protein and carbohydrates

Protein was estimated by Folin Lowry's method while carbohydrates content was analyzed by DNSA method.

Chemical analysis of the dehydrated powder

The chemical composition, macronutrient content of the tested samples was determined by EDS (Energy Dispersive Spectroscopy) analysis.

RESULTS

Alternative media supported the growth of fungi. No variation was observed in the growth of Fungi *Trichoderma sp* as well as *Aspergillus sp*. when grown on alternative media. Optimum growth was obtained after 42 hrs of incubation. On further incubation, sporulation was seen in culture inoculated on alternative media. Thus, on alternative media, sporulation took place within 48hrs same as on Potato Dextrose agar sporulation was seen after 48hrs. Comparative growth profile of *Trichoderma sp*., *Aspergillus sp.* on PDA and various formulations. Fungal growth measured in terms of diameter was compared to growth on different formulations and control. Formulation DP showed 4.5cm diameter which was equal to fungal diameter on control. Other formulations DS, CPFO-A, CPFO-B, CPFO-C, CPFO-D, CPFO-E, CPFO-F showed diameter 2cm, 2.5cm, 4cm, 3cm, 4cm, 3cm, 2cm respectively. Fungal growth measured in terms of diameter was compared to growth on different formulations and control. Formulation DP showed 4cm diameter which was equal to fungal diameter on control. Formulation DS, CPFO-A, CPFO-B, CPFO-C, CPFO-D, CPFO-E, CPFO-Fshowed diameter 2cm, 2cm, 4cm, 2cm, 3.5cm, 3cm, 3.5cm respectively.

Protein and sugar estimation

The protein and sugar composition was estimated by standard method and showed presence of sugar and protein. Maximum sugar and protein was found to be present in media CPFO-B and CPFO-C respectively. Thus being rich in these components, the media is able to support growth of microorganisms.

Chemical analysis of the dehydrated powder

To determine the elemental content of formulated media, EDS (Electron Dispersive Spectroscopy) analysis was done. In B formulation Oxygen (O), carbon (C), Potassium (K), Molybdenun (Mo), Magnesium (Mg), Sodium (Na), Nikel (Ni), Silicon (Si) and Aluminium (Al) were found. In D formulation Oxygen (O), carbon (C), Potassium (K), Chlorine (Cl), Sodium (Na), Calcium (Ca), Nitrogen (N), Magnesium (Mg), Silicon (Si) and Aluminium (Al) were found. In DP formulation Oxygen (O), carbon (C), Potassium (K), Chlorine (Cl), Sodium (Na), Calcium (Ca), Nitrogen (N), Magnesium (Mg), Silicon (Si) and Aluminium (Al) were found. The cost of Alternative media is drastically less than the commercially available liquid media.

DISCUSSION

Our investigation was aimed at replacing synthetic nutrient media with vegetable and fruit waste. Previously carrot, tomato, cabbage, pumpkin waste was used in formulation of

media (Deivanayaki *et al.,* 2012) but in present investigation, cauliflower, drum stick, orange peel, potato peel and fenugreek stem was used for formulation of media. The cost of alternative media is considerably less than commercial media. Cost of commercial liquid media is 17,980 Rs/100L but cost of our media is ranging from 1150-1607 Rs/100L in different formulations. Chemical analysis of formulations was done by Electron Dispersive Spectroscopy.

Conclusion

The formulated medias CPFO-A to CPFO-F supported growth of fungi such as *Trichoderma* and *Aspergillus.* In preliminary study it was found that *Trichoderma* grew well in DP and CPFO-B formulation in 48 hours. *Aspergillus* grew well in DP formulation in 48 hours EDS analysis was done to find the elements present in DP, CPFO-B and CPFO-D formulations. Alternative media could be used as cheap media for routine experiment in laboratory. On comparison with Potato dextrose broth, our formulated media DP and CPFO-B gave results similar as that of commercial media. Formulated media was found to be highly cost effective.

REFERENCES

Adesemoye, A.O. and Adedire, C.O. 2005. Use of Cereals as basal medium for the formulation of alternative culture media for fungi, *World J Microbiol & Biotech,* 21: 329-336.

Adoki, A. 2008. Factors affecting yeast growth and protein yield production from orange, Platinum and banana waste processing residues using *Candida sp.* African. *J. Biotechnology,* 7(3), 290-295.

Akharaiyi, F. C. and Abiola, M. A. 2016. Isolation and cultivation of fungi with agrowastes formulated media, *Der Pharma Chemica,* 8(9):56-62.

Anupama and Ravindra, P. 2000. Value added Food: single cell protein, Biotechnology advances, 18, 459- 479.

Asad, M. J., Asghan, M., Yaqub, M. and Shahzad, K. 2000. Production of single cell protein, 2000. Delignified Corn Cob By Arachniotus species, *Pak. J. Of Agric. Sci.,* 2000. 37, 3-4.

Basu, S., Bose, C., Ojha, N., *et al*. 2015. Evolution of bacterial and fungal growth media. *Bioinformation*, 11(4):182-184. doi:10.6026/97320630011182

Bhattacharya, S., Vijayalakshmi, N. and Parija, S.C. 2002. Uncultivable bacteria: Implications and recent trends towards identification. *Indian J Med Microbiol,* 20:174-7

Deivanayaki, M. and Iruthayaraj, A.P. Alternative vegetable nutrient source for microbial growth, *Inter J Biosci,* 2012; 2: 47-51.

Dr. Chanda V. Berde and Dr. Vikrant B. Berde, 2015. Vegetable waste as alternative microbiological media for laboratory and industry, *World Journal of Pharmacy and Pharmaceutical Sciences,* Volume 4, Issue 05, 1488-1494.

Famurewa and David, O.M. 2008. Formulation and Evaluation of Dehydrated Microbiological Media from Avocado Pear (*Peasea americana* Cmill). *Research Journal of Microbiology, 3: 326-330.*)

Jamel, P., Alam, M. Z. and Umi, N. 2008. Media optimization for bio proteins production from cheaper carbon source. *J. of Engi. Sci. and Techno.,* 3(2) 124-130.

Janssen, P.H., Yates, P.S., Grinton, B.E., Taylor, P.M. and Sait, M. 2002. Improved culturability of soil bacteria and isolation in pure culture of novel members of the divisions Acidobacteria,Actinobacteria, Proteobacteria, and Verrucomicrobia.. Appl Environ Microbiol.;68:2391–6.

Kasanadze, A.K. 2000. Replacement of Agar by cassava flour in microbial media. BSc Dissertation University of Malawi, Bunda College of Agriculture, Lilongwe.

Konstantinidis, K.T. and Tiedje, J.M. 2005. Genomic insights that advance the species definition for prokaryotes. *Proc Natl Acad Sci,* USA;102(2567–2572):99.

Kuria, P., Demo, P., Nyende, A.B. and Kahangi, E.M. 2008. Cassava starch as an Alternative gelling agent for the in vitro micro propagation of potato (Solanum tuberosumL.). *African Journal of Biotechnology,* 7: 301–307.

Laleye, S.A. Tedela, P.O. Adesua, B. and Famurewa, O. 2007. Growth of Some Microorganisms on Media Formulated from Local Raw Materials. *Research Journal of Microbiology,* 2: 545-549.

Leach, H.W., McCowan, L.D. and Schoch, T.J. 1959. Swelling and solubility patterns of various starches. Cereal Chemistry, 36: 534-544.

Lucyszyn, N., M.Quoirin, M. M. Homma and M. R. Sierakowski, 2008. Agar/galactomannan gels applied to shoot regeneration from tobacco leaves.. Biol. Plant. 51(1):173-176.

Maliro, M.F.A. and Lameck, G. 2004. Potential of cassava flour as a gelling agent in media for plant tissue cultures. *African Journal of Biotechnology,* 3: 244-274.

Mbanaso, E.N.A. 2008. Effect of multiple subcultures on Musa shoots derived from cassava starch-gelled multiplication medium during micropropagation. *African Journal of Biotechnology,* Vol 7, No 24 (2008)

Mohamed, M. A. H., Alsadon, A. A. and Al Mohaidib, M. S. 2009. Corn and potato starch as an agar alternative for Solanumtuberosum micro propagation. *Afr. J. Biotechnol.* 9(1): 012- 016.

Murashige, T. and Skooga, F. 1994. A revised medium for rapid growth and bio assays with the tobacco tissue cultures. *Physiol. Plant.* 15: 473-497.

Naik, P. S. and Sarker, D. 2001. Sago An Alternative Gelling Agent For Potato In Vitro Culture. *Biol. Plant.* 44(2): 293.

Najafpur, Ghasem D. 2007. Single Cell Protein. Biotechnology advances. *Biochemical Engineering and Biotechnology Advances,* 332-347.

Nambiar, V.S. and Bhdalkar K. Daxini, 2003. Drumstick Leaves As Source Of Vitamin A In ICDS-SFP. *Indian Journal of Pediatrics.*70: 383-387.

Nambiar, V.S. and Seshadri, S. 1998. Beta Carotene Cotent Of Green Leafy Vegetables Of Western India By HPLC. *Journal of Food Science and Technology,* 35: 365-367.

Nambiar, V.S., Mehta, R. and Daniel, M. 2005. Polyphenol Content of Three Indian Green Leafy Vegetables 2005. *Journal of Food science and Technology,* 42: 312-315.

Nichols, D. 2007. Cultivation gives context to the microbial ecologist. *FEMS Microbiol E.coli.,* 60:351–7.

Ozel, C. A., Khawar, K. M. and Arslan, O. 2008. Comparison Of The Gelling Of Isubgol, Agar And Gelrite On In Vitro Shoot Regeneration And Rooting Of Variety Samsun Of Tobacco (Nicotiana tabacuml.). *Scient. Horticul.* 2008. 117(2): 174-181.

Pandhre, G.R., Satwase, A.N. and Hashmi, S.I. 2011. Effect Of Different Pretreatments And Drying Methods On Color Characteristics Fenugreek Leaves, *Int J Cur Sci Res,* 1: 115-119.

Pham, V.H.T. and Kim, J. 2012. Cultivation Of Unculturable Soil Bacteria, *Trends Biotechnology*. 30:475–84.

Porndarun, H. Vichai, And Walairut, C. 2010. Department of product development, Faculty of agro industry, Kasetsart University, Bangkok 10900,Thailand, 100-106.

Priadi, D., Fitriani, H. and Sudarmonowati, E. 2008. The growth of cassava (Manihotesculenta Crantz)on various alternative gelling agents. *Biodiversitas,* 9(1): 9-12.

Prokash, S., Hoque, M. I. and Brinks, T. 2000. Culture media And Containers. Biotechnology and Eco Development Research Foundation, Bangalore, India.

Ravathie, A., Sevvel, P., Nirmala, R. and Kularajany, N. 2012. *Journal of natural product and plant resources,* 2; 697-700.

Ravimannan, N., Arulanantham, R., Pathmanathan, S. and Niranjan, K. 2014. Annals of Biological Research, 5(1), 36-39.

Sathiya Vimal, S., Vasantha Raj, S., Senthil kumar, R.P. and Jagannathan, 2013. *Natural Sources of Gooseberry Component used for Microbial Culture Medium Journal of Applied Pharmaceutical Science*, Vol. 3 (11), 040-044.

Shipra Jha, S.N. Dikshit 2017. Alternative culture media for fungal growth using different formulation of plant material, *Int J Pharm Bio Sci.,* 2017 Jan; 8(1): (b) 445 – 452.

Tharmila, S. Jeyaseelan, E. C. and Thavaranjit, A. C. 2011. *Archives of Applied Science Research,* Vol.3 (3):389-393.

Vartoukian, S.R., Palmer, R.M. and Wade, W.G. 2010. Strategies for culture of 'unculturable' bacteria. *FEMS Microbiol Lett.* 309:1–7.

Wasas, A.D., Huebner, R.E. and Klugman, K.P. 1999. Use of Dorset Egg Medium for Maintenance and Transport of *Neisseria meningitidis* and *Haemophilus influenzae* Type b. *Journal of Clinical Microbiology*, 37(6):2045-2046.

Zapata, A. 2001. Cost Reduction In Tissue Culture Of Banana, (Special Leaflet), International Atom Energy Agency Labs., Agric. And Biotech. Lab., Vienna, Austria.

Zhe, W. R., Debasis, C. Hahn, E. J. and Paek, K. Y. 2005. Growth of Doritaenopsis In Peat substituted Growing Medium. J. *Korean Soc. Hort. Sci.* 46(1): 76-81.

****