Advanced Studies in Biology, Vol. 8, 2016, no. 1, 39 - 52
HIKARI Ltd, www.m-hikari.com
http://dx.doi.org/10.12988/asb.2016.626

Design and Construction of Household Composter and Detection of Extended Spectrum β-lactamase Producing *Pseudomonas aeruginosa* and *Enterobacteriaceae* in Compost

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Abstract

Today, generation of waste is increasing and despite the advancement of science and technology, its management has changed into a complex problem. The aim of this study was to reduce solid waste from the source by designing and building home composter and also detect of extended spectrum β-lactamase (ESBL)-producing *Pseudomonas aeruginosa* and *Enterobacteriaceae* isolates in composting processes by phenotypic and molecular methods. AUTOCAD, Photoshop and 3ds MAX were used to model and design the components of device. Composter was built using galvanized iron sheet with the dimensions 35 × 36 × 32 cm and its defects were resolved step by step. The effect of produced compost was determined on the growth of selected plants. Gram negative bacteria were isolated from the materials undergoing composting, the leachate, and mature compost in two repetitions. Composting process took long 38 days.

Produced compost significantly increased the growth of cranesbill and wheat in comparison with the growth hormone and chemical fertilizer ($p<0.05$). The frequency of bacteria isolated in the initial waste was larger than in the maturity stage. Antibiotic resistance was low among isolates and none of them were ESBL-producer. Colony PCR revealed that all isolates harbored the *opr*L and *exoA* genes. The results showed that the built composter had a good efficiency and the produced compost could be used as a suitable alternative to chemical fertilizers.

Keywords: Household composter, Composting, *Pseudomonas aeruginosa*, Extended spectrum β-lactamase

Introduction

The population is increasing at an exponential rate leading to significant increases in waste generation (Phiri, 2012). Increasing of the volume produced wastes and diminished capacity of landfill sites along with the low advancement for the programs of converting waste to energy result in environmental pollution (Goldstein, 2001). In order to solve these problems, it is necessary to convert the waste to compost at the site of generation. In sustainable agricultural systems, application of biological fertilizers bears high significance in increasing production of crops and preserving sustainable fertility. With its properties, compost can lead to enhanced ion exchange, preservation of water, soil consistency, enzymatic activities such as phosphatases, microbial activities, maintaining the elements cycle, and provision of the plants essential elements (Doroudian *et al.,* 2006). Compost may harbor pathogens including bacteria, viruses, fungi, protozoa and helminths that are able to cause infections in humans, animals or plants (Deportes *et al.,* 1998). Most opportunistic and obligate pathogens are mesophilic bacteria and can be destroyed during the thermophilic phase of composting (Nakhaei Moghaddam, 2006).

Pseudomonas aeruginosa (PA) is an aerobic non-fermentative Gram-negative bacterium that has an intrinsic resistance to antimicrobial agents. Penicillin, cephalosporin, and monobactam have beta-lactam ring. Resistance to these agents can occur with the catalysis of this ring by beta-lactamases (Poole, 2011). This bacterium is an opportunistic pathogen, which can be associated with various diseases such as respiratory system infection, meningitis, ear and eye infections, skin and soft tissue infections, and nosocomial infections (Okkotsu *et al.,* 2014). *Enterobacteriaceae* family is a group of Gram-negative bacteria in human's intestine and is one of the most important causes of urinary tract infections, pneumonia, and sepsis. The major genera in the *Enterobacteriaceae* family include *Escherichia*, *Klebsiella*, and *Enterobacter* (Kram *et al.,* 2015). Little information is available about the fate of these bacteria in compost. Presence of these organisms in compost, their virulence and antibiotic resistance might be a concern for individuals (Edrington *et al.,* 2009).

Cereals are considered the basis of nutrition in human life, providing 70% of food of Earth's population. Cereals have the highest significance in human nutrition, among which wheat plays the most important role (Alimohammadi *et al.,* 2005).
The aim of this study was to develop a household composter as well as detection of *P. aeruginosa* and *Enterobacteriaceae* beta-lactamase producers in compost through phenotypic and molecular methods. Also the effect of produced compost was investigated on the growth of cranesbill as an ornamental apartment plant and wheat as a farming plant.

Materials and Methods

The outline of the household composter was evaluated considering the available information, followed by assessment of the composter overall schema and designing the accessories of device. To model and investigate the components of the device, AUTOCAD, Sketchup and 3ds MAX were used. The interior chamber (25×35 cm) and the external chamber (35×45 cm) were built out of galvanized iron sheets with a thickness of 2 mm. The bottom layer of the lid was built by a thickness of 2 mm, whose edges 1 cm wide were bent by 90 degrees downwards. The top later and the peripheral walls of the lid were fabricated out of Neopane wood with a thickness of 1 cm. In order to minimize the heat energy loss, glass wool heat insulator was used between the walls and also, between the bottom and top layers. To allow entrance of oxygen to the composter and outflow of carbon dioxide from the composter, a bifunctional air pump was employed. To measure the temperature of various stages of the composting process, a digital temperature was used. To mix the materials and circulate the air, a stirrer was designed and installed on the composter. This stirrer was fabricated out of an iron rebar with the 6 oars on it. A hole with a diameter of 1 cm was created in the center of the bottom base on the interior chamber for emission of produced leachate. A metal tube (internal diameter of 1 cm, length of 15 cm) was attached from one end to the external embouchure of the hole using a carpit welding, while the other end of the tube was conducted out of the lateral walls of the external chamber with a slope of 20 degrees in relation with the other end. To prevent blockage of this duct, several holes were made at certain intervals on a galvanized iron sheet with dimensions of 20×21 cm. Next, this sheet was attached to the lateral walls of the internal chamber 2 cm above the top of the bottom base of the internal chamber.

The composting process

The residuals of kitchen food such as vegetables, fruits, food remnants were collected and further placed by a height of 25 cm inside the composter chamber. The foods contained 35% nitrogenous and 65% carbon compounds. Thermal changes were controlled and recorded during composting process. The process was replicated three times, being terminated after 38 days once the temperature reached 25°C.

The effect of produced compost on the growth of plants

The experiments were done on 45 cranesbill plants (*Pelargonium zonale*) as an apartment plant and 45 wheat plants (*Triticum* spp. cultivar Alvand) as the farming plant. For the cranesbill plants, the experiment was done in the form of fully random design with three treatments (5 plants for each treatment) and three replications in the form of vase experiment with size of 14×14 cm. The treatments include the control (without compost and plant growth hormone), the produced compost (50% of the vase volume), and plant growth hormone (HB-101, Flora Co., Japan- 1 drop for each irrigation period). The preliminary shrubs for planting all were similar and 10 cm tall. For the wheat plants, the experiment was performed in a fully random design with three treatments (5 plants for each treatment) and three replications in the form of vase (16×20 cm) experiment. The treatments included the control, the produced compost (4 kg/m^2), and urea chemical fertilizer (solid and pure form of nitrogen, containing 59% urea, by Lordegan Co., Iran) (15 g/m^2). All of the compost and one third of the chemical fertilizer were well mixed with soil some days before the cultivation. The rest of the fertilizer was consumed during the stages of tillering, heading and stem elongation.

Collection and identification of the bacteria

Gram negative bacteria were isolated from the materials undergoing composting, the leachate, and mature compost from three thermal phase of the composting (initial mesophilic, thermophilic and final mesophilic phases) in two replications. The samples were inoculated on EMB (Eosin methylene blue) agar and Cetrimide agar by streak culture method. Cetrimide agar plates were placed in a 42°C incubator for isolation of PA (Brown *et al.*, 2012). The bacteria were then identified by using conventional biochemical tests (Tille, 2013).

Antibiotic susceptibility testing and phenotypic ESBL detection

Antibiotic susceptibility testing was done using agar disk diffusion by Kirby Bauer method (Bauer *et al.,* 1966). The antibiotic disks (Mast Co. in England) of meropenem (10 μg), gentamicin (10 μg), ticarcillin (75 μg), ciprofloxacin (5 μg), imipenem (10 μg), Kanamycin (30 μg), and tobramycin (10 μg) were used for PA. The antibiotic disks of co-trimoxazol (25 μg), amoxicillin (25 μg), tetracycline (30 μg), Amikacin (30 μg), and ceftazidime (30 μg) were used for *Enterobacteriaceae*. All experiments were repeated three times. Identification of the ESBL- producing isolates was conducted using double disk confirmatory test by ceftazidime/clavulanate (10 + 30 μg) combination disk and ceftazidime (30 μg) disk (Cormican *et al.,* 2001).

Detection of *oprL* and *exoA* genes with PCR colony

The presence of *oprL* (peptidoglycan-associated lipoprotein OprL) and *exoA* (exotoxin A) genes were detected for identification of PA (Xu *et al*., 2014). To detect these genes, PCR colony method and the specific primers according to Table 1 were used. The reaction mixture with the final volume of 25 μl was prepared

according to Table 2. PCR was carried out using Kyratec thermocycler (Korea) according to the thermal cycles mentioned in Table 1. *P. aeruginosa* ATCC 1074 was used as positive control. The products of the experiment were electrophoresed on 1.5% (w/v) gel agarose containing ethidium bromide for 45 min with a voltage of 90 V.

Table 1. Primers and thermal conditions for amplifying *oprL* and *exoA* genes by colony-PCR

Primers	Sequence (5´ to 3´)	Denaturation	Annealing	Size (bp) -Reference
oprL F*	ATGGAAATGCTGAAATTCGGC	94°C (1 min**)	60°C (1 min)	504- Xu *et al.*, 2014
oprL R*	CTTCTTCAGCTCGACGCGACG			
exo A F	GACAACGCCCTCAGCATCACCAGC	96°C (1 min)	55°C (1 min)	396- Xu *et al.*, 2014
exo A R	CGCTGGCCCATTCGCTCCAGCGCT			

*F: Forward, R: Reverse, **min: Minute

Statistical analysis

The statistical analysis of the data (standard deviation ± mean) was done using MSTATC software. Comparison of the means was carried out using LSD test and the P-value ≤ 0.05 was considered significant.

Results & discussion

The final and overall plan of the composter was modeled using Sketchup, AUTOCAD, and 3d max (2015) softwares, as shown in Fig. 1. After numerous changes in various components of the household composter, this device was built, as shown in Fig. 2.

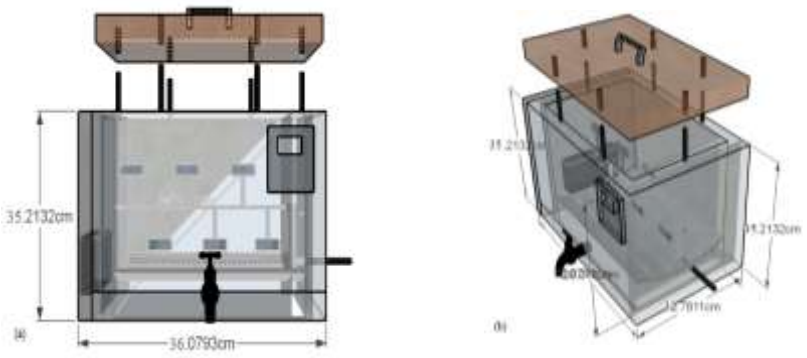

Figure 1- Design of household composter from two different angles by software

Figure 2- Household composter made in this study, (a) from the top, (b) the side view

It took 38 days from the time when the materials were mixed until they changed into mature compost. As seen in thermal diagram (Fig. 3), three phases for the compost process could be considered; initial cold phase around 11 days, thermophile phase of about 25 days, and final cold phase of approximately 2 days. Fig. 4 indicates a sample of mature compost produced by the built household composter. The mature compost was brown in color and had particles with a size of 0.5-1.5 cm. Growth of plants treated by the compost produced were significantly greater than those of the control ($p<0.05$).

By comparing the results (Table 3), it can be implied that the number of leaves and the height of cranesbill plants treated by produced compost were significantly greater than the ones treated with growth hormone ($p<0.05$). The height of plants, the number of leaves and spikes, and the number of grains in the spikes in plants treated by the produced compost was significantly more than control plants (Table 4) ($p<0.05$).

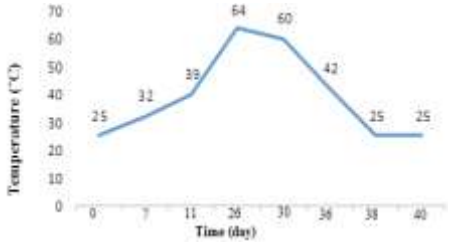

Figure 3- The average temperature changes in composting process in household composter made

Figure 4- Mature compost produced by household composter

Table 3. The mean (± SD) of plant height, number of stems and leaves in cranesbill plants treated by compost*

Plants	Plant height (cm)	Number of stems	Number of leaf
Control	25.00 ± 4.72^{e}	2.26 ± 6.23^{e}	16.00 ± 2.62^{e}
Treated with growth hormone	47.86 ± 0.15^{ab}	4.13 ± 4.21^{cd}	23.08 ± 4.84^{ab}
Treated with compost	57.02 ± 2.17^{cd}	5.20 ± 1.31^{bcd}	27.07 ± 3.54^{cd}

*Means, in each column, followed by at least one letter in common were not significantly different using LSD test.

Table 4. The mean (± SD) of studied traits in 3 group of wheat plants (controls, plants treated by compost, and plants treated by chemical fertilizer)*

Treatment	Plant height (cm)	Number of spike per plant	Number of grain per spike	1000-grain weight (gr)	Number of leaf per plant
Control	62.70 ± 0.97^{e}	2.92 ± 0.28^{d}	34.23 ± 2.86^{e}	38.89 ± 0.56^{g}	35.27 ± 0.19^{e}
Treated by fertilizer urea	70.32 ± 2.15^{cd}	3.48 ± 4.21^{cd}	38.42 ± 0.19^{de}	41.78 ± 0.47^{fg}	40.00 ± 0.35^{d}
Treated by compost	71.52 ± 1.20^{cd}	3.56 ± 1.67^{cd}	41.71 ± 3.24^{bcd}	43.11 ± 5.17^{efg}	40.61 ± 4.47^{cd}

*Means, in each column, followed by at least one letter in common were not significantly different using LSD test.

Bacteria were isolated from raw materials, materials undergoing composting (psychrophilic, thermophilic, and maturity phases) and leachate. Samples were taken from two repetitions of composting processes, so that 24 isolates of PA and 48 isolates of *Enterobacteriaceae* were collected. The number of bacteria was lowest during the maturity phase, possibly due to generation of heat in thermophilic phase resulting in lower microbial load. All of PA isolates were non-fermentative, mobile, oxidase and catalase positive and negative in indole, methyl red, Voges-Proskauer, H_2S, and urease tests. *Enterobacteriaceae* isolates were oxidase negative and catalase positive, and examined for other chemical properties according to reference tables. Antibiotic susceptibility tests showed that all PA isolates were sensitive to gentamicin, with the greatest resistance to kanamycin (Fig. 5). All isolates of *Enterobacteriaceae* were sensitive to co-trimoxazole, tetracycline, and amikacin, where the greatest resistance (27.08%) was observed to amoxicillin (Fig. 6). ESBL phenotypic tests indicated that all of isolates were not ESBL- producing. Colony PCR experiments for *oprL* and *exoA* genes showed that all isolates of PA had the same bands as positive controls (Figs. 7 and 8).

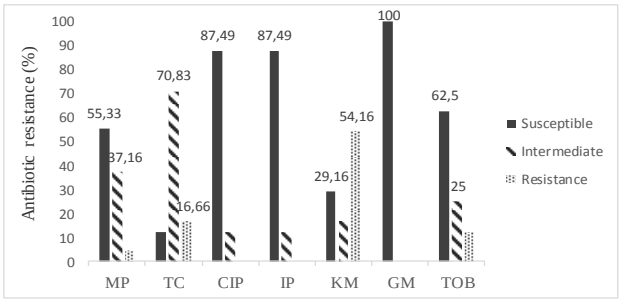

Figure 5- Antibiotic sensitivity pattern of 24 isolates of *P. aeruginosa* collected from composting process (MP: Meropenem, TC: Ticarcillin, CIP: Ciprofloxacin, IP: Imipenem, KM: Kanamycin, GM: Gentamicin, TOB: Tobramycin)

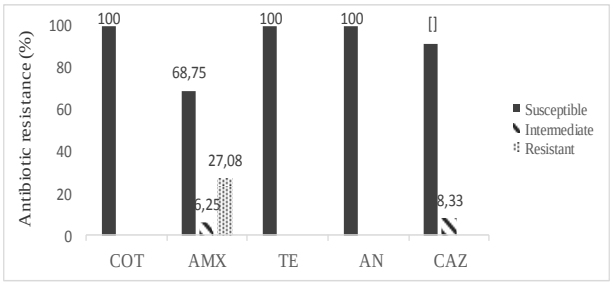

Figure 6- Antibiotic sensitivity pattern of 48 isolates of *Enterobacteriaceae* collected from composting process (COT: Co-trimoxazole, AMX: Amoxicillin, TE: Tetracycline, AN: Amikacin, CAZ: Ceftazidime)

Figure 7- Gel electrophoresis of colony PCR product of *oprL* gene in *P. aeruginosa* isolates (-: Negative control, +: Positive control, L: Standard marker, 1-11: Samples)

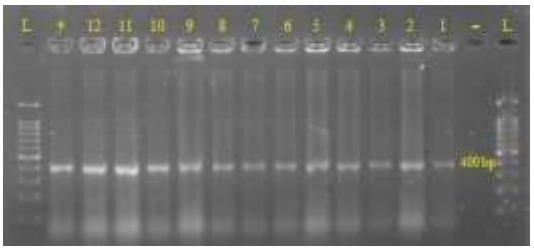

Figure 8- Gel electrophoresis of colony PCR product of *exoA* gene in *P. aeruginosa* isolates (-: Negative control, +: Positive control, L: Standard marker, 1-12: Samples)

Wastes such as vegetables, fruits, and foods account for an average of 70% of the total wastes of residential areas. By collecting and modification them via aerobic biological operations, these perishable compounds change into compost, a useful substance for farming and garden soil (Diaz *et al.,* 1993).

The present study was among the first attempts for modeling and building household composter in Iran, carried out with minimum facilities inexpensively. After solving the problems, developing of this sample of household composter was accomplished with dimensions of 35×36×32 cm with a cost of around 10 million Rials (295 USD). The American company, Nichermill, manufactures three models of household composters with dimensions of 50×30×50 cm. The Models Neo, Metro, and Ultra are sold as 249, 299, and 399 dollars, respectively (http://naturemill.net/products.html). The American company Denby, manufactures household composter in dimensions of 37×35×32 cm with a price of 213.99 dollars (http://wayfair.Com/Danby-2lb-Protable-Ice-Maker-DIM2500SSDB-DAN1236.html). Comparison of the results indicated that the manufacturing cost of the composter developed in this research is less than that of the similar samples in other countries. But comparison of the appearance and facilities of devices revealed that the composter developed in this research requires some changes in order to be built with a better appearance with more facilities. Mass production of this device might lower the costs. In this research, the time required for producing the mature compost took 38 days including 11 days in the mesophilic phase, 19 days in the thermophilic phase, and 8 days in the maturity phase, with the highest elevation of temperature being related to day 26 of 65°C. Papadopoulos *et al* designed and developed a type of non-rotating household composter. They achieved mature compost using food wastes within 35 days (Papadopoulos *et al.,* 2009), 3 days shorter than this research. Singh *et al*. designed and developed a rotational composter. They produced mature compost by mixing cattle manure, rice husk and as raw materials in 20 days (Singh *et al.,* 2012). It seems that the difference between the times of compost production can be related to the type and ratio of the raw materials. In any case, development of household composter can have a high significance to be used in houses or gardens in order to reduce waste volume (Nakhaei Moghaddam, 2006). In sustainable agricul-

tural systems, application of biological fertilizers would be valuable in increasing the production of crops and maintaining the fertility. Water contamination by discharging municipal wastes in the environment and flow of surface waters into different regions is resulted in dissemination of pollution. In order to solve these problems, it is necessary that we change wastes to compost at the site of production (Hoveidi, 2011).

Ensuring the quality and absence of contamination of obtained compost is essential Gram-negative bacteria are more present in the raw materials introduced to the device. Over time, during the stages of preparing the compost, their number declined, so that they were seldom isolated in mature compost. Beta-lactamases are the most important defense factor of Gram-negative bacteria against beta-lactam antibiotics. As PA and *Enterobacteriaceae* are found in food wastes (Abbasi *et al.,* 2012, Xu *et al.,* 2012), thus detecting and measuring their antibiotic sensitivity in composting process is crucial. The highest antibiotic resistance of the PA isolates belonged to kanamycin (54.16%) and ticarcillin (16.16%). None of the PA isolates were resistant to gentamicin, ciprofloxacin, and imipenem. The highest antibiotic resistance among the 48 isolates of *Enterobacteriaceae* was related to amoxicillin (27.08%). None of the isolates were resistant to amikacin, tetracycline, co-trimoxazol, and ceftazidime. In a study in 2011 on 25 isolates of PA isolated from the compost obtained from plant material in Hungary, none of the isolates were resistant to cefepime and fluoroquinolone. Resistance to ceftriaxone, cefotaxime, and ceftazidime was 48, 16, and 8%, respectively. The frequency of the isolates carrying *exoA*, similar to this study, was 100% (Kaszab *et al.,* 2011). In a study done in 1393 on 106 isolates of PA isolated from different clinical samples in the West of Iran, antibiotic resistances to meropenem, ciprofloxacin, and imipenem was 82.1, 91.5 and 84.9%, respectively (Alikhani *et al.,* 2014). In a study performed in 2014 on the isolates of *Enterobacteriaceae* isolated from treated wastewater in Portugal, antibiotic resistance to ceftazidime, co-trimoxazole, and tetracycline were 16.2, 21.1 and 18.2%, respectively (Amador *et al.,* 2015), where in comparison with this research the frequency of the antibiotic resistance was greater than all of the three antibiotics.

In this research, to investigate the quality of the produced compost, its effect was studied on the growth of 45 cranesbills and 45 wheat plants. The results suggested that the effect of the produced compost on the growth of both plants was greater than the chemical fertilizer ($p<0.05$). These results are in accordance with data reported by Demelash *et al.* (2014) and Saikia *et al.* (2015). Some studies, similar to this research, have showed that the effect of compost on the growth and other morphological properties of cranesbill plants have been significant (Borji *et al.,* 2014, Tullio *et al.,* 2012).

Conclusion

We were successful to build a household composter using inexpensive instruments and raw materials in this research. It seems that the device has a reasonable efficiency and the compost that was produced in it was characterized

by high quality. Results indicated to the reduction of Gram-negative bacteria in the mature compost, lower antibiotic resistance, and no ESBL- producers among isolates. The production and development of these composters can be a step towards decreasing the volume of wastes, producing valuable products from waste for gardens and houses.

Acknowledgements. This work was conducted in the department of Biology, Mashhad Branch, Islamic Azad University and the authors would like to express their gratitude to the authorities of this University.

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Received: March 11, 2016; Published: May 15, 2016