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MICROBIAL ENRICHMENT OF VERMICOMPOST THROUGH EARTHWORM *Eisenia fetida* (SAVIGNY, 1926) FOR AGRICULTURAL WASTE MANAGEMENT AND DEVELOPMENT OF USEFUL ORGANIC FERTILIZER

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Major challenge in agriculture industry is lack of proper management and recycling of waste of major crops like wheat and rice as it contributes a lot in smog and methane production that causing adverse impact on human health as well. Vermicomposting by earthworms (*Eisenia fetida*) is an emerging approach to on-farm management of nutrients such as NPK, beneficial soil microbes, actinomycets, phosphate solubilizing bacteria, nitrogen-fixing, humus, micronutrients, growth hormones like auxins, cytokinins and gibberlins. A year round study was conducted at Student Research Farm, Department of Agronomy, University of Agriculture, Faisalabad, by treating dung and crop residue with earthworms to prepare vermicompost. Nine treatments were used to compare various vermicompost *i.e.*, rice straw, wheat straw and cow manure in different concentrations. Vermicomposting through cow dung + microbial strains and rice straw + microbial strains responded increase the earthworm population, nutrients, and number of cocoons while lowest was recorded in wheat straw vermicompost. Total nitrogen contents in vermicompost ranged 0.90-2.2% that recorded higher than 0.23-1.09 percent in raw material, depicted conversion of harmful waste into valuable fertilizers. It was concluded from the current study that FYM (cow dung) is a best source to increase the availability of both macro and micro nutrients. While rice straw assumed to be a good feed for earthworms for their higher production. However, wheat straw is a poor source for vermicompost and also it is not easily available.

Keywords: Cow dung, *Eisenia fetida*, microbial strains, rice straw, vermicomposting, wheat straw.

INTRODUCTION

Vermicomposting is termed as a procedure which involves action of earthworms tending to change solid agriculture and kitchen waste into stable form which is darkish in colour and provide rich source of macro and micronutrients (Singh and Sharma, 2002; Nurhidayati *et al*., 2020). Humification of raw material is done through a non-thermophilic process, (Elvira *et al.,* 1998) and stable form of organic matter is formed through vermicomposting (Atiyeh *et al.,* 2001; Aslam *et al.,* 2020; Ahmad *et al.,* 2021).

Highest benefit methodology of vermicomposting is that it can be carried outdoors as well as inside room accordingly permitting year corpulent composting. It also offers flat residents with a means of producing high quality compost. Vermicomposting consents gaining foundations of plant growth elements for use in agriculture in comparatively fewer period that are substantially, biochemically and nutritionally developed. The process of vermicomposting is termed as a stumpy charge knowledge scheme for treating or management of agriculture or kitchen waste material (Hand *et al.,* 1988; Bellitürk *et al.,* 2020; Aslam *et al.,* 2020).

An enormous amount of decomposable agriculture waste that produced regularly in urban areas causing difficulties in waste storage and transportation particularly in less settled countries like Pakistan. Those enormous quantities of waste material could be a cradle for numerous agricultural and industrial sectors. In Pakistan, there exist approximately 72 million cows and buffaloes, around 81 million tons yearly crop residues, and around 785 million poultry or birds. They all yield about 158.3 million tons waste in a year mutually (GOP, 2013). It is also predictable that around 18 million ton weight from rice (husk and straw), wheat, maize (autumn and spring), cotton (waste, ginning and sticks), sugarcane (bagasse and tops) is created yearly merely from Faisalabad, Jhang, Hafizabad, Sahiwal, Chiniot, T.T. Singh, Sheikupura and Okara districts (Personal communication). If it is hypothetical that only 10% is accessible, straight then 1.8 million ton/acre can be made accessible for vermicomposting. The waste formed by animal excreta and dung is significant replacement to use as an organic fertilizer for crop fields. Though, if not used appropriately, it becomes lethal to environment and human. Eutrophication overflow is also a key source of noxious microorganisms like *P fiesteria piscicida* (Lynn *et al.,* 2001). If we do not use the waste of animal properly, we are misplacing the impending cause of nutrients. Odour problem is another distress from waste of animal (Reinecke *et al.*, 1992).

This waste could be transformed into the cherished vermi-compost by utilizing technology of vermicomposting. This method decreases contamination and delivers an appreciated ancillary for synthetic fertilizers. This procedure is gainful at every point of procedure, deliveres suitable development constraints are sustained.

Vermicomposting technology can support nonstop in resolving the issues of agriculture, food safety by contribution of bio-fertilizers which can be used for fulfilling the nutritional requirements of plants. Agricultural wastes like medicinal plants and vegetables processed by earthworms during vermicomposting causes significant increase in crop productivity. This experiment is probably to have substantial influences on the climate, individual income and also security of food.The vermicompost application has controlled the agriculture wastes in urban areas and increased the agriculture productivity on all regions in the past decade.

Various wastes of agriculture could be utilized for vermicomposting by diverse earthworm's species including wheat straw (Shilpa *et al.*, 2016); rice straw (Yvonne *et al.* 2018; Aslam *et al.,* 2020); cow dung (Bansal and Kapoor. 2000); urban waste material (Gajalakshmi*et al.*, 2002) poultry droppings (Garg and Kaushik, 2005); water jacinth (Gupta and Garg, 2008); waste of sheep (Edwards *et al.,* 1985); horse waste (Garg *et al.*, 2005); leaves of apple (Talashilkar*et al.,* 1999); dog waste (Edwards *et al.,* 1998); Buffalo slurry (Hand *et al.,* 1988); city leaf litter and food wastes (Singh and Sharma, 2002), swine manure (Atiyeh *et al.,* 1999), paper waste (Alves and Passoni, 1997); residues of crops decomposition (Kamergam*et al.,* 1999; Rehman *et al.,* 2020), waste of turkey (Edwards *et al.,* 1998); sugarcane pulp and sewage sludge (da Silva *et al.,* 2002). The cattle manure also used for the production of vermicompost (Benitez *et al.,* 2004; Aslam *et al.,* 2020). Manures animals comprise important amounts of plant accessible nutrients which could enhance yields of crop (Banerje, 1997; Aslam *et al.,* 2020). However, there is less research work done on the industrial sludge for vermicomposting. Kaushik and Garge (2003) has found that using *Eisenia fetida*vermicompost of slurry of textile mill. Orozco *et al.* (1996) have found that using of earthworm *E. fetida*production of vermicomposting by pulp of coffee. Suthar (2007) by using of *Perionyx sansibaricus* has described the guar gum and industrial waste can also be undergo through vermicomposting. Butt (1993) presented that under laboratory conditions *Lumbricusterrestis* feed solid paper mill sludge and excreta is used as vermi-fertilizer for crops. The earthworms have no harmful effects through sludge, while progression percentage was reduced. The intensity of nitrogen (0.5%) was low reflected as restraining influence. It was shown that mill paper and sludge vermicomposting utilizing *Eisenia fetida* under field and laboratory condition Elvira *et al.* (1998). Gupta and Garg (2008) under laboratory conditions found that vermicomposting of vineyard waste using *E. fetida.* The species of earthworms differ in procedure how they acquire food, and live diverse portion of the soil, and has slightly diverse impacts on the soil media. They drop into the 3 different biological sets founded on nourishing and burrowing routines.

1- Litter dwelling (Epigeic) earthworms alive and eat in litter present on surface. Their movement is horizontally over litter along-with slight absorption of or digging in the soil environment. These type of worms are typically small in size and are not available in low amounts of organic matter in the soil. *Eisenia fetida* is a sample specimen of litter dwelling species. Tripathi and Bhardwaj (2004) found that these type of earthworms can rush the process of compost production to an important degree along construction of better excellence of composts, as compared to those which are produced by outdated methods (Garg *et al.*, 2006).

2- Shallow dwelling (Endogeic) earthworms are vigorous in inorganic topsoil deposits. They generate a 3-D network of holes while ingesting large amounts of soil. The genus *Aporrectodea* and *Diplocardia* have endogenic life modes.

3- Deep burrowing (Anecic) earthworms living in everlasting, approximately perpendicular holes that may spread numerous feet in the soil environment. They eat on surface deposits and pull out them into their holes. *Eisenia fetida* and *Lumbricusterrestris* is examples of a deep burrowing species (Dominguez and Edwards, 2011).

The most communal category of earthworms utilized for process of vermicompost production is (*Eisenia foetida*) and red wigglers *(Lumbricusrubellus*) or red worms. Frequently discovered in ancient compost heaps, they commonly have flashing and healthy red stripes. The field earthworm *Allolobophoracaliginosa* are not to be disorganized with red wigglers or red worms.

The basic to somewhat neutral pH of feeding material is favorable for earthworm's health. Liming in vermicompost box usually improve bacterial populace along earthworm actions. Hence, it could be an exciting studies that variations in enzymatic activity and micro-nutrient contents of vermicompost due to lime adding to biological wastes (Reinecke *et al.,* 1992).

Organic agriculture necessities continued maintenance of bio fertilizers and decent ways of organic wastes. Vermifertilizer gets approachable environment reprocessing of organic wastes and produces the basis for involvement elevated nutrients assessment compost for organic agriculture.

Keeping in view above facts, the trial was performed with the following objectives:

1. To develop Vermi fertilizer by using earthworm species *Eisenia fetida*
2. To study the bio waste *i.e.*, wheat straw, rice straw and cow dung for suitability as raw material for vermicomposting and
3. To check the role of certain ecological factors in the production of vermicompost.

MATERIALS AND METHODS

The trial was performed at the Plant and Microbial Ecology Laboratory, and Student Research Farm, Department of Agronomy, Faculty of Agriculture, University of Agriculture, Faisalabad, during 2018-19.

***The vermicomposting system study*:**

Collection of material: Wheat straw, rice straw and cow dung were gathered from Student Research Farm and Dairy Farm, UAF.

Crushing of material: After collection, materials were dried. Wheat straw and rice straw were dried in sunlight for 10 days. All the samples were crushed in a big grinder one by one. Small quantities of materials were carefully added into crushing machine for better quality (0.1-1.0 cm). The crushed material was collected in polythene bags carefully. Separate polythene bags were used for each sample.

Earthworm types: The most commonly used earthworm for vermicomposting, *Eisenia fetida* (an epigeic species), were used as the test species (Edwards *et al.*, 1998). These earthworms were collected from a farmer, Malik Ejaz Billu from Kohat. Pre-mature non-clitellated specimen of *E. fetida*, weighing 200-250 g were selected randomly from several stock cultures, maintained in laboratory with different test materials as culturing material (wheat straw, rice straw and cow dung).

***Culturing conditions*:** Earthworms were given certain attention for their survival on the daily basis. The environmental factors that affect earthworm growth, fitness and replication were sustained as follows: the earthworms were kept at 25°C for breeding and joyful life. The temperature for vermicompost was maintained at 25 to 35°C (using Air Conditioner) for growth and action. The 60 to 85% moisture was maintained by using humidifier. The oxygen was sustained by decreasing the percentage of wetness, retrenchment of feed, and rotating the pile through altered hand tools. By turning the materials in wooden boxes after every 5-10 days may comfort to keep the boxes airy. The pH was maintained in 6.0-7.5. The pH readings were taken at dissimilar levels in the boxes, *i.e.*, the top, 3 and 8 inches depth by pH meter. To remodify the conditions of acidic, $CaCO_3$ was mixed in the boxes and then sprinkled water. To remodify alkalinity, dry peat moss was spread and mixed into the boxes until pH readings optimize up to range of 6.8 to 7.2. The pH of heap was determined through continuous sampling and analysing using pH meter.

Feeding of earthworm: Wheat straw, rice straw, and farm yard manure (cow dung), were used in feeding the earthworms. The epigiec earthworm consumes half of their body weight per day.

Pre-composting the raw materials: The fresh organic material was pre-composted in wooden boxes for 20 days to remove all the toxic gases. It is proved that pre-composting helps to increase the survival rate of worms (Garg *et al.*, 2012). During the pre-composting period, the material was turned over on days 4, 8, 12, 16 and 20.

Vermicompost system watering and aeration: Jute bags were spread on wooden boxes to possess the material wet during summer weather. 60-70 percent moisture contents by irrigation every two to three days across warm days and every seven days during winter conditions. During vermicomposting, turning the wooden boxes was done every one to three days to prevent it from anaerobic conditions depend on the season to offer the aeration.

Enrichment of VF resources with functional bacteria: The most of the agricultural biomass waste is mainly lignocellulose in origin. The cellulosic material has obtained an important position in the world due to its availability and potential for transforming it into sugars, chemical feed stock and fuels. To select the best VF having good characteristics of more nutrient availability and functional microbiota, there were enrichment of cellulose degrading, Zn and phosphorus solubilising bacteria to different VF resources (compared with control) to enhance the VF process and to increase the Zn availability to the crops. The bacteria were acted as inoculants in this process. These bacteria were isolated from the gut of different earthworm species (Shankar *et al*., 2011; Goteti*et al*., 2013;Gupta *et al*., 2012). For screening of active bacteria, they were isolated in half- nutrient agar media by using serial dilution method at 28□C. Out of 200 strains; only 10 strains were active for cellulose degradation, Zn solubilization and P-solubilization. Out of them only three most active strains, C-03, Z-05 and P-01 were cultured in large quantity in broth media and were inoculated the pre-composting material.

***How to make growth medium for functional bacterial growth*:** 4.5g nutrient broth and 11-15g agar were added in beaker container and added 700 ml distilled water. The nutrients were weighed in aluminum foil. Distilled water was added in beaker, mixed nutrients in water and stirred it with magnet on stirrer. Placed it in autoclave for 15 min at 121°C and took outside. Sterilized the laminar air flow with ethanol mixture (70% ethanol: 30% water). After cooling at around 60°C, then pour the medium into Petri dishes.

Dilution method: The excreta of one earthworm was diluted in 1ml/900 microlitre double distilled water in test tube. Stirred it at vortex. Took 10 microliter from the first tube and further allowed to dilute it in 1ml/900 microliter double distilled water in other test tube (named as 2nd test tube). 10µl from second test tube was taken and diluted it in 3rd test tube with 1ml/900microliter. Then, streaking was done from 2nd and 3rd test tubes by taking 3 plates from each.

Isolation of bacterial strains from gut of earthworm: The central gut of earthworm was opened with dissecting box. Then, 0.5g earth excreta was taken out and added to 10ml double distilled water. This solution was vortexed (10^{-1}).1 ml solution from 1st tube was taken and added it in 2nd tube containing 9ml double distilled water that was also vortexed (10^{-2}).The same procedure was repeated to make $10^{-3}, 10^{-4}, 10^{-5}$ of *E. fetida* species. Total tubes were 10. 100 µl solutions were taken from each 10^{-3} and 10^{-5} of both tubes and put on agar medium plates. Two plates were made from each one and streaking was done afterwards. Zinc solubilizing bacteria were isolated by using the media given in table 1.

Table 1. Nutrient agar for Zn solubilizing bacteria Composition.

Chemicals	Quantity (g/l)
• $ZnO/ZnCO_3$	12.0
• Glucose	10.0
• Ammonium sulphate	0.5
• Potassium Chloride	0.2
• Megnisium Sulphate	0.1
• Manganese sulphate	0.5
• Ferris sulphate	0.5
• Yeast extract	0.5
• Pure agar	0.5
• Distilled water	15.0
	1.0 liter

(Saravanan *et al.*, 2007)

Cellulose degrading bacteria:

Requirements: Sterilized CMC/Carboxy Methyl cellulose (Table. 2) agar plates. 1% Congo red (aq). 1M NaCl.

Table 2. Composition of CMC Agar.

Components	Amount (g/l)
❖ NaCl	4.0
❖ Ammonium sulphate	1.0
❖ KH_2PO_4	0.5
❖ K_2HPO_4	0.5
❖ $MgSO_4$	0.1
❖ $CaCl_2$	0.1
❖ CMC	0.1%

Stewart *et al*. (1982)

Procedure: Took the material in petri dishes. Streaking/spreading of bacteria. Incubated for 72 hours. After incubation, flood the plates with 1% cognate/ Congo red (Table. 3) at least 10 minutes. Removed the excess Congo red and washed the plates with 1M NaCl. After washing observed zone of phydrolysis around the colony which indicates cellulose degradation by bacteria.

Table 3. Preparation of Congo red solution composition (1%)

Composition	Amount (g/l)
✓ Congo red	1.0 gm
✓ Distilled water	100 ml

Teather and Wood, (1982)

Procedure

- Mixed it completely.
- Prepared

Isolation of phosphate solubilizing bacteria: Weight the contents of pihovaskya's agar (Table 4.)

Table 4. Composition of Pihovaskya's agar.

Components	Amount (g/l)
▪ Glucose	10.0
▪ Calcium phosphate	5.0
▪ Ammonium sulphate	0.5
▪ NaCl	0.2
▪ $MgSO_4.7H_2O$	0.1

Subba (1977)

Procedure: Added the contents of Pihovaskya's to distilled water. Kept it in autoclave. Poured media aseptically. Took bacteria from medium and spread on Pihovaskya's. Kept the plates for incubation in incubator.

Number of earthworm in different feeding material: The number of earth worms were counted from different feeding sources which were replicated and then average was taken.

Figure 1. *Eisenia fetida* earthworm's counting

***Weight of earthworms (g)*: ** After counting the earth worms from different feeding sources the weight of earthworms was calculated in average.

Figure 2. Average weight of earthworms, Number of cocoons.

The number of cocoons were calculated when fully matured vermicompost was prepared from different feeding sources (wheat straw, rice straw and cow dung) which was in replication and it's average taken.

Harvesting and sieving of vermicompost: The watering process was stopped one week before start of harvest of vermicompost. The worms were spread around the trench come in close and penetrated each one other in the type of ball in 3 or 4 places. The composting material was heaped by eradicating the ball and placed them in a container. After compilation of compost from the top layers, the food material was once again replaced and process of compost formation was rearranged. The compost material was filtered in 2mm sieve, the raw material passed through the sieve is termed as vermicompost which was stored in bags.

Figure 3. Calculating number of cocoons

ANALYTICAL METHODS

The pH of earthworm media: For pH determination of vermicompost and raw material, solution was made using a vermicompost and material to water ratio of 1:2.5 for 30 minutes, the ingredients were permitted to balance and the pH was determined using a standardized pH metre (Arthur, 1982).

The EC of earthworm media: EC of the vermicompost and material was concluded by formulation of suspension with 1:2 compost to water ratio. Equilibrize of contents was done for 40 minutes and the electrical conductivity was documented (Richards, 1954).

Raw material and vermicompost analysis: Digestion of vermicompost and raw material for assessment of total P, K, Mg and Ca. Placed 0.6 g vermicompost and 0.5 g raw material in container and moistened with some droplets of H_2SO_4 and then putted 1 ml $HClO_4$ and 3 ml HNO_3. Vermicompost, raw material and acid mixture were heated on hot plate until smokes of $HClO_4$ looked and then it was chilled and put 6 ml of HF. Put the container in bath of sand and enclosed about 9/10 of pot top with a Pt lid until desiccation. Lasting dyes burnt with Meker burner and oxidized. 5 ml, 6 N HCl and 5 ml of water were added after cooling. Boiled the solution slightly through heating the container. After fully deposits mixed in HCl poured this in 100 ml volumetric flask, after washing the filter paper dilute to volume. Total P, K, Ca and Mg was determined by using this solution (Jackson, 1958).

Total N: Took 2.0 g vermicompost and raw material into digestion tube and weighed. In every digestion tube 4.4 mL mixture of digestion ($H_2O_2$40%, Lithium sulfate and concentrated H_2SO_4) was added. Seven spaces were also made for amendment of the interpreting. The tubes of digestion were placed on digestion block for 2 h at 360°C. The digested material after cooling was added in a volumetric flask and 50 mL distilled H_2O was also added and permitted to again cool. Solution was prepared up to the volume, assorted well and then permitted to settle until a perfect solution was made. 50 mL of this was shifted to the distillation flask and 25 mL of the mixture of alkali was added into it. 5 mL used as pointer in an appropriate flask progressed at 30 mL to gather the distillate. Distillation started immediately and sustained till 25 mL distillate composed. The distillate was titrated with 0.01 MHCl till the color was changed from green to pink. (Bremner and Mulvaney, 1982).

Total P: Aliquot was taken from the fully digested solution in volumetric flask (50 ml) through the pipette as stated in digestion method. The sample was diluted to 40 ml with distilled water, then 8 ml of newly made diluted the ascorbic acid was added up to the mark and shaked well. After 10 min, the absorbance was noted at 880 wavelength using 1 cm cuvette. The known concentrations of KH_2PO_4 solution was used to prepared the standard curve (Watanabe and Olsen, 1965).

Total K: Aliquot was taken from the fully digested solution in the bottle through the pipette as mentioned in the method of digestion. Flame photometer model JENWAY PFP 7 was used to determine the K contents. The solution of identified concentration of K_2SO_4 was prepared and for creation of standard curve, solution was run on spectrophotometer and reading was noted. (Bansal and Kapoor, 2000)

Total Ca: As indicated in the digestion method, an aliquot of completely digested solution was placed in a 250 ml volumetric flask and distilled water was added to the 150 ml quantity. Then 10 drops of HCl, KCN, NH_4OH, TEA (Triethanolamine) $K_4(Fe)CN_6$ were added and 1 ml of 10% NaOH was applied to boost the pH to 12. The mixture was turned from red to blue when uniform EDTA disodium was applied and titrated by 10 drops of Colcon indicator (Shapiro and Brannock, 1955)

Total Cu, Fe, Zn: Atomic absorption spectrophotometer was used for determination of minerals *i.e.* Copper (Cu), Iron (Fe) and Zinc (Zn) in the prepared sample (Hitachi Polarized Zeeman AAS, Z-8200, Japan) following the conditions described by AOAC (1990). The instrumental operating conditions for the above said elements are summarized in Table 5:

Table 5. Operational conditions employed in the determination of Fe and Zn by atomic absorption spectrophotometer.

Parameters	Set Value	
	Zn	**Fe**
Lamp Current (mA)	10.0	10.0
Flame	Air-C_2H_2	Air-C_2H_2
Wavelength (nm)	213.9	248.3
Slit Width (nm)	1.3	0.2
Burner Head	Standard type	Standard type
Oxidant gas pressure (Flow rate) [kpa]	160	160
Fuel gas pressure (Flow rate) [kpa]	6	6
Burner Height (mm)	7.5	7.5

Standard Preparation: Standardized specifications, in the form of an aqueous solution (1000 ppm), were set from a readily available stock solution (Applichem ®). All the glass equipment used in the experimental work process were immersed in 8N HNO_3 overnight and cleaned prior to use with de-ionized water many times.

Statistical Analysis: All the experiments were repeated thrice and data was summarized. The recorded data was statistically analyzed via Fisher's analysis of variance (ANOVA) technique (Steel *et al*., 1997). LSD test was used ($p \leq 0.05$) to compare the treatments means using Statistic version 8.1 (Analytical Software ©, 1985-2005).

RESULTS AND DISCUSSION

Change in vermicompost pH is substrate dependent and dynamic process. So, the pH shift during the vermicomposting depends upon chemical characteristics of feedstock. Decrease or increase in pH is result of production of ammonia and organic acids during vermicomposting. In current study, overall minor increase in pH (near to neutral) of materials were observed in almost all treatments at the end of vermicomposting. The pH ranged between the 6.84 and 7.95 in mature vermicompost (Table 6). These results are in range of best quality vermicompost as stated by Pandit *et al.* (2012). Whereas, further studies quantified that pH values of final vermicompost ranged from alkaline to acidic (Garg *et al.,* 2006). The precipitation of calcium carbonates and degradation of fatty acids (short chains) uplifts the pH (Lim *et al.* 2011). More shift of pH was observed in those treatments in which cow dung was used as feeding stock while less increase was perceived in wheat straw feeding material. This pH variation among different treatments was due to the

Table 6. Characteristics of raw material, mature vermicompost prepared from wheat straw, rice straw, FYM and vermicompost produced by the addition of microbes using *Eisenia fetida*.

Treatments	EC (dS/m)	pH	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (ppm)	Iron (ppm)	Copper (ppm)	Zinc (ppm)
Wheat straw	3.26e	6.24f	0.23g	0.29g	0.47g	1917.3g	1353.3b	5.27g	20.20h
Rice straw	4.32d	6.76e	0.34g	0.26g	0.79f	1267.3h	1158.0d	5.57g	18.97i
FYM	9.63b	8.00b	1.09e	0.78f	1.03e	777.3i	1491.7a	14.00e	37.10g
Wheat straw vermicompost	2.56e	7.53c	0.90f	1.05e	1.04e	1987.7f	1063.1e	17.27c	63.57f
Rice straw vermicompost	6.61c	7.06d	1.25d	2.02c	1.23d	2222.7e	1462.0a	11.77f	105.60a
FYM vermicompost	17.91a	8.26a	1.51c	2.05c	1.33c	5087.3b	1304.1c	19.47b	80.43e
Wheat straw + microbial strains vermicompost	3.40e	7.43c	1.48c	1.84d	1.35c	3927.3c	769.6f	16.70d	84.17c
Rice straw + microbial strains vermicompost	5.74c	7.35c	1.89b	2.33b	1.58b	3022.3d	1351.6b	11.60f	111.17b
FYM + microbial strains vermicompost	9.52b	7.84b	2.20a	2.52a	2.02a	6532.7a	1355.1b	96.53a	82.90d
LSD value at p <0.05	0.905	0.247	0.123	0.043	0.041	3.53	41.66	0.338	0.779

Table 7. Earthworm population, cocoons production and average weight of *Eisenia fetida* in different feeding mixtures during vermicomposting.

Treatments	No. of cocoons	No. of earthworms	Avg. weight of earthworm (g)
Wheat straw VC♦	5.00 f	124.00 f	0.13 c
Wheat straw + microbial strains VC	8.00 e	186.00 b	0.16 b
Rice straw VC	45.00 b	175.00 c	0.20 a
Rice straw + microbial strains VC	128.00 a	436.00 a	0.12 c
FYM VC	15.00 d	156.00 d	0.20 a
FYM + microbial strains VC	19.00 c	149.00 e	0.16 b
LSD value at p <0.05	1.779	1.779	0.018

♦ Vermicompost

use of different types of materials which exaggerated the mineralization. All the treatments indicated adequate pH for earthworm growth (Ndegwa and Thompson, 2000).

Electrical conductivity reveals the salinity of organic material, in which more salt contents cause the phyto-toxicity. So, the EC is best indicator of safety and suitability of vermicompost for the agriculture purposes. Generally, EC of mature vermicompost presented slight increase, however, slight decrease in EC of wheat straw vermicompost was observed as compared to initial values. This decrease in EC values was due to production of metabolites *e.g.*, ammonium (NH^{4+}) and salt precipitation (Lim *et al.*, 2012). In FYM, the EC was significantly higher in vermicompost as compared to original material and it might be due to mineralization. When microbial strains were used in excess, there was reduction in EC and it might be due to usage of soluble salts by microbes. This reduction in EC showed the activeness of microbes involved in vermicomposting.

Data related to changes in total Kjeldahl Nitrogen (TKN) of final vermicompost are summarized in Table 6. During vermicomposting process, nitrogen content in wheat straw, rice straw and FYM increased significantly but to different amount. Whereas, vermicompost of these substrates (rice straw, wheat straw and FYM) plus application of phosphate and Zinc solubilizing bacteria enhanced the nitrogen contents manifold than without microbes application. The nitrogen contents in vermicompost ranged from 0.90-2.2% which was higher than initial nitrogen contents that ranged between 0.23-1.09 percent. The total nitrogen concentrations in all examined treatments were in suggested range of good teas as described by Siddiqui *et al.* (2009). Higher the nitrogen contents in vermicompost than feedstock was probably due to decrease in organic carbon and accumulation of nitrogen by the earthworms in the form of excretory nitrogenous substances, mucus, growth encouraging hormones, rhizobium and enzymes (Tripathi and Bhardwaj, 2004; Malik *et al.*, 2014).

The improved concentration of nutrients that are essential for growth of plants, in vermicompost made them appropriate growth promoter for the plants (Singh and Kalamdhad, 2016). Total potassium and phosphorus content were higher in vermicomposts than the feeding materials after vermicomposting. Phosphorus contents were significantly higher in vermicompost as paralleled to the initial feeding substrates. Initial phosphorus contents was 0.26% in rice straw which increased to 2.02% in rice straw vermicompost and 2.33% in vermicompost prepared from rice straw with microbial application showing overall significant increase in matured vermicompost. Higher phosphorus concentration in rice straw vermicompost due to phosphorus mineralization by microorganism. The best results were in case of FYM where P and K increased exponentially higher. The aging of casts increase mineralization process (Suthar, 2008). Phosphorus contents in wheat straw and FYM vermicompost increased also. This increment in phosphorus contents probably due to phosphatases enzymes present in earthworm cast (Suthar, 2008).

The contents of iron, zinc, copper and calcium were found higher in vermicompost as compared to initial material. The more iron contents were found in rice straw vermicompost from 1155 ppm (initial material) to 1462 ppm (vermicompost). However, there is slight decrease in Fe contents in FYM vermicompost. This may be attributed to higher pH as higher the pH value, lower will be Fe availability. Zinc contents in feeding material ranged from 20 ppm to 37 ppm while in vermicompost, it ranged between 63.56 ppm and 111.17 ppm. However, higher Zn concentration was observed in those treatments which were supplied with phosphorus and zinc solubilizing bacteria (Shahzad *et al*., 2020). Increase in Zinc contents of FYM from 37.10 ppm (initial contents) to 82.90 ppm (final vermicompost) which was an excellent result and it can be used in Zn-bio fortification process.

Cu contents were higher in final vermicompost after 100 days of worm's action. The Cu content of original wheat straw was 5.26 ppm which was increased to 17.26 ppm in wheat straw vermicompost. Whereas, higher concentration of Cu (96.53 ppm) was observed in the treatments which were supplied with phosphorus and zinc solubilizing bacteria. Whereas, smaller increase of Cu contents in rice straw vermicompost. Initial rice feedstock contains 5.56 ppm of Cu which was boosted during the vermicomposting process to 11.76 ppm in rice straw vermicompost which is almost double the original amount. This increase in metal contents maybe due to organic matter degradation and mineralization that helped in reduction of feedstock volume resulted in concentrate the composting material consequently boosted the metal contents (Sharma and Garg, 2017).

Table 6 shows that maximum increase in calcium contents as compared to initial values (1917.3 ppm) ranged from 1987.7 ppm to 3927.3 ppm in wheat straw vermicompost. Higher calcium contents were observed in farm yard manure (FYM). The initial contents of calcium in original FYM was 777.33 ppm whereas, this boosted up to 5087.3 ppm in FYM vermicompost and treatment with FYM + zinc and phosphorus solubilizing bacteria showed higher calcium contents (6532.5 ppm) after vermicomposting. This rise in calcium content was attributed to the calcium metabolism in the gut of earthworm, which increased the Ca contents in vermicasts. When Ca^{2+} is released and absorbed in calciferous area of earthworm gut by using energy from calcium oxalate, then calcium carbonate is formed (Spiers *et al.*, 1986). The additional bicarbonates in vermicasts improved the Ca content of final vermicompost. Furthermore, during the mineralization, organic carbon is converted to CO_2. The fixation of this carbon dioxide produced calcium carbonate due to carbonic anhydrase catalytic activity and led to increase in Ca contents in vermicompost (Das *et al.*, 2012).

Vermicomposting has many benefits and it can increase the availability of macro and micronutrients. At the same time, there is another byproduct; mass production of earthworms which can be used in different industries including poultry and fish farming and fishing. During the first year experiments, the data about earthworm population, average body weight and number of cocoons expressed very interesting information (Table 7). It showed that highest earthworm population was in treatment where rice straw was inoculated with bacterial strains at the time of pre-composting while minimum was in treatment of wheat straw vermicompost but they increased significantly in treatment when the wheat straw was inoculated with microbial strains. Khucharoenphaisan and Sinma (2018) obtained the similar results while using soybean meal as vermicompost source. The maximum average weight was obtained in rice straw vermicompost treatment while lowest was in wheat straw VC which was at par with treatment where rice straw was inoculated with bacterial strains. This might be due to high population rate and less nutrients or food competition. The number of cocoons were higher in treatment where rice straw was inoculated with bacterial strains whiles lowest were in wheat straw VC. It is clear from this Table 8 that earthworm population increased exponentially higher when they were fed with the rice straw which were pre-inoculated with active bacterial strains (consortia of cellulose degrading, Zn-solubilizing and phosphorus solubilizing bacterial strains) at the time of pre-composting. This all data was established in 75 days. Microbial inoculation concept was introduced by other researchers and they also presented similar results (Pérez-Godínez *et al*., 2017). There are two main points that first you have to select your objective of vermicompost and secondly you have to select biomass type according to your objective. In this research, it is very clear that if you want good high quality verimicompost, then use FYM (cow-dung) as VC source and if you want to do vermiculturing (growing earthworm population) then select rice straw and add selective microbial strains.

Conclusion: It can be concluded from this study that *Eisenia fetida* is the most appropriate decomposer for agricultural wastes. Vermicompost was prepared through organic material like FYM and other farm waste like rice and wheat straw and evaluated for constancy, early maturity, and acceptable palatability. Vermicompost egested from earthworms containing cellulose degrading, phosphate and zinc solubilizing bacteria in its gut supplies enormous amount of nutrients compared to VC produced only from earthworms. This kind of research also established a view that which organic waste source is the best for vermicomposting and its utilization for different crop deficiencies. Among many agricultural waste materials, the FYM (cow dung) is a best source to increase the availability of both macro and micro nutrients. While rice straw assumed to be a good feed for earthworms for their higher production. However, wheat straw is a poor source for VC and also it is not easily available.

Recommendations: Following are the suggestions and recommendations in the light of present study;

- Pakistan is served with different climatic and soil resources, there is need of study for identifications of epigeic local earthworm species like *Eisenia fetida,* better research and efficient vermicomposting.
- Recorded data must be confirmed by the projects. Agronomic crops and high value vegetables should be evaluated through the use of vermicompost and hydroponics vermicompost tea. Various aspects of vermicompost tea must also be studied.
- Various other wastes should be assessed for maturity, quality and stability of vermicompost and their deliciousness for the earthworms.

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