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Co-cultivation of Fungal inoculants for an effective Biodegradation of poultry feathers and preparation of compost

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Abstract

Environmental pollution by the Poultry feathers is one of the major nuisance to the environment because of its bad odour and transmission of various microbial diseases. Keratinolytic fungi have a great importance in the biodegradation of Poultry feather wasted and its improvement of livestock feed and production of protein hydrolysates. The present research deals with the Co-cultivation of Fungal inoculants for an effective biodegradation of Poultry feathers and preparation of Compost material for an agricultural purpose. The present study was also aimed to prepare the Compost fertilizer by using a Poultry feathers wastes. The collected feathers are degraded with the help of selected fungal isolates and converted into Compost fertilizer. Waste feathers of Broiler chicken were collected from the Tirupattur poultry farm, Proteolytic fungi were isolated by Serial dilution technique and, identified by Lactophenol cotton blue (LPCB) staining and Platting in Sabouraud's Dextrose Agar (SDA). Three fungal isolates *viz*., *Aspergillus niger*, *Trichoderma viride* and *Beauveria bassiana* were isolated and identified in the present research. The three fungal isolates were screened for its Proteolytic activity and efficient isolates were selected for the Poultry feather degradation. The growth of the three fungal isolates were optimized under different pH, Temperature and Culture conditions for studying the best growth conditions for the Poultry feather degradation process. The effect of *Aspergillus niger, Beauveria bassiana* and *Trichoderma viride* on Poultry feather degradation was determined in the present research by Single and Co-cultivation treatment methods.

In conclusion, Co-cultivation method showed an excellent result than the Single cultivation method. Among the six treatments studied in this present research, an effective Poultry feather degradation was noticed in the combination of *Trichoderma viride* and *Beauveria bassiana*, and less feather degradation was observed in the treatment containing *Beauveria bassiana* alone.

Key words : Poultry feather, Co-cultivation, Fungi, *Aspergillus niger, Beauveria bassiana* and *Trichoderma viride*.

Due to its flavour and lower cost, poultry meat is one of the foods that is enjoyed by people of all walks of life worldwide. The production of commercial poultry has expanded globally, but it is also regarded as one of the main issues facing the whole globe. The poultry sector is making a substantial contribution to the battle against poverty, hunger, and unemployment[15]. Large volumes of waste chicken feathers are generated almost everywhere in the globe, where they are often burned, dumped in landfills, or used as subpar animal feed. Poultry feather wastes are produced in large amounts and release foul odours into the environment on a daily basis, contributing to pollution[2]. The improper disposal of feather debris contaminates water and air sources, which is a serious environmental risk. An alternate process for turning chicken feathers into usable byproducts is required to remedy this. The use of microbial keratinase treatment to feathers has garnered attention because of its nutritional worth and possible application as a source of protein in food and feed additives. Like other proteases, keratinase manufacturing is being progressively targeted by the global market because of its potential as a workable substitute for conventional techniques[13].

Fungi are widely used in bioremediation and waste recycling because they are necessary microorganisms for the production of hydrolytic and industrial enzymes. Protease and Keratinase, which are necessary for the deterioration of keratinaceous chicken feathers, are especially well-produced by them. These feathers are good for agricultural usage since they contain nutrients that promote plant development. Research data expressed that the composting produces the plant nutritious final product and, it is an appropriate way to recycle the chicken feather waste. It is beneficial to focus on utilising chicken feathers treated with fungus for agricultural purposes[4]. Several fungal isolates including, *Aspergillus niger, Beauveria bassiana,* and *Trichoderma viride* have been identified to synthesize the proteolytic enzyme keratinase[14,18,21]. They regularly colonise different keratinous substrates, breakdown them, and increase the soil's mineral content, keratinophilic fungi have frequently been isolated from soil[17].

Waste materials may be bioremediated into valuable end products *via* the process of Composting. In addition to being an Aerobic waste management method, it eliminates soil contamination, greenhouse gas emissions, and other forms of environmental pollution in this universe. Composting, the resultant product, was used as an organic fertiliser for sustainable agriculture[10,16]. The compost is a rich source

of micronutrients and macronutrients which is very helpful for improving the physico-chemical and biological properties of the soil[12,19]. For managing the Poultry feather waste, Composting process might be a cheap and easy way for recycling and agriculturally beneficial utilization for crop nourishment[9]. The present study was focused on the fungal degradation of the Poultry feathers by using Keratinolytic fungal isolates (*Aspergillus niger, Beauveria bassiana* and *Trichoderma viride*) and preparation of Compost for the benefit of Agricultural crops[7].

Collection of Poultry feather contaminated soil :

A soil sample was taken from the Poultry feather contaminated areas in Tirupattur, Tamil Nadu, India, in order to isolate the fungus that degrade feathers. In a sterile container, a soil sample was taken between three and five centimeters below. The collected Poultry feather sample was marked, appropriately packaged, and sent to the laboratory within 24 hours for further process and research.

Isolation of Poultry feather degrading fungi :

The fungi that degrade the Poultry feather was isolated by Serial Dilution Method (Pour plate method). Proteolytic fungi that degrade the Poultry feather was degraded by Lactophenol cotton blue (LPCB) staining and platting in the Sabouraud's dextrose agar (SDA) plates. The sub-cultured fungal isolates were stored as a slants in the refrigerator at 4 °C for further research.

Screening of Fungi for its proteolytic activity :

The proteolytic activity of the identified fungal isolates was measured quantitatively by Plate assay. The fungal cultures (*Aspergillus niger, Trichoderma viride* and *Beauveria bassiana*) were inoculated into Skimmed milk agar and incubated for 6 days. The formation of the clear halo zone and the diameter of the fungal colony were observed on the third and sixth days of incubation.

Optimization of culture conditions for Poultry feathers degradation :

The ability of the selected fungal isolates (*Aspergillus niger, Trichoderma viride* and *Beauveria bassiana*) on decomposing the chicken feathers was optimised by taking into account by several important factors, including pH levels (pH 4, pH 5, pH 6, pH 7, pH 8, and pH 9), temperature (20 ºC, 27 ºC, 30 ºC, 37 ºC and 40 ºC), and incubation duration (3 days, 6 days, 9 days, 12 days and 15 days).

Determination of the ability of Fungi for Poultry feathers degradation :

Fungal inoculants and Co-cultivation treatments for Poultry feather degradation:

Fungal inoculants (*Aspergillus niger, Trichoderma viride* and *Beauveria bassiana*) were used for Poultry feather degradation as both Single inoculant and Combined inoculant as Six treatments given below

a) FD_1 – *Aspergillus niger* alone
b) FD_2 – *Trichoderma viride* alone
c) FD_3 – *Beauveria bassiana* alone
d) FD_4 – *Aspergillus niger* + *Trichoderma*

viride

e) FD_5 – *Aspergillus niger* + *Beauveria bassiana*

f) FD_6 – *Trichoderma viride* + *Beauveria bassiana*

Biodegradation of Poultry feathers (Weight Loss Method) :

Poultry feather biodegradation was done in a 500 ml Erlenmeyer flask. Feather was weighed using an electronic balance and recorded before to being placed in the Culture media. The identical circumstances applied to the control group that did not receive a fungal inoculate. In order to degrade the feathers, one millilitre of each fungal isolate as a single or combined inoculum was added to a 500 millilitre Erlenmeyer flask that held 200 millilitres of Sabouraud's dextrose broth[1]. The flasks were incubated for 15 days at room temperature while being shaken in a Shaking incubator at 160 rpm. Following a 15 days incubation period, the leftover feathers were removed from the Sabouraud's dextrose broth by straining it using Whatman filter paper and stored for the further examination. The protein content was estimated by using Lowry's technique on the supernatant. Feather degeneration was assessed visually, and the percentage of feather degradation was recorded[6]. By analysing the amount of feather weight loss, the percentage of Fungal isolate degradation of Poultry feathers was as calculated. The percentage of weight loss was calculating by the formula

Percentage of Feather Degradation = Initial weight of the Feather – Final weight of the Feather /Initial weight of the Feather × 100

Preparation of Poultry feather compost fertilizer :

In order to prepare the Poultry feather compost, an amount of 5 g, 10 g, 20 g, and 30 g of Poultry feathers were combined with 2 kg of sterile soil in each plastic bin and pre-autoclaved for 15 minutes at 121 °C [16]. Next, a 200 ml culture solution of fungal inoculants (*Aspergillus niger, Trichoderma viride,* and *Beauveria bassiana*) (in single and combination) that had been cultured for three days was evenly added to the Compost preparations aseptically, and labelled properly[11]. Additionally, a second control was kept in place without addition of fungal culture. For thirty days, the feathers were stored for deterioration. Following a 30 days degradation period, treatments exhibiting feather deterioration of at least 50% were chosen for physico-chemical parameter analysis[5].

Analysis of Physico-chemical parameters of the Treated Poultry Feather Compost material :

The pH of Compost material was analyzed in Digital pH meter. The Total organic carbon (TOC) content was estimated by using the method proposed by Abdullah and Chin[1]. Total Nitrogen content was estimated by following the Micro Kjeldahl method[20]. Total Phosphorus content was determined by using the Colorimetric method[3]. Total Potassium was estimated by Flame Photometer. Sulfur, Zinc, Boron, and Iron contents were measured by using the diacid digest using an Atomic Absorption Spectrophotometer[4].

Identification of Fungi isolated from Poultry feather contaminated soil :

The fungi isolated from the Poultry feather contaminated soil was identified by Lactophenol cotton blue (LPCB) staining and platting on Sabouraud's dextrose agar (SDA). Three different fungal species identified are *Aspergillus niger, Beauveria bassiana* and *Trichoderma viride*. The characteristics of the identified Fungal isolates is given below.

Characterization of Aspergillus niger :

Microscopic examination :

Conidiophore are smooth walled, hyaline or pigmented in nature. Vesicles are sub-spherical in shape and conidial heads are radiate. Conidiogenous cells biseriate in arrangement. Medulla twice as long as the phialides. Conidia are brown in colour, ornamented with warts and ridges. Hyphae were septate in nature.

Colony morphology on SDA plate :

Colonies are black in colour and consisting of a dense felt of conidiophores all over the plate.

Characterization of *Beauveria bassiana :*

Microscopic examination :

Conidia was distinctive and appear as blue spore balls and composed of clusters of conidiogenous cells. The conidiogenous cells are short in size, ovoid shaped and terminate in a narrow apical extension called a rachis. The rachis elongates after each conidium was produced resulting in a long zig-zag extension.

Colony morphology on SDA plate :

Colonies are grown in white colour with hyaline hyphae and conidiogenous cells densely clustered and whorls, colorless, short, ovoid with base globose and extending apically and repeated branching a short distance below each of several apically – formed conidia.

Characterization of *Trichoderma viride :*

Microscopic examination :

Conidiophores are highly branched, thin walled, loosely or completely tufted, often formed in distinct concentric rings along the aerial hyphae. Main branches of the conidiophores produce lateral side branches that may be paired or not. Phialides arising directly from the main axis near the tip.

Colony morphology on SDA plate :

Colonies at first transparent or white. Mycelium typically not obvious. Conidia typically forming within one week in compact or loose tufts in shades of green or yellow or less frequently white.

Screening of Fungi for its Proteolytic activity :

The three isolated Fungal species (*Aspergillus niger, Beauveria bassiana* and *Trichoderma viride*) were screened for its Proteolytic activity because Poultry feathers are protein in nature and Proteolytic activity is very essential for the Feather degradation. In Skimmed milk agar, all the three fungal isolates showed the zone of clearance and confirms the Proteolysis activity. According to the

previous reports, the three distinct fungal species that have been isolated have the ability to create proteolytic enzymes, such as Keratinase and Protease, which are capable of breaking down feather proteins. The discovered fungal isolates were chosen for additional feather degradation due to their proteolytic activities.

Optimization of culture conditions for poultry feathers degradation :

Effect of pH *on the growth of poultry feather degrading fungi :*

The effect of pH on the growth of three Poultry feather degrading fungi (*Aspergillus niger, Beauveria bassiana* and *Trichoderma viride*) was studied at pH 4, pH 5, pH 6, pH 7, pH 8 and pH 9, and the results were given in the Table – 1. The growth of all the three fungal species were very good and luxuriant at pH 6 and pH 7, and moderate at pH 4 and pH 5. No fungal growth was observed at pH 8 and pH 9.

Table–1. Effect of pH on the growth of Poultry Feather Degrading Fungi

pH	Fungal Growth		
	Aspergillus niger	*Beauveria bassiana*	*Trichoderma viride*
4	+	+	+
5	+	+	+
6	++	++	++
7	++	++	++
8	-	-	-
9	-	-	-

(++ Good and luxurious growth; + Moderate growth and - No growth)

Effect of Temperature on the growth of Poultry feather degrading fungi :

The effect of Temperature on the growth of three Poultry feather degrading fungi (*Aspergillus niger, Beauveria bassiana* and *Trichoderma viride*) was determined at 20 °C, 27 °C, 30 °C, 37 °C and 40 °C, and the results were furnished in the Table–2. The growth of all the three fungal species were very good and luxuriant at 27 °C and 30 °C, and moderate at 20 °C and 37 °C. No fungal growth was observed at 40 °C.

Effect of Incubation period on the growth of Poultry feather degrading fungi :

The effect of Temperature on the growth of three Poultry feather degrading fungi (*Aspergillus niger, Beauveria bassiana* and *Trichoderma viride*) was determined at 3rd Day, 6th Day, 9th Day, 12th Day and 15th Day, and the findings were presented in the Table – 3. It was observed that the growth of all the three fungal species were very good and luxuriant at 6th Day, 9th Day, 12th Day and 15th Day, and moderate at 3rd Day.

Table-2. Effect of Temperature on the growth of Poultry Feather Degrading Fungi

Temp-erature (°C)	Fungal Growth		
	Asper-gillus niger	*Beauve-ria bassiana*	*Tricho-derma viride*
20	+	+	+
27	++	++	++
30	++	++	++
37	+	+	+
40	-	-	-

(++ Good and luxurious growth; + Moderate growth and - No growth)

Table-3. Effect of Incubation time on the growth of Poultry Feather Degrading Fungi

Incubation Time (Days)	Fungal Growth		
	Aspergillus niger	*Beauveria bassiana*	*Trichoderma viride*
3	+	+	+
6	++	++	++
9	++	++	++
12	++	++	++
15	++	++	++

(++ Good and luxurious growth; and + Moderate growth)

Determination of the ability of Fungi for Poultry feathers degradation as Single and Co-cultivation Treatments (Weight loss method) :

The effect of three fungal isolates (*Aspergillus niger, Beauveria bassiana* and *Trichoderma viride*) on Poultry feather degradation was determined in the present research by Single and Co-cultivation treatment methods. The Weight loss method was employed to study the Poultry feather degradation by Fungi. The findings of the present study were given in Table–4 to Table–8. The feather degradation was done for 30 days and the Weight loss was analyzed during every 6 days interval on 6th Day, 12th Day, 18th Day, 24th Day and 30th Day. Interestingly, the degradation of Poultry feather was increased while the days were increased. As expected, Co-cultivation method showed an excellent result when compared to the Single cultivation method. Good feather degradation was observed at FD – 6 (*Trichoderma viride* + *Beauveria bassiana*) followed by FD – 4 (*Aspergillus niger* + *Trichoderma viride*), FD – 5 (*Aspergillus niger* + *Beauveria bassiana*), FD – 1 (*Aspergillus niger* alone) and FD – 2 (*Trichoderma viride* alone). Less feather degradation was noticed in FD – 3 (*Beauveria bassiana* alone).

Table–4. Effect of Fungi on Poultry feathers degradation as Single and Co-cultivation Treatments (Day – 6)

Treatments	Initial weight (g)	Final weight (g)	Percentage of Feather degradation (%)
FD 1	310	307.86	0.690
FD 2	310	308.05	0.629
FD 3	310	309.09	0.293
FD 4	310	297.18	4.135
FD 5	310	303.77	2.009
FD 6	310	297.33	4.087

(FD_1 – *Aspergillus niger* alone; FD_2 – *Trichoderma viride* alone; FD_3 – *Beauveria bassiana* alone; FD_4 – *Aspergillus niger* + *Trichoderma viride;* FD_5 – *Aspergillus niger* + *Beauveria bassiana* and FD_6 – *Trichoderma viride* + *Beauveria bassiana*)

Table–5. Effect of Fungi on Poultry feathers degradation as Single and Co-cultivation Treatments (Day – 12)

Treatments	Initial weight (g)	Final weight (g)	Percentage of Feather degradation (%)
FD 1	310	302.67	2.364
FD 2	310	308.15	0.596
FD 3	310	309.23	0.248
FD 4	310	295.71	4.609
FD 5	310	296.77	4.267
FD 6	310	294.15	5.112

(FD_1 – *Aspergillus niger* alone; FD_2 – *Trichoderma viride* alone; FD_3 – *Beauveria bassiana* alone; FD_4 – *Aspergillus niger* + *Trichoderma viride;* FD_5 – *Aspergillus niger* + *Beauveria bassiana* and FD_6 – *Trichoderma viride* + *Beauveria bassiana*)

Table–6. Effect of Fungi on Poultry feathers degradation as Single and Co-cultivation Treatments (Day – 18)

Treatments	Initial weight (g)	Final weight (g)	Percentage of Feather degradation (%)
FD 1	310	303.55	2.080
FD 2	310	307.11	0.932
FD 3	310	308.20	0.580
FD 4	310	296.63	4.312
FD 5	310	300.55	3.048
FD 6	310	295.45	4.693

(FD_1 – *Aspergillus niger* alone; FD_2 – *Trichoderma viride* alone; FD_3 – *Beauveria bassiana* alone; FD_4 – *Aspergillus niger* + *Trichoderma viride;* FD_5 – *Aspergillus niger* + *Beauveria bassiana* and FD_6 – *Trichoderma viride* + *Beauveria bassiana*)

Table–7. Effect of Fungi on Poultry feathers degradation as Single and Co-cultivation Treatments (Day – 24)

Treatments	Initial weight (g)	Final weight (g)	Percentage of Feather degradation (%)
FD 1	310	302.00	2.580
FD 2	310	308.02	0.638
FD 3	310	309.23	0.248
FD 4	310	294.96	4.851
FD 5	310	296.20	4.451
FD 6	310	294.45	5.016

(FD_1 – *Aspergillus niger* alone; FD_2 – *Trichoderma viride* alone; FD_3 – *Beauveria bassiana* alone; FD_4 – *Aspergillus niger* + *Trichoderma viride;* FD_5 – *Aspergillus niger* + *Beauveria bassiana* and FD_6 – *Trichoderma viride* + *Beauveria bassiana*)

Table–8. Effect of Fungi on Poultry feathers degradation as Single and Co-cultivation Treatments (Day – 30)

Treatments	Initial weight (g)	Final weight (g)	Percentage of Feather degradation (%)
FD 1	310	301.66	2.690
FD 2	310	308.00	0.645
FD 3	310	309.00	0.322
FD 4	310	294.00	5.161
FD 5	310	295.80	4.580
FD 6	310	293.00	5.483

(FD_1 – *Aspergillus niger* alone; FD_2 – *Trichoderma viride* alone; FD_3 – *Beauveria bassiana* alone; FD_4 – *Aspergillus niger* + *Trichoderma viride;* FD_5 – *Aspergillus niger* + *Beauveria bassiana* and FD_6 – *Trichoderma viride* + *Beauveria bassiana*)

Physico-chemical parameters of the Treated Poultry Feather Compost :

Physico-chemical parameters of the Fungal treated Poultry feather compost was analyzed during every 6 days intervals for 30 days. The compost parameters are given in Table–9. Increase in days showed an increase in the improvement of Physico-chemical characteristics of the compost. At the final stage (Day – 30), the pH was 8.1, Temperature recorded was 25 °C, Nitrogen content was 8.20 %, Phosphorous content was 1.85 %, Potassium content was 4.58 %, Sulphur content was 14.88 %, Iron content was 7.5 %, Zinc content was 5.30 %, Boron content was 1.92 % and Organic matter content was 13.20 %.

Table–9. Physico-chemical parameters of the Treated Poultry Feather Compost

Physico-chemical properties	Poultry Feather Compost					
	Day-0	Day-6	Day-12	Day-18	Day-24	Day-30
pH	8.5	8.3	8.8	8.4	8.2	8.1
Temperature (°C)	17	18	20	21	22	25
Nitrogen (%)	1.25	3.33	5.89	6.36	7.22	8.20
Phosphorous (%)	0.30	0.88	1.09	1.53	1.78	1.85
Potassium (%)	2.15	3.40	3.90	4.03	4.25	4.58
Sulphur (%)	11.02	13.69	13.72	13.94	14.80	14.88
Iron (%)	5.3	6.6	6.8	7.0	7.1	7.5
Zinc (%)	1.77	2.03	2.75	3.35	4.28	5.30
Boron (%)	0.40	0.56	0.65	0.87	1.15	1.92
Organic matter (%)	15.26	14.80	14.30	14.00	13.80	13.20

Since poultry feathers are a serious environmental contaminant, they require the right kind of treatment. The biological treatment of poultry feather waste using fungi offers a sustainable, economical, and environmentally friendly alternative to traditional treatment methods such as physical and chemical treatments. From this present study, it was observed that the Fungal isolates like *Aspergillus niger*, *Beauveria bassiana* and *Trichoderma viride* are the appropriate decisions for the decomposition of poultry feather wastes. In contrast to the single culture method, the co-cultivation method produced great results. Among the six treatments, effective feather degradation was observed at FD – 6 (*Trichoderma viride* + *Beauveria bassiana*) and less feather degradation was recorded in the treatment FD – 3 (*Beauveria bassiana* alone). Despite promoting plant growth, it keeps different waste products from contaminating the soil. Rich in macro, micro, and macronutrients as well as nitrogen, potassium, and phosphorus, feather compost helps with organic farming and crop cultivation.

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