Effects of Vermicomposting on Microbiological Flora of Infected Biomedical Waste

Umesh B Mathur*, Lalji K Verma**, Jitendra N Srivastava***
*Principal Medical Officer, HQ Central Air Command, Indian Air Force, & Former Senior Adviser & Professor (HOD) Department of Pathology, Air Force Hospital, Bangalore - PI of the project.
**Former Director General Medical Services Indian Air Force - Progenitor of the Concept/Hypothesis.
***Former Deputy Director Medical Services Indian Air Force Co-worker in the Project.

ABSTRACT

As an offshoot of WHO aided pilot project on hospital waste management in Bangalore, India, composting of bio-degradable part of infected hospital waste using earthworms (Eisenia fetida and Eudrilus eugeniae) was conceived and carried out in Command Hospital (CHAFB). The aim was to study whether vermicomposting was an effective method for rendering infected waste to non-infective, the end product being eco-friendly as well and to assess the efficacy of vermicomposting. Results are suggestive of prospects of use of Vermicomposting to convert infected biodegradable waste containing various pathogenic microorganisms to an innocuous waste containing commensals like citrobactor frundii and aerobic spore bearing microorganism usually found in the soil all over. The paper highlights detailed methodology of a systematic attempt towards examining a hitherto less exposed option.

Keywords: Vermi-Composting, Biomedical Waste, Microbiological Flora

Hospital wastes are hazardous and need to be disinfected before disposal. One out of eleven WHO aided pilot projects on Hospital Waste Management in India during biennium, 1999-2000 was allotted to the Command Hospital, Air Force, Bangalore (CHAFB). Project commenced at CHAFB on 10 Jan 1999 as Air Mshl (then AVM) Lalji K Verma as principal investigator to lay down a proper hospital waste management system (Verma and Srivastava, 2000). As an offshoot of this project composting of bio-degradable part of infected hospital waste using earthworms (Eisenia fetida and Eudrilus eugeniae) was conceived and carried out. It was conceived that bio-degradable component of the waste could be disinfected with process of vermicomposting without causing any adverse impact on the environment & human health. Vermi composting as an effective method to treat bio-degradable municipal or household waste is an age old proven methodology. The same concept was applied for treating infected bio-degradable component of hospital waste.

Vermicomposting refers to production of compost by growing/breeding earthworms as these worms in the process of feeding on waste cause biooxidation by relentless turning, fragmentation and aeration of waste by devouring resulting in homogeneous and stabilized humus like product which is an ideal nutrient for plants thus used as manure. Vermicomposting of biodegradable Municipal Solid Waste & household waste is in vogue in many places and instances but there is no available literature on use of vermicomposting technique for treatment and disposal of infected bio medical waste. The aim was to see whether vermicomposting was an effective method for rendering infected waste to non-infective, the end product being eco-friendly as well. To assess the efficacy of vermicomposting in this regard microbiological cultures (aerobic cultures) of vermicompost were carried out.
MATERIAL AND METHODS

Preliminary studies were carried out from vermin-composting beds, each measuring 3.5ft \times 3.5ft \times 1ft., lined by a single layer of earthen tiles made on a cemented floor in a few rooms having asbestos sheets ceiling. Twelve such beds were used for studies. These beds were charged with infected biodegradable waste comprising of cotton/gauze pieces soiled with blood, pus and body fluids etc. In many cases it would have been admixed with antiseptics/antibiotics used for dressing of wounds etc. It was covered with dry leaves after watering and admixing with an adequate number of earthworms (Eisenia fetida and Eudrilus eugeniae). They were left undisturbed for vermicomposting and compost thus formed later was subjected to aerobic microbiological cultures at periodic intervals till full composting took place (Photograph I).

<table>
<thead>
<tr>
<th>Vermicomposting Beds</th>
<th>Microorganism isolated from vermicompost of different duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bed No I</td>
<td>Esch coli, Staph aureus, Aerobic spore, Citrobacter sp Aerobic spore, Citrobacter sp Aerobic spore, Citrobacter sp Aerobic spore</td>
</tr>
<tr>
<td>Bed No II</td>
<td>Esch coli, Staph aureus, Citrobacter sp, Citrobacter sp, Citrobacter sp, Citrobacter sp</td>
</tr>
<tr>
<td>Bed No III</td>
<td>Aerobic spore, Aerobic spore, Esch coli, Proteus sp Aerobic spore, Citrobacter sp Aerobic spore, Citrobacter sp Aerobic spore, Citrobacter sp Aerobic spore</td>
</tr>
<tr>
<td>Bed No IV</td>
<td>No microorganism Isolated, No microorganism, Citrobacter sp Aerobic spore, Citrobacter sp Aerobic spore, Citrobacter sp Aerobic spore, Citrobacter sp Aerobic spore</td>
</tr>
<tr>
<td>Bed No V</td>
<td>Staph aureus, Esch coli, Pseudomonas, Proteus, Esch coli, Citrobacter sp Aerobic spore, Citrobacter sp Aerobic spore, Citrobacter sp Aerobic spore</td>
</tr>
<tr>
<td>Bed No VI</td>
<td>Esch coli, Pseudomonas, Esch coli, Pseudomonas, Proteus, Pseudomonas, Citrobacter sp Aerobic spore, Citrobacter sp, Citrobacter sp</td>
</tr>
</tbody>
</table>

Table 1: Microorganisms isolated from vermicompost of different duration.

The details of microorganisms isolated from various vermicomposting beds (No I to VI) at different intervals is shown in Table 1.

Samples from beds VII to XII showed similar findings. Preliminary studies of initial samples
from different vermicomposting beds showed growth of different bacterial flora on aerobic culture. Microorganisms isolated were Esch coli and Staph aureus, Esch coli and aerobic spore bearing microorganism, Esch coli and Proteus sp, Pseudomonas sp along with aerobic spore bearing microorganism and Citrobacter sp while samples from some vermicomposting beds had an admixture of all these pathogens. In a few beds initially no microorganism was isolated and this could have been due to effects of antiseptics/antibiotics in the compost sample collected for culture. As the vermicomposting process advanced it was observed that mainly Citrobacter sp was isolated (except in a few cases where aerobic spore bearing microorganism and Citrobacter sp. were isolated) from later samples. It is interesting to note that even in beds where initially no microorganism was isolated, Citrobacter sp were isolated in later samples with continued vermicomposting process. As infected biodegradable waste was used for vermicomposting, initial cultures from immature compost showed isolation of Esch Coli, Staph aureus, Pseudomonas sp and Proteus sp. This was as expected as these are usual pathogens found in purulent material. Strains of Esch coli and related coliform bacteria are predominant among the aerobic commensals flora present in the gut of human beings and animals and are also widely distributed in the environment. All persons have a rich flora of Esch coli in the lower ileum and in the colon. Esch coli is incriminated as a pathogen outside the gut and particularly in the urinary tract and in wounds where the infection may be endogenous or acquired from exogenous source. It grows in human beings and animals mainly in intestine, and excreted in very large number in faeces. It can contaminate the environment, including the soil widely, and bacilli may survive without growth for several days to a few weeks outside the body. When it is found in a water supply, it indicates that the supply has recently been subjected to contamination with human or animal faeces.

Following vermin-composting, it was observed that instead of Esch coli, Citrobacter frundii and aerobic spore bearing microorganisms were isolated. Pathogens such as Staph aureus, Proteus and Pseudomonas sp were not isolated from the samples. Citrobacter frundii was isolated on repeated cultures after vermicomposting irrespective of duration of vermicomposting, from 3 weeks to 18 weeks. Incidentally Citrobacter is one of the coliform bacteria and it commonly grows in soil, vegetation, and water outside the body. Aerobic spore bearing microorganisms were also isolated in the compost. These organisms are usually found in atmosphere and are non-pathogens.

Findings as described above during the preliminary studies required further studies. It was therefore decided to conduct the study under strict controlled environmental conditions for drawing any conclusion. It was necessary that cultures were done on samples of vermicomposting which has been processed under controls.

A research project was undertaken with an aim to find out the effect of vermicomposting on microbiological flora of infected biodegradable biomedical waste by carrying out microbiological cultures (aerobic cultures) of infected biomedical waste at the beginning of vermicomposting process, and at intervals of three weeks till maturity of compost. The protocol adopted for microbiological cultures (aerobic cultures) in this study were strictly followed to avoid variations due to extraneous physical factors. Since the preliminary studies revealed growth of Citrobacter bacteria on the culture of vermicompost, special care was taken to avoid contamination of vermicompost sites with refuse and dust generated at time of cleaning and sweeping. Besides material used for making bed for earthworms, dried leaves and water used for vermicomposting throughout was checked for coliform microorganisms to ensure that it is free of all microorganisms.
To prevent contamination of beds vermicompost of infected biodegradable waste was carried out in fabricated metallic vermicomposting containers, each measuring 3 ft x 1.5 ft x 1.5 ft having a sliding lid duly perforated for watering and dissipation of heat generated during the composting. Besides, the containers had a few small perforations, each measuring 3 mm in diameter at the base and sides to prevent accumulation of toxic nitrogenous waste but small enough to prevent entry of rodents/reptiles. These containers were labeled and kept on metallic stands. Legs of the stand were kept inside bowls containing water duly covered with a layer of kerosene oil so as to prevent entry of crawling insects into the vermicomposting containers. Three containers were prepared as follows:

1. Test Container
2. Positive Control Container
3. Negative Control Container

Each container was duly prepared after coating and maturation of earthworm beds (made up of coconut husk & shredded papers weighing approximately 14 Kg). The Test container was thereafter charged with 6.5 kg of infected biodegradable waste generated in various wards/departments, comprising of waste soiled with blood, pus and body fluids, and admixed with 750 gm of earthworms (Eisinia foetida and Eudrilus eugeniae) for composting. The Positive Control Container was charged with 6.5 Kg of waste prepared in biological laboratory containing biodegradable waste prepared by soaking cotton/gauze swabs with blood containing microbes in large numbers viz. Esch coli, Proteus vulgaris, Staph aureus, Pseudomonas pyocyaneae and aerobic spore bearing microorganism initially by repeated cultures till nine weeks of vermicomposting. Later tests revealed that above mentioned pathogenic microorganisms were not isolated and Citrobactor frundii and aerobic spore bearing microbes only were isolated. Microbiological cultures of compost generated in container used as a positive control revealed the growth of Staph aureus up to three weeks of vermicomposting while Esch coli and Pseudomonas pyocyaneae could be isolated up to nine weeks and twelve weeks respectively. From ninth week Citrobactor frundii and aerobic spore bearing microorganisms only were isolated. Microbiological cultures of compost generated in container used as a positive control revealed the growth of Staph aureus up to three weeks of vermicomposting while Esch coli and Pseudomonas pyocyaneae could be isolated up to nine weeks and twelve weeks respectively. From ninth week Citrobactor frundii and aerobic spore bearing microbes were also isolated but beyond twelve weeks, Citrobacter frundii and aerobic spore bearing microbes only were isolated. Examination of infected biodegradable waste charged in container used as negative control revealed no composting of waste in the absence of earthworms till eighteen weeks of study. The waste appeared decayed but still discernible, as cotton
fibers could be identified. Microbiological cultures of specimens from negative control vermicomposting container revealed growth of a wide range of pathogenic microorganisms viz. Esch coli, Staph aureus, Klebsiella aerogenes, Proteus vulgaris and Pseudomonas pyocyaneae till eighteen weeks of study. Aerobic spore bearing microorganisms could also be isolated after nine weeks of study. Citrobacter frundii was not isolated from this container on repeated cultures.

Appearances of Citrobacter frundii in compost after vermicomposting led us to dissect the earthworms (Eisiniia fetida and Eudrilus eugeniae) and to carry out microbiological cultures from its alimentary canal. It revealed pure growth of Citrobacter frundii on repeated cultures form different earthworms. The results obtained indicate that:

- There is a marked decrease in the net content of waste after vermicomposting and the total content in test as well as in positive control container decreased from 20.5 kg to 4.5 kg each with the formation of compost that could be used as plant nutrient.

- The pathogenic microorganisms present in infected biodegradable waste initially viz. Esch coli, Staph aureus, Proteus vulgaris and Pseudomonas pyocyaneae were not isolated from the compost after twelve weeks of vermicomposting. However these pathogens were replaced by commensals like Citrobacter frundii and aerobic spore bearing microorganisms.

- Citrobacter frundii was isolated repeatedly from the alimentary canal of the earthworms used for vermicomposting.

- Earthworms used for composting hasten the process of composting. The earthworms in each container (test & positive control containers) increased from 750 gms to 1.250 kg.

- There was no appreciable change in microbiological content or weight in the

<table>
<thead>
<tr>
<th>Vermicomposting Containers</th>
<th>Microorganism Isolated from vermicompost of different duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Container</td>
<td>3 Wks 6 Wks 9 Wks 12 Wks 15 Wks 18 Wks</td>
</tr>
<tr>
<td>Positive Control container</td>
<td>Esch coli Proteus vulgaris Staph aureus</td>
</tr>
<tr>
<td>Negative Control container</td>
<td>Esch coli Staph aureus Pseudomonas pyocyaneae</td>
</tr>
</tbody>
</table>

Similar findings were obtained by repeated cultures carried out later on.

Table - II
negative container.

DISCUSSION

Dominguez et al (1997) studied the similarities and differences in composting systems that can be operated in open or in vessel system with or without worms. They found that organic waste can be broken down and fragmented rapidly by earthworms, resulting in a stable nontoxic material with good structure. Their preliminary research showed that vermicomposting involves great reduction in populations of pathogenic microorganisms. After 60 days of vermicomposting, fecal coliform bacteria in bio-solids dropped from 39000 MPN/g to 0 MPN/g. In that same period, salmonella sp. dropped from <3 MPN/g to <1 MPN/g. They stated that as an aerobic process, composting lead to a nitrogen mineralization and the use of earthworms in vermicomposting increases and accelerate this nitrogen mineralization rate. The humidification processes that take place during the maturation stage of composting are greater and faster during vermicomposting. It also decreases heavy metals and the final product may contain hormone like compounds that accelerate plant growth. Our research project revealed that human pathogens did not survive vermicomposting and microorganisms like Staph aureus, Proteus vulgaris, Pseudomonas pyocyanaeae and Esch coli were not isolated after twelve weeks of vermicomposting. However Citrobacter frundii and aerobic spore bearing microorganism were isolated. Incidentally both are the commensals found in soil and the alimentary canal of earthworms. Besides, it established that earthworms not only hasten the process of composting but also activate composting and helps reducing the net content of infected biomedical waste remarkably with the resultant manure formation that could be used as a plant nutrient thus economically beneficial for the organization.

CONCLUSION

The importance of biomedical waste management by safe, cheap and easy methods need no further emphasis. Hospital wastes are hazardous thus before it leaves the hospital it should be safe for humans, animals and environment. Composting as one of the simple, cheap and easy methods of management of animal organic wastes, and has been in vogue since time immemorial. This is still a common method of generating manure all over the world. Vermicomposting is more efficient method of management of waste where in biooxidation and stabilization of organic matter is carried out with the help of a breed of earthworms (Eisenia fetida and Eudrilus eugenia) which are sturdy, have tremendous appetite and reproduce at a fast rate. It has been found that besides resulting in marked decrease in the net content of waste, vermicomposting converts the infected biodegradable waste containing various pathogenic microorganisms to an innocuous waste containing commensals like citrobactor frundii and aerobic spore bearing microorganism usually found in the soil all over.

REFERENCES


Waste that cannot be incinerated

Chlorinated plastics, volatile toxic wastes such as mercury.

Patient-contaminated plastics, non-plastics and infectious laboratory wastes may be treated by steam sterilization in autoclavable bags or microwave treatment. Shredding should follow both these methods. In case of non-availability of the above, chemical treatment with 1% hypochlorite or a similar disinfectant is recommended. However, excessive use of chemical disinfectants may be a health and environmental hazard.

Source: WHO Website