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ROLE OF MICROORGAINSMS AND THEIR ENZYMES IN COMPOSTING PROCESS FROM PLANT RESIDUES

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ABSTRACT

Newly reclaimed sandy soils are considered one of the main items of agricultural expanding policy in Egypt. Therefore we need to continuous addition of organic matter to increase soil fertility and agricultural production. For this reason, eight heaps were prepared each heap involves four plant residues. One set of four heaps was supplemented with organic manure. Another set was supplemented with cellulose decomposers. Some physical, chemical and microbiological properties were determined during composting process. The important obtained results are as follows: Temperature inside the compost heaps began to rise from the third day of composting and reached maximum values at the third week of the process. From the fourth week there was a gradual decrease in temperature till the end of composting. The pH values of the eight heaps tended slightly to acidic at early intervals of composting process till the 20th day, then the pH values increased to reach neutral or slightly alkaline by the end of the process. The Gradual increases in EC and bulk density values during the process. The obtained results showed a gradual decrease in organic carbon and organic matter by time and increase in total-N of all composted heaps during the different intervals of composting process. The results showed remarkable decrease in C/N ratio with the progress of the composting process.

At the beginning of the composting, total number of mesophilic bacteria was increased, however it was markedly decreased in the third week of composting. Then it was highly increased at the end of composting. The same pattern of the results was also observed with mesophilic fungi and actinomycetes. On the other hand, thermophilic bacteria were sharply increased at the third week and then obviously decreased at the end of the composting process (cooling phase). The same trend was also observed with thermophilic fungi and actinomycetes. It has been also observed that no marked differences were obtained in the total microbial counts of different compost heaps for both mesophilic and thermophilic organisms.

In concerning the enzymes activities of common selected fungal, bacterial and actinomycets strains the obtained results were: In regarding to Cellulase enzyme activity, it was obvious that *Trichoderma reesei*, *Bacillus stearothermophilius* and *Streptomyces antibioticus* recorded the highest activity in culture filtrates. In regarding to dehydrogenase enzyme activity, *Trichoderma reesei*, *Bacillus subtilis* and *Streptomyces roseus* exhibited the highest activity. In case of protease activity and also soluble protein the fungus *Trichoderma ressei*, *Bacillus subtilis*, and *Streptomyces antibioticus* showed high protease activity and also high soluble protein content.

Keywords: Composting, plant residues, organic matter, cellouse decomposers, cellulase, dehydrogenase, protease.

INTRODUCTION

A great part of Egyptian soil is classified as arid soil. It is usually deficient in organic matter, nitrogen and micronutrients. Intensive using of chemical

fertilizers led to increase soil, water and food pollution, besides the high cost of chemical fertilizers, all those directed the researchers to pay attention in using alternative sources such as the natural sources of fertilizers via using the organic manures and biofertilizers. Thus the composting of plant residues is considered to be the essential alternative source of organic matter in such arid conditions. Composting is a widely used technique for recycling all kinds of organic matter. The compost material may be applied to agricultural or horticultural soils and used for soil amelioration or recultivation. While gradually decomposting, compost-released minerals also may serve as plant nutrients in crop production. The utilization of compost (as slow release) fertilizer has been in focus of many studies (Ehrig, 1992, Diaz *et al*., 1995, Larchevêque *et al*., 2006 and Plaza *et al*., 2007).

The list of materials appropriate for composting is almost endless. Plant residues are considered to be an important source of C in soil and nutrients for crops in agricultural system. The decomposition of crop residues and green manures is often applied to sustain soil organic matter at acceptable level and hence soil C content. Maximum efficiency of nutrients utilization occur when they released from organic residues with the plant demand (Whitbreed *et al.*, 1999). Decomposition of plant remains takes place spontaneously and transformed into humus by microbial action.

The gathering of plant remains into heaps for decomposition is called composting (Gray, 1967). In biological concept, composting is the aerobic decomposition of organic materials by microorganisms under controlled conditions. It is also, non polluting and safe method disposal through converting organic matter (wastes) into resources that provide nutrients to crop and enhance the tilth, fertility and productivity of soil. Nutrients are more available to plants while pathogens destroyed. (Parr and Colacicco, 1987, Crecchio *et al*., 2004 and Sellami *et al.*, 2008).

The microbiological process where decomposition of organic materials in compost is carried out by different kinds of organisms including bacteria, fungi and actinomycetes. A large variety of mesophilic, thermotolerant and thermophilic aerobic microorganisms plays an important role in composting process, including several bacterial genera and species, i.e. *Alcaligenes fabaclis, Bacillus brevis, B. circulans, B. coagulans, B. licheniformis, B. megaterium, B.pumilus, B. sphaericus, B. stearothermophilus, B. subtilis,* and *Clostridium thermocellum.* Some fungal species such as (*Aspergillus fumigatous, Humicola grisea and Sporotrichum thermophile*) and some actinomyces species (*Microbispora bispora, Nocardia* sp*., Streptomyses* sp*., Streptomyces roseus,* and *Thermoactinomyces virids*) (Palmisano and Bartez 1996 and Stefan *et al.,* 2004).

Bacteria tend to flourish especially in the early stages of composting before the easily degraded materials are consumed, while, fungi and actinonmycetes flourish near the end of composting. Bacteria are mostly hemicellulolytic, while fungi are better at decomposting woody substances and other decayed resistant materials. Therefore they are responsible for decomposition of many complex plant polymers in soil and compost (Bowen, 1990). Fungi are able to breakdown tough debris enabling the bacteria to continue the decomposition process. They spread and grow vigorously by

producing many cells and filaments, and they can attack organic residues that are too dry, acidic or low in nitrogen (Bowen, 1990 and Charest *et al*., 2004). Actinomycetes play an important role in composting process through degrading complex organic materials such as cellulose, lignin, chitin and proteins. Their enzymes enable them to chemically breakdown tough debris such as woody stems, bark or newspaper. Some species appear during the thermophilic phase and other become important during the cooler curing phase, when only the most resistant compounds remain in the last stages of humus formation (Bowen, 1990).

The destruction of human, animal and plant pathogens is not only caused by heat but it's a combination of factors including: Competition for food from compost microorganisms, inhibition and antagonism by compost microorganisms, consumption by compost organisms and biological heat generated by microorganisms. (Charest *et al*., 2004, Baeta *et al.,* 2005 and Raviv *et al.,* 2005).

The main objectives of the present study are:

1. Evaluation of the physical and chemical properties of plant residues through composting.
2. Studying the microbial population during composting process.
3. Studying the microbial enzyme activities of common isolated and inculated microorganisms through composting.

MATERIALS AND METHODS

1. **Preparation of compost starters:** Equal plant residue samples (rice, wheat, maize, bean and white clover straw) were mixed together, grinded, moistened to (60-70%) and then the mixture was inoculated with cellulose decomposers strains of (*Trichoderma reesei, Bacillus subtilis* and *Streptomyces antibioticus*).
2. **Preparation of compost heaps:** Eight heaps (1 x 1 x 0.7 m) were prepared from the shredded rice, wheat, faba bean, maize and clover straw (cut into 1-2 cm pieces and mixed at equal ratio) supplemented with organic manure or cellulose decomposers.

   The complete ingredients of the prepared heaps were listed in the following:

   B_1: Rice, wheat, faba bean and maize straw with organic manure.
   A_1: Rice, wheat, faba bean and maize straw with cellulose decomposers
   B_2: Rice, wheat, clover and maize with organic manure
   A_2: Rice, wheat, clover and maize with cellulose decomposers.
   B_3: Rice, faba bean, clover and maize with organic manure
   A_3: Rice, faba bean, clover and maize with cellulose decomposers.
   B_4: Rice, wheat, clover, faba bean and maize with organic manure
   A_4: Rice, wheat, clover, faba bean and maize with cellulose decomposers

   Each type of the prepared compost was inoculated with compost starter (1.0kg/ton) to initiate composting process in one set of the experiment, while the other set was mixed with animal organic manure. The moisture

of each compost heap was adjusted to 60-70%. The heaps were turned up each 10 days.

3. Monitoring of composting process: Some physical, chemical and microbiological properties were detemined including: bulk density (Vomocil, 1965) moisture, pH and EC (Abad *et. al.,* 2002) organic matter (OM) and organic carbon (OC) (Abad *et al.,* 2002) total nitrogen (Jackson, 1973) and C/N ratio. Temperature heaps were recorded daily at depths of 15, 30 and 50 cm. Also, moisture content and bulk density were measured just before each turning.

Total microbial counts of different microbial group (mesophilic and thermophilic) were determined after mixing the samples of two compost types that have the same contents of row materials and different in supplementation to obtain a representative sample as following:

Compost A: Representative sample from heaps B_1 and A_1
Compost B: Representative sample from heaps B_2 and A_2
Compost C: Representative sample from heaps B_3 and A_3
Compost D: Representative sample from heaps B_4 and A_4

Total bacteria and fungi were determined in these samples according to the method mentioned by (Reinhold *et al*., 1985). Total actinomycetes were determined according to Allen (1950). Three replicates from each heap were collected at 0, 3, 20, 40, 60 and 80 days for chemical and microbiological determinations.

4. Purification and identification of selected microbes:

The dominant microbial species, which are isolated and identified by using different media as follows: Nutrient agar medium for purification and identification of bacteria (Difco, 1984), Jenson's medium for actinomycetes (Allen, 1950) and Dox medium for purification and identification of fungi (Difco, 1984). These microorganisms isolate were kindly identified through specialists in Faculty of Science, Cairo University, Egypt, for fungi isolates by the methods described by Gilman (1957), Ainsworth *et al.* (1973) and Raper and Fennel (1963 & 1977), the identification of the isolated fungi were confirmed by Prof. Dr. M. I. Ali, Biotechnology and Applied Microbiology Center (BAMC), Al-Azhar University, Cairo, Egypt for bacterial isolates and Microbiology Department, National Research Center, Cairo; Egypt for actinomycetes isolates.

5. Estimation of cellulase activity:

The dominant isolated fungal species were cultured on basal nutrient medium and incubated at 40°C for 4 days using a rotary shaker at speed of (200rpm). Developed mycelia were harvested by filtration and the supernatant was assayed for enzymatic activities. (Kvachadze and Yashvili, 1996). The dominant isolated bacterial species were cultivated in liquid medium and then incubated at 30°C for mesophilic spp. and at 55°C for thermophilic ones for 2 days according to (Moatza *et al*., 1998). The dominant isolated actinomycetes species were cultured in the basal medium described by (Waksman, 1961) and incubated on a rotary shaker (200 rpm) at 30°C for mesophilic and 55°C thermophilic for for five days. Culture supernatants from individual isolates were assayed for the presence of cellulolytic activity using CMC as a substrate according to (Schinner and

Mersi, 1990). Release reducing sugars after the incubation of fungi, bacteria and/or actinomycetes with CMC was determined according to Somogyi (1952).

6. Estimation of protease activity:

Culture supernatants due to the individual isolates were assayed for the protease activity according to **Ladd and Butler (1972)** based on determination of amino acids released after incubation of the microbial isolates with sodium caseinate for 2h at 50°C using folin ciocalteu reagent.

7. Determination of soluble proteins:

Soluble protein in microbial filtrates was determined according to (Lowry *et al*., 1951) as follows:

8. Dehydrogenase activity:

Dehydrogenase activity in compost microbial filtrates was determined according to **Skujins (1976)** based on the use of 2, 3, 5 triphenyltetrazolium chloride (TTC) as an artificial electron acceptor. Nearly all microorganisms reduce TTC to tri-phenylformasan (TPF), which can be estimated after incubation at 30°C for 24h.

RESULTS AND DISCUSSION

1. Changes in the temperature of different compost piles at different intervals during composting process:

During the composting process an elevated temperature can be created by microorganisms and subsequently, most pathogens can be inhibited. Therefore, changing temperature during the composting stage could be used as a good sign for composting operation. The changes in the temperature of heaps at different depths (0, 30, 50cm) from the first day (initial time) to 80 days are shown in Figure (1). Data showed in general, that temperature degrees varied during time intervals. It was conducted in the period (August to October) in which the ambient temperature variations through this period ranged between (20-25°C) at nights and from (25-39°C) at daytime. At the beginning of the composting process data recorded (zero time) 30°C - 30.5°C at the surface, 31.1°C - 31.8°C at 30cm depth and recorded 31.5°C - 32.6°C at 50cm depth for all heaps, respectively.

At the third day of the composting process, the data represented a high increase in the temperature where it was (30.5-31.5°C) at the surface, while at depth of 30cm

it recorded (33.7-35.8°C) for all heaps. While, at the depth of 50cm the temperature exhibited an increase between 36.5-38°C for all heaps. A marked increase of the temperature was also recorded for all heaps at 20 days of the process where it ranged between 33-33.5°C at the surface set, 55-57.5°C at 30 cm depth and 68-69°C at 50.0cm depth.

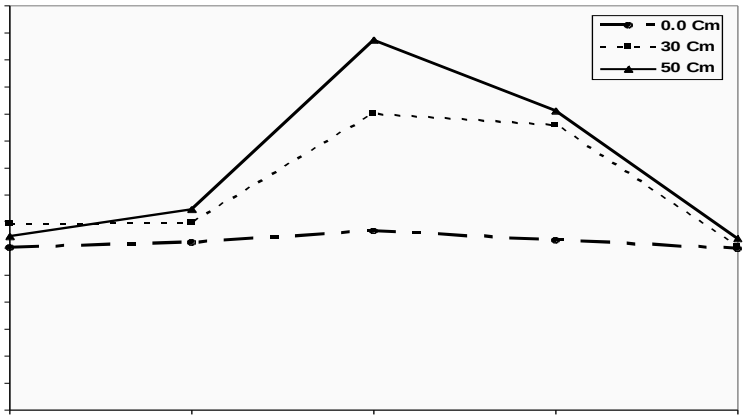

Fig. (1): The changes in temperature of compost heaps at different time intervals during composting processes.

At 20 days of composting period all thermophilic microorganisms were enhanced and became more active, meanwhile all the pathogens might be completely inhibited and the composting process occurred in an ideal way and directed to maturation. Moreover, a slight decrease in temperature was obtained at 40 days of composting, where the temperature ranged between 31-31.6°C at the surface set, 52-53.6°C at 30cm depth and 54.5-56.2ºC at 50 cm depth in all piles, respectively.

On the other hand, at 80 days of composting the temperature values markedly decreased and became more favorable for mesophillic microorganisms where its values ranged between 30-31°C, 36-37.2°C and 39.7-40.4°C at depth of 0, 30 and 50 cm, respectively, for all piles. More decreases in temperature values occurred at 80 days of the composting process and the piles became cool again where its temperature values ranged between 29.5-30°C at the surface set, 30.1-30.6°C at 30 cm depth and 31.5-32.6°C at 50 cm in all piles, respectively. It was also observed that the temperature of the heaps treated with organic manure were slightly higher than the temperature of the piles treated with cellulose decomposers. It was also remarkable that, there was a gradual increase in temperature with increasing the amount of the used raw materials. During the composting process in compost heaps, the temperature evaluation is considered to be a reflection of the metabolic activity of the microbial population involved in the process and in many studies temperature has been shown to be a critical determination of composting efficiency (Miller, 1992, Namkoong and Hwang, 1997). Results indicated that temperatures between 35 and 40°C maximized the microbial diversity in the composting process, those between 45 and 55°C maximized the biodegradation rates and only the thermophilic range between 55 and 65°C is sufficient to destroy pathogens.

This finding is in agreement with the results of Bach *et al*. (1984), Mckinley and Vestal (1984) and Venglovsky *et al.* (2005).

2. Changes in pH:

The changes in piles pH values recorded during the different time intervals of composting process are presented in (Table 1). Irrespective of treatments, generally the pH values were slightly decreased in early intervals of composting process. The pH values increased afterwards to reach to neutral value. pH values were changed toward acidity over the first week, the average of pH of all piles during this period ranged between 7.70 and 6.68, while as at the third week of the composting process there was a slight increase in the pH values. The average pH of the heaps exhibited 7.01, this period represented the neutral period. The pH values afterwards were changed to alkalinity and the average of pH values of the compost piles recorded the maximum at 60 days where it was 7.46. This was followed by slight alkalinity in all heaps to pass the neutral phase during composting till the end of the period (80 days), where the average pH values at this period recorded 7.2.

The decreasing of pH values at the early periods may be due to production of simple organic acids causing further acidification. Elvira *et al.* (1998) showed that the decreasing of pH values was due to nitrification process, which converted ammonia to nitrate. Whereas, on going the composting process the pH values shifted toward neutrality. This pattern was reported by Kaloosh (1994) who found that the pH values showed a little decrease in the first month then, it raised to neutral during composting process.At the end of composting process in the present study the pH was shifted toward neutral due to degradation of composted materials, which, led to the release of some organic acids and CO_2 as a result of microbial activity (Ginting *et al.*, 2003 and Venglovsky *et al.,* 2005).

3. Changes in EC (dS/m) during the compost process:

Regarding to electrical conductivity (EC) changes in compost heaps, (Table, 1) showed in general that its values were increased gradually till the end of the process where the average of EC values at the beginning of the process exhibited 6.70dS/m and reached to 13.3dS/m at the end of composting process.

The EC values of different compost heaps have been found to increase during the composting process, this may be due to the increase of both soluble mineral and organic ions. Similar findings were obtained by Kaloosh (1994) and El-Nadi *et al*. (1995) who found that EC values increased to 10.0-22.5 for compost mixture (municipal refuse, rotted vegetables and fruits) and chicken manure, respectively. They attributed the increase in EC to the released soluble inorganic ions during mineralization of organic materials. However, it has been found that, the increase in EC of the all composted heaps could be related to the high concentration of ammonia released during the rapid mineralization of organic matter (Abdel-Wahab, 1999 and Benito *et al.,* 2005).

Table (1): Change in pH, EC and bulk density values during the composting process in different heaps

Compost heaps	pH						EC (dS/m) *					
	Composting periods (days)						Composting periods (days)					
	0	3	20	40	60	80	0	3	20	40	60	80
A_1	7.60	6.50	6.99	7.40	7.40	7.20	6.50	9.50	11.12	11.50	12.30	13.33
A_2	7.70	6.70	7.01	7.50	7.37	7.15	6.70	9.60	12.03	13.63	10.50	13.99
A_3	7.65	6.70	7.03	7.10	7.41	7.19	6.75	9.65	12.37	13.20	13.00	13.35
A_4	7.75	6.80	7.07	7.60	7.62	7.40	6.80	9.68	12.49	13.50	13.10	13.70
B_1	7.60	6.51	6.96	7.00	7.20	7.05	6.55	9.52	11.15	11.75	12.30	12.80
B_2	7.69	6.75	6.99	6.80	7.50	7.30	6.71	9.60	12.13	12.61	12.90	12.11
B_3	7.72	6.71	7.01	7.20	7.55	7.35	6.77	9.63	12.00	13.15	13.70	13.40
B_4	7.75	6.81	7.05	7.20	7.61	7.31	6.81	9.70	11.24	12.25	13.62	13.60
	Bulk density (g/cm^3) *											
	Composting periods (days)											
A_1	0.090	0.100	0.120	0.140	0.180	0.320						
A_2	0.092	0.120	0.130	0.150	0.185	0.350						
A_3	0.095	0.110	0.120	0.150	0.185	0.340						
A_4	0.100	0.120	0.140	0.170	0.200	0.360						
B_1	0.097	0.100	0.110	0.130	0.176	0.240						
B_2	0.098	0.120	0.125	0.155	0.195	0.350						
B_3	0.100	0.250	0.134	0.160	0.200	0.310						
B_4	0.110	0.130	0.137	0.165	0.230	0.370						

(*) Each value is a mean of 3 replicates.
A_1 = Rice, wheat, faba bean and maize straw + cellulose decomposers.
A_2 = Rice, wheat, clover and maize straw + cellulose decomposers.
A_3 = Rice, faba bean, clover and maize straw + cellulose decomposers
A_4 = Rice, wheat, clover, faba bean and maize straw + cellulose decomposers
B_1 = Rice, wheat, faba bean and maize straw + organic manure.
B_2 = Rice, wheat, clover and maize straw + organic manure.
B_3 = Rice, faba bean, clover and maize straw + organic manure.
B_4 = Rice, wheat, clover, faba bean and maize straw + organic manure.

4. Changes in bulk density (g/cm^3) of compost materials during the composting process:

In respect to bulk density data in Table (1) indicated that there was an increase in bulk density of all studied heaps. It increased when the decomposition was progressed by time from the beginning of the process till the 80th day. The average values of the bulk density increased from 0.098 g/cm^3 to 0.33g/cm^3.

Increasing bulk density with the time during the composting process were presumably due to the reduction of the volume of raw materials as a result of breaking down the composted materials. These results are in harmony with those obtained by Rynk *et al*. (1992) who found that composting process led to a volume reduction of one quarter to more than one-half of the initial of this volume. Part of this reduction represents the loss of CO_2 and water vapour to the atmosphere. They also cleared that, the bulk density was greatly affected by initial C/N ratio of raw materials. While, Fahmy *et al.* (2000) stated that addition of amendments as different types of manures to composted heaps led to accelerate the biological activity which causes an increase in the volume reduction.

5. Changes in organic matter (%) during the compost process:

Results obtained in Table (2) showed a gradual decrease in organic matter during the intervals of the time composting process particularly from the day twenty to 80th where the values of organic matter fluctuated from 69.08% to 48.85%. The loss of organic matter also varied according to the type of the raw materials, which represent each pile.

The above mentioned results are in accordance with those of Ehsan *et al*. (1990) who reported that the rate of decomposition of organic matter was rapid during the first period and then continued at comparatively slower rates during the last period of composting. They also indicated that, the decreasing of organic matter during the composting process may be due to oxidation effect of the organic acids obtained under aerobic conditions. These results are also in agreement with those reported by Paredes *et al.* (2002) and Zbtniewski and Buszewski (2005).

Table (2): Change in organic matter, organic carbon, total nitrogen and C/N ratio during the composting process in different heaps

Compost heaps	Organic matter (%)						Organic carbon (%)					
	Composting periods (days)						Composting periods (days)					
	0	3	20	40	60	80	0	3	20	40	60	80
A_1	81.55	81.33	69.30	54.00	51.75	51.00	47.30	47.17	40.19	31.30	29.71	29.58
A_2	77.60	76.00	73.00	54.00	50.00	50.00	45.00	44.08	42.34	31.30	29.00	29.00
A_3	80.57	80.45	52.30	52.61	48.27	48.27	46.73	46.66	30.34	30.20	28.00	28.00
A_4	91.49	90.20	80.75	64.00	61.00	60.34	53.06	52.32	46.84	37.10	35.30	35.00
B_1	81.27	80.60	68.23	56.05	54.00	54.00	47.14	46.75	39.58	32.50	31.30	31.20
B_2	78.39	78.15	67.00	65.17	47.00	47.00	45.47	45.33	38.86	37.80	27.00	27.26
B_3	80.75	80.57	75.30	43.27	43.27	43.18	46.84	46.74	43.67	25.50	25.10	25.05
B_4	90.57	90.23	66.74	38.00	37.00	37.00	52.53	52.33	38.71	22.00	21.20	21.46
	Total nitrogen (%)						C/N ratio					
	Composting period (days)						Composting period (days)					
	0	3	20	40	60	80	0	3	20	40	60	80
A_1	0.80	0.83	0.90	1.10	1.16	1.20	59.13	56.83	44.66	28.45	25.60	24.65
A_2	0.66	0.67	0.70	1.15	1.15	1.15	68.18	65.79	60.49	27.21	25.21	25.13
A_3	0.60	0.65	0.65	1.10	1.37	1.30	77.88	71.78	46.68	27.45	22.04	21.53
A_4	0.75	0.75	0.78	1.30	1.25	1.30	70.75	69.67	60.65	28.53	28.24	26.92
B_1	0.85	0.85	0.90	1.00	1.13	1.13	55.46	55.00	43.97	32.50	31.23	27.61
B_2	0.65	0.65	0.70	0.90	1.14	1.14	69.95	69.73	55.51	30.66	27.69	23.09
B_3	0.67	0.68	0.70	0.95	1.21	1.21	69.91	68.72	62.39	26.84	22.50	20.70
B_4	0.86	0.86	0.90	0.90	0.95	0.95	61.80	60.85	43.01	42.44	22.31	22.59

6. Changes in organic carbon during the composting process:

Concerning the changes in organic carbon during the composting process, data in Table (2) showed that the percentage of organic carbon gave a gradual decrease in all types of compost heaps during all intervals. The percentages of organic-C during the composting intervals from the first day to the eighty day of the process fluctuated between 48.01 and 28.32 and the loss of carbon reached 41.01. The loss of carbon varied according to the sort and ratio of the raw materials, which formed each heap.

The gradual decrease in organic carbon was mostly due to the loss of carbon as CO_2 owing to microbial oxidation during the composting

process. These findings are similar to those found by Kaloosh (1994) who attributed the loss of carbon as CO_2 is also due to the presence of cellulolytic fungi, which may accelerate the rate of decomposition and declined the organic carbon. Acceleration of composting when using microorganisms as a bioactivators may reduce the time of composting as well as enhancing the biological activity in the compost product, these were discussed by many investigators (Diaz *et al*., 1993, Desoki, 2000 and Zmora-Nahum *et al*., 2005).

7. Changes in total nitrogen (%) of compost material during the compost process:

Data presented in Table (2) showed changes in total nitrogen occurred during the different time intervals of composting process. The percentage of total nitrogen increased during the composting process.

The average percentage of total nitrogen increased during the composting process from the first day to the 80^{th} and ranged between 0.6 and 1.3. Data also indicated that there were remarkable increases in total nitrogen from the period of 20 days to 40 days then, there was a slight increase in total nitrogen percentages till the end of the composting process. Data also exhibited a variance in total nitrogen percentage among piles.

Nitrogen percentage in compost was increased as a percentage of bulk composted materials gradually decreased. Similar findings were reported by Kaloosh (1994) who indicated that the increase in total nitrogen during the composting process may be due to the loss in weight of composted materials and stimulation of N_2 fixers activity grown on the products of cellulose decomposition. In addition, gathering of some raw materials varied in chemical composition, and mixing them with organic manures and biofertilizers may lead to improve the nutritional status of produced compost particularly their nitrogen content. Similar findings were obtained by Gaur (1987) who indicated that inoculation of compost heaps with cellulolytic fungi led to improve the nitrogen content of the compost. These results were also in harmony with those obtained by Rynk *et al*. (1992), Diaz *et al.* (1993) and Abd El-Wahab *et al.* (2003).

8. Changes in C/N ratio of compost material during the compost process:

Data presented in Table (2) showed that the C/N ratio decreased markedly with the progress in the composting process. The C/N ratio sharply decreased from the period of 3 to 20 days, where its average ranged from 64.79 to 52.17. From the period of the day twenty to the period of forty days, the ratio was markedly decreased. From the forty days till the end of composting process the ratio decreased slightly, where its average recorded 24.03 at 80 days of composting process.

From the obtained results, it was concluded that the narrowing of C/N ratio with the progress of the composting process is a common feature and an indicator for maturation.

As a result of high depletion of organic-C, the total nitrogen was increased hence, the C/N ratio decreased. It tended to be narrow with time in compost heaps. This may be due to the loss of carbon as CO_2, while the nitrogen remained more tightly bounded in organic combination as long as

the C/N ratio is wide. These results are in agreement with those obtained by Gaur (1987), Bernal *et al*. (1998) and Wong *et al.* (2001) who found that addition of some amendments such as inoculation with cellulololytic microorganisms led to decrease organic-C and cause narrow C/N ratio of composted materials. Moreover, the C/N ratio was more narrow in heaps of high faba bean straw content than the other ones which were free from faba bean straw. Low original ratio of faba bean straw as compared to wheat straw could be the reason.

9. Effect of different incubation periods on total microbial count in different compost heaps (-x10^5 cfu/g):

Composting of plant residues was mainly due to the activity of various microbial communities such as (bacteria, fungi and actinomycetes. At the beginning of the aerobic composting cycle, the total number of mesophillic bacteria was increased. However, it was markedly decreased in the third week of composting. Then it was highly increased at the end of composting process (cooling phase) as shown in (Table, 3) and illustrated in Figs. (2).

Table (3): Effect of different incubation periods of composting on total microbial counts in different compost heaps (-- x 10^5 cfu/g dry weight of compost)

Compost Type*	Incubation period (days)	Incubation temperature (ºC)					
		30ºC			55ºC		
		Bacteria	Fungi	Actinomycetes	Bacteria	Fungi	Actinomycetes
A	0	150	20.0	50.0	0.085	0.05	0.08
	3	180	17.0	60.0	1.2	0.12	0.10
	20	10.0	13.0	2.2	1.90	20.0	50.0
	40	13.0	18.0	6.5	15.0	1.0	4.0
	80	250	30.0	90.0	0.006	0.001	0.002
B	0	150	25.0	52.0	0.090	0.06	0.10
	3	200	20.0	70.0	1.40	0.15	0.12
	20	10.7	1.5	3.0	200	23.0	52.0
	40	13.5	2.2	6.5	14.0	1.1	4.2
	80	260	33.0	100	0.0075	0.001	0.002
C	0	165	25.0	55.0	0.087	0.07	0.13
	3	200	21.0	70.0	1.47	0.15	0.13
	20	10.0	1.7	3.2	210	25.0	55.0
	40	15.0	2.0	7.0	15.0	1.0	4.0
	80	300	33.0	100	0.003	0.003	0.0023
D	0	170	25.0	58.0	0.008	0.09	0.12
	3	210	20.0	85.0	1.50	0.15	0.15
	20	11.0	1.5	3.3	230	27.0	55.0
	40	16.0	2.0	7.5	15.0	1.2	4.2
	80	300	32.0	110	0.0035	0.004	0.0023

***Compost A: Representative sample from heaps B_1 and A_1**
Compost B: Representative sample from heaps B_2 and A_2
Compost C: Representative sample from heaps B_3 and A_3
Compost D: Representative sample from heaps B_4 and A_4

The same pattern of the results was also observed with mesophilic fungi and actinomycetes. On the other hand, few numbers of thermophilic bacteria at the beginning of composting process were observed, while as at

the third week, they were sharply increased and then obviously decreased at the end of the composting process (cooling phase). The same trend was also observed with thermophilic fungi and actinomycetes in all types of compost heaps.

It has been also observed that no marked differences were obtained in the total microbial counts of different compost heaps for both mesophilic and thermophilic organisms.

10. Cellulase activity of common fungal, bacterial and Actinomycetes species from different compost heaps:

Results in Table (4) show the diversity of cellulase activity among the microbial species on cellulose substrates such as carboxymethyl cellulose. The screening of eleven fungal species for cellulase potentiality revealed that *Trichoderma ressei, T. viride* and *A. niger* represented the highest CMC-ase activity. Their activities exhibited 3.9, 3.5 and 2.8 µmole/ml/min, respectively. On the other hand, the other fungal species showed lower activity.

Seven selected bacterial species were screened for cellulase activity. Data revealed that the thermophilic bacterial species, *Bacillus strearothermophilus* showed high cellulolytic activity where its activity was 2.90 µmole/ml/min. It was observed that *B. straerothermophilus* was the best bacterial isolates for cellulase activity, its activity represented 1.87, 2.2 and 2.3 fold higher than in *B. subtilis, B. badius* and *Thermus* spp., respectively.

Cellulase activity of seven selected actinomycetes species were studied. *Streptomyces antibioticus, Thermoactinomyces dichotomicus* and *Thermoactinomyces vulgaris* exhibited high cellulase comparing with other tested *Actinomycetes* spp.

Obviously, there is a variation among the microbial cellulolytic activity whereas the fungal species displayed high cellulolytic activity such as, *Trichoderma reesei, T. viride* and *Aspergillus niger* also recorded cellulase activity higher than the bacterial and actinomyces species as shown above.

From the results, the activity of *Trichoderma reesei* and *Trichoderma viride* for cellulase production, were considered to be the best cellulase producers than several other fungi, which, were also studied by Srivastava *et al.* (1987) such activity was attributed to the ability of these species to degrade hemicellulose and cellulose if they were abundant during decomposition of plant residues as also mentioned by Kang *et al*. (2004). The production of cellulase by bacterial species was less than fungal species and more than *Actinomyceties* species, which indicate that bacteria are considered to be cellulitic and hemicellullitic according to their enzyme profiles (Charest *et al*., 2004 and Baeta-Hall *et al.,* 2005).

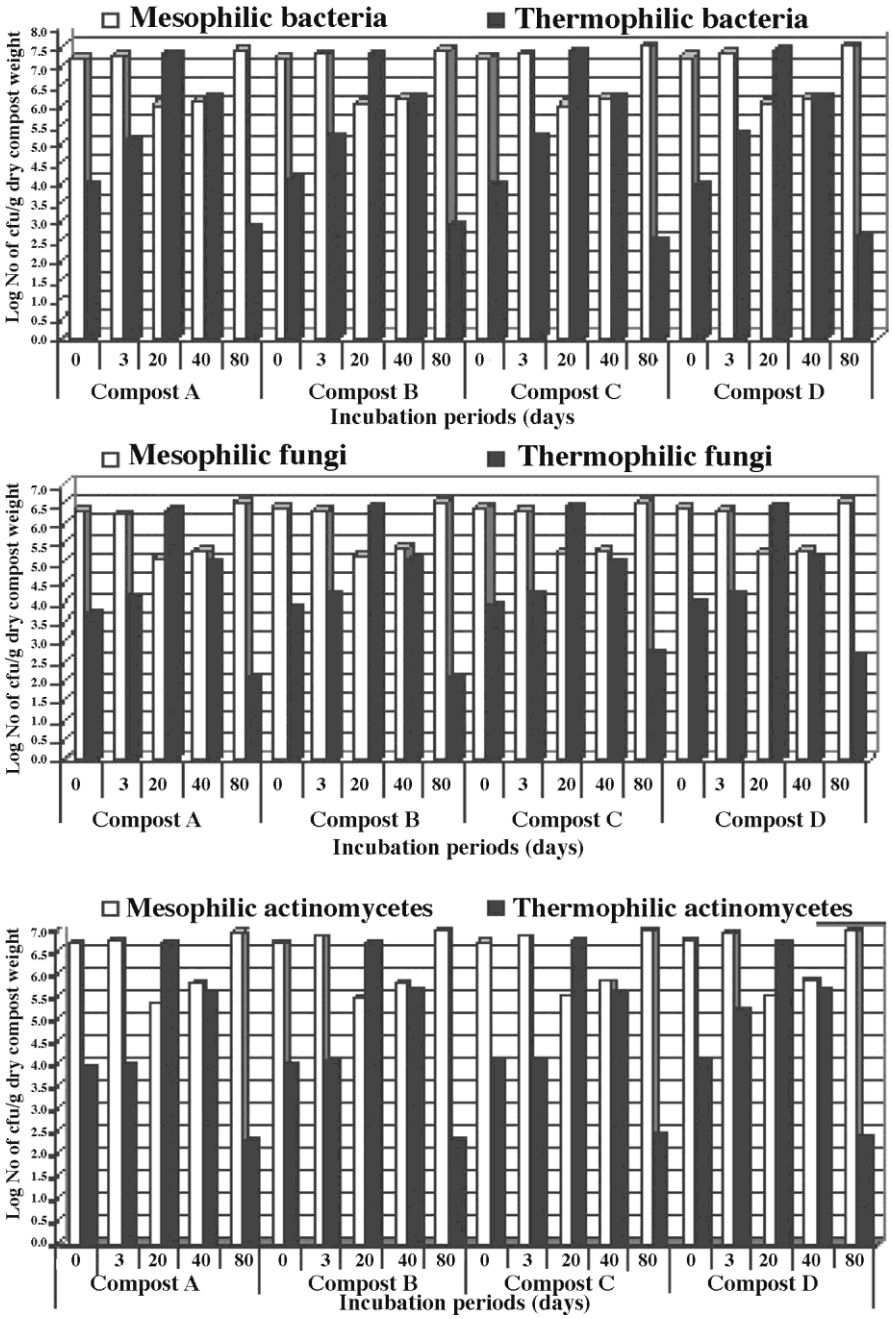

Fig. (2): Effect of incubation period (days) for different compost types on total bacterial fungi and actinomycetes counts (mesophillic and thermophillic) (Data are expressed as Log No. of cfu./g dry weight of compost).

11. Dehydrogenase activity of culture filtrate of some common microbial species isolated from different compost heaps:

Data in Table (4) show that there is a diversity of dehydrogenase activity among the microbial species as an indicator of microbial activity through the oxidation of organic compounds. The activity of eleven selected fungal species revealed that *Trichoderma reesei* was the best fungal species for dehydrogenase activity, (165.2 µg TPF/ml culture filtrate/day). This was followed by *Aspergillus niger*, (163. µg TPF/ml/day). *Trichoderma viride* exhibited also high dehydrogenase activity compared to both fungal species mentioned above.

Table (4): Cellulase and dehydrogenase enzymes activities of common fungal, bacterial and actinomycetes species isolated from different compost heaps (Data are expressed as µmol reducing sugar/ml/min and µg TPF/ml/day)

Strain No.	Isolates	Cellulase (µmol/ml/min)	Dehydrogenase (µg TPF/ml/day)
Fungal spp.			
1	*Penicillium citrinum*	1.36	21.60
2	*Rhizopus nigricous*	1.63	33.30
3	*Aspergillus flavus*	1.04	48.00
4	*Fusarium moniliforme*	0.8	163.30
5	*Aspergillus niger*	2.81	28.00
6	*Trichoderma viride*	3.50	165.20
7	*Aspergillus fumigatus*	0.96	155.40
8	*Trichoderma reesei*	3.90	39.30
9	*Aspergillus fumigatus var elipticus*	1.36	33.30
10	*Talaromyces thermophilus*	1.10	40.30
11	*Talaromyces* spp.	1.00	38.30
Bacterial spp.			
1	*Bacillus badius*	1.30	57.47
2	*Bacillus subtilis*	1.55	67.94
3	*Bacillus polymyxa*	1.00	23.94
4	*Bacillus brevis*	1.00	56.56
5	*Bacillus* spp.	0.95	64.18
6	*Bacillus stearothermophilus*	2.90	27.65
7	*Thermus* spp.	1.25	25.059
Actinomycetes spp.			
1	*Streptomyces antibioticus*	1.33	299.13
2	*Streptomyces roseus*	0.96	298.50
3	*Streptomyces cinnaborinus*	0.72	36.03
4	*Streptomyces griseus*	0.60	26.56
5	*Thermoactinomyces dichotomicus*	1.20	15.03
6	***Streptomyces aureofaciens***	0.88	57.166
7	***Thermoactinomyces vulgaris***	1.00	18.660

Seven bacterial species isolated from compost were screened for dehydrogenase activity (Table, 4). Data reveal that *Bacillus subtilis* exhibited the highest activity for dehydrogenase compared to the other Bacterial spp., this was followed by *Bacillus* spp. where their activity gave 67.94 and 64.180 TPF µg TPF/ml/day, respectively.

Results showed that *Actinomycetes* species, *Streptomyces roseus* and *streptomyces antibioticus* exhibited the highest activity for dehydrogenase since they recorded 298.50 and 290.13 µg TPF/ml/day, respectively, while other actinomycetes species showed little activity.

12. Total soluble protein and protease activity in culture filtrate of different common microbial species isolated from different compost heaps:

Data in (Table, 5) indicated that among eleven fungal spp., isolated from different compost heaps and tested for their extraction to soluble protein and activity for protease enzyme, *Trichoderma reesei* resulted the highest crude soluble protein content (288.640µg/ml) as well as the highest protease activity (0.0133 unit/ml/min). This was followed by the activity of *Trichoderma viride* which gave 276.980 µg/ml soluble protein content and 0.01275 unit/ml/min protease activity. Contrarily *Aspergillus fumigatus* var. *elipticus* recorded lowest protein content and protease activity (271.150µg/ml protein content and 0.01250 unit of enzyme). On the other hand, other fungal strains also exhibited lower soluble protein content and enzyme activity.

Results also showed that all isolated bacterial species produced high amounts of crude soluble protein and also represented high protease activity. *Bacillus subtilis, Bacillus polymyxa* and *Bacillus* spp**.** represented the highest bacterial isolates for soluble protein production and protease activity. They recorded 472.33, 460.66 and 408.18 µg/ml soluble protein respectively and obtained 0.02173, 0.02119 and 0.010107 unit/ml/min, respectively.

Results also showed that *Streptomyces antibioticus*, *thermactinomyces vulgris* and *Thermoactinomyces dichotomicus* represented the highest soluble protein production and also the highest protease activity. They recorded 204.092, 174.94 and 147.638 µg/ml for soluble protein production, respectively. While, they were scored 0.0094, 0.00687 and 0.0081 unit/ml/min for protease activity, respectively.

The obtained results indicated the ability of common fungi, bacteria and actinomycetes to produce extracellular protease and soluble protein content when they do bioconvert the agricultural byproducts through composting as sources of sole carbon and nitrogen. It was obvious that this represented bacteria, particulary *Bacillus subtilis,* is considered to be the optimum for producing soluble protein and protease activity more than both of fungi and actinomycetes. These findings are the same with that obtained by Moatza *et al.* (1998) and Young *et al.* (2002) who reported that, aquatic microbes synthesize exo-enzymes which were identified as inducible catabolic enzymes, and both *Bacillus* spp. and *Bacillus subtilis* are known to produce stable extracellular protease. Hanaa and Laila (1998) also added that these alkaline proteases from alkalophilic microorganisms have excellent characteristics not only in activity but also stability, and the enzymes produced by *Bacillus* species find a wide variety of applications.

On the other hand, thermophillic bacteria are able to produce thermostable proteases and *Bacillus sterothermophilus* is considered as the best in producing thermostable protease and soluble proteins. These findings are in agreement with Young *et al.* (2002) who reported the use of *Bacillus sterothermophilus* for enhancing the reduction of wastes. They also added

that this protease is considered to be a parameter for a biological treatment by using the agricultural wastes microorganisms.

Table (5): Soluble protein and protease activity in culture filtrate at different isolated fungal, bacterial and actinomycetes species isolated from different compost heaps [Data are expressed as µg/ml and Unit/ml (µmole tyrosine/ml/min)]

Strain No.	Isolates	Crude soluble protein µg/ml	Protease activity Unit/ml
Fungal spp.			
1	*Penicillium citrinum*	145.78	0.00671
2	*Rhizopus nigricous*	204.09	0.00940
3	*Aspergillus flavus*	151.61	0.00692
4	*Fusarium moniliforme*	233.32	0.01075
5	*Aspergillus niger*	174.94	0.00810
6	*Trichoderma viride*	276.98	0.01275
7	*Aspergillus fumigatus*	236.16	0.01086
8	*Trichoderma reesei*	288.64	0.01330
9	*Aspergillus fumigatus var elipticus*	271.15	0.01250
10	*Talaromyces thermophilus*	131.20	0.00603
11	*Talaromyces* spp.	119.54	0.00550
Bacterial spp.			
1	*Bacillus badius*	381.94	0.01731
2	*Bacillus subtilis*	472.33	0.02173
3	*Bacillus polymyxa*	460.66	0.02119
4	*Bacillus brevis*	221.00	0.01020
5	*Bacillus* spp.	408.18	0.01017
6	*Bacillus stearothermophilus*	376.11	0.01730
7	*Thermus* spp.	341.12	0.01570
Actinomycetes spp.			
1	*Streptomyces antibioticus*	204.092	0.00940
2	*Streptomyces roseus*	93.300	0.00430
3	*Streptomyces cinnaborinus*	96.215	0.00443
4	*Streptomyces griseus*	104.960	0.00480
5	*Thermoactinomyces dichotomicus*	147.638	0.00687
6	*Streptomyces aureofaciens*	64.140	0.00157
7	*Thermoactinomyces vulgaris*	174.94	0.0081

Concerning soluble protein and proteases produced by the isolated fungi, the obtained results indicated that *Trichoderma reesi* was the most active fungal spp. that produces high soluble protein and also protease enzyme. These results are in accordance with those found by Griffen *et al.* (1997).

Concerning soluble protein and protease activity, the data exhibited the ability of *Streptomyces antibioticus* to produce highl protein and protease more than other actinomycetes species. These results are agreement with those obtained by (Mansour, 1985) who stated that the enzyme producion capacity of various micro-organisms was found to depend on medium composition and / or cultural conditions.

In conclusion, composting is a microbiological process through contribution of fungal (*Trichoderma reesei* and *Trichoderma viride*), bacterial (*Bacillus subtillus* and *Bacillus polymyxa*) actinomycetes (*Streptomyces antibioticus*) activities in rapid production of compost at limited periods. Composting of agricultural wastes such as rice, maize and faba bean straw lead to: increased its agronomic effectiveness, reduced the pollution problem associated with these wastes and reducing the reliance on chemical fertilizers and decreases the cost of production. Also, composts proved to be effective agents in reclaiming sandy soils through improving soil chemical properties and consequently increasing crops production.

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دور الميكروبات وإنزيماتها فى عملية كمر المخلفات النباتية

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مركز البحوث الزراعية - معهد بحوث الأراضى والمياه والبيئة - قسم بحوث الميكروبيولوچيا الزراعية

يعتبر استصلاح الأراضى الرملية واحد من أهم أهداف السياسة الزراعية للحكومة المصرية ، مما يتطلب الإمداد المستمر بالمادة العضوية لزيادة خصوبة وإنتاجية تلك الأراضى ؛ لهذا السبب جُهزت ٨ كومات ، كل كومة مكونة من ٤ أنواع من المخلفات النباتية ، نصف هذه الكومات عومل بالسماد البلدى ، والنصف الآخر عومل بمحللات السليولوز ، وتم تتبع التغير فى بعض الخواص الطبيعية والكيميائية والميكروبيولوچية لهذه الكومات أثناء عملية الكمر ، كانت أهم النتائج المتحصل عليها ما يلي :

بدأت درجة حرارة الكومات فى الارتفاع من اليوم الثالث ، ووصلت أعلى قيمة لها فى الأسبوع الثالث للكمر ، حدث انخفاض تدريجى لها من الأسبوع الرابع وحتى نهاية عملية الكمر . قيم الـ pH للكومات اتجهت للحموضة فى الفترات الأولى حتى ٢٠ يومًا من بداية عملية الكمر ، ثم ارتفعت حتى وصلت إلى التعادل ثم الميل للقلوية فى نهاية عملية الكمر ، وكان هناك زيادة تدريجية فى قيم EC، وكذا density Bulk خلال عملية الكمر ، وبمرور الوقت وجد انخفاض تدريجى فى الكربون العضوى ، وكذا فى المادة العضوية ، وزيادة فى النسبة المئوية للنيتروچين الكلى فى كل الكومات ، مما أدى إلى انخفاض ملحوظ فى قيم N/C ratio خلال عملية الكمر .

كان هناك زيادة ملحوظة لأعداد البكتيريا الميزوفيلية فى بداية الكمر ، ما لبثت أن انخفضت فى الأسبوع الثالث ، ثم حدث زيادة عالية فى أعدادها حتى نهاية عملية الكمر ، نفس الاتجاه لوحظ مع الفطريات والأكتينومايسيتات الميزوفلية . من جهة أخرى زاد أعداد البكتيريا الثرموفيلية زيادة ملحوظة فى الأسبوع الثالث من الكمر ، ثم انخفضت الأعداد حتى نهاية عملية الكمر ، نفس الاتجاه لوحظ مع الفطريات والأكتينومايسيتات الثرموفيلية ، وقد لوحظ عدم وجود اختلاف ملحوظ فى أعداد الميكروبات الميزوفيلية والثرموفيلية بين الكومات المختلفة .

بالنسبة للنشاط الإنزيمي للسلالات التى كانت سائدة أثناء عملية الكمر (والتى تم تنقيتها والتعرف عليها) ، بصفة عامة سجلت السلالات :

Streptomyces and *stearothermophilius Bacillus* ،*reesei Trichoderma antibioticus*

أعلى نشاط لإفراز إنزيم السليوليز ، وسجلت السلالات :

roseus Streptomyces and *subtilis Bacillus* ،*reesei Trichoderma*

أعلى نشاط لإفراز إنزيم الديهيدروچينيز ، وسجلت السلالات :

antibioticus Streptomyces and ،*subtilis Bacillus* ،*ressei Trichoderma*

أعلى نشاط لإفراز إنزيم البروتييز ، وكذا البروتين الذائب .