Journal of Applied and Natural Science
12(3): 411 - 414 (2020)
Published online: September 7, 2020
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Chicken feather waste degradation by *Alternaria tenuissima* and its application on plant growth

Mannu Kumari
Department of Microbiology, Dolphin PG College of Science and Agriculture, Chunni kalan Fatehgarh sahib-140307, (Punjab), India
Jitendra Kumar*
Department of Botany, Dolphin PG College of Science and Agriculture, Chunni kalan Fatehgarh sahib-140307, (Punjab), India

*Corresponding author. E. mail: jeetmicro@gmail.com

Article Info
https://doi.org/10.31018/jans.v12i3.2345
Received: August 5, 2020
Revised: September 1, 2020
Accepted: September 4, 2020

How to Cite
Kumari, M. and Kumar, J. (2020). Chicken feather waste degradation by *Alternaria tenuissima* and its application on plant growth. *Journal of Applied and Natural Science*, 12(3): 411 - 414.https://doi.org/10.31018/jans.v12i3.2345

Abstract
The use of chicken as food is rising day today and as byproduct 8.5 billion tons, poultry feathers are produced worldwide, whereas India contributes about 350 million tons per annum. It is a waste product of poultry industries, are considered a potential high-quality protein supplement owing to their crude protein content of more than 85%. In the present study *Alternaria tenuissima* a keratinophilic fungus was used for feather degradation in submerged state fermentation and soil. Total Protein releases were studied in submerged state fermentation by *A. tenuissima* are 122µg/ml and 238µg/ml in 15 and 25 days respectively. Lysine, Methionine, Cysteine and Valine were found 15.8, 6.8, 20.2, 7.5 µg/ml in 25 days, respectively. Chicken feathers were mixed with soil and inoculated with spore suspension for degradation of complex keratin protein into simpler organic forms. *A. tenuissima* degraded feathers in soil and enhances nutritional value. Five-gram feathers in 250 g soil mixtures were found better growth enhancers and increased height. This work will reduce the solid waste generated in the form of feathers from the poultry industry, and convert it into a simpler organic form that can be used by plants.

Keyword: Biofertilizer, Feather compost, Keratinophilic fungi, Keratinase

INTRODUCTION

Feathers are biological waste produced in large volumes by the poultry processing industry, and their microbial degradation is for the generation of end products with benefits to the manufacturers in setting up ecological and economic strategies (Kim and Patterson 2000). Traditional approaches have restrictions for producing digestible feather meal force the use of microbial degradation of feather wastes as environmentally safe, low-cost methods (Gupta and Ramnani 2006). Keratin degradation is attracting biotechnological attention since it might provide a substitute way of waste management as well as the production of valuable products (Brandeli and Riffel 2006, Pasupuleti *et al*., 2010). *Bacillus* was used for their keratinases production, optimization and characterization (Sahoo *et al*., 2012, Dong *et al*., 2017, Arokiyaraj *et al*., 2019). Tiwary and Gupta (2012) used *Bacillus licheniformis* to study the conversion of the chicken feather into feather meal. Recycled keratin wastes and other organic wastes can be used as soil alterations and nourishments to provide organic matter for biologically vital and productive soils (Zheljazkov 2005). Soil samples treated with feather found variation in physiochemical composition to control (Ibrahim *et al*., 2014). The protein-rich concentrates feather meal formed for poultry feed can also use as a semi-slow release of nitrogen fertilizer (Kumar *et al*., 2017). Bacillus sp. was used to develop chicken feather compost from feathers waste and to promote plant growth activity (Nagarajan *et al*., 2017, Tamreihao *et al*., 2019). Keratinophilic fungi were isolated for their taxonomical aspect by several workers (Deshmukh and Verekar 2006, Deshmukh *et al*., 2010, Sharma 2016, Bairwa and Sharma 2020). However, few records are available in which fungi were used for the application of fertilizing potential of feather waste for enhancing plant growth as *Chrysosporium tropicum* and *Malbranchea* sp. (Kumar *et al*., 2017), *Aphanoascus keratinophillus* and *C. tropicum* (Bohacz *et al*., 2020). In the present study, *A. tenuissima,* a keratinolytic fungus was used to degrade feathers into submerged state fermentation and soil. The effect of feather waste supplemented soil was studied on the growth of Chickpea, *Cicer arietinum*.

MATERIALS AND METHODS

Microorganism: *Alternaria tenuissima* (NCBI accession no MN027926, named as *A. tenuissima* isolate A1) was used in the present study. Preserved fungi were subcultured on Potato dextrose Agar (Fig 1).

Degradation of feathers in submerged state fermentation: The media were prepared by the method of (Kumar and Kushwaha, 2014) containing sets of flasks that were marked as a Fungal control (FC), Keratin control (KC), and tests (T). The tests were inoculated with a disc of fungus *Alternaria tenuissima*. The flasks were incubated at 28˚C for 5, 10, 15, 20, and 25 days in shaking conditions.

Estimation of protein and amino acids: Culture filtrate from each flask was centrifuged, and the supernatant was used for protein assay (Lowry *et al*., 1951) and amino acid (McGrath, 1972). The results of protein estimation are expressed as total protein i.e. the sum of the measured value of the test sample, fungus control, and keratin control.

Preparation of feather compost: Feather compost was developed by Sekar *et al*., (2015) as follows: The soil was collected from the agricultural field adjacent to Dolphin College Chunni kalan Punjab and chicken feathers were collected from poultry shops. Collected feathers were washed with detergent and tap water, air dried, and autoclaved. The control contained only soil, no feathers were added and marked as sample A, while samples B, C, D, and E were mixed with 2, 3, 4, and 5g autoclaved feathers with soil respectively. All samples were inoculated with 20ml of aqueous spore suspension of *A. tenuissima* of 5day old culture. Feather compost preparations were kept for degradation for 25 days. The box was covered regularly moistened with sterilized water and mixed. The morphological change was analyzed.

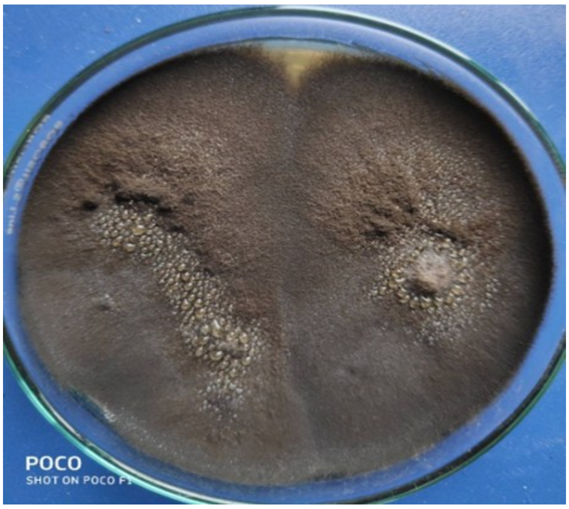

Fig. 1. *Growth of Alternaria tenuissima on potato dextrose agar medium.*

***In vitro* experiments on plants growth:** Pod experiments were performed by the method of Nagarajan *et al*., (2017) with some modifications that were as follows: The seeds of Chickpeas were sowed to pods A, B, C, D, and E. The pods were grown in triplicate at temp 25-15^{0}C, relative humidity 65-70-%. The pods were watered every day to keep water holding capacity. The morphological analysis of the plant was done at intervals of the 5 day.

RESULTS AND DISCUSSION

Estimation of total protein release and amino acid: The *A. tenuissima* moderately degraded feathers. The protein content released into the supernatant due to the degradation of feathers is given in Table 1. Total protein release in submerged state fermentation was 122µg/ml and 238µg/ml in 15 and 25 days incubation respectively. Cysteine, Lysine, Methionine and Valine were released as 20.2, 15.8, 6.8 and 7.5µg/ml in 25 days. The release of protein and amino acid in submerged state fermentation represented that degraded feather improved soil nutritional value by adding amino acids and oligopeptides. Parihar and Kushwaha, (2000) observed 107.66µg/ml protein release by *Chrysosporium indicum*. Gousterova *et al*., (2005) studied examined fungi for the estimation of protein and amino acid in submerged state fermentation and found maximum by *C. tropicum* 432.66µg/ml. Bohacz *et al*., (2020) studied chicken feathers degradation by *Aphanoascus keratinophilus* and *C. tropicum* and recorded 400µg -1500 µg in 6 weeks. Lysine and cysteine production by *C. tropicum* and *Malbranchea* sp. was 12.98 and 23.4 µg/ml in 20 days respectively (Kumar *et al*., 2017).

Preparation of soil feather compost: Feathers were found degraded in 25 days. Throughout the composting process, the keratinous material was observed for morphological changes that occurred due to the colonization of fungus. The processing of compost initially resulted in an increase in pH and fungi degraded the feathers in soils. Gousterova *et al*., (2005) used Thermoactinomycetes and used it in feather composting purposes and degraded feathers in submerged and exposed soil.

***In vitro* experiments on plants growth:** The effect of feather compost on the growth of Chickpea seed was observed on control (without feather compost) and treated plants (containing feather compost) as shown in Fig.2 & 3. A regular rise in height was observed in feather compost potted plants except for pod B may be due to contest for nutrients between fungus and plant sample containing less amount of feathers. The pod length observed in Chickpea seeds was as follows E>

Table 1. Total protein and amino acids release in submerged state fermentation.

	Protein and amino acid release µg/ml (Incubation period in days)				
	5	**10**	**15**	**20**	**25**
Total protein**	089.40	107.70	122.00	217.70	238.00
Lysine*	11.4	12.2	14.3	15.1	15.8
Methionine*	5.2	5.7	6.3	6.4	6.8
Cystien*	15.1	18.3	19.2	19.8	20.2
Valine*	6.3	6.5	6.8	7.2	7.5

Total protein (sum of keratin control + Test sample) ** readings mean of triplicate*

Table 2. Growth of Chickpea plants in feather supplemented soil.

	Sample				
	A	**B**	**C**	**D**	**E**
Feather Condition	No feather	Colonization occurred	Moderate degradation	Degradation	Degradation
Height (cm)	10.2	9.1	11.5	15.3	16
Growth condition	Slow	Stunted	Normal	Good	Good
Root development	Normal	Stunted	Moderate	Good	Maximum

D> C> A> B. Length of the shoot was 1.4 times increased in compassion to control (Table 2). Feather supplemented pot showed greener leaves and root length of the plant were increased. The number of leaves was 50 % increased in feather supplemented soil. The effectiveness depends on the degradation capability of microorganisms. Adetunji *et al*., (2012) used organic fertilizers at a rate of 30 % and found good growth and yield, and least susceptibility to diseases, as compared for control in the case of cowpea. Kumar *et al*., (2015) found better growth in pea plants supplemented with decomposed feathers. Feather compost controls plant growth after complete degradation although undegraded feather not supporting the plant growth. Degraded feather treated samples found enhanced root and shoot (Nagarajan *et al*., 2017). Oluwaseun *et al*., (2018) studied biodegradation of feather waste by keratinase enzyme and reported its positive effect on tomato plants. Feather protein degradation enhances the protein and amino acid contents which increases water retention capacity and moister content of the soil which is favourable for abundant growth. All workers supported that feather compost supplemented plant showed better growth.

Conclusion

The present study concluded that *A. tenuissima* was found efficient for feather degradation and increased the nourishing value of the soil by adding proteins (238 µg/ml) Cysteine (20.2 µg/ml), Lysine (15.8 µg/ml), Methionine (6.8 µg/ml) and Valine (7.5µg/ml) in 25 days. The quality of nutrients in the soil depended on the keratin degradation capability of keratin degrading fungi. An accurate amount of fungal inoculums and soil feathers mixture is still an issue. Work on these fungi could solve the environmental issue of poultry waste management and the production of low-cost fertilizers.

Fig. 2. *Chick pea growth on feather compost.*

Fig. 3. *Uprooted chick pea sample demonstrating shoot length.*

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