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Effect Of Fungicides On Feather Degradation And Keratinolytic Ability

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Abstract

Keratinophilic fungi that decompose keratinous substrate have got interest in recent days and are an important part of the soil microbiome. Soil represents the major pool of these fungi, where there is more keratin substrate. Feathers are the major by-product from the poultry industry which is chiefly composed of keratins. From poultry industry vast amount of feather is produced which could become pollutant. The aim of this study was to focus on the feather degrading ability of *C. keratinophilum* and *M. gypseum* at different concentration of fungicides – 10 ppm, 20ppm, 30ppm and 40ppm. The fungicides used were Bavistin, Aureofungin and Dithane M-45. All the fungicides showed suppressive effect overall and the keratinase production was decrease with increase in fungicidal conc. Bavistin and Dithane M-45 were highly effective. After evaluation of these fungicides at different conc. help us to standardize the best fungicides viz. Bavistin and Dithane M-45 against *C. keratinophilum and M. gypseum.*

KEYWORDS: Keratinolytic fungi, fungicides, feather, pollutant, poultry industry, keratinolytic ability

Introduction

Feathers are the major by-product from the poultry industry which is chiefly composed of keratins. From poultry industry vast amount of feather is produced which could become pollutant. Feathers are rich in amino acids, so it is an excellent source of fertilizers and animal feeds. Numerous bacteria and fungi have potential to degrade these feathers with the help of enzymes- Keratinases. Evidences shows that feather waste can be renewed into value added products. Untreated feather waste can sustain many pathogenic micro –organisms and release various pollutants such as nitrous oxide, ammonia, and hydrogen sulphide which are a risk to the environment and people's health [1]. In the environment, keratinous waste accumulates day by day, generated from different industries. India is the major contributor in production of keratinous waste material. Feather which accounts for 5-8% of the total weight of mature chickens are produced in large amount as a waste by – product from poultry industry. Millions of tons of feathers are produced annually worldwide [2]. For recycling of such keratinous waste biotechnological techniques are develop to hydrolyze those materials to soluble form through specific enzymes called Keratinases.

Keratinases– a proteolytic enzyme, have potential to hydrolyze insoluble keratin. It has enormous potential application in the processing of waste of poultry industry, leather industry and other industries. Keratinases are used in cosmetics, leather, medicine, detergent production and so many other industries. It is produced mainly by micro –organism such as saprophytic and parasitic fungi and actinomycetes.

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Keratinase is produced only in the presence of keratin substrate. Keratinolytic enzymes are widespread in nature, especially in microbial world. A vast variety of bacteria, actinomycetes and fungi are known to be keratin degraders [2, 3, 4]. Use of keratinolytic fungi for feather degradation is an economical, environmentally friendly alternative. As physiological and nutritional factors greatly affect feather degradation and keratinase production, the effect of fungicides on feather degradation by selected fungi was reviewed in this study.

2. MATERIAL AND METHOD:

2.1 Collection of soil samples: Soil samples were collected from different indoor animal habitats (Aviary, Serpentarium, Rabbit habitat, Guinea pig habitat, and Nocturnal house) of Kanpur Zoological Park.

2.2 Isolation, Purification and Identification: Keratinophilic fungi were isolated by Vanbreuseghem hair bait technique [5]. Soil were placed in sterile petridishes and moistened with sterile water and baited with sterile human hairs. The petridishes were incubated at room temperature and examined after one week. After observing the growth, isolates were cultured on SDA medium at 28 ± 2^0 c for up to one week. When fungal colony is seen it is transferred to other dishes for purification. Then isolates were examined and identified on the basis of morphological characters and microscopic characters.

2.2 PREPARATION OF KERATIN SUBSTRATE (FEATHER MEAL): Chicken feather was purchased from local market and brought to the laboratory. Feather were washed many times with distilled water and dried. The dried feather were defatted by soaking in diethyl ether for 24 hours, and washed thoroughly with distilled water followed by air drying and cut into short fragments of 1-2 cm. now the processed feathers called as feather meal.

Preparation of inoculums and inoculation procedure

250ml conical flask containing 50 ml of SDA broth medium and 1 gm of feather meal as a keratin substrate were autoclaved at 15 labs pressure for 10 minutes and pH was adjusted to 7 before sterilization. These flasks were inoculated with 6 mm disc 0f 7-8 days old culture of *C. keratinophilum and M. gypseum* previously grown on SDA medium.

2.3 DETERMINATION OF FEATHER DEGRADATION: The utilization of feather keratin was assessed by following method of Garret (1962). Feather meal (1% feather) incubated with loop full of fungal spores in SDA broth medium for 10 days. After incubation the residual feather was determined by filtering the culture broth by taking the weight of filter paper before and after filtration. Percent reduction of feather was calculated by the method of Kim et. al, (2011) [6]

$$\text{Degradation \%} = \frac{TF - RF}{TF} \times 100$$

Where, TF is the total feather and RF is the residual feather.

2.3 EFFECT OF FUNGICIDES ON FEATHER DEGRADATION: To determine the effect of fungicides- Bavistin, Aureofungin, Dithane M-45 were taken in the conc. 0f 10, 20, 30, 40 ppm and the flasks were incubated at 28 ± 2^0c for 10 days in static condition and filtered.

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3. RESULTS AND DISCUSSION:

3.1 ISOLATION OF FUNGI One hundred seventy isolates yielded12 genera and 23 species of keratinophilic fungi and related dermatophytes from different indoor animal habitats of Kanpur zoological park. *Chrysosporium keratinophilum* and *Microsporum gypseum* were the most dominating species.

3.2 EFFECT FUNGICIDES ON FEATHER DEGRADATION: The effect of fungicides on feather degradation is shown in Table 1, 2 and fig 3, 4, that in case of *C. keratinophilim*, the maximum percent reduction of feather was at 10 ppm conc. of Dithane M-45 i.e. 56.86 percent followed by 53.90 and 52. 94 percent at 10 ppm conc. of Aureofungin and Bavistin respectively. In case of *M. gypseum*, the minimum percent of feather degradation was at 40 ppm conc. of Dithane M-45 and keratinase production was 8.24 U/ml. Aureofungin also gives the good result in inhibition of keratinolytic ability. In the presence of Aureofungin maximum inhibition of keratinase production was exhibited at 40 ppm i.e. 12.43 U/ml and feather degradation was 11.76%.

All the fungicides showed suppressive effect overall and the keratinase production was decrease with increase in fungicidal conc. Bavistin and Dithane M-45 were highly effective and achieving 100% decrease over control. Pandey and Chakrabarti (2004) investigated that carbendazim inhibited the growth of germ tubes of conidia and also reduced the rate of infection.[7] However Chakarbarti and Gaur (2009) reported that Mancozeb and carbendazim were the most effective in inhibition of mycelia growth of *F. mangiferae*. [8]. Kaul and Sumbali (1999) investigated that the higher content of N in poultry soil ranging from 1.1- 1.8% [9]. Awasthi (2010) proved it to be an excellent slow release of N fertilizers in soil to increase the nitrogen [10]. Evaluation of these fungicides helped to standardize the best fungicides viz. Bavistin and Dithane M-45 against *C. keratinophilum and M. gypseum* in the present study.

Table-1 Effect of fungicides on keratinolytic ability of the isolated *C. keratinophilum* culture

Fungicides	Conc. (PPM)	Residual feather with filter paper (gm)	% reduction of Feather	Keratinase production (U/ml)
Bavistin	10	0.48 ± 0.011	52.94	43.74
	20	0.61± 0.034	40.19	35.23
	30	0.71 ± 0.026	30.39	21.33
	40	0.85 ± 0.01	16.66	10.54
Aureofungin	10	0.47 ±0.02	53.90	43.24
	20	0.58 ± 0.04	43.13	37.01
	30	0.68 ± 0.01	33.33	22.32
	40	0.83 ±0.011	18.62	13.83
Dithane M-45	10	0.44 ±0.05	56.86	46.56
	20	0.56 ±0.03	45.09	32.72
	30	0.7 ± 0.047	31.37	20.20
	40	0.84 ± 0.045	17.64	14.60

Value is mean of three replication± Standard deviation (SD)

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Table- 2 Effect of fungicides on keratinolytic ability of the isolated *M. gypseum* culture

Fungicides	Conc. (PPM)	Residual feather with filter paper (gm)	% reduction of feather	Keratinase production (U/ml)
Bavistin	10	0.556 ± 0.011	46.07	40.40
	20	0.72± 0.05	29.41	23.45
	30	0.89 ± 0.045	12.74	18.76
	40	0.92 ± 0.02	9.80	10.32
Aureofungin	10	0.53 ±0.026	48.03	41.25
	20	0.73 ± 0.03	28.43	27.43
	30	0.84 ± 0.01	17.64	21.67
	40	0.9 ±0.034	11.76	12.43
Dithane M-45	10	0.56 ±0.02	45.09	40.87
	20	0.74 ±0.01	27.45	24.42
	30	0.88 ± 0.04	13.72	15.65
	40	0.93 ± 0.047	8.82	8.24

Value is mean of three replication± Standard deviation (SD)

Fig:--3 Effect of fungicides on keratinolytic ability of the isolated *C. keratinophilum* culture

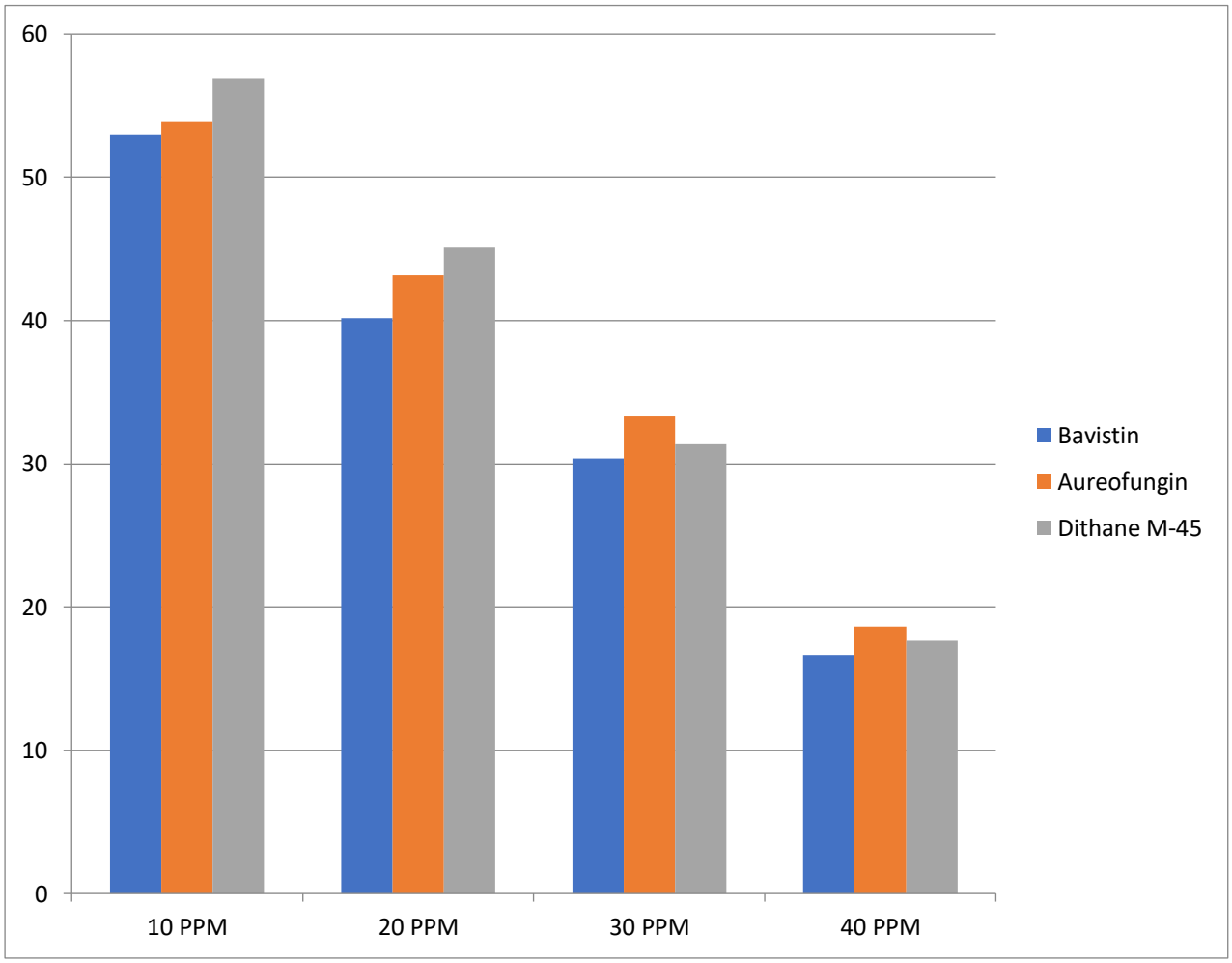

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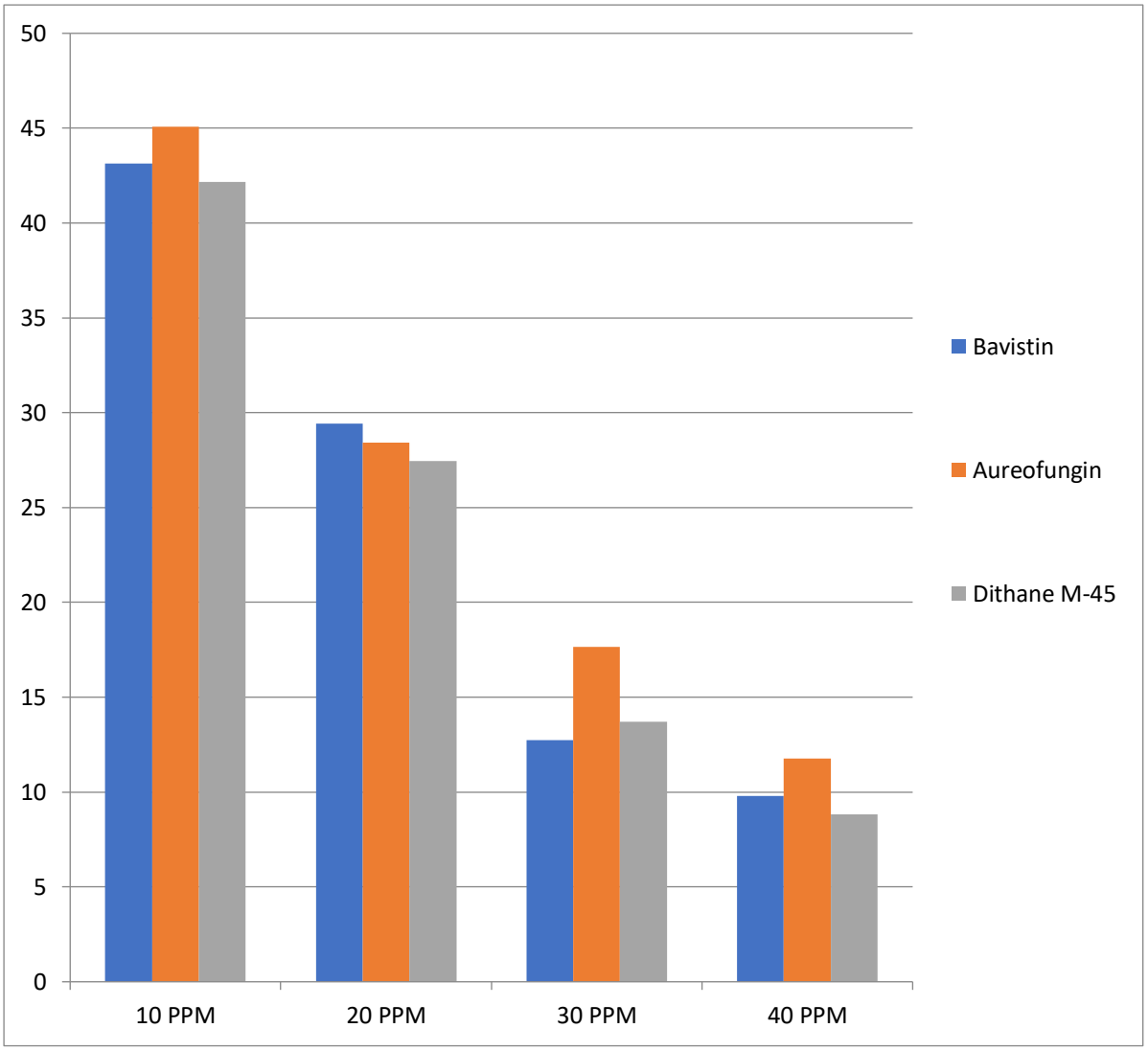
Fig:--4- Effect of fungicides on keratinolytic ability of the isolated *M.gypseum* culture



4.

CONCLUSION Production of feather in large amount from poultry industry is one of the major form of pollution. Traditional feather degradation reduces the overall quality of proteins and destroys essential amino acids. Biodegradation of feathers is found to be an efficient, eco-friendly, cost effective method for bioconversion of feather waste into useful products. The use of keratinolytic fungi to degrade poultry waste feather has emerged as a sustainable and alternative tool to meet this challenge.

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