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MICROBIAL ACTIVITY ON FICUS LEAVES WASTES BY MODIFIED COMPOSTING SYSTEM

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ABSTRACT: *Heaps experiment was technically designed, in November 2004. to investigate microbial activity and bioconversion of ficus leaves waste (Ficus nitida L.) to compost under ordinary and modified composting condition. Four forms of heaps as treatments were tested. No bacterial inoculation and modification was applied to the first heap (control). Second one was inoculated with agriculture waste effective microorganisms (EM). Third one was inoculated with a combination of nitrogen fixating bacteria (Azotobacter chrococcum & Azospirillum brasilinse) while the fourth heap was modified by covering it with transparent plastic sheet (0.5mm) at regular interval periods (week after week). After establishing heaps, the moisture content was conserved at 60% of water holding capacity (WHC). Microbial activity, physical and chemical properties of deferent samples taking from the fermented heaps were determined at (0, 2, 50, 80 and 100 days) from the start of fermentation. Some dominant colonies of bacteria, mesophilic actinomycetes and fungi were isolated, then identified and tested ability to cellulolytic, amilolytic, protolytic & lipolytic. The effectiveness of isolates were examined for biomass production using submerged culture condition by growing on chopped leaves at 1, 2, and 4% concentration .*

The obtained results demonstrated that population size of different microbial groups was not a limited factor in this composting process. The modification of heap gave the best results compared to the others treatments, either compost mature nor microbiological, physical and chemical properties .On the other hand fungi isolates gave a high assimilation rate on chopped leaves and biomass production more than bacteria and mesophilic actinomycetes .

key words : *agriculture -wastes - biodegradation -compost – amilolytic microorganisms Thermophilic – mesophilic- actinomycetes - cellulolytic - protolytic .*

INTRODUCTION

Fermentation process of agricultural waste is very important for environmental, sanitary, agricultural economic and agriculture biodynamic sustainability insurance. (Biddlestone *et al.* (1987) . Bioconversion of agriculture waste by microorganisms to valuable product known as compost has a beneficial effect on soil fertility

Inoculation of heaps with bioactive microorganisms leads to specific

microbial enrichment and rapid compost maturation (Ganesh &Thakre (2002), Cronje *et al.* (2003) and Smith &Hughes (2004). In this respect the heap can be enriched , by adding a combination between symbiotic N- fixing bacteria as *Azotobacter* & *Azospirillum* and cellulolytic bacteria (Rasal *et al.*,1988, Perestelo *et al.*, 1994, Ezelin *et al.*, 1996). Thermophilic cellulolytic microorganisms has important role in the same trend (Stutzenberger *et al.*, 199, Zanetta *et al.*,1994 and Suarez *et al.*, 2003) . Fungal population has a main role in cellulolytic and other organic materials decomposition (Kelley &Paterson,1997 , Hart *et al.*, 2002, and Suarez *et al.*,2003) . Actinomycetes population also has a vital known activity as scavenger in biodegradation of cellulose, hemicelluloses and other compounds .Microbial communities and activities, during composting of organic waste was increased and succeeded according to the waste type and fermenting conditions (Agamuthu *et al.*, 2000, Tiquia *et al.*, 2002, and Pedro *et al.*, 2003).

This work was conducted to study the effect of regular partially cover with transparent plastic sheet as a modified condition, and full homogenization after cover elimination on microbiological, physical and chemical properties of heaps , comparison with ordinary condition (without covering) . Moreover effect of inoculation with agricultural waste as effective microorganisms (EM) separately, and with a nitrogen fixing bacteria (*Azotobacter* &*Azospirillum*) on heap properties, also biomass production with test microorganisms by use chopped leaves as a nutrition source.

MATERIALS AND METHODS

Materials :

Various materials were: dry leaves of ficus trees (*Ficus nitida L*), was collected from a college farm (note these was collected and seasonally cenirated as wastes).

The activation mixtures are: organic manure was carried out from animal farm by rate 100kg/ ton , nitrogen source as ammonium nitrate (nitram) 33.5% by a rate 20 kg /ton ,Calcium supper phosphate($P_2 O_5$) 16.5% 4 kg /ton, cell suspension of effective microorganisms inoculants was carried out by personal communication , with colleagues of agric. , research center, while a symbiotic nitrogen fixing bacteria , cell suspension of *Azotobacter chrococcum* , *Azospirillum brasilense* one liter of each organism, CFU were (370 , 298 x 10^6 /ml^1) respectively . Isolated from fertile Egyptian soil, identified and performance was tested from previous studies.

Methods:

Heaps were technically designed by use Ficus leaves waste and other previous materials, in four heaps treatments. The heaps were done in different reciprocal layers (layer elevation nearly 20cm) and pressed by legs. Moistures content was conserved at 60% of WHC for long time composting process. The treatments of heaps were as follow:

-First heap without bacterial inoculation or any modification (control)
-Second heap inoculated with effective microorganisms (EM) separately.
-Third heap inoculated with combination between Azotobacter *chrococcum* and *Azospirillum brasilense* together, one liter of each microbe.
-Fourth heap without any inoculation, but modified with regular partially transparent plastic sheet cover (0.5mm), at reciprocal periods (week after week) and full homogenized after each cover elimination.

Activity of heap microorganisms:

All heaps microorganisms were determined as a biodynamic of (total bacteria, mesophilic & thermophilic actinomycetes and fungi population) at interval periods (0, 21 ,50 , 80 and 100 days) from fermentation process. Determination was done by pour plate methods , and using suitable media for each group as follow :Total bacteria were cultured on (SYA) , mesophilic and thermophilic actinomycetes were cultured on (ISSA) ,and fungi were cultured on (PDA) , and take care of incubation condition & periods of each group . Microbial colonies were counted and mean was calculated for each group.

Isolation, Identification and enzymatic activity of test organisms:

From microbial determination previous stage, dominant colonies were chosen and purified by streaking more time on the suitable media mentioned above under aseptic condition, then examined microscopically to insure purification.

These purified isolates were tested on ability cellulolytic, amilolytic, protolytic and lipolytic in laboratory bioassay. Different isolates were identified as genera by characterization review as bacteria (Bergys Manual , 1984) , actinomycetes (Szabo,1974) and fungal population (Booth,1971).

Biomass production of test organisms:

From ability of enzymatic activity, the effective isolates were examined on use chopped leaves as nutrition source at 1,2 and 4 % concentration by submerged culture condition , after elimination of base carbon source with or without inoculation as control . Single cell protein (SCP) known as biomass were measured after 10 days of each test organisms as dry weight/ 100 ml liquid medium,

Chemical & Physical properties of Heaps :

Physical and chemical properties of heaps samples were analyzed at different periods (0, 21, 50, 80 and 100days) of waste composting process. pH, EC, K and soluble cations and anions were determined according to

Jackson (1973), organic matter and CEC to Page *et al.* (1982), available P to Olsen *et al.* (1965) , and available N to Onken and Sunderman (1977).

RESULTS AND DISCUSSION

Activation of heap microorganisms:

Data presented in Table (1), indicated that in the first , second and third heaps treatments, microorganisms groups were different and increased however, the modified one (fourth heap) showed a decrease in some microbial groups at different periods (0, 21 , 50 , 80 and 100 days) . In the beginning, the native microbial activity was very low, and then proliferated gradually follow heap condition. In general, microbial activities were continuously increased. The maximum augmentations were recorded at100 days as a percentage for each microorganisms groups. These percentage were :227 ,229 ,210 161 % for total bacteria compared to the control at 0 period, while the increases for mesophilic actinomycetes were 368 ,346 , 300, 164 % and for thermophilic actinomycetes were 440 , 590 , 679 , 921 % . The increases of fungi were 328 , 426 , 375 , 236 % for first , second , third and fourth heap respectively .These results revealed an obvious increase in activation of thermophilic actinomycetes ,that may be due to the partial regular cover with plastic sheet other than ordinary condition .Wherever the increase percentage in the fourth heap approximately reached to nine fold at 100 days in comparison with zero time and other heap treatments. These results are in partial agreement with Godden and Penninckx (1984), Insam *et al.* (1996) and Tiquia *et al.* (2002).

Physical & Chemical properties changes of heaps :

Data in table (2) showed the influence of microbial inoculation by EM and cooperation with native waste microorganisms groups, also inoculation with combination between *Azotobacter chrococcum* and *Azospirillum brasilense* together and the period of composting on physical and chemical properties of the different heaps. An obvious decrease in EC, pH, OM, and C/N ratio were occurred with the period of composting in the different heaps. However a considerable enhancement in CEC, N, P and K were observed. Likewise the data revealed that the cations and anions was remarkably affected with composting period. Wherever Na^{+} , Mg^{++} and Cl^{-} markedly decreased, K^{+}, Ca^{++} and $SO4^{--}$considerably promoted. This changes in physical and chemical properties may be ascribed to more activity of thermophilic actinomycetes by modified condition and growing of some algae on surface layer as a result of enough light and moisture retention by partially covering . These findings are in consonance with Fermor (1993) ,Sarojini and Mathur (1994), Ezelin *et al.*(1996), Anshu *et al* (2002) and Smith and Hughes (2004).

Table (1) Heaps microorganisms groups activation and dominant percentage at different composting periods. (mean count CFU / g dry weight) .

Composting. periods	%	Total bacteria Cfu x 10^6 Heaps				Mesophilic Actinomycetes Cfu x 10^5 Heaps				Thermophilic Actinomycetes Cfu x 10^3 Heaps				Fungi Cfu x 10^4 Heaps			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0		165	171	185	163	95	115	135	131	25	22	19	26	35	41	44	36
	%	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
21		295	365	375	275	185	231	245	195	48	57	65	95	80	95	131	61
	%	179	213	203	169	195	200	181	148	192	259	342	365	228	231	297	169
50		325	375	383	285	230	251	275	205	60	95	71	151	95	120	131	71
	%	197	219	207	175	242	218	203	156	240	431	373	580	271	292	297	197
80		365	385	399	281	290	340	370	230	85	120	89	215	110	169	145	75
	%	221	225	215	174	305	295	274	175	304	545	468	826	314	412	329	208
100		375	392	390	260	350	398	405	215	110	130	129	250	115	175	165	85
	%	227	229	210	162	368	346	300	164	440	591	679	962	328	427	375	236

Table (2): Heaps Physical and Chemical properties changes at different composting periods.

Heaps	Periods composting	Physical properties					Chemical properties											
											Cations mq/l				Anions mq /l		
		E.C d/m	PH 1:10	O.M %	O.C %	N %	C/N ratio	P %	K %	ECC mq/ 100g	Na^{+}	K^{+}	Ca^{++}	Mg^{++}	$So4^{=}$	$Hco3^{-}$	CL^{-}
1	0	5.9	7.7	59.0	34.4	1.0	34.3	0.17	0.75	26	4.11	0.65	3.0	40	8.76	10	65.0
	21	5.4	7.8	60.5	35.17	1.0	35.17	0.18	1.0	25	3.47	0.86	4.5	50	6.33	3.0	45.0
	50	5.45	7.4	44.0	25.28	0.9	28.42	0.22	1.75	30	1.95	1.52	25	25	24.97	1.0	27.5
	80	3.50	7.3	39.0	22.67	0.9	25.18	0.24	1.75	78	1.30	1.52	5.0	30.1	19.32	1.0	17.5
	100	3.40	7.1	36.12	21.0	1.0	21.0	0.25	1.76	79	1.04	1.61	4.9	28.1	20.1	1.1	16.1
2	0	5.9	7.7	59.0	34.0	1.0	34.0	0.17	0.75	26	4.11	0.65	30	40	8.76	10	65.0
	21	5.4	7.7	58.0	33.72	1.0	33.72	0.21	2.1	27	2.6	1.82	24	26	18.42	1.0	35.0
	50	4.5	7.2	40.0	23.25	1.0	23.25	0.24	1.55	65	1.86	1.34	10	30	15.7	2.0	22.5
	80	3.9	7.3	38.0	22.09	1.0	22.09	0.27	2.0	85	1.55	1.73	13	27	24.78	1.0	17.5
	100	3.8	7.1	35.0	20.34	1.1	18.49	0.30	2.1	88	1.6	1.9	12	28	23.9	1.0	16.0
3	0	5.9	7.7	59.0	34.0	1.0	34.0	0.17	0.75	26	4.11	0.65	30	40	8.76	10	65.0
	21	6.5	7.5	55.0	31.97	1.0	31.97	0.19	2.05	28	3.26	1.78	35	25	23.04	2.0	40.0
	50	5.5	7.3	45.0	26.16	0.9	26.16	0.23	1.5	35	2.0	1.30	5.0	47	23.8	2.0	29.5
	80	3.8	7.2	37.0	21.51	1.0	21.51	0.28	1.65	85	1.55	1.52	8.0	27	20.57	1.0	16.5
	100	3.6	7.0	33.0	19.24	1.1	17.49	0.32	2.1	89	1.75	1.93	15	21	19.3	2.0	14.5
4	0	5.9	7.7	59.0	34.0	1.0	34.0	0.17	0.75	26	4.11	0.65	30	40	8.76	10	65.0
	21	3.4	7.3	48.76	28.34	1.0	28.34	0.23	2.25	71	2.6	1.95	15	15	19.45	20	12.5
	50	3.4	7.2	45.5	26.45	1.1	24.04	0.24	2.27	75	2.5	1.91	14.5	16	20.1	10	11.6
	80	3.2	7.1	43.2	25.11	1.2	20.93	0.28	2.29	82	2.2	1.89	14.1	14.5	25.5	8.0	9.4
	100	3.1	6.99	32.5	18.89	1.3	14.53	0.35	2.5	89	2.0	1.82	13.65	13.1	29.2	9.0	8.6

Identification & Enzymatic activety of test organisms :

The purified isolates of microorganisms groups (bacteria, mesophilic actinomycetes and fungi) as a dominant colonies were performed on cellulolytic ,amilolytic ,protolytic and lipolytic decomposition. After that purification related to genera characterization, 18 bacterial isolates belonging to six genera as shown in Table (3) were identified. These genera were arranged descendingly according to its dominant percentage as Bacillus 28%, *Pseudomonas* 22% , *Enterobacter* 11%, *Micrococcus* 11%, *Cytophaga* 17% and *Sporocytophaga* 11%. The bioactivities of these isolates was given marks as (-) non-active, (+) weak, (++) moderate and (+++) good active. The results indicated that isolate of *Cytophaga* and *Sporocytophaga* was more effective in cellulolytic than other genera, while isolate of *Pseudomonas* was more than other genera in lipolytic .

About 52 mesophilic actinomycetes were isolated. Data in Table (3) indicated that these isolates were belonging to a five series and its dominant percentage were : *St. antibioticus* 38% , *St.aureofaciens* 29%, *St. albonige*r 12% , *St. griseus* 13% and *St. albus* 8% . This results revealed that series *antibioticus* isolates were more bioactive on tested substance (+++) , while *albus* series was les bioactive and dominant (+) other than series .

About 50 fungal isolates belonging to 10 genera were identified with some unknown isolates. Data presented in Table (3) declared that, the highest bioactive and dominant percentage of fungi are: *Fusarium sp.* 20% (+++) of cellulolytic and amilolytic , followed be *Rhizocotonia sp.* 14% (+++) .On the other hand unknown isolates represent 4% (++) was low percentage and bioactive . These results are in agreement with Godden and Penninckx (1984), Agamuthu *et al.* (2000), Desai and Shah (2002), Ganesh and Thakre (2002), Dey *et al.* (2002) and Pedro *et al.* (2003).

Biomass production of effective test organisms:

The ability of good bioactive isolates on cellulolytic , amilolytic , protolytic and lipolytic of each group were examined using a waste (chopped leaves) with 1, 2, and 4% as carbon source, only one bioconversion to biomass , compared with base carbon source inoculated or non as (control) . Data presented in Table (4) indicated that there are variations between dry weight of bacteria, actinomycetes and fungi. Bacteria, *Cytophaga* gave a higher biomass than *Sporocytophaga* and *Bacillus* at concentration 1%. These increments were 1.211, 1.113 and 1.011 g/100ml liquid medium. However these results excelled that of 2 and 4% concentration , it were less than the control. Mesophilic actenomycetes showed the same trend. *St. antibioticus* series gave the higher dry weight (2.960 g/100ml liquid medium) more than the other series at concentration waste 1%. This superiority was obviously occurred with the concentration 2% and 4%. On the other hand the effective fungi gave higher assimilation rate of waste (chopped leaves) compared

Table (3): Identification genera , dominant percentage and enzymatic activity on cellulolytic , amilolytic , protolytic and lipolytic of test organisms (Bacteria , mesophilic Actinomycetes and Fungi) .

Test organisms	%	cellulolytic				amilolytic				Protolytic				Lipolytic			
		-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++
A. Bacteria:																	
Bacillus sp.	28	-	-	5	-	-	-	-	5	-	-	-	5	-	5	-	-
Psedomona sp	22	-	4	-	-	-	4	-	-	-	-	4	-	-	-	-	4
Enterobacter sp.	11	2	-	-	-	-	-	2	-	-	-	2	-	-	2	-	-
Micrococcus sp.	11	2	-	-	-	-	-	2	-	-	-	2	-	-	2	-	-
Cytophaga sp.	17	-	-	-	3	-	-	3	-	-	3	-	-	3	-	-	-
Sp.Sp.ppppsorocytophaga	11	-	-	-	2	-	-	2	-	-	-	2	-	2	-	-	-
Total	100	4	4	5	5	-	4	9	5	-	3	10	5	5	9	-	4
B.Actinomycetes:																	
St. antibioticus	38	-	-	-	20	-	-	-	20	-	-	20	-	-	10	5	5
St.*aureofaciens*	29	-	-	-	15	-	-	-	15	-	-	15	-	-	10	5	-
St. alboniger	12	-	-	-	6	-	-	-	6	-	-	6	-	-	-	6	-
St.griseus	13	-	-	-	7	-	-	-	7	-	-	7	-	-	7	-	-
St. albus	8	-	-	4	-	-	-	-	4	-	-	4	-	-	-	4	-
Total	100	-	-	7	45	-	-	-	52	-	-	52	-	-	25	22	5
C.Fungi :																	
Aspergillus sp.	8	-	-	1	3	-	-	-	4	-	-	4	-	-	4	-	-
Penecillium sp.	10	-	-	-	5	-	-	-	5	-	2	2	1	-	-	1	4
Rhizopus sp.	4	-	-	2	-	-	-	-	2	-	-	2	-	-	2	-	-
Muccur sp.	8	-	-	4	-	-	-	-	4	-	-	-	4	-	-	4	-
Alternaria sp.	6	-	-	-	3	-	-	3	-	-	3	-	-	2	1	-	-
Fusarium sp.	20	-	-	-	10	-	-	-	10	-	-	10	-	-	10	-	-
Rhizocotonia sp.	14	-	-	-	7	-	-	-	7	-	5	2	-	5	2	-	-
Vertecillium sp.	6	-	-	-	3	-	-	-	3	-	-	3	-	-	3	-	-
Cheatomium sp.	10	-	-	-	5	-	-	-	5	-	5	-	-	3	2	-	-
Trichoderma sp.	10	-	-	-	5	-	-	-	5	-	-	5	-	2	-	3	-
Unknown	4	-	-	2	-	-	-	-	2	-	2	-	-	2	-	-	-
Total	100	-	-	9	41	-	-	3	47	-	17	28	5	14	28	8	4

Where : (-) non enzymatic active (+) weak , (++) moderate , and (+++) good active .

Table (4): Biomass production by effective of test microorganisms growing on chopped leaves at 1 , 2 and 4% concentration by submerged culture condition .

Test organisms	Mean of dry weight biomass g / 100ml liquid medium			
	control	1%	2%	4%
A . Bacteria :				
Non inoculation	0.332	1.232	2.212	4.212
Bacillus sp.	1.112	1.011	0.811	0.612
Cytophaga sp.	1.335	1.211	1.011	0.901
Sporocytophaga sp.	1.331	1.113	0.911	0.899
B.Actinomycetes :				
Non inoculation	1.211	2.110	3.010	5.890
St. *antibioticus*	1.989	2.960	3.870	6.330
St.aureofaciens	1.870	2.760	3.500	6.211
St.alboniger	1.911	2.781	3.620	6.100
St. griseus	1.710	2.630	3.610	6.050
C.Fungi :				
Non inoculation	1.511	2.611	3.712	5.811
Penicillium sp	3.575	3.411	3.511	3.911
Fusarium sp.	4.631	4.761	4.911	5.315
Rhizocotonia sp.	3.995	4.561	4.871	5.331
Cheatomium sp.	3.643	3.890	4.110	4.516
Trichoderma sp.	4.718	5.211	5.732	6.321

with actinomycetes and bacteria . The results revealed that *Trichoderma sp. Fusarium sp. ,Rhizocotonia sp. ,Cheatomium sp.* and *Penicillium sp.*were achieved the highest biomass production at 4% concentration of chopped leaves added. Those augmentations were 6.321, 5.315, 5.331, 4.516 and 3.911 g /100ml, respectively, although these increases of biomass dry weight at 2 and 4 % waste added were less than the biomass of the control (with or without inoculation). These findings are in coincidence with Insam *et al.* (1996) and Gattinger *et al.* (2004).

Conclusion

Heap modified by partially regular covering with transparent plastic sheet was suitable approach in this time year(winter season) . Hence it gave a better results for microbiological, physical and chemical heap properties . This condition enhanced thermophilic actinomycetes activity consequently; the compost maturation would be more rapid than the ordinary condition. Likewise it gave a chance for some algae to grow on the surface layer of the heap, make a homogenization and enhancing nitrogen content. On the other hand the results revealed that population size of microbial groups were not a limiting factor in this composting process. Moreover the addition of waste analytic effective microorganisms (EM) and a symbiotic nitrogen fixation in individually treatments, separately, lead to improve the heap characters. Also Fungi showed a more activation in assimilation rate of waste other than bacteria and actinomycetes, as a biomass production and these can be benefit in several fields.

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النشاط الميكروبى على مخلفات أوراق الفيكس بتحوير نظام الكمر

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اصبح الاهتمام بتدوير المخلفات بواسطة الميكروبات مطلب بيئ و صحى و اقتصادى وضمان لاستمرار الزراعة البيوديناميكية لذلك صممت تجربة كومات (معاملات) لكمراوراق اشجارالفيكس لانتاج سماد الكمبوست العضوى **compost**. وكانت المعاملات للكومات كالآتى :

- الكومة الأولى بدون اى تلقيح ميكروبى أو تحوير فى ظروف الكمر (كونترول).
- الكومة الثانية لقحت بالميكروبات النشطه فى تحليل المخلفات النباتيه منفردة دون التحوير فى ظروف الكمر.
- الكومة الثالثه لقحت بواسطة البكتريا المثبتة لأزوت الهواء الجوى لاتكافليا (بكتريا الازوتوباكتر كروكوكم و الازوسبيريللم برزيلنس) بالاشتراك معا , و بدون التحوير فى ظروف الكمر .
- الكومة الرابعة بدون تلقيح ميكروبى ولكن حورت بالتغطية الجزئية على فترات منتظمة (اسبوع واسبوع) بالبلاستيك الشفاف قطر ٠,٥مليمتر مع التقليب التام للكومة بعد رفع الغطاء .

قدر النشاط الميكروبى بالعد الكلىللبكتريا - الاكتينوميسيتا ت المحبة للحرارة العالية والمتوسطة وكذلك الفطريات مع تقدير الخواص الفيزيائية والكيماوية على فترات (صفر، ٢١ ، ٥٠ ، ٨٠ ، ١٠٠يوم) من بدايةالكمر للكومات الاربعة تحت الدراسة .

في مرحلة سابقة تم عزل وتنقية وتعريف عدد ١٨ عزلة بكتيريا , ٥٢ عزلة استربتوميسيتات محبة للحرارة المتوسطة ,٥٠ عزلة فطر و قد تم اختبار القدرة الانزيمية (النشاط) للعزلات السابقة على تحليل وتمثيل مواد السليلوز , النشا , البروتين , والزيوت .ثم

انتخبت العزلات الاكثر نشاطا من المرحلة السابقة وتم اختبار قدرتها على تمثيل مطحون الاوراق بتركيزات ١ ، ٢، ٤ % لانتاج الكتلة الحيوية (وزن الخلايا الجاف جم /١٠٠ملل) بواسطة مزارع الغمر.

وقد أوضحت نتائج البحث الاتى :

- أدت عملية التحوير فى ظروف الكمر الى زيادة نشاط الاكتينو ميسيتات المحبة للحرارة العالية وتحسين الصفات الفيزيائية والكيماوية للكومة بالمقارنة بظروف الكمر العادية وثبت بالنتائج ان العبرة بنوع المجموعات الميكروبية وليس بحجمها وان ارتفاع نسبة النتروجين ربما تعود الى نشاط الميكروبات السابقة بالاضافة لنمو الطحالب لتوفر الضوء وحجز الرطوبة بالتغطية الجزئية المنتظمة بالبلاستيك الشفاف .
- أدى التلقيح بالميكروبات الفعالة فى تحليل المخلفات النباتية منفردة الى تحسين صفات الكومة وكانت فى المرتبة الثانية . كما تحسنت صفات الكومة الملقحة بالبكتيريا المثبتة لازوت الهواء الجوى بصورة مشتركة وكانت فى المرتبة الثالثة بالمقارنة بالكومة الاولى (الكنترول) .
- تفوقت الفطريات على الاكتينوميسيتات المحبة للحرارة المتوسطة والبكتيريا فى معدل تمثيل مطحون الاوراق وانتاج الكتلة الحيوية وكان معدل الاضافة ١% افضل من ٢ ، ٤% على التوالى .