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Composting of Agricultural Waste Residues by Effective *Streptomyces rameus* KAC3 and *Streptomyces mutabilis* KAC6 Isolates for the Mass Production of Vermicompost

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ABSTRACT

Agricultural sector after yield harvest remains plant parts huge environmental pollution issues because not suitable technology makes composting process. In this point of view, present investigation rigorously scrutinizes the agricultural waste decomposition process facilitated successfully completion in 40 days duration compost maturation by use effective of *Streptomyces rameus* KAC3 and *Streptomyces mutabilis* KAC6 to increase nutrient availability. Different agricultural crop waste is being used in this composting technology process which stem, root and leaves from multiple crops. The parameters reveled which include temperature, pH, moisture content, microbial populations, and nutrient analysis at various composting maturation stages, Based on findings emerge during degradation process the temperature fluctuation days 10th (34.6±0.06), 20th (49.37±0.14), 30th (62.4±0.06) and end of 40th day (32.7±0.15) and alongside pH elevations increases ranged from 5.2 to 6.53. The 30th day marks the zenith of microbial populations reaching 71.23±0.2 (10^9 CFU) and 9.28±0.09 (10^9 CFU), respectively. Furthermore, examination of vermicompost nutrient availability values reveals its superior profile compared to effective microorganism (EM) compost, with organic carbon (12.17%), nitrogen (1.24%), phosphorus (1.39%), potassium (0.76%). Finally, the research concluded that the plant nutrient availability was enhanced through incorporation of effective actinomycetes KAC3 and KAC6 to convert Agro-waste material to mature compost for further processing of vermicompost.

Keywords: Compost; Vermicompost; Biofertilizer; Waste management; Organic farming

NOMENCLATURE

EM	: Effective microorganism
CFU	: Colony forming unit
KARE	: Kalasalingam academy of research and education
FYM	: Farmyard manure
ANOVA	: Analysis of variance
SD	: Standard deviation
NPK	: Nitrogen, phosphorus and potassium
UKM	: Universiti kebangsaan malaysia
EC	: Electrical conductivity
MSW	: Municipal solid waste

1. INTRODUCTION

The increase in human population indirectly contributes to the increasing demand for larger area cultivation for making more food grains. Therefore, there will be more plant waste and as a result of the large production of agro-industrial-based products[1]. Among various waste management methods, composting is a key component of sustainable waste management and supporting the organic farming[2]. It is an aerobic biological process that turns biodegradable organic materials into a substance that resembles humus by using naturally occurring microbes. Aerobic microorganisms require oxygen, water, and food for their breakdown process with controlling key parameters including temperature, moisture, pH, and oxygen demand is crucial for producing high-quality compost[3]. Microorganisms can produce enzymes and metabolites that help organic waste decompose faster and improve soil humus quality[4]. Bacteria, fungi and actinomycetes isolates to make consortia formulation with advanced tools such as nanoparticle and drone through large scale management of Agriculture waste, this successful technology will apply to Municipal Solid Waste (MSW), Agricultural, Horticulture, solid waste and industrial waste[5]. This method of collecting a variety of organic waste materials from crops such as finger millet, sorghum, brinjal, cowpea, and lawn grass clippings. These items, acquired from various locations, are meticulously gathered for composting, which reduces waste occupying area while enriching the soil with humus[6]. Similarly, MSW management by co-mixture of effective microorganism with cow dung to accelerate better stability decomposition[7].

The composting process required frequent watering and the strategic turning of the compost bed to reduce the more heat and safe for decomposition process. The

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material is moved to a vermicompost bed when the decomposition process is finished, where earthworms (*Eisenia fetida*) added to create vermicompost process. Biofertilizer potential inoculum such as *Azospirillum brasilense and Rhizobium leguminosarum* are highly viable condition to drive quality of vermicompost material. The derived vermicompost act as carrier material for application in the agricultural field because inoculum maintain 10^7 population in active nature up to 5 to 6 month shelflife[8]. Compost made by earthworms contains a variety of enzymes, hormones, vitamins, antibiotics, and other critical nutrients are essential for plant growth. It also helps to improve soil structure and water-holding capacity, as well as boosting crop yield and quality[9-10]. Vermicompost promotes high porosity, aeration, drainage, water retention, and microbial activity[11].

To summarize, the composting process starts with the careful gathering of various organic waste components segregation and enrichment of effective organism (KAC3 and KAC6) decomposed material easily uptake the earthworm and produce rich organic nutrients with water soluble nature at the end of the process. The vermicompost using as biofertiliser carrier material also for long time sustainability, which contributes to sustainable and environment friendly farming practices.

2. MATERIALS AND METHODS

2.1 Collection of Agricultural Waste

Crop waste materials generated from crop cafeteria experimental farm, Department of Agriculture, Kalasalingam Academy of Research and Education (KARE) shown in Fig. 1. Different crop residue waste such as finger millet, sorghum, brinjal, cowpea, lawn grass clippings, and the substrate of cow dung was used in the vermicompost process.

Figure 1. Aerial view of crop cafeteria experimental farm.

2.2 Effect on Actinomyces KAC3 and KAC6 Isolates

The isolated (*Streptomyces rameus* KAC3 and *Streptomyces mutabilis* KAC6) from different barren land soil environment and characterised by cultural, molecular level with validated NCBI Blast. Enzyme qualitative screening involved for *in-vitro* degradation ability assessment using pure culture were streaked on Petri dishes containing appropriate medium then incubated at 28 °C for 3- 4 days of EM actinomycetes (KAC3 and KAC6). Enzymatic activities like xylanase, pectinase, carboxy methyl cellulase, lipase, protease and amylase analysed on selective media as per[12]. Xylanase and pectinase activity were detected by method[13,14]. Other cellulose[15, 16], protease[17], amylase[13] and lipases[18] was performed.

2.3 Preparation of Compost Pile

The overall dimensions of the bed measure 120×45×40 inches. The 1st layer enriched mixed trimmed with crop residues pile to a height 0.32 feet and 2nd, 3rd and 4th layer prepare similar height with enrichment of cow dung slurry and EM. This layering technique (Table 1) is repeated until the bed reaches a height of 40 inches and maintained physical characters like density by tapping method as described by[19] and water holding capacity described by Keen Raozkowski box method[20] inside the compost bed followed as per[18].

Table 1. Microbial bed quantification

Bed size	Particulars	Qty. (Kg)
12ft×4ft×1.3ft	Cow dung	100
	FYM	60
	Grass waste	400
	Crop residues	440
	Total	1000

2.4 Enrichment of Culture Application to Compost Pile

Streptomyces rameus KAC3 and S*treptomyces mutabilis* KAC6 isolates at 10^6 CFU concentration spray application through three days intervals and maintain the population in compost pile by *in-vitro* assay. The entire pile is consistently monitored for moisture levels, with regular watering adjustments to maintain optimal moisture conditions to manage excess heat generated during decomposition, the bed undergoes two turns during the composting period, facilitating the escape of heat and promoting a balanced composting environment. The decomposing materials were frequently watered and turned according to the method[21-22].

2.5 Physio-Chemical Analysis of Microbial Compost

The compost piles under regular monitoring by employing a thermometer for temperature assessment[23]. Furthermore, the compost's electrical conductivity and pH levels were assessed using a pH meter and conductivity meter through potentiometric method[24]. Moisture content analysis by oven-dried at 110 °C for 24 hours and accuracy assessed using digital balance for precise weighing[25]. The organic carbon content of the compost was assessed through Walkley-Black titration method described[26]. Calcium, Magnesium, Sodium, Iron, Potassium, and Manganese content was estimated by Calorimetric method[27]. Nitrogen content was determined using the Kjeldahl method[28]. Phosphate were determine using UV spectrophotometer, Zinc, copper were estimated by Atomic Absorption Spectrophotometer[29].

2.6 End Product of Microbial Compost

After degradation of agricultural waste by action of KAC 3 and KAC6 isolates and end of mature compost should be sifted through mesh (about 4-5 mm). The transfer of sieved compost material to the vermicompost process tank[12].

2.7 Preparation of Vermicompost Tank

Well constructed cemented pits (10x4x1.4ft) with cooling shed covered and surround 7 years old tamarind tree covered shade vermicompost tank, and well-ventilated shed to create an optimal environment for the earthworms multiplication in fast manners. At the base of the pit, 1:0.3 ratio of actinomyces degraded agricultural waste product was sieved by 3 mm size of mesh and wet cow dung enriched. The earthworm of *Eisenia fetida* to release in the pit 100 mature earthworm/kg actinomyces degraded compost and top layer fully covered dry leaves with manually sprinkle water to maintain moisture[30-31]. The vermicompost was started to harvest in 10 days interval from vermicompost tank (Table 2). The mature vermicompost is dark brown and very porous and is very porous, granulated, and free of any foul smell[32]. This mature vermicompost is sieved (2 mm) to separate the earthworms.

Table 2. Details about vermicompost tank

S. No.	Bed size	Ingredients	Qty. (Kg)
1.	8x4x1.4ft	Sieved compost (The end product of microbial degradation)	850
		Cow dung	150
		Earthworm (*Eisenia fetida*)	1.5

2.8 Statistical Analysis

For experiments, IBM SPSS statistics was used as the statistical software for analysis of variance (ANOVA-version 29.0.2.0) and means separated using Duncan's multiple range test (P=0.05). All experiments were performed in triplicate, the data are presented as the mean values ± standard deviation (SD), correlation with significant at a 95 % confidence interval (P=0.05).

3. RESULTS

3.1 Determination of Degrading Ability by Collected Isolates

The actinomyces isolates KAC3 and KAC6 were subsequently examined for their growth pattern at 28 °C and confirmed to be gram-positive in nature by Gram staining method. The cultural characteristics of aerial mycelium were white, whereas the substrate mycelium exhibited yellow in KAC3 and white in KAC6 when grown on actinomycetes isolation agar and nutrient agar (Fig. 2). The degradation ability exhibited by KAC3 and KAC6 is notably superior in amylase, cellulose, and lipase, while showing an average performance in xylanase, protease, and pectinase (Table 3).

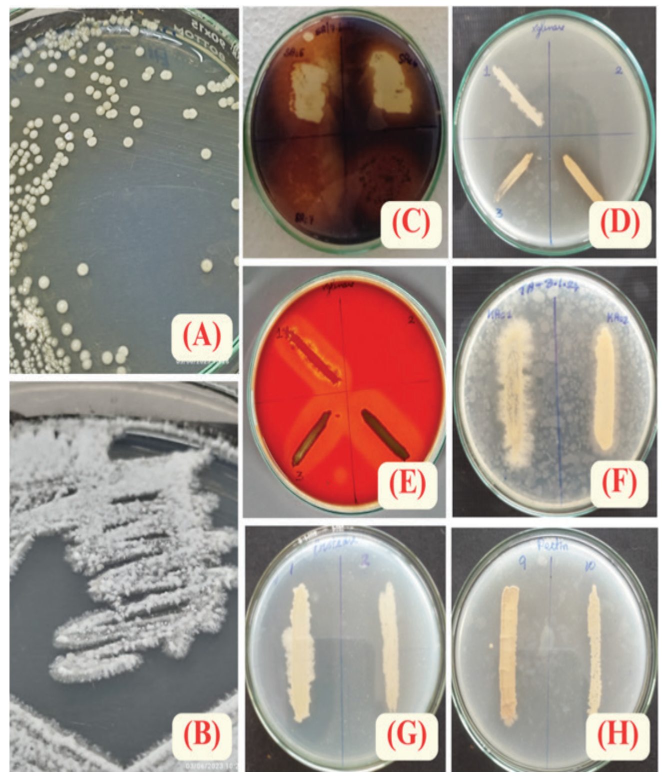

Figure 2. (A) Plate view of streptomyces rameus¬ KAC3 isolates in AIA medium (B) Plate view of streptomyces mutabilis KAC6 isolates in AIA medium (C) Starch hydrolysis (Amylase activity) (D) Xylanase activity (E) Cellulase activity (F) Lipase activity (G) Protease activity and (H) Pectinase activity.

Table 3. Morphological and Enzymatic activities of collected isolates

Isolates	Culture characteristics	Pigment nature		NCBI accession number	Amy.	Cel.	Xyl.	Lip.	Pro.	Pec.
		Aerial	Substrate							
Streptomyces rameus KAC3	Raised sphere, Soft powdery	Dull white	Dark yellow	PP550146	+++	+++	++	+++	++	++
Streptomyces mutabilis KAC6	Irregular raised soft powdery	Dull white	Yellowish white	PP177364	+++	+++	++	+++	++	++

Notes: Amy. – Amylase; Cel. – Cellulase; Xyl. – Xylanase; Lip. – Lipase; Pro. – Protease; Pec. – Pectinase; (++) 0.6-0.9 cm; (+++)>1cm.

3.2 Yield of Compost and Vermicompost

The compost bed details with dimensions 12 ft × 4 ft × 2 ft, when prepared using 1000 kg of agricultural waste residues, yields approximately 480 kg of compost. Similarly, a vermicompost bed of the same size, prepared with 200 kg of compost material with cow dung substrate 10 kg, typically produces a yield of 100–110 kg of vermicompost (Table 4). The process adapted using the effectiveness of microbial activity (KAC3 and KAC6 isolates), and the ambient parameters maintained during the composting process to achieved the yield (Fig. 3).

Table 4. Biomass of end products yields

Bed size	Agri-waste residues (Kg)	Yield (Kg)
Compost pile		
12ft×4ft×2ft	1000	480
Vermicompost tank		
12ft×4ft×2ft	200	100-110

Figure 3. Agricultural residues primed with EM solution.

3.3 KAC3 and KAC6 Isolates Viability

Table 5, data illustrates the changes in various parameters across different composting phases: mesophilic, thermophilic, and maturation. Temperature peaked at 62.4 °C during the thermophilic phase, indicating optimal conditions for microbial activity, followed by a decline in the maturation phase. Moisture decreased progressively from 47.33 % to 18.9 %, reflecting water loss during composting. The pH transitioned from acidic (5.28) to slightly alkaline (7.36), aligning with microbial decomposition processes.

Table 6 illustrate the microbial population status that total bacterial population increased significantly, peaking at 88.5 CFU during maturation, signifying a resurgence of mesophilic bacteria. Similarly, fungal populations peaked in the maturation phase, suggesting their critical role in breaking down complex organics. KAC3 and KAC6 isolate populations showed notable activity, with KAC6 exhibiting consistent growth across all phases, peaking during maturation. The observations of degradation were dynamic shifts in temperature, moisture, pH, and microbial activity essential for efficient composting and nutrient stabilisation. As compost matures, microbial populations stabilize but remain significantly higher compared to initial levels. Fig. 4, 5 and 6 represents the physical parameters and fig. 7 represents the microbial population counts in compost pile.

3.4 Nutritional Status of Compost and Vermicompost

A Nutritional comparison of compost and vermicompost is shown in (Table 7), which highlights notable differences in nutritional content. Vermicompost exhibited higher levels of organic carbon (12.17 %) compared to compost (10.23 %), reflecting enhanced organic matter stability in vermicompost. Key nutrients like calcium (4.8 %), nitrogen (1.24 %), phosphorus (1.39 %), and potassium (0.76 %) were significantly higher in vermicompost, underscoring its superior nutrient enrichment due to earthworm activity and microbial interactions. Conversely, compost showed greater sodium (0.31 %) and iron (1.2 8%) concentrations, which may result from specific decomposition pathways or raw material composition. Micronutrients such as zinc (0.58 %) and copper (0.0032 %) were more abundant in vermicompost, emphasizing its improved micronutrient bioavailability. Fig. 8 shows the NPK comparison of compost and vermicompost.

Table 5. Characteristics of physical properties of compost pile

S. No.	Parameters	10th day	20th day	30th day	40th day
		Mesophilic phase	Thermophilic phase		Maturation phase
1.	Temperature (°C)	34.6±0.06	49.37±0.14	62.4±0.06	32.7±0.15
2.	Moisture (%)	47.33±0.33	39.5±0.11	21.6±0.06	18.9±0.1
3.	pH	5.28±0.04	6.62±0.06	6.11±0.06	7.36±0.01

Notes: The values at each parameters in different treatment days were compared with the analysis of variance (P=0.05). P=0.05 was considered significant.

Table 6. Characteristics of different microbial properties of compost pile

S. No.	Parameters	10th day	20th day	30th day	40th day
		Mesophilic phase	Thermophilic phase		Maturation phase
1.	Total bacterial population (CFU)	40.2±0.5[b]	76.7±0.31[b]	71.23±0.2[a]	88.5±0.23[d]
2.	Total fungal population (CFU)	8.7±0.02[a]	11.87±0.26[a]	9.28±0.09[a]	14.87±0.12[a]
3.	KAC3 isolate population (CFU)	54.03±0.38[d]	63.87±0.12[c]	57.47±0.2[a]	60.53±0.09[b]
4.	KAC6 isolate population (CFU)	52.83±0.39[c]	63.6±0.32[c]	58.53±0.09[a]	61.6±0.25[c]

Notes: Data in the table represents the mean of bacterial and fungal population counts compare with our isolates at different treatment days were compared with the analysis of variance (P=0.05) and ± symbol represent the values for standard error of means. Different letters represent the significant difference between treatments, using Ducan's multiple range test (P=0.05).

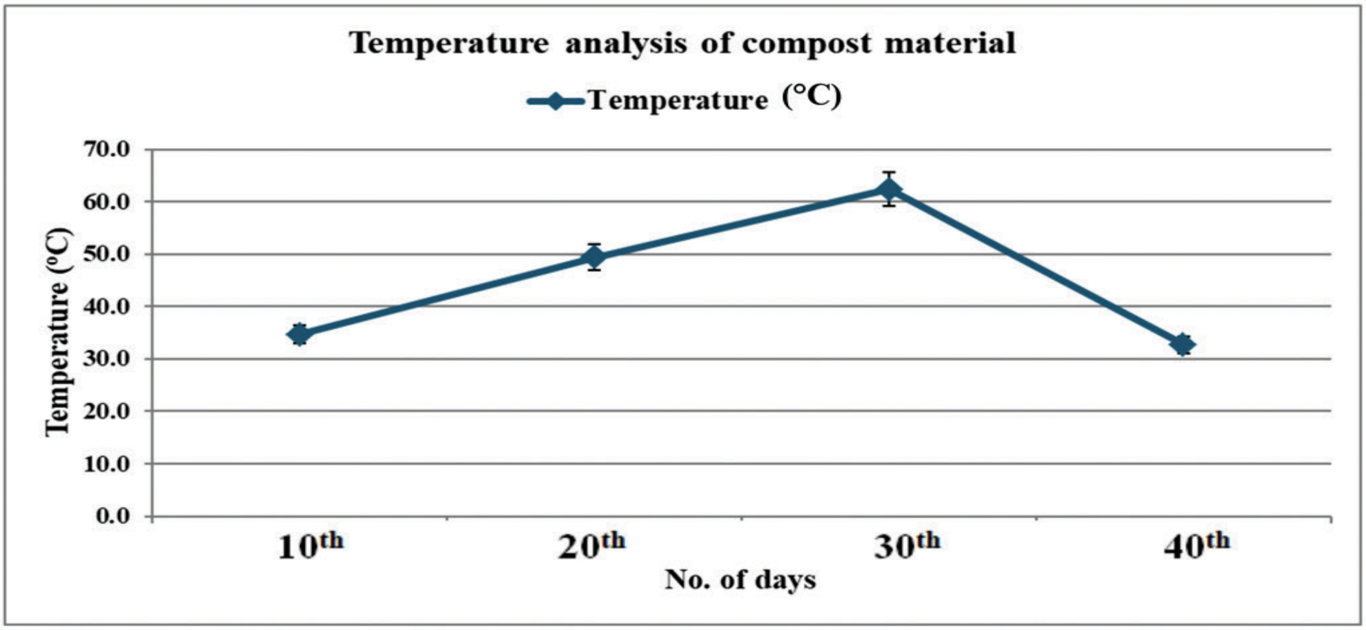

Figure 4. Physical parameter of temperature analysis in compost pile.

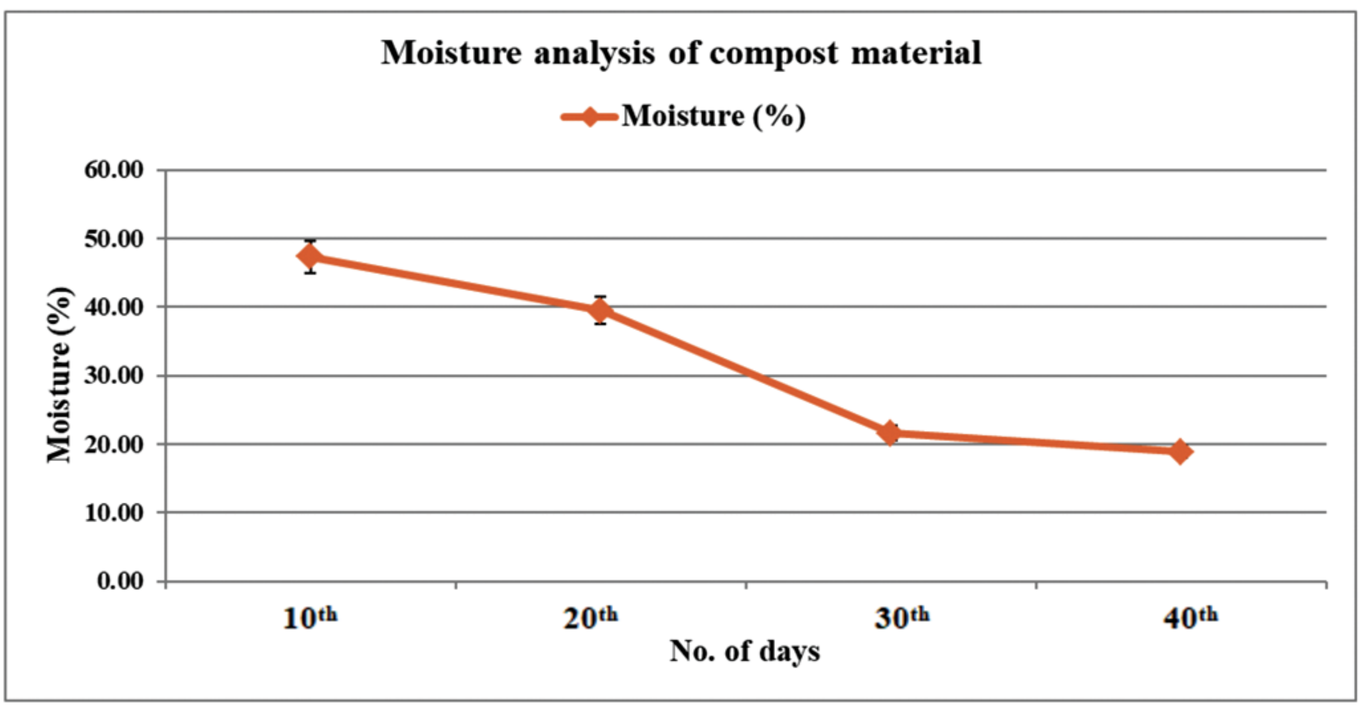

Figure 5. Physical parameter of moisture analysis in compost pile.

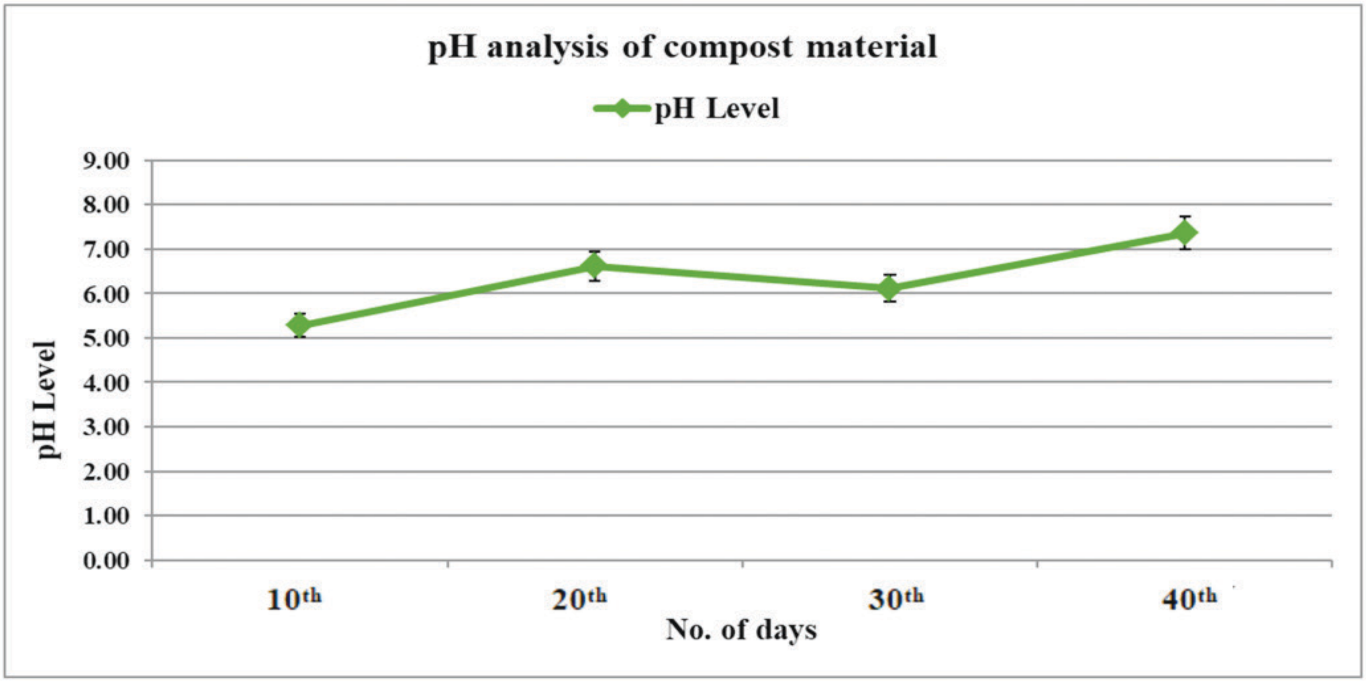

Figure 6. Physical parameter of pH analysis in compost pile.

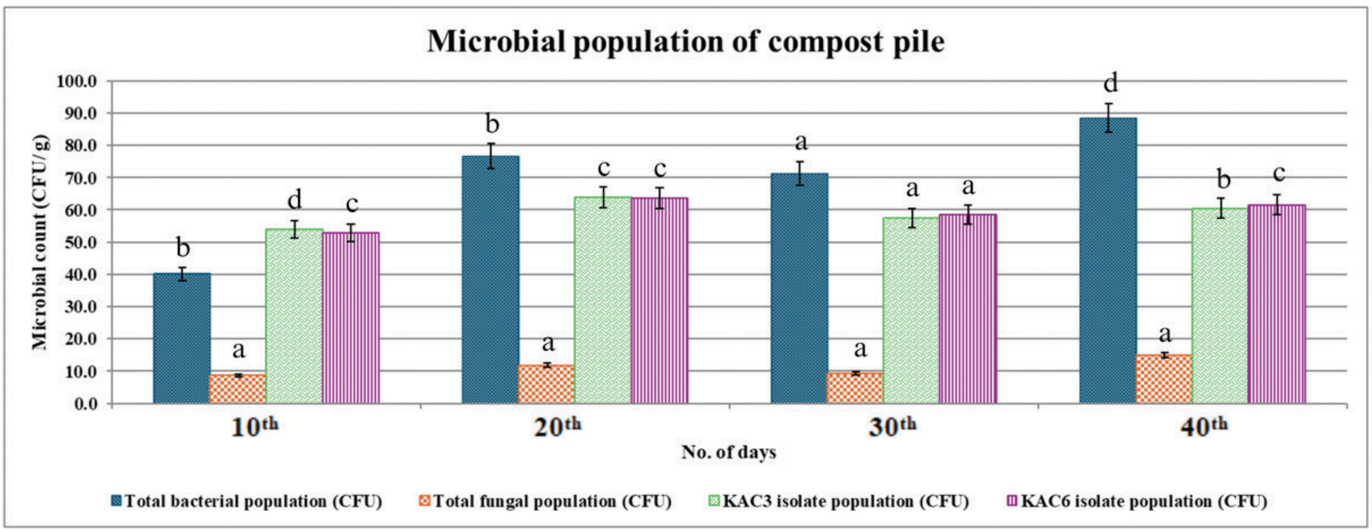

Figure 7. Microbial population of compost pile in degradation activities.

Table 7. Comparison of compost and vermicompost products

Nutrient element	Compost (%)	Vermicompost (%)
Organic carbon	10.23±0.03[g]	12.17±0.23[e]
Calcium	2.34±0.03[f]	4.8±0.25[d]
Magnesium	0.59±0.01[cd]	0.69±0.01[c]
Sodium	0.31±0.24[b]	0.07±0.00[ab]
Zinc	0.0012±0.00[a]	0.58±0.26[bc]
Copper	0.0017±0.00[a]	0.0032±0.00[a]
Iron	1.28±0.11[e]	0.46±0.08[abc]
Manganese	0.05±0.00[a]	0.02±0.01[a]

Notes: Data in the table represents the mean of nutritional status compare with compost and vermicompost by analysis of variance (P=0.05) and ± symbol represent the values for standard error of means. Different letters represent the significant difference between treatments, using Ducan's multiple range test (P=0.05).

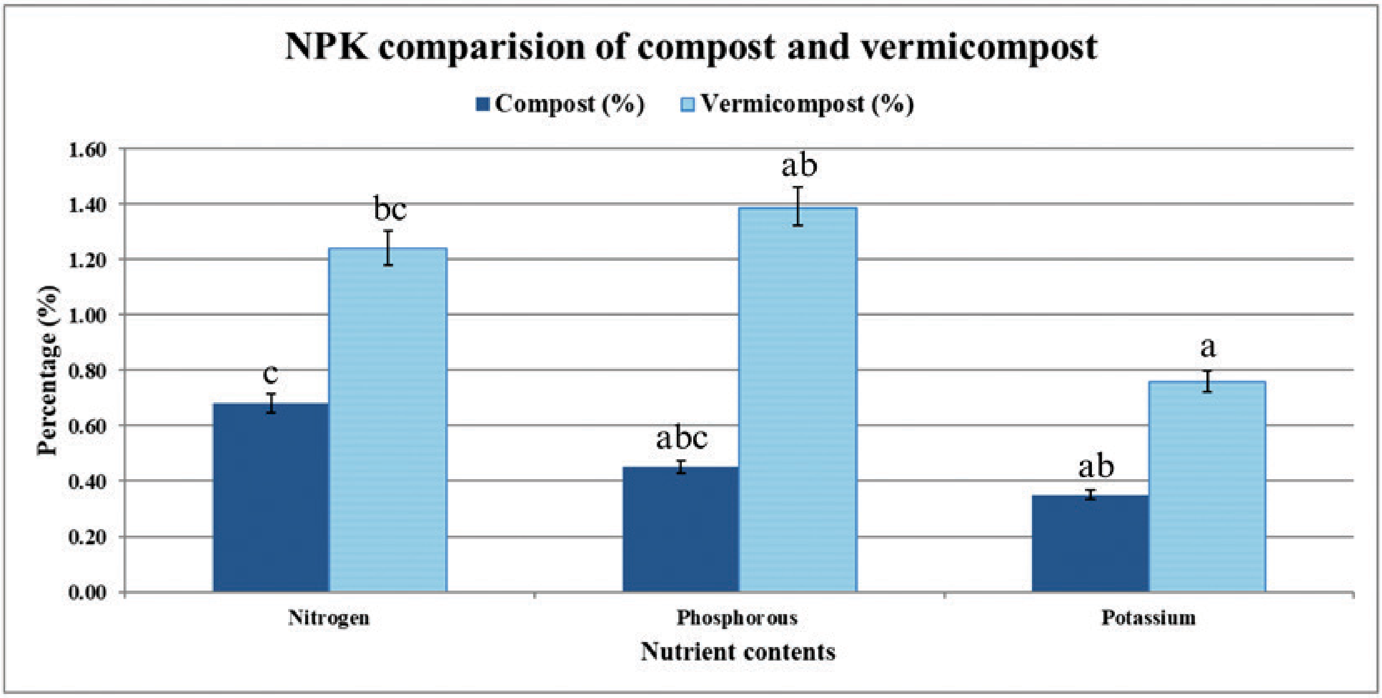

Figure 8. NPK comparison of compost and vermicompost.

Table 8 illustrates the comparative analysis between compost and vermicompost, elucidating notable disparities in their physical attributes. Vermicompost exhibited higher bulk density g cm^{-3} (1.97±6.36) and water-holding capacity % (39.95±0.17) compared to compost bulk density 1.43±3.75 and water-holding capacity 27.77±0.35 respectively. This indicates vermicompost's superior physical properties, enhancing soil structure and moisture retention, making it more effective for sustainable agricultural applications (Fig. 9 and 10).These distinctions imply that vermicompost boasts superior water retention capabilities and enhanced soil aeration, attributes conducive to fostering improved soil structure and facilitating optimal plant growth.

Table 8. Physical properties from compost and vermicompost.

Properties	Compost	Vermicompost
Bulk density (g cm^{-3})	1.43±3.75^{b}	1.97±6.36^{a}
Water holding capacity (%)	27.77±0.35^{b}	39.95±0.17^{a}

Notes: Data in the table represents the mean of physical properties compare with compost and vermicompost by analysis of variance (P=0.05) and ± symbol represent the values for standard error of means. Different letters represent the significant difference between treatments, using Ducan's multiple range test (P=0.05).

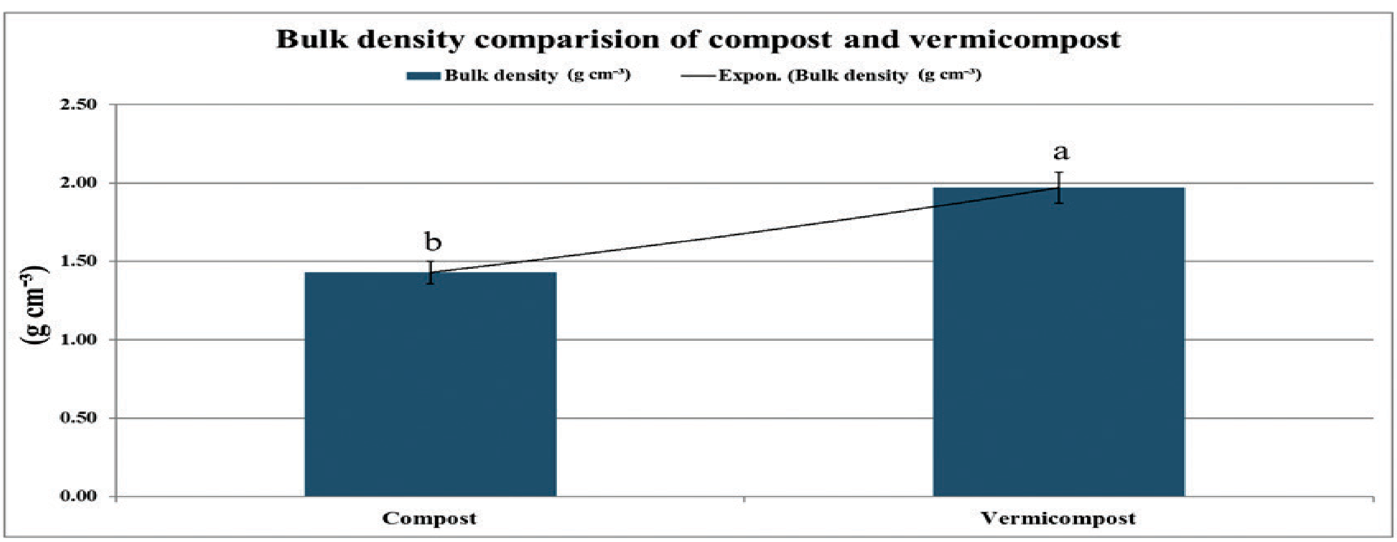

Figure 9. Bulk density comparison of compost and vermicompost.

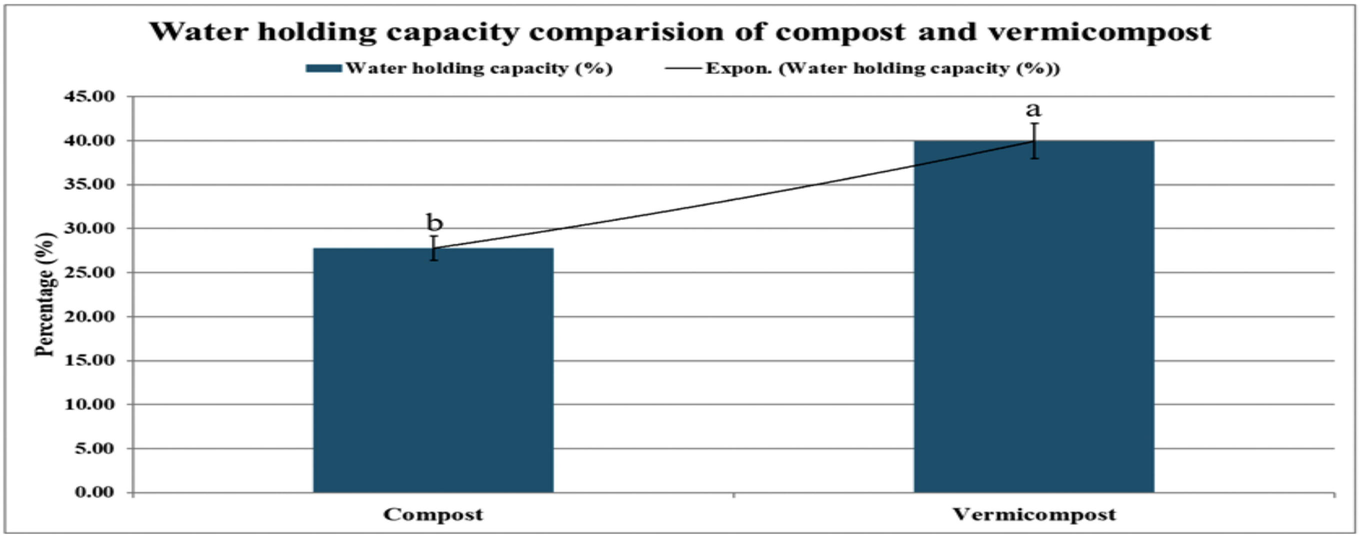

Figure 10. Water holding capacity comparison of compost and vermicompost.

4. DISCUSSION

The present studies involves in the waste management's using the actinomyces isolates *Streptomyces rameus* KAC3 and *Streptomyces mutabilis* KAC6. Involvement of these isolates shows the high degradation activities *in-vitro* and *in-vivo* conditions. The previous studies are elaborated with bacteria and fungi for degradation of different waste materials[33]. Current work illustrate the *Streptomyces* sp survival in extreme condition and able to involve in various enzyme activities to enhance for degradation process. Rice straw waste material[34-35] successfully obtain degradation process at 76 % in 30 days using an actinomyces *Streptomyces griseorubens* JSD-1 due to release a series of enzymes, including cellulase, pectinase, xylanase and ligninase simultaneously.

Similary in our study KAC3 and KAC6 qualitative assessment for enzyme production superior level. Reported bacterial strains by[36] such as *Brevibacillus borstelensis, Bacillus cereus and Paenibacillus sp.* higher zone inhibition of cellulose activity at 37 °C, pH 7.0 with 6 % inoculum volume. Analyzed by[24,27-29] soil types (clay, sand, red) and found variations in NPK, pH, and EC across locations and depths. Incorporating vermicomposting can enhance soil fertility, stabilize nutrient variability, and support a sustainable cropping pattern tailored to soil and nutrient conditions. Previous studies shows that the smaller compost beds (2 kg) with 60 % moisture, 20 mm particle size, and a 28:1 C/N ratio degrade faster, achieving a degradation rate constant of 0.0503 day^{-1}. All beds reached neutral pH (7), reduced total organic carbon (<25 %), and demonstrated effective mesophilic phase composting36,14. The findings align with numerous studies indicating the nutrient-rich nature of vermicompost due to the involvement of earthworms by input material of microbial decompose fine particles[37,36]. Analysed[38] without composting pre-process of waste, he enable that 90-days vermicomposting study using Eisenia foetida, mustard residues, and sugarcane trash with cattle dung, vermicomposting significantly reduced the C:N ratio and increased mineral nitrogen. Microbial activity peaked at 60 days. Total nitrogen increased with earthworm inoculation, while total phosphate, potassium, and copper showed no significant differences. With slight modification the present studies were make the pre-process of waste degradation using the actinomyces isolates KAC3 and KAC6 the nutrients level were high impact that loses the density of waste to simple form in 40 days and the actinomyces degraded compost were processed with earthworm were high nutritional value. Food trash and yard garbage[3] are produced in enormous quantity at Universiti Kebangsaan Malaysia (UKM). Takakura EM was the most effective waste composting process in large scale level because it produced high-quality nutrient content of compost in shorter time than Fruit Waste EM.

5. CONCLUSION

The study concludes by highlighting the significance of composting process optimised for agricultural solid waste management. Keep the isolates of *Streptomyces rameus* (KAC3) and *Streptomyces mutabilis* (KAC6) active in the waste residue degradation process. Short days produced fine powder of compost particles and partially decomposed compost with very low levels of accessible nutrients. Earthworms (*Eisenia foetida*) are used in this compost material to improve the availability of nutrients with water solubility. Isolates of Streptomyces are essential for managing agricultural waste on a large scale level and for reducing the degradation duration it takes for trash to decompose. The degrading potential of KAC3 and KAC6 isolates presents a viable way to turn agricultural waste into useful resources of quality organic fertiliser for environmentally friendly waste management.

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