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FEATHER MEAL PREPARATION FROM CHICKEN FEATHERS AND ITS APPLICATION

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Abstract– Feather meal was prepared in powdered form from chicken feather waste, by defatting process. For the overall growth of the wheat crop feather meal used as a bio-fertilizer. In the presence of feather meal, the height of test leaves (39cm), number of wheat plant leaves (T) (213) was found to be increased as compared to standard (36.5cm), (123) respectively, dry weight of plant (T) roots was also improved by 0.8 g. *In vivo* and in vitro, KS (Keratin azure)-hydrogel and FM (feather meal) hydrogel was prepared and effectively involved in wound healing, tissue aggregation (regeneration). Chemical composition of hydrogel was analyzed by FTIR. Hence, in the present work, environmental non-biodegradable pollutant, i.e. Chicken feathers were positively transformed into eco-friendly valuable product as a bio-fertilizer and therapeutic agent for the wound healing.

INTRODUCTION

Keratin is the major structural fibrous protein occurs in nature mainly in the form of hair, horn, nails. Feather waste is poorly recycled in nature and has limited utility due to the chemically non-reactive nature of keratin. Chicken feathers are high protein resource consist of 90% keratin. Keratin proteins that have α-helix or β-sheet structure are linked by disulphide and hydrogen bond. The structure and linkage by keratin is resistant to degradation by common proteolytic enzymes such as trypsin, papin, pepsin. The alternative method to overcome abundant feather waste is by the utilization of keratinolytic microorganisms able to synthesize keratinase for feathers degradation (Satish *et al.,* 2019). Chicken feather waste forms considerable environmental problem because it is a non-biodegradable material and produced in enormous amounts. Keratin protein is a natural source of essential amino acid, peptidase and minerals that can be used in animal feed supplements and as a bio-fertilizer.

Keratin fibers extracted from chicken feather are eco-friendly, non-abrasive, and biodegradable, having good mechanical properties, hydrophobic behavior, low density and finally cheapest protein source (Niranjana *et al.,* 2019).

The structure of feather is complex to break down. Some bacteria in the presence of keratin-contained substrates are proficient to produce the keratinase enzyme to hydrolyze keratin (Somayeh Khodayari *et al.,* 2018). The mechanism of degradation of keratin by keratinolysis involves the steps as sulfitolysis, proteolysis and deamination. A group of these enzymes can be produced by variety of insects, various species of different bacteria (*Bacillus, Streptomyces, Pseudomonas* etc), fungi (*Aspergillus sp. Fusarium oxysporum*) and some actinomycetes**.** Keratinases can degrade feathers and other keratin substances efficiently, thus it can be employed for provident generation of animal feed and fertilizers. The application can be extended to leather, soap, cloth, medicinal, cosmetics industry, prion decontamination and biogas product and also to advance the quality of silk and hair (Mariam *et al*., 2020; Udenigwe *et al*., 2020; Preczeski *et al.,* 2020).

Hydrogels are three-dimensional polymer nets that can absorb large amounts of water by various natural and synthetic polymers. Hydrogels have revolutionized the approaches on the modern wound dressing and drug delivery systems, which

(Assistant Prof. and Students)

they could allow to oxygen to permeate, absorb tissue exudates, prevent wound dehydration, create better healing condition and controlled release of drug. PVP (poly-vinylpyrroliodone) is also bio-degradable, water soluble and non-toxic synthetic polymer which is used in hydrogel preparation. Keratins have been widely used to develop in wound healing applications owing to their special properties, such as biocompatibility and biodegradability (Husain Mohaed *et al.,* 2019).

The purpose of this study was to prepare eco-friendly, cheapest protein (feather meal) from Chicken feather wastes and use it as bio-fertilizer and in hydrogel preparation.

MATERIALS AND METHODS

Materials

Keratin azure (Sigma- aldrich), poly-vinylpyyro-liodine (Hi-media), Dimenthylsulphoxide (DMSO), Hepes buffer, Acetone, Methanol, chloroform, etc. All chemicals used are of analytical grade.

Feather meal Preparation

Chicken feathers were collected from slaughter house of different areas like Kannad, MGM road, Palashi, Aurangabad (Maharashtra, India) The feather meal was prepared from native chicken feathers as described by (Kate and Pethe, 2014) with modifications. The feathers were cut into small pieces and washed several times with tap water. Defatting of feather pieces was done by soaking in a mixture of chloroform: methanol (1:1) for 2 days followed by chloroform: acetone: methanol (4:1:3) for 2 days. The solvent was replaced every day. The feathers were finally washed several times, dried at 40±2 °C for 2-3 days, grinded using electrical mixer blender (Kenstar) and obtained powder used as feather meal.

Feather meal as Bio-fertilizer

Two different pots were taken. In one pot 2g feather meal was mixed with soil, labeled as T (test) and in second pot only soil was added, labeled as S (standard). In both pots, 10 g wheat (*Triticum aestivum*) seeds were sowed. After sprouting, height of plant, number of leaves was recorded at interval of 3 days and amount of proteins, carbohydrate estimation is carried out on 40th Day of plant growth. Experiment was carried out in duplicates (Fahadul Haque *et al.,* 2021).

Hydrogel preparation from std. keratin azure and feather meal

Preparation of PVP solution

2 g of PVP added in 20 ml of DMSO, heated and stirred the solution at 98 °C for 3hrs in shaking boiling water bath.

Keratin solution

Keratin solution was prepared by adding 0.18 g of feather meal and keratin azure in 10 ml of Hepes buffer (pH 8, 100 mM), heated and stirred it at 50 °C for 4 hrs.

Preparation of Hydrogel and its efficiency

KS (Keratin azure)-hydrogel and FM (feather meal) hydrogel made by mixing keratin azure and feather meal (25 ml) in PVP solution (20 ml) by heating at 50 °C for 30 min in shaking condition then melted starch solution (10 ml) was added and further proceed for freeze-thaw method. The mixture was exposed to three cycles of freezing at -20 °C overnight and thawing at 25 °C for 4 hr to form KS- and FM hydrogels. The efficiency of hydrogel is analyzed *In-vivo* and *In-vitro* condition (Arun *et al*., 2021). For *In vivo* evaluation FM (feather meal) hydrogel was applied on injured skin and check its effect. *In vitro* evaluation (analysis), 0.5 g goat liver was chopped in cold PBS, to that 0.5 gm of hydrogel was added and referred as test while in control chopped goat liver in PBS was taken and kept at room temperature for 48 hrs. After incubation, morphological changes was observed under inverted microscope and checked for aggregation and healing properties of hydrogel (Wang *et al*., 2012).

Characterization of KS (Keratin azure)-hydrogel and FM (feather meal) hydrogel by FTIR

KS (Keratin azure)-hydrogel and FM (feather meal) hydrogel and starch PVP solution characterization was done by Fourier Transform Infrared Spectroscopy (FTIR), spectra were at 4000 cm^{-1} to 500 cm^{-1} to know chemical composition (functional groups) of keratin hydrogel (Mohamed *et al.,* 2021).

RESULTS AND DISCUSSION

Feather meal preparation from collected Chicken Feathers

In present study, feather meal as a source of keratin was prepared in powder form (Fig. 1) from chicken feather waste used as a bio-fertilizer for wheat plant

growth as well as in hydrogel preparation while Saibabu *et al.* (2013); Pethe *et al.* (2014) used feather meal which was prepared from chicken feather waste as a source of keratin for isolation of keratinolytic microbes, Ummikrishnan Gayathri and Vijayarghavan (2020) reported extraction of keratin from human hair and used as bio-fertilizer for Okra seedlings (*Abelmoschus esculentus*) and Kim *et al.*, (2019) designed human hair derived keratin based hydrogel.

Fig. 1. Feather meal

Feather meal as Bio-fertilizer

From first day of sprouting, wheat plant leaves height in test pots (39±2.8 cm) was found to be more than standard pot leaves (36.5±0.7 cm) (Fig. 2) indicates that feather meal provide additional nutrient supplement for wheat plant growth. Standard and test plants leaves number were measured every after 3 days till the height of leaves become constant (40 days) (Table 1 and Fig. 3). Total no. of leaves in test pot and standard pot after 40 days was found to be 213±7 and 124±13 respectively (Table 2.) similar result was reported by Nagarajan *et al*. (2018) for Green gram crop in presence of feather meal while the number of leaves in *Gima kalmi* plant increased with treatment of Poultry feather waste and the highest plant height recorded was 34.06cm while the shortest was 29.33 cm in control (Joardar

Fig. 2. Wheat plant standard and test pots

et al., 2018). In present work, dry weight roots of test (1.11 gm) were also found to be more than that of standard roots (1.22 g) (Fig. 4 and Table 3) in presence of feather meal.

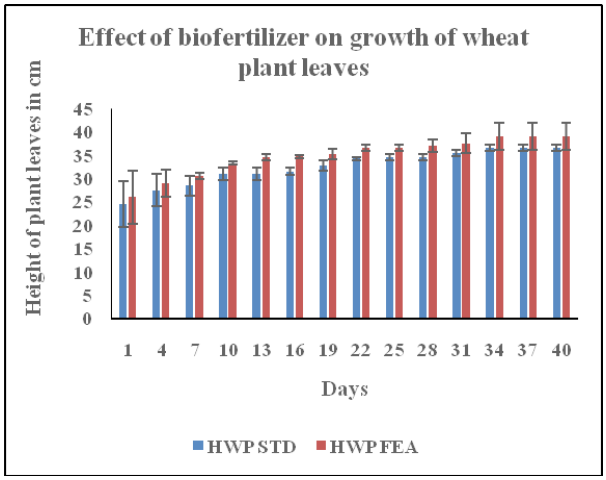


Fig. 3. Effect of biofertilizer on growth of wheat plant leaves

Fig. 4. Dry Weight roots of wheat plant (std. and test)

In test, standard plant leaves protein concentration was found to be 1185.56 µg/ml and 910.56 µg/ml and carbohydrate concentration was found 1% in std. and 1.5% in test respectively experimental outcomes proved potential of feather meal as a biofertilizer.

Characterization of KS (Keratin azure)-hydrogel and FM (feather meal) hydrogel by FTIR

FTIR analysis was carried out to know structural bonding of compounds used in KS-hydrogel FM (feather meal) hydrogel, starch PVP solution. Fig. 5. represents the FT-IR spectra to know the functional groups present in hydrogels. The strong peak was observed at 3352.28 cm^{-1}, 3379.29 cm^{-1} may be due to presence of –OH or –NH group, i.e. primary and

Table 1. Height of Standard and Test wheat plants leaves

Sr. No.	Date	Day	Height of wheat plant							
			Standard (without feather meal)				Test (wiith feather meal)			
			S1	S2	Average	Std. Dev.	T1	T2	Average	std. dev.
1	24-Jan-22	1	28	21	24.5	4.949747	30	22	26	5.656854
2	27-Jan-22	4	30	25	27.5	3.535534	31	27	29	2.828427
3	30-Jan-22	7	30	27	28.5	2.12132	33	30	31.5	2.12132
4	02-Feb-22	10	32	30	31	1.414214	35	33.5	34.25	1.06066
5	05-Feb-22	13	32	30	31	1.414214	35	34	34.5	0.707107
6	08-Feb-22	16	31	32	31.5	0.707107	36	34.5	35.25	1.06066
7	11-Feb-22	19	33.5	32	32.75	1.06066	37	34.5	35.75	1.767767
8	14-Feb-22	22	34	34.5	34.25	0.353553	37	36	36.5	0.707107
9	17-Feb-22	25	34	35	34.5	0.707107	38	36	37	1.414214
10	20-Feb-22	28	34	35	34.5	0.707107	39	36	37.5	2.12132
11	23-Feb-22	31	36	35	35.5	0.707107	41	37	39	2.828427
12	26-Feb-22	34	37	36	36.5	0.707107	41	37	39	2.828427
13	01-Mar-22	37	37	36	36.5	0.707107	41	37	39	2.828427
14	04-Mar-22	40	37	36	36.5	0.707107	41	37	39	2.828427

Table 2. No. of Standard and Test wheat plant leaves

Sr. No.	Day	No. of wheat plant Leaves					
		Standard (without feather meal)			Test(with feather meal)		
		S1	S2	Average	T1	T2	Average
1	40	134	115	124.5	218	208	213

Table 3. Dry weight roots of Standard and Test wheat plant

Sr. No.	Dry Weight roots of wheat plant					
	S1	S2	Average	T1	T2	Average
1	1.13	1.09	1.11	1.19	1.25	1.22

secondary amines and amides. The peak at 1643.35 cm^{-1}, 1653 cm^{-1} 1010.70 cm^{-1}, 1012.63 cm^{-1} and 947.05 cm^{-1} revealed the presence of –CN,C=O,-CO,-CH bond in FM and KS-hydrogel respectively. In starch PVP FTIR (Fig. 6) strong peaks was observed at 2914.44 cm^{-1}, 2848.86 cm^{-1}, 2179.56 cm^{-1}, 1737.86 cm^{-1}, 1463.97 cm^{-1}, 721.38 cm^{-1} indicates the presence of N-H stretching, -CH (aldehyde), Ca–C,C=O,-CH (alkane) and aromatic –CH respectively. Hence, the presence of these functional groups in hydrogel indicates that we can use these hydrogel as a therapeutic agent without any toxic effect to living cells similar peak assignment was reported by Husain *et al*. (2021).

Efficiency of KS (Keratin azure)-hydrogel and FM (feather meal) hydrogel

Prepared KS (Keratin azure) and FM (feather meal) hydrogel (Fig. 7) was checked for its efficiency. In vivo, healing of injured skin was fast by hydrogel as compared to natural healing, initially after applying hydrogel on injured skin little itching sensation followed by cooling effect was noticed by patient (Fig. 8.) and In vitro, after incubation at RT, chopped goat liver cells with hydrogel was found to be aggregated (Fig. 9.) with no putrid smell while in control, cells were separated with intense putrid smell. This indicates that keratin hydrogel can be used as a therapeutic agent for wound healing after optimization. Kim *et al*., 2019 demonstrated that their human hair derived keratin based hydrogels accelerated re-epithelization and wound healing process in a full-thickness animal. Shuai Wang (2012) used keratin extracted from human hair in hydrogel preparation for soft tissue regeneration. Amin Shavandi (2017), used sheep hair as keratin source for tissue regeneration of nerve, bone and skin.

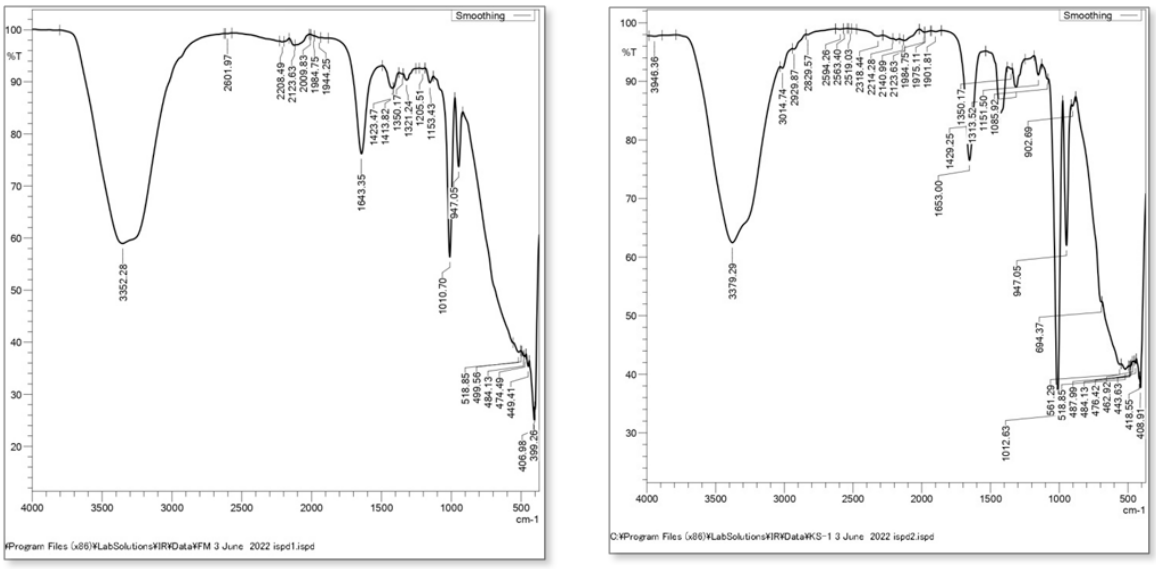


Fig. 5. FTIR (Fourier Transform Infrared Spectroscopy) of FM and KS hydrogel

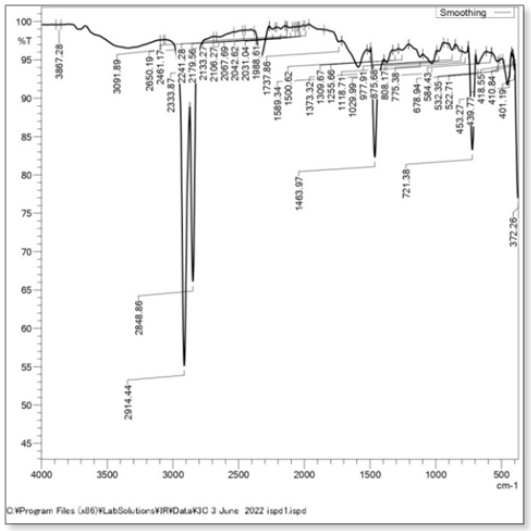


Fig. 6. FTIR (Fourier Transform Infrared Spectroscopy) of starch PVP solution

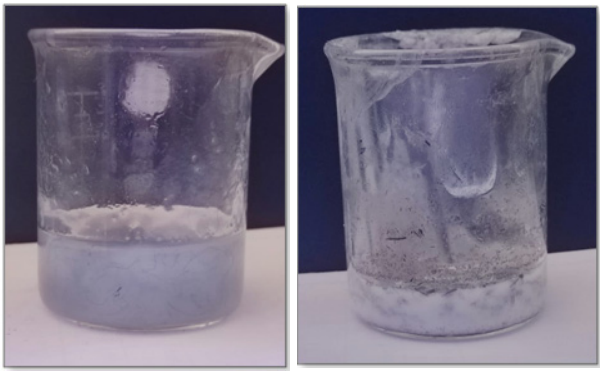
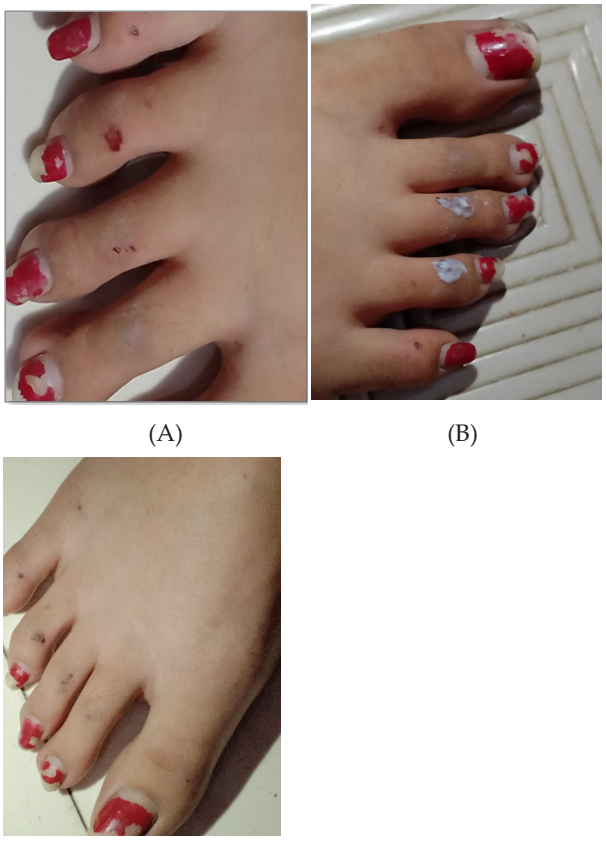


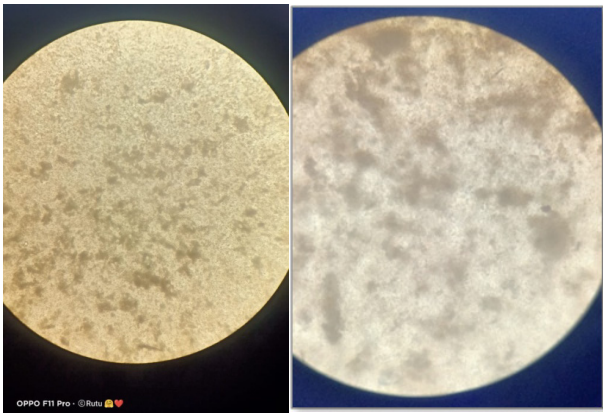
Fig. 7. KS (Keratin azure)-hydrogel and FM (feather meal) hydrogel



(A) (B)

(C)

Fig. 8. *In vivo* healing process (A) Injured skin, (B) FM Hydrogel applied on injured skin, (C) healed skin



(A) **(B)**

Fig. 9. *In vitro*, (A) Control (without Hydrogel), (B) Test (with Hydrogel)

CONCLUSION

From chicken feather waste, feather meal was prepared and used as a biofertilizer for wheat plant growth. In presence of feather meal as bio- fertilizer height of leaves was found to be increased by approx. 3cm as compared to standard, also No. of leaves increased tremendously by 90 leaves and dry weight of roots also enhanced by 0.8 g. By FTIR analysis of both hydrogels, the strong peak was observed at 3352.28 cm^{-1}, 3379.29 cm^{-1} indicates the presence of –OH or –NH group which are not toxic to living cells. *In vivo* and *in vitro*, KS (Keratin azure)-hydrogel and FM (feather meal) hydrogel was effectively involved in wound healing, tissue aggregation (regeneration) with no putrid smell in the test sample while in control, cells were separated and with intense putrid smell, hence that can be used as therapeutic agent for wound healing after optimization.

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