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Microbiological dynamics of different poultry waste disposal methods

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ABSTRACT

The improper handling and disposal of animal excrement, especially that from chickens and other animals, has historically resulted in serious environmental and societal difficulties. This research study outlines the method for improving the usage of rotational compost bins in the disposal of poultry waste. The objective was to study the microbiological dynamics of different poultry waste disposal methods. Fresh poultry manure, together with dead bird carcasses and paddy straw (i.e. aerobic composting-T1 group) was composted and compared to T2 group in which additionally yeast was incorporated @7.5%. Keeping moisture at 45-50%, the initial C:N ratio was set at 30:1 in both treatment groups and the study was carried for 90 days. The effect of introducing yeast probiotic (T2-probiotic consortium assisted aerobic composting) on compost temperature, odour and structure was assessed and their impact on total microbial load and actinomycetes were compared to that of T1 group. The decrease in total bacterial count varied non significantly in both the treatment groups with initial decline in number in group T2 as compared with T1. The *E. coli* count of the composting material varied non significantly at both initial and final stage of composting. The number of fungal colonies in T1 and T2 did not differ significantly initially but significant difference was observed in later stages. The actinomycetes colonies start developing towards the last phase of composting period in both groups and differ significantly. The quality of the manure compost was evaluated at various phases of operation. Inoculating yeast as a probiotic into the composting process increased the quality of the compost, transforming it into a stable and odor-free finished compost.

Keywords: Actinomycetes, Aerobic composting, Microbes, Poultry waste, Probiotic, Temperature

Poultry waste is largely organic in nature which necessitates its cost-effective and sanitary disposal (Beohar and Srivastava 2011). Furthermore, poultry waste has higher nitrogen (N) content than the dung of large animal (Wilkinson 1979). Direct field application of poultry litter could harm environment, human as well as animal health (Kyakuwaire *et al.* 2019). Williams *et al.* (1999) envisaged that based on the average daily live weights throughout the birds' production cycle, the amount of manure generated by 1000 birds per day was predicted as 120 kg for layer hens, 80 kg for meat chickens, 200 to 350 kg for turkeys and 150 kg for ducks. Turan (2008) demonstrated that the composting method, when combined with some natural zeolites, might increase the quality of poultry litter. Similarly, Guerra-Rodriguez *et al.* (2001) showed that when combined with chestnut burr and leaf litter, there is potential for poultry manure disposal by composting. Moreover, when composted with carbon rich feed stock, the final product was a high-quality compost in terms of stability, organic matter content, C:P ratio and C:N ratio. Poultry manure is better suited for use as a plant fertilizer rather than a soil conditioner due to quick nitrogen mineralization and low organic matter content (Alsanius *et al.* 2016). For this, it is essential that the most common pathogenic bacteria, if present at levels higher than recommended, found in poultry waste are detoxified during thermophilic phase, alongwith alternative methods of compost treatment. Some researchers attempted studies on the agronomic assessment, microbial contamination, and nutrient composition of compost (Sánchez Monedero *et al.* 2019), omitting to address the reasons for extended composting times, potential reductions in pathogen, and odour emissions, as well as the usefulness of compost for small-scale farmers. Therefore, present experiment was designed to study the microbiological dynamics of different poultry waste disposal methods.

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MATERIALS AND METHODS

Place of study: Experiment was conducted at the Poultry Research Farm, Department of Livestock Production Management, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab. Trials were conducted in the open area

of Poultry Research Farm. The geographical position of the experimental area is as following: Latitude: 30°54' North Longitude: 75°48' East and at the height of 246 meters above the mean sea level. Ludhiana experiences a semi-arid climate with three major seasons i.e. summer (April to June), monsoon (July to September) and winter (December to February). The maximum and minimum temperature of summer, monsoon and winter seasons are 45±2°C and 27±2°C, 30±2°C and 22±2°C, 12±2°C and 4±2°C, respectively.

Source of compost materials: Poultry waste was collected from the local poultry farm. The poultry waste was sun dried for 2 days to standardize moisture content between 45-50%. The carbonaceous material used in the experiment was paddy straw collected from agricultural fields. Dead bird carcass was collected from Hatchery and Post- Mortem Hall of the university. Yeast, used as the probiotic consortium (@7.5% with the concentration of 3x 10^9 *Saccharomyces cerevisiae* per gram) in the experiment, was purchased from the local market. The initial carbon-nitrogen ratio was fixed at 30:1 based on the proximate analysis (AOAC 2000) of the initial substrate used.

Design of the rotational compost bins: The experimental trial was carried out in polyvinyl drum with capacity of 210 L (Dimensions: height = 0.9 m and diameter of cylindrical portion = 0.5 m). The drums were modified by providing six layers of 10 mm diameter holes at 3 cm spacing from each other at the upper window to facilitate natural air circulation. One window/lid was provided in all the drums at top portion of the drum for ease in sample collection and for aeration. Along with those modifications, an iron pipe was inserted through the side of each drum which passed from the centre of drum. Two drums for each treatment were placed parallel to each other and a single iron pipe passes the longitudinal axis of both drums. From one end the iron rod was installed with a handheld pulley and handle for turning the drums mechanically.

Experimental design: There were two treatment groups with two replications of each viz. T1: Aerobic composting of poultry manure in rotational bins and T2: Aerobic composting of poultry manure with probiotic consortium. Bins for each treatment group were filled sequentially with carbonaceous material, dead carcass, and poultry litter. The fresh compost samples were taken fortnightly and microbiological parameters were monitored till 90 days after which compost was left on its own for one month curing. Thereafter, maturity parameters were determined following the procedure mentioned by Grewal (2020).

Recording of parameters

Temperature: Temperature was recorded with the help of a digital compost micro thermometer with probe. Using this thermometer, the temperature in all four drums of two treatment groups were recorded daily from the top, middle, bottom, and sides of the compost drums. The average of all readings was used as the final value for each replicate.

Aeration and turning: The mixture of compost materials in each treatment bins was turned twice weekly by rotating the hand-held pulley installed at the side of each treatment bin. This rotation performs dual activity of turning as well as exchange of air between the layers of the compost through the holes over the bin. Moreover, the window of bins was left open for the passage of air into the bins. Holes of size 10 mm were kept over the top of the drum for proper aeration.

Change in colour: At each sampling period, the composting mixture in T1 and T2 treatment groups was visualized through naked eye by a team of four members to determine the colour change.

Odour: Fear of odour is one of the major environmental problems causing a menace in composting of poultry farm waste. Hence odour analysis was carried out on monthly basis through olfactory judgement. A score card with hedonic scale was designed as per procedure mentioned by Baba (2014) (Table 1).

Table 1. Odour hedonic scale

Attribute	Score
Extremely Undesirable	1
Highly undesirable	2
Moderately Undesirable	3
Undesirable	4
Desirable	5
Moderately Desirable	6
Highly Desirable	7
Extremely Desirable	8

Total microbial load: It was calculated with the help of pour plate method. Initial compost suspensions were made by combining 10 g (wet weight) compost samples with 90 ml of PBS (w/v) in a centrifuge tube. Those suspensions were shaken at 200 rpm for 30 minutes at 21°C. Those initial suspensions were serially diluted in PBS solution. Aliquots of each dilution were placed in petri plates containing the appropriate medium. Per dilution, five plates were utilized. Hundred µl of dilution from 4^{th} & 5^{th} for MLA and 6^{th} & 7^{th} dilution for Hichrome *E. coli* agar from corresponding was poured into petri-plate. The petri-plate was then swirled thoroughly for uniform mixing of suspension with media. The plate was cooled to solidify the contents and then, incubated for 24 hours at 37°C for the bacterial colonies to develop. Those bacterial colonies were counted on a colony counter and the total number of bacterial load was calculated on the basis of serial dilution and the final value was expressed in Log_{10} values/g of fresh sample. The Hichrome *E. coli* agar media (HiMedia® Mumbai) was used for identification of *E. coli* (Blue colonies) from non-*E. coli* (White colonies) bacteria. Total bacterial count (white colonies) was determined by using Brain Heart Infusion agar media (HiMedia® Mumbai) and Nutrient Agar (HiMedia® Mumbai).

Total fungal count: The total fungal load was calculated using pour plate method. The determination of total fungal

count was done by using specific media. The Rose Bengal Agar (HiMedia® Mumbai) was used for determination of total fungi in the compost samples. 100 µL of dilution from 3rd & 4th for Rose Bengal Agar dilution from corresponding was poured into petri-plate.

Actinomycetes: The total actinomycetes were calculated using pour plate method. 100 µL of dilution from 4th & 5th for Starch Caesin Agar was used for determination of total actinomycetes. The plate was cooled to solidify the contents and then, incubated for 24-48 hours at 24°C for actinomycetes colonies to develop. The following equation was used to calculate the number of colony forming units (CFU) per gram from the original aliquot/ sample:

CFU per gram = Average number of colonies for a dilution × dilution factor

Statistical analysis: The data recorded on various parameters were analysed for statistical differences by the analysis of variance (Snedecor and Cochran 1994). The treatment means were compared by using the Duncan's multiple range test (Duncan 1995).

RESULTS AND DISCUSSION

Colour assessment: Initially, as the compost comprises of all the ingredients in raw state layered sequentially one over the other, the color of top layer (i.e. paddy straw) of compost was often yellowish in all the three treatment groups. The finished compost was pale brown to dark brown to black coloured in T1 and T2 treatment groups; which were free from any bad odour. This change in colour might be due to degradation of organic matter, by the action of microorganisms, and by the effect of periodic turning and aeration in the compost. The composting process converts some of the original organic matter into humus like material, which was responsible for the color change in the finished compost. The matured compost obtained was a rich source of organic matter, dark in color and easily crumbled, with an earthy aroma (not an aroma of decaying organic material).

Odour assessment: Odour was the most effective and simple indicator of whether the pile condition was aerobic. Strong putrid odour that sometimes smells of sulphur indicated anaerobic activity, particularly when odour was accompanied by low temperature (USDA-NRCS 2000). In this study moderately undesirable odour (Table 2) was observed in the vicinity of the experimental area at the beginning of the experiment. The odour started becoming desirable to highly desirable as the composting period advanced. Towards approaching maturity of the compost, there was no bad odour observed which indicates that the paddy straw acted as biofilter. Also, indicating that paddy straw, poultry waste and dead carcass had sufficient amount of carbon to promote enhanced microbial activity and creating an environment that deters the access of insects and birds to the compost piles. Furthermore, this process not only accelerates the decomposition of organic matter but also serves as a means of deodorizing the gases emitted from decomposing carcasses at ground level, which are typically malodorous. Similar data were reported by Murphy (1988) and Sanabria-León (2006). Their study reported no obnoxious odour from dead bird and slaughter waste compost. Next to odour, fly menace is the second and important problem while composting bio-wastes. The finished mature compost obtained at the end of composting period exhibited a more neutral to pleasant odour indicating a substantial improvement in odour quality.

Table 2. Odour scale (Mean ± SE) of compost samples in different treatment groups

Days of composting	T1	T2
1	2.25±0.31	2.1±0.23
30	3.25±0.31	3.50±0.27
60	4.75±0.16	5.25±0.31
90	5.87b±0.13	6.63a±0.26
120	6.75±0.25	7.00±0.19

Figures with different small letters row wise differ significantly

The periodic mixing and turning of the compost bins improved the oxygen supply inside the compost mixture in both T1 and T2 groups, also providing better activity of probiotic consortia (yeast in T2 group). At the end of maturation period (120 days of composting), earthy smell was noticed owing to the scale of moderate desirable (in T1 group) to highly desirable (in T2 group) odour. Similar findings were observed by Sarkar *et al*. 2011 that composting with effective microorganism had reported to reduce the malodour.

Total microbial count assessment: There was decline in the total bacterial count in both the treatment groups. The number of bacteria reduced as the conditions in the compost bin turned thermophilic as depicted from Table 3. The decrease in total bacterial count varied non significantly in both the treatment groups with earlier decline in number in group T2 as compared with T1. It was also noted that microbial flora was inactivated within 24 hours as the temperature reached around 50°C during an aerobic thermophilic phase (Bicùdo and Goyal 2003). The *E.coli* count of the composting material varied non significantly at both initial and final stage of composting. In the initial phase, *E. coli* count was numerically higher in T1 than T2 treatment group. Among the treatment groups, T2 had numerically lowest count of *E. coli* as compared to T1 at the end of compost period maturity (day 120). Coliforms could grow in adverse environments which was characterized by low pH and low temperatures. The high temperature generation might be the reason for the reduction in bacterial count. According to Gradel *et al.* (2003) and Bukhari (2017), microbial load was decreased with the increased duration of composting process.

Total fungal count and total actinomycetes count assessment: The appearance of fungi and actinomycetes in the later stages of composting is a natural progression that reflects the increasing complexity of the composting process. Their presence signifies the breakdown of tough, complex materials and indicates that the compost is nearing

Table 3. Total microbial count (Mean ± SE) of compost samples in different treatment groups

Days	Parameter	T1 (CFU in log_{10})	T2 (CFU in log_{10})
15	TBC on BHI	9.35±0.02	9.41±0.01
	TBC on Nutrient Agar	9.18±0.00	9.18±0.01
	Salmonella and *Salmonella* like microbes	7.11±0.08	6.93±0.03
	E. coli	4.96±0.02	4.67±0.04
30	TBC on BHI	9.35±0.07	9.13±0.03
	TBC on Nutrient Agar	9.17±0.01	9.10±0.02
	Salmonella and *Salmonella* like microbes	7.10±0.09	6.99±0.01
	E. coli	4.86±0.01	4.94±0.02
45	TBC on BHI	9.22±0.01	9.03±0.02
	TBC on Nutrient Agar	9.14±0.12	8.95±0.01
	Salmonella and *Salmonella* like microbes	6.98±0.01	6.68±0.14
	E. coli	4.77±0.02	4.85±0.03
60	TBC on BHI	9.10±0.01	8.94±0.01
	TBC on Nutrient Agar	9.04±0.05	8.89±0.03
	Salmonella and *Salmonella* like microbes	6.79±0.07	6.28±0.28
	E. coli	4.72±0.03	4.68±0.04
75	TBC on BHI	8.95±0.02	8.87±0.02
	TBC on Nutrient Agar	8.77±0.04	8.81±0.06
	Salmonella and *Salmonella* like microbes	6.63±0.12	6.41±0.03
	E. coli	4.63±0.05	4.56±0.03
90	TBC on BHI	8.70±0.02	8.74±0.01
	TBC on Nutrient Agar	8.61±0.06	8.64±0.02
	Salmonella and *Salmonella* like microbes	6.25±0.08	6.41±0.05
	E. coli	4.39±0.03	4.31±0.07
120	TBC on BHI	8.27±0.15	8.53±0.02
	TBC on Nutrient Agar	8.18±0.04	8.35±0.07
	Salmonella and *Salmonella* like microbes	5.82±0.34	5.96±0.37
	E. coli	0.88±0.19	0.77±0.07

TBC: Total Bacterial Count; BHI: Brain Heart Infusion Agar; CFU: Colony Forming Unit

maturity. As these organisms work, they contribute to the overall stability, nutrient availability, and quality of the compost, ensuring that the final product is safe, nutrient-rich, and beneficial for soil health. This results in the formation of stable organic compounds, which is crucial for achieving mature, high-quality compost.

The number of fungal colonies in T1 and T2 did not varied significantly initially but only numerical difference in the total fungal count was observed between the two treatment groups. The appearance of fungi at 45th day indicates that the compost is nearing maturity. The actinomycetes colonies start developing towards the later phase of composting period. As the compost reaches its final stages, the microbial community shifts towards organisms

Table 4. Total fungal count and actinomycetes count (Mean ± SE) of compost samples in different treatment groups

Days of compost	Total fungal count (Log_{10} cfu/mL)		Total actinomycetes count (Log_{10} cfu/mL)	
	T1	T2	T1	T2
15	ND	ND	ND	ND
30	ND	ND	ND	ND
45	4.89 ± 0.05	4.84 ± 0.06	ND	ND
60	5.24 ± 0.13	5.17 ± 0.02	7.20^{a} ± 0.01	7.00^{b} ± 0.03
75	2.34^{b} ± 0.01	4.99^{a} ± 0.04	7.33^{b} ±.01	7.46^{a} ± 0.01
90	2.15^{b} ± 0.03	4.72^{a} ± 0.12	7.42^{b} ± 0.04	7.50^{a} ± 0.02
120	ND	4.60 ± 0.01	7.40^{b} ± 0.01	7.56^{a} ± 0.02

Figures with different small letters row wise differ significantly

(i.e. actinomycetes and fungi) capable of decomposing the last remaining complex substances. In the present study, the number of actinomycetes starts appearing at day 60 onwards with higher count in T1 group and numerically lowest number in T2 treatment group. The actinomycetes count in both treatment groups varied significantly, although numerical difference was also noticed between them. The highest number of actinomycetes were observed in T2 group (probiotic assisted treatment group) indicating good matured compost as compared with T1 (Table 4).

Furthermore, the growth of fungi and actinomycetes helps reduce unpleasant odours that can arise from decomposing proteins and other organic compounds. These microbes contribute to the stabilization of the compost, reducing volatile gases. The mycelial network of fungi also plays a key role in improving the physical structure of the compost, contributing to the formation of aggregates that give the compost a loamy texture.

From the above experiment, it was concluded that probiotic added aerobic composting of poultry wastes disposal method not only achieved earlier peak temperature in compost but also improved the quality of compost rendering it into a stable and odour free produce.

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