USE OF *BACILLUS CIRCULANS* AS BIO-ACCELERATOR ENRICHING COMPOSTED AGRICULTURAL WASTES

I- Identification and utilization of the microorganism for compost production

ABSTRACT

A hundred and twenty eight *Bacillus circulans* like isolates were collected from soil apart or the rhizosphere of a range of host plants grown in seven Egyptian governorates. All isolates were examined for qualitative enzymatic expression of amylase, pectinase, cellulase and phosphatase. The six most expressive isolates were subjected to further examination of chitinase production and then identified by Biolog identification system. Quantification of cellulose production along with P and K mobilization capacity as well as nitrogenase activities were then determined for the four isolates identified as *Bacillus circulans*. Those strains were used as bio-accelerating mixture in composting of broad bean stalks, maize stalks and rice straw. The physical, chemical and microbiological changes during composting of the 3 wastes were compared under the application of individual and variable combinations of biological, organic and inorganic accelerators. Data of this study showed that densities of total fungi, total bacteria and K mobilizers were gradually increased up to the 6th week of composting where they gave maximum records in maize stalks and rice straw received the inorganic + organic accelerator, the 3 mixed accelerators and the organic accelerator, respectively. Spore forming bacteria, however, showed similar response but up to the 3rd week in rice straw amended with the 3 accelerators. In all treatments, the peaks of microbial densities were followed by pronounced decreases at the 9th week of composting. Temperature, on the other hand, showed a maximum increase during the 2nd to 4th week of composting and then gradually decreased up to maturity (20°C). C/N ratios also showed a gradual decrease with time, and gave the lowest records at maturity. On the other hand, available P and K were gradually increased particularly with the application of mixed accelerators.

Key words: *Bacillus circulans*, Enzymatic production Agricultural wastes, composting, Inorganic, organic and bio accelerator.

INTRODUCTION

As the potential of plant growth promoting rhizobacteria (PGPR) is realized, researches into their applications have increased dramatically over the last few decades. A diverse array of bacterial species including *Azotobacter, Azospirillum, Bacillus, Pseudomonas, Klebsiella* and *Enterobacter* has been shown to enhance growth and plant productivity by different mechanisms. **(Bertrand *et al.*, 2001, Mayak *et al.*, 2004, Tilak *et al.*, 2005)**

Bacteria of the genus *Bacillus* are one of the most diverse and also useful group of microorganisms. Many species of this genus are widely distributed in soil, air and water. The metabolic diversity of *B. circulans* in particular together with its low reported incidence of pathogenicity, has led to the fact that many representatives of this group are being used in a wide range of applications. Among the pronounced metabolic activities of *B. circulans* are the ability to (1) produce a range of enzymes, **(Travino, *et al.* 1989; Kim and Kim, 1993; Kyu, *et al.*1994; Wiwat, *et al.* 1999)**, (2) solubilize pounded nutrients e.g. silicate **(Savostin, 1972)**, tricalcium phosphate

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(Gaind and Gaur. 1991), (3) degrade organic wastes **(Kubo, *et al.* 1994)** along with the N_2 fixing ability of some strains **(Berge, *et al.*, 1990; Heulin, *et al* 1991).**

Organic fertilizers refer to fertilizers derived from plant and animal residues and they are crucial for fertile soil in organic cropping system. Composting represent a strategy of organic waste treatment that is fully compatible with sustainable agriculture. The process is defined as a microbial degradation of heterogeneous organic material under moist, self heating, aerobic conditions. New trends in composting of crop residues indicate the possibility of using accelerating factors, for shortening the composting period without deterioration of compost quality **(Abdel-wahab, 1999).**

Due to its multiple biological activities, *B. circulans* seem to be a good candidate to be used as bio-accelerator in new approaches for compost production. Therefore, the experimental work reported in this study aimed to assess the enzymatic expression along with physiological activities of *B. circulans* and utilize effective strains as bioaccelerators for composting of 3 different agricultural wastes.

MATERIALS AND METHODS

Materials

Sources of isolates

Representative samples were collected from soil apart and the rhizosphere of different plants grown in nine locations representing seven Egyptian governorates to be used for *B. circulans* isolation. Results of physico-chemical analyses of the soil samples used for *B. circulans* isolation are presented in Table (1)

Agriculture wastes

About 5 tons of agricultural wastes, i.e. broad bean stalks, maize stalks and rice straw, were obtained from El-Bosaily farm, El Behaira governorate, to be used for compost production. The used materials along with their chemical analyses are presented in Table (2).

Accelerators

Bio-accelerators

A mixed liquid culture of four effective *B. circulans* strains were used as a bio-accelerator for compost production.

Inorganic accelerators

A range of mineral nutrients in different forms were used as inorganic accelerator, i.e. Ammonium sulfate (20.5%N), super phosphate (15.5%P_2O_5), potassium sulfate (48 %K_2O), rock phosphate (20% P_2O_5) and feldspar (10 % K_2O).

Table (1): Physico-chemical analyses of soil used for isolation of *B. circulans*.

Physical analysis				Chemical analysis							
Governorate	Soil texture	pH	E.C mmhos/cm	Cation (meq/L)				Anion (meq/L)			
				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{-2}	HCO_3^{-}	Cl^{-}	SO_4^{-2}
El-Behaira	Sandy soil	7.0	1.50	10.9	1.6	1.72	1.86	0.0	2.7	3.0	10.3
Alexandria	Loamy sand	7.2	3.41	6.1	3.0	1.20	15.1	0.0	2.9	15.0	7.50
El-Kalubia	Clay soil	7.7	2.93	1.5	0.7	7.06	0.35	0.0	3.5	2.5	3.61
El-Monufia	Clay	7.6	0.96	2.8	0.2	3.62	1.68	0.0	3.8	3.0	1.50
Matrooh	Sandy soil	9.5	0.42	0.2	0.3	6.96	0.86	0.0	4.0	4.0	0.32
Giza	Sandy soil	8.4	0.62	1.6	0.4	2.70	0.60	0.0	1.0	2.5	1.80
El-Wadi El-Gadid	Sandy soil	7.6	0.82	13.0	8.4	9.53	1.68	0.0	4.0	11.0	17.6

Table (2): Chemical analysis of three agricultural waste materials used for compost production.

Waste material	Organic. carbon (%)	Total N (%)	C/N ratio	Total P (%)	Avail-able P (ppm)	Total K (%)	Avail-able K (ppm)
Broad bean stalks	31.41	0.75	41.9	0.30	0.011	0.38	0.09
Maize stalks	33.50	0.65	51.5	0.26	0.093	0.87	0.11
Rice straw	37.6	0.48	78.3	0.23	0.011	0.79	0.13

Organic accelerators

Animal manure obtained from El-Bosaily farm situated at El –Behaira governorates, was used as an organic accelerator for compost production. It contained 38.41% organic carbon, 2.66% total N, a C/N ratio of 14.44:1, 0.80% total P and 3.16% total K.

Experimental techniques

Sampling and isolation of *B. circulans*

Soil auger was used to collect free soil samples at 15 cm depth while whole plants representing the vegetative stage of clover, maize, soybean, chickpea, potato and sow thistle weed were put into plastic bags and directly transferred to the laboratory for *B. circulans* isolation. Soil apart or rhizosphere soil samples were serially diluted and all dilutions were used for isolation using pour plate method with incubation at 30°C. Colonies with typical appearance of *B. circulans*, i.e. having transparent protuberant mucous appearance which gave the shape of tears were isolated, alternatively streaked on nutrient agar and Aleksandrov's solid medium **(Zahra, 1969)** and then checked for purity. Pure cultures were kept on nutrient agar slants at 4°C

Assessment of some physiological activities of *B. circulans* isolates.

For this purpose, each of the *B. circulans* isolate was examined for the following metabolic activities.

a- Amylase production: based on the formation of colorless zone around the organisms grown on starch agar medium **(Collins and Patricia, 1984)** supplemented with iodine after 24 hours of incubation at 30°C.

b- Pectinase production: based on hydrolysis in potato slice by the tested organisms after 3 days incubation at 30°C.

c- Cellulase production and activity according to **Halliwall (1958)** using carboxy methyl cellulose (CMC) as a substrate.

d- Chitinase production: based on molecular weight identification using SDS-PAGE according to the method described by **Laemmli (1970).**

e- Phosphatase production and activity: based on the formation clear zone around the organism grown on modified Buntt and Rovira agar medium **(Abd El-Hafez, 1966)** and the method described by **Jackson (1973)**, respectively.

f- Potassium solubilization on modified Aleksandrov's medium **(Zahra, 1969**) with shaking at 100 rpm /30°C/ week. Shaked culture was used to determine soluble K using flame photometer as described by **Jackson (1973**).

g- Nitrogenase activity according to **Hardy *et al* (1973)**

Identification of some selected *B. circulans* isolates.

The most metabolically expressive isolates were identified based on Bergy's Manual of Systematic Bacteriology, 1986 and 1994 **(Sneath, 1986)** and Biolog system. The differential characterization in the Biolog system is based on carbohydrate fermentative ability of the tested isolate.

Compost preparation

Twenty one piles, 300 Kg each, were prepared from shredded wastes (rice straw, maize stalks and broad bean stalks) using either of seven different treatments representing, inorganic accelerator, organic accelerator, bio accelerator as an individual treatment or as double or triple combined applications that gave a C/N ratio of 30:1.Each pile was built up in successive layers (75cm in thickness), moistened with water up to 60%, tamped well, covered with a plastic sheet and turned over every 3 weeks. Moisture content was maintained at 60 % during composting period.

Parameters measured

Chemical and physical characteristics of composted materials

pH, total nitrogen, available phosphorus and available potassium were determined in raw or composted waste materials according to **Jackson (1973).** Organic carbon was determined according to **Walkley and Black (1934)**

Temperature and moisture contents

Temperature was measured in the piles at different depths by using thermometer, and moisture content was measured by oven drying at 70°C until reaching a constant weight.

Microbiological determination

Densities of total count of mesophilic, thermophilic and sporforming bacteria were determined by plate count on soil extract agar medium **(Clark, 1965)** with incubation at 30±2°C. The total count of mesophilic fungi were determined by plate count on potato dextrose agar (PDA) medium **(Ahmed, 2001)** with incubation for 5 days at 25±2°C. Counts of K mobilizing bacteria were determined by plate count on modified Aleksandrov's medium **(Zahra, 1969)** with incubation for 5 days at 30±2°C.

RESULTS

Isolation of *B. circulans* like organisms

A total of 128 isolates with typical appearance of *B. circulans* on modified Aleksandrov's medium, were collected. Thirty isolates were originated from soil apart and 98 were obtained from the rhizosphere of maize, soybean, clover, potato, chickpea or the sow-thistle weed grown on clay, sandy or loamy sand soil.

Assessment of enzymatic expression and metabolic activities of *B. circulans* like isolates

Considerable variations were generally recorded among collected *B. circulans* like isolates regarding their capabilities for enzymatic expression of amylase, pectinase, phosphatase and cellulase. The frequency distribution of high enzymatic expressions among those isolates was evaluated based on soil type and soil cultivation (Table 3). Data showed that, the average frequency distributions of high enzymatic expression were descendingly arranged as clay (9%), sandy soil (6.3%) and loamy sand (10%).On the other hand, the recorded averages of frequency distribution due to soil cultivation was 7.6, 5.8 % for high enzymatic expression of isolates originated from the rhizosphere and soil apart (or uncultivated soil), respectively.

The six isolates, AT13, AT21, SD6, SD29, SD33 and MM1, showed effective qualitative expression of amylase, pectinase and cellulase, were further subjected to chitinases production test using SDS-

Table (3): Frequency distribution of isolates showed high levels of enzymatic expressions among *B. circulans* like isolates as influenced by soil type and soil cultivation

Enzyme	High frequency distribution (%)				
	Soil type			Soil cultivation	
	Clay	sandy	Loamy sand	Soil apart or uncultivated soil	Cultivated plant rhizosphere
Amylase	12	17.6	22.9	20.0	17.3
Pectinase	12	0	2.9	0	1
Cellulase	12	7.4	11.4	3.3	11.2
Phosphatase	0	0	2.9	0	1
Average	9	6.3	10	5.8	7.6

Polyacrylamide gel electrophoresis (Figure.1). Chitinase production was detected in 3 out of the 6 tested strains. The isolates MM1 produced chitinases with molecular weights of about 50, 44 and 25 kDa. while SD33 had chitinases with molecular weights of about 51, 43.4 and 40.2 kDa. SD29 produced chitinase with molecular weight of 51 and 46 kDa. reference strain (*B. circulans* 1258) also produced chitinases with 51, 43.4 and 40.2 kDa.

Identification results of the 6 most effective *B. circulans* like isolates indicated that the 2 isolates AT13 and AT21 were strains of *Penibacillus (Bacillus) polymyxa* and *P. Pabuli*, respectively. The other 4 isolates, i.e. SD6, SD29, SD33 and MM1 represented *B. circulans* strains.

The quantitative assessment of the metabolic activities of the four identified strains of *B circulans* is given in Table (4). Data showed that while all strains were able to produce cellulase, fix atmospheric N_2 and mobilize K, only one (the SD6) was capable of mobilizing P (7.44 ppm) with 29.4% efficiency of the total added P (25.25 ppm). However, other strains showed variable degrees of metabolic effectiveness. While the SD29 actively produced cellulase, the MM1 (Figure 2) and SD6 were relatively superior in nitrogenase activity and K mobilization. The recorded figures were 70.06 µg protein, 3.88 µmole C_2H_4/ml/h and 8.85 ppm K, respectively. Variable lower records were observed regarding other parameters but, the SD33 strain showed an intermediate rank as it produced 31.89µg protein cellulase, reduced acetylene at a rate of 0.47µmole C_2H_4/ml/h and mobilize K at 64.26% efficiency, i.e. 5.72 ppm from a total of 8.90 ppm.

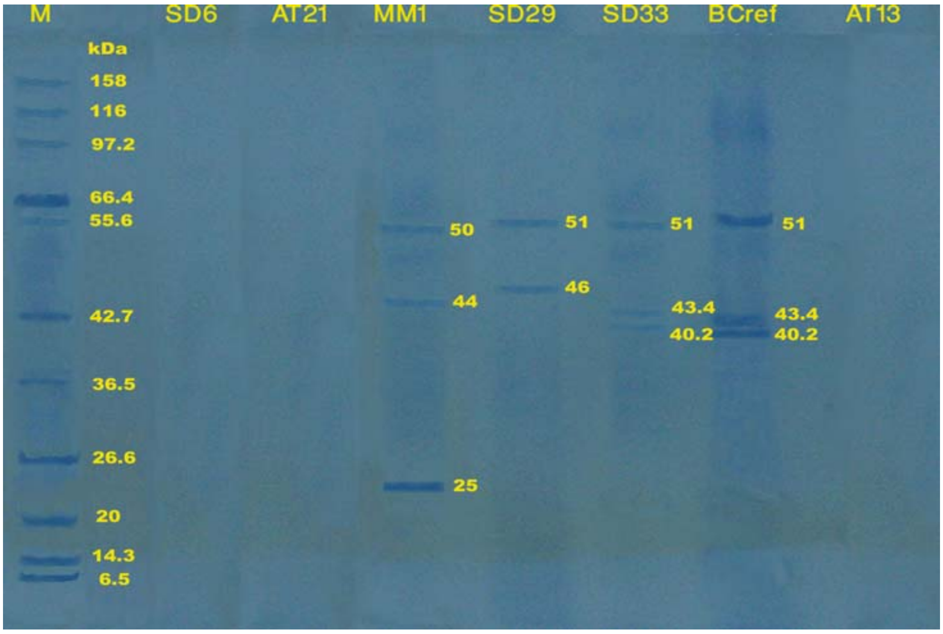

Figure (1): SDS-Polyacrylamide gel (10 %) electrophoresis of *B. circulans* 1258 and 6 selected isolates

a) b)

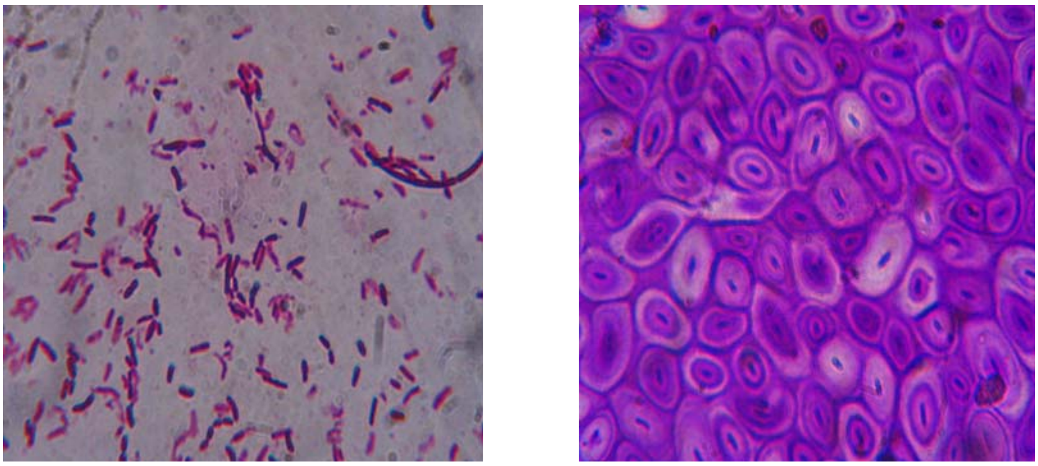

Figure (2): Morphological characteristics of gram stained *Bacillus circulans* strain. MM1 grown on nutrient broth (a) and on modified Alexsandrov's agar medium (b).

Table (4): Assessment of some metabolic activities of four strains of *B. circulans*

Parameter	*B. circulans* strains			
	SD6	SD29	SD33	MM1
Cellulase production (µg protein)	4.50	70.06	31.89	11.66
Nitrogenase (µmole C_2H_4/ml/h)	0.11	0.30	0.47	3.88
[a] Available Phosphate (ppm)	7.44	0.0	0.0	0.0
[b] Available potassium (ppm)	8.85	5.50	5.72	3.16

[a]Total phosphate was 25.25 ppm
[b]Total potassium was 8.90 ppm

Microbiological, physical and chemical changes during composting of three agricultural wastes amended with variable accelerators

Microbiological changes

Data in Figures (3 &4) showed that as a general trend, the microbial densities were generally increased up to the maximum level at the 3rd or 6th week of composting and then declined. Rice straw and broad bean stalks showed their maximal fungal colonization with the inorganic and organic accelerator, respectively. The recorded figures were 145 x 10^4 and 114 x10^4 cfu/ g composted material at the 6th week of composting. Only, maize stalks harbored the highest total fungal densities when the inorganic and organic accelerator were combined being 175 X 10^4 cfu/g composted material. Broad bean stalks, rice straw and maize stalks on the other hand, showed maximum colonization with bacteria when inorganic + biological accelerator, organic + biological and the 3 accelerators were combined, respectively. Being 297, 292 and 298 X 10^6 cfu/g composted materials, respectively.

Maximum densities of thermophilic bacteria colonizing the 3 tested waste materials were observed at the 3rd week of composting particularly with the application of organic accelerator (Figure 5). In this respect, little differences were generally observed among the recorded figures being 114, 118 and 129 X 10^5 cfu/g composted materials for broad stalks, maize stalks and rice straw, respectively. Combined accelerators generally, gave lower effect on densities of thermophilic bacteria than single accelerator.

Densities of spore formers and K mobilizers were either maximally enhanced at the 3rd week as shown with maize stalks or at the 6th week as shown with broad bean stalks and rice straw (Figure 6 and 7). However, maximum colonization with the two groups of bacteria was always observed with the application of combined accelerators. This finding was true for broad bean stalks amended with mineral + bio-accelerator, maize stalks or rice straw amended with organic + biological accelerator and rice straw amended with the 3 accelerators. The recorded figures were 292, 286, 284 and 276 X 10^4 cfu/g composted materials in the same above–mentioned respective order.

Physical changes

pH

The pH records of composted wastes did not give particular pattern in most cases, they were about the level of neutrality. This finding was generally observed with the single or combined accelerators.

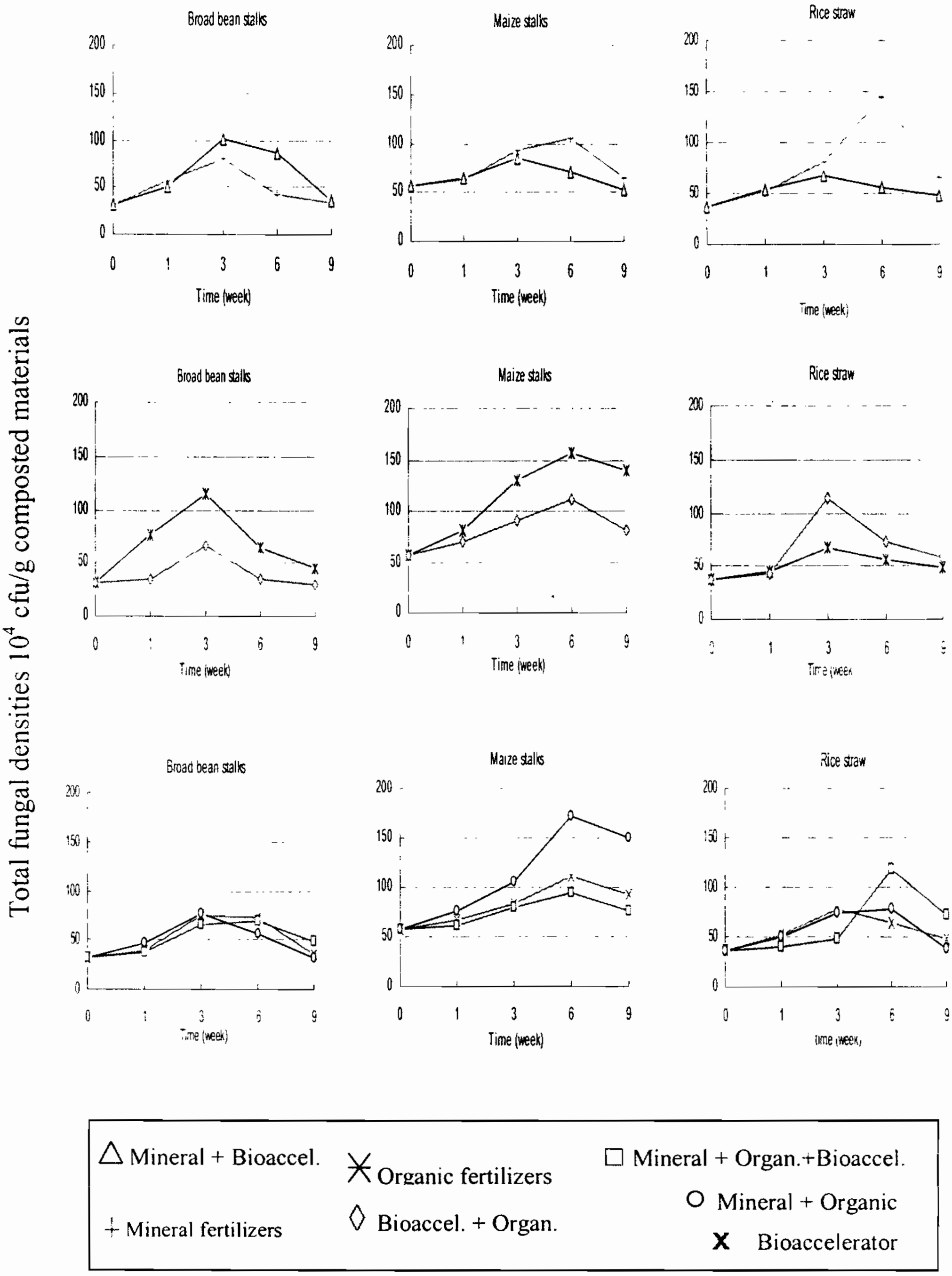

Fig (3): Changes in total fungal densities during composting of broad bean stalks, maize stalks and rice straw under different types of accelerators for 9 weeks.

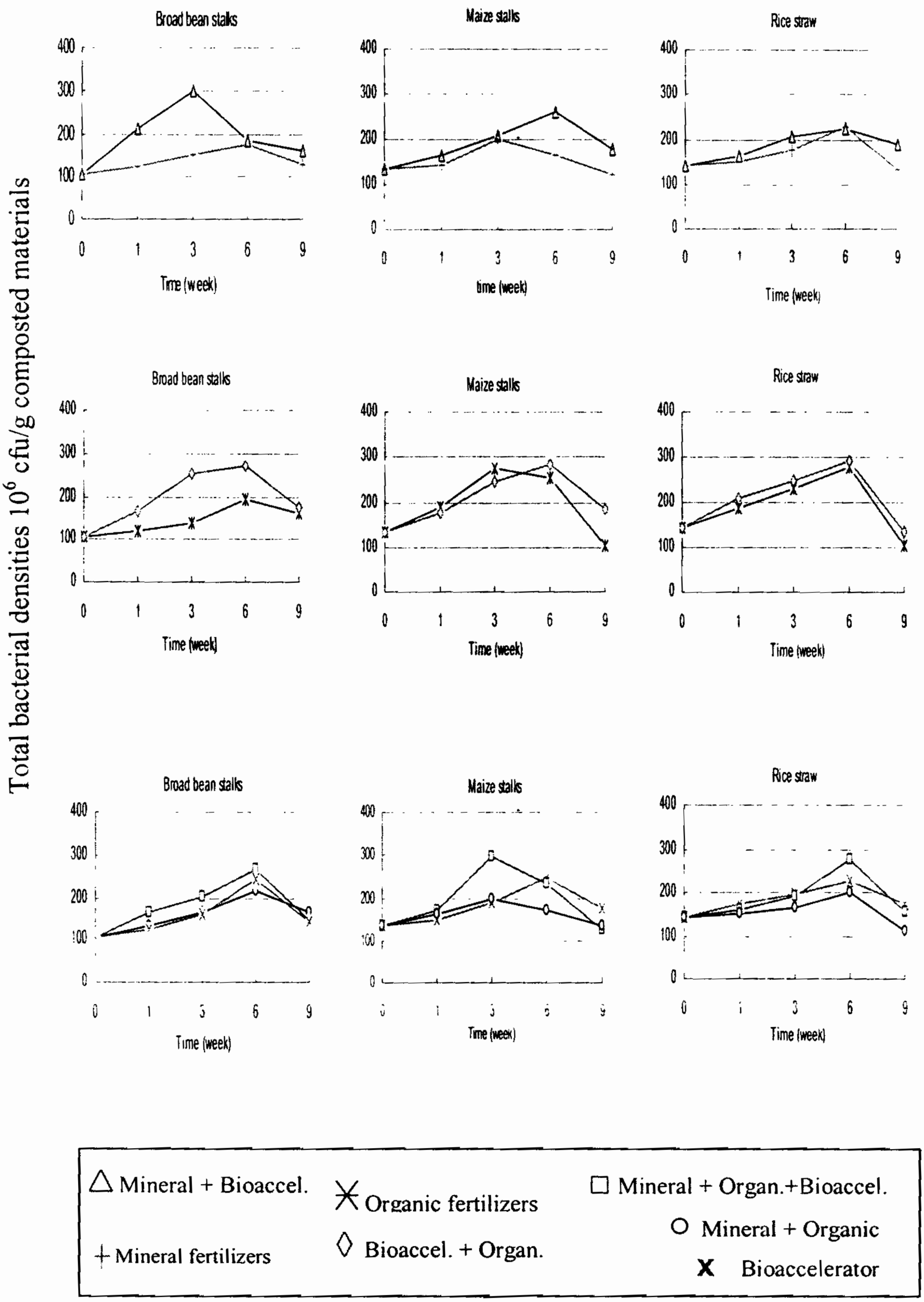

Fig (4): Changes in total bacterial densities during composting of broad bean stalks, maize stalks and rice straw under different types of accelerators for 9 weeks.

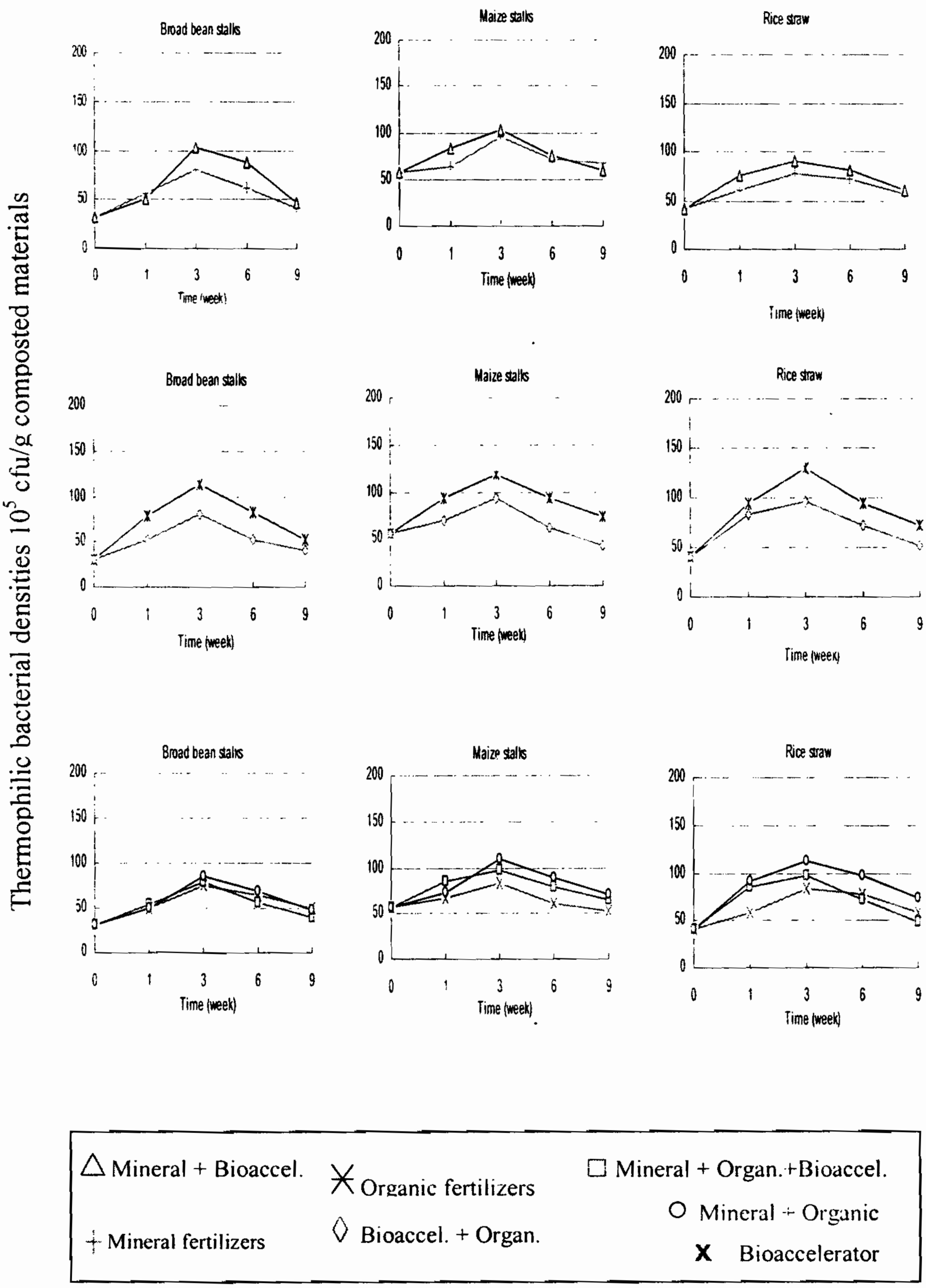

Fig (5): Changes in thermophilic bacterial densities during composting of broad bean stalks, maize stalks and rice straw under different types of accelerators for 9 weeks.

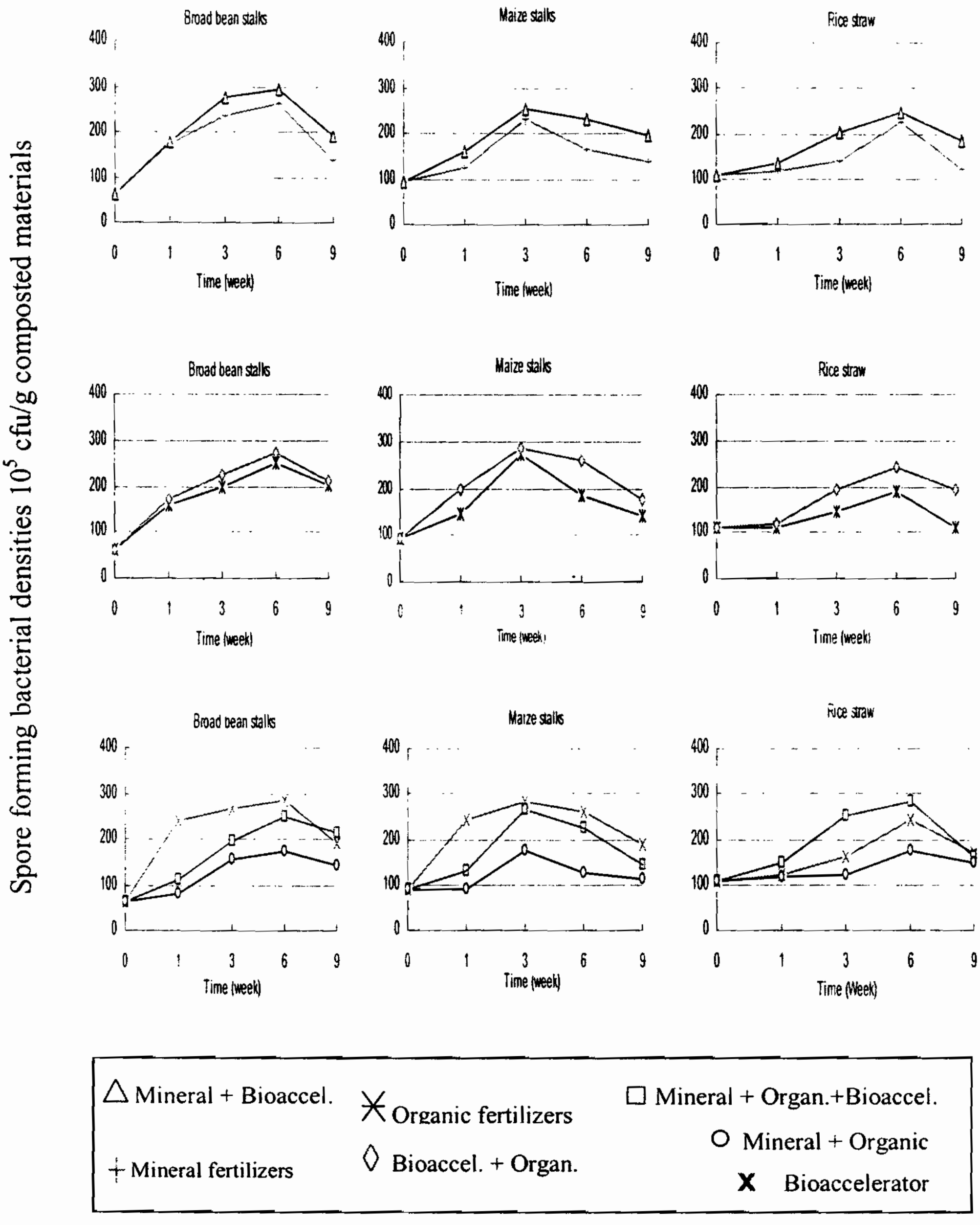

Fig (6): Changes in spore forming bacterial densities during composting of broad bean stalks, maize stalks and rice straw under different types of accelerators for 9 weeks.

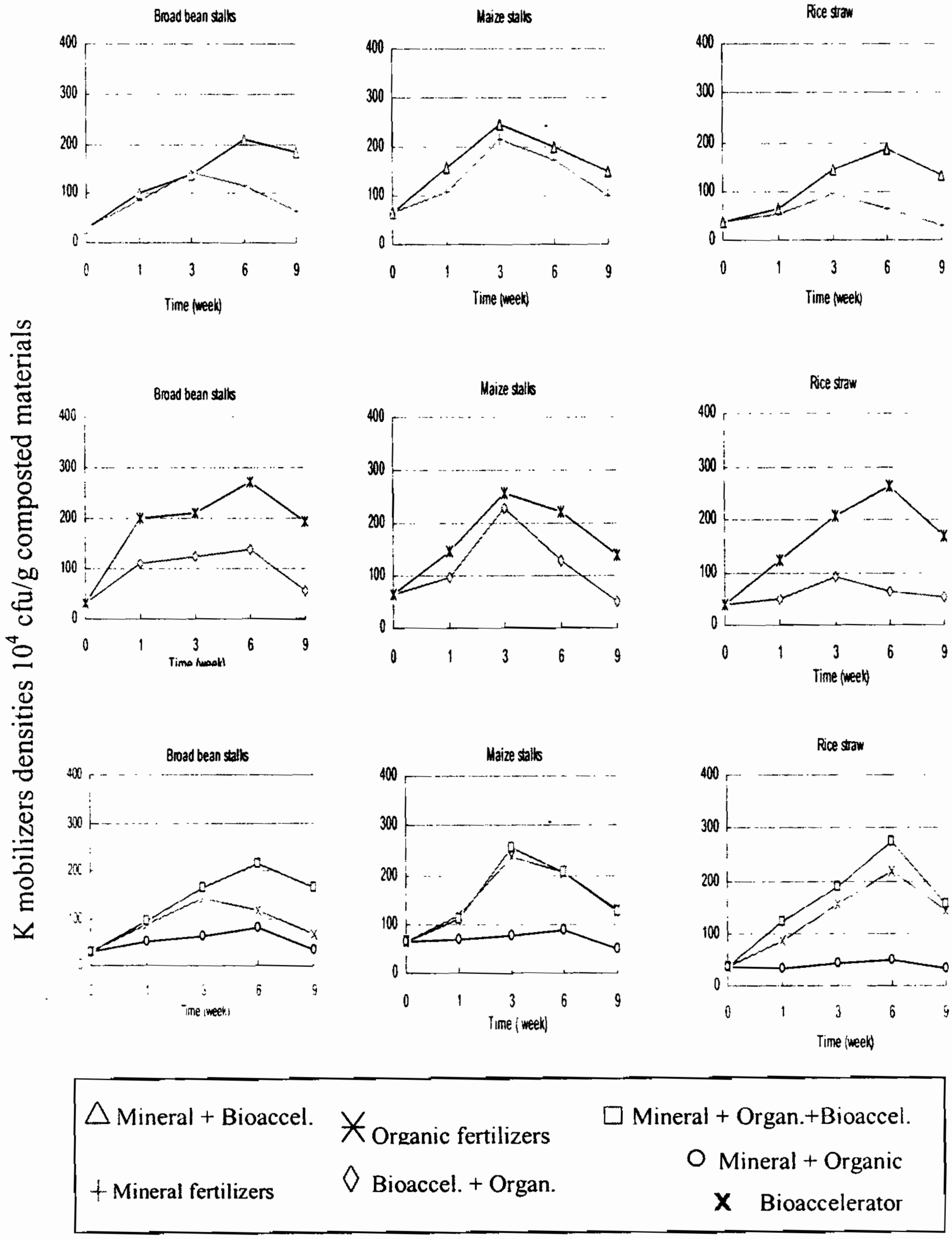

Fig (7): Changes in K mobilizers densities during composting of broad bean stalks, maize stalks and rice straw under different types of accelerators for 9 weeks.

Temperature

Temperature appeared to be elevated to the maximal level at the 2nd week (60-67°C), slowly decreased up to the 4th week and then gradually declined up to the end of composting period (Figure 7) giving the lowest records (20°C).

Chemical changes

The calculation of carbon / nitrogen ratios of composted wastes showed gradual decreases to variable levels in response to applied accelerators (see Figure 8). The decrease in that ratio could be exemplified by the drop in that parameter from 78.37, 51.53 and 41.88 at zero time to 19.95, 16.76 and 16.27 at the 3rd week for rice straw, maize stalks and broad bean stalks amended with mineral accelerator, and maize stalks amended with mineral + organic accelerator, respectively. The lowest records of C/N ratios were obtained at the 9th week of composting.

Gradual increases in the percentage of available P and K were detected with composting time (Figures 9 and 10). The dual or triple application of the tested accelerators seemed to have the most pronounced effects on those parameters. At the end of the composting period, the highest records of available P and K were generally reported. Broad bean stalks and rice straw received combined organic + biological and mineral + biological accelerators showed nearly similar content of available P being 0.31 and 0.30%, respectively. The combined 3 accelerators were more effective regarding available K content of broad bean stalks (0.39%). Available K content was also particularly higher in rice straw received the organic accelerator (0.52%).

DISCUSSION

Composting of agricultural wastes is one of the most common methods for production of organic manures. Enrichment of compost by different materials is often desired in order to a) improve chemical composition and physical structure of the heap, b) to supply desirable microorganisms and c) to reduce nitrogen losses. There are different natural substances (e.g. organic matter) or artificial ones (e.g. chemical fertilizers) which can be used to increase the nitrogen and other nutrients content of the heap **(Martin and Gershuny, 1992).** At present there are increasing interests in biological accelerator (e.g. microbial strains) to enhance breaking down of organic wastes. Studies dealt with inorganic, organic or bioaccelerators emphasized the importance of microorganisms in this concern. **Gaur (1986)** noted that enrichment of compost with *Azotobacter* and phosphate dissolving bacteria or fungi improved the nitrogen content and increased the availability of phosphates.

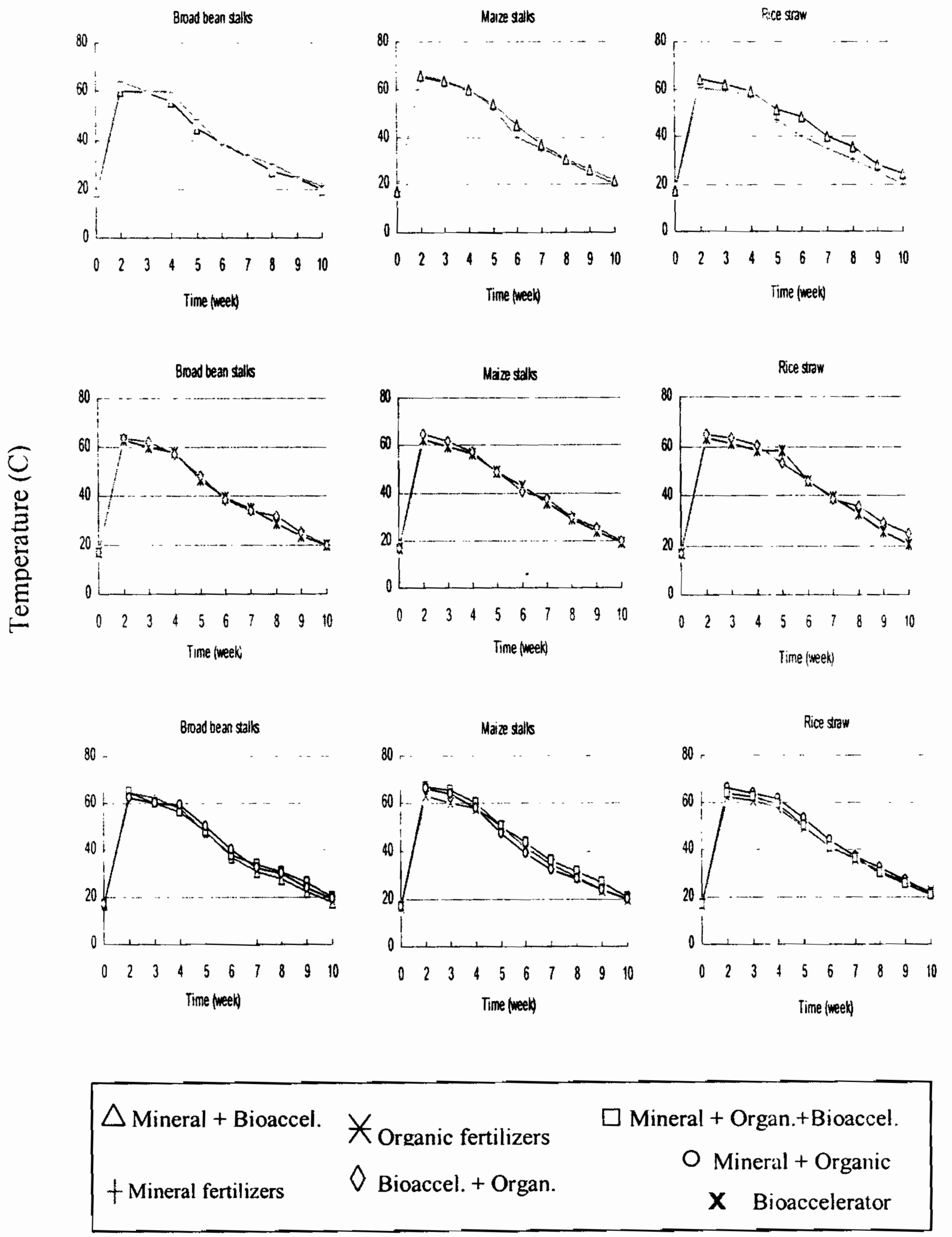

Fig (8): Changes in temperature during composting of broad bean stalks, maize stalks and rice straw under different types of accelerators for 10 weeks.

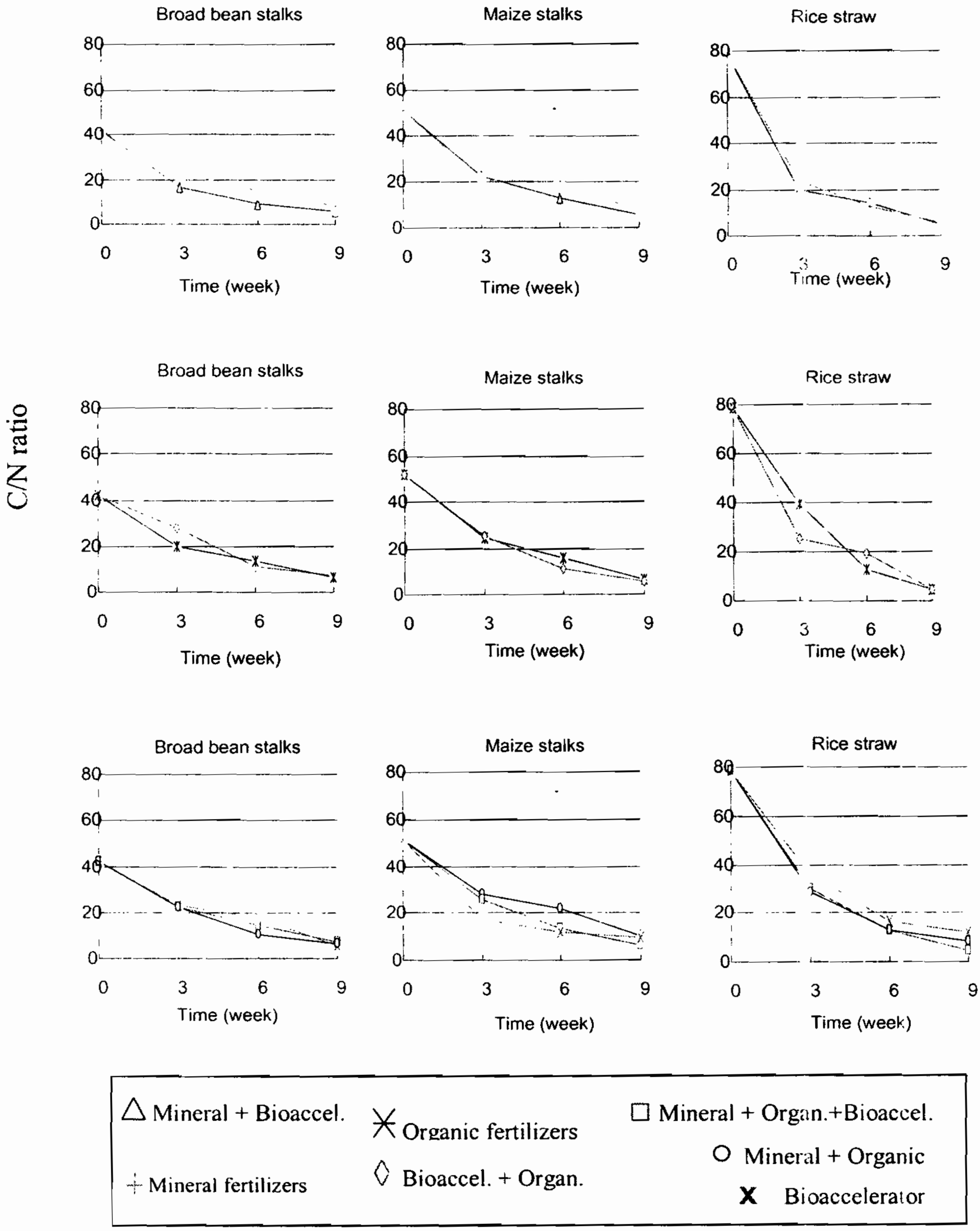

Fig (9): Changes in C/ N ratios during composting of broad bean stalks, maize stalks and rice straw under different types of accelerators for 9 weeks.

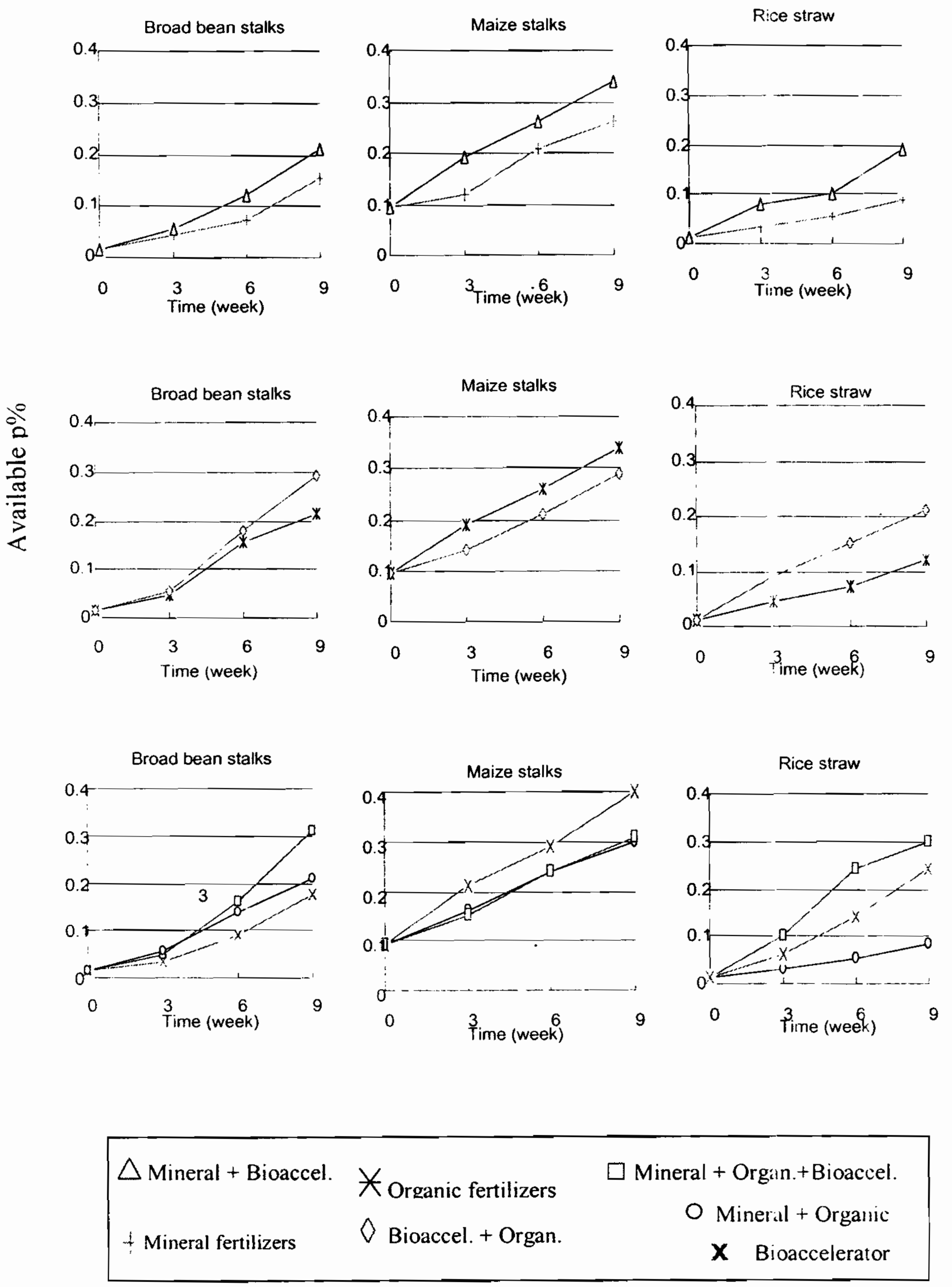

Fig (10): Changes in available P during composting of broad bean stalks, maize stalks and rice straw under different types of accelerators for 9 weeks.

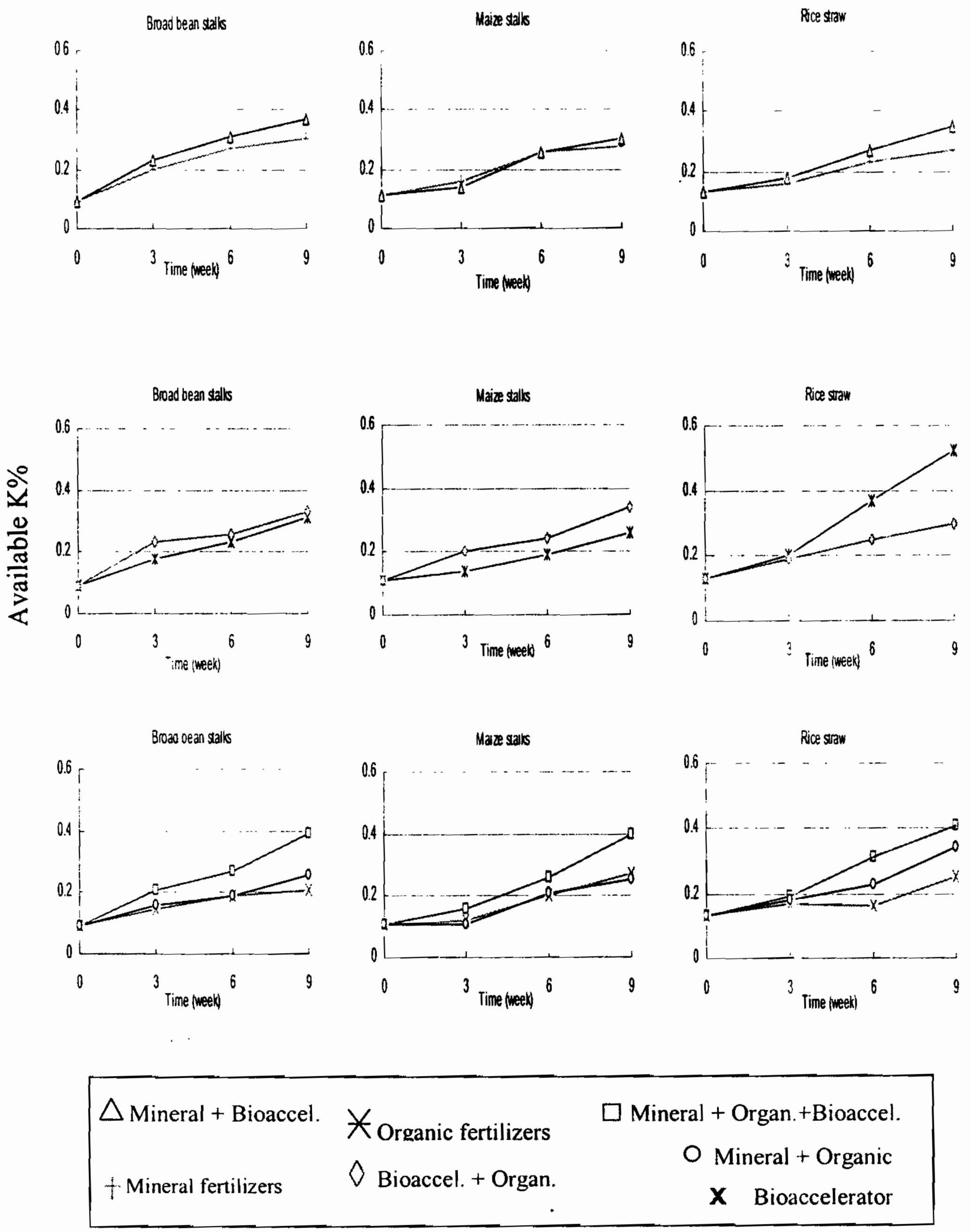

Fig (11): Changes in available K during composting of broad bean stalks, maize stalks and rice straw under different types of accelerators for 9 weeks.

Dalzell et al. (1987) reported that inoculating a compost heap with a suitable strain of *Azotobacter* in the presence of rock phosphate led to significant increase in N content of the mature compost. **Abdel-wahab (1999)** also showed that *Azolla* application and inoculation with cellulytic fungi may accelerate the rate of decomposition and led to a more decline in organic carbon of treated than in the untreated heaps. **Desoki (2000)** reported that when composted heaps of peanut hulls, saw dust and pith (waste of sugar cane factory) were amended with biogas manure, vermiculite, manganese ore and rock phosphate in addition to biofertilizers, the rate of decomposition was accelerated and high quality compost was produced.

Utilization of *B. circulans* as biological accelerator effectively degrading plant materials for waste composting and also as a biofertilizers actively multiplying and thus enriching the composted material were two combined purposes aimed from this study. The idea was derived from the fact that *B. circulans* is quite effective in production of a wide range of enzymes degrading plant materials such as amylase (**Kawn** ***et al.*****, 1993**), cellulase **(Kim and Kim, 1993)** and chitinases **(Wiwat** ***et al.*****, 1999**). Moreover, although the organism was traditionally utilized for years as potassium solubilizer **(Mansour** ***et al.*****, 1984; Groudeva and Groudev 1987 and Galgoczy, 1990)**, some studies showed that it is also capable of improving phosphate content (**Gaind and Gaur, 1991**) and nitrogen content (**Gaind and Gaur, 1991**) of the hosts. Those multiple activities were thought to be utilized for composting of 3 agricultural wastes, i.e. broad bean stalks, maize stalks and rice straw. Conclusively, *B. circulans* appeared to be an effective bio-accelerator for composting of the above mentioned wastes.

REFERENCES

Abd El-Hafez, A.M (1966). Some Studies on Acid Producing Microorganisms in Soil and Rhizosphere with Special Reference to Phosphate Dissolvers .Ph.D. Thesis, Fac. Agric., Ain Shams Univ. Egypt, pp. 29-30.

Abdel-Wahab, A.F.M. (1999): Iron – Zinc – Organic Wastes Interactions and Their Effects on Biological Nitrogen Fixation in Newly Reclaimed Soils. Ph.D. Thesis, Fac. Agric., Ain-Shams Univ., Egypt. pp. 54-62.

Ahmed. M. A. (2001). Dictionary of the Fungal Terminology. Academic Book Shop. 230-231.

Berge, O.; J. Fages; D. Mulard and J. Balandreau (1990). Effect of inoculation with *Bacillus circulans* and *Azospirillum lipoferum* on crop-yield in field grown maize. Symbiosis, 9: 259-266.

Bertrand, H.; R. Nalin; R. Bally and J.C. Cleyet-Marel (2001). Isolation and identification of the most efficient plant growth promoting bacteria associated with canola (*Brassica napus*). Biol. Fertil. Soils. 33: 152-156.

Clark F.E. (1965). Agar Plate Methods for Total Microbial Count in Methods of Soil analysis, Part 2. Agronomy, Am. Soc. of Agron. Inc, Madison, Wis., USA, 1460-1466.

Collins, C.H. and M.L. Patricia (1984). Identification methods. In Microbiological Methods. Butterworth, borough Grean, Seven oaks, Kent, England. 5^{th} Ed., 56-113.

Dalzell, H.W.; A.G. Bidlestone; K.R. Gray and K. Thurairajan (1987). Compost Production and Use in Tropical and Subtropical Environments. **FAO, "Soil Management" Bulletin** 49:18-36.

Desoki, A.H. (2000). Utilization of Some Agricultural Wastes for the Production of Rhizobial Inoculants and Soil Amendments. M.Sc. Thesis, Dept. Agric. Sci., Institute of Environmental Studies& Research, Ain Shams., Univ., Egypt.

Galgoczy, G.F. (1990). Country report (National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary) presented in the workshop on aluminosilicate minerals biodegradation. In proc. Of the 5th European congress on Biotechnology, Copenhagen, Denmark, 201-202.

Gaind, S. and A.C. Gaur (1991). Thermotolerant phosphate solubilizing microorganisms and their interaction with mungbean. Plant and Soil, 133:141-149.

Gaur, A.C. (1986). Recent trends in recycling of crop residues. Maharashtra Agric. Univ., 11: 127-133.

Groudeva, V.I. and S.N. Groudev (1987). Aluminosilicate biodegradation in the soil. Proc. of the 9th Int. Symp. on Soil Biology and Conservation of the Biosphere. J. Szegi. (Ed.), Akademiai Kiado Budapest, pp. 621-628.

Halliwall, G. (1958). A micro determination of cellulose in studies with cellulase. Biochem. J., 68: 605-613.

Hardy, F.W.E; R.G. Burnus and R.D. Holsten (1973). Application of the acetylene assay for measurement of nitrogen fixation. Soil. Boil. Biochem., 5:47-81.

Heulin, T., O. Berge; J. Balandreau (1991). Diversity of diazotroph population in the rhizosphere in the rhizosphere of maize (*Zea mays* L.) growing on different French soils. Biol. Fert. Soils, 11(3): 210-215.

Jackson, M.L. (1973). Soil Chemical Analysis Prentice Hall of India Private Limited, New Delhi India, pp. 183-192.

Kawn, H.S.; K.H. So; K.Y. Chan and S.C. Cheng (1993). Production of thermotolerant beta amylase by *Bacillus circulans*, World J. Microbiol. Biotech., 9(1): 50-52.

Kim, C.H. and D.S. Kim (1993). Extracellular celluolytic enzymes of *Bacillus circulans* are present as two multiple protein complexes. Appl. Biochem. Biotechnol., 42:83-94.

Kubo, M.; J. Okajima and F. Hasumi (1994). Isolation and characterization of soybean waste degrading microorganisms and analysis of fertilizer effects of the degraded products. Appl. Environ. Microobiol., 60(1): 243-247.

Kyu, K. h.; K. Ratanakhanokchai; D. Uttapap and M. Tanticharoen (1994). Induction of xylanase in *Bacillus circulans* B6 .Bioresource-Technol., 48(2):163-167.

Laemmli, U.K. (1970). Cleavage of the structural proteins during assembly of the head of bacteriophage T4. Nature., 227: 680-685.

Mansour, F.A.; M.A. Shady and Aida H. Afifty (1984). Microbial dissolution of silicon and alluminium from primary minerals. Egypt J. Bot., 27(1-3):1-15.

Martin, D.L. and G. Gershuny (1992). The rodale book of composting. Rodale press, Emmaus, Pennsylvania.USA.

Mayak, S.; T. Tsipora and G.R. Bernard (2004). Plant growth promoting bacteria confer resistance into tomato plant to salt stress. Plant Physiol. Biochem., 42: 565-672.

Savostin, P. (1972). Micro transformation of silicates. Z. pflanzenern. Bodenk., 132: (1) 37-45.

Sneath, P. H. A. (1986). Endosporforming ram positive rods and cocci In Beggys manula of systematic bacteriology.William R. Hensyl. 2: (13) 18-30

Tilak, K.V.; N. Ranganayaki; K.K. Pal; R. De; A.K. tripathi and B.N. Johri (2005) Diversity of plant growth and soil health supporting bacteria. Cur. Sci. 89: 139-150.

Travino, O.; W. Rodarte and L. Munguia (1989). An endoglucanase from an isolated strain of *B. circulans*. Appl. Microbiol. Biot., 31:(2) 146-149.

Walkley, A. and I.R. Black (1934). An Examination of the Degtijarelf for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci., 37: 29.

Wiwat, C.; P. Siwayaprahm and A. Bhumiratana (1999). Purification and characterization of chitinase from *B. circulans* No.4.1. Current Microbiol., 39:(3) 134-140.

Zahra, M.K. (1969). Studies on Silicate Bacteria. M Sc. Thesis, Fac. Agric, Cairo Univ., Egypt, pp. 44, 71-73.

الملخص العربي

استخدام الباسلس سيركيولانس كمسرع حيوى يثرى المخلفات الزراعية المستخدمة في انتاج الكومبوست

I – تعريف والاستفادة من الكائن الدقيق في انتاج الكومبوست

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تم عزل ١٢٨ عزلة من البكتريا الشبيهة ب *Bacillus circulans* من التربة وريزوسفير بعض المحاصيل النامية في ٧ محافظات مختلفة مع اختبار قدرة العزلات المتحصل عليها علي انتاج عديد من الانزيمات مثل الاميليز, السليوليز , البكتينيز والفوسفاتيز ثم انتخبت افضل ٦ عزلات في هذا الصدد واختبرت من حيث قدرتها علي انتاج الشيتينيز. ثم تم تعريف الست عزلات باستخدام نظام البيولوج التعريفي بالاضافة الي مرجع Berry's manual 1986 حيث اظهرت النتائج ان اربعة من الست عزلات المختبرة هي سلالات للنوع *Bacillus circulans* حيث اختبرت من حيث الانتاج الكمى لانزيم السليوليز و قدرتها علي تثبيت الازوت وتيسير الفوسفات والبوتاسيوم. تم استخدام خليط من هذة السلالات كمنشط حيوي في انتاج الكومبوست من ثلاث انواع من المخلفات الزراعية مع اضافة كلا من المنشطات الكيماوية والعضوية والحيوية. في معاملات فردية و زوجية وثلاثية وقد اظهرت النتائج حدوث زيادة في اعداد الفطريات والبكتريا الكلية وميسرات البوتاسيوم اثناء التحلل حيث وصلت الي اعلي زيادة لها في الاسبوع السادس مع استخدام المنشط العضوي والكيماوي مع حطب الذرة واستخدام المنشط العضوى والحيوى و باضافة الثلاث منشطات معا علي التوالي واظهرت البكتريا المتجرثمة نفس الاستجابة حيث وصلت لاقصي اعداد لها في الاسبوع الثالث مع استخدام الثلاث انواع من المنشطات وفي كل المعاملات تلي ذلك حدوث انخفاض في الاعداد عند تمام النضج (الاسبوع التاسع) ومن ناحية اخري سجلت درجات الحرارة اقصي ارتفاع لها في الاسبوع الثالث ثم اخذت في الانخفاض تدريجيا حتى وصلت في نهاية الكمر الي درجة حرارة ٢٠ درجة مئوية . كما حدث انخفاض ملحوظ في نسبة الكربون : النيتروجين مع زيادة ملحوظة في قيم الفوسفور والبوتاسيوم الميسر خصوصا مع استخدام خليط من المنشطات الثلاث.