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Organic Waste Composting with Bacterial Consortium and its Effect on Plant Growth Promotion

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ABSTRACT

The present study was aimed to isolate and develop cellulolytic microbial consortium for preparing compost from various crop residues. Further, the effect of leaf litter compost was tested for plant growth promotion along with different levels of Recommended Dose of Fertilizer (RDF). Leaf litter compost and Farm yard manure samples were used for isolation of cellulolytic bacteria. Isolates were screened based on cellulase and xylanase activities and identified by 16S rDNA sequencing. Microbial consortium was developed and tested their efficiency for composting of different cellulosic waste materials. Effect of leaf litter compost on Black gram growth and yield was tested with different doses of recommended fertilizer. Based on xylanase and cellulase activities two bacterial isolates (ACC52 and ACCA2) were selected from the total of 15 isolates. Based on 16S rDNA sequence analysis ACC52 and ACCA2 isolates were identified as Bacillus safensis and Enhydrobacter aerosaccus, respectively. Use of these two isolates as a consortium in the composting of leaf litter resulted in higher nutrient release and lower C:N ratio compared to other agricultural wastes composted using the same bacterial consortium. Recommended dose of leaf litter compost with 75% of the RDF showed higher germination rate (95%), plant growth (27.7 cm) and number of pods (31 pods per plant) of Black gram compared to the application of 100% RDF and compost. Results of this study illustrated that the leaf litter compost developed by the microbial consortium mediated decomposition could reduce the fertilizer usage by 25% without compromising the yield.

Keywords: Black gram, Cellulolytic microorganisms, Compost, Microbial consortium

INTRODUCTION

The crop residues are abundant with cellulose, hemicellulose, lignin, pectin and also contains low amount of diverse group of substances like protein and fatty acids. The major portions of crop residues were burnt in the field itself. Burring of crop residues in the field affecting soil C:N ratio and biota; on the other hand burning of waste leads to emission of greenhouse gases [1]. Bio-degradation of agricultural waste into compost and incorporation into soil may enhance the nutrient recycling and improves soil. However, higher lignocellulosic content in the waste materials slow down the microbial degradation process. Effective utilization of lignocellulosic crop residues requires physical or chemical pre-treatments which may not be a cost effective for the farmers. Use of microorganism (lignocellulolytic microorganisms) may be low cost method of lignocellulosic crop residue utilization [2]. No single microorganism has the capability to completely degrade the lignocellulolytic materials, there is need to use a consortium of microorganisms which can act synergistically for bioconversion of agricultural residues.

Compost is the rich source of nutrients that contains high level of organic matter contents. Use of compost improves the soil physico-chemical properties and increases the plant productivity. Soil physical properties such as bulk density, porosity, water permeability and hydraulic conductivity improved by compost applications. In general, microorganism involved in composting process can be grouped in to Mesophilic and thermophylic microbiota. Mesophilic microorganism initiate the degradation of crop residues and the thermophilic microorganisms dominates when the compost temperature rises to 60–65˚C. More rapid decomposition of crop residues occurs during thermophilic stage which is achieved with in the first week of composting process. Even though the crop residues naturally harbored by thermophilic microorganism, their degradation efficiency may not be effective due to the lack of enzymes which are required for cellulose and lignin degradation. It is always required the additional inoculation of thermophilic cellulolytic microorganism for effective compost preparation. Quality and the nutrient content of the compost are determined by the microbial activity.

Kumar et al. [3] reported that a thermophilic consortium, consists of *Aspergillus nidulans*, and *Scytalidium thermophilum* was effective in converting high silica paddy straw into nutritionally rich compost. Many workers demonstrated that fungi are capable of degrading lignocellulosic materials [4]. Very limited literatures are reported that degradation of lignocellulosic wastes by bacteria [5]. Hence, the present study was formulated to isolate lignocellulose degrading bacteria and to develop a microbial consortium for effective degradation of farm wastes. Further, compost obtained through effective microbial consortium was tested on Black gram growth promotion under pot culture condition.

MATERIALS AND METHODS

Selective isolation and screening of cellulase producing microorganisms

Totally twenty four farm yard manure and leaf litter compost samples were collected from Agricultural College and Research Institute, Madurai, (9°58'11.18" N; 78°12'15.54" E) Tamil Nadu India for the selective isolation of cellulase enzyme producing bacteria. The samples were serially diluted and plated on CMC (Carboxyl Methyl Cellulose) agar medium for isolation of cellulolytic bacteria. Pour plate technique was used and the plates were incubated at 30ºC for 5 days. Bacterial colonies appeared on the CMC agar plates were purified further. In order to select an efficient cellulose degrading microorganism, the cellulase activity was tested in a plate assay using the CMC medium supplemented with 2% agar. Ten microliter of each bacterial isolate were spotted on CMC agar plate and incubated at 30ºC for 3 days. At the end of incubation period, the plates were flooded with an aqueous solution of 0.1% Congo red for 15 min and washed with 1M NaCl. To indicate the cellulase activity of the organisms, diameters of clear zone around the colony and colony diameter was measured. The experiment was repeated three times and results are expressed as cellulase efficiency=clear zone diameter/colony diameter*100 [6].

Quantitative estimation of microbial cellulase activity

The bacterial cultures were inoculated into nutrient broth supplemented with 0.1% CMC and incubated at 30°C for 2 days. The culture filtrate was drawn at 48 h by centrifugation at 13,000 rpm for 5 min. The cellulase activity was measured by mixing 0.1 mL of culture filtrate with 0.1 mL of 1.0% (w/v) CMC in 10mM sodium phosphate buffer, pH 7.0 at 37º C for 60 min. The reaction was stopped by adding 1.0 mL 3, 5-dinitrosalicylic acid (DNS) reagent. The mixture was boiled for 10 min cooled in ice and its optical density at 546 nm was determined using an UV spectrophotometer (DU-64, Shimadzu, Japan). The standard curve was generated using glucose. One unit of Carboxymethyl-Cellulase (CMCase) was defined as the amount of enzyme that released 1 µmol of glucose min^{-1}.

Xylanase activity

The xylanase activities of isolates were determined by following the method of Bailey et al. [7]. Briefly, the cultures were inoculated into nutrient broth supplemented with 0.1% birch wood xylan and incubated at 30°C for 2 days. The culture filtrate was drawn at 48 h by centrifugation at 13,000 rpm for 5 min. Culture filtrate of 0.1 mL was incubated with 0.1 mL xylan and 0.2 mL of the acetate buffer at 40°C for 30 min then the reaction was stopped by addition of 0.5 mL alkaline copper reagent. Then tubes were placed in boiling water bath for 10 min and cooled, and then 0.2 mL of arsenomolybdate was added and incubated for further 15 min. The supernatant was diluted fivefold with water and measured in UV spectrophotometer at 495 nm (DU-64, Shimadzu, Japan). The standard was prepared with xylose and one enzyme unit was expressed as 1 µmol of xylose released mL^{-1} of enzyme min^{-1}.

Biochemical characters of the efficient bacterial isolates

Cellulolytic bacterial isolates were streaked on nutrient agar supplemented with 1% starch and incubated for 48 h at 30°C. Then petri plates were flooded with Lugol's iodine solution for 30 seconds and drained. The positive starch hydrolysis was indicated by the formation of yellow zone around the colonies against dark blue background. Catalase test of the bacterial isolates were performed by adding 1 mL of 0.5% hydrogen peroxide to agar slant culture and observed for appearance of gas bubbles. The citrate utilization characters of the isolates were performed by streaking

bacterial isolates in Simmon's citrate agar slants and incubated at 30°C for 48 h. The change in color of the medium from green to blue after incubation was positive for test.

Indole production of the isolates was done using standard protocol. Bacterial isolates were inoculated into glucose tryptone broth and incubated for 48 h. Then 0.3 mL of Kovac's reagent was added and mixed well. Positive result for indole production of bacterial isolates was indicated by reddening of alcohol layer within few min. Methylred-Voges Proskauer test, Urease test and Nitrate reduction tests were done by following standard methods.

Molecular characterization and phylogenetic analysis

The bacterial isolates were cultivated in nutrient broth and DNA was extracted using bacterial genomic DNA purification kit (Hi media, India). The bacterial 16S rDNA was amplified through PCR with forward primer 27f: 5′-AGAGTTTGATCCTGGCTCAG-3′ and reverse primer 1492r: 5′-GGTTACCTTGTTACGACTT-3′. Nearly complete 16S rDNA sequences from automatic sequencer were aligned and bacterial identities deduced by using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/); [8] to ascertain their closest relatives. Phylogenetic tree, multiple sequence alignments were performed using the CLUSTAL W. The method of Jukes and Cantor was used to calculate evolutionary distances; phylogenetic dendrograms were constructed by neighbor-joining [9] methods using the program MEGA 6 [10] and bootstrap values were calculated on the basis of 1000 replications.

Temperature tolerance of the bacterial isolates and consortium development for composting

Based on the Cellulolytic and Xylanase activity ACC52 and ACCA2 isolates were selected for the further experiments. To test the thermotolerant nature of the isolates ACC52 and ACCA2 were grown in wide range of temperatures (25°C-60°C). Developments of visible colonies on the agar plates were considered as tolerant and absences of visible colonies were considered as sensitive to that particular temperature. To test the compatibility between ACC52 and ACCA2 isolates cross streak assay was performed and the absences of inhibition zone were considered as the isolates were compatible to each other. The bacterial isolates ACC52 and ACCA2 were inoculated in liquid CMC medium and incubated at 30°C for 3 days. After incubation the broth was centrifuged at 10000 rpm and the pellets were dissolved in phosphate buffer (pH 7.2), no. of viable cells in one mL broth was adjusted to $1x10^9$ cfumL^{-1}. To develop microbial consortium the above mentioned two cultures were mixed in equal proportion and used as inoculum for composting [11].

Composting

Agricultural wastes such as paddy straw, sugarcane trash and leaf litter used for composting were collected from Agricultural College and Research Institute, Madurai, and chopped into 10–15 cm pieces. About 20 kg of each substrate mixture was filled in the compost pit and inoculated with microbial consortium @1% (v/w) (1 x 10^6cfu/ mL) as per treatments (T1-Paddy straw +Microbial consortium; T2-Sugarcane trash+Microbial consortium; T3-Leaf litter+Microbial consortium; T4-Paddy straw+Conventional method; T5-Sugarcane trash+Conventional method; T6-Leaf litter+Conventional method; T7-Paddy straw+Uninoculated; T8-Sugarcane trash+Uninoculated; T9-Leaf litter +Uninoculated). The inoculum was mixed with the substrates and the mixture was allowed to decompose for three months during summer season under natural conditions. Simultaneously natural methods (uninoculated) of composting were performed to compare the decomposition efficacy of microbial consortium developed in this study.

In conventional method of decomposition, the fresh cow dung 10 kg was mixed with 100 liters of water and thoroughly mixed with sugarcane trash, paddy straw and leaf litter. After mixing all the inputs, heap was formed with a minimum height of 4 feet. This height was required to generate more heat in the composting process, and the generated heat was retained long time inside the material. The compost material was turned periodically once in 15 days to allow more aeration inside the material.

The samples were drawn from compost pit at 0^{th}, 15^{th}, 30^{th}, 45^{th}, 60^{th}, 75^{th} and 90^{th} days for nutrient analyses. Samples were dried at 60ºC and grinded to pass through 2 mm sieve. The powdered samples were analyzed for organic carbon total nitrogen and available potassium by following the standard method. Available phosphorus was estimated by the method of Olsen et al. [12]. One gram of representative samples was taken in above mentioned interval and total bacterial count was measured. Starch iodine test was conducted to test the efficiency of the compost process [13].

Effect of leaf litter compost on plant growth

A pot culture experiment was conducted to study the effect of leaf litter compost plus inorganic fertilizer on the growth of Black gram cv., VBG6 (Vigna mungo) with nine treatments (T1-Control;T2-100% RDF; T3-100% RDF+ 100% Leaf litter compost; T4-75% RDF+100% Leaf litter compost; T5-50% RDF+100% Leaf litter compost; T6-25% RDF+100% Leaf litter compost; T7-100% Leaf litter compost; T8-100% Farm yard manure; T9-100% Vermi compost). Black gram seeds were obtained from National Pulse Research Centre, Indian Council of Agricultural Research, Vamban, Pudukkottai district, Tamil Nadu, India. Two seeds of Black gram were sown in pot filled with red soil and five pots were maintained for each treatment (9°58'11.41" N; 78°12'12.42" E). Further, each treatment was replicated thrice. The pots were arranged in a completely randomized block design and appropriate cultivation

practices were followed. The physio-chemical properties of experimental soil as follows; pH-7.72, EC (dSm^{-1}) -0.25, OC-0.67(%), available nitrogen-25.93 mg/kg of soil, available phosphorus-24.6 mg/kg of soil, and available potassium-0.24 cmol/kg of soil. Seed germination percent was measured on day six; Shoot, root lengths and yield parameter of Black gram were measured on 60th day after sowing.

Statistical analysis

Microbial count and plant parameter data were subjected to analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) was performed using the SAS package, Version 9.1.3 (SAS Institute Inc., Cary, NC, USA), tested significant differences at 0.05.

RESULTS

Enzymatic activity and biochemical characteristics of the bacteria

Among fifteen cellulolytic bacterial isolates, ACCA2 and ACC52 isolates exhibited higher cellulolytic efficiency compared to other isolates. In line with this the quantitative data showed 4.9 U mL^{-1} of cellulase activity by ACCA2 isolate, second highest was exhibited by ACC52 isolate (Figure 1). Similarly, the xylanase enzyme production was found to be higher in ACCA2 and ACC52 isolates. These two isolates scored positive for growth in wide range of temperatures (25°C to 60°C). All the six cellulolytic bacterial isolates had positive results for catalase, citrate utilization, reduced the nitrate concentration and scored positive for methyl red test.

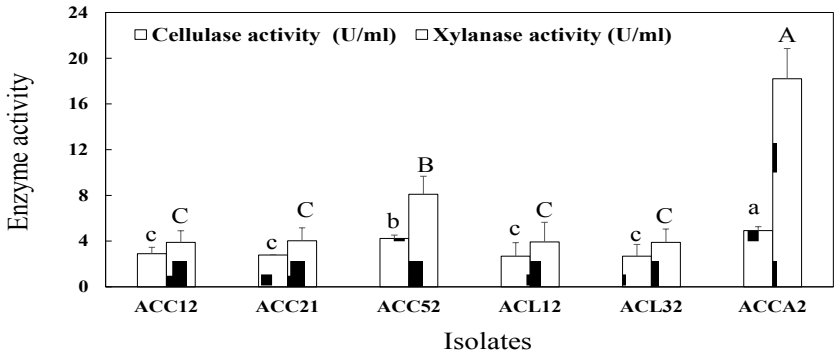

Figure 1: Quantitative estimation of Cellulase and Xylanase activities of the isolates. Values are means ± SE (standard error) of three replications. Same letters on columns are not significantly different at P≤0.05 DMRT.

Molecular characterization of selected microorganisms

Phylogenetic tree of the bacterial isolates and their closest neighbors were presented in Figure 2. The 16S rDNA sequence result revealed that selected cellulolytic microorganisms belonged to two different genera. Among the 6 isolates, three isolates namely ACC12, ACC52 and ACL32 exhibited 100% similarity to the Bacillus safensis. Isolates ACC21 and ACL12 showed 99.87 sequence similarities with Bacillus subtilis. Isolate ACCA2 showed 100% sequence similarity with Enhydrobacter aerosaccus. The sequence of 16S rDNA obtained in this study has been submitted to Genbank under accession numbers JX042466-JX042470 and JX042472.

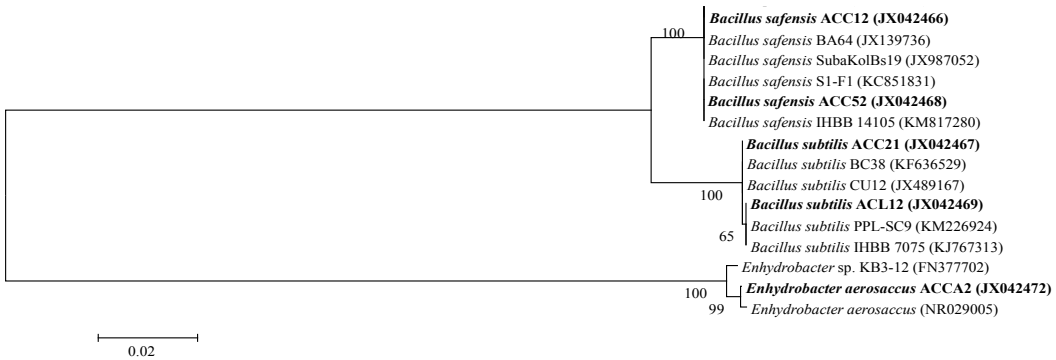

Figure 2: Neighbor-joining phylogenetic tree shows the relationship among the cellulase enzyme producing bacteria (highlighted in bold) and their closest neighbours. Tree was constructed using near full length sequences of bacterial 16S rDNA sequences with 1000 bootstraps values.

Microbial count and nutritional status changes during composting

Numerically higher bacterial population was registered in cellulolytic microbial consortium applied compost pit compared to conventional compost pit and uninoculated compost pit. Total bacterial population reached a maximum of $42x10^6$ g^{-1} in dry leaf litter and microbial consortium applied pit followed by paddy straw and microbial consortium applied pit on 90th day (Table 1). Starch iodine test showed yellow colour with little precipitate for leaf litter composted with microbial consortium treatment whereas other treatments showed dark colour with more precipitates which indicates poor composting.

Table 1: Total bacterial population in different treatments during compost making

Treatment		Days						
		0th	15th	30th	45th	60th	75th	90th
		x 10^6 cfu g^{-1} dry of compost						
T1	Paddy straw+Microbial consortium*	5	19	27	36	41	36	29
T2	Sugarcane trash+Microbial consortium	6	18	25	36	39	32	25
T3	Leaf litter+Microbial consortium	5	18	27	39	42	39	32
T4	Paddy straw+Conventional method	5	15	21	26	30	28	25
T5	Sugarcane trash+Conventional method	6	10	19	27	30	27	25
T6	Leaf litter+Conventional method	5	9	16	21	29	25	21
T7	Paddy straw+Uninoculated	5	8	10	12	9	7	5
T8	Sugarcane trash+Uninoculated	6	8	11	13	12	10	8
T9	Leaf litter+Uninoculated	5	7	8	10	8	8	6
LSD (P=0.05)		**0.25**	**0.81**	**0.90**	**0.79**	**0.79**	**1.40**	**0.87**

*Microbial consortium–consortium of *Bacillus safensis* ACC52 and *Enhydrobacter aerosaccus* ACCA2.

Incorporation of cellulolytic microbial consortium in farm waste showed increase in total nitrogen content. Nitrogen content was observed maximum of 2.87% in leaf litter amended with cellulolytic microbial consortium followed by paddy straw amended with cellulolytic microbial consortium (2.47%) (Figure 3A). The higher phosphorus content (1.25%) was recorded in leaf litter incorporated with cellulolytic microbial consortium followed by sugarcane trash incorporated with cellulolytic microbial consortium (1.23%) on 90th day after the initiation of compost processing (Figure 3B). Significant increase in potassium was observed in leaf litter amended with cellulolytic microbial consortium followed by paddy straw, sugarcane trash with cellulolytic microbial consortium on 90th day of composting (Figure 3C). Microbial consortium received treatments registered the lower organic carbon compared to natural and control compost pit (Figure 3D). The narrow C:N ratio (10:1) was observed in leaf litter amended with microbial consortium on day 90 (Table 2).

Table 2: Estimation of C:N ratio during compost making processes.

Treatment					Days			
		0th	15th	30th	45th	60th	75th	90th
T1	Paddy straw+Microbial consortium*	52:1	45:1	39:1	32:1	27:1	26:1	24:1
T2	Sugarcane trash+Microbial consortium	43:1	33:1	26:1	20:1	17:1	14:1	12:1
T3	Leaf litter+Microbial consortium	30:1	27:1	22:1	17:1	14:1	12:1	10:1
T4	Paddy straw+Conventional method	52:1	48:1	43:1	39:1	35:1	34:1	30:1
T5	Sugarcane trash+Conventional method	43:1	37:1	33:1	27:1	16:1	16:1	14:1
T6	Leaf litter+Conventional method	30:1	25:1	21:1	19:1	16:1	15:1	13:1
T7	Paddy straw+Control	52:1	49:1	47:1	31:1	30:1	28:1	26:1
T8	Sugarcane trash+Control	43:1	40:1	38:1	36:1	32:1	30:1	29:1
T9	Leaf litter+Control	30:1	28:1	25:1	24:1	23:1	22:1	20:1

*Microbial consortium–consortium of ACC52 and ACCA2

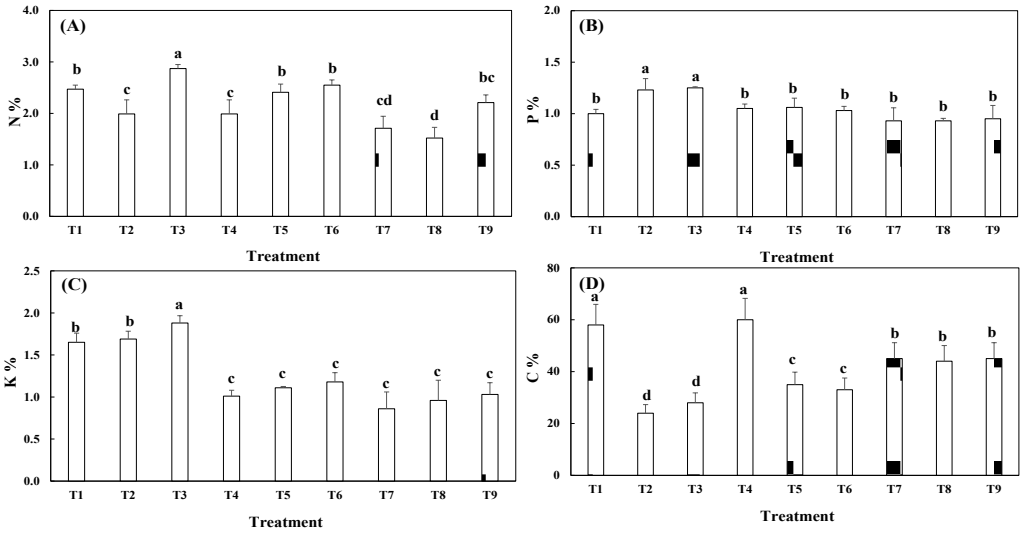

Figure 3: Nutritional status of compost after 90 days of decomposition. (A) Nitrogen, (B) Phosphorus, (C) Potassium and (D) Carbon. T1-Paddy straw+Microbial consortium; T2-Sugarcane trash+Microbial consortium; T3-Leaf litter+Microbial consortium; T4-Paddy straw+Conventional method; T5-Sugarcane trash+Conventional method; T6-Leaf litter+Conventional method; T7-Paddy straw+Uninoculated; T8-Sugarcane trash+Uninoculated; T9-Leaf litter+ Uninoculated. Values are means ± SE (standard error) of three replications. Same letters on columns are not significantly different at $P \leq 0.05$ DMRT.

Effect of leaf litter compost on germination percentage, root and shoot lengths and yield parameter of Black gram under pot culture condition.

The germination percentage was observed higher in 75% of RDF plus 100% leaf litter compost followed by the treatment received 100% RDF plus 100% leaf litter compost. The application of 100% leaf litter compost with 75% RDF showed significant increase in root length compared to 100% RDF application after 60 days of sowing (DOS) (Table 3). Significantly higher number of nodule (12 plant^{-1}) and pods (31 pod plant^{-1}) were registered in the treatment received 100% leaf litter compost with 75% leaf litter compost compared to the 100% RDF application (Table 3).

Table 3: Influence of leaf litter compost on growth and yield parameters of Black gram.

	Treatment	Germination (%)	Root length(cm)	Shoot length(cm)	No of nodules plant^{-1}	No of seed pod^{-1}	No of pod plant^{-1}	100 grain weight (g)
T1	Control	66.3 ± 1.5	12.3 ± 0.2	20.0 ± 0.27	5 ± 0.09	4 ± 0.07	21 ± 0.05	3.2 ± 0.02
T2	100% RDF	90.0 ± 0.7	14.1 ± 0.08	24.0 ± 0.06	9 ± 0.25	5 ± 0.14	25 ± 0.31	3.4 ± 0.08
T3	100% RDF+100% leaf litter compost	94.0 ± 1.4	15.3 ± 0.23	26.3 ± 0.21	10 ± 0.23	5 ± 0.07	27 ± 0.80	4.2 ± 0.10
T4	75% RDF+100% leaf litter compost	95.0 ± 4.2	15.7 ± 0.15	27.7 ± 0.72	12 ± 0.13	5 ± 0.10	31 ± 0.11	4.4 ± 0.08
T5	50% RDF+100% leaf litter compost	93.0 ± 2.6	14.3 ± 0.29	24.3 ± 0.11	11 ± 0.28	5 ± 0.10	25 ± 0.54	3.8 ± 0.19
T6	25% RDF+100% leaf litter compost	85.3 ± 1.3	12.3 ± 0.91	26.3 ± 0.19	10 ± 0.02	5 ± 0.07	27 ± 1.11	4.0 ± 0.17
T7	100% leaf litter compost	88.3 ± 1.3	13.7 ± 0.13	26.7 ± 0.17	12 ± 0.26	4 ± 0.07	29 ± 1.20	3.9 ± 0.05
T8	100% FYM	90.0 ± 1.0	13.7 ± 0.37	27.0 ± 0.25	9 ± 0.09	4 ± 0.20	28 ± 0.65	3.9 ± 0.03
T9	100% Vermicompost	90.0 ± 0.3	13.7 ± 0.32	25.7 ± 0.06	10 ± 0.37	5 ± 0.14	26 ± 0.98	4.0 ± 0.07
	LSD (P=0.05)	**16.08**	**0.49**	**4.63**	**0.55**	**NS**	**1.25**	**NS**

Values in each column are means of three replication one experiments ± standard deviation. (100% RDF=25:50:25 kg NPK ha-1; 100%; compost=2.5 ton ha-1); NS–Non Significant. RDF–Recommended dose of fertilizer; FYM–Farm yard manure; NS–Non significant.

DISCUSSION

In recent years, higher amount of lignocellulolytic waste are increasingly realized as an environmental problem and

the utilization of these wastes has become a welcome issue. lignocellulolytic waste is the most abundant natural waste substance and has become one of the most important raw materials for compost preparation. There has been much research focused on isolation of microorganisms which produces cellulase enzymes with higher specific activities and greater efficiency [14]. However there are only few studies which focus on the utilization of these cellulolytic enzyme producing bacteria on compost preparation and subsequent usage [15]. Hence, in this study we have isolated the bacteria with high cellulase and xylanase producing capacity to prepare compost using agricultural waste materials and the effect of the compost was tested on Black gram growth and pod production.

Among fifteen isolates obtained from the farm yard manure and leaf litter compost the isolate ACCA2 and ACC52 showed higher cellulase production. Similarly, Afzal et al. [16] and [17] isolated higher cellulase producing bacterial isolates from waste. Venkata et al. [18] reported that the Bacillus genus are the promising bacteria which produces higher amount of cellulase. However in our study, genus Enhydrobacter was found to produce higher amount of cellulase compared to *Bacillus genus*. In this study, we have reported higher xylanase producing capabilities of *Bacillus safensis* and *Enhydrobacter aerosaccus*. Previously, xylanase activity was reported in other bacterial species such as *Bacillus pumilis* and *Pseudomonas sp*. WL11 [19, 20].

The microbial consortium applied during compost preparation showed higher nitrogen, phosphorus and potassium content compared to natural method. Savy and Banzatto [21] reported that the application of castor crop residues to the soil aids in recycling of nutrients and increased organic matter N, P and K. Initial C:N ratio was found to be higher in all the treatments during the initial stage of compost preparation. However, the treatments received microbial consortium recorded lower C:N ratio (10:1). This may be due to the action of microorganisms on the fast degradation of cellulose and hemicelluloses present in the waste. The above findings were concurrent with the findings of Kumar et al. [3] who have reported the lower C: N ratio (14.6:1) in paddy straw amended with microbial consortium. Eiland et al. [22] reported that the improvement in nitrogen and lowering of carbon as an important criteria to assess the maturity of compost. Similarly, microbial consortium mediated compost preparation using agricultural waste resulted in the lowest C:N ratio (9.54:1) [3].

Chemical fertilizer treated plants exhibited rapid plant grow that the initial two weeks of plant growth compared to organic manure treated plants. Because the rate of nutrients release are higher in the inorganic fertilizers since they provide major nutrients at the early stage of plant growth and development [23]. Consequently, plants exhibited enhanced growth than that of compost treated plants. However, no differences were observed between the organic manure and inorganic fertilizer treated plants in the later (flowering) stages of plant growth. This may be due to the differences in the nutrient availability to the plants from the organic and inorganic fertilizers. Organic manures activate many beneficial microorganisms which release phytohormones and may enhance the plant growth and absorb nutrients from soil [24]. Therefore combined use of inorganic fertilizers and compost may facilitate the continuous flow of available nutrients to the plants. In addition the requirement of inorganic fertilizer can be reduced to the certain level when applying compost as an additional nutrient without affecting the yield of the plants.

Use of compost and top soil was found to enhance the plant growth compared to control plants [25]. Application of biodegraded sunflower extract at 2.5% to 10% increased growth, protein and chlorophyll contents of Green gram and Chickpea [26]. In the present study, the effect of compost on germination and growth of Black gram was investigated. The germination percentage, root length, shoots length of Black gram varied with the application of leaf litter compost amended with recommended RDF.

Application of RDF plus leaf litter compost resulted in increased shoot length and root length. Higher shoot length (27.7 cm) and root length (15.17 cm) were recorded in the treatment applied with 100% leaf litter compost plus 75% RDF compared to other treatments after 60 days of sowing. Similarly application of leaf litter compost was found to increase the root growth [25]. Composts application reduces pH and EC in the rhizosphere soil due to high nutrient supply [27, 28] and resulted in improved plant growth. The soil pH determines the nutrients accessibility in plants with the changes in soil pH as increase or decrease in pH alters the solubility of nutrients and has direct effect on plant growth and development. The yield of the plant was found higher in the treatment which received 75% RDF and compost. In contrast with our study, application of 75% RDF and 2 tons of compost showed significantly higher bulb yield than that of 100% RDF applied treatment [23].

CONCLUSION

This experiment demonstrated the effects of leaf litter compost and inorganic fertilizers in the yield of Black gram. Overall this work indicates that leaf litter compost with different levels of recommended fertilizer resulted in good

plant growth and yield of Black gram. From this result, it was found that 75% of recommended inorganic fertilizers and compost at the rate of 2.5 t ha^{-1} could give profitable yield and this combination could possibly reduce the cost of production in the Black gram cultivation.

REFERENCES

1. Badarinath KVS, Kiran Chand TR, Krishna Prasad V. Agriculture Crop Residue Burning In The Indo- Gangetic Plains: A Study Using IRS-P6 Awifs Satellite Data. *Current Science*, **2006**. 91: 1085–1089.
2. Maki ML, Idrees A, Leung KT, Qin W. Newly Isolated and Characterized Bacteria with Great Application Potential for Decomposition of Lignocellulosic Biomass. *J Mol Microbiol Biotechnol*, **2012**. 22: 156–166.
3. Kumar A, Sunitagain M, Latanain R. Evaluation of thermophilic fungal consortium for paddy straw composting. *Biodegradation*, **2008**. 19: 395–402.
4. Jadhav AR, Girde AV, More SM, More SB, Khan S. Cellulase Production by Utilizing Agricultural Wastes. **Res** *J Agri Fores Sci*, **2013**. 1: 6–9.
5. Ariffin H, Abdullah N, UmiKalsom MS, Shirai Y, Hassan MA. Production and characterization of cellulase by Bacillus pumilis EB3. *Int J Eng Technol*, **2006**. 3: 47–53.
6. Anandham R, Choi KH, Indira Gandhi P, Yim WJ, Park SJ, et al. Evaluation of shelf life and rock phosphate solubilization of Burkholderia sp. in nutrient-amended clay, rice bran and rock phosphate-based granular formulation. *World J Microbiol Biotechnol*, **2007**. 23: 1121–1129.
7. Bailey MJ, Biely P, Poutanen K. Interlaboratory testing of methods for assay of xylanase activity. *J Biotechnol*, **1992**. 23: 257–270.
8. Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, et al. Introducing Ez Taxon-e: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol*, **2012**. 62: 716–721.
9. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol*, **1987**. 4: 406-425.
10. Kumar S, Tamura K, Nei M. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform*, **2004**. 5: 150-163.
11. Mirdamadian SH, Khayam SM, Nekoui H, Ghanavathi H. Reduced of fermentation time in composting process by using a special Microbial consortium. *World Acad Sci Eng Technol*, **2011**. 76: 12–19.
12. Olsen S, Cole C, Watanabe F, Dean L. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular Nr 939, US Gov. Print. Office, Washington, D.C **1954**.
13. Lossin RD. Compost studies Part I., Compost Sci, **1970**. 11: 16-21.
14. Subramniyan S, Prema P. Cellulase-free xylanases from Bacillus and other microorganisms. *FEMS Microbiol Lett*, **2000**. 183: 1–7.
15. Premalatha N, Gopal NO, Arul Jose P, Anandham R, Kwon SW. Optimization of cellulase production by Enhydrobacter sp. ACCA2 and its application in biomass saccharification. *Front Microbiol*, **2015**. l6: 1046.
16. Afzal I, Shah AA, Makhdum Z, Hameed A, Hasan F. Isolation and characterization of cellulase producing Bacillus cereus MRLB1 from soil. *Minerva Biotecnol*, **2012**. 24: 101–109.
17. Mukesh Kumar DJ, Poovai CL, Puneeth Kumar M, Sushma Saroja Y, Manimaran A, et al. Optimization of Bacillus cereus MRK1 cellulase production and its Biostoning activity. *Der Pharmacia Lettre*, **2012**. 4: 881–888.
18. Venkata Naga Raju E, Divakar G, Rajesh T, Ghazi A, Pourgharashi A. A Screening and isolation of cellulase producing Bacteria from dump yards of vegetable wastes. *World J Pharm Pharm Sci*, **2013**. 3: 428–435.
19. Battan E, Sharma EJ, Kuhad RC. High-level xylanase production by alkaliphilic Bacillus pumilusASH under solid-state fermentation. *Bioresour Technol*, **2006**. 12: 42–48.
20. Zheng HXU, Ling BY, Song SJ, Wen TAO. Production of alkali-tolerant cellulase-free xylanase by Pseudomonas sp. WLUN024 with wheat bran as the main substrate. *World J Microbiol Biotechnol*, **2005**. 21: 575–581.

21. Savy FA, Banzatto NV. Crop residue of castor as a nutrient source. *J Am Oil Chem Soc*, **2006**. 121: 151–157.

22. Eiland FM, Klamer A, Lind N, Leth M, Baath E. Influence of initial C: N ratio on chemical and microbial composition during long term composting of straw. *Microb Ecol*, **2004**. 41: 272–280.

23. Seran TH, Srikrishnah S, Ahamed MMZ. Effect of different levels of inorganic fertilizers and compost as basal application on the growth and yield of onion (Allium cepa l.). *J Agri Sci*, **2010**. 5: 64–70.

24. Arisha HME, Gad AA, Younes SE. Response of some pepper cultivars to organic and mineral nitrogen fertilizer under sandy soil conditions. *Zagazig J Agri Res*, **2003**. 30: 1875–1899.

25. Riaz A, Younis A, Ghani I, Tariq U, Ahsan M. Agricultural waste as growing media component for the growth and flowering of Gerbera jamesonii cv. hybrid mix. *Int J Recycl Org Wast Agri*, **2015**. 4: 197–204.

26. Kaya Y, Engul M, Hatice OT, Algur OF. The possibility of useful usage of biodegradation products of sunflower plants. *Biores Technol*, **2006**. l97: 599–604.

27. Bi L, Zhang B, Liu G, et al. Long-term effects of organic amendments on the rice yields for double rice cropping systems in subtropical China. *Agri, Eco Environ*, **2009**. 129: 534–541.

28. Diacono M, Montemurro F. Long-term effects of organic amendments on soil fertility. A review. *Agron Sustain Dev*, **2010**. 30: 401–422.