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Original Article

Fungal conversion of chicken-feather waste into biofortified compost

Conversão fungosa de resíduos de pena de frango em composto biofortificado

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Abstract

Poultry industry is amongst highly developed industries of Pakistan, fulfilling the protein demand of rapidly increasing population. On the other hand, the untreated poultry waste is causing several health and environmental problems. The current study was designed to check the potential of keratinolytic fungal species for the conversion of chicken-feather waste into biofortified compost. For the purpose, three fungal species were isolated from soil samples. These strains were pure cultured and then characterized phenotypically and genotypically. BLAST searches of 18S rDNA nucleotide sequence of the fungal isolates revealed that the two fungal isolates belonged to genus *Aspergillus* and one belonged to genus *Chrysosporium*. Optimum temperature for *Aspergillus flavus*, *Aspergillus niger* and *Chrysosporium queenslandicum* was 29, 26 and 25 °C, respectively. *A. flavus* showed maximum (53%) feather degradation, *A. niger* degraded feather waste up to 37%, while *C. queenslandicum* showed 21% keratinolytic activity on chicken feathers at their respective temperature optima. The degradation potential of these fungal species showed their ability to form compost that has agro-industrial importance.

Keywords: biodegradation, biofortified compost, keratinase, keratinolytic fungus, keratinous waste, microbial biotechnology.

Resumo

A indústria avícola está entre as indústrias altamente desenvolvidas do Paquistão, atendendo a demanda de proteína da população em rápido crescimento. Por outro lado, os resíduos de aves não tratados estão causando diversos problemas de saúde e ambientais. O presente estudo foi desenhado para verificar o potencial de espécies de fungos queratinolíticos para a conversão de resíduos de penas de frango em composto biofortificado. Para tanto, três espécies de fungos foram isoladas de amostras de solo. Essas cepas foram cultivadas puramente e, em seguida, caracterizadas fenotipicamente e genotipicamente. As pesquisas do BLAST da sequência de nucleotídeos do rDNA 18S dos isolados de fungos revelaram que os dois isolados de fungos pertenciam ao gênero *Aspergillus* e um pertencia ao gênero *Chrysosporium*. A temperatura ótima para *Aspergillus flavus*, *Aspergillus niger* e *Chrysosporium queenslandicum* foi de 29, 26 e 25 °C, respectivamente. *A. flavus* apresentou degradação máxima de penas (53%), *A. niger* degradou resíduos de penas em até 37%, enquanto *C. queenslandicum* apresentou 21% de atividade queratinolítica em penas de frango em suas respectivas temperaturas ótimas. O potencial de degradação dessas espécies de fungos mostrou sua capacidade de formar composto de importância agroindustrial.

Palavras-chave: biodegradação, composto biofortificado, queratinase, fungo queratinolítico, resíduos queratinosos, biotecnologia microbiana.

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1. Introduction

Massive amounts of environmental wastes are produced in various countries which contain significant amount of carbon and protein complexes (Park and Son, 2009). One category of environmental pollutants is keratinous wastes (Werlang and Brandelli, 2005; Kumawat et al., 2016). Many industries like slaughterhouse, food industry, wool and leather industry and poultry industry are constantly generating million tons of keratinous wastes. China, USA and Brazil are the chief producers of keratin containing biomass, producing about 40 million tons of keratinous waste annually (Sharma and Gupta, 2016). According to the poultry meat production data of 2014, the total production of meat in 2012 was 776,000 tons and increased up to 1.6% in 2014. In the total body weight of chicken, about 5–7% is the weight of feathers. So, it can be evaluated that every year in Pakistan thousand tons of feather waste is produced (Riffel and Brandelli, 2006). The keratinous wastes are usually incinerated, land filled, dumped or buried (Fellahi et al., 2016). These techniques can cause air, soil and water pollution. Feather waste is responsible for many human diseases like fowl cholera and chlorosis (Williams et al., 1991).

Microbial degradation is the most remarkable process because it is environment friendly, no secondary products are released, less energy is consumed and most importantly it is cost-effective (Vasileva-Tonkova et al., 2009). Composting is the biological transformation of organic solid waste into useful end products like fertilizers. High biological activity and high organic contents make the compost very operational in various applications like bioremediation, control of erosion, revegetation and biofilteration (Alexander, 1999). Microbes play an important role in the process of composting. The ratio of biomass of fungus to prokaryotes is 2:1 (Sparling et al., 1982; Wiegant, 1992). Fungus uses various carbon sources especially lignocellulosic polymers which can tolerate extreme conditions. They are generally accountable for maturation of compost (Miller, 1996). Because of the capacity to degrade the extensive range of organic waste, *Penicillium, Thermomyces, Acremonium, Malbranchea, Aspergillus, Pseudallescheria* and *Cladosporium* genera are the most common in composting material (Miller, 1996).

Meanwhile different materials are related with different fungal species and these fungal communities are monitored to estimate the quality of compost and its applications in field (Peters et al., 2000). Different studies are performed on feather composting and the main focus of these studies was on usage of some keratinolytic bacterial species and actinomycetes which were isolated from feathers (Ichida et al., 2001; Tiquia et al., 2005; Tiquia, 2005). In compost, the mineralization of organic sulphur and nitrogen is associated with keratinolytic fungus (Bohacz and KornihowiczKowalska, 2009). Latest studies show that fortification of compost material with feathers produced operational proteolytic activity which is non-specific initial in the process of compost. It also stimulated the keratinolytic fungal growth which caused feather degradation in late stages of composting (Bohacz and Kornihowicz-Kowalska, 2009; Kornihowicz-Kowalska and Bohacz, 2010).

The advantages of compositing include easy management and reduction in solid waste for disposal (Steuteville, 1992; Lowe and Buckmaster, 1995). Compost can potentially reduce the use of fertilizers and also increase the moisture retention when applied to the land (Crobe, 1994). Also, in the process of composting, the mature compost can be used as bulking agent, moisture absorbent or compost activator (Riggle, 1989). Focus of the present study is isolation, phenotypic and molecular characterization of keratinolytic fungal species and its implication for the viable conversion of chicken feather waste into agro-industrially important product i.e. biofortified compost.

2. Materials and Methods

2.1. Sample collection

For the isolation of keratinolytic fungal strains soil samples were collected from different feather dumping sites at University of Veterinary and Animal Sciences, UVAS, Ravi Campus, Pattoki and Tollinton market Lahore, Pakistan. These samples were transported to the Applied and Environmental Microbiology Laboratory, Department of Wildlife and Ecology, UVAS, Ravi Campus, Pattoki for further processing.

2.2. Isolation and pure culturing of keratinolytic fungal strains

2g of each soil sample was added to 1000mL of distilled water. 10 dilutions of each sample were made from these suspensions. Then these soil-suspended dilutions were spread over Feather Meal Agar (FMA) on isolate keratinolytic fungal strains and incubated for 6 days in incubator at 28 °C. Every dilution was processed in triplicates. After 6 days of incubation isolated fungal strains were pure cultured on Malt Extract Agar (MEA).

2.3. Preparation of feather meal powder

Chicken feathers were collected from different sampling sites. These feathers were soaked in detergent for 2h and washed thoroughly with water. These washed feathers were dried in hot air oven at 171 °C for 96 h. To prepare feather meal these dried feathers were crushed in ball mill and passed through small-mesh net to eliminate the coarse particles.

2.4. Phenotypic characterization

Pure cultures of fungal strains were identified on the basis of macroscopic and microscopic analysis of fungal colonies. Isolated fungal strains were identified on the basis of morphology up to genus and species level. Macroscopic features like color, shape, texture and size of colony were observed. Microscopic features like vesicle, septation of hyphae, conidiophore, metulae and phialides, were examined. This examination was carried out through the staining of fungus with lacto-phenol cotton blue.

2.5. Genotypic characterization

Molecular identification of the fungal isolates was carried out by DNA extraction from freshly grown fungal cultures. The DNA was extracted using Dneasy® plant mini kit (Qiagen, Hilden, Germany). 18S rRNA gene was then amplified using forward (5´-TACTGTGAAACTGCGAATGGCTC-3´) and reverse (5´-TGATCCTTCCGCAGGTTCACCTA-3´) primers (Simon et al., 2000; Krienitz et al., 2001). The amplification reaction was conducted in thermal cycler for 35 cycles (Hamburg 22331, Germany). Initiation was the first step at 95 °C for 5 minutes. Denaturation step was conducted at 90 °C for 30 seconds. Annealing temperature was about 56.3 °C for 45 seconds at 72 °C for 30 seconds and termination step was conducted 72 °C for 5 minutes. The PCR products was separated on 1% (w/v) agarose gel using ethidium bromide in TAE buffer and purification was done with the help of Gene Purification Kit (Fermentas). The sequencing was conducted using Big Dye Terminator v3.1 cycle sequencing ready reactions (Macrogen) Korea. Homology searches were conducted with the help of BLAST (NLM, 2021).

2.6. Fungal conversion of feather waste into biofortified compost

The ability of the fungal species to degrade feather waste was determined by using 5% (v/v) inoculum of the each isolated fungal strain (1 day old culture) individually into a 13g heap of artificially prepared feather meal; experiment was conducted in triplicates. For the maintenance of aeration, mixing of heaps was done periodically. One uninoculated group was acted as control with same experimental conditions i.e. placed at room temperature. The estimation of waste degradation was measured by withdrawing the sample periodically after ten days up to fifty days (Sarkar and Chourasia, 2017).

2.7. Estimation of organic waste degradation

Three sets containing 13g of artificially prepared organic waste (feather meal) were prepared. These three sets were inoculated individually by three different strains (that were previously isolated) with 5% (v/v) inoculum to estimate the capability of these strains for the degradation of waste material. One set was inoculated with *A. flavus,* second set was inoculated with *A. niger* and the third set was inoculated with *C. queenslandicum.* Aeration was maintained by mixing the heap periodically. One set was served as control group and kept uninoculated under the same experimental conditions. Periodically, the sample were taken for the estimation of waste degradation at the regular intervals of ten days for fifty days and the waste material degradation was estimated on the basis of the following factors (Sarkar and Chourasia, 2017).

2.7.1. Determination of moisture content in waste

Decrease in moisture content is linked with waste deprivation. 2g of compost sample was carried into the china dish and it was placed into the hot air oven for 4 h at 120 °C. After this china dish was kept into the desiccator for cooling process. After cooling the weight of the sample was estimated (Sarkar and Chourasia, 2017). The assessment of moisture content was done according to the given Formula 1:

$$\text{Moisture}(\%) = \frac{\text{W1} - \text{W2X100}}{\text{Weight of Sample}} \quad (1)$$

Where
W2 = final weight of crucible + sample
W1 = initial weight of crucible + sample

2.7.2. Estimation of pH

The solution of compost was made in 1:10 ratio by the addition of distilled water. This solution was kept for 2 hours for the salt dissolution. The electrode of pH meter was dipped into the solution of compost and when it was stabilized the reading was noted (Sánchez-Monedero et al., 2001).

2.7.3. Estimation of electrical conductivity

The solution of compost was made in 1:5 ratio by addition of distilled water. The conductance cell was rinsed with distilled water. Afterwards, this cell was dipped into the compost solution. The stabilized reading was noted on EC meter. Reading was displayed in mS/cm (Arnold, 2004).

2.7.4. Change in temperature

Three levels were selected for the estimation of change in temperature; upper surface, middle layer, bottom. Holes were made in the wall of bucket for the detection of temperature change of all these three levels along with waste degradation (Sarkar and Chourasia, 2017).

2.7.5. Physical observation of waste

Organic waste was observed for the fluctuations in odor and color after 30 days of incubation. Selection of best consortium was done after the thirty days experiment used for degradation of waste material (Sarkar and Chourasia, 2017).

2.7.6. Estimation of organic matter

For the estimation of ash, an empty and clean crucible was placed into the muffle furnace for 1h at 600 °C. After cooling it was weighted and noted as (W1). 2g of sample was taken into the crucible (W2). Then it was placed into the muffle furnace for 4h at 550°C. The indication of complete oxidation of all organic matter was the appearance of white grey matter and the weight of the ash in crucible was noted as (W3). The calculations of organic matter was done with given Formula 2 (Sarkar and Chourasia, 2017):

$$\text{Ash}(\%) = \frac{\text{Difference in weight of ash} \times 100}{\text{Weight of sample}} \quad (2)$$

Where
Difference in weight of ash = W3−W1

2.7.7. Determination of waste mass degradation

By using the density Formula 3, the mass of degrading material was estimated at an interval of 10 days up to 50 days (Sarkar and Chourasia, 2017).

$$P = mass \ / \ volume \quad (3)$$

2.8. Chemical analysis of the compost

Total nitrogen and carbon contents were determined by combustion in a CHN-600 LECO analyzer in accordance with the ASTM D-5373 standard. Oxidizable carbon was analyzed by the Walkley-Black method (Walkley and Black, 1934).

2.9. Statistical analysis

Data collected at different stages both in the laboratory and field experiments for different fungal strains were analysed using descriptive statistics (mean and standard errors). A one-way analysis of variance (ANOVA) was performed using SAS 9.1 to compare fungal growth rates and product yields at lab and commercial scales for various fungal species.

3. Results

3.1. Identification of fungal species

The present research work was designed for the isolation of keratinolytic fungal species from the soil samples. The species were identified on the basis of their phenotypic and genotypic characterization up to genus and species level.

3.2. Phenotypic characterization

Three fungal strains were isolated on Feather Meal Agar (FMA). Their morphological characters were as follow.

3.2.1. A. flavus

Colony color was pale yellow roughened, shape of colony was circular having powdery texture and showed maximum growth at 29 °C. By staining of fungal hyphae smooth conidiophore bearing vesicles were observed. Hyphae were non-septate, conidia were terminal, and vesicles were globose, and loosely radiated biseriate phialides were present all over the vesicle. Metulae was absent and conidia were directly attached with vesicles.

3.2.2. A. niger

Second strain showed slightly blackish colored colony with large sized biseriate phialides and 26 °C was the optimum temperature for its growth. Conidial shape was globose with very rough irregular surface. This strain was identified as *A. niger*.

3.2.3. C. queenslandicum

Third strain showed maximum growth at 25 °C and colony color was white creamy with flat cotton like appearance. Hyphae were septate, conidia were hyaline, one-celled, smooth or rough walled. This strain was *C. queenslandicum*.

3.3. Genotypic characterization

BLAST search of 18S rDNA nucleotide sequence of the fungal isolate revealed that the two fungal isolates showed close resemblance with genus *Aspergillus* and one with *Chrysosporium*.

3.4. Fungal conversion of feather waste into biofortified compost

Different parameters of the compost were calculated as follow:

3.5. Estimation of moisture content in compost

In composting process, water is a very critical factor. Initially, the moisture content was uniform in all the groups which indicated that the reduction of moisture was due to evaporation or drying. 50% of moisture reduction was calculated by *A. flavus*. 51% and 58% decrease in moisture content was recorded by *A. niger* and *C. queenslandicum*, respectively (Table 1 and Figure 1).

3.6. Estimation of pH

The measurement of hydrogen ion activity is pH and it depends on hydrogen and metallic ion absorbent. Initially the pH was same i.e. 7.1. With the passage of time, slight increase in pH was observed (Table 2). At the termination of experiment the calculated pH of *A. flavus* was 8.02. However, 8.1 and 8.08 pH was recorded by *A. niger* and

Table 1. Percentage of moisture reduction following periodic incubation of 10 days using chicken feathers as growth substrate.

Fungal species	Incubation period (days)				
	10	20	30	40	50
A. flavus	77.00	73.42	67.10	58.32	50.21
A. niger	77.00	70.32	61.32	58.11	51.15
C. queenslandicum	77.00	72.12	65.23	62.13	58.12
Control	77.00	75.23	74.14	73.21	72.11

Values represent moisture% and are means ±S.E. of three replicates. Those not sharing a common alphabet within a respective column are significantly different from each other. Single factor analysis of variance at $P<0.05$.

Table 2. Pattern of pH following periodic incubation of 10 days using chicken feathers as growth substrate.

Fungal species	Incubation period (days)				
	10	20	30	40	50
A. flavus	7.1^{a}±0.01	7.43^{b}±0.12	7.74^{a}±0.05	8.06^{a}±0.28	8.02^{a}±0.07
A. niger	7.1^{ab}±0.05	7.34^{c}±0.11	7.54^{bc}±0.34	7.97^{b}±0.32	8.10^{b}±0.05
C. queenslandicum	7.1^{ab}±0.03	7.44^{a}±0.16	7.61^{b}±0.16	7.95^{c}±0.11	8.08^{bc}±0.03
Control	7.1^{a}±0.01	7.12^{d}±0.13	7.13^{d}±0.25	7.13^{d}±0.41	7.13^{d}±0.01

Values represent pH and are means ±S.E. of three replicates. Those not sharing a common alphabet within a respective column are significantly different from each other. Single factor analysis of variance at $P<0.05$.

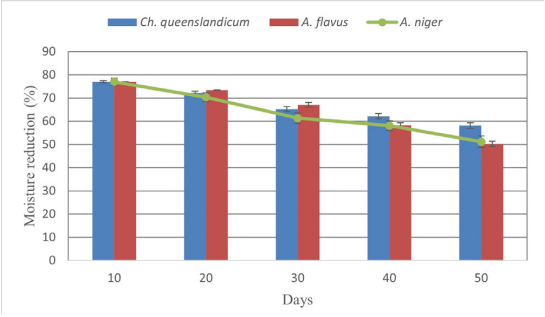

Figure 1. Reduction of moisture (%) in post-culture medium following periodic incubation of 10 days.

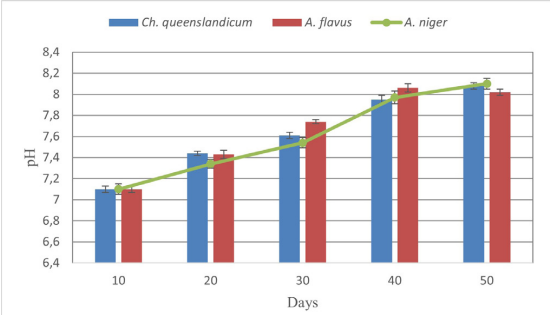

Figure 2. pH changes in post-culture medium following periodic incubation of 10 days.

C. queenslandicumin experimental groups respectively. While, there was no significant difference in control groups (Figure 2).

3.7. Change in temperature

Change in temperature was estimated after 10 days interval. A rapid increase in temperature was observed in *A. flavus* after twenty days. At the termination of experiment 53 °C was recorded by *A. flavus*. While 49 °C and 40 °C temperature was recorded by *A. niger* and *C. queenslandicum*, respectively at the termination of experiment (Table 3 and Figure 3).

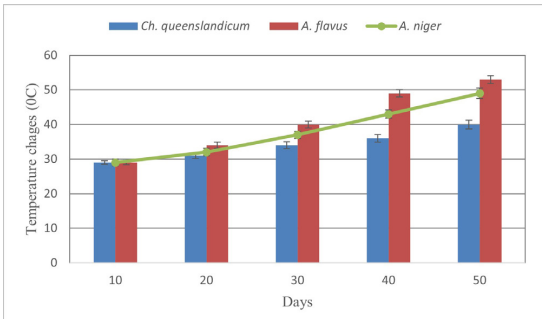

Figure 3. Change in temperature in post-culture medium following periodic incubation of 10 days.

3.8. Estimation of electrical conductivity

The ability to transfer the charge is electrical conductivity. The value of electric conductivity was high at the beginning of the experiment. But at the termination of experiment the value of electric conductivity of *A. flavus* was 0.13 (ms/cm). While *A. niger* and *C. queenslandicum* showed 0.22 and 0.32 (ms/cm) electric conductivity, respectively (Table 4 and Figure 4).

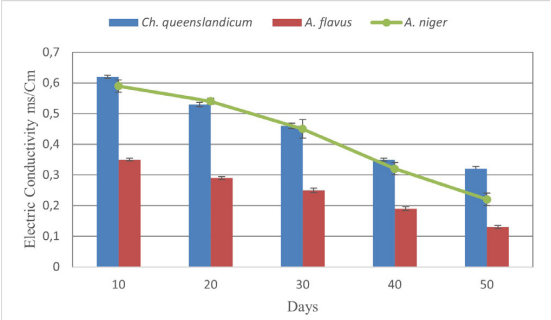

Figure 4. Electric conductivity changes in post-culture medium following periodic incubation of 10 days.

3.9. Estimation of organic matter

Organic matter is important for maintaining the water holding capacity, availability of nutrients and structure of soil. The absolute amount of organic matter is not fixed. It may range from 30-70%. But organic matter in compost must be above 30% (US Composting Council, 2003). Initially, the value of organic matter was low. But with the passage of time it started increasing (Table 5). At the

Table 3. Pattern of temperature (°C) change following periodic incubation of 10 days using chicken feathers as growth substrate.

Fungal species	Incubation period (days)				
	10	20	30	40	50
A. flavus	29^{c}±0.19	34^{ab}±0.03	40^{d}±0.09	49^{cd}±0.06	53^{a}±0.04
A. niger	29^{b}±0.11	32^{a}±0.17	37^{a}±0.03	43^{ab}±0.24	49^{b}±0.01
C. queenslandicum	29^{b}±0.02	31^{c}±0.21	34^{b}±0.43	36^{b}±0.67	40^{bc}±0.72
Control	29^{ab}±0.27	29^{bc}±0.56	29^{ab}±0.18	30^{a}±0.53	30^{cd}±0.05

Values represent temperature °C and are means ±S.E. of three replicates. Those not sharing a common alphabet within a respective column are significantly different from each other. Single factor analysis of variance at $P<0.05$

Table 4. Pattern of electric conductivity (ms/Cm) following periodic incubation of 10 days using chicken feathers as growth substrate.

Fungal species	Incubation period (days)				
	10	20	30	40	50
A. flavus	0.35^{a}±0.05	0.29^{b}±0.01	0.25^{c}±0.01	0.19^{bc}±0.12	0.13^{a}±0.05
A. niger	0.59^{ab}±0.03	0.54^{a}±0.16	0.45^{b}±0.08	0.32^{b}±0.03	0.22^{ab}±0.03
C. queenslandicum	0.62^{c}±0.08	0.53^{b}±0.05	0.46^{a}±0.10	0.35^{a}±0.07	0.32^{c}±0.08
Control	0.92^{d}±0.21	0.84^{c}±0.26	0.78^{d}±0.02	0.70^{c}±0.10	0.65^{d}±0.01

Values represent electric conductivity and are means ±S.E. of three replicates. Those not sharing a common alphabet within a respective column are significantly different from each other. Single factor analysis of variance at $P<0.05$.

Table 5. Percentage of organic matter (ash %) reduction following periodic incubation of 10 days using chicken feathers as growth substrate.

Fungal species	Incubation period (days)				
	10	20	30	40	50
A. flavus	30.01	39.00	42.21	50.14	58.01
A. niger	29.12	32.11	37.27	43.13	49.12
C. queenslandicum	26.11	30.13	33.00	36.32	40.11
Control	8.02	9.01	9.21	11.31	12.02

Values represent ash% and are means ±S.E. of three replicates. Those not sharing a common alphabet within a respective column are significantly different from each other. Single factor analysis of variance at $P<0.05$.

termination of experiment the value of organic matter by *A. flavus* was 58%. While, *A. niger* and *C. queenslandicum* contained 49% and 40% of organic matter, respectively (Figure 5).

3.10. Estimation of mass degradation and physical changes in compost

A rapid decrease was observed in all three experimental groups as well as in control groups in initial two weeks. However, the *A. flavus* experimental group showed maximum decrease in waste mass as compared to *A. niger* and *C. queenslandicum* experimental groups. After the trial of fifty days, 53% reduction of waste mass by *A. flavus* was calculated. Also, 37% and 21% of waste mass reduction was observed by *A. niger* and *C. queenslandicum*, respectively. Though, only 0.1% of decrease in waste mass was calculated in control groups (Table 6). The odor of compost was woody earthy and the color was blackish brown.

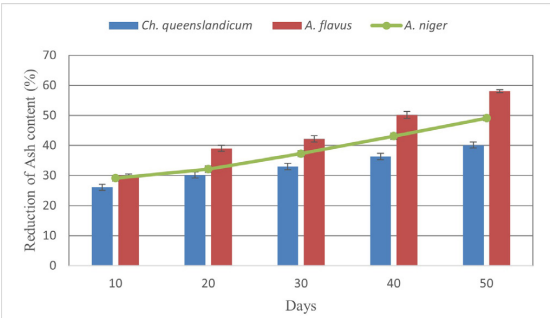

Figure 5. Reduction of organic matter (ash%) in post-culture medium following periodic incubation of 10 days.

3.11. Estimation of chemical analysis of compost

In the process of composting the end product was analyzed and the percentage of micro and macro nutrients was estimated. This analysis revealed the presence of all

Table 6. Pattern of feather weight (g) reduction after the incubation period.

Fungal species	Initial weight	Final weight
A. flavus	13^{bc} ±0.18	6.11^{d}±0.01
A. niger	13^{ab}±0.12	8.19^{c}±0.34
C. queenslandicum	13^{c}±0.21	10.27^{b}±0.02
Control	13^{a}±0.04	12.9^{a}±0.01

Values represent weight (g) and are means ±S.E. of three replicates. Those not sharing a common alphabet within a respective column are significantly different from each other. Single factor analysis of variance at $P<0.05$.

kinds of essential primary, secondary and trace elements with increased proportions (Table 7).

4. Discussion

The current study was designed to check the potential of isolated keratinolytic fungal strains for the formation of biofortified compost. On the basis of phenotypic characterization one of the isolated strain showed pale yellow to greenish colony color with biseriate phialides. Conidial shape was globose with smooth conidiophore surface. This strain was identified as *A. flavus*. These features were almost same with the results of Hedayati et al. (2007) who reported that *A. flavus* is either uniseriate or biseriate and yellow to green in color. Conidia are globose to sub-globose. Second isolated strain was recognized as *A. niger* showing slightly black colony color with large sized biseriate phialides. Conidial shape was globose with very rough irregular surface. Our findings about *A. niger* were in accordance with Zulkifli and Zakaria (2017). Third strain showed resemblance with *C. queenslandicum* exhibiting white creamy colony color with flat cotton like appearance. Hyphae were septate. Conidia were hyaline, one-celled, smooth or rough walled. Our findings about *C. queenslandicum* were almost same with previous literature about *Chrysosporium* sp. (Larone, 1995; Sutton et al., 1998; de Hoog et al., 2000). Genotypic characterization also showed maximum similarity of our isolated strains with *A. flavus, C. queenslandicum* and *A. niger*. *A. flavus* and *A. niger* showed maximum growth at 28 °C and 26 °C, respectively. While maximum number of colonies of *C. queenslandicum* were observed at 29 °C. The optimum temperature range for *A. flavus* reported by Samapundo et al. (2006) was 25 to 30 °C i.e. in accordance to our results.

Syed et al. (2009) reported that microbial degradation is eco-friendly and the most cost effective technique. *A. flavus* showed maximum degradation that was about 53%. *A. niger* degraded 37% and *C. queenslandicum* degraded 21% of chicken feathers. Hargerty et al. (1999) reported that *Aspergillus* species were dominant fungal species during the process of composting due to their thermophilic characteristics. For the successful degradation, various physical factors are accountable which have direct or indirect effect on the activities of fungus (Taiwo and Oso, 2004). The microbial activity and mineralization

Table 7. Chemical properties of feather compost.

Parameter	Fungal species		
	A. flavus	***A. niger***	***C. queenslandicum***
Total N (%)	6.84	5.32	3.21
Total C (%)	42.34	39.23	30.34
C/N Ratio	6.23	5.23	3.54
Calcium (ppm)	4028	3254	2365
Phosphorous (ppm)	5.99	4.32	2.43
Iron (ppm)	3.43	2.12	1.22
Copper (ppm)	Nil	Nil	Nil
Zinc (ppm)	<0.1	<0.1	<0.001
Magnesium (ppm)	0.006	0.004	0.001
Sulphate (ppm)	0.078	0.052	0.043

was high at the initial stages. Due to which the soluble salt concentration and electric conductivity (EC) were high. The value of EC increased with the increase of pH (Huang et al., 2004). These studies showed similarities with our results i.e. in the present study the pH was increased during the experiment. Initially, it was 7.1 but at the termination it reached 8.08. Kaul and Sumbali (1999) reported that due to the elimination of excess nitrogen the medium becomes alkaline. According to na Mona (2003) at the end of the composting the range of pH must be from 6.9–8.3.

Adegunloye et al. (2007) also reported that the fungal population was reduced near the maturity of compost. *A. flavus* was predominant among all the isolated strains. According to Korniłowicz-Kowalska (1997), 65–80% of substrate mass loss, solubilization of 25–60% of amino groups and 50% of peptides was caused by the keratinolytic fungal strain after the 10 days of inoculation in which feathers were used as the source of C, N, S and energy. According to Muhsin and Hadi (2002) *A. flavus* degraded 32% of chicken feathers. Kumawat et al. (2016) reported the maximum degradation of chicken feather i.e. by 39.2% by *C. queenslandicum* with the increase of pH from 7.0 to 8.15. Koutb et al. (2012) stated that *A. niger* degraded the chicken feathers after seven days of inoculation. According to Kannahi and Ancy (2012) 19.2% chicken feathers were degraded *by A. flavus*. Previous literature showed that strains isolated in our study are highly keratinolytic comparatively.

5. Conclusion

Economically and environmentally, composting is one of the best methods for the safe recycling of nutrients from waste material. Among all the three keratinolytic fungal strains (i.e. *A. flavus, A. niger* and *C. queenslandicum*), *A. flavus*

was the most efficient species because it degraded 53% of chicken feathers. Composting of keratinous waste by fungal species is not only an eco-friendly technique of getting rid of poultry waste but also its by-product can solely be used as fertilizer or can be added with other fertilizers to enhance the crop production and yield.

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