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Monitoring of Dynamic Fluctuations in Microbiological and Bio-Physico-Chemical Properties of Multi-Functional Inoculated Triad Organic Mixtures during Different Aerobic Composting Systems

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ABSTRACT

The main objective of the present work aimed to produce, on a large scale, high quality stabilized matured microbial co-composts from the cheapest organic residues and maximizing their soil manuring values for using as soil conditioners and bio-organic fertilizers for bio-organic reclamation and rehabilitation of agricultural sandy and calcareous soils, and consecutively alleviate the environmental pollution and encourage them to replace the expensive agro-chemical fertilizers. The aerobic co-composting bioprocesses for bioconversion from the activated triad organic mixtures agricultural organic low-input cheapest residues rice straw (R), cotton stalks (C) and saw dust (S) were conducted on open site of the Experimental Research of Agricultural farm (un-controlled) Windrows (piles) conditions and on laboratories of Soil & Water Department (controlled) conditions Bin (PVC-barrels) conditions, Fac. Agric., Kafrelsheikh University. These experiments were started during summer season on June 2017 extended till and of September (120 days). For realizing these purposes, the following subjects were entirely achieved: 1- Preparation of the triad components RCS-mixtures by mixing rice straw (R), cotton stalks (C) and saw dust (S) on the basis 1:1:1 v/v preparation their activated organically O-RCS mixtures with chicken manures, fast release mineral activated with N,P,K and S mineral fertilizers mf-RCS-mixtures and slow release mineral activated ms-RCS-mixtures with rock-p, k-feldspar and mineral sulfur-s° for adjusting their elementary relative ratios C/N=30, C/P=100 and C/S=200. 2- Preparation and stowing 3 piles and charging 8 PVC-barrels with different activated RCS-mixtures. 3- Isolation, purification, enrichment and characterizations of the most potent isolates of cellulolytic microorganisms, phosphate and silicate solubilizing bacteria and non-symbiotic N_2 fixer Azotobacter and preparation their liquid culture inoculants. Molecular genetic characterization of cellulolytic isolates was performed by PCR. These microbial liquid inoculants were incorporated and inoculated the barrels and piles at mesophilic and maturity biophases at two doses for each biophase. Dynamic variations in temperature-degrees and pH-values Four typical co-composting thermal biophases were monitored throughout the aerobic co-composting incubation period elongated 120 days as follows: * Short mesophilic biophase (30 days, normal temperature) its grand mean (GM) Temp. 28 °C and 34 °C within barrels and piles respectively, acidic conditions (acid generation) its GM.pH value (6). * Thermophilic biophase (50 days, highly temperature) 43 °C, 55 °C within barrels and piles respectively, more alkaline conditions (NH_4^+-evolution) its GM-pH value (8.7). * Cooling biophase (15 days, transmissive temp. conditions, median state conditions) GM. temp 34.5 °C and 36 °C respectively median alkaline conditions, its GM-pH value (8). * Maturation biophase (25 days, normal temp. conditions) GM. temp 26 °C and 25 °C respectively and normal acidic-alkaline media, its GM.pH-value (7.15) which represent stabilized final product. Effects of these selected studied parameters on the dynamic fluctuations in the chosen of co-composting characteristics in the course of entirely co-composting incubation period were monitored, examined and elucidates. Generally, the obtained analytical and statistical results on the average conditions of all other selected studied parameters in the course of all co-composting incubation periods, could be summarized as thefollowing:1- Effect of co-composting biophasesAll the obtained data were reported on the basis of average conditions of all studied other parameters. Maturation biophase (25 days, normal pH 7.15 and moderately temp.). * Generally, the activity of the selected studied predominant enzymes have realized their superiority values at this biophase over there controls as: Dehydrogenases activity: 305.5 (control 38.5 and general grand mean activity during co-composting period G.G.M 162) as µg 1, 3, 5 TPF produced. $g^{-1}_{d.w}$ co-compost. $hour^{-1}$. Endoglucanase activity: 1073 (control 567 and G.G.M 776.5) µmoles glucose equivalent. $g^{1}_{d.w}$ co-compost. $(24\ hour)^{-1}$. β-glucosidase activity: 252 (control 91.5 and G.G.M 172) µg PNP released. $kg^{-1}_{d.w}$ co-compost. $hour^{-1}$. * Total bacterial (TBC) and fungal (TFC) counts $logCFU.g^{-1}_{d.w}$ co-compost. Commonly, TBC realized the predominancy (8.54) over TFC 5.45. TBC and TFC displayed greater values in comparison with their controls as TBC: 9.78 (control 7.79 and G.G.M 8.54) TFC: 6.15 (control 5.63 and G.G.M 5.45). * Temperature-degrees and pH-values realized the lowest values at this biophase in relation to their controls. Temp.degrees: 25.5 °C (control 29.20 °C, ambient temp. 25.75 °C and G.G.M 35.3 °C) pH-values: 7.15 (control 7.10 and G.G.M 7.5). The absolutely lowest temp. degree (average 22) was registered at this biophase after 120 days from co-composting initiation. Themophilic biophase (50 days) * Activity of studied enzymes displayed the lowest values at this biophase (more alkaline conditions pH 8.7 highly temp. degree 49.25) as (91.5, 620.5 and 120) in comparison with their controls (38.5, 567 and 91.5) respectively. * Total microbial counts recorded the lowest values (5.67 and 4.84) for TBC and TFC, which were more lesser than their controls 7.78 and 5.63. * Temperature-degrees and pH-values displayed the highest magnitude values at the biophase in comparison with their controls as: Temp.degrees: 49.25 °C (control 29.2 °C, ambient temp. 25.75 °C and G.G.M 35.3 °C). pH-values 8.7 (control 7.10 and G.G.M 7.5). The absolutely highest temp. degree was registered at this biophase 62 °C after 85 days from the initiation. Meanwhile, the highest pH-values (9.2) were measured after 65 days form initiation. However, the absolutely lesser value (pH 5.1) was measured at the mesophilic biophase after 15 days from co-composting initiation.2- Effect of multi-functional microbial co-inoculationEnzymes activity of the co-inoculated co-composted mixtures with multi-functional microbial co-inoculants (two phases) realized the highest enzymes activities in accordance with their values as 96, 445 and 213 respectively, in comparison with non-inoculated mixtures which showed as 75, 332 and 143 respectively. Total bacterial and fungal counts displayed higher values for inoculated mixtures in comparisons with non-inoculated mixtures as TBC (9.28 and 7.8) and TFC (5.61 and 5.45) respectively. Co-inoculated mixtures realized higher temp. degrees and pH-values as (38.15 °C and pH 8.9) in comparison with non-inoculated mixtures (33.4 °C and pH 8.5).3- Effect of aerobic co-composting systemsStudied enzymes activity have realized their predominancy values under Windrows piles system 169, 942 and 218 in comparison with Bin (PVC-barrels) system which realized the lesser values 155, 611 and 126 respectively. Total bacterial and fungal counts gave the highest values 8.94 and 5.74 under Windrows piles system in relation to their values 8.14 and 5.16 under Bin (barrels) system respectively. Temperature-degrees within piles announced higher values than those obtained within barrels as 37.6 °C and 33 °C for piles and barrels respectively. However, pH-values registered magnitude higher values within barrels system than those obtained under piles system as 9.4 and 7.5 respectively.

Keywords: Aerobic co-composting, Time Domain Reflectometer (TDR apparatus), CFU-colony forming units, xenobiotics, stabilization, manuring value, composting maturation, soil conditioners, biofertilizers, Bin (PVC-barrels) system, Windrows (piles) system, sandy and calcareous soils.

Enzymes: dehydrogenases, β-glucosidase, endo- and exoglucanases.

INTRODUCTION

In the present technoeconomic era, the energy and environmental crises developed due to huge amount of cellulosic and other organic materials are disposed as wastes. Unscientific disposal causes an adverse impact on all components of the environment and human health. Microorganisms perform their metabolic processes rapidly and with remarkable specificity under ambient conditions, catalyzed by their diverse enzyme – mediated reactions (Gautam *et al.,* 2010 and 2012). An enzyme alternative to harsh chemical technologies has led to intensive exploration of natural microbial biodiversity to discover enzymes. There is a wide spectrum of microorganisms which can produce the variety of enzymes like cellulase, phosphatases, β-glucosidase and dehydrogenases under appropriate conditions. In particular, enzyme activities are very significant because of their major contribution to the ability of the soil to degrade organic matter.

Overuse of agrochemical fertilizers alarmingly causes deterioration in soil health and soil – flora. Persistence of these agrochemicals exerts detrimental effects on environment, potentially inducing toxic effects on human health, this pronouncing an urgent need for a substitutes. The intensification and expansion of modern agriculture is amongst the greatest current threats to worldwide biodiversity. An alternative to this is bio-organic agriculture which promotes and enhances agro – ecosystems health, including biodiversity, biological cycles and soil biological activity (Maleva *et al.,* 2017). Semiarid Mediterranean soils are often subjected to severe degradation processes accompanied by a decline of the soil organic matter content, which contributes to a loss of soil fertility. One method to reverse this degradation in soil quality is the addition of selected organic residues as a soil conditioners and fertilizers. There is growing evidence that soil microbiological and biochemical parameters may play a potential role as early and sensitive indicators of soil ecological stress and restoration (Garcia *et al.,* 2000).

Composting helps in managing large quantilies of organic wastes in a sustainable manner. It is one of the technologies of integrated waste management strategies, used for the decomposition of organic materials into a useful product (Inbar *et al.,* 1990 and Gigliotti *et al.,* 2005). Recently, great efforts have been made to the recycling of organic wastes, encouraging them to replace the expensive chemical fertilizers. Soil application of organic wastes and residues of any nature requires, however, that these materials by previously subjected to appropriate treatments. Composting of organic wastes is a spontaneous biooxidative process in a predominantly aerobic environment involving the mineralization and partial humification of the organic matter, leading to stabilized final product, free of phytotoxicity pathogens and plant seeds with certain humic properties (hygienic humus- rich product) and implies the volume reduction of the wastes called compost and can be beneficially applied to be used as a soil conditioner and an organic fertilizer to agricultural land. (Zucconi and de Bertoldi, 1987; Sylvia *et al.,* 1998 and Bernal *et al.,* 2009).

However, the evolution of the stabilization of organic matter during the composting has primodial importance for the control of the efficiency of this process. In this sense, several physical, chemical and biological parameters have been proposed for the evaluation of the compost stability and maturity (Jeris and Regan, 1973; Droussi *et al.,* 2006). Complementary information on safety and environmental aspects related to manure composting are reviewed by Moral *et al*. (2009), in this special issue, including the suppressive effect against phytopathogens of compost and recent techniques to determine. Therefore, co-composting bio-processes of the organic wastes should delineate the unfavourable impacts on the environment and allows a more complete conservation of residual energy than do other disposal and reclamation methods (zucconi *et al.,* 1981).

Decomposition processes of co-composted materials were actually enhanced via inoculation by beneficial microorganisms acted, themselves or their metabolites as plant growth promoting rhizobacteria (PGPR) (Brinton, 1995 and Mehesen *et al.,* 2013).

Bowen and Rovira (1999) reported that PGPR strains benefit the plants through production of antibiotics, siderophores and hormone-like substances, or through antagonistic activity against the soil-borne pathogens. Some micro-organisms active in the biodegradation of lignocellulose such as the genus *Bacillus*, *Cellulomonas* and *Asperigillus* can accelerate the humification of lignocelluloses when they are inoculated (Faure and Deschamps, 1991).

So, on the light of the abovementioned information, the main ideal of the present investigation was to carry out, study and evaluate the effect of selected studied parameters on the fluctuations in the co-composted materials characterizations throughout the aerobic co-composting bioprocesses under Bin (barrels) and Windrows (Piles) systems. For realizing these purposes, the following subjects were achieved.

* Isolation, purification and detection enzymatic activities of these isolates, identification as well as enrichment the most potent bacterial and fungal strains in liquid cultures as microbial inoculants.
* Selection and characterization of the cheapest organic residues, used as basic ingredients for preparating organic mixtures.
* Preparation and characterization of triad components mixtures (RCS) on the basis 1:1:1 (v/v) without activation and adjustment (as control).
* Adjustment and activation of the RCS-mixtures to achieve wanted C/N, C/P and C/S ratios at 30, 100 and 120 at as these composition sequence 30:1:0.3:0.15 organically (O-RCS)-mixtures and also { minerally (fast release (mf-RCS)-mixtures and (slow release (ms-RCS)-mixtures supplemented with natural rocks as rock-P, feldspar-K and elemental-S^0}.
* Co-inoculation the co-composted materials with multi-functional bacterial and fungal liquid inoculants, plant growth promoting microorganisms PGPR strains and multi- functional effective micro-organisms (basic EM1) solution at mesophilic and maturation biophases during aerobic co-composting period. Moreover, addition of multi-moistening solution for arriving moisture content 50-65 % W.H.C.
* Preparation and charging of the co-composting bioreactors (8 PVC-barrels each 120 L)under controlled laboratory conditions as well as construction and stowing the co-composting (3 piles each 4.5 m^3) under field un-controlled conditions
* Detection of pathogenic coliform numbers during the aerobic co-composting period and evaluation of the matured co-composts hygiene (*Salmonella* and *Shigella*).
* Finally, monitoring the alterations in microbiological, biochemical and some physico-chemical parameters at different intervals of incubation periods throughout the whole aerobic co-composting bioprocesses elongated 120 days.

MATERIALS AND METHODS

The aerobic fermentations for bioconversion of agricultural organic cheapest residues into highly quality microbial, matured co-composts were conducted on open site of the Experimental Research of Agricultural Farm (un-controlled conditions) and at the laboratories of Soil &Water Sciences Department, Faculty of Agriculture, Kafrelsheikh University, Egypt under Windrows (piles) and Bin (barrels) systems. These experiments were started, during summer season, on June 2017 extended for four months till end of September elongated 120 days.

3.1: Preparation and characterizations of triad components mixtures

Select basic ingredients

Composite samples of each cotton stalks (C), rice straw (R) residues were collected from natural habitat

Messear village. Saw dust (S) which used as source of organic-C and bulking agent was obtained from the industrial zone. Fresh (chicken and cattle manures), which used as organic nutrients and microbial activators were immediately obtained from Poultry House-Animal Production, Research Station Farm, Sakha. Cotton stalks and rice straw residues were spread air dry and mechanically chopped into about 2-5 segments using crashing machine. Other ingredients were freshly used without treatments. All these organic residues were homogenized mixed.

Triad components mixtures (RCS) were made by mixing thoroughly the three basic ingredients together on the basis of 1:1:1 (v/v) oven dry weights at 70 °C for 18 hours without activation and thenafter adjusting their C/N, C/P and C/S ratios before beginning aerobic co-composting bioprocesses. Their elementary composition were determined and mathematically calculated according to the results of the initial analysis of basic ingredients and their dry weights using median weights equations.

Organic activated triad components mixtures (O-RCS): were made by adjusting their relative ratios C/N = 30, C/P = 100 and C/S = 200 in the following composition sequence (C: N: P: S) equal to 30: 1: 0.3: 0.15 before the co-composting processes. To achieve the wanted ratios for adjusting, the (RCS) mixtures were organically activated using the appropriate calculated median weights of chicken manure (CM) which was used as organic activator to realizing C/N ratio = 30.

Elementary composition (total-C, N, P and S %) of (O-RCS) mixtures were mathematically calculated using the median equations according to the elementary concentrations (%) of each basic organic ingredients and their dry weights. C/P and C/S ratios of (O-RCS) mixtures were mathematically adjusted to C/P = 100 and C/S = 200 ratios by adding the calculated appropriate quantities from triple superphosphate-P and elemental-S.

Mineral activated triad components mixtures (m-RCS): were prepared by adjusting their relative ratios of RCS-mixtures to the wanted C/N = 30, C/P = 100 and C/S = 200 in the following composition sequence (C: N: P: S) equal to 30: 1: 0.3: 0.15 using urea-N, triple superphosphate-P and potassium sulfate-S for mineral fast release triad components mixtures (mf-RCS). Also mineral slow release triad components mixtures (ms-RCS) were prepared by adjusting their relative C/N, C/P and C/S ratios of the RCS-complexes in the following sequence 30: 1: 0.3: 0.15 to the wanted C/N = 30, C/P = 100 and C/S = 200 using urea-N, rock phosphate-P, feldspar-K and elemental-S.

3.2 : Aerobic co-composting bioprocesses

Preparation and stowing Windrows (piles) system

Windrows piles form co-composting is a commonly used processing method. Composting heaps pyramidical shape were built up under aerobic un-controlled field –scale conditions for co-composting bioprocesses (Golueke, 1972; Kuhlman, 1990 and Eide, 2007). Their dimensions were 1.5×2×3 m (height, width and length) respectively, and their horizontal surface area and internal volumes were 6 m^2 and 4-5 m^3.

In windrows system three pyramidical shape piles were built up. 791 Kg on oven dry weight basis at 70 °C for 18 hours of each homogenized co-inoculated triad components mixtures namely [(O-RCS)+2); (ms-RCS)+2) and (mf-RCS)+2)] were moistened with fresh multi-moistening solution and their moisture contents were adjusted to 65% of W.H.C.

The multi-functional co-inoculants liquids of the chosen micro-organisms were applied by spreading proper volumes cultures (0.5% v/w) of these micro-organisms over the surface and between these layers. After the piles were built up, they were thoroughly, covered with plastic sheets and kept under uncontrolled field conditions for aerobic co-composting for 4 months. Moisture content % of the co-composted materials within piles were directly measured *in situ* using TDR apparatus (Time Domain Reflectometer) every two days, at which calculated volumes of multi-moistening solution must be done immediately for arriving at 50-60% W.H.C to overcoming the progressively decreases until maturation biophase during the whole aerobic co-composting period. The piles were moistened 2-3 times weekly during this summer co-composting period, moreover, during the turning-over bioprocesses.

For aeration-out, five turning-over bioprocesses were achieved at different intervals every two weeks during the whole aerobic co-composting bioprocesses after 20, 35, 50, 65 and 80 days from the initiation with keeping the moisture within the range of 50- 60 % W.H.C. along the composting process. Multi-moistening solution was added to obtained suitable moisture (50-60 % W.H.C).

Preparation and charging PVC-Bin-bioreactors

Aerobic fermentation of controlled co-composting bioprocesses was performed under laboratory-scale Bin barrels conditions as used by Hamoud (2001). Eight barrels were used for co-composting of different triad components mixtures. Barrels are PVC-cylindrical open plastic containers used as bioactivators. Their dimensions are 40 cm diameter, 95 cm depth as well the internal volume and cross-section area 120 liter and 1257 cm^2 respectively, obtained from local market. For realizing a good aeration, each barrel was provided with three cylindrical perforated plastic tubes having 4 cm diameter and 105 cm length and internal cross-section area 12.57 cm^2. These tubes were vertically fixed in each barrel 95 cm from its bottom and 10 cm above the surface. Each barrel was divided theoretically into four sections, each has 24 cm height and 30 L volume.

In Bin barrels system eight different types of homogenized co-inoculated and non-co-inoculated triad components mixtures were moistened with fresh multi-moistening solution and their moisture contents were adjusted to 65% W.H.C. These organic co-composted materials were mixed thoroughly immediately before charging into the eight barrels. The aerobic co-composting operational practicles were achieved just in the same manner for windrows piles system. These mixed moistening co-composted materials were used in three portions and charged into the barrels in three successive layers each (30 L volume and 24 cm height).

Each layer was provided with equal volumes of these materials and received the same suitable moistening solution. Moisture content was directly measured in situ using TDR apparatus as mentioned before.

Microbial inoculants liquids were applied by spreading proper volumes (0.5% v/w) on the surface and between the layers. Thenafter the barrels covered and kept under laboratory controlled conditions. The co-composted materials were agitated and turned-over five times during the co-composting bioprocesses for aeration-out the barrels, then recharged and recovered again as well as moisture contents were readjusted to 50-60 % W.H.C. at the same manner for piles co-composting.

Multi-functional microbial co-inoculation

Co-inoculation of multi-functional bacterial and fungal liquid inoculants was carried out during the aerobic co-composting bioprocesses at two co-composting biophases under Bin (barrels) and Windrows (piles) systems at the same time.

The first addition was achieved through the first mesophilic biophase (30 days incubation period) in two doses at the beginning initiation (zero time) and after 20 days (incubation period) during the first turning-over bioprocess.

The second application was done within the maturation biophase (the second mesophilic biophase) 25 days incubation period also at two doses after 105 and 115 days incubation periods from the initiation.

3:Microbiological methods of co-composts

Isolation, Purification, Enrichment and Identification of some most potent microorganisms

1. Cellulolytic microorganisms (cellulolases producing bacteria and fungi)

Representitative different composite samples from fertile soils, matured composts and natural decomposed

agricultural residues were collected from natural habitate in different locations at Kafr El-sheikh city. These samples were used for isolation of cellulolytic meso-and thermophilic bacteria and fungi in this present study. A loopful (hundred µl) of each dilution suspended solution was used to inoculate the surface of Mandel's medium onto avicel- or CMC agar plates. This medium (mineral salt agar) containing 1% carboxy methyl cellulose (CMC) or avicel as a sole source of carbon (Mandels *et al.,* 1976) (PH 7; for bacteria and PH 5; for fungi). Petri dishes were then incubated at 30 °C (3 days for mesophilic bacteria), at 50 °C (3 days for thermophilic bacteria), and at 30 °C (5 days for mesophilic fungi) to obtain bacterial and fungal growths.

Agar streak plate method was used for purification of bacterial and fungal isolates under investigation. All colonies of different forms and colors showing separate growth on cellulolytic agar medium were picked up and re-streaked onto the agar surface of plates of the same media. The plates were incubated at 30 °C (3 days for mesophilic bacteria), at 50 °C (3 days for thermophilic bacteria) and at 30 °C (5 days for mesophilic fungi). Distinct shape and color of single separate colonies were picked up and re-streaked again for several consecutive times onto the surface of agar plate to ensure its purity. Purity was checked up microscopically and morphologically using simple Gram's stain. Pure isolates were subcultured on slants of nutrient agar (NA) medium for bacteria and potatoes dextrose agar medium (PDA) for fungi and kept for further investigation at 4-5 °C. The purified colonies were prepared to be used for identification and preparation the microbial inoculants. The isolated colonies were maintained on respective media slants as described by Belal *et al*. (2014) and used by Elmasry (2017).

Detection of cellulolytic activity of the isolates

In this experiment, exoglucanase (avicel PH 101 substrate) commercial micro crystalline cellulose (Alpha Chemika) and endoglucanase (CMC substrate) [commercial carboxymethylcellulose amorphous cellulose, completely water-soluble polymer: Sigma C4888 medium viscosity (viscosity of 2% aqueous solution at 25 °C : 400-800 cps)] producing isolates were qualitatively investigated. Main fermentation broth media Mandel's medium (Mandels *et al.,* 1976) contained the main ingredients mineral salt medium (mineral medium) supplemented with cellulose substrate (1% CMC or avicel) separately as a sole carbon source, and 1.5% agar were used. The PH was adjusted at 7 for bacteria and 5 for fungi. The media were sterilized by autoclave at 121 °C and 1.5 atm for 20 minutes. Agar streak plate method was used for purification of bacterial and fungal isolates under investigation. Mandel's medium agar plates were prepared and spotted in the center with an isolates of cellulolytic micro-organisms as used by Alrefaey (2015) and Elmasry (2017).

After cooling at 45 C°, cellulolytic avicel and CMC agar media were distributed into sterile Petri dishes with equal amounts under aseptic conditions. The bacterial and fungal co-inoculants were used. Each plate was Inoculated with a loopful in its center with bacterial and fungal isolates. The plates were incubated at 30 °C (72 hours for mesophilic bacteria and fungi) and at 50 °C (72 hours for thermophilic bacteria) to obtain bacterial and fungal growth.

After incubation, endoglucanase and exoglucanase activities were visualized by flooding the avicel and CMC–agar plates with congo red dye C.I.22120; $C_{32}H_{22}N_6O_6S_2Na_2$ Mol.wt 696.7 Sigma C6767. So, for appearance of cellulolytic activity, plates were stained with 0.1% (w/v) congo red dye for 15 min., followed by de-staining with 1*M* NaCl solution for 13 to 20 min (Apun *et al.,* 2000). For cellulolytic activity observation, the appearance of clear zone around bacterial and fungal growth i.e. non-stained areas, where the CMC or avicel hydrolyzed, were measured in diameter and taken as a criteria for determing the exo- and endo-glucanases activity (Belal,2013).

Cellulolytic ratios (C.R) were calculated as:

$$\text{C.R} = \frac{Total\ diameter - Growth\ diameter}{Growth\ diameter} = \frac{Y-X}{X}$$ **for bacteria and** $\frac{Y}{X}$ **for fungi**

The most potent isolates, which showed the most efficient results were selected and maintained at 4-5 °C on CMC and avicel agar media slants for further experiments and used for multi-functional co-inoculation of the triad components mixtures under Bin and Windrows systems.

Characterization and identification of cellulolytic bacterial isolates.

Identification of the most potent cellulolytic bacterial isolates to species level was based mainly on 16 S rDNA sequence analysis according to Ausubel *et al*. (1987) and van Berkum and Fuhrmann (2000). This genetic characteristics was complemented by study, growth pattern and microscopic features morphological, physiological and recommended biochemical characteristics of microbial colonies and cells using the identification keys according to Parry *et al*. (1983) and Williams *et al*. (1989).

Morphological characterization growth pattern and microscopic features including colony appearance (colour, shape, elevation, edge, surface and optical features), of the colonies and cells appearance (Gram reaction of the colonies) shape and arrangement cells in smears of the purified bacterial isolates as described by Krieg and Holt (1984) and Cheesbrough (1991). Isolates fungi identified according to Alexopoulos and Mims (1979) and Domsch *et al*. (1980).

Gram reaction of the purified bacterial isolates was demonstrated using Gram's stain solution method was given as mentioned by Shushan *et al*. (1981) and Claus (1992).

Spore formation was investigated by flooding the bacterial slide using 5% aqueous malachite green and 0.5% aqeous safranin as described by Schaeffer and Fulton's (1933). The following recommended tests were carried out after activation the isolates by growing them overnight on nutrient agar (catalase and oxidase activities); gas from glucose; (nitrate reduction, methyl red, voges-proskauer, methyl red, citrate utilization, indole and 3% KOH solution.

Moleccular characterization of selected cellulolytic isolates

The identification method was performed by Polymerase Chain Reaction (PCR) at Sigma Scientific Services Co., Giza, Egypt and then 16 S rDNA gene sequencing technique for bacterial strains and 18 S rDNA for fungal strains by ABI 3730 × 1 DNA sequencer at GATC Company, Germany, using forward and reverse primers by combining the traditional Sanger technology with the new 454 technologies.

2. Isolation and purification of phosphate dissolving bacteria

Isolates of inorganic phosphate dissolving bacteria from the rhizosphere under the most economic field crops grown in Desoque and El-Hamoul districts at Kafr El-Sheikh, were isolated. A soil suspension of each rhizosphere (1:100 w/v) was prepared and inoculated in Bunt & Rovira agar medium (Bunt and Rovira, 1955) as modified by Abdel-Hafez (1966).

The isolated microorganisms were purified using the streaking technique on modified Bunt and Rovira medium. During purification, the most efficient and promising strains were chosen for further detailed studies (preparation the microbial inoculants). They were selected according to their capabilities to produce wide clear zones around their growth when streaked on modified Bunt & Rovira agar plates as used by Mahmoud (1999). The efficiency of the selected identified isolates of phosphate dissolving bacteria in solubilizing tri-calcium phosphate was measured in a liquid culture Bunt and Rovira modified by Abdel-Hafez (1966) and Mahmoud (1999).

3. Isolation and purification of silicate solubilizing bacteria (SSP)

Silicate and phosphate solubilizing bacteria can play an efficient role not only solubilizing insoluble forms silicates but also potassium and phosphates, hence increasing fertility and thereby enhancing plant productivity as reported by Han and Lee (2005); Han *et al*. (2006) and Tripti *et al*. (2017). The SSP bacterial isolates were isolated on standared

Zak-Alexandrov broth medium as described by Maleva *et al*. (2017).

The pure culture of silicate solubilizing bacteria (*Bacillus sp*.)was isolated from clay as a substrate, cultivated on Zak-Alexandrov agar medium by dilution plates technique under aseptic conditions. The typical silicate and phosphate solubilizing bacterial colonies were frequently examined. The isolated bacteria was purified using the streaking technique on Zak-Alexandrov agar medium. The most potent isolates from the isolated set of silicate dissolving bacterial were exposed to detailed examination. Pure typical colonies were picked up and restreaked again for several consecutive times onto surface of agar plates, to ensure its purity. Pure isolates were subcultured on slant Zak-Alxandrov agar medium.

The efficiency of the selected strains of SSB in solubilizing insoluble forms of silicates was measured in a broth cultures. The release of potassium by bacterial activity was followed during 18 days incubation period at 28 °C. Available-K and P as well as pH-values were determined periodically. Inoculated flasks were shaking incubated (150 rpm) at 28 °C for 7 days. Total count was estimated and its concentration was adjusted to (0.6×10^8 CFU ml^{-1}) using distilled water. Thereafter, it was added to the co-composted materials as microbial liquid culture at rate of 0.5% (v/w) to accelerate the decomposition of silicates rate and improve the potassium status during and after the aerobic co-composting bioprocesses barrels and heaps conditions.

4. Isolation and purification of Azotobacter species

Non- symbiotic-N_2 fixer *Azotobacter sp.* was isolated from fertile soils, composted materials in different stages. Isolation was done on modified Ashby's Mannitol Phosphate Broth (Ashby's M.P.B) medium as described by Abd El-Malek and Ishae (1968) by dilution plates technique. Typical Azotobacter colonies were frequently examined. Typical mucoid colonies were isolated onto the appropriate medium tested for purity after 48 hours by making lugol hanging drop preparations and Gram stain smears.

Pure typical mucoid colonies were picked up and restreaked again for several consecutive times onto the surface of agar plates to ensure its purity. The isolated strains were stocked on (Ashby's M.P.B) medium slants for further studies. Total count was estimated and its concentration was adjusted to (10^8 CFU ml^{-1}) using distilled water. Thereafter, it was applied to the co-composted materials as liquid microbial Azotobacter inoculant at rate of 0.5% (v/w) to improving available nitrogen status during and after the aerobic co-composting bioprocesses under barrels and piles conditions.

Counting of the microbial populations

To enumerate the micro-organisms in co-composted materials during the aerobic co-composting bioprocesses, under Bin (barrels) and Windrows (piles) systems, total viable counts of bacteria and fungi were determined using plate count method as described by (Nakasaki *et al.,* 1992). Nutrient agar (NA) and potato dextrose agar (PDA) media were used for bacteria and fungi respectively. Homogenized composite co-composted materials were weekly collected in duplicates from 4 regions within barrels or piles. These regions are high temp., anaerobic lower, cool lower and cool outer regions. The incubation temperatures were 30 °C for isolation of mesophiles (3 days) and 50 °C for thermophiles (2 days) as well 7 days for fungi mesophilic and thermophilic. Microbial counts were calculated on the oven dry weights basis.

Multi-functional basic effective micro-organisms (EM)

In this investigation, commercial activated EM (EM.1) which contains a mixture of lactic acid bacteria, yeast, actinomycetes , fungi and phototrophic bacteria, was used.

Photosynthetic bacteria: include the main species as *Rhadopseudomonas plustris* (ATCC 17001), *Rhadobacter sphacrodes* (ATCC 1723).

Lactic acid bacteria: *Lactobacillus plantaru* (ATCC 8014), *Lactobacillus easei* (ATCC 7469), *Streptococcus lactis* (IFO 12007).

Yeasts: *Saccharomyces cerevisiae* (IFO 0203).

Multi-functional effective micro-organisms locally produced by Afforestation and Environment Administration, Ministry of Agriculture, Egypt, in association with (EMRO) Organization Okinawa, Japan. The basic EM solution is required for the production of EMAS. EMAS is actually an activated EM suspension in a mixture of molasses (sugar cane) and non-chlorinated water or rice rinse water (which provides the minerals for the multiplication of the microorganisms. For the activation of EM.1 one part EM.1 microbial inoculants and one part of molasses were mixed with 20 parts of chlorine –free water. This solution was then stored for three to five days in an air tight expandable container for fermentation.

Moistening-solution was then added until the moisture content reached 60% W.H.C. in each composting mixture (Rashad *et al.,* 2010 and Jusoh *et al.,* 2013). To retain the moisture and prevent excessive loss of heat, the heaps of co-composting materials were then covered using plastic sheets.

Multi-moistening-solution:

For preparation multi-extracts solution, different kinds of water extracts were obtained and used to moistening co-composted materials immediately after each turning-over and different intervals during the co-composting process, which elongated 120 days. These water extracts were:

1. Fresh cattle manure-water extract (1:5 w/v).
2. Fresh chicken manure-water extract (1:5 w/v).
3. Cultivated fertile luxuriant soil-water extract (1:5 w/v).

These extracts were mixed (1:1:1 volumetrically) to obtain the fresh multi-moistening solution.

Detection of pathogenic bacteria

Total-bacterial coliform numbers were determined by the Most Probable Number (MPN) according to: standard methods of APHA, AWWA and WEF (1998) as used by Kocaer *et al*. (2004) using brilliant green bile broth. The pH value was adjusted at 7.1 before sterilization. The medium was sterilized at 121 °C for 20 min.

Results were recorded as logarithmic total coliform numbers (log MPN.100 ml^{-1}) of different co-composted materials. Representative subsamples were in duplicates collected daily from the same three selected multi-functional co-inoculated co-composted materials (a, b and c) from barrels or piles within 4 regions of each barrel or pile. [See notes Table (6)].

Evaluation of co-composts hygiene

Occurence, growth of *Salmonella* and *Shigella*

Concerning pathogen destruction during the co-composting processes, the relative efficiencies of the co-composting bioprocesses were evaluated using *Salmonella* and *Shigella* sp as a biological indicator. The isolation method was carried out on the finished matured co-composts only. In this method, matured co-compost samples were suspended in buffered peptone water by 1:10 (w/v) and shaken for 15 min.

Ten fold serial dilutions were made in buffered peptone water and 0.1-ml from each dilution was spread on sterilized Salmonella-Shigella agar medium (Bess, 1999 and Bess *et al.,* 2002). The incubation time was 3 days at 37 °C as reported by Hussong *et al*. (1984 and 1985) and used by Khalil (1996). Representative subsamples were daily collected in duplicates from the same three selected co-composted materials (a, b and c) from each barrel or pile within 4 regions.

3.4:Biochemical methods of co-composts

Enzymes activities of co-composted materials

The activities of the selected dominant enzymes in different co-composted materials and their matured co-composts during the co-composting bioprocess were assayed at their optimal pH and temp. in oven dry equivalents weights of field-moist samples by the methods described by Tabatabai (1994); Nannipieri (1995) and Nannipieri *et al*. (1996).

Dehydrogenase enzymes activity:

Dehydrogenase activity (high specific) was determined using the procedure reported by Tabatabai (1994). This method involves colorimetric determination of 1, 3, 5-triphenyl tetrazolium formazan (TPF) produced by the reduction of 2, 3, 5- triphenyl tetrazolium chloride (TTC) by the dehydrogenases. TPF was extracted after incubation at 37 °C and pH 8 for 24 hours by methanol CH_3OH. Results were recorded as µg 1, 3, 5-triphenyl tetrazolium formazan produced (or H_2 consumed) per gram co-composted materials per one hour at 37 °C and pH 8.

β-glucosidase activity (EC 3.2.1.21)

This enzyme catalyzes the hydrolysis of β-D-glucopyranosides. The principle method is based on the colorimetric determination of ρ-nitrophenol (PNP) released and detection by β-glucosidase when the samples of co-composted materials are incubated at 37 °C for 90 min. with buffered 0.05 *M* pH 6 of ρ-nitrophenyl β-D-glucopyranoside solution (PNG, 0.05 *M*) [$C_{12}H_{15}NO_8$, mol. wt. 301.3 Sigma (N 7006)] as chromogenic substrate and toluene (Eivazi and Tabatabai, 1990). The reaction was stopped with tris-hydroxymethyl aminomethane THAM according to Tabatabai (1982). The amount of PNP was determined by spectrophotometry at 398 nm. β-glucosidase activity was expressed as µg PNP released.$Kg^{-1}_{d.w}$ co-compost. $hour^{-1}$.

Endoglucanase activity (EC 3.2.1.1.4)

Carboxymethyl-cellulase activity was determined according to the method described by Schinnar and von Mersi (1990). The determination was carried out via the substrate solution of CM-cellulose sodium salt (0.7% w/v in 2 *M* acetate buffer). The reaction was performed at pH 5.5 and 50 °C for 24 hours. The reducing sugars (as glucose equivalents) were measured colorimetrically after reaction a potassium ferric hexa-cyanide reagent.Activities of endoglucanase are expressed as: [µmoles glucose equivalents.$g^{-1}_{d.w}$ co-compost. $(24\ hours)^{-1}$] at 50 °C pH 5.5.

Generally, controls were made in the same ways, without the substrates. All the obtained results were calculated on dry weights basis at 70 °C for 18 hours. The selected predominant enzymes activities were determined weekly in duplicates at different intervals during the aerobic co-composting bioprocesses period and also at the terminal matured co-composts at the end of co-composting elongated 120 days from 4 regions within heaps or barrels and tabulated as an average of 2weeks.

3.5:Physio-chemical characterizations of the co-composted materials

Composite samples of the selected cheapest organic residues were collected, air-dried for three days, then oven-dried at 70 °C for 18 hours, grounded and pulverized with stainless steel mill to pass through 0.2 mm sieve screen.

Total organic-C% of the selected organic residues and their triad components mixtures was determined according to the wet combustion as described by Nelson and Sommers (1982). Diluted wet digested acidic solutions were prepared as described by Carter (1993).

Total-N% was determined according to Bremner and Mulvaney (1982). Total-P% was estimated as described by Olsen and Sommers (1982). Total-K% was determined as reported by Knudsen *et al*. (1982) and Simard (1993). Total-S% was determined as described by Tabatabai (1982) and Guthrie and Lowe (1984) respectively. Their C/N, C/P and C/S ratios were calculated.

Available-N, available-P and available-K as mg nutrient.$Kg^{-1}_{d.w}$ organic residues were determined as described by Keeney and Nelson (1982); Olsen and Sommers (1982); Knudsen *et al*. (1982) and Simard (1993) respectively. The obtained results were calculated on oven dry weights basis at 70 °C for 18 hours.

The moisture content was frequently checked. Before moistening the co-composted materials, the calculated volumes of applied multi-moistening –solutions must be done immediately for arriving first at 65% W.H.C. Thenafter was measured *in situ* using TDR apparatus (Time Domain Reflectometer). This apparatus enable us to measure accurately the moisture content *in situ* of co-composted materials, at limit timing of moistening. The quantity of deficit moistening-solutions must be applied to reach its 60% W.H.C was estimated throughout the co-composting period by frequent checking. The moisture level optimal for biological activity is usually 50-70% of W.H.C.

pH-values of the co-composted materials

pH- values of three different types of triad components mixtures (a, b and c) and their matured co-composts throughout the aerobic co-composting bioprocesses were immediately measured in 1:10 w/v co-composted materials : water suspension using single probe combination pH electrode (Beckman pH meter) and standard buffer solutions of pH (4, 7 and 12) as described by Rhoades (1982). Representative subsamples were weekly collected in duplicates from three-activated, multi-functional co-inoculated co-composted materials (a, b and c) from each pile within 4 regions as mentioned before.

Temperature-degrees observation of the co-composted materials

Temperature-degrees observation of the selected multi-functional co-composted and non-co-composted (a, b and c) materials were daily measured under Bin (barrels) and Windrows (piles) conditions from four regions within these systems under the surface area, at the center and bottommost parts of barrels and heaps, using metal probe thermometers (1-100°C) and digital thermometers according to Poincelot and Day (1973) throughout the co-composting bioprocesses period. These 4 regions represent the variation of thermal conditions within the co-composting heaps or barrels. These regions are high temperature, cool outer, cool lower regions and anaerobic lower region. Ambient air temperature degrees around barrels and piles were daily measured three times (mornings, afternoon and evenings). Frequent five turning-over were achieved at different intervals (15 days)during the aerobic co-composting bioprocesses after 20; 35; 50; 65 and 80 days from the initiation.

RESULTS AND DISCUSSION

Degradative ability of the dominant cellulolytic isolates

The ability of bacterial and fungal isolates to produce cellulolytic glucanohydrolases [endoglucanase (CMC-medium) and exoglucanase (avicel-medium)] were examined. Inoculated (9 cm) plates were incubated at 30°C and 50°C for mesophilic and thermophilic microorganisms respectively. The incubation period elongated to 72 hours for the detection of endo- and exocellulases activity. The growth diameter (X) of each colony and that of hydrolyzed area (clear zone diameter (y) were scored. The cellulolytic ratio ($C.R=\frac{y}{x}$) was then taken as an indication for the ability of these isolates to hydrolyze the tested substrates (see page 8).

The degradative abilities of the studied bacterial and fungal isolates as detected by hydrolysis of various cellulase substrates (avicel and carboxymethylcellulose) are shown in Table (2 and 3). It was clearly apparent as delineated in these Tables, that all the mesophilic and thermophilic bacterial isolates were able to hydrolyze the tested substrates (avicel and CMC). Also, the mesophilic fungal isolates have the greatest degradative ability to hydrolyze these selected substrates. However, these bacterial and fungal isolates have differed in their hydrolytic abilities.

These isolates were qualitatively determined as cellulase producers by showing a zone of clearance onto surface of avecil and carboxymethylcellulose agar plates, flooded with congo red dye (C.I 22120) or hexadecyltrimethylammonium bromide ($C_{32}\ H_{22}\ N_6\ O_6\ S_2\ Na_2$ Mol wt. 696.7 Sigma C6767) or Gram's iodine solution, after incubation period, indicating enzymes production. Concerning relative ability of the predominant mesophilic and thermophilic bacterial isolates to hydrolyze CMC onto agar medium, the obtained results listed in Table (5) show that 25 bacterial isolates were primary selected as the most potent cellulases bacterial producers. Of those 20 mesophilic and 5 thermophilic bacterial isolates. These isolates showed endoglucanase activity on CMC-agar medium and also revealed exoglucanase activity on avicel-agar medium at 30° and 50°C respectively.

Table 2. Relative ability of the dominate mesophilic and thermophilic bacterial isolates to hydrolyze carboxymethylcellulose (endoglucanase) and avicel (exoglucanase) using the substrates CMC and avicel media

Isolates		CMC medium (Endoglucanase)		Cellulolytic ratio (C.R)	Avicel medium (Exoglucanase)		Cellulolytic ratio (C.R)
No.	code	Growth diameter (X) (cm)	Total diameter (Z) (cm)		Growth diameter (X) (cm)	Total diameter (Z) (cm)	
Mesophilic Bacteria							
1	B1	1.3	3.1	1.4	0.4	1.5	2.6
2	B2	1.8	2.7	0.5	0.8	2.1	1.8
3	B3	1.4	2.7	0.9	0.9	2.8	2.0
4	B4	1.2	3.0	1.5	0.8	2.5	2.8
5	B5	0.5	2.3	3.6	0.4	2.7	5.8
6	B6	0.5	2.0	3.0	0.7	2.3	2.3
7	B7	1.2	2.8	1.3	1.0	2.0	2.0
8	B8	1.5	3.0	1.0	0.5	2.0	3.0
9	B9	1.2	2.6	1.2	0.4	2.2	4.0
10	B10	0.8	2.2	0.8	0.4	2.0	4.7
11	B11	1.5	2.0	0.3	1.5	2.3	0.5
12	B12	0.5	2.0	3.0	0.5	1.7	2.4
13	B13	1.0	2.3	1.3	0.5	2.4	3.8
14	B14	1.3	2.1	0.6	0.7	1.0	0.4
15	B15	1.5	2.5	0.7	0.5	1.8	2.6
16	B16	1.1	3.2	1.9	0.7	1.7	1.3
17	B17	1.7	2.4	0.4	1.2	2.4	1.0
18	B18	1.0	3.1	2.1	1.1	2.5	1.3
19	B19	0.7	2.0	1.9	0.6	2.4	3.2
20	B20	0.9	2.3	1.6	0.7	2.0	1.9
Mean		1.03	2.52	1.45	0.6	2.11	2.46
Thermophilic Bacteria							
1	S1	0.6	3.0	4.0	0.4	2.6	5.5
2	S2	0.9	2.5	1.8	1.2	3.5	1.9
3	S3	1.8	2.7	0.5	1.5	3.0	1.0
4	S4	1.7	3.1	0.8	0.8	3.0	2.8
5	S5	0.5	1.6	2.0	0.7	2.8	3.0
Mean		1.92	2.58	1.82	0.77	2.98	2.87

Notes:1. Values are the means of three replicates in incubated (9 cm) plates.

2. x means: Growth diameter (cm) z means: Total diameter y means: Clear zone diameter (z-x)

3. Cellulolytic ratio (C.R)= $\frac{\text{Clear zone diameter (cm)}}{\text{Growth diameter(cm)}}$ **C.R for bacteria=** $\frac{z-x}{x}$**, for fungi =** $\frac{Y}{X}$

Table 3. Relative ability of the dominate mesophilic fungal isolates to hydrolyze carboxymethylcellulose (endoglucanase) and avicel (exoglucanase) using the substrates CMC and avicel media

Isolates		CMC medium (Endoglucanase)		Cellulolytic ratio (C.R)	Avicel medium (Exoglucanase)		Cellulolytic ratio (C.R)
No.	Code	Growth diameter (X) (cm)	Clear zone diameter (Y) (cm)		Growth diameter (X) (cm)	Clear zone diameter (Y) (cm)	
1	F1	1.0	3.0	3.00	1.1	3.7	3.36
2	F2	1.3	2.8	2.15	0.9	3.5	3.89
3	F3	1.7	3.0	1.76	1.2	3.4	2.83
4	F4	1.1	2.1	1.91	1.1	2.9	2.64
5	F5	1.3	3.0	2.31	1.2	3.8	3.17
6	F6	0.8	3.5	4.38	0.7	3.8	5.43
7	F7	1.1	3.3	3.00	1.3	2.7	2.08
8	F8	1.2	2.8	2.33	0.8	3.3	4.13
9	F9	1.0	2.6	2.60	1.2	4.1	3.42
10	F10	1.3	3.2	2.46	1.3	4.2	3.23
11	F11	1.7	2.1	1.24	1.9	2.8	1.47
12	F12	1.5	3.2	2.13	1.0	3.1	3.11
13	F13	1.3	2.6	2.00	1.3	2.5	1.92
14	F14	1.3	2.8	2.15	1.1	3.4	3.09
15	F15	1.1	2.5	2.27	1.2	3.6	3.00
Mean		1.19	2.83	2.38	1.08	3.39	3.13

On the average conditions, the relative ability of the predominant mesophilic bacterial isolates to produce and hydrolyze avicel (exoglucanase) C.R 2.46 in avicel-agar medium were greater than those obtained in carboxymethylcellulose-agar medium (endoglucanase) C.R 1.45 (average 20 isolates). It is noteworthy to say that, mesophilic bacterial isolate (B5) exhibited the highest CMCase and avicelase activities at 30˚C, since their C.R values were 3.6 and 5.8 respectively. Meanwhile, the isolate (B11) realized the lowest C.R values over all the mesophilic isolates, which C.R values were 0.3 (CMCase) and 0.5 (avicelase) respectively.

In regard to the thermophilic bacteria, the isolate (S1) realized the highest cellulolytic ratios (C.R) 4.0 and 5.5 (average 5 isolates) for CMCase and avicelase, meanwhile the isolate (S3) revealed the lowest values 0.5 and 1.0 respectively.

Regarding relative ability of the predominant mesophilic fungal isolates to hydrolyze CMC (endoglucanase) and avicel (exoglucanase), data listed in

Table (6) reveal that 15 fungal isolates were preliminary chosen as the most potent cellulases fungal producers. These fungal isolates showed also exoglucanase and endoglucanase activities on avicel- and carboxy methylcellulose media at 50˚C. Commonly, relative ability of the dominant thermophilic bacteria were displayed the highest CMCase and avicelase activities at 50˚C, since C.R 1.82 and 2.87 respectively in comparison with those obtained by mesophilic bacteria which C.R were 1.45 and 2.46 respectively. It is obviously that, mesophilic fungal isolate (F6) realized the highest CMCase and avicelase activities since, (C.R) values 4.38 and 5.43, overall the mesophilic fungal isolates. However, mesophilic fungal isolate (F11) pronounced the lowest CMCase and avicelase activities, which C.R values were 1.24 and 1.47 respectively.

Relative ability of the predominant mesophilic fungal isolates to hydrolyze CMC and avicel were higher than those obtained from mesophilic and thermophilic bacterial isolates.One of the most important purposes of the present research was to isolate; characterize; purify and identify potential cellulase-producing bacterial and fungal strains for efficient utilization of plant biomasses and application of these strains in co-composting bioprocesses of some treated agricultural organic cheapest residues in order to shorten the co-composting maturation time and increase the product quality.

Accordingly, the mesophilic bacterial isolate (B5) and the thermophilic bacterial isolate (S1) as well as the mesophilic fungal isolate (F6) exhibited the highest avicelase and CMCase activities over all isolates, indicating their potential as a good source for endoglucanase and exoglucanase production. These isolates were selected for further studies.

Identification of the most efficient cellulolytic microorganisms

(1) Morphological and biochemical characterizations:

Bacterial isolates characteristics

Two dominant bacterial isolates which showed the highest cellulolytic activities were subjected to Gram staining, morphological, physiological and biochemical identification tests according to Bergey's Manual Parry *et al*. (1983); Williams *et al*. (1989) and Cheesbrough (1991).

Colonies and cells morphology: The preliminary properties of the selected isolates showed the following results.

Colonies appearance included the following properties: colour (white yellow); shape (circular); elevation (flat); edge (entire); surface (smooth) and optical features (translucid).

Cells appearance: the preliminary properties of the selected predominant isolates displayed negative reaction with 3% KOH test. Therefore all strains were Gram-positive bacteria and their cell shapes were bacilli (rods) with endospores as well as single arrangements.

Biochemical characteristics:

All isolates were catalase positive bacteria indicating their aerobic respiration and have the opposite behaviour with oxidase. All the selected isolates displayed positively results with the following tests: methyl red; gelatin, nitrate reduction, starch hydrolysis and citrate utilization. Oppositely, the isolates have negatively results with Voges-Proskauer and indol tests.

These characteristics are coefficient with that of the genus *Bacillus* sp. (proposed genus). According to Fermor *et al*. (1979); Miller (1992) and Khalil (1996) bacterial genera reported commonly during composting include *Bacillis* and *Pseudomanas*.

Fungal isolates characteristics

The fungal isolates were identified based on the microscopic characters which were assessed after staining with lactophenol cotton blue. The morphological characteristics of the most efficient fungal isolates were recorded as described by Alexopoulos and Mims (1979), Domsch *et al*. (1980) and Fawole and Oso (1988). The colonies colors were observed by naked eye on the surface of agar plates. The mycelia colors of colonies were white at the edge in early culture stages then became pitchy at the head of mycelium of fungal isolate (F6) and green for fungal isolate (F4) and the mycelia colors of colonies were white in the reverse side in agar plate.

The dominant fungal isolates were found to belong to the genera *Trichoderma*, *Aspergillus* and *Penicillium* as proposed genera. According to Fermor *et al*. (1979) fungal genera reported commonly during composting include *Aspergillus; Penicillium; Trichoderma* and *Mucor*. Large number of different species of fungi has been reported for composted materials.

(2) Molecular characterizations of selected cellulolytic isolates:

The identification method was performed by Polymerase Chain Reaction (PCR) and carried out at Sigma Scientific Services Co., Giza, Egypt, and then 16S rDNA gene sequencing technique for bacterial strains and 18S rDNA for fungal strains by ABI 3730×1 DNA sequencer at GATC Company, Germany, using forward and reverse primers by combining the traditional Sanger technology with the new 454 technologies. The phylogenetic analyses were conducted in MEGA6 (Tamura *et al.,* 2013).Phylogenetic analysis of bacterial strains (B5 and S1) with related species according to 16S rDNA gene sequence revealed that the bacterial strain (B5) was most closely related to *Bacillus nakamurai* strain NRRL B-41091 16S ribosomal RNA gene with 90.3% [(identities 485/537) and (0% Gaps 1/537)] sequence similarity and (S1) was most closely related to *Anoxybacillus rupiensis* strain R270 16S ribosomal RNA gene with 98.2% [(identities 703/720) and (0% Gaps 7/720)] sequence similarity. Phylogenetic analysis of fungal strain (F6) with related species according to 18S rDNA gene sequence revealed that the fungal strain (F6) was most closely related to *Trichoderma viride* strain with 98% sequence similarity.

Dynamic changes in temperature degrees of the co-composts during aerobic co-composting bioprocesses.

Temperature is an important factor that influences the enzyme production and activity (Pandey, 2003; Immanuel *et al.,* 2006 and Alrefaey, 2015). Temperature of the substrates solid state fermentation process is critically affects the growth of the microorganisms; germination, spore formation and end product formation.

Regarding fluctuations in temperature degrees (Temperature profiles C˚) of both multi-functional co-inoculated and non-co-inoculated co-composted materials during aerobic co-composting bioprocesses within controlled Bin (barrels) and uncontrolled Windrows (piles) conditions as well as ambient air temperatures around barrels and piles systems as affected by the co-composting biophases and their incubation periods (days), the obtained results listed in Table (4) and illustrated graphically in Fig.(1), clearly demonstrate that, four typical co-composting biophases were monitored throughout the aerobic co-composting period elongated 120 days as follows: short mesophilic (30days) followed by thermophilic (50 days), cooling (15 days) and maturation 25 days biophases. Tabulated data reveal also, on average conditions of all other studied parameters that, these fluctuations in temperature-degrees in the course of co-composting period (120 days) could be sequenced in the following arrangement as:

Barrels (a+b+c)	**Ambient Temp. 23**	**thermophilic biophase 43.5**	**>**	**cooling biophase 34.5**	**>**	**mesophilic biophase 28**	**>**	**Maturity biophase 26**	**General grand mean (G.G.M) 33**
Piles (a+b+c)	28.5	55	>	36	>	34	>	25	37.6
Grand mean	25.75	49.25	>	32.25	>	31	>	25.5	35.3

Table 4. Fluctuations in temperature degrees of both multi-functional co-inoculated and non-co-inoculated co-composted materials during aerobic co-composting bioprocesses under controlled Bin (barrels) and uncontrolled Windrows (heaps) conditions as well as ambient air temperatures.

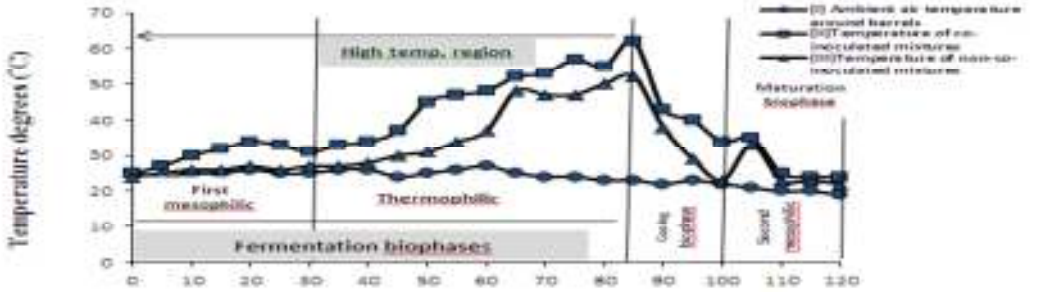
Co-composting		Barrels (Bin system)			Heaps (Windrows system)			Co-composting biophases	
Incubation period (days)	Bioprocesses	Ambient air temperature (I)	Co-Inoculated (II)	Non-co-inoculated (III)	Ambient air temperature (I)	Co-inoculated (II)	Non-co-inoculated (III)		
			Mean of (a)+(b)+(c)			Mean of (a)+(b)+(c)			
0		25	25	24	30	30	30		
5		24	27	25	31	32	31		
10		25	30	26	30	35	31		
15		25	32	26	32	37	32		
20	Turning over (1)	26	34	27	33	38	33	30 days Mesophilic biophase	Fermentation biophase (80 days)
25		25	33	26	32	33	28		
30		25	31	27	33	47	36		
Mean		25	30	26	32	36	32		
Grand mean			28			34			
35	Turning over (2)	26	33	27	34	50	38		
40		26	34	28	33	56	47		
45		24	37	30	31	53	46		
50	Turning over (3)	25	45	31	30	53	47		
55		26	47	34	32	55	49		
60		27	48	37	34	60	53		
65	Turning over (4)	25	52	48	32	60	56	50 days Thermophilic biophase	
70		24	53	47	31	58	57		
75		24	57	47	30	60	57		
80	Turning over (5)	23	55	50	28	65	50		
85	Higher Temp.	23	62	52	28	75	60		
Mean		25	48	39	31	59	51		
Grand mean			43.5			55			
90		22	43	38	27	55	45		
95		23	40	29	27	34	27	15 days cooling biophase	
100		22	34	23	26	30	25		
Mean		22	39	30	27	40	32		
Grand mean			34.5			36			
105		21	35	33	25	35	27		
110		20	25	23	24	25	25		
115		20	24	23	23	23	22	25 days Maturation biophase	
120	Lower Temp.	19	24	22	22	22	20		
Mean		20	27	25	24	26	24		
Grand mean			26			25			
General grand mean (GGM)		23	33		28.5	37.6			

Notes: 1. Each value due to (I) is a mean of 15 variables (1 × 3 times × 5 days).

2. Each figure due to (II), (III) is a mean of 60 variables [1 × 4 regions × 3 (barrels or piles) × 5 days].

3. The four regions which representing the thermal conditions in heaps and barrels are: high temperature region, anaerobic lower region, cool lower region and cool outer region as mentioned elsewhere.

4. Four typical biophases of the co-composting bioprocesses were monitored throughout the aerobic co-composting period elongated 120 days: short mesophilic (30 days); followed by thermophilic (50 days); cooling (15 days) and maturation (25 days) biophases.



Temperature profile under Windrows (piles) system (non-controlled conditions)

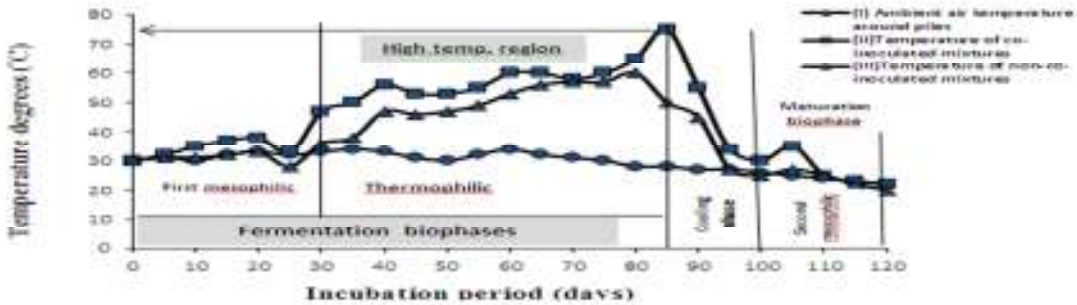


Fig. 1. Alterations in temperature degrees (temperature profiles) of selected three co-inoculated and non-co-inoculated triad components mixtures throughout the aerobic co-composting bioprocesses elongated 120 days within Bin (barrels) and Windrows (piles) systems. Temperature profile under Bin (barrels) system (controlled conditions)

Notes: The selected three triad components mixtures (RCS mixtures) were as the following:

1. Organic activated (O-RCS mixtures) include co-inoculated O-RCS+2 mixtures (two phases) and non-co-inoculated O-RCS+1 mixtures (one phase).

2. Mineral fast release activated (mf-RCS mixtures) include co-inoculated mf-RCS+2 mixtures (two phases) and non-co-inoculated mf-RCS+1 mixtures (one phase).

3. Mineral slow release activated (ms-RCS mixtures) include co-inoculated ms-RCS+2 mixtures (two phases) and non-co-inoculated ms-RCS+1 mixtures (one phase).

The co-composting bioprocesses were carried out under Bin (barrels) and Windrows (piles) systems.

As concerns dynamic fluctuations in temperature-degrees of different activated co-composted materials at various stages in the course of aerobic co-composting bioprocesses as affected by the co-composting fermentation systems, data show, on average conditions that, temperature-degrees differed with the co-composting conditions under Bin (barrels) and Windrows (piles) systems. The differences could be arranged in the following sequences:

General grand mean (G.G.M) of temp. degrees of activated RCS mixtures under Windrows (piles) system		General grand mean (G.G.M) of temp. degrees of activated RCS mixtures under Bin (barrels) system
37.6 °C	>	33 °C

Concerning oscillations in the temperature-degrees (C°)of the activated co-composted materials at various stages in the course of aerobic co-composting bioprocesses within Bin (barrels) and Windrows (piles) systems in comparison with ambient air temperatures (outside) around barrels and piles systems as affected by multi-functional microbial co-inoculants, data listed in Table (5) and illustrated graphically in Fig.(2) display, on average conditions that, generally, multi-functional co-inoculated, activated (a+b+c) RCS+2 mixtures (two phases) realized higher temperature-degrees than those obtained by non-co-inoculated, (a+b+c) activated RCS+1 mixtures in comparison with the ambient air temperatures. These changes could be arranged in the following arrangements as:

Barrels (a+b+c)	Ambient Temp. 23	Temperature-degrees °C of multi-functional co-inoculated, activated RCS+2 mixtures (two phases)		Temperature-degrees °C of multi-functional non-co-inoculated, activated RCS+1 mixtures (one phase)	General grand mean (GGM)
		36 °C	>	30 °C	33
Piles (a+b+c)	28.5	40.3 °C	>	34.8 °C	37.6
Grand mean	25.75	38.15 °C	>	32.4 °C	35.3

In this connection, analytical data also elucidate, on average conditions that, temperature-degrees of co-inoculated RCS activated mixtures registered the highest degrees in comparison with the ambient temperature at the thermophilic biophase after 85 days within barrels and piles systems from the co-composting initiation as the following arrangements:

Barrels (a+b+c)	Ambient Temp. 23	Temperature-degrees °C co-inoculated RCS+2 mixtures (two phases)		Temperature-degrees °C non-co-inoculated RCS+1 mixtures (one phase)
		62 °C	>	52 °C
Piles (a+b+c)	28	75 °C	>	60 °C
Grand mean	25.5	68.5 °C	>	56.0 °C

However, at the end of maturation biophase, temperature-degrees of non-co-inoculated RCS activated mixtures registered the lowest degrees after 120 days within barrels and piles from the co-composting initiation as the following sequences:

Barrels (a+b+c)	Ambient Temp. 19	Temperature-degrees °C non-co-inoculated RCS+1 mixtures (one phase)		Temperature-degrees °C co-inoculated RCS+2 mixtures (two phases)
		22 °C	<	24 °C
Piles (a+b+c)	22	20 °C	<	22 °C
Grand mean	20.5	21 °C	<	23 °C

Concerning dynamic changes in temperature-degrees of different activated co-composted materials at various stages during the aerobic co-composting bioprocesses as affected by the (a, b and c) triad components mixtures types, data reveal, on average conditions of all selected parameters that, temperature-degrees differed obviously with the types of activated co-composted materials. These differences could be arranged in the following sequences as:

	Temperature grand means					General grand Mean (GGM)
	O-RCS mixtures (a)		ms-RCS mixtures (b)		mf-RCS mixtures (c)	
Barrels (a+b+c)	36.1	>	34.0	>>	29.9	33
Piles (a+b+c)	43.8	>	37.6	>>	31.3	37.6
Grand mean	39.95	>	35.8	>>	30.6	35.3

Dynamic fluctuations in pH–values of the co-composts during aerobic co-composting bioprocesses

pH is one of the most important environment parameters affecting cell growth, enzymes, respiration activities and production. The pH values of the fermentation medium plays a critical role for the optimal physiological performance of the microbial cell and transportation of various nutrient components across the cell membrane aiming at maximizing the enzyme yields (Bacha *et al.,* 2013).

The effects of pH on the cell growth and product formation vary not only with the microbes but also with the operation conditions (Hu *et al.,* 2006).

Considering dynamic changes in pH–values (pH–profile) of different activated co-composted materials at various stages in the course of aerobic co-composting bioprocesses within three selected co-inoculated and non-co-inoculated triad components mixtures (a, b and c) [see footnotes Fig.(1) as mentioned elsewhere] under Windrows (piles) conditions as affected by the four co-composting biophases and their incubation periods (days), data displayed in Table (5) and illustrated graphically in Fig.(2), elucidate, on average conditions of all studied parameters that, pH–values varied markedly with the co-composting biophases. So, the grand means changes of pH–values could be arranged in the following descending order as:

Control	thermophilic biophase		cooling biophase		Maturity biophase		mesophilic biophase	General grand mean (G.G.M)
	8.7	>	8.0	>	7.15	>	6.0	7.5
7.1	more alkaline conditions						more acidic conditions	terminal pH-value

Table 5. Changes in pH values of both multi-functional co-inoculated and non co-inoculated co-composted materials throughout the aerobic co-composting bioprocesses under Windrows (piles) conditions.

Co-composting		Heaps (Windrows system)		Co-composting Biophases	
Incubation period (days)	Bioprocesses	Co-inoculated activated RCS mixtures (II)	Non-co-inoculated activated RCS mixtures (III)		
		Mean of (a)+(b)+(c)			
0		6.3	7.0	30 days Mesophilic biophase	Fermentation biophase (80 days)
5		5.5	6.7		
10		5.2	6.4		
15		Lower pH 5.1	6.2		
20	Turning over (1)	5.3	6.2		
25		5.4	6.3		
30		5.8	6.7		
Mean (1)		5.5	6.5		
Grand mean		6.0			
35	Turning over (2)	6.5	7.4	50 days Thermophilic biophase	
40		7.5	7.8		
45		8.2	8.2		
50	Turning over (3)	9.0	8.5		
55		9.2	8.8		
60		9.7	9.0		
65	Turning over (4)	High pH 9.8	9.1		
70		9.5	9.0		
75		9.4	9.0		
80	Turning over (5)	9.7	8.6		
85		8.7	8.5		
Mean (2)		8.9	8.5		
Grand mean		8.7			
90		8.2	8.4	15 days cooling biophase	
95		7.8	8.2		
100		7.5	8.0		
Mean (3)		7.8	8.2		
Grand mean		8.0			
105		7.3	7.5	25 days Maturation biophase	
110		7.0	7.3		
115		6.9	7.2		
120		6.8	7.1		
Mean (4)		7.0	7.3		
Grand mean		7.15			
General grand mean (GGM)		7.3	7.7	average 7.5	

Notes:
1. **pH values of different co-composts types were weekly estimated in 1:10 w/v (1 gm of composted materials: 10 ml of distilled water) during the aerobic co-composting bioprocesses prolonged 120 days.**
2. **Each pH-value is a mean of 48 variables (1× 2 replicates× 4 regions× 3 piles×2 weeks).**
3. **The four regions which representing the thermal conditions in a compost heap are: high temperature region, anaerobic lower region, cool lower region and cool outer region as mentioned elsewhere.**
4. **The selected three a, b and c piles were chosen as: multifunctional co-inoculated (O-RCS); (mf-RCS) and (ms-RCS) mixtures of co-composted materials.**
5. **pH-profile under Bin (barrels) conditions of the same selected a, b and c barrels have been taken the same behavior trend**

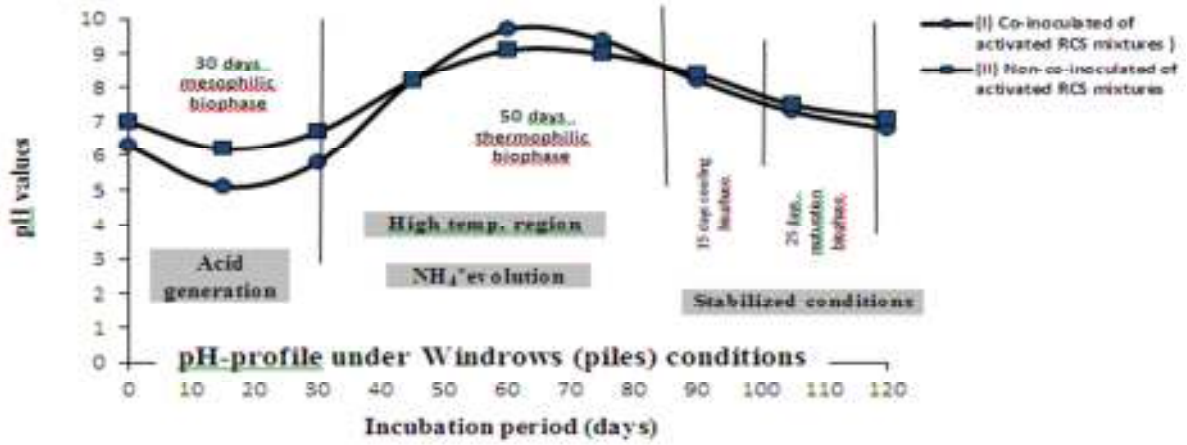


Fig. 2. Variations in pH-values of three different types of triad components mixtures of multi-functional co-inoculated and non-co-inoculated activated RCS throughout the aerobic co-composting bioprocesses under piles conditions elongated 120 days.

Notes:
1. **pH values of different co-composts types were weekly estimated in 1:10 w/v (1 gm of composted materials: 10 ml of distilled water) during the aerobic co-composting bioprocesses prolonged 120 days.**
2. **Each pH-value is a mean of 48 variables (1× 2 replicates× 4 regions× 3 piles×2 weeks).**
3. **The four regions which representing the thermal conditions in a compost heap are: high temperature region, anaerobic lower region, cool lower region and cool outer region as mentioned elsewhere.**
4. **The selected three a, b and c piles were chosen as: multifunctional co-inoculated (O-RCS); (mf-RCS) and (ms-RCS) mixtures of co-composted materials.**
5. **pH-profile under Bin (barrels) conditions of the same selected a, b and c barrels have been taken the same behavior trend.**

From the previous results as displayed in Fig.(2), it may be generally concluded that, thermophilic (NH_4^+–evolved) biophase (more alkaline conditions) realized higher pH–values than those obtained at mesophilic (acidic generation) biophase more acidic conditions.

Indeed, this conclusion could be attributed to the increment in indigenous microbial bacterial and fungal populations and their activities at these appropriate medium of mesophilic biophase, moreover multi-functional microbial and effective microorganisms co-inoculation and sequentially increasing the organic acids production which led to increasing the acidic conditions.

Oppositely, thermophilic biophase led to the deepest decreasing in organic acids production in consequence of the highly decrement in microbial populations, moreover the evolution of NH_4^+ in the course of this thermophilic biophase which led to increasing pH–values.

With regard to dynamic fluctuations in pH–values (pH–profile) of different activated co-composted materials at various stages throughout aerobic co-composted production under Windrows (piles) system as affected by multi-functional microbial co-inoculants, data displayed in Table (5) and Fig.(2) reveal, on average conditions of all selected studied parameters that, grand mean (GM) values of microbial co-inoculated (a,b,c) activated RCS-mixtures under piles realized lower pH–values than those obtained by non-co-inoculated (a,b,c) activated RCS–mixtures and can be arranged as the following:

Grand mean (GM) of microbial co-inoculated (a+b+c) RCS mixtures under Windrows (piles) system (acidic conditions) 7.3	>	**Grand mean (GM) of non-co-inoculated (a+b+c) RCS mixtures under Windrows (piles) system (alkaline conditions) 7.7**		**General grand mean (G.G.M) 7.5**

it is worthy note that, pH–values of co-inoculated RCS activated mixtures recorded higher values than those obtained by non-co-inoculated RCS activated mixtures at thermophilic biophases as the following:

Mean of co-inoculated RCS mixtures 8.9	>	**Mean of non-co-inoculated RCS mixtures 8.5**	**Grand mean (GM) 8.7**

However, pH–values of non-co-inoculated RCS activated mixtures at mesophilic; cooling and maturation biophases realized pH–values higher than those obtained at corresponding co-inoculated RCS activated mixtures as arranged in the following sequences:

	mean of pH-values Non-co-inoculated RCS-mixtures		**mean of pH-values Co-inoculated RCS-mixtures**	**Grand mean (GM)**
Mesophilic biophase	**6.5**	>	**5.5**	**6.0**
Cooling biophase	**8.2**	>	**7.8**	**8.0**
Maturation biophase	**7.3**	>	**7.0**	**7.15**

Results in the same Table and Figure indicate also that, on the average conditions the lowest pH–values were realized at the mesophilic biophase (30 days) after 15 days from the co-composting initiation which represented as the following arrangement:

pH values of co-inoculated (a, b and c) activated RCS mixtures 5.1	<	**pH values of non-co-inoculated (a, b and c) activated RCS mixtures 6.4**

On the other hand, the highest pH–values were realized at the thermophilic biophase 50 after 65 days from the co-composting initiation as the following sequence:

pH values of co-inoculated (a, b and c) activated RCS mixtures 9.8	>	**pH values of non-co-inoculated (a, b and c) activated RCS mixtures 9.0**

As regards dynamic oscillations in pH–values of different activated co-composted materials at various stages in the course of aerobic co-composting bioprocesses as affected by the (a, b and c) triad components mixtures types, obtained data reveal, on average conditions of all selected studied parameters that, pH–values differed obviously with the types of co-composted materials under Windrows piles system. These variations in pH–values during aerobic fermentation period 120 days could be arranged in the following descending order as:

mf - RCS mixtures (c) 8.3	>	**ms - RCS mixtures (b) 7.4**	>	**O- RCS mixtures (a) 6.8**	**General grand mean (GGM) 7.5**
(alkaline conditions)				**(acidic conditions)**	

It was noticed, on the average conditions of all other studied variable parameters that, descending order direction of the dynamic fluctuations of different activated co-composted materials at various stages in temperature-degrees have displayed the entirely opposite direction of changes in pH-values in the course of the composting period 120 days under Bin (barrels) and Windrows (piles) systems.

These findings mean that, organic activated triad components mixtures (a) (O-RCS) have higher acidic and thermic conditions than the others [ms-RCS (b) and mf-RCS (c) mixtures].

Detection of pathogenic bacteria

Total-coliform numbers (MPN)

Total-coliform numbers were tabulated and illustrated as logarithmic of total coliform numbers (log MPN. 100 ml^{-1}). The most probable number (MPN) method permits estimation of population density without an actual count of single cells or colonies. It is sometimes called the method of ultimate or extinction dilution or, less descriptively simply the dilution method. The MPN technique is based on a determination of the presence or absence of microorganisms in several individual portions of each several consecutive dilutions of soil or other materials as revealed by Alexander (1982).

Concerning occurrence and changes in pathogenic coliform numbers detected throughout the co-composting bioprocesses as affected by the incubation period under Bin (barrels) and Windrows (piles) systems, data listed in Table (6) and illustrated graphically in Fig.(3) reveal, on average conditions of all selected studied parameters that, logarithmic numbers of coliform were sharply decreased with increasing the incubation period until the terminal mesophilic biophase from 6.75 to 0.7 (4.1 general mean) after 30 days from initiation. Thereafter, the coliform numbers were approached to stationary state at almost constant lowest value with average (1.0) after 45 days from

initiation. Total-coliform numbers were absolutely not detected in the following co-composting biophases.

Obtained analytical data show also that, on average conditions of all studied parameters that, co-composted mixtures under barrels (Bin system) (average 4.95) displayed greater values than those obtained under piles (Windrows system) (average 3.24) at the mesophilic biophase during co-composting bioprocesses. From the previous results, it may be concluded that, the obtained matured co-composts are pathogens-free as indicated by total-coliform-bacteria.

Pathogen survival of co-composting bio-process

Concerning pathogen destruction, the relative efficiencies of the aerobic co-composting bioprocesses was evaluated on the basis of the occurence of *Salmonella* and *Shigella* sp. The obtained results were negative for this test for all types of matured co-composts. This meant that these co-composts went through the thermal cycle, mature and safe for handling as reported by Khalil (1996). The obtained results showed that the matured co-composts were pathogens-free, as indicated by the salmonella and Shigella test.

These obtained data are confirmed with the results those obtained by Hussong *et al*. (1985) and Khalil (1996).

They stated that, high concentrations of soluble organic nutrients present in immature composts support growth of *Salmonella* sp. and other pathogens, which depend on the free nutrients for growth, since concentrations of these available nutrients decrease as composts mature. So, in properly matured co-composts, regrowth of nutrient-dependent pathogens is not possible as reported by Inbar *et al*. (1990) and Khalil (1996).

Table 6. Logarithmic total coliform numbers (log MPN.100 ml^{-1})

	Mesophilic biophase								Thermophilic biophase			
No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**mean**	**8**	**9**	**10**	**mean**
Incubation periods (days)	**Initial**	**5**	**10**	**15**	**20**	**25**	**30**		**35**	**40**	**45**	
Piles	6.25	6.0	5.0	2.0	1.5	1.2	0.7	3.24	0.7	0.6	0.6	0.6
Barrels	6.5	6.75	6.5	5.8	4.5	3.0	1.6	4.95	1.4	1.4	1.4	1.4
General mean				4.1							1.0	

Notes: 1. Representative subsamples were collected in duplicates from the same three activated, multi-functional co-inoculated co-composted materials (a, b and c) within 4 regions in barrels or piles.

2. These regions are high temp., anaerobic lower, cool lower and cool outer regions.

3. Total-coliform numbers were estimated every 5 days in duplicates during the co-composting bioprocesses.

4. Each value is a mean of 48 variables [1×2 replicates×3 barrels (or 3 piles)× 2 multi-functional co-inoculated co-composts and non-co-inoculated× 4 regions within each barrel or pile].

5. MPN: Most Probable Number of microbial population.

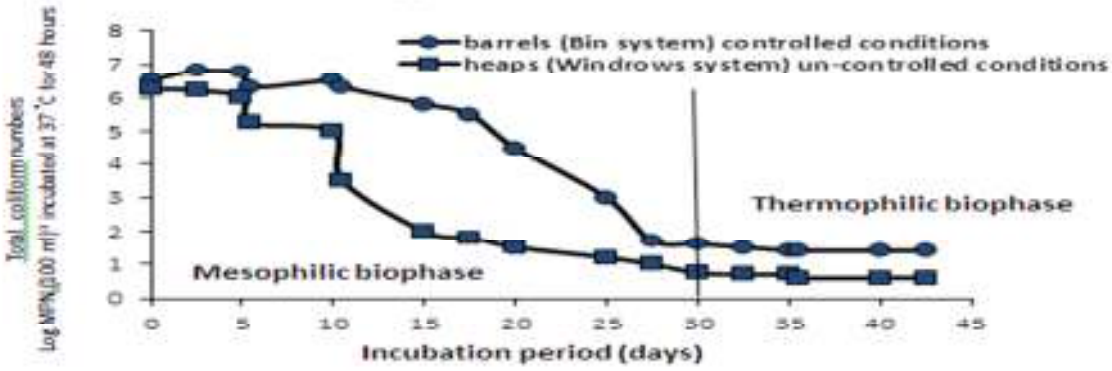


Fig. 3. Changes in pathogenic coliform numbers detected throughout the mesophilic and thermophilic biophases of the same three activated, multi-functional co-inoculated co-compsted materials a, b and c of barrels or piles from 4 regions during aerobic co-composting bioprocesses under Bin (barrels) and Windrows (piles) systems.

Dynamic changes in bacterial and fungal counts

Total bacterial and fungal counts were estimated weekly induplicates and the obtained data were calculated as a logarithmic of total microbial numbers (CFU) per gram co-composted materials, on dry weights basis at 70 ° C for 18 hours (log CFU. g^{-1} co-compost). With regard to changes in total counts of bacteria and fungi of co-composted materials at different intervals throughout aerobic co-composting bioprocesses under Windrows (piles) and Bin (barrels) systems as affected by the four co-composting biophases and their incubation periods (days), data represented in **Table (7)** demonstrate, on the average conditions of the other selected studied parameters that, the fluctuations in total bacterial count could be arranged in the following descending order as:

Total bacterial count (TBC): log CFU. g^{-1} co-compost

Barrels (a+b+c)	**Control (zerotime) 7.81**	**Maturity biophase 8.98**		**cooling biophase 8.49**		**mesophilic biophase 8.28**		**thermophilic biophase 5.54**	**Median weights bacterial (MBC) 8.14**
Piles (a+b+c)	7.77	10.60	≥	10.03	>>	8.27	>>>	5.79	8.94
General grand mean	7.79	9.78	≥	9.40	>	8.75	>>>	5.67	8.54

On the other hand, the alterations in total fungal count have been taken the following sequences as:

Total fungal count (TFC): log CFU. g^{-1} co-compost

Barrels (a+b+c)	**Control (zero time) 5.61**	**Maturity biophase 6.05**		**cooling biophase 5.88**		**mesophilic biophase 5.60**		**thermophilic biophase 4.25**	**Median weights fungal (MFC) 5.16**
Piles (a+b+c)	5.65	6.25	>	5.79	>	5 .36	>>	4.71	5.74
General grand mean	5.63	6.15	>	5.84	>	5.48	>>	4.48	5.45

As concerns, the alterations in total bacteria and fungi of co-composted materials at different intervals during aerobic co-composting bioprocesses under piles and barrels conditions as affected by activated triad components mixtures types and their following sequence of elementary composition C: N: P: S at 30: 1: 0.3: 0.15 respectively on the basis of (1 : 1 : 1 (v/v) mixtures) as elucidated in detailed elsewhere, obtained results as median bacterial total count (MBC) and fungal total count (MFC) declare, on average conditions that, the changes in total bacterial count could be sequenced gradually in the following arrangements as:

Median weights of logarithmic total bacterial count (MBC)

(log CFU. g^{-1} co-compost)

Barrels plus Piles (a+b+c)	**O - RCS mixtures**		**ms - RCS mixtures**		**mf - RCS mixtures**		**control - RCS mixtures**
	7.79	**>**	**7.46**	**>**	**7.29**	**>**	**7.15**

Median weightsof logarithmic total fungal count (MFC)

(log CFU. g^{-1} co-compost)

Barrels plus Piles (a+b+c)	**mf- RCS mixtures**		**ms - RCS mixtures**		**O - RCS mixtures**		**control - RCS mixtures**
	5.65	**≥**	**5.53**	**≥**	**5.40**	**>**	**5.16**

Table 7. Changes in total bacterial and fungal counts of different types of co-composted materials at different intervals during aerobic co-composting bioprocesses in Bin (barrels) and Windrows (piles) systems elongated 120 days.

Co-composting bio-phases / **Types of co-composts Mixtures**	**Initial Zero time**	**Co-composting period (120 days)**				**Median total count**
		Mesophilic bio-phase	**Thermophilic bio-phase**	**Cooling bio-phase**	**Maturation bio-phase**	
		30 days	**50 days**	**15 days**	**25 days**	
		Logarithmic of total bacterial count (MBC) Log cfu.$g^{-1}_{d.w}$ co-compost				
Mean of (RCS) barrels (1+2)	7.18	7.26	6.86	7.59	7.82	7.15
Mean of (O-RCS) barrels (3+4)	7.78	8.28	7.01	8.15	8.57	7.79
Mean of (mf-RCS) barrels (5+6)	7.49	7.71	6.73	7.45	7.79	7.29
Mean of (ms-RCS) barrels (7+8)	7.60	7.83	6.84	7.68	8.11	7.46
Average mean of barrels (a+b+c)	7.81	8.28	5.54	8.49	8.98	8.14
Average mean of heaps (a+b+c)	7.77	8.27	5.79	10.03	10.60	8.94
		Logarithmic of total fungal count (MFC) Log cfu.$g^{-1}_{d.w}$ co-compost				
Mean of (RCS) barrels (1+2)	5.14	5.21	4.92	5.33	5.50	5.16
Mean of (O-RCS) barrels (3+4)	5.46	5.48	4.90	5.43	5.96	5.40
Mean of (mf-RCS) barrels (5+6)	5.82	5.73	4.99	5.62	5.88	5.65
Mean of (ms-RCS) barrels (7+8)	5.80	5.77	5.16	5.67	5.88	5.53
Average mean of barrels (a+b+c)	5.61	5.60	4.25	5.88	6.05	5.16
Average mean of heaps (a+b+c)	5.65	5.36	4.71	5.79	6.26	5.74

Notes: 1. Each value listed in this table represents the median weights of total bacterial count as well total fungal count of each biophase equal to :

$$= \frac{\Sigma\ total\ microbial\ count\ of\ each\ biophase \times incubation\ days\ of\ each\ biophase}{Total\ co-composting\ incubation\ period\ of\ each\ biophase}$$

2. (MBC) or (MFC) =

$$\frac{\Sigma\ Total\ microbial\ counts\ of\ all\ co-composting\ biophases \times co-composting\ incubation\ (days)\ of\ each\ biophase}{Total\ co-composting\ incubation\ period\ (120\ days)}$$

On the average conditions of all selected studied parameters, bacterial total count (MBC) and fungal total count (MFC) of co-inoculated activated RCS-mixtures realized higher values than those obtained by non-coinoculated RCS-mixtures as the following arrangements:

	Co-inoculated RCS+2 mixtures (two phases)		**Non-coinoculated RCS+1 mixtures (one phase)**	**General grand mean**
	Total bacterial count (TBC): log CFU. g^{-1} co-compost			
Barrels (a+b+c)	8.83	>	7.45	8.14
Piles (a+b+c)	9.73	>	8.15	8.94
Grand mean	9.28	>	7.80	8.54
	Total fungal count (TFC): log CFU. g^{-1} co-compost			
Barrels (a+b+c)	5.37	>	4.95	5.16
Piles (a+b+c)	5.85	>	5.63	5.74
Grand mean	5.61	>	5.29	5.45

Total microbial counts of bacteria and fungi generally differed with the co-composting conditions under Bin (barrels) and Windrows (piles) systems. These differences could be sequenced as the following:

	Grand mean of total microbial count under Piles system		**Grand mean of total microbial count under Barrels system**	**General grand mean**
Total bacterial count (TBC)	8.94	>	8.14	8.54
Total fungal count (TFC)	5.74	>	5.16	5.45

Generally, examination of data listed in the abovementioned Table, appear appropriately, on the overall average conditions of the selected studied parameters that, total bacterial count (TBC) of the co-composted (a,b and c) mixtures realized the predominancy over the total fungal count (TFC). This domination could be arranged in the following descending order as:

General grand mean of bacterial count		General grand mean of fungal count
8.54	>>	5.45

Concerning co-composting biophases, there are negative correlations between fluctuations in total microbial bacterial count (MBC) as well as total microbial fungal count (MFC) and the changes in pH-values ($r = - 0.89^{++}$) and alterations in temperature – degrees ($r = - 0.96^{++}$).

Dynamic fluctuations in predominant enzymes activity

1- Deheydrogenases activity

Results were recorded as (µg 1,3,5 – TPF produced. $g^{-1}_{d.w}$ co-compost. $hour^{-1}$) at 37 °C pH 8. Biological oxidation of organic compounds is generally a dehydrogenation process and there are many dehydrogenases (enzymes catalyzing dehydrogenation) which are highly specific. The dehydrogenase enzyme systems apparently fulfill a significant role in the oxidation of organic matter as they transfer hydrogen from substrates to the acceptors (Tabatabai, 1982).

Concerning fluctuations in dehydrogenases activity values of different co-composted materials at different intervals throughout aerobic co-composting bioprocesses 120 days under Bin (barrels) and Windrows (piles) systems as affected by the four co-composting biophases and their incubation periods (days), obtained results listed in Table (8) reveal clearly, on average conditions of the other studied parameters that, changes in dehydrogenases activity trend could be arranged in the following descending order as follows:

Barrels (a+b+c)	Control	Maturity biophase		cooling biophase		mesophilic biophase		thermophilic biophase	Median weights activity(MEA)
Mean	**37**	**294**	**>**	**272**	**>>**	**93**	**>**	**88**	**155**
Piles (a+b+c) Mean	43	317	>	297	>>	106	>	95	169
General grand mean	38.5	305.5	>	284.5	>>	99.5	>	91.5	162

Generally, all these dehydrogenases activities were recorded the highest values under aerobic co-composting bioprocesses in comparison with controls 37 and 43 for barrels and piles respectively. Tabulated data displayed generally that, dehydrogenases activity was sharply decreased at thermophilic biophase during co-composting period in comparison with other biophases. However maturation and cooling biophases showed higher values than those obtained at mesophilic and thermophilic biophases under the two co-composting systems. It was noticed that, fluctuations in delydrogenases activity has entirely taken the opposite direction in relation to pH and temperature-profiles of the co-composted materials.

From the previous results it may be concluded that, dehydrogenases activity was increased with decreasing in pH–values (acid medium) and oppositely decreased with increasing. pH-values (alkaline medium). Dehydrogenases activity had the maximum values at pH 7 and reached its minimum values at pH 8.5. Analytical data declared obviously also that, there are negative correlation between changes in dehydrogenases activity and alterations in temperature degrees (temperature profiles) as illustrated in Fig (1) and Table (4) during co-composting period. Statistically, there are a negative correlation between fluctuations in dehydrogenases activity and changes in pH-values (r = -0.78**) [see Table (5) and fig (2)]. It was found that the dehydrogenases activity was greatly decreased with increasing temperature degrees and vice versa and had the maximum activity values at temperature degrees between 20-30 °C in the course of aerobic co-composting period under the two co-composting systems. However, it reached its minimum values at temperature above 65-75 °C at the thermophilic biophases under both co-composting system

Table 8. Activity of the predominant enzymes of different types of co-composted materials at different intervals during aerobic co-composting bioprocesses within Bin (barrels) and Windrows (piles) conditions elongated 120 days.

Co-composting bio-phases / Types of co-composts Mixtures	Dehydrogenases activity						Endoglucanase activity					
		Co-composting period (120 days)						Co-composting period (120 days)				
	Initial Zero time	Mesophilic bio-phase	Thermophilic bio-phase	Cooling bio-phase	Maturation bio-phase	Median weights enzyme activity (MEA)	Initial Zero time	Mesophilic bio-phase	Thermophilic bio-phase	Cooling bio-phase	Maturation bio-phase	Median weights enzyme activity (MEA)
		30 Days	50 days	15 days	25 Days			30 Days	50 days	15 Days	25 Days	
Mean of (RCS) barrels (1+2)	13	45	50	136	172	85	215	343	274	479	544	373
Mean of (O-RCS) barrels (3+4)	36	91	81	244	280	145	465	587	395	635	875	573
Mean of (mf-RCS) barrels (5+6)	33	77	73	225	245	129	372	419	330	692	941	525
Mean of (ms-RCS) barrels (7+8)	34	88	85	265	296	150	315	509	412	598	838	548
Average mean of barrels (a+b+c)	37	93	88	272	294	155	461	586	427	723	943	611
Average mean of heaps (a+b+c)	43	106	95	297	317	169	673	930	814	960	1203	942
		β – glucosidase activity										
Mean of (RCS) barrels (1+2)	49	81	60	85	107	78						
Mean of (O-RCS) barrels (3+4)	82	119	89	142	168	120						
Mean of (mf-RCS) barrels (5+6)	82	112	89	111	119	104						
Mean of (ms-RCS) barrels (7+8)	63	115	99	148	157	121						
Average mean of barrels (a+b+c)	85	124	102	150	160	126						
Average mean of heaps (a+b+c)	98	189	156	271	344	218						

Notes: 1. All selected enzymes activities were determined weekly during the aerobic co-composting bioprocesses period elongated 120 days.

2. Each enzyme activity value is a mean of 32 variables (1×2 replicates × composite samples from 4 regions within the heaps or barrels×2 weeks×2 co-inoculated and non-coinoculated).

3. The obtained results were calculated on oven dry weights basis at 70 °C for 18 hours.

4. (a) means that: organic activated O-RCS+1 and O-RCS+2 one phase and two phases mixtures under Bin (barrels) or Windrows (piles) systems.

(b) means that: Mineral fast release activated mf-RCS+1 and mf-RCS+2 one phase and two phases mixtures under Bin (barrels) or Windrows (piles) systems.

(c) means that: Mineral slow release activated ms-RCS+1 and ms-RCS+2 one phase and two phases mixtures under Bin (barrels) or Windrows (piles) systems.

5. Median weights enzyme activity of the four co-composting bio-phases equal to:

$$MEA = \frac{\Sigma \text{ enzyme mean activities of each biophase} \times \text{composting days of each biophase}}{\text{Total co-composting incubation period (120 days)}}$$

Regarding changes in dehydrogenases activity values during aerobic co-composting bioprocesses as affected by different types of activated triad components mixtures (RCS) and their sequence adjusted elemental composition C: N: P: S at 30: 1: 0.3: 0.15 respectively i.e. C/N = 30; C/P =100 and C/S =200 on the basis of (1 : 1 : 1 (v/v) mixtures) oven dry weights at 70 °C for 18 hours, data shown in **Table (8)** reveal, on average conditions of all studied parameters that, changes in dehydrogenases activity values could be arranged in the following sequences under barrels and piles as:

Barrels plus Piles	ms - RCS mixtures		O - RCS mixtures		mf - RCS mixtures		control – RCS mixtures
	150	≥	145	>	129	>>	85

From the previous results, it could be concluded that, activated mineral slow release triad components mixtures (ms–RCS mixtures) and also activated organic (O–RCS mixtures) realized higher dehydrogenases activity values than those obtained by activated mineral fast release mixtures (mf–RCS mixtures) in comparison with the control (RCS-mixtures).

With regard to the evolutions in dehydrogenases activity values of different activated types of triad components mixtures as affected by multi-functional microbial co-inoculation during aerobic co-composting bioprocesses, data demonstrate on average conditions of all studied mixtures, co-inoculated activated triad components mixtures RCS+2 mixtures (two phases) exhibited greater dehydrogenases activity values than those obtained by non-inoculated RCS+1 mixtures (one phase).

Grand means of the median weights enzyme activity (MEA) of these mixtures can be generally arranged in the following sequence as:

Co-inoculated activated RCS+2 mixtures (two phases)		Non-coinoculated activated RCS+1 mixtures (one phase)
96	>>	75

Furthermore, obtained data declare also that, dehydrogenases activity values were more pronounced under Windrows (piles) system than those obtained under Bin (barrels) system in the course of co-composting bioprocesses period 120 days as:

MEA of piles system		MEA of barrels system
169	>	155

2- Endoglucanase activity (EC 3.2.1.1.4)

There are three principle cellulases synergistically act to the complete hydrolysis of cellulose. One of them is 1,4-β-D-glucan-4-glucanohydrolase, (EC 3.2.1.1.4). Common names include endoglucanase and carboxymethylcellulase. It hydrolyze the internal 1,4-β-D-glucosidic linkages in cellulose and cereal β-D-glucans. Therefore, it hydrolyze glycosidic bonds at the amorphous sites of the cellulose generating long chain oligomers (non-reducing ends) for the action of exoglucanases. Therefore, endoglucanase breaks internal bonds to disrupt the crystalline structure of cellulose and expose individual cellulose polysaccharide chains (Juturu and Wu, 2014).

Activity of endoglucanase are expressed as µmoles glucose equivalent. $g^{-1}_{d.w}$ co-compost. (24 hours)$^{-1}$ at 50 °C pH 5.5. Regarding changes in endoglucanase activity values of different types of activated co-composted materials at different intervals in the course of aerobic co-composting bioprocesses period (120 days) under Bin (barrels) and Windrows (piles) systems as affected by the four, co-composting biophases and their incubation periods (days), data shown in Table (8) reveal, on the average conditions of all the other studied parameters that, the behaviour of fluctuations in endoglucanase activity values could be arranged in the following sequence as:

Barrels (a+b+c)	Control	Maturity biophase		cooling biophase		mesophilic biophase		thermophilic biophase	Median weights activity(MEA)
Mean	461	943	>	723	>>	586	>	427	611
Piles (a+b+c) Mean	673	1203	>	960	>	930	>	814	942
General grand mean	567	1073	>	841.5	>	758	>	620.5	776.5

Analytical data concerning changes in endoglucanase activity values as affected by the types of co-composted materials and their adjusted elemental compositions in the sequence of C: N: P: S at (30: 1: 0.3: 0.15 respectively) as before mentioned, are given in Table (8). Data clearly indicate, on average conditions of all other studied parameters that, the behaviour pattern of median weights of enzyme activity MEA can be sequenced in the following arrangement under barrels and piles systems as :

Barrels plus Piles	O - RCS mixtures		ms - RCS mixtures		mf - RCS mixtures		control – RCS mixtures
	573	>	548	>	525	>>	373

This arrangement has entirely the same trends which were obtained by dehydrogenases activity in the course of the co-composting period.

Respecting alterations in endoglucanse activity values as affected by the multi-functional microbial co-inoculation, obtained data demonstrate also, on average conditions that, multi-co-inoculated, activated triad components mixtures realized generally endoglucanase activity values higher those obtained by non-co-inoculated activated triad components mixtures as the following sequence:

MEA of co-inoculated activated RCS+2 mixtures (two phases)		MEA of non-co-inoculated activated RCS+1 mixtures (one phase)
445	>>	332

Generally, endoglucanase activity values were higher under Windrows (piles) system than those obtained under Bin (barrels) system during the co-composting bioprocesses period (120 days) as:

MEA of piles system		MEA of barrels system
942	>	611

Statistically, from the previous results, it may be concluded on average conditions that, there are a negative correlations between fluctuations in endoglucanase activity values and changes in pH-values (r = -0.603**) as well as in consideration of the effect of co-composting biophases fluctuations in temperature-degrees (r = -0.835**) respectively in the whole course of aerobic co-composting bioprocesses period (120 days).

However, obtained results reveal also that, there are a positive correlations between the changes in dehydrogenases activity values and the alterations in endoglucanase activity value (r = +0.725**).

3- β-D-glucoside glycohydrolase activity (EC 3.2.1.21) Commonly known as β-Glucosidase

Among the glycosidases, α- glucosidase (obsolete name maltase, EC 3.2.1.20), which catalyzes the lydrolysis of α-D-glucopyranoside, and β-glucosidase (obsolete name gentiobiase or cellobiase, EC 3.2.1.21) which catalyzes the hydrolysis of β-D-glucopyranosidase, are involved in hydrolysis of maltose and cellobiose, respectively. The hydrolysis products of β-glucosides are believed to be improtant energy sources for microorganisms (Tabatabai,

1982). It hydrolyze cellobiose and cause the removal of glucose from non reducing ends of cello-oligosaccharides and glycosyl transfer to cellobiose. β-glucosidase activity was expressed as µg ρ-nitrophenol released.$Kg^{-1}_{d.w}$ co-compost.$hour^{-1}$ at 37 ˚C pH 6.

Concerning alterations in β-glucosidase activity values of different types of co-composted materials at different intervals during aerobic co-composting bioprocesses under Bin (barrels) and Windrows (piles) systems as affected by the four co-composting biophases and their incubation periods, data listed in Table (8) reveal, on average conditions that, behavior of variations in β-glucosidase activity values could be sequenced in the following arrangement as:

Barrels (a+b+c)	Control	Maturity biophase		cooling biophase		mesophilic biophase		thermophilic biophase	Median weights activity (MEA)
Mean	**85**	**160**	**>**	**150**	**>**	**124**	**>**	**102**	**126**
Piles (a+b+c) Mean	98	344	>	271	>	189	>	156	218
General grand mean	91.5	252	>	210.5	>	156.5	>	129	172

As concerns the changes in β-glucosidase activity values as affected by different types of activated triad components mixtures and their sequence adjusted elemental composition C: N: P: S at 30: 1: 0.3: 0.15 respectively i.e. C/N = 30; C/P =100 and C/S =200 on the basis of (1 : 1 : 1 (v/v) mixtures) oven dry weights at 70 ˚C for 18 hours. The behavior pattern of the medium weights of enzyme activity (MEA) under barrels and piles systems has taken the following descending order as:

Barrels plus Piles	ms - RCS mixtures		O - RCS mixtures		mf - RCS mixtures		control – RCS mixtures
	121	**=**	**120**	**>**	**104**	**>>**	**78**

In this connection, on the average conditions of all the other studied parameters that, multi-functional co-inoculated activated RCS mixtures displayed β-glucosidase activity values higher than those obtained by non-co-inoculated RCS mixtures as the following sequence:

MEA of co-inoculated activated RCS+2 mixtures (two phases)		MEA of non-co-inoculated activated RCS+1 mixtures (one phase)
213	**>>**	**143**

Obtained results reveal also, on average conditions that, β-glucosidase activity values under Windrows (piles) system were higher than those obtained under Bin (barrels) system.

MEA of piles system		MEA of barrels system
218	**>>**	**126**

Statistically, there are positive correlations between fluctuations in β-glucosidase activity values as affected by the four biophases in the course of aerobic co-composting period at different intervals and the fluctuations in dehydrogenases activity values (r = + 0.94**) and endoglucanase activity (r = + 0.81**). However, there are negative correlations between fluctuations in β-glucosidase activity values and changes in pH-values and alterations in temperature-degrees as (r = + 0.98**) and (r = + 0.68**) respectively.

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رصد التغيرات الديناميكية في الخصائص الميكروبيولوجية والبيو-فيزيائية الكيميائية للمخاليط العضوية الثلاثية الملقحة بلقاحات متعددة الأغراض أثناء معالجتها بالكمر الهوائي تحت نظم مختلفة

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الهدف الرئيسى للبحث هو محاولة إنتاج مكمورات عضوية صناعية ميكروبية (أنواع كمبوست ميكروبى) ناضجة عالية الجودة على نطاق واسع فى فترة قصيرة، من المخلفات العضوية الزراعية منخفضة القيمة والفائدة زراعيا وخاصة قش الأرز، علاوه على تعظيم وزيادة قيمتها السمادية لإستخدامها كمحسنات ومخصبات عضوية- حيوية للتربة الزراعية بالأراضى الرملية والجيرية وإحلالها جزئيا محل الكيماويات الزراعية وبالتبعيه تقليل التلوث البيئى. * إستخدم نظامين للكمرالهوائى المشترك : نظام المصفوفات (الأكوام) بمزرعة كلية الزراعة (ظروف طبيعية غير متحكم فيها) ونظام الإناء (البراميل) بمعامل قسم الأراضى والمياه بكلية الزراعة (ظروف متحكم فيها) أثناء الموسم الصيفى أربعة أشهر (يونيو - سبتمبر) 2017 – لتحويل المخاليط العضوية المعالجة إلى مكمورات عضوية ميكروبية. * أستخدمت لقاحات ميكروبية سائلة متعددة الأغراض من سلالات بكتيرية وفطرية نقية ذات قدرات عالية وذلك على دفعتين فى المرحلة الميزوفيلية (30 يوم) لإسراع عمليات تحلل وتحطيم المواد العضوية إلى مكوناتها الوسطية حتى يتم بلمرة وتكثيف هذه المكونات وتكوين المواد العضوية الدبالية والأحماض اليورونية فى المراحل اللاحقة فى عملية الكمر الهوائى المشترك للمخاليط العضوية الثلاثية المنشطة داخل البراميل والأكوام. * أضيفت أيضا لقاحات ميكروبية سائلة أخرى متعددة الأغراض على دفعتين فى مرحلة النضج لتحسين خواص وجودة الكمبوست الميكروبى الناتج فى نهاية عملية التخمر الهوائى كما أضيف أيضا المخصب الحيوى للكائنات الحية الدقيقة النافعة EM_1. وأتحقيق هذه الأغراض تم تجهيز وتنفيذ الآتى:1- معالجة المخاليط العضوية الثلاثية لقش الأرز وحطب القطن ونشارة الخشب الناعمة وخلطها على أساس الحجم 1:1:1 وتنشيطها عضويا ومعدنيا (سريعة الإنطلاق بإستخدام أسمدة معدنية) وبطيئة الإنطلاق (بإستخدام مواد طبيعية: صخر الفوسفات والفلسبارات والكبريت المعدنى) لضبط القيم النسبية للعناصر على أساس C/N=30, C/P=100, C/S=200 لهذه المخاليط.2- تجهيز وتعبئة وتأهيل عدد 3 كومات ، 8 براميل بهذه المخاليط وإجراء عملية الكمر الهوائى لمدة 120 يوم تحت ظروف هوائية.3- معمليا: تم عزل وتنقية وإكثار وتعريف العزلات الميكروبية الأكثر كفاءة مورفولوجيا وبيوكيميائيا وإختبار كفاءتها وقدرتها الإنزيمية على تحلل وإذابة المواد العضوية وغيرالعضوية. وبها تم تجهيز اللقاحات الميكروبية السائلة لإستخدامها فى تلقيح الكومات والبراميل وهى سلالات بكتيرية وفطرية فعالة للميكروبات المحللة للسليلوز المتوسطة والعالية التحمل للحرارة والبكتيريا المذيبة للفوسفات المعدنية والمذيبة للسيليكات وإنطلاق البوتاسيوم والبكتيريا المثبتة للأزوت الجوى اللاتكافلية (الأزوتوباكتر)، علاوه على التعريف والتوصيف الجينى الوراثى للسلالات المحاله للسليلوز. بصفة عامة يمكن تلخيص النتائج التحليلية والإحصائية المتحصل عليها من هذا البحث كالآتى: أولا: تأثير مراحل الكمر الهوائى على خواص وصفات المواد المكمورة خلال إجمالى عملية الكمر الهوائى المشترك جميع القيم كمتوسط عام لتأثير باقى المتغيرات الدراسية على الصفات المدروسة. 1- مرحلة النضج (25 يوم): حقق نشاط جميع الإنزيمات المدروسة أعلى القيم مقارنة بالكنترول كالآتى: Dehydrogenases: 305.5 (control 38.5 and median weights enzymes activity MEA 162) µg 1, 3, 5 TPF product. $g^{-1}_{d.w}$ co-compost. $hour^{-1}$. Endoglucanase: 1073 (control 567 and MEA 776.5) µmoles glucose equivalent. $g^{-1}_{d.w}$ co-compost. 24 $hour^{-1}$. β-glucosidase: 252 (control 91.5 and MEA 172) µg PNP released. $kg^{-1}_{d.w}$ co-compost. $hour^{-1}$. Log total bacterial (TBC) and total fungal (TFC). $g^{-1}_{d.w}$ co-compost. TBC: 9.79 (control 7.78 and general grand mean G.G.M 8.54) TFC: 6.15 (control 5.63 and G.G.M 5.45). Temp.degrees: 25.5 °C (Temperature-degrees) و رقم pH-values أقل القيم فى هذه المرحلة مقارنة بالكنترول *حققت قيم درجات الحرارة (control 29.20 °C, ambient temp. 25.75 °C and G.G.M 35.3 °C) pH-values: 7.15 (control 7.10 and G.G.M 7.5) وقد تحققت فى هذه المرحلة أقل درجة حرارة بعد 120 يوم من بداية الكمر المشترك بمتوسط 22 درجة مئوية. 2- المرحلة الثيرموفيلية (50 يوم) *حققت قيم نشاط الإنزيمات السابقة أقل القيم فى هذه المرحلة مقارنة بالكنترول ومرحلة النضج 91.5 (control 40 and MEA 162); 620.5 (control 567 and MEA 776.5) and 129 (control 91.5 and MEA 172). لإنزيمات الدى هيدروجينيز، الاندوجلوكانيز وبيتا- جلوكوسيديز على الترتيب. *حققت قيم الأعداد الكلية للبكتيريا والفطريات أقل القيم مقارنة بالكنترول ومرحلة النضج.TBC 5.7 (control 7.78 and G.G.M 8.54) and TFC 4.48 (control 5.63 and G.G.M 5.45) *حققت قيم درجات الحرارة ورقم pH أعلى القيم فى هذه المرحلة مقارنة بالكنترول ومرحلة النضج. Temp.degrees: 49.25 °C (control 29.2 °C, ambient temp. 25.75 °C and G.G.M 35.3 °C). pH-values 8.7 (control 7.10 and G.G.M 7.5). وقد تحققت أعلى قيمة مطلقة لدرجة الحرارة 62 درجة فى هذه المرحلة بعد 85 يوم من بداية الكمر فى حين تحققت أعلى قيمة مطلقة 9.2=pH فى هذه المرحلة بعد 65 يوم من بداية الكمر فى حين تحققت أقل قيمة مطلقة لـ 5.1=pH فى المرحلة الميزوفيلية. ثانيا: تأثير التلقيح الميكروبى متعدد الأغراض على خواص وصفات المواد المكمورة خلال إجمالى عملية الكمر الهوائى المشترك. *أوضحت النتائج أن قيم نشاط الانزيمات المدروسة حققت قيم عالية للمخاليط العضوية الملقحة بالميكروبات متعددة الأغراض مقارنة بتلك المخاليط غير الملقحة 213) and (445 and 332); (96 and 75) and 143) لنشاط الانزيمات بالترتيب السابق للمخاليط العضوية الملقحة وغير الملقحة على الترتيب. *حققت قيم الأعداد الكلية للبكتيريا والفطريات أعلى القيم للمخاليط العضوية الملقحة مقارنة بالمخاليط غير الملقحة (5.61 and 5.45) TFC (9.28 and 7.8) TBC للمخاليط الملقحة وغير الملقحة على الترتيب. *حققت قيم درجات الحرارة والـ pH للمخاليط الملقحة أعلى القيم مقارنة بتلك غير الملقحة للمخاليط العضوية الملقحة وغير الملقحة على الترتيب.38.15) (33.4 °C and pH 8.5) and (°C and pH 8.9 ثالثا: تأثير نظم الكمر الهوائى المشترك على خصائص وصفات المواد المكمورة *حقق نشاط الانزيمات المدروسة أعلى القيم تحت نظام المصفوفات (الأكوام) مقارنة بنظام الاناء (البراميل)218 and 942, 169 على الترتيب كما سبق تحت نظام الأكوام مقارنة بتلك الأقل قيم تحت نظم البراميل 155, 611 and 126*حققت قيم الأعداد الكلية للبكتيريا والفطريات زيادة عالية تحت نظام المصفوفات.(5.74 and 8.94) للبكتيريا والفطريات أعلى من تلك المتحصل عليها تحت نظام البراميل 5.16 and 8.14 على الترتيب. *سجلت درجة الحرارة داخل الكومات أعلى درجات الحرارة مقارنة بدرجة الحرارة داخل البراميل 37.6 °C and 33 °C for piles and barrels أما قيمة ال pH فقد تحققت داخل الكومات أقل منها داخل البراميل 7.5 and 9.4 for piles and barrels على الترتيب. توجد علاقات إرتباط موجب عالى المعنوية بين صفات وخصائص المكمورات العضوية المدروسة بعضها وبعض علاوة على ذلك توجد علاقات إرتباط سالب عالى المعنوية بين كل خاصية من هذه الخواص مع كل من الـ pH والحرارة.