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# Effect of Various Composting Methods on the Concentration and Viability of *Ascaris suum* Eggs in Organic Fertilisers

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## ABSTRACT

The process of composting supports biological methods and management practices, such as vermicomposting and microbial inoculation, to enhance soil quality using biodegradable materials. However, the use of manure from animals poses potential risk for soil-transmitted helminths (STH) contamination. This study is aimed to determine the influence of various composting methods on the concentration of artificially-inoculated *Ascaris suum eggs.* There were three treatments (vermicomposting, composting with lactic acid bacteria, sun dry-composting) and a control (composting alone) which were artificially inoculated with *A. suum* eggs. Composting was done for a period of 31 days. *A. suum* percent recovery was determined on the 10$^{th}$ and 31$^{st}$ day of the composting process. Results revealed no significant differences in the percent recovery of *A. suum* in the various composting methods ($p$>0.05). Meanwhile, on the 31$^{st}$ day, the control (11.09%±6.40) and sundry-composting (9.03%±3.04) showed the highest percent recovery, followed by composting with lactic acid bacteria (7.62%±4.41). No *A. suum* eggs were recovered for vermicomposting on the 31$^{st}$ day. However, statistical analysis revealed no significant difference among treatments and control ($p$>0.05). Nevertheless, the present results suggest that the various methods of composting showed a 93.07% mean reduction of *A. suum* egg concentration in the organic fertilizers produced, and that composting rendered mechanical damage to eggs leading to reduced viability. Nevertheless, the presence of some fertilised eggs that could develop into infective embryonated eggs could still be a potential threat of viable eggs contaminating the organic fertilisers.

*Keywords: Ascaris suum*, composting, food safety, organic fertilizers

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## INTRODUCTION

Soil-transmitted helminths (STH) infects approximately 24% of the world's population, affecting areas suffering from poverty (WHO, 2015). Diseases caused by these organisms range from asymptomatic to detrimental depending on the parasitic load of the host (Cook & Zumla, 2009). The presence of STHs in agricultural crops could be an alarming threat to food safety (Gajadhar, 2015). *Ascaris suum* is the most commonly used STH model species because of its characteristic thick egg shell. *A. suum* can only reproduce within its host, the pig. However, recent studies have revealed cases of ascariasis in humans caused by *A. suum* (Miller et al., 2015) and in some individuals, the disease concerns both *A. suum* and *A. lumbricoides* (da Silva Alves, et al., 2016; Leles, Gardner, Reinhard, Iñiguez, & Araujo, 2012). *Ascaris spp.* Has also been reported to exhibit the highest STH prevalence from soil samples in Laguna, Philippines (Horiuchi, Paller, & Uga, 2013).

Organic farming is being promoted by the Philippines government to the agricultural sector by virtue of the Republic Act 10068, also known as the Organic Agriculture Act of 2010 (Congress of the Philippines, 2010). Raw materials of fertilisers used in place of chemical fertilisers usually consist of biodegradable farm wastes, which are mainly made up of animal manure. There are various methods of composting which include bacteria inoculation, vermicomposting and sun-drying of animal manure. The manure can be contaminated with STHs if it comes from an infected animal. Anthelminthic treatment is usually not administered on animals in organic farms, thereby increasing the risk of STH infection, contamination, and transmission. Though various methods on how to enhance composting have been developed, scientific evidence that deals with the effect of these methods on pathogens, such as *Ascaris* eggs, are still lacking. Hence, this study aims to determine the effect of various composting methods namely, microbial inoculation, sun drying and vermicomposting on the concentration and viability of STH, using *A. suum* as a model species.

## METHODS

### Experimental Design

The protocol was adapted from the practices of organic farms and organic fertiliser producers in Laguna, Philippines. A total of three experimental set-ups (lactic acid bacteria composting, vermicomposting, and sun-dry composting) and a control set-up (composting alone) were used in the present study. The control set-up and treatments were replicated with six beds, giving a total of 24 samples. Each bed contained two kilograms of swine manure and three kilograms of washed chopped banana trunk (total of five kilograms). The chopped banana trunks were washed thoroughly to wash out any possible parasite contamination. Banana trunk is commonly used in composting by local farmers in the Philippines as it is reported to produce better yields and improve organic fertiliser

properties such as increase availability of micronutrients and soil moisture. The swine manure were obtained from local farms, and the swine were dewormed prior to collection of manure samples. Prior to inoculation, fecal samples were tested through Formalin-Ether Concentration technique (FECT) for possible presence of *A. suum* eggs. Positive fecal samples were not used in the experiments.

**Treatments and Control**

The lactic acid bacteria composting set-up was prepared through inoculation with lactic acid bacteria serum (LABS) prepared at a proportion of distilled water and LABS at a ratio of 995:5 ml. Application of 200ml of LABS to each bed was done on alternate days until the end of the composting process. For the sundry-composting set-up, the manure used was sundried for one week, exposed under the sun from 07:00 until 17:00. For vermicomposting set-up, approximately 300g of *Eudrilus eugeniae* (African nightcrawlers) were inoculated into vermicomposting bins after stabilising the compost for 10 days. Vermicomposting took place for another 21 days to complete the process. This protocol has been practised and recommended by organic farms and vermicompost producers. While the control set-up constituted manure and chopped banana trunks only, regular watering (every other day) of all set-ups were done to keep a moist mixture but not too wet, except for lactic acid bacteria composting which were watered with LABS concoction.

The mean number of eggs inoculated for the various treatments was about 10,904 ± 974 *A. suum* eggs, isolated from female gravid *A. suum.* Adult *A. suum* worms were collected from intestines of infected pigs from a slaughterhouse. Female worms were dissected two centimetres from its posterior end to obtain the uteri (Nordin, Nyberg, & Vinnerås, 2009) and were macerated with 0.1% HCl (to prevent growth of molds). The washings were subsequently placed in a 13-ml vial. The eggs were counted by obtaining 0.1 ml from the stock which was examined to account for the number of eggs under a light microscope.

Temperature and pH readings were recorded daily from all set-ups. A thermometer was placed at least five inches into the bin for five minutes, while pH was obtained using a pH meter that was placed in a soil-water (2 g : 1ml) paste for two minutes (Paller & de Chavez, 2014). The composting process for all treatments lasted for 31 days.

***Ascaris suum* Concentration and Developmental Stage Determination**

**Stabilization Period (0-10$^{th}$day) Sample Processing.** Humus samples obtained on the 10$^{th}$ day were processed through FECT as the compost at that time was still predominantly swine manure and was not homogenous yet. Two samples were obtained from each replicate bin during the collection on the 10$^{th}$ day. One gram of the sample was mixed with at least 7 ml of 10% formalin, and sieved through a 3-layered surgical gauze into a

test tube. Three milliliters of diethyl ether were added into the test tube and then covered with an electrical tape before shaking vigorously for 10 seconds. Then, the tubes were centrifuged at 1,500 rpm for five minutes. Formalin and ether were decanted, leaving a sediment layer at the bottom. The sediment was pipetted from the tube, placed over a glass slide and covered with a coverslip, and examined under the light microscope at 100x and 400x magnification. The number and stage of eggs recovered were recorded.

**Composting Period (10th-31st day) Sample Processing.** The humus samples obtained on the 31st day were air dried for at least 24 hours. Dried samples were strained through a 125 $\mu$m sieve, and two grams of these were placed in a test tube. Six milliliters of distilled water were added into the tube and was vortexed thoroughly to mix the soil and water. Following that, the tubes were centrifuged at 1800 rpm for 10 minutes. The liquid was decanted and 8 ml of 1.2 gravity sucrose solution was added into the sediment. The tube was again vortexed and then centrifuged at 1800 rpm for 10 minutes. Using a 10-ml syringe, 1.3 gravity sucrose solution filled the tubes up to the brim. A coverslip was used to transfer the upper portion of the solution into the glass slide. The slides were labeled and viewed under a light microscope at 100x and 400x. The number and stage of eggs recovered were recorded.

The size of specimens were identified using a microscope camera (OptixCam, China) and its software, ToupeView. The following equations (as modified from Gnani Charitha, Rayulu, Kondaiah, & Srilatha, 2013) were also used in the present study:

$$\text{Proportional recovery} = \frac{(\text{no. eggs seeded} \times \text{sample mass})}{\text{batch mass}}$$

$$\text{Percent recovery} = \frac{\text{number of A. suum recovered}}{\text{Proportional recovery}} \times 100$$

### Statistical Analysis

All data were analysed in SPSS 20. Proportional recovery and percent recovery values were analysed using Shapiro-Wilk test to assess normality of sample sizes of less than 50. The parametric data gathered were subjected to One-Way ANOVA at $\alpha$= 0.05 following Tukey's Post-Hoc test for multivariate comparisons.

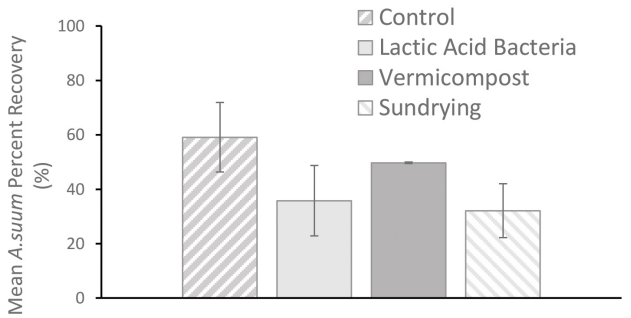
## RESULTS AND DISCUSSION

### Percent Recovery Analysis

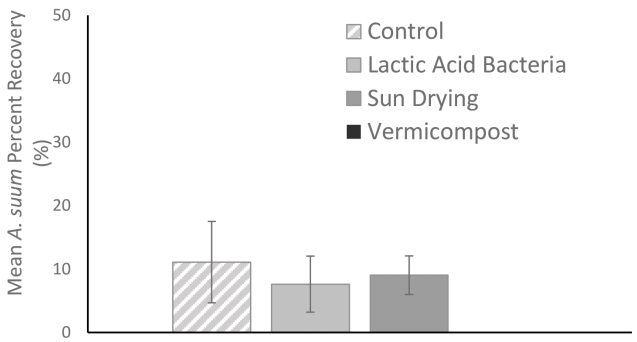
**Stabilization Period (0-10th day) Recovery.** As shown in Figure 1.0A, the control set-up (composting) showed the highest mean percent recovery (59.13%±12.79%), followed by vermicomposting (49.73%±0.28%), lactic acid bacteria composting (35.80%±12.95%), and sundry-composting (32.11%±9.89%). However, statistical analysis revealed that there were no significant differences between the control and treatment groups. All set-ups at this

stage underwent initial composting and thus may not show significant differences on percent recovery.

**Composting Period (10th-31st day) Recovery.** From the samples obtained on the 31st day, the control set-up exhibited the highest percent recovery (11.09%±6.40%). However, this time, it was followed by the sundry-composting (9.03%±3.04%) and the lactic acid bacteria composting (7.62%±4.41%). There were no eggs recovered from the vermicomposting set-ups (Figure 1.0B). Statistical analysis showed no significant difference between the control and treatment groups. However, the percent recovery of all four set-ups exhibited a mean reduction rate of 93.07% in *A. suum* concentration. Related studies by Bowman, Liotta, McIntosh, and Lucio-Forster (2006) and Hill, Lalander, and Baldwin (2013) also reported that despite the control set-up having higher percent recovery than the vermicomposting set-up, statistical differences were not significant enough to show that vermicomposting could reduce the concentration of *A. suum* egg and other pathogens in soil.




(a)




(b)

*Figure 1*. Mean *A. suum* percent recovery of various composting methods on: (a) 10th day, and (b) 31st day
N=24; 6 replicates each treatment (T bars represent standard deviations)

In contrast, the study for the formulation of USEPA guidelines for composting (Eastman et al., 2001), *A. suum* concentration experienced a reduction of 98.87% through vermicomposting, and 74.24% through composting alone, in 144 hours. Hait and Tare (2011) also presented a similar conclusion in their study where *A. suum* concentrations gradually decreased throughout the composting process (144 days), and were not detectable after vermicomposting. On the other hand, a study by Sypula, Paluszak, Ligocka, and Skowron (2013) revealed that sun-drying during the spring (average 18°C) did not reduce and inactivate *A. suum* eggs, and could therefore pose as health hazard if applied to crops as fertiliser. In the present study, average temperature observed in all set-ups was 27.87 °C±0.94°C, higher than those reported in the studies mentioned above.

**Viability Determination**

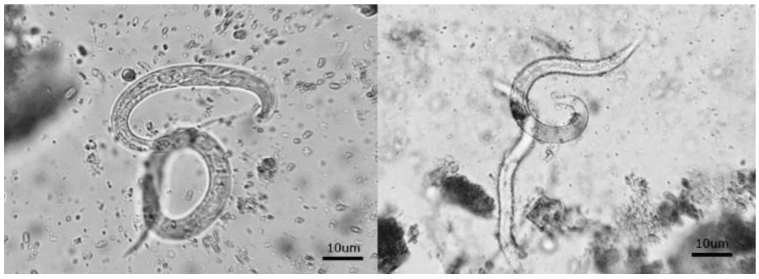
**Viability of Recoveries from the Stabilisation Period (0-10$^{th}$ day).** The presence of embryonated eggs is an indication of development to an infective stage. However, in the study, no intact embryonated eggs were recovered, instead the eggs hatched into larvae. Fertilised eggs were also recovered from the samples. During the 10$^{th}$ day (Table 1.0A), the control (composting), lactic acid bacteria composting, and sundried compost recovered 50% fertilised eggs and 50% hatched larva, while the vermicomposting set-up contained 100% hatched larva. Based on these data, the fertilised eggs arrested their development while the hatched larva were embryonated eggs that hatched from their shells. Nevertheless, the presence of fertilised eggs that could develop into infective embryonated eggs could still be a potential threat of viable eggs contaminating the organic fertilisers. Jones, Crewe, and Owen (1979) mentioned that helminth eggs ingested by earthworms could experience mechanical damage within the earthworm gut and this could trigger hatching into larvae. He further suggested that hatching could also be triggered by mechanical damage that could be inflicted by fungi and microbes during the composting process. Nevertheless, the *A. suum* eggs ingested by African nightcrawlers in the present study exhibited mechanical damage on its shell. The tendency to be subjected to mechanical damage while in the earthworm gut can also be influenced by the fact that the egg is corticated or not.

**Viability of Recoveries from the Composting Period (10$^{th}$ – 31$^{st}$ day).** On the 31$^{st}$ day (Table 1.0), egg recoveries from the control group (composting) and lactic acid bacteria composting comprised 100% hatched larva while the sundried set-up maintained its 50% fertilised eggs and 50% hatched larva recovery composition (Figure 2.0). There were no *A. suum* recovered from the vermicomposting set-up. The only set-up that had a modified environment compared to the 10$^{th}$ day was the vermicomposting set-up, where 300 g of *Eudrilus euginea* (African nightcrawlers) were inoculated.

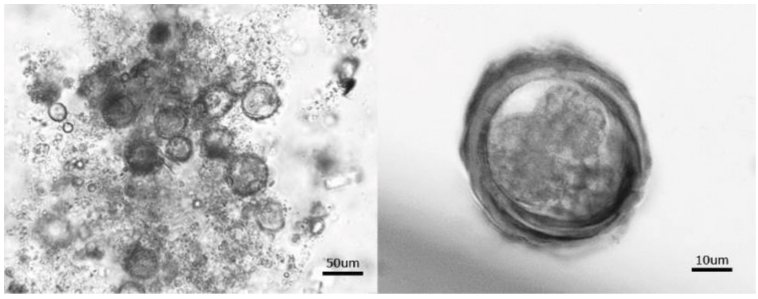
Table 1
*Number (n) and percent recovery (%) of A. Suum showing the proportion of developmental stages during the 10th and 31st day of composting process (n=24)*

| *Set-up* | *Fertilised egg n±SD (%)* | *Embryonated egg n (%)* | *Hatched larvae n±SD (%)* |
|---|---|---|---|
| *10th day* | | | |
| Control | 3715.25 ± 803.63 (50) | 0 | 3715.25 ± 803.63 (50) |
| Lactic acid bacteria | 2249.40 ± 813.68 (50) | 0 | 2249.40 ± 813.68 (50) |
| Sun drying | 2017.55 ± 621.41 (50) | 0 | 2017.55 ± 621.41 (50) |
| Vermicomposting | 0 | 0 | 6249.31 ± 35.19 (100) |
| *31st day* | | | |
| Control | 0 | 0 | 1393.62 ± 804.25 (100) |
| Lactic acid bacteria | 0 | 0 | 957.56 ± 554.181 (100) |
| Sun drying | 1134.75 ± 191.01 (50) | 0 | 1134.75 ± 191.01 (50) |
| Vermicomposting | 0 | 0 | 0 |

Note that no (0) embryonated eggs were recovered.




(a) (b)




(c) (d)

*Figure 2*. (a) and (b) *A. suum* larva recovered from the composting set-up on the 10th day (400x); (c) fertilized eggs recovered on the 10th day (200x); (d) fertilized egg recovered on the 31st day (400x)

In a study by Katakam, Thamsborg, Kyvsgaard, Dalsgaard and Mejer (2014), incubation upon recovery proved to add more information regarding the developmental state of the egg. They reported that fertilised eggs may remain in this stage for as long as six months in the soil without development but could resume development to embryonated stage (infective stage) at room temperature (26°C). This information suggests that fertilised eggs accounted in the present study cannot be directly considered as inviable as these may still resume embryonation. On the other hand, *A. suum* larvae that have hatched in the soil are no longer viable. *A. suum* egg shells are one of the most resistant from the helminths. Typical helminth egg shells would only consist of three layers: a vitelline layer, a middle chitinous layer and an inner lipid layer, while an *A. suum* egg shell has a total of five layers with additional uterine layers over the three basic layers (Perry & Wharton, 2011)

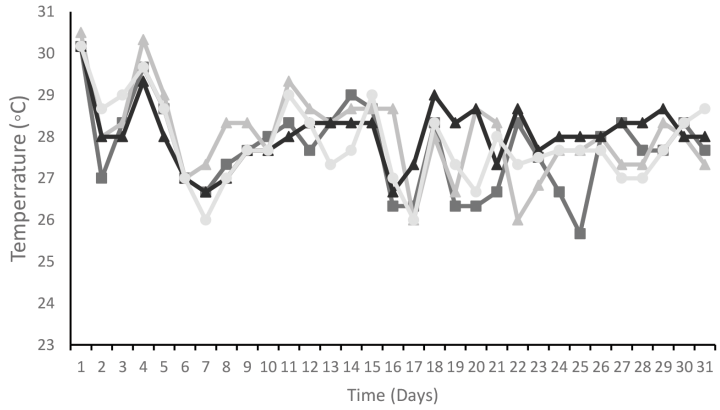
**Physicochemical Factors Analysis**

**Temperature during the Stabilization Period (0-10$^{th}$ day).** As shown in Figure 3.0A, none of the set-ups exhibited a drastic increase of temperature (mean=27.87°C±0.94°C), nor reached the minimum *Ascari*s inactivation temperature of 45°C (Kone et al., 2007). This temperature is expected to reach on the 14$^{th}$ day of composting (Goyal, Dhull, & Kapoor, 2005) in large-scale organic composts which are usually covered. This allows carbon dioxide to build up, thus, the high temperature. However, the temperatures exhibited by the set-ups, with minimum of 25°C and a maximum of 30.50°C, were ideal for the *E. euginea* (African nightcrawlers) development and productivity. Similarly, Viljoen and Reinecke (1992) reported that *E. eugeniae* were most maturing, developing, and productive in temperatures 22°C to 29°C, and that temperatures beyond 30°C could be detrimental for them.

Bacterial pathogens and coliforms could be eliminated after several weeks of composting because of thermophilic changes that occur throughout the process. However, helminths are considered the most resistant (Davies, 2011). Since temperature plays a crucial role in composting, there are varying accounts as to the temperature and duration that helminth ova can be inactivated. Haug (1993) states that 55°C to 60°C for one or two days is enough to deactivate enteric bacteria, virus and helminth ova while Kone et al. (2007) suggested that 60+/-30 days is considered the optimum composting period to reduce all (90-100%) helminth eggs at 45°C.

Meanwhile, Ratasuk, Boonsaner, and Hawker (2012) reported that swine manure is sun dried in South East Asian countries as a low cost pre-application treatment. *Ascaris* ova are considered the most ultraviolet-resistant waterborne pathogen as reported by Brownwell and Nelson (2006). Decorticated *Ascaris* eggs were inactivated by 98.4% upon exposure to UV radiation indicating that their shells were truly resistant to UV exposure.

**pH During the Vermicomposting Period (10th – 31st day).** In the same way as the temperature, the pH (Figure 3.0B) did not exhibit significant changes (mean=7.15±0.61) except for the sudden pH drop on Day 2. This result supports McKinley, Parzen, and Guzmán (2012) who have stated that low pH should be expected in the early stages of the composting period and is primarily accounted to *Lactobacilli,* a microbe which is normally found in composting ecology that induces acidic environment and high temperature. Close to neutral readings were observed in the vermicompost, which sets a favourable condition for the worms. Acidic environment could be detrimental for the worms (Cekmecelioglu, Demirci, Graves, & Davitt, 2005) while alkaline soil pH hinders plant development (Valdez-Perez, Fernandez-Luqueno, Franco-Hernandez, Flores-Cotera, & Dendooven, 2011). Available studies relate nitrogen from increased ammonia concentration to low pH (4.60±0.01) (Hill et al., 2013).

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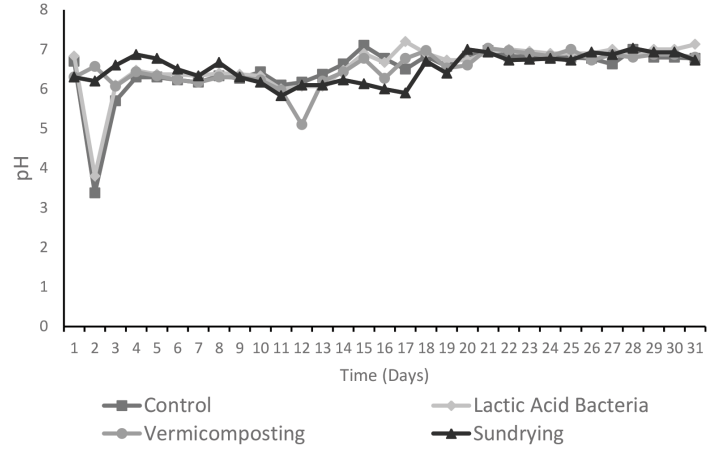


(b)

*Figure 3*. (a) Daily temperature (°c) and (b) ph readings of the different composting methods during the 31-day observation period

## CONCLUSION

The effect of various composting methods (vermicomposting, composting with lactic acid bacteria, sun dry-composting) on the reduction of *A. suum* eggs was insignificant ($p>0.05$) compared with the control group. However, the study revealed that there was a 93.07% mean reduction of *A. suum* eggs observed in control and treatment groups, revealing that composting process is an effective way to reduce the number of parasite eggs in organic fertilisers. No embryonated eggs were recovered from the 10th and the 31st day of composting; fertilised eggs and hatched larvae were recovered instead. The development of the eggs from fertilised to embryonated stage could still occur and thus could still pose a threat as potential source of contamination. The longevity of infective *A. suum* eggs represents an important public health issue because of the widespread use of pig manure as a fertiliser. This study used *A. suum* as the model pathogen but there could be other parasite eggs that may be present in animal manure used in composting, although some reports have demonstrated that *A. suum* infects humans frequently. Thus, when animal waste is reused in agriculture, measures should be taken to ensure inactivation of pathogens. This study emphasises the importance of good farming practices to reduce the risk of harmful parasite eggs for food safety.

## ABBREVIATIONS

ANOVA – Analysis of variance
ATI-DA – Agricultural Training Institute – Department of Agriculture
FECT – Formalin-ether concentration technique
LABS – Lactic acid bacteria serum
STH – Soil-transmitted helminths
USEPA – United States Environmental Protection Agency
WHO – World Health Organisation

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