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Formulation Of *Moringa Oleifera* Seed Powder And Ferrous Sulfate Heptahydrate Into A Matrix To Control Coliform Bacteria And Odor In Poultry Manure

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Abstract

A blend was formulated from a mixture of *Moringa Oleifera* seed powder (MOSP) and ferrous sulfate heptahydrate crystals, each at blend ratios of 2%, 5% and 10% via factorial experiment in order to optimize efficacy of the blends for odor inhibition. It involved the two components as factors vize: factor A (Ferrous sulfate) at three levels each i.e a_1, a_2, a_3 representing 2%, 5% and 10% and factor B (moringa oleifera) also at three levels each i.e b_1,b_2, b_3 representing 2%, 5%, and 10%. This is a 2x3 factorial experiment from which a total of 9 treatment combinations were obtained. The products were coded F1 – F9 and each applied to a 300 g fixed weight of poultry waste. Their odor reduction potential was evaluated on weekly basis up to 5 weeks incubation period using an odor panel. Results showed significant differences in their odor reduction potentials ($p > 0.05$). F2, F5 and F7 were particularly impressive and gave overall odor reductions of 53.6%, 53.2% and 52.8%% respectively. It is concluded that the formulations suppressed manure odors at both short and long term incubation periods in contrast to most commercial products. Therefore they show promise of pre-land application use.

Key words: Formulations, matrix, coliforms, manure, odors

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Introduction

Over the years, there has been increased participation in rearing of livestock both in the rural and urban centres. The ultimate is mainly food and income and manure for crop cultivation. Livestock production and the waste generated can pose a threat to soil, water and air quality and to human health. Some of the more serious problems with livestock waste include nutrient enrichment of soil and water, emission of offensive odors and green house gases, as well as presence and transmission of pathogenic microorganisms (Vincent, 2002; varel et al, 1999). Livestock farming have continued unregulated particularly in the poultry sector, with little or no regards to the wide range of the adverse effects of these facilities. This is because the legal and regulatory frameworks required to impose compliance are reported to be weak and in most cases uncertain on the statutory responsibilities and duties of the government with regard to environmental management and protection to guaranty clean air (Ogunba, 2004).

The offensive odor is the immediate impact of livestock production on the human population and is partly the result of incomplete anaerobic decomposition of plant fibre and protein in stored manure (Spoelstra, 1980; Hammond, 1989). Results from studies of different researchers have characterized these odorous substances. A list of 150 different compounds in swine manure was presented (Spoelstra, 1980). Another list of 168 volatile compounds related to animal production was reported (O'Niell and Phillips, 1992). Schiffman et al, (2001) also gave a list of 331 volatile substances sampled from lagoons and air at swine production facilities. About 35 t0 73 decomposition products from diary manure were equally reported (Sunesson et al, 2001, Raband et al, 2003, Filipy et al, 2006). The products reported have far reaching environmental, health and socioeconomic concerns.

In recent years, many treatments such as biogas production, anaerobi or aerobic waste conversion and solids separation have been available to farmers for managing livestock odors and manure. Other modern abatement technologies and additives that reduce odors from animal units have equally been developed and categorized into digestive agents, oxidizers, adsorbents masking agents and disinfectants (McCrory and Hobbs, 2001., Mackie et al, 1998., Ritter et al, 1975., Warburton et al, 1980).

While some of these methods have proven effective, particularly for short term odor control, the results of many have been mixed and generally unsatisfactory especially for long term odor reduction. Their use may equally be limited by cost and /or operative expertise and unavailability of products (Ritter et al, 1989, Eileen et al, 2011). Besides having variable effects, no single treatment or amendment has been successful for short or long term odor control. Odor abatement technologies are often targeted towards other livestock systems, but limited data exist for poultry waste odor management (Ulman's et al, 2004). Therefore nuisance odor generation from poultry farms has continued unabated and is a global concern. Due to the health and environmental issues associated with odorous emissions from the highly proliferated poultry farms and waste storage facilities, continued efforts and search for effective, cheap and available materials for its control is an urgent imperative.

This work is therefore a report of the synergistic effects of $FeSO_4.7H_2O$ crystals and Morringa Oleifera seed powder used to inhibit microbial activities and reduce odors from poultry waste. The short term effect of $FeSO_4.7H_2O$ as pH modifier in livestock waste has been mentioned (More et al, 1996). Morringa Oleifera, a natural biopolymer, is also well reported for its broad based antimcrobial activities (Raheela et al, 2008), but never tested for inhibition of odor causing microbial activities in poultry waste odor control. This is expected to provide both short and long term solution to odor problems in the vicinity of poultry houses in the developing countries and hence ensuring clean and healthy environment.

Materials and methods

Manure collection and preparation:

Poultry fecal waste was collected from caged broilers grown on a modern farming skill acquisition center in Yola, Adamawa state of Nigeria, after being excreted over night. A clean 8 x 6ft nylon sheet was placed underneath the cages to avoid contamination of manure from the floor. This was carefully wrapped and transferred to the laboratory for further processing.

Formulation of the matrix:

The biopolymer based matrix was formulated from a mixture of MOSP and ferrous sulfate heptahydrate crystals by physical blending. The materials were first oven dried according to standard methods, ground to fine powder and sieved using 200 mm mesh size. A factorial design approach was used to optimize efficacy of the formulation of the biopolymer based matrix for odor inhibition. The strategy is to study the effects of ferrous sulfate hepta-hydrate and MOSP at different ratios as variables on odor inhibition. It involved the two components as factors vize: factor A (Ferrous sulfate) at three levels each i.e a_1, a_2, a_3 representing 2%, 5% and 10% and factor B (MOSP) also at three levels each i.e b_1,b_2, b_3 representing 2%, 5%, and 10% (Table 1). This is a 2x3 factorial experiment from which a total of 9 treatment combinations were obtained and were coded F1 – F9.

Table 1: Treatment combinations of variables

Factors	levels		Treatment combinations	
A	a_1 (2%)		a_1b_1	a_1b_1
	a_2 (5%)	a_1	a_1b_2	a_1b_2
	a_3 (10%)		a_1b_3	a_1b_3
			a_2b_1	a_1b_1
		a_2	a_2b_2	a_2b_2
			a_2b_3	a_2b_3
B	b_1 (2%)		a_3b_1	a_3b_1
	b_2 (5%)	a_3	a_3b_2	a_3b_2
	b_3 (10%)		a_3b_3	a_3b_3

Quantity of waste used = 300 g

Treatment of Farm Manure with Matrix

The two components and the various formulations obtained were added to about 300 g each of the poultry waste and thoroughly mixed together using a motorized mixer. This was transferred into 1-L capacity wide mouth glass jars each and sealed with covers underneath which was cotton swatches, as odor absorbents. A controlled jar with no addition was included. The jars were painted black to minimize the visual impact of manure during sampling and analysis. The jars were transferred into the sniffing room and incubated at room temperature. The contents of the flask were sampled at weeks 1, 2, 3, 4, and 5 for analysis.

Microbial load count

The microbial load in the samples was estimated using method outlined by El-Jalil *et al*, 2008. Ten **(**10) grammes of each sample was blended in 90 cm^3 of saline water (0.9 % W/V NaCl) with a Warring blender to prepare the initial dilution. Colony forming units (CFU) were determined by standard pour plate methodology. Decimal dilution for total viable counts was made in (0.85% W/V saline solution) and 1 cm^3 was placed in duplicate on standard plate count agar - Enterobacteria were enumerated on MacConkey Agar (Leininger, 1976). The plates were incubated at 30°C for 24 h.

Analysis of Odor

Analysis of odor was performed according to the procedures authored by Sobel, (1972); Sweteen and Akanbi, (1994). A panel of 10 members was presented with the odor samples who rated the samples on a given scale of 0 – 5 over a 5 week period. The ‘0’ represents no odor while ‘5’ represents maximum odor levels. Observations were taken by lifting up the lids of the jars with a gloved hand and smelling the treated and controlled samples at a distance of 6 – 8 inches and odor levels were rated accordingly.

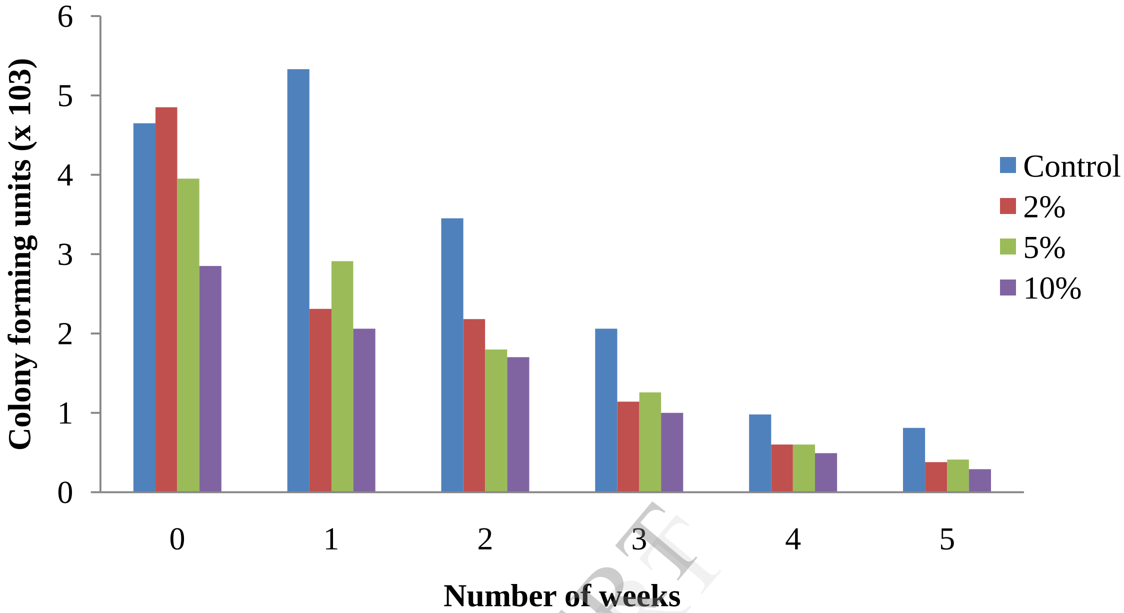
Statistical analysis

All treatments were in duplicate and data in figures are expressed as means. Statistically significant differences at $p< 0.05$ probability level were obtained from ANOVA tests.

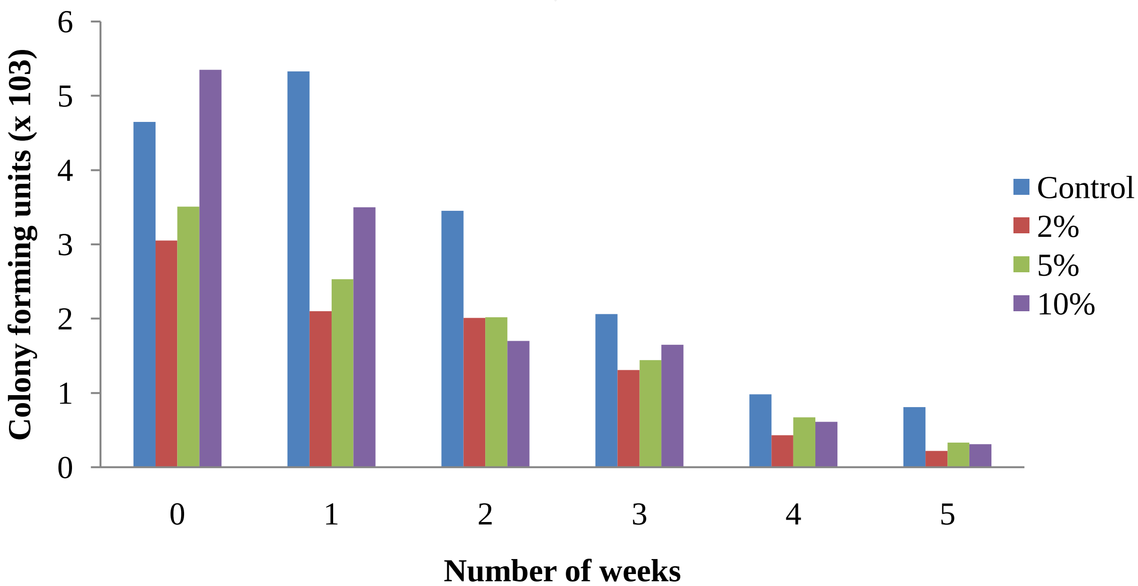
Results and discussion

Fecal coliforms were enumerated from the controlled and treated samples. The no addition treatment initially contained 4.65×10^3 CFU ml^{-1}, which increased to 5.33×10^3 CFU ml^{-1} in 7 days before declining to a level below the initial concentration at the end of 35 days (0.81×10^2 CFU ml^{-1}). The steady decline in the concentration of coliform bacteria may be due to increase in toxic secretions and degradative acidic products which became lethal to a select population of organisms in the control. When compared to the no addition treatment (control), the $FeSO_4.7H_2O$ and MOSP treatments significantly reduced the number of viable coliform bacteria as shown in figure 1a & b ($p < 0.05$).

From Fig.1a, the 2%, 5% and 10% concentrations of ferrous sulfate hepta hydrate all effectively suppressed the populations of the coliforms in the waste. The 10% $FeSO_4.7H_2O$ treatment was seen to be more effective as it accelerated the decline of the viable populations from 2.85×10^3 CFU ml^{-1} at the start of the incubation to 0.29×10^1 CFU ml^{-1} after 35 days period. This effect may be due to the hostile nature of the highly acidic $FeSO_4.7H_2O$. The MOSP treatment was however more effective at the lowest concentration of 2% (Fig.2). The population of viable coliforms decreased from 3.05×10^3 CFU ml^{-1} initially to 0.22×10^1 at the end of the 35 days incubation. MOSP seed is a natural biopolymer and the carbohydrate content at the higher concentrations of 5% and 10% may increase nutrient for microbial growth rather than inhibition, thereby limiting the antimicrobial strength evidenced by the low values.


Colony forming units (x 103)
6
5
4
3
2
1
0
0
1
2
3
4
5
Number of weeks
Control
2%
5%
10%

(a)


Colony forming units (x 103)
6
5
4
3
2
1
0
0
1
2
3
4
5
Number of weeks
Control
2%
5%
10%

(b)

Figure1: Effects of Ferrous Sulfate heptahydrate (a) and MOSP seed powder (b) on viable coliforms

The efficacy of the combination of the two additives at the various concentrations on fecal coliforms was also investigated (Fig.2). All the formulations exhibited improved performance in comparison with the single component treatments. Although the odor control formulations did not completely eliminate the microbial mass in the treated samples, they however accelerated the death of the coliforms.

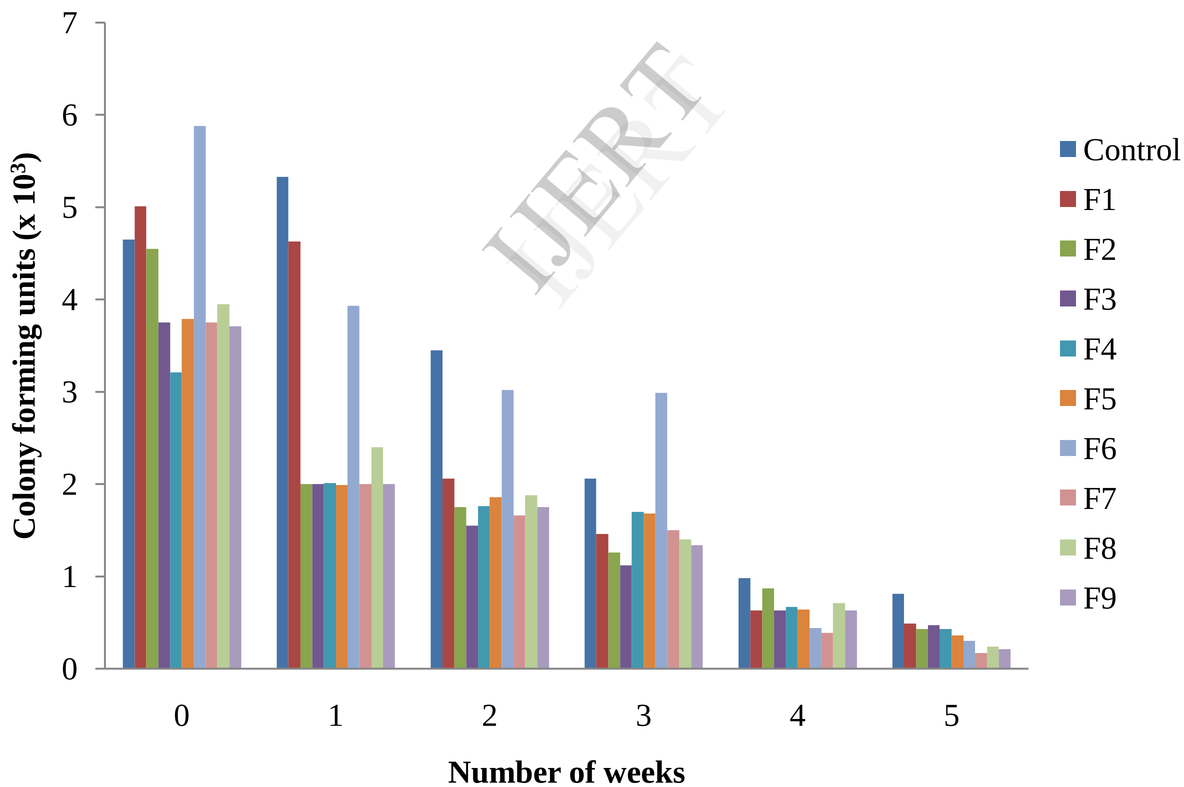


Figure 2: Effect of Formulations on viable fecal coliforms

The performance of these treatments compared well with, and in some cases, proved better than a number of commercial products that claim to eliminate coliform bacteria from manure. Varel et, al (2004) reported a total CFU of 0.02 x 10^5 ml^{-1} after 14 days of treating cattle waste with thymol, a plant derived oil. In another study, N- (n-butyl) thiophosphoric triamide (NBPT) at 20 and 80 mg/kg^{-1} gave 0.4 x 10^5 and 0.13 x 10^5 CFU ml^{-1} respectively after 42 days of waste treatment (Varel, et al 2006). Their actions is predicated on the capacity to reduce or eliminate microbial pathogens, the odor causing agents in manure. Clearly, reducing the concentrations of potentially pathogenic bacteria in manure is a key to reducing manure odors and is an attractive feature of odor controlling agents (Della *et al*, 2002).

Consequently, an odor assessment of the treated samples was conducted to ascertain the odor reducing potential of the formulations throughout the treatment period. The odor control effectiveness of the formulations on weekly basis is shown in Figure 3.

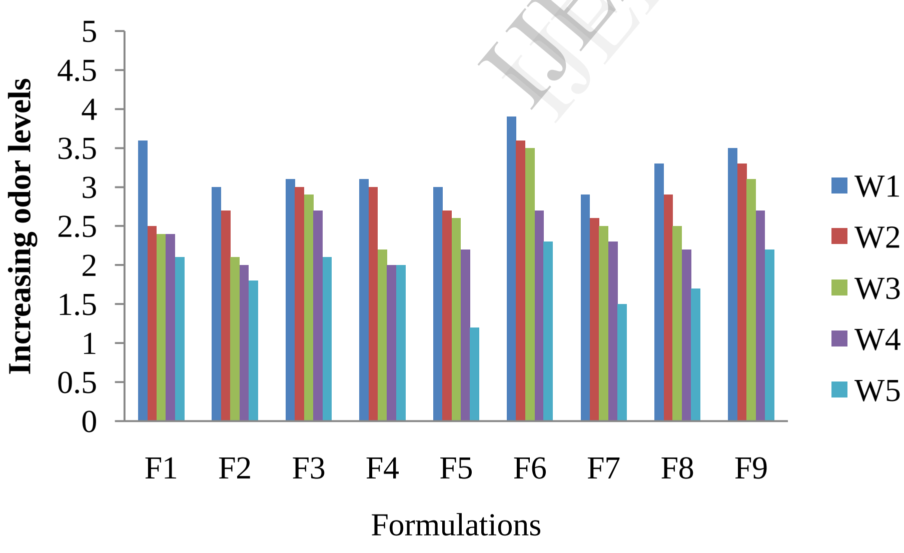
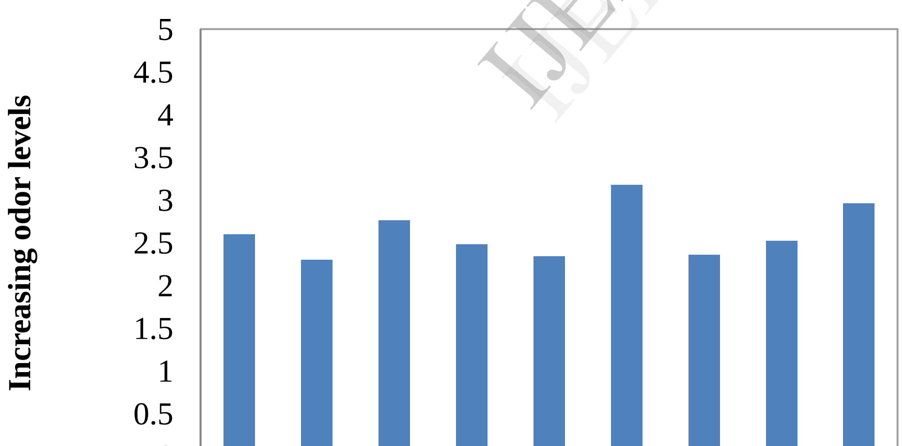


Figure 3: Weekly effectiveness of formulations for odor inhibition

From Figure 3, the odor levels generally decreased with time. From the first week, all the formulations indicated positive effect on the odor. F6 gave minimum reduction of 22% and F7 showed the highest value of 42% reduction. By the end of the treatment period of 5 weeks, F7, F5, F2 gave impressive results of 70%, 76% and 82% respectively.

The overall effectiveness of the formulations over the entire period of treatment is depicted in Figure 4. The results showed that F6 was the least with odor levels reduced by 36%, while F2 was the best with odor levels of 53.6% reduction.



	F1	F2	F3	F4	F5	F6	F7	F8	F9
Odor levels	2.6	2.3	2.76	2.48	2.34	3.18	2.36	2.52	2.96

Figure 4: Overall formulations effectiveness for odor inhibition

From the nine formulations, only F2, F5 and F7 reduced the overall odor levels by more than 50%. F6 seemed to be the least effective with odor reduction of 36%. The level of effectiveness of the remaining formulations was in-between 36% and 50%. F2, F5, and F7 were particularly impressive and can therefore be used to effectively inhibit malodor generation from poultry waste.

The performances compared well with the efficacy of some commercial odor reducing products reported in the literature. Amon *et al.,* (1995) used saponin at 6.2g/day for 60 days to treat hog waste and reported 26% odor reduction. A pH modifier, AMGUARD (an organic acid), applied to waste for 8 months, gave 42% reduction in odor levels (Hendriks *et al.*, 1997). In another study, a digestive agent used over 22 month's period inhibited odor generation only by 30% (Hendriks *et al.,* 1997). Wheeler *et al* (2011) investigated the efficacy of other 22 commercial manure amendments covering five classes (microbial, chemical, adsorbent, masking and disinfectant) for both short and long term periods. Three products showed significant short term odor control. A microbial digest/ enzyme (MAF) showed a 33% reduction in odor, another, a disinfectant (H_2O_2) with a 45% reduction and the third a masking agent (*Hyssopus officinalis*) essential oil with a 27% reduction.

These products were reported to offer potential for use prior to the transition from storage to land application by providing short term odor reduction. Reductions of odor emission by 22 or 17% respectively were also reported in manure treated with peppermint oil and basil oil. The efficacy of zeolite was also tested and was found to reduce odors by only 11%. Odor emission rates were reduced by 48% following the addition of digestive / facultative MBR and chemical only as a frequent re-application to diary manure. However, some of these compounds tested were found to influence odor generation instead. Manure treated with odorless glycerol, a product of biodiesel production, increased odor emission by 27% (Wheeler *et al*, 2011). In contrast to its performance at 3 days, H_2O_2 increased odor by 46% after 30 days incubation period, Zeolite increased by 177% after 30 days.

On the whole, they concluded that none of the 22 manure amendments significantly reduced odor emission from animal manure for both 3 and 30 days incubation periods. This agrees with the report that a large percentage of the commercial odor reducing compounds are not effective, besides being costly and scarce (Ritter *et al*, 1989; Wheeler *et al*, 2011). This is an indication that short, medium and long term odor control from poultry houses remains challenging. Comparatively, the effects of the formulations reported in this work showed significant coliform reduction and consequent odor inhibition at both short (7 days) and long term (35 days) incubation periods. However, none of the products was able to totally eliminate odors.

Conclusion

Results suggest that both the single components and their formulations were generally effective at both short and long term durations, therefore may require no repeated additions and can be used to inhibit malodor generation from poultry waste. However, studies under field conditions are expected to verify claims of efficacy at the optimized concentrations.

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