www.sospublication.co.in Journal of Advanced Laboratory Research in Biology

We- together to save yourself society e-ISSN 0976-7614

Volume 4, Issue 1, January 2013 Research Article

Enhancement of Cotton Stalks Composting with Certain Microbial Inoculations

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Abstract: Effect of inoculation with *Phanerochaete chrysosporium* and *Azotobacter chroococcum* microbes on cotton stalks composting was studied in an attempt to achieve rapid maturity and desirable characteristics of produced compost. Composting process was maintained for 16 weeks under aerobic conditions with proper moisture content and turning piles. The C/N ratio of the mixtures was adjusted to about 30:1 before composting using chicken manure. Temperature evolution and its profile were monitored throughout the composting period. Mineralization rates of organic matter and changes in nitrogen content during composting stages were evaluated. Total plate count of mesophilic and thermophilic bacteria, cellulose decomposers and *Azotobacter* were determined during composting periods. The treatment of cotton stalks inoculated with both *P. chrysosporium* and *Azotobacter* gave the most desirable characteristics of the final product with respect to the narrow C/N ratio, high nitrogen content and high numbers of *Azotobacter*. The phytotoxicity test of compost extracts was evaluated. The use of *P. chrysosporium* in composting accelerated markedly decomposition process, so that 16 weeks composting enough to produce a stable and mature compost suitable for use as fertilizer while the fertilizer obtained by composting cotton stalks mixed with chicken manure and inoculated with microorganisms is highest quality Compost.

Keywords: Composting, Composting cotton stalks, Chicken manure, *Azotobacter chroococcum*, *Phanerochaete chrysosporium.*

1. Introduction

Cotton is the most important commercial fiber crop in Egypt. The main wastes arising from cotton are cotton stalks as agricultural residue, which are generally used for fuel purposes or burnt in the field for disposal causing real harmful environmental implications. Thus, recycling part of such accumulated stalks through composting, which are one of the more economical and environmentally the safest methods of recycling the agricultural wastes? So composting is an aerobic, bioprocess by which organic materials are degraded through the activities of successive groups of microorganisms including bacteria, actinomycetes and fungi; to produce organic fertilizer or soil conditioner (Gajdos, 1992; Rebollido *et al*., 2008). EL-Sharawy *et al*., (2003) studied the effect of the composts of some plant residues as rice straw and cotton stalks on some physical and chemical properties of the sandy soil. Application of these composts significantly improved the physical properties of the tested soil as bulk density hydraulic conductivity and moisture constants (Francis *et al.*, 2006). Lignocellulose usually constitutes an important fraction of the total organic matter in agricultural waste, as it is slowly decomposed by the microbes naturally present in the wastes (Shi *et al*., 2006; Yu *et al*., 2007). According to (Requena *et al*., 1996; El-Din and Abo-Sedera, 2001 and Ferial *et al*., 2010), the inoculation with lignocellulolytic microorganisms is a strategy that could potentially enhance the lignocellulose degradation and accelerate the composting process as shown for crop residues (Singh and Sharma, 2002) and sugarcane residues (Kumar *et al*., 2010). Some species of basidiomycetes designated as white-rot fungi are able to break down all components of lignocellulose, including lignin, the polymer more refractory to microbial attack (Huang *et al*., 2008). Therefore, White-rot fungus, *Phanerochaete chrysosporium*, is a lignin degrader (Asamudo *et al*., 2005). The combination of *P. chrysosporium* and *T.*

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reesei was found best in terms of lignocellulosic decomposition (Kumar and Shweta, 2011). A lot of work has been carried out to study the effect of inoculation during composting (Kapich *et al*., 2004; Phil and Dan, 2007; Zeng *et al*., 2007). The most active ligninolytic organism described to inoculation with *P. chrysosporium* was usually done during the first fermentation phase of composting in the previous researches. Feng *et al*., (2011) observed that ligninolytic enzymes increased the degradation ratio of lignin and hemicellulose, and indicated that the presence of ligninolytic enzymes could improve lignocellulose waste composting and enhance the activity of microorganisms. *Azotobacter chroococcum* and some strains of *Bacillus cereus* are able to fix nitrogen (Abdel-Wahab, 1980; Seldin *et al*., 1984). These microorganisms considerably reduced the pre-decomposition time of wastes (Asamudo *et al*., 2005; McMahon *et al*., 2009). The promoting effect due to application of *Azotobacter chroococcum* not only due to the nitrogen fixation but also to the production of plant growth promoting substances, production of amino acids, organic acids, vitamins and antimicrobial substances as well, which increase soil fertility, microbial community and plant growth (Russell, 1989). On the other hand, (Gaur, 1987 and Estafanous, 2003) reported that enrichment of compost material with *Azotobacter* and phosphate solubilizers increased the nitrogen and humus content and decreased the C/N ratio of the produced compost.

The present work aims at studying the effect of inoculation with *Phanerochaete chrysosporium* and *Azotobacter chroococcum* on the rate of decomposition and quality of the compost produced from cotton stalks and improving the product for agronomic propose.

2. Materials and Methods

2.1 Materials

Cotton stalks and chicken manure were obtained from Dimo surrounding farm, Fayoum Governorate, Egypt. Cotton stalks were air dried and chopped into small pieces (3-5cm) before compost preparation to give a large surface for liquid adhesion and direct contact with microorganisms.

Microorganisms: Microbial strains of effective microorganisms; *Phanerochaete chrysosporium* and *Azotobacter chroococcum* were obtained from Department of Microbiology, Faculty of Agriculture, Fayoum University, Egypt.

2.2 Methods

2.2.1 Preparation of inoculum

The inoculant of *A. chroococcum*: Flasks contained with 150ml of Ashby's medium for *Azotobacter* (Allen, 1959) were sterilized and inoculated with the above-mentioned strain. Incubation was carried out on a rotary shaker (180 rpm) at 28-30^{0}C for 5 days. Fresh preparation of the inoculant was prepared by centrifugation the culture. The obtained cells were resuspended in the same volume of saline solution and diluted with distilled water in the ratio of 1:50 (v:v) to sprayed on compost heaps.

2.2.2 Production of *P. chrysosporium* inoculum

Pure culture of lignocellulolytic fungi *P. chrysosporium* grown on Potato Dextrose Agar medium (P.D.A) in Petri plates, (Difco, 1976) at 30^{0}C for 7 days before used as spore suspensions or inoculation disks for spawn preparation.

Spores suspension of *P. chrysosporium*: were prepared by washing the surface of Petri plate culture with 10ml sodium acetate buffer (50mM, pH 4.5) and 0.1% tween 80, spore counts were determined. The obtained spores were resuspended in the same volume of saline solution and diluted with distilled water in the ratio of 1:50 (v: v) to sprayed on compost heaps.

Spawn preparation of *P. chrysosporium*: sorghum grains were used as substrate for growth of fungi strain, the inoculant was prepared in polypropylene bags, each contained 2% Calcium carbonate and 450g of the wet substrate after washing 2-3 times and surface sterilized with Clorox 1% for 1 min., then washed several times, soaked and boiled in tap water for 2 hrs and 30 min. respectively, bags were autoclaved for 30 minutes for 3 days, then inoculated after cooling with the above-mentioned strain was taken from one-week-old PDA culture and aseptically introduced 5 disks into each bag and allowed to colonize sorghum grains for three weeks incubation at 30^{0}C in the absence of light. The bags containing the complete colonization of the grain surfaces by the fungal mycelia were kept for use as inoculum, was added to compostable materials at a rate of 10% (w/w).

2.2.3 Experimental procedures

The experiment for bioconversion of cotton stalks into compost was carried out in the Consultative Center for Studies and Agricultural Services, Faculty of Agriculture, Fayoum University, Egypt.

2.2.4 Compost heaps construction and enrichment materials

The weights of the raw materials used for every particular compost heap were 180kg of shredded cotton stalks and 100kg of air-dried chicken manure. This preparation was chosen to give a C/N ratio of 30:1 on dry weight basis. Ammonium sulfate 20.5% N (3.5 Kg/ton) and super phosphate 15.5% P_2O_5 (7.0 Kg/ton) also, 35kg calcium carbonate per ton dry matter was mixed with all heaps as amendment.

Four composts heaps were set up as follow:

C1: Cotton stalk + Chicken manure
C2: C1 + *P. chrysosporium*
C3: C1 + *A. chroococcum*
C4: C1 + *P. chrysosporium* + *Azotobacter*

Compost heaps were constructed by laying several layers of shredded cotton stalks one over the other; the dimensions of each pile at the beginning were about 1.0m length, 0.75m width, 0.50m height. Approximately equal parts of the air-dried chicken manure were distributed on the surface of each layer and moistened with water and diluted spores suspension of *P. chrysosporium* (and moisture were maintained at around 60%). After heaping, each heap was covered with a perforated plastic sheet to keep up the moisture and to help in the decomposition work by increasing temperature. Compost heaps (C2 and C4) were inoculated with diluted fungal spores suspension within heaps construction, but the inoculation of compost heaps (C2 and C4) with the spawn of *P. chrysosporium* and (C3 and C4) with the nitrogen fixer (*A. chroococcum*) were done after stage of active decomposition when the temperature had stabilized around 30-35^{0}C (4 weeks of composting period). The piles were turned for aeration and moistening were done regularly biweekly for aerobic compositions as well as no percolation of compost exertion for 16 weeks. Samples were taken from five different spots of each heap at zero time and after 3, 7, 14, 28, 56, 84 and 112 days of composting period. A composite sample was prepared and subject to physicochemical, microbiological analysis and germination index test.

Physicochemical analysis: The most important factors affecting the composting include temperature, moisture content, C/N ratio and the physical structure of the waste material. Temperature degrees were recorded daily at the center of each heap using a thermocouple thermometer. Moisture content, organic matter, organic carbon and total nitrogen of raw materials and composting mixtures were determined according to the standard methods of Page *et al*., (1982).

Microbiological determinations: Mesophilic and thermophilic viable bacterial count were estimated by the standard plate count method using soil extract agar medium (Allen, 1959). Plates were incubated at 30^{0}C for growing mesophilic and 55^{0}C for thermophilic bacteria. Total viable counts of cellulose decomposers and *Azotobacter* were determined by Most Probable Number method (MPN) using Dube's and Ashby's liquid media (Allen, 1959) for cellulose decomposers and *Azotobacter* respectively. At the beginning and at the end of the composing process, *Salmonella* detection was conducted by inoculating a bottle of Selenite Cystine Broth medium with 10g of the sample and incubated at 37^{0}C for 24 h. Full loop from this bottle was streaked onto plates of *Salmonella-Shigella* (SS) agar medium, plates were incubated at 37^{0}C for 24–48 h.

Germination index test: The germination index test of compost extracts was evaluated by the seed germination technique (Zucconi *et al.*, 1981 and Tiquia *et al*., 1996). Cress seeds (*Lepidium sativum*) were surface sterilized by immersion in 75% alcohol for three minutes followed by transferring in 0.1% $HgCl_2$ solution for two minutes with periodical agitation and finally thoroughly washed with sterilized distilled water to get rid of toxic chemicals (Rovira, 1956). A water extract of each compost pile was prepared by shaking the samples with distilled water at 1:10 w/v ratio for 1 hour and then filtered, 10ml of filtrated water compost extract was applied to sterilize filter paper in a Petri dish and ten seeds were then placed on the filter paper. All experiments were run in triplicate under a septic condition. The Petri dishes were sealed with parafilm to minimize water loss while allowing air penetration and then were incubated in the dark for 72 hours at room temperature, the seed germination percentage in the extracts were determined and the length of the longest root produced by the seeds were measured after 72 h in all the extracts and they were compared with those of the distilled water was used as a control. The germination index (GI) was obtained by multiplying germination (G) and root elongation (R), both expressed as a percentage (%) of control values. This index has proven to be the most sensitive parameter, capable of detecting low levels of toxicity, which affect root growth, as well as high toxicity levels, which affect the germination (Tiquia and Tam, 1998). The germination index was calculated according to (Zucconi *et al*., 1981) as follows:

The germination index: GI = (G% × R%) / 100.

Where, G% = (number of seeds germinated in a sample/number of seeds germinated in control) ×100.
R% = (mean root length in sample / mean root length in control) × 100.

3. Results and Discussion

3.1 Chemical composition of cotton stalks and chicken manure

In order to start with composting the appropriate mixture of cotton stalks and chicken manure as composting material, the C/N ratio of the mixture has to be optimized to maximize microbial decomposing activities within the heaps. Therefore, the chemical composition of both wastes was determined (Table 1).

Table 1. Chemical composition of raw materials (*On dry weight basis).

Raw materials	Analysis %				
	Total solids	Total nitrogen*	Organic matter*	Organic carbon*	C/N ratio
Cotton stalks	92.60	0.62	85.16	49.39	79.66
Chicken manure	64.80	2.82	64.66	37.50	13.69

As expected, chicken manure contained more nitrogen than cotton stalks. However, chicken manure contained more organic matter than cotton stalks. This might explain the wider C/N ratio found in cotton stalks compared to chicken manure. Certain organic materials are resistant to microbial attack because of the wide C/N ratio and the high content of lignocellulose (Zhu, 2007). Predetermined quantities of cotton stalks and chicken manure were mixed in order to start with a composting material at a C/N ratio of 30:1 as explained in Materials and Methods section.

3.2 Profile of temperature evolution

Temperature changes during the composting period for the four piles of cotton stalks are shown in Table (2). The ambient temperatures throughout the composting period (Aug. to Nov. 2011) were ranged between 35 to 25^0C at daylights and from 25 to 16^0C at night times. The composting process for the four piles exhibited the classical temperature pattern, where it is possible to distinguish three different phases: mesophilic, thermophilic and cooling down phase, which continues later as compost maturation stage. In all piles, the temperature increased to over 60^0C during the first 10 days, thermophilic phase of composting period, then gradually decreased to around 32^0C-34^0C after 42 days, Rebollido *et al.*, (2008) revealed that during composting under aerobic conditions, temperature is the major selective factor for populations and determines the rate of metabolic activities. The rise of temperature during composting is due to the active evolution of heat, which accompanies the microbial decomposition of the readily decomposable constituents of plant residues. Results are generally in agreement with those of (Dalzell *et al*., 1991 and Dewes, 1994) who reported that the microorganisms present in the raw materials multiply rapidly during the first stage of composting and cause the rise of temperature.

The temperature of the pile C4 which was inoculated with both microbes show temperature had risen during composting 51^0C until the third week revealed to the microbial activity compared with other piles, Zeng *et al.,* (2010), mentioned that the inoculation during the second phase of composting increased the temperature in the compost heaps. Jouraiphy *et al*., (2005), reported that more heat output had probably been the result of biological activity in the compost. So, the result in this study indicated that the biological activity in the compost was improved when the inoculants was present. Thereafter, the temperature of all compost piles regularly dropped off to equal the ambient level become more or less constant around 28-30^0C and remained constant during the maturation phase till the end of composting period. Obtained results (Table 2) are generally in harmony with those of (El-Housseini *et al*., 2000; El-Din & Abu-Sedra, 2001; Rebollido *et al.,* 2008; Ferial *et al.,* 2010 and Zeng *et al*., 2010). Moreover, Kaloosh, (1994) noticed that the pile temperature can be used as an indicator for maturation of compost when the pile temperature curve has definitely leveled off, the temperature goes down below 50^0C and does not rise again upon moistening and turning up, and this can be considered as an indication of compost maturity.

3.3 Changes in dry and organic matter

Table (3) shows the changes in dry matter during composting of cotton stalks. The dry matter content of heaps decreased gradually during the whole period of composting. The use of *P. chrysosporium* inoculums enhanced composting by promoting the decomposition process, especially during the first 4 weeks. Over the composting period the losses in dry matter content were found to be in the order of 49.49 and 48.41% in the inoculated compost either by *P. chrysosporium* alone or *P. chrysosporium* and *Azotobacter*, respectively compared to 37.04% in the uninoculated heaps. This finding is similar to those obtained by El-Din & Abo-Sedera (2001) who found that the use of cellulose decomposers inoculants during composting of sugar beet haulms accelerated decomposition process especially during the first 50 days of composting period, also Huang *et al.*, (2008) resulted that lignin was degraded to 41% and 31% by *P. chrysosporium* and *Streptomyces badius* during rice straw composting.

Data in Table (3) shows the loss in organic matter and organic carbon due to the decomposition of cotton stalks which was parallel to the loss in dry matter in all treatments throughout the composting time, these results are similar to those obtained by Ferial *et al.,* (2010). The highest rate of decomposition took place in composts during the first 4 weeks, which was characterized by high temperature. Later on, the decomposition process proceeded slowly until the end of the experiment (16 weeks). On the other hand, fungi inoculant increased the carbon loss in composted material due to the acceleration of the decomposition process. These results are in full agreement with those obtained by (El-Housseini *et al*., 2000; Singh and Sharma, 2002; Estafanous, 2003; Zeng *et al.,* 2010 and Kumar & Shweta, 2011).

Table 2. Changes in the temperature (°C) inside the heaps during composting.

Treatments	The temperature (°C) of composting (days)											
	0	3	7	10	14	21	28	35	42	56	84	112
C1	27	44	62	61	56	45	34	34	33	28	30	28
C2	27	46	64	63	58	48	35	33	32	29	28	29
C3	27	45	62	63	55	46	34	32	32	28	28	28
C4	27	47	65	64	60	51	37	38	34	30	28	28

Table 3. Changes in dry matter, organic matter and organic carbon during composting of cotton stalks.

	Treatments	Time of composting (weeks)						Loss %
		0	2	4	8	12	16	
Dry matter (kg)	C1	158.4	145.80	124.46	111.31	104.9	99.10	37.04
	C2	158.4	123.44	105.98	91.26	82.30	79.51	49.49
	C3	158.4	147.48	126.07	110.67	101.77	99.71	36.65
	C4	158.4	123.25	108.35	91.67	83.87	81.20	48.41
Organic matter (%)	C1	76.16	74.28	69.87	66.31	64.25	62.18	-
	C2	76.58	70.14	65.22	59.61	55.21	53.64	-
	C3	76.22	74.62	70.31	66.18	63.22	62.46	-
	C4	75.68	68.94	64.67	58.24	54.36	52.86	-
Organic carbon (%)	C1	44.17	43.08	40.52	38.46	37.26	36.06	-
	C2	44.42	40.68	37.83	34.57	32.02	31.11	-
	C3	44.21	43.28	40.78	38.38	36.67	36.23	-
	C4	43.89	39.99	37.51	33.78	31.53	30.65	-
Organic matter (kg)	C1	119.88	108.30	86.96	73.81	67.40	61.65	48.57
	C2	120.54	86.58	69.12	54.40	45.44	42.65	64.62
	C3	119.97	110.05	88.64	73.24	64.34	62.28	48.09
	C4	119.12	84.97	70.07	53.39	45.59	42.92	63.97

3.4 Changes in total nitrogen

Data presented in Table (4) show changes occurred in total nitrogen (T.N.) percentage during different intervals of the composting process. Data shows that irrespective of treatment, the percentage of T.N. gradually increased over composting process. In spite of these increases, nitrogen content, (Table 4) showed a gradual decrease during the composting period. The increase in nitrogen percentage in various heaps might be due to a concentration effect caused by active degradation of the easily decomposable carbonaceous substrates, which resulted in a decrease in the weight of the composting process. On the other hand, the continuous decrease in total nitrogen during the bio-oxidation period could be attributed to its volatilization as ammonia especially during the thermophilic phase and or/by leaching as nitrate after moistening the piles, these results are similar to those obtained by Ferial *et al.*, (2010).

Enrichment of compost with *P. chrysosporium* and *A. chroococcum* led to increases in nitrogen content as compared to the control treatment (Table 5). Inoculation with *P. chrysosporium* alone resulted in an increase of 17.78% over control, while inoculation with *Azotobacter* alone gave an increase of 8.89%. The highest increase in nitrogen content was observed in the treatment inoculated with both microorganisms (C4) which showed an increase of 20% in nitrogen over the control. The obtained results might be explained by non-symbiotic nitrogen fixing activities caused by *Azotobacter* and N_2-fixing *Clostridia* which were found to increase markedly during the first 48 hrs of the mesophilic phase (Kapoor *et al*., 1983). In addition, total nitrogen can also be increased by the activities of nitrogen-fixing bacteria at the end of the composting process (Bishop and Godfrey, 1983). These results are in agreement with those reported by (Gaur, 1987; El-Din & Abo-Sedera, 2001 and Estafanous, 2003) who indicated that inoculation of compost heaps with cellulolytic fungi and *Azotobacter* led to an improvement in nitrogen content of the compost as compared to non-inoculated heaps.

3.5 Change in C/N ratio

The changes in the C/N ratio during the composting period are also shown in Table (4). The values of C/N ratios showed a gradual reduction during the composting process to reach its maximum reduction at the end of composting period. This finding was true for all pile mixtures under this study. The C/N ratio at the end of the composting period reached 14.67 and 14.17 for inoculated treatments (C2 & C4) respectively in return to 20.03 in nontreated compost (C1) or 18.48 in the inoculated compost with *Azotobacter* only. This indicates that the rate of organic matter decomposition and carbon loss was higher in both inoculated treatments with *P. chrysosporium* + *A. chroococcum* than the untreated compost. Similar findings were obtained by (Kaloosh, 1994; El-Din & Abo-Sedera, 2001 and Estafanous, 2003); also, (Singh and Sharma, 2002; Kumar *et al*., 2010) reported that rapid decomposition of sugarcane waste and crop residues respectively, with a mixture of cellulolytic fungi along with nitrogen fixing bacteria *A. chroococcum.* On the other hand, these results are in agreement with those obtained by Zing *et al.*, (2010), how to observe that the C/N ratio in the heaps that were inoculated with *P. chrysosporium* decreased from 30 to 16 during the composting process.

3.6 Changes in the total bacterial viable counts

Changes in the numbers of mesophilic and thermophilic bacteria throughout the composting period for the four different treatments of cotton stalks heaps are shown in Tables (6). Mean counts of mesophilic bacteria in all the composted materials showed a marked increase during the first 3 days followed by a marked decrease after 7 days of composting. The mesophilic bacteria in the inoculated treatments showed a high increase in their counts as compared to the non-

inoculated one. The counts of these bacteria fluctuated and did not show any noticeable changes from the initial counts. Similar results were obtained by (El-Housseini *et al.,* 2000; El-Din & Abo-Sedera, 2001 and Rebollido *et al.,* 2008). The importance of mesophilic microorganisms in starting of composting stems is due to the fact that these microorganisms develop rapidly and attack the readily decomposable constituents of the compost materials resulting in the production of heat and creating a suitable environment for the thermophilic microflora.

Mean counts of thermophilic bacteria in all the composted materials showed marked increases during the high temperature phase (50-65^{0}C) of composting (Table 6). A gradual decreased was seen in the fall in temperature till the end of the composting period with the final counts much higher than the initial one. It is likely that the changes in temperature of the composted heaps govern the types and activity of microorganisms taking part in the decomposition process. While mesophilic organisms are active at the lower temperature of the process, the thermophilic flourish at high temperature, (Taha *et al*., 1968; El-Din & Abo-Sedera, 2001 and Rebollido *et al*., 2008).

Table 4. Changes in total nitrogen and C/N ratio during composting.

	Treatments	Time of composting (weeks)					
		0	2	4	8	12	16
Total nitrogen (%)	C1	1.46	1.52	1.67	1.71	1.73	1.80
	C2	1.45	1.75	2.01	2.06	2.10	2.12
	C3	1.45	1.54	1.71	1.88	1.94	1.96
	C4	1.46	1.76	2.02	2.10	2.14	2.16
Total nitrogen (kg)	C1	2.30	2.22	2.08	1.90	1.81	1.78
	C2	2.28	2.16	2.13	1.88	1.73	1.69
	C3	2.28	2.27	2.16	2.08	1.97	1.95
	C4	2.30	2.17	2.19	1.93	1.79	1.75
C/N ratio	C1	30.25	28.34	24.26	22.49	21.54	20.03
	C2	30.63	23.25	18.82	16.78	15.25	14.67
	C3	30.49	28.10	23.85	20.41	18.90	18.48
	C4	30.06	22.72	18.57	16.09	14.73	14.19

Table 5. Weight loss and nitrogen content in finished compost material.

Treatments	Weight of raw material (kg)	Final weight of compost (kg)	Weight loss (%)	Total nitrogen			
				produced compost (%)	(kg)	per ton produced compost (kg)	Increase over Control (%)
C1	158.4	99.15	37.01	1.80	1.78	18.00	-
C2	158.4	79.51	49.49	2.12	1.69	21.20	17.78
C3	158.4	99.71	36.65	1.96	1.95	19.60	8.89
C4	158.4	81.20	48.41	2.16	1.75	21.60	20.0

Table 6. Total bacterial viable counts during composting of cotton stalks.

Treatments	Time of composting in days							
	0	3	7	14	28	56	84	112
	Mesophilic (cfu x $10^7 g^{-1}$ fresh weight)							
C1	12	86	9.4	5.6	11	76	96	114
C2	17	110	12	7.6	24	84	48	104
C3	15	124	11	8.4	22	68	54	68
C4	21	116	16	14	38	108	45	58
	Thermophilic (cfu x $10^5 g^{-1}$ fresh weight)							
C1	0.2	2.2	80	112	36	24	4.6	2.6
C2	0.3	2.6	92	163	48	18	3.8	4.4
C3	0.3	2.4	74	144	62	34	6.8	5.2
C4	0.2	2.7	118	186	94	42	4.2	6.4

3.7 Changes in cellulose decomposing microbial count

Changes in numbers of mesophilic cellulose decomposers throughout the composting period of the four different treatments of cotton stalks composting are shown in Table (7). Mean counts of mesophilic cellulose decomposer in all the composted materials showed a marked increase during the first 3 days followed by a marked decrease after the 7 days of composting period. On the other hand, the counts of these microorganisms were higher in the inoculated compost than those of the non-inoculated one. In this concern, Taha *et al.,* (1968) showed that aerobic cellulose decomposers increased within the first two

days then decreased during the high temperature phase. They added that the densities of these microorganisms increased during the first period of composting owing to the presence of easily utilizable carbon and energy constituents in the residues undergoing composting. In the second mesophilic phase of composting period, the mesophilic cellulose decomposers in all treatments showed gradual increase till the 12th week of the composting period followed by a slight decrease at the end of the experiment. In this respect, De Bertoldi *et al*., (1983) attributed the importance of the mesophilic phase to the spectacular development of mesophilic eumycetes, which were developed very actively in further degradation of cellulose. On the other hand, Feng *et al*., (2011) studied the assess of the ligninolytic enzymes potential to improve lignocellulose waste composting, The results showed that the treatment increased the degradation ratio of lignin and hemicellulose by 5.24% and 11.74%, respectively and indicated that the presence of ligninolytic enzymes could enhance the activity of microorganisms.

3.8 Changes in total viable counts of *Azotobacter*

The counts of *Azotobacter* in the compost material after inoculation with these bacteria are given in Table (8). Generally, these bacteria recorded an appreciable number in all studied heaps. This could be attributed to the presence of simple organic and inorganic substances in the matured compost, which resulted from early decomposition process. Moreover, data show that the highest value for the viable cell counts of *Azotobacter* bacteria was found in the inoculated treatments with *Azotobacter* alone or in combination with *P. chrysosporium*. The obtained results indicate the importance of inoculation of composted material with *Azotobacter* for the purpose of production of bioorganic fertilizers. Similarly, (Gaur, 1987; Abdel-Wahab, 1998; Estafanous, 2003 and Abd El-Gawad, 2008) reported that inoculation of compost with *Azotobacter chroococcum* resulted in an increase in their numbers than the non-inoculated one. Microbial examination of the obtained compost revealed the increase in numbers of beneficial microorganisms like *Azotobacter*, phosphate dissolving bacteria, aerobic cellulose decomposers and total microbial counts, despite absence of pathogenic microorganisms. These results are incompatible with Abd El-Gawad, (2008). Therefore, the result in this study indicated that the biological activity in the compost was improved when the inoculant was present.

3.9 *Salmonella* detection

Salmonella was detected at the beginning of composting process only in all samples taken from the piles and after 16 weeks of bioprocess. All treatments were they completely disappeared totally free of *Salmonella* without differences between treatments Ferial *et al*., (2010).

Table 7. Changes in total viable counts of aerobic cellulose decomposing microorganisms during composting of cotton stalks. (Numbers x 10^3 g^{-1} fresh weight).

Treatments	Time of composting in days							
	0	3	7	14	28	56	84	112
C1	2.6	14.8	0.7	2.8	44	160	330	140
C2	28	82	16.0	24	66	380	510	340
C3	2.4	13.0	0.8	2.1	51	220	420	210
C4	32	120	18	26	68	440	620	360

Table 8. Changes in total viable counts of *Azotobacter* during composting.

Treatments	Time of composting (weeks)			
	4	8	12	16
	(Cells x 10^3 g^{-1} fresh weight)			
C1	0.24	1.20	0.80	1.40
C2	0.74	2.60	1.10	0.90
C3	8.20	13.00	12.0	7.80
C4	10.00	18.00	14.0	9.60

Table 9. The percentage of germination index of final produced compost (*Mixed samples).

Parameters	Time of composting (weeks)				
	0	16			
	Initial*	C1	C2	C3	C4
G %	56	78	82	88	94
R %	32	69	76	78	82
GI %	17.92	53.82	62.86	68.64	77.26

3.10The phytotoxicity test of compost extracts

The most popular phytotoxicity test of compost extracts was evaluated by researchers is based on the germination bioassay with seeds of cress (*L. sativum* L.), developed by Zucconi *et al*., (1981) was carried out. The responses of cress plant (*Lepidium sativum*) to the toxicity of the compost water extract at the beginning and end of the composting process in term of the relative seed germination. At the beginning of composting, the GI% of cress plants was very low with values of 16.7% compared to the control using distilled water, probably due to the phytotoxic effects of ammonia and low molecular weight organic acids (Zucconi *et al*., 1981 and Wong *et al*., 2001) but as composting proceeded, the GI value increased. At the end of composting period, in this study, the GI values were higher for inoculated heaps than uninoculated.

In general, the decrease of phytotoxicity during composting results from the degradation of phytotoxic substances by microorganisms, Zucconi *et al*., (1981) were considers that the degree of compost maturity with a GI near to 50 are phytotoxin-free composts and, as a consequence, their application should not injure plants. In the present study, the GI value of the initial reached 17.92 due to the contents of raw materials. However, after composting proceeded, the GI value increased to greater than 60% revealing that the phytotoxicity in these inoculated heaps was eliminated after composting. The elimination of phytotoxicity has also been widely used as a measure of compost maturity Meunchang *et al*., (2005). These results suggested that GI as an indicator of the disappearance of phytotoxicity and maturity of compost. Therefore, was defined that GI up to 50% and nearly to 80%. That 50% of GI was near maturity and more than 80% were completed maturity, Selim *et al*., (2012). The result showed that GI in uninoculated piles was 53.82% after 16 weeks (112 days) and the value were reached around (62.86–77.26) in the case of inoculated piles of produced compost. The GI value in uninoculated piles compost was relatively lower than those of others during the maturing procedure but up to 50%. In all compost, surprisingly, GI value was higher than 50% at the beginning of the maturation process. This result suggested that all compost piles were nearly maturated at the timeand suitable for use as fertilizer.

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