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Bisma Rashid
Division of Basic Sciences and Humanities, Faculty of Agriculture, Wadura, SKUAST-K, Jammu and Kashmir, India

Mushtaq Ahmad Malik
Division of Basic Sciences and Humanities, Faculty of Agriculture, Wadura, SKUAST-K, Jammu and Kashmir, India

Malik Asif Aziz
Division of Basic Sciences and Humanities, Faculty of Agriculture, Wadura, SKUAST-K, Jammu and Kashmir, India

Shahid Ahmad Padder
Division of Basic Sciences and Humanities, FOH, Shalimar, SKUAST-K, Jammu and Kashmir, India

Inayat Mustafa Khan
Division of Soil Science and Agricultural Chemistry, Faculty of Agriculture, Wadura, SKUAST-Kashmir, Jammu and Kashmir, India

Fehim Jeelani Wani
Division of Agricultural Economics and Statistics, Faculty of Agriculture, Wadura, SKUAST-Kashmir, Jammu and Kashmir, India

Tanveer Ahmad Wani
Division of Plant Pathology, Faculty of Agriculture, Wadura, SKUAST-Kashmir, Jammu and Kashmir, India

Saima Shafi
Division of Basic Sciences and Humanities, Faculty of Agriculture, Wadura, SKUAST-K, Jammu and Kashmir, India

Azra Khan
Division of Genetics and Plant Breeding, Faculty of Agriculture, Wadura, SKUAST-Kashmir, Jammu and Kashmir, India

Corresponding Author:
Bisma Rashid
Division of Basic Sciences and Humanities, Faculty of Agriculture, Wadura, SKUAST-K, Jammu and Kashmir, India

Characterization of *Bacterial species* involved in solid waste management in Kashmir Valley

Bisma Rashid, Mushtaq Ahmad Malik, Malik Asif Aziz, Shahid Ahmad Padder, Inayat Mustafa Khan, Fehim Jeelani Wani, Tanveer Ahmad Wani, Saima Shafi and Azra Khan

Abstract
The objective of the current study was to evaluate the variety of *Bacterial species* utilized in Kashmir Valley's various districts for solid waste management. Using different media, various bacterial isolates from the samples were isolated from agricultural waste dump sites throughout the Kashmir Valley. The production of extracellular enzymes by the bacterial strains as well as synergistic activities within the strains was documented. Gram staining and biochemical tests were used to isolate, culture, and characterize individual colonies. Apart from these various enzymatic activities such as amylase, cellulase, protease, and xylanase production were evaluated. These findings have expanded the possibility of finding agricultural waste dump-associated bacteria that are important for industry, and these isolates may be a key source for the identification of industrially relevant enzymes and molecules.

Keywords: *Bacterial species*, characterization, enzymatic activities, solid waste management, Kashmir Valley

Introduction
Microorganisms are ubiquitous in nature where they have a variety of essential functions. Microorganisms play important roles in the maintenance of many natural and man-made phenomenon's in the environment. They serve positive functions that make life easier and better for man. Microbes also play an essential role in the natural recycling of living materials. All naturally produced substances are biodegradable, that is, they can be broken down by living organisms such as bacteria. Microorganisms have been invaluable in finding solutions for several problems mankind has encountered in maintaining the quality of the environment. They have, for example, been used to positive effect in human and animal health, genetic engineering, environmental protection, and municipal and industrial waste treatment. Microorganisms have enabled feasible and cost-effective responses which would have been impossible via chemical or physical engineering methods. More so, microbial technologies have successfully been applied to a wide range of environmental problems, especially waste management issues.

Waste management is the collection, transportation, processing, treatment, recycling or disposal of waste materials to reduce their adverse effects on human health or amenities. The type of waste management techniques that should be applied for proper management of waste depend on the composition of waste. Although composting is the appropriate for all organic wastes: wastes such as plastic metals and glasses are better handled through recycling. Waste management technique take place in many ways viz., landfill, incineration, pyrolysis and gasification, composting and anaerobic digestion (Adewale *et al.* 2011) [1]. Sanitary landfills represents a common, economical and environmentally acceptable method for the disposal of solid waste even with implementation of waste reduction, recycling and transformation technologies disposal of residual solid waste in landfills still remains an unavoidable component of an integrated solid waste management strategy (Chugh *et al.* 1999) [4]. Several alternative methods are available for proper disposal of MSW. Composting is one of the alternatives for recycling and ecofriendly management of MSW. Composting is an aerobic biological decomposition of organic solid substrates with putrescible materials converted to establish end product, the compost by microbial action. This process is in widespread use as a means of treating organic wastes including sewage sludge animal and agricultural residues and household refuse (Mena *et al.* 2003 and Vanderroot *et al.* 1997) [7, 11].

Through composting organic matters undergoes partial mineralization and to a varying degree transformation in to humus like substances (Majumdar, K. and N. Singh 2007) [6]. Thus compost can be used directly in agriculture an organic amendant to enhance soil and fertility. According to a current trend in many other countries, composting is gaining particular consideration (Gautam *et al.* 2010) [5]. Even though, the composting technology is efficient there are still many parameters to be improved some of them including reduction in overall time taken for composting and developing efficient decomposing microorganisms (Sivarajan *et al.* 2005) [10]. Enzyme activities have been indirectly used as an index of microbial population on organic matter decomposition. Enzyme activities are due to the enzyme present within a living or dead cells, cell debris and free enzyme (Nannipieri *et al.* 2002) [8].

The potential to identify efficient bacterial strains from waste dump sites with useful applications is enormous given the significance of biodegradable solid waste decomposition in temperate environments. To meet the demand for new organisms with production-enhancing qualities. An ongoing effort has been made to isolate novel bacteria from various environments in the hopes of discovering novel enzymes or molecules for use in the agro industrial sector and waste degradation. In light of this, the current study's primary goals were to investigate bacterial strains from waste dump sites in order to eventually degrade waste and find novel bioactive compounds for use in agro-industrial applications.

Materials and Methods

Collection of samples

Various waste samples were gathered from various districts of the Kashmir Valley's agricultural waste disposal sites. In order to maintain aseptic conditions, the samples were collected in sterile zip-lock plastic, stored at 4 °C, and marked with the source and location of the sample. In order to isolate bacteria, the collected samples were taken to a lab, where the samples' moisture content and pH were recorded.

Isolation of bacteria from waste samples

The isolation of bacteria was accomplished using serial dilution methods. By adding waste (1g) to 10 ml of sterile water (the stock) and vigorously shaking the mixture for at least a minute, sample suspension was created. After that, the diluted substance was briefly sedimented. Blanks for sterile dilution were serially marked from stock and 10^{-1} to 10^{-4}.Using a brand-new, sterile pipette, one ml of the stock was transferred to the 10^{-1} dilution blank. For each subsequent step, one ml from the 10^{-1} dilution was transferred to the 10^{-2} tube and from the 10^{-2} to the 10^{-3}, then 10^{-4} after the 10^{-3}. A total of 0.1 ml of the dilution fluid from each dilution tube was added to the culture medium before being incubated for 24 hours. Pure cultures of bacteria were sub cultured in NA slants and incubated at 4 to 10°C to achieve vigorous growth after successful microorganism growth (Table 1).

Table 1: Isolation of bacteria involved in solid waste decomposition

Bacterial Isolates		
CAM Medium	**LB Medium**	**LB Medium**
CAMW2	LBW3	KBW1
CAMS1	LBS2	KBS2
CAMP2	LBP1	KBP3
CAMB1	LBB1	KBB2
CAMN2	LBN2	KBN1
CAMSu3	LBSu3	KBSu2
CAMK3	LBK2	KBK1
CAMH1	LBH3	KBH2
CAMKr1	LBKr3	KBKr3
CAMSA3	LBSA1	KBSA2
CAMPr2	LBPr2	KBPr2
CAML3	LBL3	KBL3
CAMM1	LBM1	KBM2
CAMC2	LBC2	KBC2
CAMG2	LBG2	KBG2
CAMGa2	LBGa2	KBGa1
CAMKa2	LBKa3	KBKa2
CAMNa1	LBNa2	KBNa3
CAMPa1	LBPa1	KBPa2
CAMR1	LBR2	KBR2
CAMPTI	LBPT3	KBPT3
CAMSh3	LBSh2	KBSh1
CAMKe3	LBKe2	KBKe2
CAMH2	LBH3	KBH3
CAMKT2	LBKT3	KBKT1
CAMBu2	LBBu2	KBBu2
CAMBb3	LBBb3	KBBb3
CAMAn1	LBAn2	KBAn1
CAMBi2	LBBi3	KBBi2
CAMD3	LBD2	KBD3

Microbiological and biochemical characteristics of isolated bacteria

Gram staining was done to examine the bacteria's cellular structure and gram nature, and the strains were also

biochemically characterised. The biochemical tests for the production of amylase, cellulase, protease and xylanase were carried out.

Table 2: Morphological characteristics of Bacterial strains isolated on CAM Medium

Isolate	Colony Features	Cell Features	Gram's Reaction	Shape
	Colour of Colony	Nature of Colony		
CAMW2	Creamy	Smooth, raised	+	Bacilli
CAMS1	Creamy	Smooth, raised, transparent	+	Bacilli
CAMP2	Creamy	Smooth, raised, transparent	+	Bacilli
CAMB1	Whitish	Raised, transparent, irregular	+	Bacilli
CAMN2	Creamy	Smooth, irregular	+	Bacilli
CAMSu3	Creamy	Raised, transparent	+	Bacilli
CAMK3	Creamy	Smooth, irregular	_	Bacilli
CAMH1	Whitish	Raised, irregular, transparent	+	Cocci
CAMKr1	Creamy	Smooth, transparent	_	Cocci
CAMSA3	Whitish	Raised, irregular	+	Bacilli
CAMPr2	Creamy	Smooth, raised, transparent	_	Bacilli
CAML3	Creamy	Raised, transparent	_	Cocci
CAMM1	Creamy	Smooth, transparent, raised	+	Cocci
CAMC2	Whitish	Raised, transparent, irregular	_	Cocci
CAMG2	Creamy	Smooth, transparent, irregular	_	Bacilli
CAMGa2	Whitish	Smooth, irregular	+	Cocci
CAMKa2	Creamy	Smooth, transparent, irregular	_	Bacilli
CAMNa1	Whitish	Raised, transparent	_	Cocci
CAMPa1	Creamy	Smooth, irregular	+	Bacilli
CAMR1	Whitish	Raised, smooth, irregular	+	Cocci
CAMPTI	Creamy	Smooth, raised	_	Cocci
CAMSh3	Creamy	Raised, smooth, irregular	+	Bacilli
CAMKe3	Whitish	Smooth, irregular	+	Bacilli
CAMH2	Creamy	Smooth, transparent	_	Cocci
CAMKT2	Whitish	Raised, irregular, smooth	_	Cocci
CAMBu2	Whitish	Smooth, transparent	_	Bacilli
CAMBb3	Creamy	Raised, smooth	+	Cocci
CAMAn1	Creamy	Smooth, raised, transparent	+	Bacilli
CAMBi2	Creamy	Raised, smooth, transparent	+	Bacilli
CAMD3	Creamy	Smooth, raised	+	Bacilli

Table 3: Morphological characteristics of Bacterial strains isolated on LB Medium

Isolate	Colony Features	Cell Features	Gram's Reaction	Shape
	Colour of Colony	Nature of Colony		
LBW3	Whitish	Smooth, raised, opaque	+	Bacilli
LBS2	Creamy	Raised, opaque	+	Bacilli
LBP1	Whitish	Smooth, raised, transparent	+	Cocci
LBB1	Whitish	Raised, opaque	_	Bacilli
LBN2	Creamy	Smooth, raised, opaque	+	Cocci
LBSu3	Light yellow	Smooth, transparent	_	Bacilli
LBK2	Creamy	Raised, transparent	+	Cocci
LBH3	Light yellow	Smooth, raised, transparent	+	Bacilli
LBKr3	Creamy	Raised, opaque	+	Bacilli
LBSA1	Whitish	Smooth, raised	_	Bacilli
LBPr2	Whitish	Raised, opaque	_	Cocci
LBL3	Creamy	Smooth, raised	+	Cocci
LBM1	Light yellow	Smooth, raised, opaque	+	Bacilli
LBC2	Whitish	Smooth, raised, opaque	+	Bacilli
LBG2	Whitish	Raised, smooth, opaque	+	Bacilli
LBGa2	Creamy	Smooth, raised	_	Bacilli
LBKa3	Light yellow	Smooth, transparent	_	Cocci
LBNa2	Whitish	Raised, opaque	+	Bacilli
LBPa1	Whitish	Smooth, raised, opaque	+	Bacilli
LBR2	Creamy	Smooth, raised, transparent	+	Cocci
LBPT3	Light yellow	Raised, transparent	+	Bacilli
LBSh2	Creamy	Smooth, raised, transparent	+	Bacilli
LBKe2	Creamy	Smooth, raised	+	Bacilli
LBH3	Creamy	Raised, opaque	+	Cocci
LBKT3	Creamy	Smooth, transparent	_	Bacilli
LBBu2	Whitish	Raised, smooth	_	Cocci

LBBb3	Light yellow	Smooth, transparent	+	Cocci
LBAn2	Whitish	Smooth, raised, opaque	+	Bacilli
LBBi3	Whitish	Raised, opaque	+	Cocci
LBD2	Whitish	Smooth, raised, opaque	+	Bacilli

Table 4: Morphological characteristics of Bacterial strains isolated on King's B Medium

Isolate	Colony Features	Cell Features	Gram's Reaction	Shape
	Colour of Colony	Nature of Colony		
KBW1	Light yellow	Smooth, raised, shiny	+	Bacilli
KBS2	Light yellow	Raised, shiny, transparent	+	Bacilli
KBP3	Creamy	Smooth, raised, opaque, shiny	_	Bacilli
KBB2	Whitish	Smooth, raised, transparent, shiny	+	Cocci
KBN1	Dark yellow	Raised, smooth, transparent, shiny	+	Bacilli
KBSu2	Creamy	Smooth, raised, shiny, opaque	+	Cocci
KBK1	Whitish	Smooth, opaque, shiny, irregular	_	Bacilli
KBH2	Light yellow	Smooth, raised, shiny, transparent	+	Bacilli
KBKr3	Light yellow	Raised, opaque, shiny	+	Cocci
KBSA2	Creamy	Smooth, raised, opaque, shiny	+	Bacilli
KBPr2	Light yellow	Smooth, irregular, transparent, shiny	_	Bacilli
KBL3	Light yellow	Raised, irregular, opaque	+	Cocci
KBM2	Dark yellow	Smooth, raised, transparent, shiny	+	Cocci
KBC2	Creamy	Raised, irregular, transparent	_	Bacilli
KBG2	Whitish	Raised, smooth, opaque	+	Bacilli
KBGa1	Light yellow	Smooth, raised, transparent, shiny	_	Cocci
KBKa2	Creamy	Smooth, raised, opaque, shiny	+	Cocci
KBNa3	Whitish	Raised, irregular, smooth, transparent, shiny	+	Bacilli
KBPa2	Light yellow	Raised, irregular, opaque, shiny	+	Bacilli
KBR2	Creamy	Smooth, raised, transparent, shiny	+	Cocci
KBPT3	Creamy	Raised, irregular, transparent, shiny	+	Bacilli
KBSh1	Whitish	Smooth, irregular, opaque, shiny	_	Bacilli
KBKe2	Light yellow	Raised, irregular, opaque	_	Cocci
KBH3	Creamy	Smooth, raised, transparent, shiny	+	Bacilli
KBKT1	Dark yellow	Smooth, raised, irregular, opaque	+	Cocci
KBBu2	Light yellow	Raised, transparent, irregular, shiny	+	Cocci
KBBb3	Creamy	Smooth, raised, shiny, opaque	_	Bacilli
KBAn1	Whitish	Smooth, raised, transparent, shiny	+	Bacilli
KBBi2	Whitish	Smooth, raised, transparent, shiny	_	Bacilli
KBD3	Light yellow	Raised, irregular, opaque	+	Bacilli

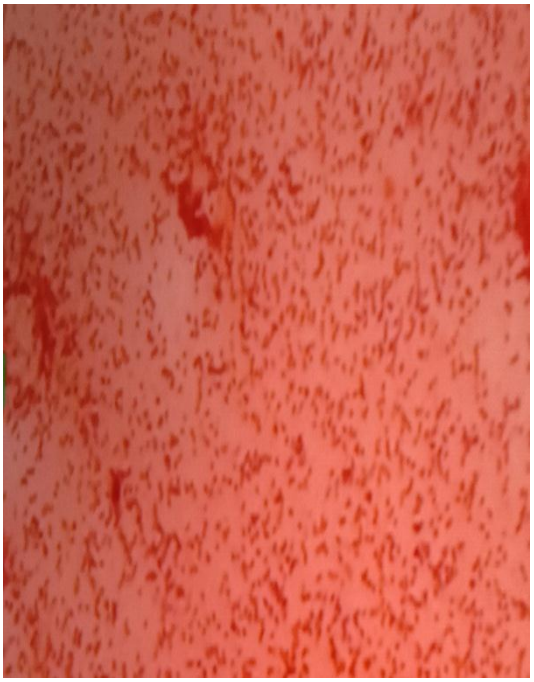

Plate 1: Gram Negative

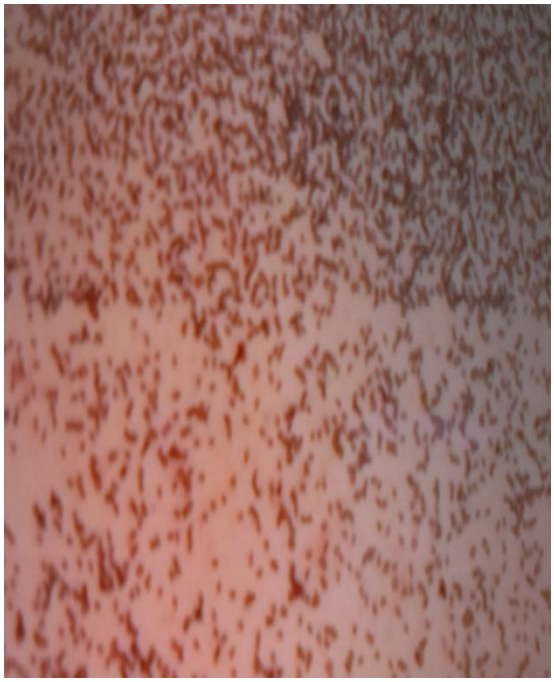

Plate 2: Gram Positive

Production of extracellular enzymes

All the isolated bacterial strains were screened qualitatively for the production of four important enzymes such as amylase, cellulase, protease and xylanase. The Petri plates were incubated overnight at 10 to 15 °C. Then the plates were flooded with indicator solution and the development of clear zone around the growth of organism was considered positive for enzyme activity.

Results and Discussion

Cultural characteristics of bacterial isolates

In our study, bacterial strains were isolated in three different media. The ideal media for ensuring the isolated strains rapid growth were found by comparing CAM, LB and King's B medium.

The chosen strains were characterised by visual and microscopic examination. Details of the bacteria's colony characteristics are noted (Table 2, 3, 4).An established and time-tested technique for observing the bacteria is gram staining. Alcohol decolorized gram-negative bacteria, causing them to lose their crystal violet-purple hue. Gram-positive bacteria remained purple (Plate 1 and Plate 2), not changing colour.

Screening and assessment of isolated bacterial cultures for their ability to decompose solid wastes using enzymatic processes

Qualitative enzyme assay

On the basis of enzymatic activities (amylase, cellulase, protease and xylanase) at low incubation temperatures, potential *Bacterial species* were chosen.

Plate 3: Amylase

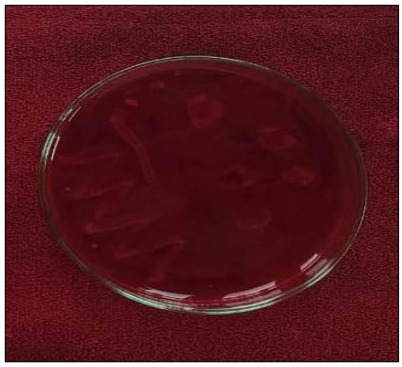

Plate 4: Cellulase

Plate 5: Protease

Plate 6: Xylanase

Isolated bacteria's enzymatic activity

Amylase synthesizing bacteria

When the isolates were tested for amylase activity, among 90 bacterial isolates, around 70% of bacterial isolates showed the highest levels of amylolytic activity. These findings are supported by the observations of Amalesh *et al.* (2013) [2] who studied the isolation of an amylase producing bacteria that can grow in the irritant municipal waste and help in their bio conversation. These isolates were further chosen for the treatment of the solid waste based on the results that suggested they produced extracellular amylolytic enzymes (Plate 3).

Cellulase synthesizing bacteria

The hydrolysis of the substrate in the basal salt medium was used to test the bacterial isolates for their Cellulase activity, and among 90 bacterial isolates, around 70% of bacterial isolates showed the highest levels of Cellulase enzyme activity. The results of the current study corroborate with the findings of Singh *et al.* (2019) [9] who studied 11 cellulose degrading bacterial strains which were isolated from water

and soil samples of hot springs in the Chumathang village, Leh and Ladakh region, India. The isolated strains were identified as *Bacillus subtilis*, *Bacillus aryabhattai*, *Bacillus stratosphericus*, *Bacillus altitudinis*, and *Brevibacterium frigoritolerans* by biochemical and molecular approaches. All the strains were evaluated for the total cellulase, endoglucanase, exoglucanase, and β-glucosidase enzyme activities (Plate 4).

Protease synthesizing bacteria

When the protease-secreting isolates were tested, they showed extremely high enzyme activity. Among the 90 bacterial isolates, around 70% of the bacterial isolates had the highest proteolytic activity when compared to one another (Plate 5).

Xylanase synthesizing bacteria

The soluble xylanase activity of the isolates was tested in a medium containing the substrate in the right amounts. Among 90 bacterial isolates, around 70% of bacterial isolates displayed the highest Xylanase activity per unit time when compared to one another. The results are in agreement with the findings of Amare Gessesse and Gashaw Mamo (1999) [3] studied *Bacillus* sp. AR-009 which produced up to 720 U/g dry bacterial bran xylanase activities when grown by using solid-state fermentation with wheat bran serving as a substrate. Xylanase production was highest at a wheat bran-to-moisture ratio of from 1:0.5 to 1:1.5 and a Na2CO3 concentration of 10% (w/w). These isolates were further chosen for the treatment of solid wastes based on the findings that they produced extracellular Xylanase enzymes (Plate 6).

Rapid organic waste degradation requires the action of micro-organisms with enhanced enzyme activity to break down complex polymers into simpler degradable molecules. Therefore, inoculation of waste with microorganisms that produce extracellular enzymes such as amylase, cellulase, protease and xylanase at higher levels enhances waste degradation, thus helping in keeping up of the waste degradation rate to that of waste dumping. This requirement is fulfilled with the co-operation of many micro-organisms, each of which contributes in secreting different enzymes. Agricultural wastes are leftovers from the production and processing of raw agricultural products and may contain materials that are useful to humans. These residues are produced by a variety of agricultural practices, including aquaculture, livestock production, and cultivation. When these wastes are properly managed by applying agricultural knowledge, waste management practices like the "3Rs" can be converted into useful materials for use in agriculture and by humans.

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References

1. Adewale Aiwo MT. Composting as a sustainable waste management technique in developing countries, Journal of Env. Sci & Tech. 2011;4(2):93-102.
2. Amalesh, Samanta, Debmalya, Mitra, Sudipendra, Nath, *et al*. Characterization and Optimization of Amylase Producing Bacteria Isolated from Solid Waste. Journal of Environmental Protection. 2013;4:647-652.
3. Amare Gessesse, Gashaw, Mamo. High-level xylanase production by an alkaliphilic *Bacillus* sp. by using solid-state fermentation. Enzyme and Microbial Technology. 1999;25:68-72.
4. Chugh S, Chynoweth DP, Clarke W. Degradation of unsorted municipal solid waste by a leach-bed process (J). Bioresource technology. 1999;69:103-115.
5. Gautam SP, Bundela PS, Pandey AK, Awasthi MK, Sarsaiya S. Composting of Municipal solid waste of Jabalpur city. Global J Environ. Res. 2010;4(1):43-46.
6. Majumdar K, Singh N. Effect of soil amendments on sorption and mobility of metribuzin in soils. *Chemosphere*, 2007;66:630-637.
7. Mena E, Garrido A, Hernandez T, Garcia C. Bioremediation of sewage sludge by composting. Commun, Soil. Sci. Plant Anal. 2003;34:957-971.
8. Nannipieri P, Kandeler E, Ruggiero P. Enzyme activities and microbiological and biochemical process in soil. In: Burns, R.G. and R. Dick (Eds.) Enzyme in the environment. Marcel Delker, New York, 2002, PP 1-33.
9. Singh Saurabh, Durgesh Kumar, Jaiswal Nallusamy, Sivakumar, Jay Prakash Verma. Developing Efficient Thermophilic Cellulose Degrading Consortium for Glucose Production from Different Agro-Residues. Frontiers in Energy Research 2019;7:61.
10. Sivarajan A, Jayanthkumar J, Srimannarayanan J. Effect of graded level of NPK along with coffee pulp compost on growth and yield attributes of Bhendi, In: National Seminar on Microbial Technology for Productive Agriculture, Oct.7-8. Tamilnadu Agricultural University, Coimbatore, Tamilnadu, India, 2005, p229.
11. Vanderroot C, Zabik MJ, Branham B, Lickfeldt DM. Fate of selected pesticidal applied to turf grass: effect of composting on residues, bull. Environ. Contam. Toxicol. 1997;58:38-45.