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Evaluation of Green Pea Pod Residue as a Carrier Substrate for the Development of Cellulolytic Fungal Starter Cultures for Composting

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Abstract: ***Agro-industrial wastes are valuable renewable resources for microbial bioprocesses. Novel organic residues have always been on the forefront of research as substrates for starter cultures for various purposes on account of their abundant availability, cost-effectivity and environmental sustainability. The present study investigates green pea (Pisum sativum L.) pod residue as potential carrier substrate for development of cellulolytic fungal starter cultures of efficient isolates belonging to four cellulolytic genera viz., Aspergillus, Penicillium, Chaetomium and Trichoderma for rapid composting. The substrate was pre-treated, characterized for its physico-chemical properties, and evaluated for fungal growth and colonization. The pre-treated green pea pod powder exhibited properties indicating its adequacy and nutritional suitability as a carrier material. The growth of the fungal isolates on the substrate and viability of the inoculum thereafter for a shelf-life period of 120 days was monitored, results of which are discussed.***

Keywords: Green pea pod residue, cellulolytic fungi, carrier material, agro-waste, starter culture

1. Introduction

Agro-industrial wastes have emerged as a growing environmental concern in recent years, primarily due to their large-scale generation and improper disposal practices. However, these residues also represent valuable renewable resources that can be repurposed for several beneficial applications [1]. The increasing emphasis on sustainable agricultural practices has intensified global interest in the bioconversion of agro-wastes into value-added products, thereby transforming potential pollutants into economically and environmentally viable materials.

Among various bioconversion strategies, composting is recognized as one of the most effective and eco-friendly methods for managing organic solid waste. This microbiologically driven process converts organic residues into nutrient-enriched compost, which serves as an excellent soil conditioner and contributes to the improvement of soil fertility and structure [2]. Composting is also considered a sustainable waste management approach that minimizes environmental hazards associated with organic waste accumulation. The efficiency of the process, however, depends largely on the activity of cellulolytic microorganisms, which play a pivotal role in the degradation of lignocellulosic materials [3]. Despite their crucial function, natural composting systems often exhibit variable microbial efficiency due to inconsistent inoculum, microbial competition and fluctuating environmental conditions.

To address these limitations, the development of starter cultures, carrier-based formulations containing active microbial inoculum has gained increasing attention. Such inoculum enhances microbial activity, accelerate organic matter decomposition, and improve the overall quality and maturity of compost. Agro-industrial residues are now being extensively investigated as potential carrier materials for these starter cultures and other biocontrol agents, either independently or in combination with conventional substrates [4,5,6].

In this study, four cellulolytic fungal isolates viz., *Aspergillus niger*, *Penicillium* sp., *Chaetomium* sp., and *Trichoderma* sp. were evaluated for their growth response on pre-treated green pea pod powder used as a carrier material. Mycelial and ascomatal development were systematically recorded over a 15-day incubation period to assess the suitability of the substrate. The long-term viability of the inoculated carrier was further monitored over 120 days to evaluate its storage stability. This work provides a scientific basis for utilizing green pea pod waste as an eco-friendly carrier for cellulolytic fungal starter cultures in composting.

2. Materials and Methodology

Collection and Pretreatment of Green Pea Pods:
Green pea pods were collected from local markets and small-scale traders in Bhiwandi, Dist-Thane. Maharashtra State before disposal as waste. The material was cleaned, air-dried, and heated for 4 hours for sterilization. It was then pulverized to a 20-mesh particle size to obtain uniform powder. The pretreated green pea pod powder was stored in sterile containers and used as a carrier material for cellulolytic fungal inoculum preparation.

Physico-chemical Analysis of Green Pea Pod Carrier Material:
The physical and chemical properties of the pretreated green pea pod powder were analyzed to assess its suitability as a carrier substrate for cellulolytic fungal inoculum. The parameters evaluated included moisture content, water-holding capacity (WHC), organic matter (OM), and total

nitrogen (N), which are essential indicators of an effective carrier base [7].

The water-holding capacity was determined by using 10 g of oven-dried green pea pod powder. The organic matter content was analyzed by the method described by [8], while total nitrogen was estimated using the Micro-Kjeldahl method as outlined by [9].

Evaluation of Fungal Growth on Green Pea Pod-Based Carrier Substrate:

The cellulolytic fungal isolates viz., *Aspergillus niger*, *Penicillium* sp., *Chaetomium* sp., and *Trichoderma* sp. were individually inoculated on pretreated green pea pod powder to assess its suitability as a carrier substrate. Fifteen grams of the carrier material was placed in 100 mL glass bottles, the moisture adjusted to 60% with Reese liquid medium, and sterilized by autoclaving at 20 lbs psi for 1 hour. The study was carried out in triplicate with control used.

After cooling, each bottle was inoculated with 2 mL of spore suspension of the respective isolate and incubated at 28 ± 2°C for 15 days. Sterilized distilled water was used instead of inoculum with control set. Daily observations on mycelial and ascomatal development were recorded, with growth expressed as '+' (poor), '++' (moderate), or '+++' (rich). This helped evaluate the growth performance of each isolate on green pea pod as a carrier material.

Storage Life and percent viability of the Starter Cultures:

After the starter cultures were stored for 120 days at room temperature, their viability was evaluated based on mycelial growth. A portion of the inoculum was transferred to fresh Potato Dextrose Agar (PDA) plates and incubated at 28°C for 5–7 days. The extent of mycelial growth and ascomatal growth was recorded and categorized as poor (+), moderate (++), or rich (+++), depending on the spread of mycelium and ascomata across the agar surface. This growth response was considered an indicator of the viability of the starter cultures after storage.

The percentage of viability was calculated using the following formulas:

$$\%\text{Viability} = \frac{\text{Number of plates with mycelial growth}}{\text{Total number of plates}} \times 100$$

$$\%\text{Viability} = \frac{\text{Number of colonies forming perithecia}}{\text{Total number of perithecia plated}} \times 100$$

3. Results and Discussion

Physicochemical Analysis of Green Pea Pod Residue as Carrier Material:

The physical and chemical parameters of the pretreated green pea pod carrier material, including colour, texture, particle size, moisture content, water-holding capacity, organic matter, organic carbon and nitrogen content (C:N ratio), were analyzed to assess its suitability for use as a carrier substrate in cellulolytic fungal inoculum development.

The pretreated green pea pod powder appeared greenish brown to light yellow-brown with a fine texture, indicating uniform pulverization. Its 20-mesh particle size provided suitable surface area for fungal colonization. The moisture content (5.2%) ensured stability, while the water-holding capacity (32.1 mL per 10 g; 321%) reflected adequate hydration without anaerobic conditions. High organic matter (76.30%) and organic carbon (44.26%) levels confirmed its richness in carbonaceous compounds and nitrogen content (25:1%) indicated a balanced C:N ratio, supporting efficient cellulolytic fungal growth. Results obtained are in confirmity with those of [10].

Table 1: Physicochemical analysis of green pea pod residue as carrier material

Physico-Chemical Characteristics	Carrier Material Green pea pods
Colour	Greenish brown to light yellow brown
Texture	Fine Powder
Particle size	20 mesh size
Moisture	5.2%
Water retained by 10gm sample (mL)	32.1
Effective water holding capacity (%)	321
Organic matter	76.30
Organic carbon	44.26
C:N Ratio	25:1

The green pea pod residue carrier material exhibited favorable physical and chemical properties suitable for cellulolytic fungal inoculum development. Its fine powder texture and uniform 20-mesh size provided an ideal surface for fungal growth and colonization. The moderate moisture content [11] and good water-holding capacity ensured adequate hydration while maintaining substrate stability. The high organic matter and carbon content indicated its richness in degradable organic compounds, essential for microbial metabolism. The balanced C:N ratio confirmed that green pea pod residue is a nutrient-rich and effective carrier substrate for cellulolytic fungi.

Evaluation of Fungal Growth on Green Pea Pod Residue as Carrier Substrate:

The cellulolytic fungal isolates viz., *Aspergillus niger*, *Penicillium* sp., *Chaetomium* sp., and *Trichoderma* sp. showed progressive growth on the green pea pod carrier over 15 days. Visible mycelial growth appeared by Day 5, *Aspergillus niger* and *Penicillium* sp. showing moderate (++) growth that reached rich (+++) levels by Day 10 and remained stable thereafter. *Chaetomium* sp. exhibited the best growth, showing both mycelial and ascomatal (++/+) development by Day 5 and achieving rich (+++/+++) dual growth by Day 10, sustained through Day 15. The results are in agreement [12]. *Trichoderma* sp. displayed strong colonization, increasing from moderate (++) at Day 5 to very rich (+++) by Day 10, and stabilizing at rich (+++) by Day 15.

Table 2: Growth of test organisms on green pea pod residue as carrier material for starter culture

Isolates	Growth in terms of mycelial/ascomatal development		
	Day 5	Day 10	Day 15
*Aspergillus niger**	++	+++	+++
Penicillium sp. *	+	+++	+++
Chaetomium sp. */**	+/+	+++/+++	+++/+++
Trichoderma sp. *	++	+++	+++

Development Pattern: '+' poor: '++ moderate': '+++' rich
*Mycelial Development
**Ascomatal Development

All four cellulolytic fungal isolates exhibited vigorous growth on the green pea pod carrier, confirming its suitability for fungal colonization. *Chaetomium* sp. showed the most pronounced development with ascomatal structures, indicating high physiological activity. *Aspergillus niger, Penicillium* sp., and *Trichoderma* sp. also demonstrated rich, sustained mycelial growth, reflecting strong compatibility with the substrate.

Storage Life and percent viability of the Starter Cultures:

Table 3: Viability of starter cultures based on different carrier materials after 120 days of storage

S. No.	Isolates	Percent Viability (%)
1	*Aspergillus niger*	70
2	*Penicillium* sp.	70
3	*Chaetomium* sp.	60
4	*Trichoderma* sp.	76

The viability of cellulolytic fungal isolates on green pea pod-based carrier material was evaluated after 120 days of storage to assess the stability and shelf life of the starter cultures. The results (Table 3) indicated that all isolates retained good viability, demonstrating the ability of the carrier to support long-term fungal survival. The findings agree with those of [10].

Among the isolates, *Trichoderma* sp. recorded the highest viability (76%), showing vigorous regrowth upon subculturing, which suggests superior adaptability and strong compatibility with the nutrient composition of the green pea pod substrate. *Aspergillus niger* and *Penicillium* sp. each maintained 70% viability, indicating stable survival during storage. *Chaetomium* sp. showed slightly reduced viability (60%), reflecting minor loss in metabolic activity over time.

4. Conclusion

The present study demonstrated that green pea pod residue (*Pisum sativum* L.) waste can be effectively utilized as a carrier substrate for the development of cellulolytic fungal starter cultures. The physicochemical analysis confirmed that the pretreated green pea pod powder possessed desirable characteristics, including high organic matter content, good moisture retention and supported C:N ratio, making it a nutrient-rich and ideal substrate for fungal growth. All selected cellulolytic fungal isolates, viz., *Aspergillus niger, Penicillium* sp. *Trichoderma* sp., exhibited excellent mycelial development and *Chaetomium* sp., exhibited excellent ascomatal development on the substrate. The green pea pod carrier supported uniform and dense mycelial colonization, confirming its compatibility with fungal proliferation. Furthermore, the storage life evaluation indicated that the carrier material could maintain substantial viability of the inoculated cultures over an extended period, demonstrating its effectiveness in preserving fungal activity.

Overall, the study establishes that green pea pod waste is a cost-effective, nutrient-rich, and eco-sustainable carrier material, suitable for large-scale production of cellulolytic fungal inoculum. Its utilization can enhance composting efficiency, promote agro-waste valorisation, and contribute to sustainable waste management practices.

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