Effect of poultry waste derived feather degraded lysate on seed germination and growth of *V radiata* L.

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Abstract:

The accumulation of feather waste from poultry operations represents an environmental challenge due to its high keratin content and resistance to natural breakdown. In the present study, microorganisms were isolated from soil collected at a poultry dumping site. Among the isolates obtained, KM1, KM7, and KM11 demonstrated feather-degrading capabilities, with KM11 showing the most pronounced activity. The feather lysate produced by KM11 was evaluated for its effects on the growth of *Vigna radiata* L. (mung bean). Seeds treated with the KM11 lysate exhibited improved germination compared to untreated controls. Plant growth analysis was done after 7 and 14 days revealed significant increases in shoot and root length, fresh and dry weight and chlorophyll content, indicating the potential of feather degraded lysate to act as a natural plant growth promoter. These results suggest that KM11 could serve a dual role in biodegrading poultry waste and promoting sustainable plant growth.

Keywords: Feather waste, Feather lysate, *Vigna radiata*, Plant Growth

Introduction-:

Nowadays, with the growth of the poultry industry, huge quantities of waste are produced reaching millions of tons annually (Da Silva et al. 2018; Verma et al. 2018). This creates complex challenges, particularly in managing the large volumes of waste it generates among these, chicken feathers constitute a significant portion. This waste poses serious environmental challenges, contributes to climate-related issues and may adversely affect human health. (Williams et al. 1990). Common disposal methods such as landfilling and incineration, not only contribute to environmental pollution but also result in the loss of valuable components like proteins and enzymes (Lasekan et al. 2013).

Chicken feathers are an abundant and low-cost source of protein, with significant potential for use as bio-fertilizers. Through microbial degradation, feathers can be broken down into a lysate rich in amino acids and peptides. This lysate acts as a biostimulant, enhancing nutrient absorption, promoting plant growth and improving resistance to both abiotic and biotic stresses, ultimately contributing to increased crop yields (Bhise et al. 2017)

Sustaining high agricultural productivity is essential to meet the food demands of the growing global population. To achieve this, synthetic fertilizers are widely used; however, their excessive application often leads to runoff into water bodies, resulting in eutrophication and environmental degradation. To mitigate the environmental risks associated with the excessive use of chemical fertilizers, organic fertilizers and biofertilizers have gained recognition as key components of sustainable agricultural practices (Abdel-Hamid et al. 2021). Among these, the development of bio-fertilizers from chicken feather waste is drawing significant attention from researchers, agronomists and environmentalists due to its potential as a sustainable and eco-friendly solution (Tiwari and Gupta 2010). Feather meal, containing approximately 15% nitrogen, is

an inexpensive and readily available nitrogen source, making it a promising candidate for use as a bio-fertilizer. Additionally, protein hydrolysate derived from feathers have shown potential in promoting plant growth, further supporting their application in agriculture. Therefore, microbial degradation of feathers offers a sustainable approach for producing slow-release fertilizers that enhance soil fertility and support long-term crop productivity (Hadas and Kautsky 1994).

In present study, we have isolated feather degrading organism from poultry dumped soil. The isolates were tested for proteolytic activity and feather degradation ability. The feather degradation was studied by evaluating released proteins and amino acids content. The effect of feather degraded lysate of KM11 on seed germination and growth of *Vigna radiata* L. was studied and plant analysis was done after 7 and 14 days of growth.

Material and methods

Collection of soil sample

The soil sample is collected from poultry feathers dumping site from Sangli and Kolhapur district nearby areas. The six representative soil samples were collected in sterile polythene bags and transported to the laboratory.

Isolation of organisms from the soil sample

One gram of soil sample was taken and it was serially diluted from 10^{-1} to 10^{-10}. 0.1 ml of soil dilution was spread on sterile Skim milk agar plates for detecting the proteolytic activity. The plates were incubated at 37°C for 24-48 hrs morphologically distinct bacterial colonies were selected and purified cultures were preserved for further study.

Feather degradation study

One loopful of bacterial culture was inoculated into the poultry feather minimal media containing chicken feathers (PFM) as sole source of carbon and nitrogen and was kept under shaking condition (150 rpm) at RT for 4-5 days. After every 24 hrs feather degradation was studied by evaluating proteins and amino acids contents of CFM (Bhise et al. 2017).

Protein Estimation

Protein estimation was performed by using Lowry method ((Lowry et al. 1951). Briefly, 0.5 ml of sample was mixed with 2.5 ml of freshly prepared alkaline copper reagent (a mixture of 2% Na_2CO_3 in 0.1 N NaOH, 0.5% $CuSO_4 \cdot 5H_2O$ and 1% sodium potassium tartrate). After a 10-minute incubation at room temperature, 0.25 ml of diluted Folin–Ciocalteu reagent was added and the mixture was incubated in the dark for 30 minutes. Absorbance was measured at 750 nm. The concentration of protein was determined using BSA (bovine serum albumin) as standard.

Amino acid Estimation

Amino acids was estimated using method of Moore and Stein (1954). Briefly, 1.0 ml of the sample was combined with 1.0 ml of 0.1% ninhydrin reagent prepared in citrate buffer (pH 5.5). The reaction mixture was incubated in a boiling water bath for 15 minutes to facilitate the formation of the Ruhemann's purple chromophore. Following incubation, tubes were cooled to room temperature and absorbance was recorded at 570 nm using a UV-Visible spectrophotometer. Quantification was performed by comparison with a standard leucine curve.

Characterization and identification of potent isolate

Morphological and biochemical characteristics of potent organism was studied to identify genus of organism.

In vitro plant growth studies

Seed germination study

The seeds of *Vigna radiata* were surface sterilized with 70% ethanol for 10 mins and washed several times with distilled water. Surface sterilized seeds were treated with feather degraded lysate (FL) and control set (CS) was treated with water and germination was studied after 7 days (Abdel-Aziz et al 2014).

$$\text{Germination \%} \quad \frac{\text{Number of germinated seeds}}{\text{Number of total seeds}} \quad \text{X } 100$$

Pot study

The effect of feather degraded lysate to promote growth of plant was tested by pot trials. Pot study was carried out in September with average humidity of 30% and temperature 32-35° C. Soil was autoclaved for sterilization. Seeds used for experiment was surface sterilized using ethanol (70%) and washed repeatedly with sterile distilled water. The surface sterilized seeds were sown into pot and FL set was inoculated separately with feather degraded lysate (5ml) of KM1, KM7 and KM11 and CS with water only. After 7 and 14 days of growth seedlings was uprooted and plant analysis was performed to evaluate effect of feather degraded lysate on growth of plant (Bhise et al. 2017).

Plant analysis

The shoot and root length, fresh plus dry weight of FL and control set seedlings were measured and noted down. The chlorophyll content of control and feather degraded lysate was estimated using method of Arnon (1949).

Statistical analysis

All the experiments were run in triplicates and data values were expressed as mean±S.D. One way ANOVA was applied using Minitab (release 19) on data to find efficacy.

Result and Discussion

Isolation of organisms from soil

Fifty-nine organisms were isolated from different soil samples. Out of these, thirteen organisms showed maximum proteolysis zone on skim milk agar plates. These thirteen organisms were used for further study.

Feather degradation study

Ability of thirteen potent organisms to degrade poultry feathers were tested using PFM. Among thirteen organisms, three organisms (KM1, KM7 and KM11) showed highest feather degradation after 72 hrs of incubation. The released proteins and amino acids were evaluate after every 24 hrs. The amount of

proteins released during feather degradation was highest after 72 hrs and reduced after 96 hrs (Fig.1). Similarly, amino acid contents was also analyzed and it was maximum after 96 hrs and declined after 120 hrs (Fig. 2). Among three isolates, KM11 was highly potent in feather degradation ability (Fig.1 and 2).

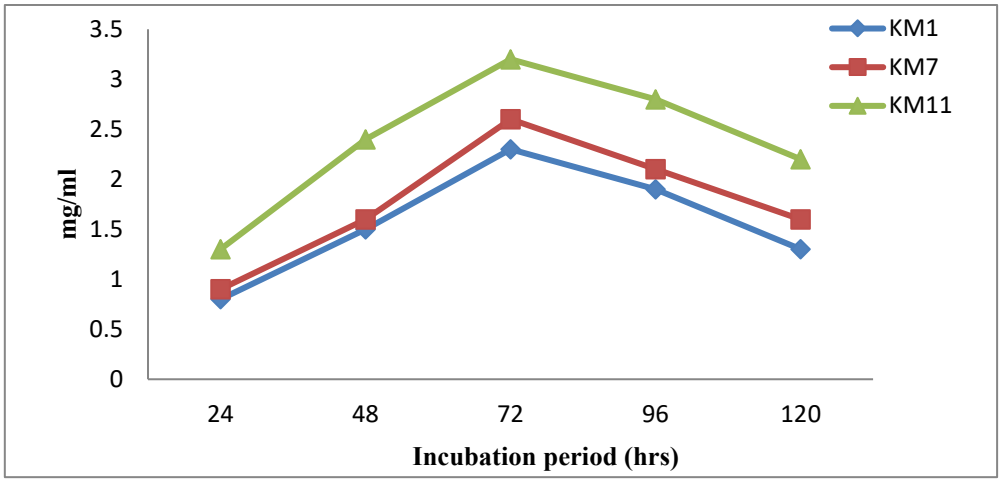

Fig 1. Estimation of protein content of feather degraded lysate

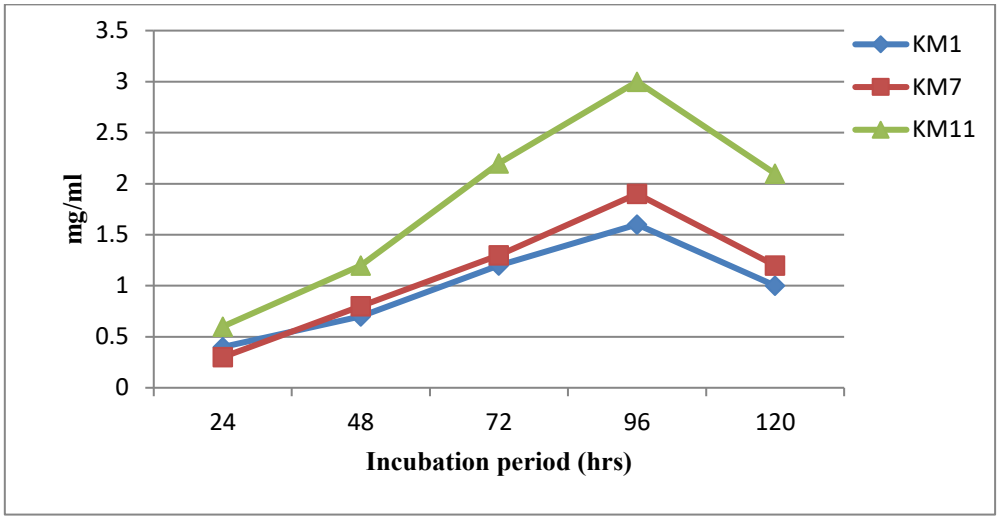

Fig 2. Estimation of amino acid content of feather degraded lysate

Characterization and identification of potent isolate

The KM11 was gram positive rod shaped motile organism. The isolate showed glucose, fructose, sucrose, maltose, mannitol, mannose and xylose sugar fermentation. It also showed catalase and gelatin hydrolysis. IMVic test was also performed and organism showed negative indole plus methyl red test while voges proskauer and citrate utilization test were positive. These results indicates that the KM11 isolate belongs to *Bacillus subtilis* genera however 16S rDNA sequence analysis is required to confirm identity of organism.

Seed germination

Feather degraded lysate inoculated seeds showed highest germination compared to control set. Germination study clearly indicated the potency of KM1, KM7 and KM11 in germinating seeds of *V. radiate.* Among these three lysates, KM11 was found to be potent in supporting seed germination after 7 days of growth (Fig.3). similarly, Paul et al. (2013) also reported improved growth and germination (87.5%) of *Cicer arietinum* supplied with feather lysate.

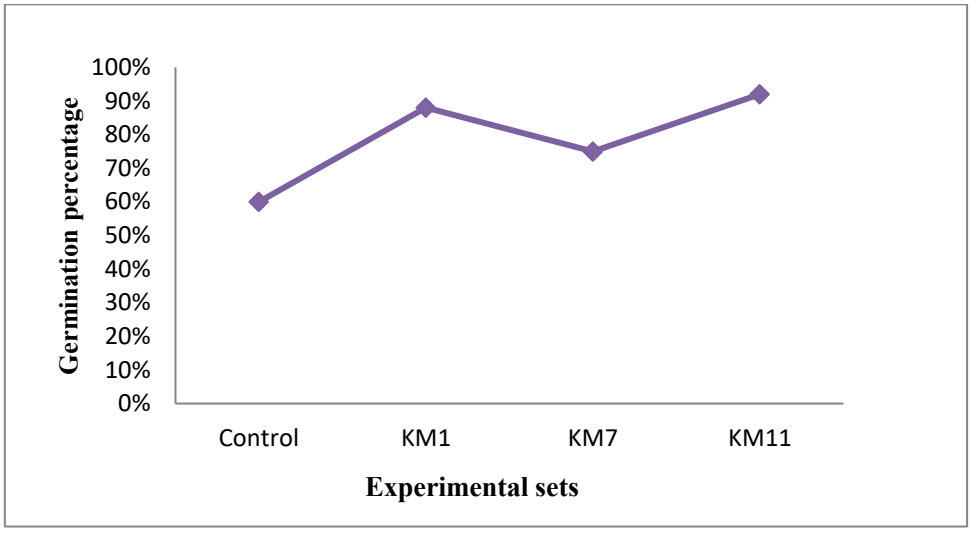

Fig 3. Effect of FL on seed germination

Plant analysis

Researchers have successfully identified various microorganisms from different habitats that possess the ability to degrade keratin, enabling the transformation of keratin-rich waste materials into commercially valuable products (de Menezes et al. 2021). The isolated microorganisms demonstrated the capability to break down keratin and keratin-rich wastes such as feathers into soluble compounds and amino acids (Callegaro et al. 2018; Bohacz, 2019; Chaudhary et al. 2021) that can supports the growth of plant.

Effect of FL on growth of plant was studied and analysis was done after 7 and 14 days. FL inoculated plants showed significantly improved root and shoot length compared to control plants (Fig. 4).

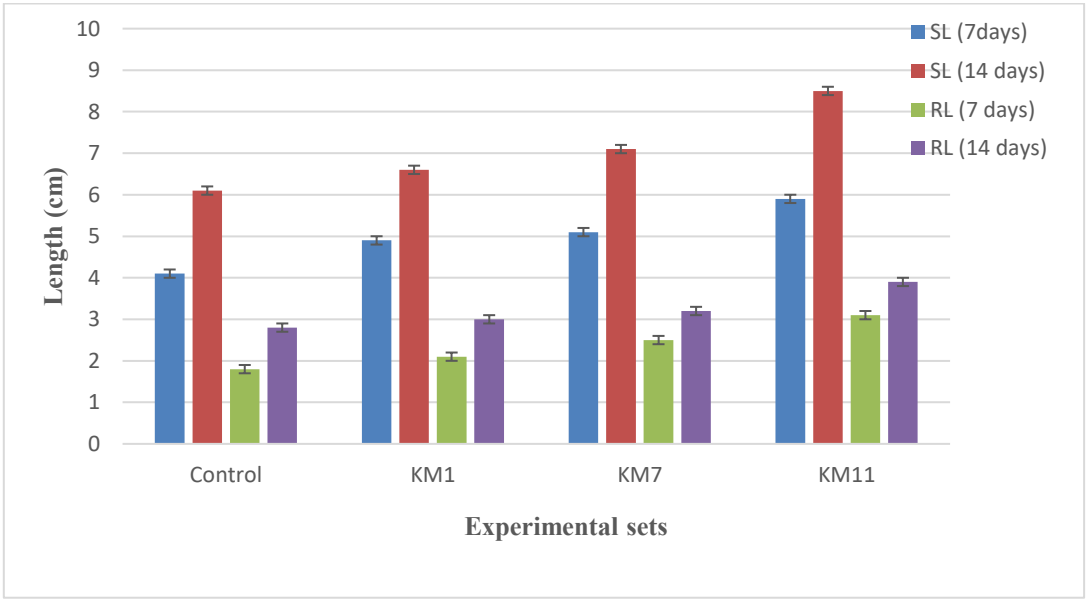

Fig 4. Effect of FL on plant length

KM1, KM7 and KM11 was effective in promoting shoot and root length after 7 and 14 days. KM11 was found to be highly effective in promoting both root and shoot length followed by KM 7 and KM1 (Fig.4). Similarly, the complete degradation of chicken feathers yielded a feather hydrolysate that significantly promoted the growth of *Vigna radiata* var. *meha* (mung bean) (Bose et al. 2014). The Peptides

present in keratin hydrolysate may exhibit hormone-like activity contributing to plant growth regulation. Additionally, these compounds can indirectly enhance nutrient uptake and utilization thereby supporting overall plant growth development (Raguraj et al. 2022).

Effect of lysate on fresh weight of plant was also studied. KM 11 improved fresh weight of seedlings by 64 % after 7 days and 57 % after 14 days of growth (Fig.5). Similarly, dry weight of seedling was increased 90 % and 70 % when supplemented with KM11 after 7 and 14 days of growth respectively (Fig. 5). The amino acids present in keratin hydrolysate can serve as a nitrogen source for rhizospheric microorganisms, influencing their activity in the root zone and thereby affecting plant growth and development. KM11 exhibited highest ability to degrade feathers hence plants inoculated with FL showed improved length and weight.

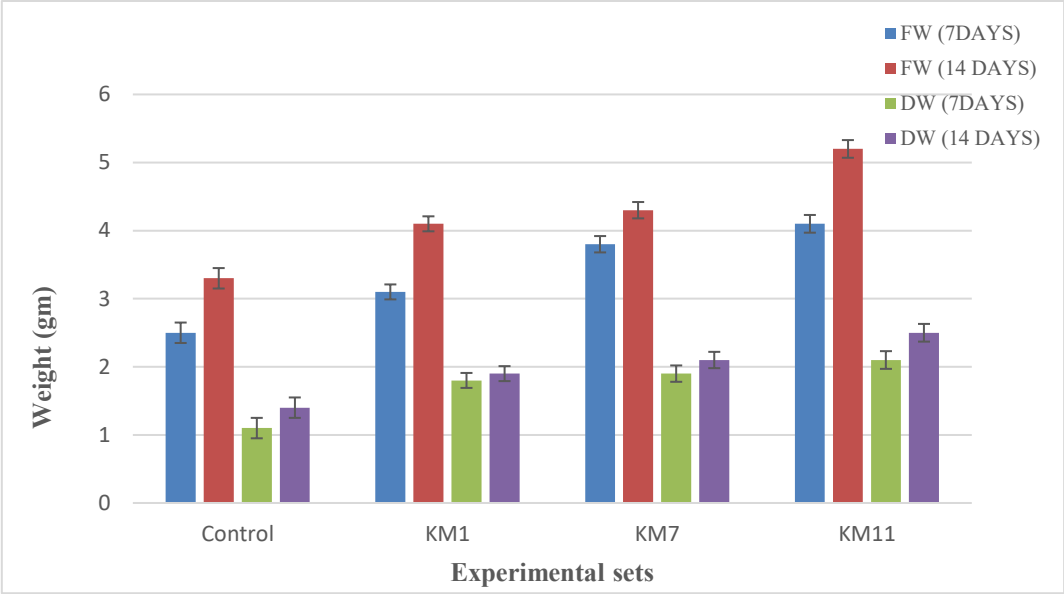

Fig 5. Effect of FL on plant weight

Studies have demonstrated that microbial keratin hydrolysate can effectively promote plant growth, making it a sustainable alternative to conventional fertilizers (Moe, 2013). Nafady et al. (2018) reported a strain of *Bacillus licheniformis* ASU with the ability to degrade chicken feathers, In addition, this strain demonstrated phosphate-solubilizing activity and produced high levels of indole-3-acetic acid (IAA). Presence of lysate along with such plant growth promoting traits accelerates plant growth.

Plants inoculated with FL also showed improved chlorophyll content as compared to control. Out of three lysates studied, KM11 was more impactful in improving photosynthesis activity compared to KM1 and KM7. KM11 improved chlorophyll content by 60 and 43 % after 7 and 14 days respectively. KM1 and KM7 improved chlorophyll content by 29 and 39 % after 7 days and 10 and 23% after 14 days of growth (Fig.6).

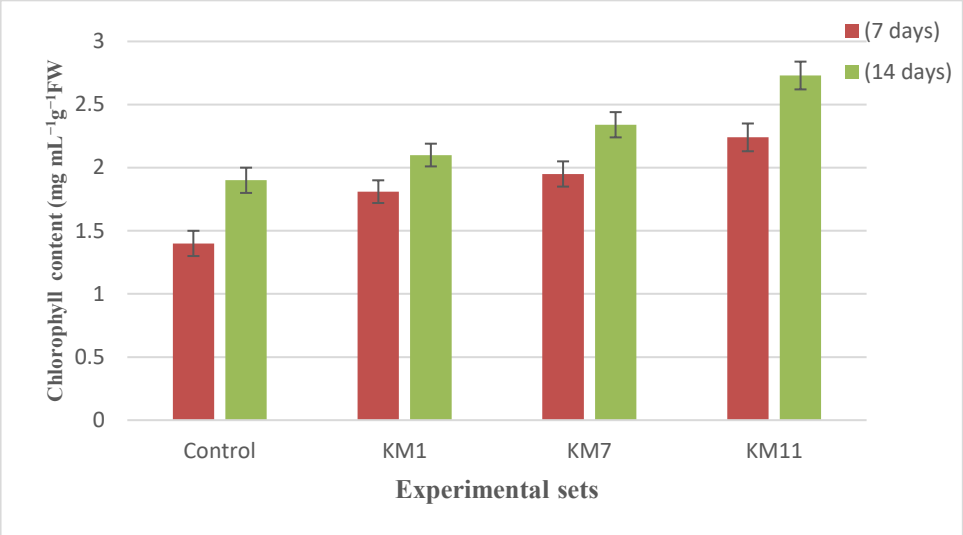

Fig 6. Effect of FL on plant chlorophyll content

Microbial keratin hydrolysate applied to soil enhance the soil microbiome by stimulating the proliferation of beneficial microorganisms involved in biological nitrogen fixation, as well as in the solubilization and mobilization of phosphate and potassium ions. Availability of such nutrients along with beneficial organism improves chlorophyll content of plants. Due to its composition of viable microbial populations combined with essential macro- and micronutrients, microbial keratin hydrolysate qualifies as a biofertilizer, contributing to improved nutrient cycling and soil fertility (Kumar et al. 2022).

Conclusion

The present study demonstrates the promising potential of feather-degraded lysate inoculum, KM11 in enhancing seed germination, plant growth and soil fertility. The enzymatic breakdown of keratin-rich feathers by microbial action not only provides a sustainable method for managing poultry waste but also generates nutrient-rich byproducts beneficial to plant development. Application of this lysate, especially when combined with beneficial microorganisms, led to improved seed germination, length, weight and chlorophyll content in *Vigna radiata* L. Moreover, the incorporation of feather hydrolysate into soil contributes to its nutrient profile, particularly by supplying nitrogen and amino acids, which are vital for plant metabolism. These findings suggest that microbial feather degradation can be effectively integrated into agricultural practices as a biofertilizer strategy, potentially leading to higher crop yields and reduced dependency on synthetic fertilizers. Continued research and field-scale validation may further establish its role in sustainable and eco-friendly farming systems

Conflict of interest

The authors declare that they have no conflict of interest

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