 ISBN : 978-81-922163-4-8

Research Paper **Poster Presentation**

Screening of Agroindustial Residue for Development of Carrier based Starter cultures of Cellulolytic inoculums

Gosavi M.C [1] and R.G.Bagool.[2]

1. Dept. of Botany, SIES College, Sion (w), Mumbai-22,

2. Adj. Prof. PG research lab, Dept. of Botany, DUB College, Dapoli, Dist. Ratnagiri, Maharashtra.

Email : mahavirgosavi04@rediffmail.com

ABSTRACT: India has a tremendous potential of local manurial resources including various agricultural and agro industrial wastes, which are available in abundant quantities. Use of carrier materials is not a new concept; conventionally used carriers, viz. peat, lignite, sterile soil, etc. require some financial inputs as well as their availability can pose a problem in future. An acute need of development of suitable carrier materials for preparation of cellulolytic inoculants has been felt since the past two decades in India. In the present investigation, six agro industrial wastes (residues) were selected as carrier materials for the development of starter cultures of cellulolytic inoculum for composting. It describes the development of starter cultures and delivery system for composting. It includes selection and pretreatment of various agro-industrial residues as the base for the starter culture and delivery system on basis of physical and chemical parameters for the cellulolytic fungi and study of their growth on different carrier materials and its storage.

KEY WORDS: Starter culture, *Chaetomium,* Composting.

INTRODUCTION: Now days, agro wastes are being tried as carrier materials for bio control agents, either individually or in combination with the conventional carriers (Vyas *et al.,* 2001; Thara & Nassema, 2002 and Bharathi *et al.,* 2003). In spite of an acute need, the mass advent of such delivery systems especially those utilizing fungal inoculum, is still awaited in the Indian scenario (Kolet, 2003).The carrier materials obtained from agro industrial wastes for developing a delivery system of starter cultures for composting could thus be considered as novel and innovative.

These wastes are available abundantly and can be collected from rural and semi urban areas. All these materials selected are easy to process and inexpensive because they are available as waste. In the present context, the lignocellulolytic nature of most of the residues makes them ideal substrates for mass production of inoculum for composting. In the present investigation six agroindustrial residues viz. cajanus percarp, groundnut shells, pea pericarp, safflower shells, soybean testa and sunflower shells were screened for their potential use as carrier based starter culture of cellulolytic inoculums.

MATERIALS AND METHODS:

1. Development of starter culture : Above agro industrial residues were collected from different sources and treated for use as carrier material for cellulolytic inoculum. The carrier materials were dried by heating to 80°C

ISBN : 978-81-922163-4-8

for 4 hours and then pulverized (2mm Mesh Size) as apart of pretreatment. (Plate I)

2. **Characteristics of the Carrier materials:** The selected carrier materials were tested for physical as well as chemical characteristics (Table: 1) The organic matter and organic carbon content were determined by the method described by Bhardwaj and Sharma (1991). Total nitrogen content was determined using micro-kjeldahl method described by Sadasivan and Manikan (1992). The organic phosphorus and organic potassium content were determined by the method determined by Bhardwaj and Sharma (1991).

3.Growth of cellulolytic fungal inoculum on different carrier materials: The Cellulolytic isolates of fungus *Chaetomium globosum* were inoculated separately on different carrier materials and this carrier based inoculum was incubated for 15 days at room temperature to develop into starter culture. Pretreated carrier material (50 g) was dispensed in glass bottles (saline bottles, GJ make, and 500ml capacity) and moisture content adjusted to 60% with Reese liquid medium. The bottles were plugged with cotton and autoclaved at 15 lbs psi for 20 minutes. On cooling each bottle was inoculated with 1ml of spore suspension of the respective isolate. The bottles were incubated at room temperature for 15 days. On daily basis, observations regarding visible mycelial and perithecial growth were recorded.

4. **No. of perithecia produced on different carrier materials:** This experiment was based primarily on the method described by Gardemann and Nicolson (1963) for Arbuscular Mycorrhiza. To estimate the no. of perithecia produced on carrier materials. 1g of starter culture was suspended in 50 ml distilled water and filtered through Whatman no.1 filter paper. The no. of perithecia was counted using binocular microscope. The readings were recorded based on 10 observations for each carrier material for every isolate.

5. Storage-life of the starter culture: The starter cultures were stored at room temperature for a period of 6 months (180days) in the same bottles in which they were prepared (Table: 2). The effectiveness of the starter culture depends on the viability of the fungal spores /propagules. The perithecia of the respective isolates from starter cultures were lifted with the needle and plated on PDA in a Petri plate. A total no. of 100 perithecia of each test organism was thus plated, in 20 plates. The ability of the perithecia to form colonies of the isolate was interpreted as viability of the starter culture for carrier based inoculum. The percentage of viability was calculated by the formula:

$$\% \text{ Viability} = \frac{\text{No. of colony forming perithecia}}{\text{Total no .of perithecia plated}} \text{ X } 100$$

OBSERVATIONS AND RESULTS:

1. Physical As Well As Chemical Characteristics:

The selected carrier materials were tested for physical as well as chemical characteristics viz, water holding capacity, organic matter content, organic carbon content, organic phosphorus content and organic potassium content which are essential properties of a good carrier materials. The physical and chemical characteristics of carrier materials are presented in table no. 1.

2. Growth of cellulolytic fungal isolates on different carrier materials:

ISBN : 978-81-922163-4-8

The isolates were inoculated separately over the different carrier materials and incubated at room temperature for 14 days. The growth pattern in terms of mycelial growth and perithecia development was recorded. Out of six potential carrier materials tested except Soybean testa, all five materials found good in terms of growth of inoculum supported by them.

3. Evaluation of Carrier materials for perithecial development :

At the end of incubation period perithecia produced on different carriers were counted. The no. of perithecia produced on different carrier materials are represented in table no. 2. The test organism produced maximum perithecia on Cajanus pericarp followed by safflower shells. The Pea pericarp also recoded good no. of perithecia. Groundnut shells and Sunflower shells found moderate in terms of perithecial count. Soybean testa failed to produce any perithecia.

4.Storage life of the Starter Culture :

The storage of the starter culture was confirmed after a storage period of 180 days (6 months). It was tested in terms of the viability of the perithecia. This was tested by plating perithecia from the different carrier materials over PDA medium in a Petri plate and counting those which formed colonies. It was observed that Groundnut shells (94.4%) supported maximum viability of perithecia followed by Safflower shells (87.3%) and Cajanus pericarp (86.32%). The moderate viability was observed on Pea pericarp (74.48%) and Sunflower shells exhibited the least viability (72.84%). These data revealed that Groundnut shells, Safflower shells and Cajanus pericarp were excellent carrier materials for the test isolates. In case of Pea pericarp and Sunflower shells the percent viability recorded after the storage period was relatively less but acceptable.

DISCUSSION:

Cajanus (Arhar) is one of the prominent legume crops in pulse growing regions. It is almost cultivated in every state (Agrikar, 1970). Cajanus pericarp is left in huge quantities after separation of seeds. The ground nut shells constitute about 30% of the weight of the pods (CSIR, 1948; Wani *et al.,* 1988 and www. ikisan. com). The shells are rich in essential plant nutrients (Prasad and Kumar, 1998) and are known to constitute around 50% cellulose, 11% hemicellulose and 30% lignin (Abd-Alla and Sorour, 2004). Pea pericarp has almost the same application as cajanus pericarp. Safflower seeds after removal shells used for oil extraction. The shell waste is conventionally burnt as fuel. Now days soybean has became highly essential and vital crop all over the world . In the present work, the de hulled waste which is generated after oil extraction was tried as carrier material to develop starter culture of cellulolytic inoculum. Sunflower is also one of the major oil crops in India. After oil extraction, shells are obtained as a waste; it represents 15% of seed weight. It is the first time powdered safflower shells, sunflower shells and soybean testa (de hulled waste) has been used as carrier material to develop starter culture of cellulolytic inoculum for composting.

All the residues are pretreated before their use; as it is recommended for effectiveness of the product (Wahah, 1998). It makes cellulosic materials more suitable for enzymatic activities as well as for microbial growth (Nakkeeran *et al.,* 1997 and Tengerdy & Szakacs, 2002). After the pretreatment, carrier materials were used for

ISBN : 978-81-922163-4-8

development of cellulolytic starter cultures (Plate I).

These results found satisfactory for all the important criteria for carrier materials. Thus, supports the suitability of agro industrial wastes as useful carrier materials. For mass production of starter cultures, cellulolytic inoculum was multiplied and stored in the carrier materials.

The study of the parameters of the substances revealed that most of the selected materials possessed several desired qualities making them suitable for their use as carrier materials. These desirable qualities were –

1. Good water holding capacity (highly absorptive).
2. Rich in organic matter content.
3. Ideal organic carbon content, organic nitrogen content, organic phosphorus content and organic potassium content.
4. Suitable C: N ratio.
5. Natural organic Substrates: Nontoxic to the inoculum.
6. Lignocellulosic residues: Natural and ideal substrate for mass cultivation and production of cellulolytic inoculum.
7. Easily available in abundant quantities.
8. Inexpensive since available as waste.
9. Easy to process.
10. Easy to sterilize.

The degradation of the substrates during growth and multiplication of the inoculum was evident from the change in colour and texture of the residues. Out of six agro industrial residues studied, cajanus pericarp and safflower shells ranked superior ones with respect to rich vegetative growth and perithecial development of inoculum. Pea pericarp, safflower shells and sunflower shells also showed moderate vegetative growth and perithecial development.

In the present investigation, the shelf life of the starter culture was confirmed after 180 days of storage. This was possible because of the presence of numerous and visible perithecia which develop and multiply in the carrier materials even during the storage period, utilizing its lignocellulosic contents. This appears to be a great advantage in the application of starter cultures of *Chaetomium globosum* isolates in composting. The results reveal that all the five agro industrial residues listed above, recorded more than 70% viability percentage after 180 days of storage and therefore excellent for use as carrier materials in developments of starter cultures. Hence, they can be recommended for use as carrier materials in development of starter cultures of cellulolytic inoculum for composting.

Instead of discarding all these lignocellulosic wastes, they can be used easily for the production of cost effective starter cultures. It would be extremely beneficial in the easy distribution of the effective cellulolytic inoculum to users for application in rapid composting.

ISBN : 978-81-922163-4-8

Table no. 1: Physical and Chemical characteristics of the carrier materials after pretreatment

Characters	Cajanus pericarp	Groundnut shells	Pea pericarp	Safflower shells	Soybean testa	Sunflower shells
Colour	Brown	Pale brown	Yellowish	White	Yellow White	Blackish brown
Texture	Fine powder	Fine powder	Fine powder	Fine powder	Fine powder	Powder
Particle size	20 mesh size	20 mesh size	20 mesh size	20 mesh size	20 mesh size	20 mesh size
Moisture	7.0%	9.0%	5.5%	7.5%	8.5%	10.0%
Water holding capacity	27%	28%	29%	32%	42%	24%
Organic matter	75%	72.5%	68.25%	82.25%	81.5%	78.5%
Organic Carbon	43.9%	44.2%	41.8%	51.19%	50.75%	48.38%
Organic Nitrogen	1.47%	1.58%	1.35%	1.54%	2.88%	1.14%
C: N Ratio	29.86	27.97	36.89	33.24	17.62	42.44
Organic Phosphorus	0.21%	0.26%	0.23%	0.28%	0.13%	0.19%
Organic Potassium	0.68%	0.72%	0.62%	0.7%	0.45%	0.58%

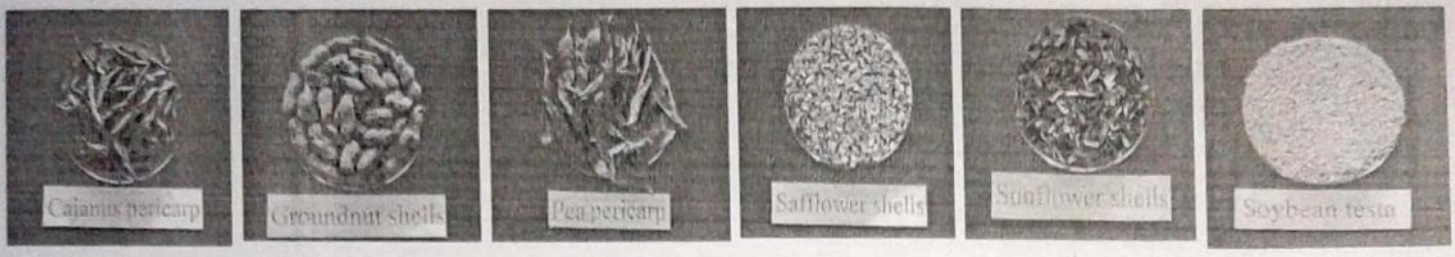
Table no.2 : Perithecia produced on different carriers after 15 days of incubation:

Isolate no.	Mean no. of perithecia / 0.1 gm carrier material					
	Cajanus pericarp	Groundnut shells	Pea pericarp	Safflower shells	Soybean testa	Sunflower shells
1	417	257.33	331.33	365.33	---	192.67
2	347.67	231	309.67	373.67	---	212.33
3	331	188	284.33	326.67	---	203
4	316.33	174.33	270	340	---	180
5	433	268.33	332.33	378.67	---	224.67

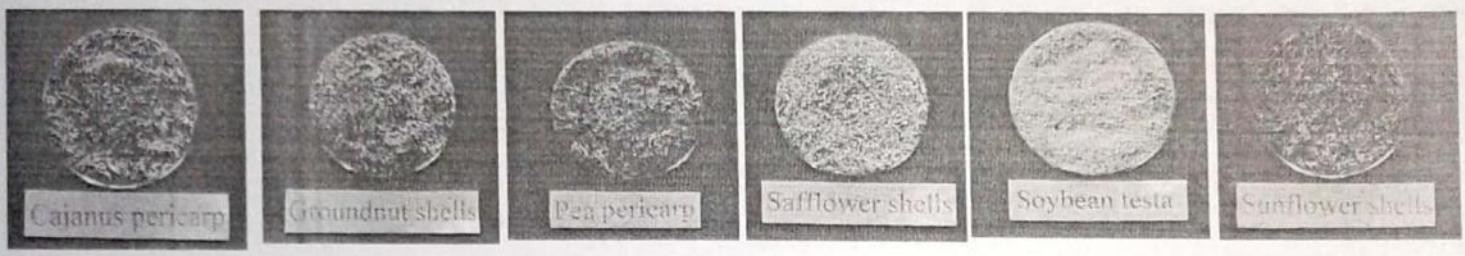
 ISBN : 978-81-922163-4-8

Plate no. I

Raw material for Carriers



(B) Carrier materials after pretreatment



REFERENCES:

Abd-Alla, M.M.S. and W.A.I. Sorour (2004)- Seed characteristics as indicators of peanut seed quality. *Annals. of Agricultural Science.* Moshtohor, 42(3): 989-1000.

Agirkar,G.D. (1970)- 'Pulse Crop of India', ICAR, New Delhi. pp. 1-2.

Bharadwaj,K.K.R.and C.Sharma (1991)- Manual of selected Chemical and Microbiological Methods. All India Co ordinated Research Project on Microbiological Decomposition and Recycling of organic Wastes, H.P. Krishi Vishvavidyalaya, Palampur. pp. 1-52.

Bharathi, R.; K. Manjunath and S.B. Sullia (2003)- Bio control of diseased Forest nursery seedling with Tricoderma formulation (Abst.). Nat. Symp.on Prospecting of Fungal Diversity and Emerging Technologies (Feb 6-7, 2003) MACS Pune. pp. 113-114.

CSIR (1948)- The Wealth of India (Raw Materials). Vol. I. Publications and Information Directorate. New Delhi. pp.102.

Gaur, A.C. (1992)- Bulky organic manures and crop residues. In: Fertilizers, Organic manures, Recyclable wastes and Biofertilizers – Components of Integrated plant nutrition. (H.L.S. Tandon, ed.), Fertilizer Development and Consultation organization, New Delhi. pp. 36-51.

Gaur, R.B.; R.N. Sharma and R.R. Sharma(2003)-Shelf life of talc base formulation of *Trichoderma* and soil application for bio control of dry root rot of chicken pea. *Indian phytopath.* 56(3): 328.

Gardemann, J.W. & T.H. Nicholson (1963)- Spores of Mycorrhizal Endogone species extracted from soil by wet- Sieving and decanting. *Trans. Br. Mycol. Soc.* 46 (2): 235-244.

ISBN : 978-81-922163-4-8

Grantzau, Erich (2004)-Ornamental plants grown in substrates with less peat. Proceedings of the International Peat Symposium. Danish Institute of Agri. Sci., Dept. of Horticulture, Aarslev. 15th May' 2004. pp. 52-53.

Nakkeeran, S.; P. Sankar and R. Jeyarajan (1997)- Standardization of storage conditions to increase the self-life of *Trichoderma* formulations. *J.Mycol. Pl.Pathol.* 27(1): 60-63.

Prasad, S. and U. Kumar. (1998)- Manures and Biofertilizers. In: Principles of Horticulture. Agro Botanica, Bikarner, India.pp.222-253.Pringshim, H. and K. Baur. 1928. *Z.Physiol.Chem.* 173-188.

Subba Rao, N.S. (1984)- Biofertilizers in agriculture. Oxford and IBH Publishing Co. New Delhi. pp. 12-165.

Subba Rao, N.S. (1997)- Biofertilizer in Agriculture and Foresty. Third Rev. Edn. ISBN 81-204-0791-1. Oxford and IBH Publishing Co. New Delhi.pp.51-165.

Tengerdy, R.R. and G. Szakacs (2002)-Bioconversion of lignocellulose in solid substrate fermentation. *J. of Chemical Technology.* 15(4). July'2002. pp. 56-58.

Thara, S.S. and A. Naseema (2002)-Mass production of *Fusarium* species, potent bio control agents of water hyacinth. *J. Mycol. Pl. Pathol.* 32(1): 94-95.

Vyas, R.V.; N.B. Patel; H.R. Patel and D.J. Patel (2001)-Search for a cheaper substrate for mass production of nematophagous fungi. Invitro. (Abstr.) *J. Mycol. Pl. Pathol.* 31: 106-107.

Wahal, C.K. (1998)- Microbial cellulose degradation and its enhancement for application in Agriculture, Biology & Industry using recombinant DNA technology.

In Micobes: for Health, Wealth & Sustainable Environment. (Ajeet Verma, Ed), Malhotra Pub. House, New Delhi. pp. 307 – 346.

Wani, B.B.; G.D. Patil and M.D. Patil (1988)- Effects of levels of single super phosphate and boronated super phosphate on yield and quality of groundnut. *J. Maharashtra Agric. Univ.* 13 (3): 302-304.

www.ikisan.com – Groundnut> Overview http: // www. ikisan.com