

Vermicomposting of De-Oiled Cake of *Brassica juncea* by *Eisenia foetida*

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De-oiled cake of *Brassica juncea* is produced in large quantities in India and Worldwide. It has no economic importance. Present experiment is an attempt to convert it in nutrients rich vermicompost through the activities of *Eisenia foetida*. The experiment was laid down with four treatments viz. Control: Buffalo dung + *Eisenia foetida*, T₁: Buffalo dung + *Brassica juncea* cake, T₂: Buffalo dung + *Brassica juncea* cake + *Eisenia foetida* and T₃: *Brassica juncea* cake + *Eisenia foetida*. The experiment was performed in above ground pits made from bricks of size (45×45×21 cm) (l×b×h). Treatments were replicated thrice. Vermicomposting material was analyzed at 0, 30, 60 and 90 days period for its various chemical properties like pH, electrical conductivity, total organic carbon, available phosphorus, potassium, sodium, lignin, cellulose, and hemicellulose and degradation of substrate was confirmed by FTIR spectroscopy. Yield of vermicompost was also recorded at maturity. Results reveal that in 90 days produced vermicompost show slightly alkaline pH, increased values of electrical conductivity, available phosphorus, potassium, sodium; and decreased values for total organic carbon, cellulose and hemicellulose compared to their initial values. This effect of treatments was found significant at 5% level for some parameters.

Keywords: Chemical changes, earthworms, microbial decomposition, vermicompost.

Rapeseed-mustard is an important oil seed crop and grown on 34.19 million hectares area with a production of 63.09 million tons in the world during 2012-13. In India it covered 19.29% acreage area and 11.127% of production in this period (http://www.drmr.res.in/about_rm.html, retrieved on 12/1/2015). The oil seeds contain Glucosinolates. When oil seeds are processed to remove oil these glucosinolates converted into thiocyanates, nitriles, isothiocyanates, epithionitriles etc. by the action of myrosinases. These products are toxic to mammalian species because they interfere with uptake of iodine causing goiter. Its de-oiled cake is commonly used as cattle feed but it is not safe due to above facts. Harmful effects of glucosinolates

are reported by many workers (Duncan, 1991; Barrett *et al.*, 1997; Papas *et al.*, 1979; Fenwick and Cruits, 1980). Therefore we tried a different approach for management of this waste through vermicomposting. Earthworm's gizzard has numerous of enzymes having capacity to rupture a number of complex molecules.

Vermicomposting is a proven technology to convert a variety of plant wastes into a best fertilizer which supports plant growth (Benitez *et al.*, 1999; Atiyeh *et al.*, 2002). *Eisenia foetida* species of earthworm is widely accepted for vermicomposting of agricultural waste.

MATERIALS AND METHODS

In the present experiment we used de-oiled cake of Mustard (*Brassica juncea*), earthworms species *Eisenia foetida* and buffalo

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dung. Mustard cake was obtained from Kukarvada, Dist: Mehsana and other materials were procured locally from sadra and Randheja village of District Gandhinagar, Gujarat. Before addition to vermicompost pits the cake was chopped into small pieces (about 1 inches long). For vermicomposting pits from bricks were prepared on above ground of our campus. These pits were 45cm long, 45cm wide and 21cm high.

Treatments

Above materials were mixed in different combinations to obtain four treatments. Control comprises Buffalo dung and *Eisenia foetida* only; Treatment T1 comprises Buffalo dung and *Brassica juncea* cake without earthworms whereas treatment T2 contains earthworms alongwith Buffalo dung and *Brassica juncea* cake. Last treatment T3 contains *Brassica juncea* cake and *Eisenia foetida* alone without dung. To minimize the experimental error all the above treatments were replicated thrice.

Filling of pits

A total of six kilogram of material was filled in each pit. In pits of control and T3 treatments 6kg buffalo dung and *Brassica juncea* cake were filled alone whereas in T1 and T2 the material was filled in layers. In layered treatments a layer of 3Kg Buffalo dung was kept at bottom of pit followed by one layer of 1Kg mustard cake which was covered by 2Kg of buffalo dung. Fermenting material was kept moist and turned after every seven days. Earthworms in respective pits were added after second turning in the amount of 18g except T1. To prevent moisture loss all pits were covered with gunny bags. The material was analysed at 30, 60 and 90 days of filling to find out

the changes in various chemical properties. At maturity (90 days) the Earthworms were removed from pits and samples were analyzed for various parameters along with yield.

Analysis of vermicomposting material

The samples at different time intervals were analysed for physico-chemical parameters including pH and electrical conductivity (Richard's, 1945), total organic carbon (Walkely and Black, 1965), nutrients like phosphorus, potassium and sodium (Jackson, 1967), and for cellulose, hemicellulose and lignin content (Thimmaiah, 1999). FTIR (Fourier-Transform Infrared Spectroscopy) analysis was also performed at Center of Excellence, Vapi, Gujarat.

Table 1. Effect of treatments on yield of vermicompost (kg)

Treatments	Yield (kg)
Control	1.60 ^a
T ₁	2.60 ^b
T ₂	2.25 ^{a,b}
T ₃	3.80 ^c
SEM (±)	0.33
CD 5%	0.75

Control: Buffalo dung + *Eisenia foetida*; T₁: Buffalo dung + *Brassica juncea* cake; T₂: Buffalo dung + *Brassica juncea* cake + *Eisenia foetida*; and T₃: *Brassica juncea* cake + *Eisenia foetida*

Alphabet ^{a,b,c} shows significance and non-significance of treatments at 5% level of significance. Treatments with same alphabet are non-significant and with different alphabet are significant.

Table 2. Effect of treatments on pH, EC, Total organic carbon (TOC) contents at various stages (0, 30, 60 and 90 days) of vermicomposting

Treatments	pH				EC (dSm ⁻¹)				TOC (%)			
	0	30	60	90	0	30	60	90	0	30	60	90
Control	6.25 ^{a, b}	6.64 ^{a,b}	6.90	7.46 ^a	0.21	0.21	0.23 ^a	0.37	14.72	13.99	13.25	13.11
T ₁	6.13 ^a	6.33 ^a	6.76	7.43 ^a	0.27	0.31	0.38 ^b	0.47	15.24	14.36	14.01	13.95
T ₂	6.33 ^{a,b}	6.53 ^{a,b}	6.73	7.83 ^{a,b}	0.26	0.28	0.33 ^b	0.46	15.31	14.99	13.97	12.41
T ₃	6.56 ^b	6.78 ^b	7.10	7.96 ^b	0.21	0.31	0.37 ^b	0.41	14.98	14.44	13.30	12.15
SEM(±)	0.14	0.14	0.16	0.19	0.03	0.05	0.04	0.05	1.82	1.93	2.57	1.69
CD5%	0.31	0.31	NS	0.45	NS	NS	0.09	NS	NS	NS	NS	NS

Control: Buffalo dung + *Eisenia foetida*; T₁: Buffalo dung + *Brassica juncea* cake; T₂: Buffalo dung + *Brassica juncea* cake + *Eisenia foetida*; and T₃: *Brassica juncea* cake + *Eisenia foetida*

Alphabet ^{a,b} shows significance and non-significance of treatments at 5% level of significance. Treatments with same alphabet are non-significant and with different alphabet are significant.

Statistical analysis

Completely Randomized Block Design was followed and significance level was found out to check the significance in obtained data at 5% level following procedure described in Gomez and Gomez, (1984).

RESULTS AND DISCUSSION

Yield of vermicompost

During vermicomposting earthworm ingest the substrate, digest it, absorb nutrients and discard undigested part as castings. If the substrate is easy to digest, contains simple forms, then more nutrients will be absorbed and eventually less part will be discarded with lower yield of product. In present experiment two substrates were used viz. buffalo dung and mustard cake. Compared to mustard cake buffalo dung is easy to degrade

because it is a partially degraded product of buffalo so it contains simple forms of compounds which can be digested easily by earthworm's gut flora. Due to this fact we would obtain lesser amount of product in this case. Our results are in tune with this fact and lowest yield is recorded in control treatment containing buffalo dung and earthworms (Table 1). As a portion of buffalo dung is replaced by mustard cake (T_1 and T_2) yield of vermicompost increased significantly and highest yield was obtained with mustard cake alone (T_3) which differs significantly from rest of the treatments (Table 1). Weight loss of vermicomposting material at final stage was also reported by Twana and Fauziah, 2012; Senthilkumar and Kavimani, 2012.

Chemical properties

Data presented in Table 2 and Table 3 reveals that pH, electrical conductivity, total organic carbon, available P, K and Na contents

Table 3. Changes in available phosphorus, available potassium and available sodium at various stages (0, 30, 60 and 90 days) of vermicomposting

Treatments	Available Phosphorus (%)				Available Potassium (%)				Available Sodium (%)			
	0	30	60	90	0	30	60	90	0	30	60	90
Control	0.023 ^a	0.028 ^a	0.033 ^a	0.041 ^a	0.053	0.075	0.085	0.099	0.024	0.032	0.036 ^{a,b}	0.041 ^a
T_1	0.025 ^a	0.029 ^a	0.034 ^a	0.041 ^a	0.054	0.066	0.079	0.098	0.022	0.027	0.031 ^a	0.039 ^a
T_2	0.033 ^{a,b}	0.038 ^{a,b}	0.048 ^b	0.061 ^b	0.057	0.072	0.086	0.100	0.030	0.035	0.043 ^b	0.059 ^b
T_3	0.044 ^b	0.051 ^b	0.061 ^c	0.074 ^c	0.050	0.074	0.089	0.096	0.021	0.026	0.032 ^{a,b}	0.040 ^a
SEM(±)	0.005	0.006	0.003	0.004	0.007	0.008	0.006	0.007	0.005	0.004	0.005	0.005
CD5%	0.012	0.014	0.007	0.009	NS	NS	NS	NS	NS	NS	0.011	0.011

Control: Buffalo dung + *Eisenia foetida*; T_1 : Buffalo dung + *Brassica juncea* cake; T_2 : Buffalo dung + *Brassica juncea* cake + *Eisenia foetida*; and T_3 : *Brassica juncea* cake + *Eisenia foetida*

Alphabet ^{a,b,c} shows significance and non-significance of treatments at 5% level of significance. Treatments with same alphabet are non-significant and with different alphabet are significant.

Table 4. Changes in lignin, cellulose and hemicellulose at various stages (0, 30, 60 and 90 days) of vermicomposting

Treatments	Lignin (%)				Cellulose (µg)				Hemicellulose (%)			
	0	30	60	90	0	30	60	90	0	30	60	90
Control	2.71 ^a	3.12 ^a	3.56 ^a	4.25 ^a	26.24 ^a	19.71 ^a	16.01 ^a	14.65 ^a	2.71 ^a	2.10 ^a	1.71 ^a	1.49 ^a
T_1	16.32 ^b	20.25 ^b	24.36 ^b	31.25 ^b	25.60 ^a	22.27 ^b	17.95 ^b	15.74 ^b	16.99 ^b	16.12 ^b	15.26 ^b	15.02 ^b
T_2	17.74 ^c	22.13 ^c	25.70 ^c	31.25 ^b	31.68 ^b	23.29 ^c	19.55 ^c	14.04 ^a	15.54 ^c	13.69 ^c	11.25 ^c	9.64 ^c
T_3	30.02 ^d	36.25 ^d	40.39 ^d	48.36 ^c	33.48 ^c	28.29 ^d	18.30 ^b	12.09 ^c	29.99 ^d	25.63 ^d	26.25 ^d	22.36 ^d
SEM (±)	0.39	0.54	0.27	0.74	0.65	0.30	0.41	0.45	0.38	0.42	0.33	0.45
CD5%	0.91	1.25	0.61	1.71	1.50	0.68	0.94	1.04	0.88	0.96	0.76	1.03

Control: Buffalo dung + *Eisenia foetida*; T_1 : Buffalo dung + *Brassica juncea* cake; T_2 : Buffalo dung + *Brassica juncea* cake + *Eisenia foetida*; and T_3 : *Brassica juncea* cake + *Eisenia foetida*

Alphabet ^{a,b,c,d} shows significance and non-significance of treatments at 5% level of significance. Treatments with same alphabet are non-significant and with different alphabet are significant

varies with treatments and except organic carbon, contents of all other parameters increased at maturity than that of initial. Further data show that at various stages of analysis the effect of treatments was significant and at some stages it was non-significant. At maturity of vermicompost the effect of treatments was found significant for pH, available phosphorus and sodium. Intestinal calcium secretion and excretion of ammonia by earthworms raise the pH of vermicompost and make it alkaline (Ismail, 1997; Ismail, 2005; Edwards and Bohlen, 1996).

Mineralization of organic substrates like cellulose, hemicellulose, lignin etc. during vermicomposting by the activities of earthworms and other microorganisms of dung releases mineral salts (Gupta and Garg, 2008; Khawairakpam and Bhargava, 2009; Kaviraj and Sharma, 2003) which results into increased values of electrical conductivity (Neuhauser *et al.*, 1988). Organic carbon is being utilized by microorganisms as energy source and the respiratory activities of earthworms and microorganisms may results in

Table 5. Specific functional group /compound present in control of raw buffalo dung and dung composted with *Eisenia foetida*

Raw buffalo dung Adsorption frequency (cm ⁻¹)	Functional group or compound	Dung composted with <i>Eisenia foetida</i> Adsorption frequency (cm ⁻¹)	Functional group or compound
3420.85	Moisture	3397.69	Heteroaromatic compound
3011.51	Aromatic C-H structure		containing N-H group
2933.28	C-H with methyl and methylene	2352.87	CO ₂
2226.58	CO ₂	1049.32	C-O starching vibration in alcohol and phenol
1487.17	Aromatic monomer	829.79	Aromatic compound region
1423.99	C-N stretching/-OH bond of carboxyl		
1296.03	Phosphate ester		
1052.37	Ethanol		
848.80	Aromatic carbon compound		

Table 6. Specific functional group /compound present in control of *Brassica juncea* and *Brassica juncea* composted with *Eisenia foetida*

Raw <i>Brassica juncea</i> Adsorption frequency (cm ⁻¹)	Functional group or compound	<i>Brassica juncea</i> composted with <i>Eisenia foetida</i> Adsorption frequency (cm ⁻¹)	Functional group or compound
3421.91	Two N-H group(primary amine and amide)	3396.59	Heteroaromatic compound containing N-H group
3009	Heteroaromatic like pyridine, parazine pyrrols, furan	3007.63	Heteroaromatic like pyridine, parazine pyrrols, furan
2924.11	C-H with methyl and methylene	2927.48	Asymmetric/symmetric stretching of methylene
2352.96	CO ₂	2208.24	Weak (C=N) stretching bond alkaline molecule, C=C.
1637.94	C-O,C-N,N-O		
1425.55	C-N stretching		
1287.52	Aromatic/ heteromatic carbon	1420.35	Aromatic/ heteroaromatic ,
848.80	Aromatic carbon compound	1036.22	C-N stretching C-O bond, C-O stretching vibrations in alcohol and phenol

reduced amount of organic carbon in final product (Curry *et al.*, 1995).

Available phosphorus, potassium and sodium

Microbial mineralization of organic phosphorus and activities of earthworms gut

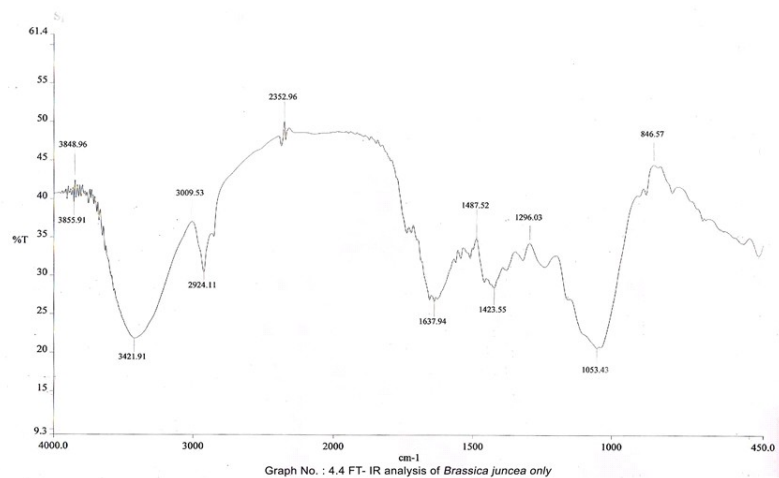
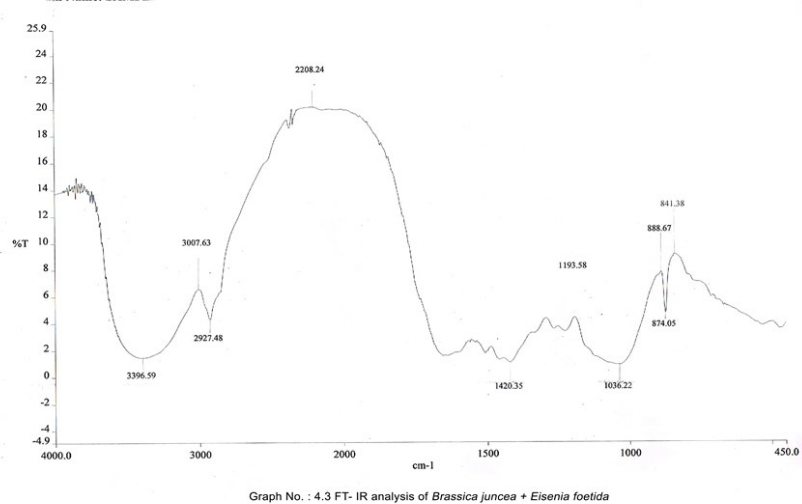
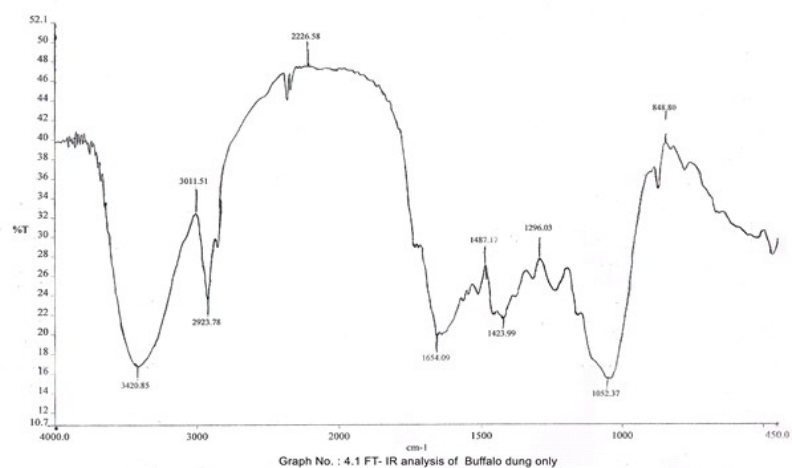
phosphatase resulted in increased amount of available phosphorus in product (Tripathi and Bharadwaj, 2004; Lee, 1992). Acid production is claimed during microbial degradation (Premuzic *et al.*, 1998) which causes solubilization of bound

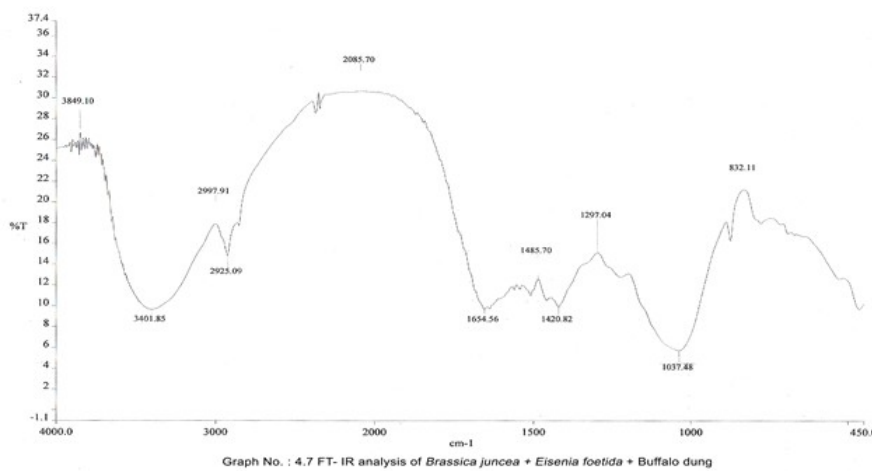
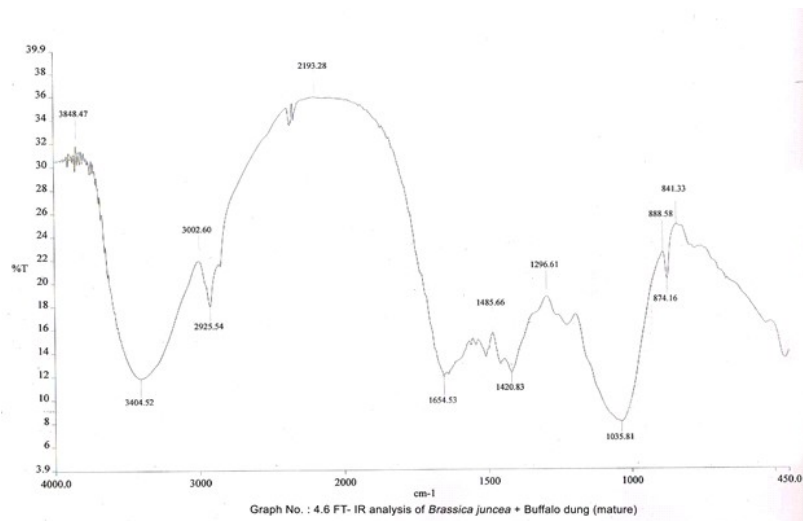
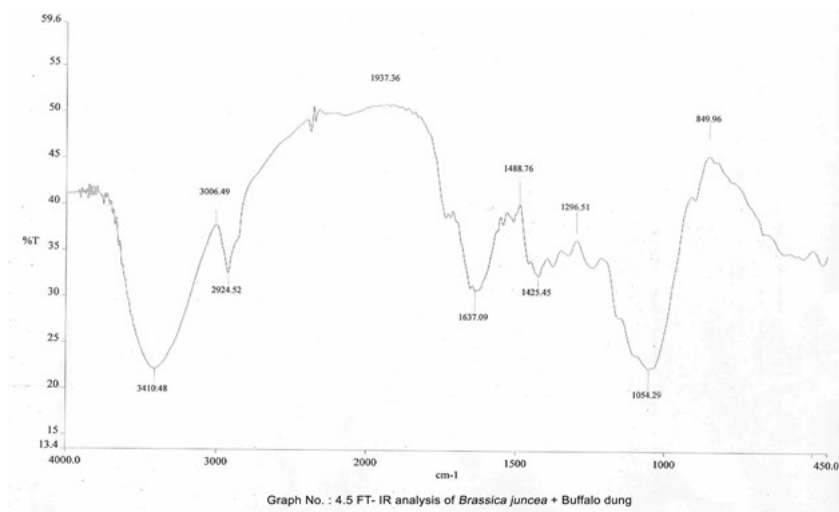
Table 7. Specific functional group /compound present in control of *Brassica juncea*+dung and *Brassica juncea* composted with dung

Control of <i>Brassica juncea</i> + dung Adsorption frequency (cm ⁻¹)	Functional group or compound	Composted <i>Brassica juncea</i> +dung Adsorption frequency (cm ⁻¹)	Functional group or compound
3410.48	Two N-H group(primary amine and amide)	3404.52	Heteroaromatic compound containing N-H group, Two N-H group(primary amine and amide)
3006.49	Heteroaromatic like pyridine, parazine pyrrols, furan	2925.54	Asymmetric/symmetric stretching of methylene
2924.32	Asymmetric/symmetric stretching of methylene	1054.53	Quinones
1937.33	C=C-CH ₂		
1637.09	C-O,C-N,N-O	3002.60	Aromatic C-H stretching
1425.48	C-N stretching		
1488.76	Aromatic/ heteroaromatic compound	1420, 1485.66	Aromatic/ heteroaromatic, C-N stretching
848.80	Aromatic carbon compound	1296	C-O,C-N, phosphate ester
		874.38,	Interaction between C=S and N,
		888.58,	Si-H Bend.
		841.33	

Table 8. Specific functional group /compound present in control of dung+ *Brassica juncea* and *Brassica juncea* composted with *Eisenia foetida* +dung

Control of <i>Brassica juncea</i> + dung Adsorption frequency (cm ⁻¹)	Functional group or compound	Composted <i>Brassica juncea</i> +dung+ <i>Eisenia foetida</i> Adsorption frequency (cm ⁻¹)	Functional group or compound
3410.48	Two N-H group(primary amine and amide)	3401.85	Two N-H group(primary amine and amide) Heteroaromatic compound containing N-H group
3006.49	Heteroaromatic like pyridine, parazine pyrrols, furan	2925.09	Asymmetric/symmetric stretching of methylene.
2924.32	Asymmetric/symmetric stretching of methylene	2997.91	C-H with methyl and methylene
1937.33	C=C-CH ₂		
1637.09	C-O,C-N,N-O	2085.70	C=C,C=N
1425.48	C-N stretching		
1488.76	Aromatic/ heteroaromatic compound	1654.56	Quinone
848.80	Aromatic carbon compound	1420, 1485.7	Aromatic / heteroaromatic C-N stretching.
		1297	C-C,C-O,C-N, phosphate ester
		1037	C-O stretching vibration in alcohol a and phenol
		832	C-C,C-O,C-N, furan ring





phosphorus, sodium and potassium. Mineralization, transformation and mobilization of nutrients from unavailable forms to plant available forms is being supported by a number of workers (Suthar, 2007; Adi and Noor, 2009; Lazcana *et al.*, 2008; Sen and Chandra, 2009; Ghosh *et al.*, 1999). Solubilization of insoluble potassium due to acid production (Adi and Noor, 2009) and increased amount of potassium in vermicompost due to higher mineralization rate of microbial activity is being well documented (Delgado *et al.*, 1995; Benitez *et al.*, 1999; Kaviraj and Sharma, 2003; Suthar, 2007).

Lignin, hemicellulose, cellulose

Cellulosic material present in substrates are decomposed by microorganisms and earthworms by secreting extracellular enzymes (Sinsabaugh *et al.*, 1992). Digestion of cellulose due to cellulolytic activity in the gut of earthworms and cellulose loss in product is well documented (Lattaud *et al.*, 1997a; Lattaud *et al.*, 1997b; Scheu, 1993; Urba' sek and Pizil, 1991; Vincelas-Akpa and Loquet, 1997; Zhang *et al.*, 2000; Zhang *et al.*, 1993). Results of present investigation are in line with above workers and showed significant decrease in amount of cellulose and hemicellulose at maturity of vermicompost compared to their initial contents (Table 4).

FTIR (Fourier Transform Infrared Spectrometer)

To prove the degradation of substrate during vermicomposting FTIR data were obtained for a wave number range of 4000-450 cm^{-1} and presented in Fig 1-7 and details of corresponding groups present is given in Tables 5-8. When Infra-red radiation is passed through sample, a portion is absorbed by the sample and remaining is transmitted. This absorption and transmittance spectrum over a wide range of wave number creates a molecular fingerprinting because each molecule gives peak at a specific wave number. Obtained peaks are than compared with spectra library of pure substances or components (Smidt and Schwanninger, 2005). Use of FTIR is very common to describe composting process (Huang *et al.*, 2006, Smidt *et al.*, 2005; Tseng *et al.*, 1996). A close examination of FTIR data obtained for Buffalo dung compared with buffalo dung + *Eisenia foetida* (T-5, Fig. 1-2); *Brassica juncea* compared to *Brassica juncea* + *Eisenia foetida* (T-6, Fig. 3-4); *Brassica juncea* + dung compared with *Composted Brassica*

juncea + dung (T-7 and Fig. 5-6) and *Composted Brassica juncea* + dung + *Eisenia foetida* (T-8, Fig. 7) clearly reveal that they are different in presence of compounds or groups which show that during vermicomposting some groups or compounds are lost and some are newly formed, hence degradation occurs.

CONCLUSION

Results of present investigation clearly direct that de-oiled cake of *Brassica juncea* can be successfully converted into nutrient rich vermicompost through the activities of *Eisenia foetida* species of earthworms. In 90 days, biomass, total organic carbon, cellulose, and hemicellulose are reduced by 36.67 to 73.33%, 8.46 to 18.94%, 38.52 to 63.89%, and 11.59 to 45.02%; whereas available phosphorus, potassium and sodium are increased by 64 to 84.85%, 75.44 to 92.00%, and 70.83 to 96.67% respectively. Further the FTIR data confirm the degradation process.

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