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Existance of Microbial Species in Vermicomposts Derived from Mixed Sesame Crust and Cow Manure Treatments

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A R T I C L E I N F O

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ABSTRACT

Introduction: The presence of pathogenic microbial agents and pathogens in organic fertilizers causes health problems and disease transmission.Therefore , the aim of this study was to identify bacterial and fungal species present in vermicompost production.

Materials and Methods: This experimental study was conducted in pilot scale in the laboratory of Public Health School in Shahid Sadoughi Univerity of Yazd. Sesame crust obtained from sesame pudding factory and cow manure mixed in three reactors with the dimension of 50 × 30 × 15 cm were used and went under the vermicompost process. Another reactor was also provided from cow manure as the control variable. Treatments were studied simultaneously during 60 days. Experiments were conducted to detect bacterial and fungal species.

Results: Totally 18 species of negative-gram bacterial species, i.e., *Salmonella typhimurium, Salmonella Paratayfi A, Acinetobacter baumannii, Escherichia coli, Proteus mirabilis, Proteus vulgaris, Providencia alkali Fasyns, Klebsiella oxy-Toka, Ponomonya Klebsiella, Citrobacter frondii, Citrobacter Diorsus, Serratia Marsns, Hafnya Olovia, pseudomalle Burkholderia, Enterobacter Peinous, Enterobacter Anrogenious, Enterobacter de Solonos*, as well as *Neisseria polysakarya*, and 3 positive-gram bacterial species, i.e., *Bacillus subtilis, Bacillus cereus, Isteria monocytogenes grew*. Overally, a total of five fungi species; *Aspergillus flavus, Aspergillus niger, Cladosporium, Penicillium, yeasts*, and Unknown fungal species grew.

Conclusion: The results of this study showed that presence of the organism in vermicompost depends on various factors, such as the action of enzymes of gut earthworms, coelomic fluid secretion, as well as competition between different groups of microorganisms.

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Introduction

Today, solid waste management, is one of the most important environmental crisis that has emerged due to rapid population growth and urbanization [1]. In addition, global approach to recycling organic waste is aimed to achieve sustainable agriculture and an environment free of contaminants. It is also necessary for sustainable agricultural development and enrichment of wastes. The majority of urban waste substances, especially in our country, are putrescible organic materials. If these organic materials be separated from the waste material compounds and go under certain conditions of biodegradation, the final product called compost fertilizer, mixed fertilizer, compound fertilizer, or humus is produced.

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In other words, the process of turning solid waste into fertilizer is known as composting process [2]. Vermicomposting is a semi -aerobic (80% moisture) process which is done by certain species of worms, fungi, bacteria, and actinomycetes. This fertilizer is a mixture of materials obtained from the growth substrate of the worm that remains in the environment after excretion of wastes from worms' digestive systems. Therefore, this material is the collection of worm waste along with decomposed organic matters and worm's corpses that has great nutritional value for plants [3]. Since organic fertilizers are mainly obtained from solid residues and municipal wastes and contamination and different pathogenic factors are always possible to exist in compost's raw material and because of short comings in the manufacturing process and increase in temperature, this issue is crucial in terms of public health, control and ensure health and safety of the produced compost. Several reports have shown changes of pathogens in vermicompost production process. In a study, Grantina et al, tried to determine the microbiological quality and biological activity in commercial samples of vermicompost with different origins. Their results showed presence of fungal pathogens such as *Aspergillus Fumigatus* and other fungi of pathogen, as well as the growth of some fungi in vermicomposting. It showed the nessecity of routine experiments to investigate the microbiological quality and biological activity of organic compost and vermicompost [4]. Further, Kui Huang et al. in 2013 studied bacterial and fungal population trend in vermicompost production process from plant wastes. The results of this study represented that after 60 days, bacterial and fungal activity in vermicompost production process with presence of earthworm species of Eisenia foetida had significant reduction compared to the control variable which was without earthworms [5]. In another study, Baoyi Lv et al, investigated the microbial community in the process of composting and vermicomposting mixed with sewage sludge and cow manure. The results indicated that, bacterial diversity in the process of vermicomposting was higher than composting process [6]. Vermicompost also contains Anonymous bacteria in terms of the material compared to compost. Generally, the bacterial community during the composting process was very different from the vermicompost production process. Iria Villar et al, also conducted a study and investigated changes of microbial population in the process of compost preparation and vermicomposting from sewage sludge. The results showed that due to the earthworms *Eisenia foetida species*' activity in vermicomposting sewage sludge (within 112 days), the microbial population (Gram-positive bacteria, Gram-negative bacteria, and fungi) has decreased in comparison with compost [7].

C.Perungkottur et al, also invetigated bacterial diversity in compost and vermicompost of cotton wastes. Five bacterial species of *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Bacillus*, and *Proteus* were examined, colonies of which in vermicomposting sample was higher than that of composting. Among the five isolated bacteria species, three species of *enterobacter aerogenes* , *Klebsiella sp*, and *Pseudomonas sp* belong to Gram-negative bacteria, while *Bacillus sp* and *Proteus species* belong to the Gram-positive bacteria [8]. G.N.Emperor et al, in another study investigated population and microbial activity (bacteria, fungi, and actinomycetes) of vermicompost in different concentrations of waste of industrial tea with household wastes and cow manure. The results of this study indicated that microbial activity of vermicompost obtained from all treatments had increased significantly. Fungal, bacterial, and actinomycetes' population has increased significantly [9]. Consequently, fertilizers produced from vermicompost process because of their nutrients are very useful for improving soil structure but can cause disease in the presence of pathogens. The aim of this study was to identify bacterial and fungal species present in in vermicomposts derived from mixed sesame crust and cow manure.

Materials and Methods

This pilot-scale study was conducted in the laboratory of Public Health Shool, Shahid Sadooghi of University of Yazd. Sesame crust obtained from sesame pudding factory and cow manure mixed in three reactors with the dimension of 50 × 30 × 15 cm were used and went under vermicompost production. And it was placed under vermicompost production process. A control reactor was also provided from cow manure. Treatments were studied simultaneously during 60 days. Vermicompost production process took place in natural pH and ambient temperature. The required samples were harvested once a week as a combination from different parts of each mass to identify the fungal species present in treatments. Moreover, during this process, the natural moisture of masses and ambient temperature were set at about 60 to 80 % and 20 to 30 ° C, respectively since they are suitable ranges for earthworms living. Different ratios of treatment combinations are listed in the following table 1.

Table 1: Different ratios of treatments

Treatments Description	Treatments
Cow manure as the control group	C
One part of cow manure: one part of sesame pudding waste	1C : 1S
One part of cow manure: three parts of sesame pudding waste	1C : 3S
Three parts of cow manure: one part of sesame pudding waste	3C : 1S

After preparation of th culture medium, 10 grams of sample was poured into a 250 ml flask and 100 ml of saline solution added to it, and then shaked for 10 minutes with shaker . Next, 0.1 ml of each diluted sample was cultivated on agar cyber dextrose medium for fungus' growing and then placed in dark place . Growth of colony on agar cyber dextrose environment, depending on the fungi species varies between three days to a week.

Colonies were evaluated in terms of their appearance, including colonies' color, shape (velvet, wool shaped, fluffy, mucoid colonies), and the color of colonies' back. Tismond method was then used to confirm species' type [10].

Furthermore, 1 ml of each diluted sample was cultured on Nutrient agar media for bacterial growth. After 24 hours, nutrient agar medias were removed from the incubator and the grown bacteria on the media were counted by colony counter. Then, each bacterium was cultivated on blood agar and EMB under sterile conditions, placed in incubator for 24 hours, and finally colored to be identifiable [11, 12].

Results

Table 2 shows bacterial species which grew during the treatment process . Totally, 18 species of negative-gram bacterial species: *Salmonella typhimurium, Salmonella Paratayfy A, Acinetobacter baumannii, Escherichia coli, Proteus mirabilis, Proteus vulgaris, Providencia alkali Fasyns, Klebsiella oxy-Toka, Ponomonya Klebsiella, Citrobacter frondii, Citrobacter Diorsus, Serratia Marsns, Hafnya Olovia, pseudomalle Burkholderia, Enterobacter Peinous, Enterobacter Anrogenious, Enterobacter de Solonos, Neisseria polysakarya* and 3 of positive-gram bacterial species: *Bacillus subtilis, Bacillus cereus, isteria monocytogenes grew.*

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Table 2: Identified bacterial species

Bacterial Species	S.No
Salmonella typhi G- ve	1
Salmonella paratyphi A G- ve	2
Acinetobacter baumannii G- ve	3
Escherichia coli G- ve	4
Proteus mirabilis G- ve	5
Proteus vulgaris G- ve	6
Providencia palcalifaciens G- ve	7
Klebsiella oxytoca G- ve	8
Klebsiella pneumoniae G- ve	9
Citrobacter freundii G- ve	10
Citrobacter diversus G- ve	11
Serratia marcescens G- ve	12
Hafnia alvei G- ve	13
Burkholderia pseudomallei G- ve	14
Enterobacter pyrinus G- ve	15
Enterobacter aerogenes G- ve	16
Enterobacter dissolvens G- ve	17
Neisseria polysaccharea G- ve	18
Bacillus subtilis G- ve	19
Bacillus cereus G- ve	20
Listeria monocytogenes G- ve	21

Table 3 shows kinds of fungi that grew in treatments during the process . Totally, five fungi species of *Aspergillus flavus, Aspergillus niger, Cladosporium, Penicillium, yeasts* and a number of unknown species grew.

Table 3: Identified fungus species

Fungal Species	S.No
Aspergillus flavus	1
Aspergillus niger	2
Cladosporium	3
Penicillium	4
Yeast	5
Saprophyte	6

Discussion

G. N. Emperor et al., conducted a study regarding population and microbial activity(bacteria, fungi, and actinomycetes) of vermicompost at varying concentrations of household waste and industrial tea residuals with cow manure. The results showed that microbial activity of vermicompost from all treatments had an increasing trend. Bacterial population, fungal population, and the population of actinomycetes were significantly greater which is consistent with this study. [9] Microorganisms are the essential part of biodiversity and play an important role in ecosystem structure and function. Microorganisms are the first parser of organic waste products. They are not only make the organic waste products into appropriate forms available for mineral plants but also can synthesize all bioactive substances. Also, they are responsible for the decomposition of biochemical organic substances. Earthworms are the most important drivers in the process of vermicomposting . They provide inherent condition for microbial activity and change biological activity through decomposition of organic waste. During the process of vermicompost, when organic materials are passing through earthworm gut, they are affected by biochemical, chemical, and physical changes due to the combined effects of earthworms' intestinal and microbial activities.

Earthworms not only help the proliferation of microbes through accelerating physical decomposition of of organic substances, but also incite other aerobic microbial activities to facilitate further analysis. The increase in microbial population may be due to the growth of bacteria and fungi while passing through the gut of the earthworm. Generally , 6 fungal species, 18 Gram-negative bacterial species, and 3 gram-positive bacterial species were identified. Species identified in the above study are consistent with this research.

In a study conducted by C.Perungkottur Selvi et al. on bacterial diversity in compost and vermicompost from cotton waste, 5 bacterial species of *Enterobacter, Klebsiella, Pseudomonas, Bacillus,* and Perteus were identified, this result is consistent with the species identified in the current study [8]. Results of an other study carried out by Grantina to identify microbiological quality and biological activity in commercial vermicompost with different origins showed that there are fungal pathogens, such as *Aspergillus fumigatus* and other fungal pathogens in vermicompost that are consistent with the current study [4].

The results of the study conducted by Iria villar and colleagues in 2015 regarding the changes of microbial population in the process of preparing compost and vermicompost from sewage sludge, showed that, Gram-positive bacteria, Gram-negative bacteria, and fungi followed a decreasing trend. This was due to the activity of Andrei izynya earthworms, microbial population, and microbial community structure which depends on the presence or absence of earthworms as well as the applied treatment. Incorporation of earthworm accelerates decomposition and deacreases large amounts of carbon and phosphorus cycles' enzyme [7]. Microbial species available in various treatments are affected by passing through the earthworm gut. Reducing the number of pathogens in vermicompost production process depends on different factors like enzyme action of *Eisenia foetida* worms ,Coelomic fluid secretion which has antibacterial properties, as well as competition between different groups of microorganisms.

Earthworms have different intestinal microorganisms that increase organic matter by producing enzymes such as amylase, protease, lipase, and biodegradation cellulase [13], Earthworms participate in the decomposition process by feeding from some micro-organisms or coexisting with microorganisms . Results of this study are in the same line with those of the present study.

Kui Huang and colleagues studied bacterial and fungal population trend on vermicompost production process from plant waste. The results showed that bacterial and fungal activity decreased after 60 days in vermicompost production process in the presence of *Eisenia foetida* earthworm that is not consistent with the present study [5]. Earthworms, essentially reduce the activity of microbial biomass, but increase microbial diversity in composting products from the vegetable wastes. Results also showed that behavior and changes in microbial populations in the process of composting and vermicompost depend on the quantity and quality of the field, the coexistence of microorganisms, and hybrid structures. So, the population and microbial diversity will change significantly in the final product.

Conclusion

In studies conducted on decomposition of organic materials of various wastes during vermicompost production process, results of the process effect on microbial and microbial biomass communities were different so that an increase and a decrease were reported. In this regard, analysis of this experiment and the previouse ones reflect that microbial changes during vermicompost processe often depen on the type of waste and organic materials, microbial species involved in the process, the interactional effects between microorganisms with earthworms (symbiotic and antagonistic), environmental conditions, and the stage of curing vermicompost.

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Conflict of interest

We have no competing interests.

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