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Vermicomposting of paper industry sludge with cowdung and green manure plants using *Eisenia fetida*: A viable option for cleaner and enriched vermicompost production

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ABSTRACT

Vermicomposting of paper mill sludge (PMS) with cowdung (CD) and green manure plants, *Tephrosia purpurea* (TEP) and *Gliricidia sepium* (GLS) in different combinations (21 days pre-decomposed) was carried out using *Eisenia fetida* (60 days). Results revealed that electrical conductivity, total Kjeldahl nitrogen, total phosphorus, total potassium and N–NO_3^- levels were higher in vermicomposts generated from PMS + CD with TEP/GLS combination than in composts prepared without earthworms; while total organic carbon, C/N ratio, C/P ratio and organic matter content depicted significant reduction. C/N and C/P of final vermicompost ranged from 13.45 to 22.33 and 15.54 to 28.56 respectively. The total microbial population was significantly higher in CD + TEP/GLS vermicomposts ($P < 0.001$). Activities of dehydrogenase, urease, acid and alkaline phosphatases during vermicomposting elevated initially and then declined, indicating vermicompost maturity. Also, the seed germination tests with maize and cowpea in vermicomposts of PMS, CD and green manure combination support the maturity and less phytotoxic nature of vermicompost produced. Growth and reproduction of *E. fetida* explained that the addition of CD along with GLS/TEP significantly created an impact on bioconversion of PMS. The PMS + CD + TEP/GLS (2:1:1 ratio) combination can be used for sustainable PMS utilization through vermicomposting, and enriched vermicompost can also be obtained by the addition of green manure plants.

1. Introduction

The biomass and renewable energy sources are now-a-days given much attention because of the concern towards the environment and cheaper utility of available resources, and suitable alternate sources for energy generation in agriculture (Esen and Yuksel, 2013). One such an alternative source is paper mill sludge (PMS) in which its generation is increasing along with the growing demand for paper by ever increasing population which poses severe disposal and environmental concerns. The production of 10.11 million tons of paper per year in India with an increase of 2.6% of world paper production approximately generates 3.033 million tons of PMS per annum (WGR, 2011; Goel and Kalamdhad, 2017). The quantity and quality of waste materials produced by anthropogenic activities create waste disposal crisis which ultimately urges promising recycling or alternate ways for resource recovery (Toghroli et al., 2018). The appropriate sustainable method to utilize industrial sludge, compared to incineration and land filling, is the stabilization with earthworms and the biotransformed end material—vermicompost, which can be used as an agricultural soil conditioner (Lee et al., 2018). The biosludge, $CaCO_3$, clay, wood fibers, and other inorganic materials are the major components in the PMS (Nurmesniemi et al., 2007) with approximately 615.17 g/kg total organic carbon (TOC), 2.39 g/kg total Kjeldahl nitrogen (TKN), and 3.19 g/kg total potassium (TK) contents (Negi and Suthar, 2013). The primary sludge from paper industry contains 19.50% ash, 27.84% Klason lignin, and 62.73% holocellulose on dry weight basis (Geng et al., 2007). Vermicomposting is regarded as an innovative discipline of vermiculture biotechnology which provides cost-effective waste management in a sustainable manner (Aalok

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Abbreviations used

ALP	alkaline phosphatase
CD	cowdung
CFU	colony forming units
DEH	dehydrogenase
DW	distilled water
GI	germination index
GLS	*Gliricidia sepium*
GP	germination percentage
OMC	organic matter content
PMS	paper mill sludge
SPA	acid phosphatase
TEP	*Tephrosia purpurea*
TK	total potassium
TKN	total Kjeldahl nitrogen
TOC	total organic carbon
TP	total phosphorus
UA	urease

et al., 2008).

As a green and clean eco-friendly technology, vermicomposting has been used to covert different biomass residues generated from paper mill (Negi and Suthar, 2018), palm oil mill (Rupani et al., 2010; Singh et al., 2011), etc. The biodegradable PMS generated by paper industries is an attractive and potential source for nutrient rich vermicompost production as a mean of organic waste recycling (Elvira et al., 1998; Negi and Suthar, 2013). PMS generated from paper mill industries required to be suitably amended with other organic materials, considering its poor nitrogen content and broad C/N ratio, for the production of compost (Simão et al., 2018), or vermicompost (Fernández-Gómez et al., 2015) with desirable qualities. To make the PMS suitable for vermicomposting and to produce enriched vermicompost, researchers worldwide have used cattle dung, leaf litter, agricultural residues, etc. For instance, Sahoo et al. (2014) added microbial consortia during pre-vermicomposting and post-vermicomposting mixtures of paper mill waste, including PMS which resulted in the enhanced nutrients with the post-vermicomposting addition of microbial consortia. Recent report by Negi and Suthar (2018) have proved that the inoculation of brown-rot fungi, *Oligoporus placenta* with the earthworm, *Eisenia fetida* during vermicomposting of PMS resulted in elevated plant nutrients. Similarly, vermistabilization of a variety of organic materials have been successfully done with the amendment of microbial agents (Das et al., 2016), biofertilizers like *Azotobacter chroococcum* and *Bacillus megaterium* (Karmegam and Rajasekar, 2012) and phosphate solubilizing bacteria, *Pseudomonas fluorescens* (Lukashe et al., 2019) apart from cattle dung for enriched vermicompost production (Guimarães et al., 2017) or vermicompost with disease-suppressive agents (Rao et al., 2017). Nonetheless, the reports on the use of green manure plants for vermistabilization of organic waste materials are scanty, and an understanding of their use in waste conversion technologies to produce enriched soil conditioner may provide further insight into the sustainable alternative for utilizing PMS.

Leguminous green manure plants are widely studied for their positive effects on soil organic matter enrichment, nutrient supply, fertility status, soil beneficial microbial community, crop growth and yield. Due to their ability to fix atmospheric nitrogen with the help of root-nodule bacteria—rhizobia, these green manure plants are either grown and mulched *in situ* or the leaves/branches are pruned and applied on soil before mulching (Agbede, 2018; Yadav et al., 2018). In the present study, *Gliricidia sepium* (Jacq.) Kunth ex Walp. (GLS) – a leafy green manure plant and *Tephrosia purpurea* (L.) Pers. (TEP) – a highly drought resistant sub-erect perennial green manure plant in South India, were used as amendments to enrich PMS vermicompost considering their abundant local availability, potential fertilizer value, rapid decomposition rate and support of microbial growth (Marin et al., 2006; Panda, 2000). Besides the crop growth, yield is significant when these green manures are applied along with other manures or organic inputs (da Silva Garrido et al., 2017; Marathe et al., 2017). However, the effect of amending the biomass of green manure plants, GLS and TEP in vermicomposting of low-nitrogen with broader C/N containing waste materials such as PMS has not been well documented. Therefore, the present study has been aimed to utilize the biomass of the green manure plants, GLS and TEP as amendments in vermicomposting of industry generated PMS along with cowdung using the earthworm, *E. fetida*.

2. Materials and methods

2.1. Collection of earthworms, cowdung, paper mill sludge and green manure plants

The earthworm, *E. fetida* was procured from a private vermiculture farm, Salem and acclimatized in the laboratory and mass cultured in partially decomposed cowdung (CD) substrate. The raw materials, PMS was collected from Paper Packaging Ltd., Chennai, India and transported to the laboratory. The CD for the study was procured from a nearby cattle shed in the fresh form and allowed to stabilize for one week and used for the study. The green manure plant, TEP was uprooted from the outskirts of Salem (Tamil Nadu, India). The soil particles adhered to the roots of TEP were cleared and the plants were transported to the laboratory. The leafy shoots of green leaf manure plant, GLS were pruned from the trees in Government Arts College (Autonomous), Salem-7 and brought to the laboratory for further processing.

2.2. Experimental design and vermicomposting

The collected green manures, GLS and TEP were separately chopped with cutter and mixed with CD and PMS as detailed below. Totally five different combinations of CD, PMS, TEP and GLS on dry weight basis with a separate treatment containing only CD (100%) as control were prepared for the vermicomposting experiments. The treatments and vermibed substrate combinations and their physico-chemical characteristics are shown in Table 1.

The treatment combinations T2 to T6 were subjected to initial decomposition in rectangular plastic containers of 45 × 35 × 15 cm size. Two kilograms of each treatment combinations in triplicates were maintained to hold 30 ± 5% moisture by sprinkling water, regularly mixed and turned for 21 days, for the pre-decomposition to effect in the environmentally controlled room. On the 21st day, the organic materials in the containers were mixed and watered to hold 65–70% moisture content and kept overnight to stabilize. Then the second generation population of the earthworm species, *E. fetida* maintained in the mass culture beds was assorted and 60 worms with an average individual biomass of 319.31 ± 5.63 mg were introduced into each treatment containers. Parallel to this, another set of each treatment without earthworms was also maintained. The treatment T4 without PMS was maintained to know whether any difference in vermiconversion in the absence of PMS. In all the experiments except T1 and T4, the proportion of PMS was maintained 50% since the proportion above 50% PMS adversely affected the earthworms (Yuvaraj et al., 2018a). All the treatments were maintained with 65–70% moisture content till the

Table 1
Physico-chemical characteristics of initial substrate mix in different treatments (Values are mean ± SEM of three replicates).

Treatments	Vermibed combinations (%)				Ratio (v/v)	pH	EC (dS/m)	TKN (g/kg)	$N-NO_3^-$ (g/kg)	TP (g/kg)
	PMS	CD	TEP	GLS						
T1	–	100	–	–	–	7.62 ± 0.20	1.25 ± 0.03	10.53 ± 0.27	0.681 ± 0.02	8.17 ± 0.21
T2	50	50	–	–	1:1	8.26 ± 0.20	0.98 ± 0.02	9.98 ± 0.24	0.346 ± 0.01	6.64 ± 0.16
T3	50	–	25	25	2:1:1	8.63 ± 0.19	0.86 ± 0.02	12.60 ± 0.27	0.190 ± 0.001	7.33 ± 0.16
T4	–	50	25	25	2:1:1	7.95 ± 0.21	1.13 ± 0.03	16.41 ± 0.43	0.415 ± 0.01	10.59 ± 0.28
T5	50	25	25	–	2:1:1	8.44 ± 0.20	0.91 ± 0.02	14.15 ± 0.34	0.273 ± 0.01	8.40 ± 0.20
T6	50	25	–	25	2:1:1	8.30 ± 0.22	1.34 ± 0.04	12.94 ± 0.34	0.300 ± 0.01	7.85 ± 0.21
Treatments	Vermibed combinations (%)				Ratio (v/v)	TK (g/kg)	TOC (g/kg)	OMC (g/kg)	C/N ratio	C/P ratio
	PMS	CD	TEP	GLS						
T1	–	100	–	–	–	13.35 ± 0.34	316.72 ± 8.14	725.11 ± 18.63	30.08 ± 0.77	38.77 ± 1.00
T2	50	50	–	–	1:1	10.70 ± 0.26	562.16 ± 13.73	781.76 ± 19.09	56.33 ± 1.38	84.66 ± 2.07
T3	50	–	25	25	2:1:1	12.33 ± 0.27	630.45 ± 13.76	805.40 ± 17.58	50.04 ± 1.09	86.01 ± 1.88
T4	–	50	25	25	2:1:1	16.17 ± 0.43	616.58 ± 16.26	766.54 ± 20.21	37.57 ± 0.99	58.22 ± 1.54
T5	50	25	25	–	2:1:1	14.50 ± 0.34	515.33 ± 12.23	819.85 ± 19.46	36.42 ± 0.86	61.35 ± 1.46
T6	50	25	–	25	2:1:1	15.49 ± 0.41	498.04 ± 13.14	827.33 ± 21.83	38.49 ± 1.02	63.44 ± 1.67

termination of the study, i.e., 60 days.

2.3. Percentage decomposition

The percentage decomposition of experimental substrates in terms of particle size reduction after 60 days of vermicomposting was calculated (Goswami and Kalita, 2000).

$$\text{Percentage decomposition} = \frac{A - B}{A} \times 100$$

Where, A = Total weight of organic substrate in the vermibed; B = Weight of decomposed material.

2.4. Physico-chemical analysis of initial substrates, compost and vermicompost

The initial substrates and the final substrates (after 60 days) i.e., worm-unworked (without earthworms, compost) and the worm-worked substrates (substrates introduced with worms, vermicompost) were analysed for various physico-chemical parameters. pH was determined by a digital pH meter, electrical conductivity (EC) by Elico conductivity meter using 1:10 (w/v) suspension and TOC by the method of Walkley and Black (1934). TKN was determined after digesting the sample with conc. H_2SO_4 and conc. $HClO_4$ (9: 1, v/v) (Tandon, 1993). The organic matter content (OMC) was derived deducing the percentage of ash content. Spectrophotometric estimation of nitrate nitrogen ($N-NO_3^-$) was done after extracting the samples with 0.01 M $CuSO_4$ solution (Jackson, 1973). The total phosphorus (TP) was analysed using colorimetric method with molybdenum in H_2SO_4 (Tandon, 1993) while the TK was analysed by the method described by Tandon (1993).

The ratio of the percentage of carbon to that of nitrogen (i.e., C/N ratio) and to that of phosphorus was calculated by dividing the percentage of carbon estimated for the sample with the percentage of nitrogen and phosphorus estimated for the same sample, respectively. The initial physico-chemical characteristics of organic materials in different treatments are given in Table 1. The percentage increase/decrease of various physico-chemical parameters in the vermicompost over the initial and worm un-worked substrates (compost) were calculated as per the method of Ramalingam and Ranganathan (2001).

2.5. Enzymatic analysis

Samples from all the treatments with and without earthworms were collected on 0 (initial), 15, 30, 45 and 60th day of experimentation and used for the analysis of the enzymes, dehydrogenase (DEH), urease (UA), acid phosphatase (SPA) and alkaline phosphatase (ALP). The activity of DEH was assayed by quantifying the mg of 2,3,5-triphenyl formazon (TPF) produced by reaction with 2,3,5-triphenyl tetrasolium chloride and expressed as μg TPF released g^{-1} sample (dry weight) h^{-1} (Cassida et al., 1964). UA activity was quantified by the rate of release of NH_4^+ from the hydrolysis of urea as per the method described by Tabatabai and Bremner (1972). The assay of SPA and ALP were done with 1 g of sample with 4 ml 0.1 mol/l of modified universal buffer at pH 6.5 SPA and pH 11.0 for alkaline phosphatase with 1 ml 25 mM p-nitrophenyl phosphate (PNP) (Tabatabai and Bremner, 1969). Then the samples were incubated at 37 °C for an hour followed by the addition of 4 ml 0.5 mol/l NaOH and 1 ml 0.5 mol/l $CaCl_2$. The concentration of *p*-nitrophenol (PNP) was determined at 420 nm absorbance with reference to the standards and the activity of SPA and ALP was expressed as mg PNP released g^{-1} sample (dry weight) h^{-1}.

2.6. Microbiological analysis

The total colony forming units (CFU) of bacteria, fungi and actinomycetes in the treatments at the beginning of the experiment (initial) and at the end of the experiment (compost and vermicompost) were enumerated using standard plate count method (Subba Rao, 1995). The total microbial population from 0th day onwards up to 60 days, and once in 15 days was carried out using the same procedure. The Petri plates with 30–300 colonies were selected for enumerating bacterial population. The bacterial, fungal and actinomycetes population was expressed as CFU per gram of the sample. One gram of each sample was taken in a sterile conical flask containing nine ml of distilled water and shaken in a vortex mixer for 30 min. From this stock, various dilutions were prepared from 10^{-1} to 10^{-7} with sterile distilled water. One ml of the diluted sample was poured into Petri plates containing nutrient agar media, Martin's Rose Bengal agar media and Kenknight's media respectively for bacteria, fungi and actinomycetes. Three replicates were maintained for each observation. The final enumeration results were $\log_{10}$ transformed and expressed as $\log_{10}$ CFU g^{-1}.

2.7. Growth and reproduction of the earthworms

For the growth and reproduction studies of earthworms, cowdung was used as a standard rearing medium which served as

control. Another batch of the same experimental setup was also maintained to observe the total biomass and the rate of reproduction of the worms in terms of their number. A separate set of experiments was maintained so as to un-necessarily disturb the vermicomposting process. To find out the biomass, the worms were hand sorted, counted, washed with water, blotted on Whatman no. 1 filter paper and then weighed in digital balance. Subsequently, the worms were immediately introduced in the respective vermibeds. This was done once in 15 days from the start of the experiment up to 60 days. The growth rate, and the number of cocoons produced were recorded for each treatment till the termination of the study. The individual biomass of the clitellate earthworms in each treatment was measured and expressed as individual mean biomass per vermibed. The number of worms was counted in the treatments according to age class-wise groups, juveniles, subadults and clitellate adults as categorized by Zorn et al. (2005).

2.8. Seed germination assay

The vermicompost maturity was also assessed by seed germination studies with maize (*Zea mays* L.) variety Co 6 and cowpea [*Vigna unguiculata* (L.) Walp.] variety VBN seeds using vermicompost extracts obtained from each treatment. For this study, extracts of fresh vermicomposts were obtained with distilled water at 1:10 w/v ratio (Tiquia et al., 1996). For each treatment double distilled water was used as control. Fifty seeds of maize and cowpea separately in 5 lots for each vermicompost were laid in Petri dishes with filter paper added with 6 ml of extract and kept for 10 days, and the number of seeds germinated per day was recorded. The germination percentage (GP) and germination index (GI) was calculated by adopting the method of UAF (2010) and Benech Arnold et al. (1991) respectively as detailed below. GI in vermicomposts was calculated as it emphasizes on both the percentage of germination and its speed. A higher GI value denotes a higher percentage and rate of germination in different treatments (Kader, 2005).

$$\text{GP}\ (\%) = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds tested}} \times 100$$

$$\text{GI} = (10 \times \text{n1}) + (9 \times \text{n2}) + (8 \times \text{n3}) \ldots + (1 \times \text{n10})$$

Where, n1, n2… n10 = No. of seeds germinated on the first, second and subsequent days until the 10th day; 10, 9… and 1 are the weights given to the number of seeds germinated on the first, second and subsequent days, respectively.

2.9. Statistical analysis

The data were expressed as mean ± standard error (SEM). The physico-chemical characteristics of initial vermibed substrates, composts and vermicomposts were statistically interpreted using ANOVA. The statistical significance of various treatments was evaluated by one-way analysis of variance (ANOVA) using SPSS version 18.0 (SPSS Inc., Chicago, USA). When there was a significant difference, Tukey's honestly significant different (HSD) multiple comparison tests were performed at $P < 0.05$ significance level. Two-way ANOVA was used to find out the effect of different treatments with reference to chemical parameters, total microbial population and enzyme activities.

3. Results and discussion

3.1. Percentage decomposition

A maximum percentage decomposition based on particle size was observed in T1 (94.33% - CD) after 60 days of vermicomposting with *E. fetida* followed by the substrate combinations, T2 (91.83%), T6 (91.07%), T4 (88.67%), T5 (86.33%) and T3 (73.17%). The decomposition rate was significantly higher ($P < 0.05$) in the substrates introduced with *E. fetida* than that of the treatments without *E. fetida*. The percentage decomposition in T2, T4 and T6 with earthworms did not differ significantly with that of control, i.e., T1 (100% CD); whereas, T3 and T5 combinations recorded statistically significant difference at $P < 0.05$ level, with CD (T1) substrate indicating the food preference of the earthworm *E. fetida* (Fig. 1). However, the percentage decomposition of all the substrate combinations without earthworms depicted an average of 46.63 ± 2.86%, which was significantly ($P < 0.05$) lower than that of the values recorded for all the treatments with earthworms after 60 days. This clearly indicates that the earthworms are contributing plenty in the degradation of PMS in combination with CD (1:1). John Paul et al. (2011) reported 70.48% of decomposition rate while vermicomposting of municipal solid waste with CD in 1:1 using the earthworm, *Perionyx ceylanensis*. Parallel studies imply that the decomposition rate depends on the efficiency of earthworm species and the nature of organic material mix used for vermicomposting apart from other varied factors (Prakash and Karmegam, 2010; Sharma and Garg, 2018a).

3.2. Physico-chemical characteristics of compost and vermicompost

The physico-chemical characteristics of the organic substrates are very important for assessing the nutrient quality of the final product and suitability of the substrate mix for vermiconversion. In the present study, the pH of the final vermicompost in different treatments showed a decrease over initial substrates and final compost where the difference in decrease was not significant ($P > 0.05$). The change in pH between treatments also showed non-

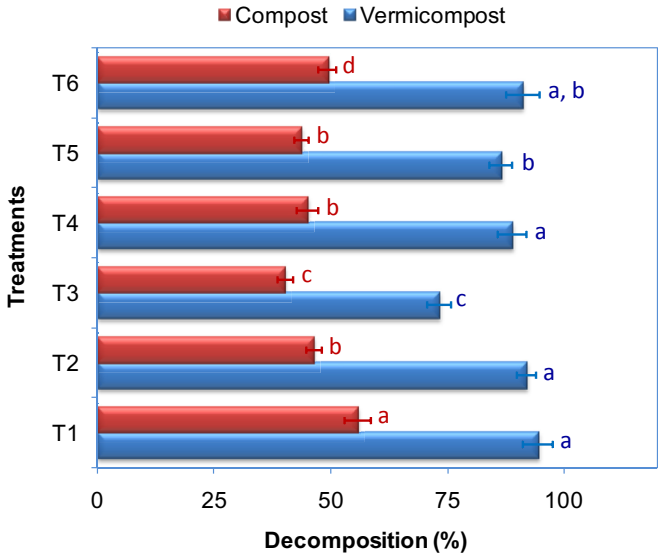

Fig. 1. Percentage decomposition of organic materials in different treatments with and without *E. fetida* (60 days). Values are mean of three replicates. Error bars indicate ± SEM. The difference in mean values between the treatments followed by the same letter are not significant at 5% level ($P < 0.05$) by Tukey's honestly significant difference (HSD) multiple comparison test.

significant difference (Table 2). The reduction of pH during vermicomposting is attributed to the release of organic acids during the bioconversion process (Yuvaraj et al., 2018b; Sharma and Garg, 2018b).

The EC in both compost and vermicompost increased from the initial levels. In all the treatments, vermicompost showed significant levels of percentage increase with a range of 74.25 (T1) – 150.43% (T5) over control. The vermicompost of T5 (PMS:CD:TEP in 2:1:1 ratio) recorded a maximum increase of EC, which was significantly higher percentage among all the other treatments with *E. fetida*. The vermicompost of PMS in combination with GLS and TEP in 2:1:1 ratio in T3 without CD also recorded a significantly higher percentage increase than 100% CD in T1 (Table 2). These observations indicate that the addition of green manure plants plays a significant role in the promotion of EC in vermicomposting. The foremost reasons for the increase of EC rely on the release of salts and purging of inorganic ions and possible increase in EC by the release of ammonium based compounds (Fang et al., 1999; Negi and Suthar, 2018). However, the levels of EC are within the safe limits of phytotoxicity (Li et al., 2012).

The TKN in vermicompost is higher than that of the compost of all the treatments. Overall, TKN, N–NO_3^-, TP and TK in vermicompost displayed a significant ($P < 0.05$) increment over compost and initial substrates. While TOC, OMC, C/N and C/P showed a decrement implying that the earthworm, *E. fetida* played a major role in vermiconversion of different treatments (Tables 2 and 3). A maximum of 31.26 g/kg TKN was recorded in T5 followed by T6 (29.01 g/kg) and T4 (28.48 g/kg), and the higher TKN content in T4, T5 and T6 did not differ significantly ($P > 0.05$) with each other. It was significantly higher ($P < 0.05$) than that of the other treatments-T1, T2 and T3. It is apparent that the treatments amended with CD and green manure plants resulted in the increased level of TKN signifying the amendment of GLS and TEP enriched the TKN in final vermicompost through vermicomposting using *E. fetida*. However, T3 (PMS + GLS + TEP in 2:1:1) showed lesser increase in TKN, insisting that CD is also to be incorporated along with GLS and TEP for better bioconversion and enrichment. A similar trend of results obtained for N–NO_3^- also supports the same (Table 3). Higher level of N–NO_3^-, TP and TK, 2.814, 24.30 and 27.48 g/kg, respectively, was found in vermicompost produced from CD and green manure amended treatments-T5, T4 and T6. The TP in vermicompost ranged from 14.38 g/kg (T3) to 24.30 g/kg (T4), whereas it was significantly lower in compost with a range between 8.08 g/kg (T2) and 12.26 g/kg (T4). The highest TK value in vermicompost from T6 (27.48 g/kg) was closely followed by T5 (26.10 g/kg) and T4 (25.76 g/kg). Though all the treatments with *E. fetida* showed significantly elevated levels of TKN, N–NO_3^-, TP and TK after 60 days comparing to the treatments without *E. fetida*, the treatments containing GLS and TEP along with CD resulted in much enhanced levels. Also, the TKN, N–NO_3^-, TP and TK contents in vermicompost recovered from T4 which consisted of 2:1:1 ratio of CD + GLS + TEP without PMS did not differ significantly ($P > 0.05$) with that of T5 and T6 which consisted of PMS and CD in combination with TEP and GLS respectively in 2:1:1 ratio. These findings clearly suggest that the addition of green manure plants, GLS and TEP along with CD for vermiconversion of the industrial waste, it was PMS which recovered major nutrient contents.

The C/N ratio of compost and vermicompost in all the treatments showed reduction from the initial levels and the difference in C/N ratio between compost and vermicompost was statistically significant at $P < 0.05$. The range of C/N decrease in vermicompost over compost was −35.12 to −60.41%. The lowest C/N was recorded in T6 followed by T5, T1, T4, T2 and T3 (Table 2). The C/P ratio also showed a parallel decrease in vermicompost over compost which ranged between −49.07% and −68.22%. The vermicompost of T1 registered the lowest C/P (15.54) followed by T4 (17.89), T6 (18.10) and T5 (19.37), and the difference in C/P among the treatments T1, T4, T5 and T6 was not significant (Table 2).

The increase of TKN and N–NO_3^- in the vermicompost is probably due to the mineralization of organic substrates and the elevated levels in treatments owing to the addition of CD and green manure plants with PMS which is well supported by the earlier works on other substrates amended with CD (Suthar et al., 2017; Negi and Suthar, 2018). Gong et al. (2018) reported an increased level of N–NO_3^- in bamboo biochar amended green waste vermicompost (0.8692 g/kg in green waste+6% biochar) where the N–NO_3^- witnessed in the present study by means of amending PMS with CD + TEP/GLS was higher (T5: 2.814 g/kg; T6: 2.360 g/kg). The increase of TP and TK has been well documented during vermicomposting of PMS in consequence of combined action of earthworms and microbial activity (Negi and Suthar, 2018; Yuvaraj et al., 2018a). The decrease of TOC and increase of nitrogen forms during vermicomposting resulted in the reduction of TOC in consequence of the organic matter degradation, mineralization and respiratory activity of earthworms and microflora. In the event of TP increment, phosphatases in the gut and as well as in the vermicasts have

Table 2
The final pH, EC, C/N ratio and C/P ratio in different treatments after 60 days of composting with and without *E. fetida*.

Treatments	pH			EC (dS/m)		
	Compost	Vermicompost	% decrease over compost ^{NS}	Compost	Vermicompost	% increase over compost*
T1	7.47 ± 0.17	7.12 ± 0.25	−4.69 ^{a}	1.67 ± 0.04	2.91 ± 0.10	74.25 ^{d}
T2	8.15 ± 0.20	7.56 ± 0.23	−7.24 ^{a}	1.21 ± 0.03	2.54 ± 0.08	109.92 ^{b}
T3	8.43 ± 0.24	7.73 ± 0.19	−8.30 ^{a}	1.04 ± 0.03	2.19 ± 0.05	110.58 ^{b}
T4	7.69 ± 0.21	7.28 ± 0.19	−5.33 ^{a}	1.45 ± 0.04	3.05 ± 0.08	110.34 ^{b}
T5	8.31 ± 0.19	7.60 ± 0.18	−8.54 ^{a}	1.17 ± 0.03	2.93 ± 0.07	150.43 ^{a}
T6	8.10 ± 0.19	7.35 ± 0.24	−9.26 ^{a}	1.80 ± 0.04	3.37 ± 0.11	87.22 ^{c}
Treatments	C/N ratio			C/P ratio		
	Compost	Vermicompost	% decrease over compost*	Compost	Vermicompost	% decrease over compost*
T1	22.79 ± 0.52	14.78 ± 0.52	−35.12 ^{c}	30.51 ± 0.69	15.54 ± 0.55	−49.07 ^{c}
T2	41.63 ± 1.01	19.63 ± 0.59	−52.83 ^{a,b}	65.07 ± 1.58	20.68 ± 0.62	−68.22 ^{a}
T3	42.63 ± 1.20	22.33 ± 0.54	−47.62 ^{b}	65.23 ± 1.84	28.56 ± 0.68	−56.22 ^{b}
T4	33.34 ± 0.92	15.27 ± 0.40	−54.21 ^{a,b}	49.38 ± 1.36	17.89 ± 0.47	−63.77 ^{a,b}
T5	31.71 ± 0.71	14.34 ± 0.34	−54.77 ^{a,b}	48.32 ± 1.08	19.37 ± 0.46	−59.92 ^{b}
T6	33.97 ± 0.81	13.45 ± 0.43	−60.41 ^{a}	48.65 ± 1.17	18.10 ± 0.58	−62.80 ^{a,b}

Values are mean ± SEM of three replicates; *, ^{NS} – the % decrease/increase values between compost and vermicompost are significantly and non-significantly different at $P < 0.05$ by ANOVA, respectively; The difference in % decrease/increase values in vermicompost over compost between treatments followed by the same letter are not significant at 5% level ($P < 0.05$) by Tukey's honestly significant difference (HSD) multiple comparison test.

Table 3
The final chemical characteristics of organic substrates in different treatments after 60 days of composting with and without *E. fetida*.

Treatments	TKN (g/kg)		$N-NO_3^-$ (g/kg)		TP (g/kg)	
	Compost	Vermicompost	Compost	Vermicompost	Compost	Vermicompost
T1	13.15 ± 0.30	18.50 ± 0.65	0.910 ± 0.02	2.132 ± 0.08	9.82 ± 0.22	17.60 ± 0.62
T2	12.63 ± 0.31	19.83 ± 0.60	0.462 ± 0.01	1.680 ± 0.05	8.08 ± 0.20	18.83 ± 0.57
T3	14.00 ± 0.39	18.39 ± 0.44	0.285 ± 0.01	0.990 ± 0.02	9.15 ± 0.26	14.38 ± 0.34
T4	18.16 ± 0.50	28.48 ± 0.75	0.609 ± 0.02	2.551 ± 0.07	12.26 ± 0.34	24.30 ± 0.64
T5	15.74 ± 0.35	31.26 ± 0.74	0.533 ± 0.01	2.814 ± 0.07	10.33 ± 0.23	23.15 ± 0.55
T6	14.02 ± 0.34	29.01 ± 0.93	0.415 ± 0.01	2.360 ± 0.08	9.79 ± 0.23	21.56 ± 0.69
ANOVA[#]	*F-value*	*P-value*	*F-value*	*P-value*	*F-value*	*P-value*
Between compost and vermicompost	24.0628	0.0045*	40.6996	0.0014*	69.9207	0.0004*
Treatments	TK (g/kg)		TOC (g/kg)		OMC (g/kg)	
	Compost	Vermicompost	Compost	Vermicompost	Compost	Vermicompost
T1	14.13 ± 0.32	22.55 ± 0.80	299.65 ± 6.80	273.52 ± 9.66	599.67 ± 13.61	460.73 ± 16.28
T2	11.87 ± 0.29	18.60 ± 0.56	525.74 ± 12.75	389.33 ± 11.76	668.64 ± 16.21	436.98 ± 13.19
T3	13.45 ± 0.38	20.33 ± 0.49	596.85 ± 16.81	410.63 ± 9.84	673.31 ± 18.96	516.45 ± 12.37
T4	17.62 ± 0.48	25.76 ± 0.68	605.42 ± 16.66	434.78 ± 11.46	605.18 ± 16.65	385.91 ± 10.18
T5	15.33 ± 0.34	26.10 ± 0.62	499.18 ± 11.12	448.40 ± 10.64	658.59 ± 14.68	432.22 ± 10.26
T6	17.06 ± 0.41	27.48 ± 0.88	476.30 ± 11.41	390.18 ± 12.55	671.54 ± 16.09	406.43 ± 13.07
ANOVA[#]	*F-value*	*P-value*	*F-value*	*P-value*	*F-value*	*P-value*
Between compost and vermicompost	149.4261	6.48E-05*	16.8569	0.0093*	109.6138	0.0001*

Values are mean ± SEM of three replicates; [#]Two-way ANOVA; *: significant difference between compost and vermicompost of different treatments.

played a major role (Parthasarathi et al., 2016). Yuvaraj et al. (2018a) in their study reported that the vermicompost of PMS + CD (1:1) prepared using the earthworm, *Perionyx excavatus* had 5.79, 11.0 and 2.99 g/kg of TKN, TP and TK respectively. While elevating the vermicomposting of PMS with the fungus, *O. placenta,* Negi and Suthar (2018) reported a maximum of 10.10, 19.19 and 3.92 g/kg of TKN, TK and TP respectively in different treatments. The results of the present study treatments incorporating CD and green manure plants with PMS showed 31.26 g/kg TKN in T5, 27.48 g/kg TK in T6 and 23.15 g/kg TP in T5, suggesting that the addition of green manure plants, GLS and TEP increased the nutritional status of vermicompost of PMS to higher levels. More significantly, the reduced C/N and C/P is well within the range of agronomic useable organic compost (Suthar, 2010). The comprehensive comparison of the important characteristics of vermicompost produced from PMS as shown in Table 4 by previous workers with that of the present study evidently depicts that the vermicompost generated from PMS in amendment with CD and green manure plants had enhanced levels of nutrients and permissible limits of C/N and C/P ratios. The improved nutrient status and qualities of vermicompost produced from PMS amended with CD and GLS/TEP indicates sustainable utility of PMS for the enriched vermicompost production.

3.3. Total microbial population

The total microbial population in different treatments with and without *E. fetida* showed increase, whereas the increase was significantly ($P < 0.05$) higher in the treatments with *E. fetida* (Fig. 2; Table 5). The total bacterial and fungal population in treatments with the earthworms showed a steady increase up to 30 days, stabilized after the 30th day and started declining towards the end, i.e., on the 60th day. While total actinomycetes population

Table 4
Comparison of characteristics of vermicompost produced from PMS.

Earthworm species used	Vermibed substrate	Ratio	EC (dS/m)	TOC (g/kg)	TKN (g/kg)	$N-NO_3^-$ (g/kg)	TP (g/kg)	TK (g/kg)	C:N ratio	References
Eisenia andrei	PMS + cattle manure	1:4	0.41	151.00	12.00	–	5.90	7.60	16.00	Elvira et al. (1998)
	PMS + dairy sludge + cattle manure	1:1:3	0.42	185.00	14.00	–	6.10	5.30	13.00	
E. fetida	PMS + CD	1:3	1.83	150.40	21.80	–	6.68	7.13	7.50	Kaur et al. (2010)
		1:1	1.80	185.50	16.30	–	3.65	4.84	10.37	
		3:1	1.73	197.20	14.90	–	2.20	4.11	13.23	
E. fetida	PMS + CD	1:3	0.41	458.10	34.33	0.359	6.29	15.92	13.34	Negi and Suthar (2013)
		1:1	0.38	454.23	30.28	0.619	6.80	12.06	15.00	
		3:1	0.34	453.40	29.24	0.417	6.27	8.04	15.51	
E. fetida	PMS + CD	1:2	0.24	467.00	14.59	–	0.37	–	31.70	Suthar et al. (2014)
		1:1	0.23	487.00	14.40	–	0.40	–	33.50	
		2:1	0.22	595.00	13.38	–	0.37	–	44.60	
E. fetida	PMS + tomato plant debris	2:1	2.60	179.00	12.60	–	3.70	13.10	14.20	Fernández-Gómez et al. (2015)
		1:1	2.20	163.00	10.60	–	3.00	9.60	15.30	
E. fetida	PMS + CD + a fungus, *O. placenta*	1:3	0.44	550.60	10.10	–	2.56	14.30	66.56	Negi and Suthar (2018)
		1:1	0.41	528.50	8.50	–	1.95	10.57	67.17	
		3:1	0.35	535.60	7.30	–	3.10	8.82	85.37	
P. excavatus	PMS + CD	1:1	1.80	13.90	5.79	–	11.00	2.99	23.25	Yuvaraj et al. (2018a)
		1:2	0.96	15.20	4.00	–	9.00	2.40	38.75	
		2:1	0.80	15.90	3.60	–	6.00	1.70	44.65	
E. fetida	PMS + CD	1:1	2.54	389.33	19.83	1.680	18.83	18.60	19.63	Present study
	PMS + TEP + GLS	2:1:1	2.19	410.63	18.39	0.990	14.38	20.33	22.33	
	PMS + CD + TEP	2:1:1	2.93	448.40	31.26	2.814	23.15	26.10	14.34	
	PMS + CD + GLS	2:1:1	3.37	390.18	29.01	2.360	21.56	27.48	13.45	

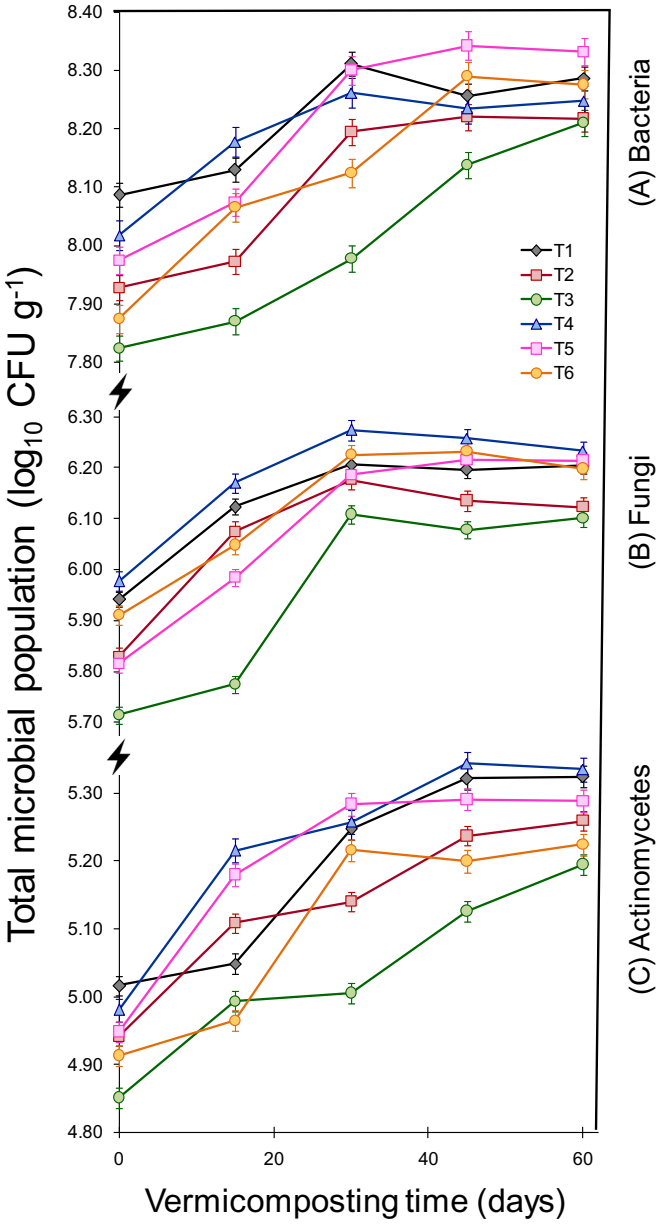

Fig. 2. Changes in total microbial population (log_{10} CFU g^{-1}) during vermicomposting (60 days): (A) Bacteria, (B) Fungi and (C) Actinomycetes. Values are log_{10} transformed mean of three replicates. Error bars indicate ± SEM.

kept on increasing after the 30th day and 45th day, and then stabilized. The total microbial population in T3 was found to be lower than all the other treatments mainly because of the non-incorporation of the CD. The treatments containing PMS amended with CD + green manure plants (TEP in T5 and GLS in T6) in the presence of *E. fetida* tremendously supported the population build-up of total bacteria, fungi and actinomycetes, which might have caused the rapid decomposition and mineralization of organic substrates. The elevated total microbial population in vermicompost between the treatments did not differ significantly ($P > 0.05$), showing that the incremental microbial activity by the action of *E. fetida* followed a uniform pattern in all treatments. However, the incremental difference in total microbial population of vermicompost with that of compost in all the treatments was found to be highly significant ($P < 0.001$), and this points out that the earthworm *E. fetida* consistently promoted the microbial population. Recent studies on vermicomposting with the earthworm, *P. excavatus* also showed similar incremental variation in total microbial population in vermicompost over compost (Ananthavalli et al., 2019a, 2019b). The microbial population maintenance is equally important to that of earthworm species used in vermicomposting, as the process of biodegradation of organic wastes is carried out by the combined action of earthworms and microorganisms (Sharma and Garg, 2018a). The chemical nature of the feed mixture influences the microbial activities, which is hiked in folds with the addition of CD in PMS vermicomposting (Negi and Suthar, 2018; Yuvaraj et al., 2018a). Also, the addition of leguminous green manure plants, TEP/GLS recorded a higher microbial dynamics due to the nutrient supply through bioconversion process. Nonetheless, the associative increment of microbial population in vermicomposting of diverse organic wastes with *E. fetida* has been reported by Suthar and Gairola (2014) and Hussain et al. (2018). Further, it has been reported that the earthworm, *E. fetida* influences the microbial profile during vermicomposting by promoting the growth of bacteria like Flavobacterium, Acidobacterium, and Planctomycetes, and by inhibiting the growth of pathogenic bacteria (Lv et al., 2018).

3.4. Enzyme activities

In vermicomposting systems, the activity of enzymes is considered as important to assess the maturation of vermicompost among the various other parameters. In the present study, the activity of the enzymes DEH, UA, SPA and ALP showed a progressive increment during vermicomposting up to 15–30 days with variation among different treatments and then started declining by the end of the study (60^{th} day) (Fig. 3). For all the enzymes studied during vermicomposting, the treatment T3 showed initially a slower increase compared to other treatments that might be attributed to the lack of CD in T3. The DEH activity in T1 was 42.55, 80.05, 68.88, 48.72 and 32.61 μg TPF g^{-1} dw h^{-1} respectively on 0, 15, 30, 45 and 60 days of vermicomposting, whereas it was 22.33, 32.43, 55.80, 68.03 and 45.69 61 μg TPF g^{-1} dw h^{-1} in T3 respectively. The UA activity in vermicompost on the 60^{th} day was 15.29,

Table 5
Variances in microbial population and enzyme activities between treatments, and between compost and vermicompost of different treatments based on two-way ANOVA.

Parameter	Between treatments			Between compost and vermicompost		
	F-value	P-value	Significance	F-value	P-value	Significance
Microbial population						
Bacteria	1.5871	0.3123	Not significant	1271.986	3.26E-07	0.1%
Fungi	1.4336	0.3512	Not significant	543.5737	2.70E-06	0.1%
Actinomycetes	0.8576	0.5649	Not significant	871.6440	8.36E-07	0.1%
Enzyme activity						
DEH	5.2145	0.0470	5%	43.1170	0.00123	1%
UA	15.7377	0.0044	1%	105.8531	0.00015	0.1%
SPA	7.8314	0.0207	5%	358.0079	7.59E-06	0.1%
ALP	21.8774	0.4994	Not significant	21.5581	0.00562	1%

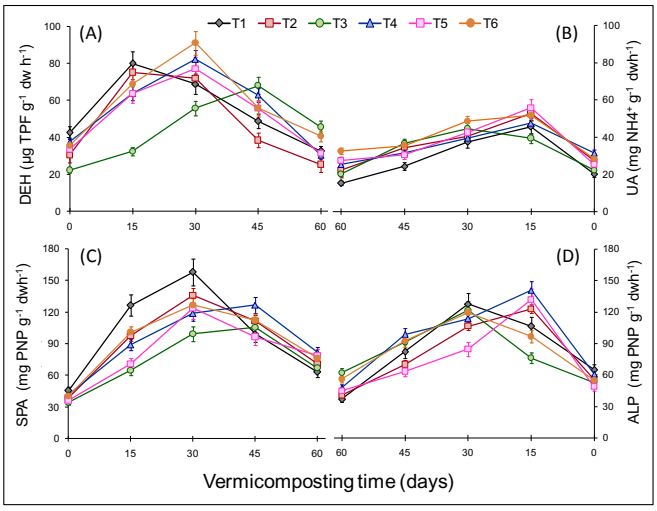

Fig. 3. Enzymatic activity in different treatments during vermicomposting (60 days) with *E. fetida*. Values are mean of three replicates. Error bars indicate ± SEM. (A) DEH –dehydrogenase; (B) UA – urease; (C) SPA – acid phosphatase; (d) ALP – alkaline phosphatase.

22.30, 20.31, 25.32, 27.35 and 32.53 mg NH_4^+ g^{-1} dw h^{-1} respectively in T1, T2, T3, T4, T5 and T6 and the values were significantly higher than the respective composts at $P < 0.001$ (Table 5). The range of SPA and ALP activity in vermicompost was 63.18–81.92 mg PNP g^{-1} dw h^{-1} and 36.87–61.98 mg PNP g^{-1} dw h^{-1} respectively. DEH, UA and SPA showed statistically significant difference between treatments, whereas ALP showed non-significant difference ($P > 0.05$). Activities of all the enzymes assayed in vermicompost were significantly different from that of the compost (DEH and ALP, $P < 0.01$; UA and SPA, $P < 0.001$). The increase of DEH during initial vermicomposting days and decrease during the later part is an indicative of compost maturity process (Alidadi et al., 2016) which is well pronounced in the present study. The elevation of phosphatases activity during vermicomposting is associated with the phosphate mineralization activity (Ghosh et al., 2018; Gong et al., 2018) and upon maturity of vermicompost, the activity showed a decline towards the end. UA activity coupled with C:N ratio of organic wastes is considered as a measure of maturity parameter (Sudkolai and Nourbakhsh, 2017), and its turn down activity after 30 days in the present study signifies the faster degradation and the start of vermicompost maturity. Overall, the enzyme activities are higher in CD and in green manure amendments (Fig. 3).

3.5. Growth and reproduction of E. fetida

The growth and reproduction of the earthworm species used for vermiconversion of organic waste materials are considered as a good sign of the effective vermicomposting process. In the current study, the growth and reproduction of the earthworm, *E. fetida* was assessed in terms of growth rate, cocoon production rate, biomass of individuals and the number of worms received at the end of the 60th day in different treatments (Fig. 4). The growth rate of *E. fetida* was higher in T6 (9.54 mg/worm/day) followed by T4 (9.33 mg/worm/day) and T5 (9.01 mg/worm/day). These values were significantly higher (T6) or slightly higher (T4 and T5) than T1 (8.81 mg/worm/day). The cocoon production rate of the earthworms ranged between 0.076 and 0.098 cocoon/worm/day (Fig. 4). The cocoon production rate was on par or higher with CD and green manure amendments. Similar kind of variation in growth rate and cocoon production rate in different feed mixtures have been

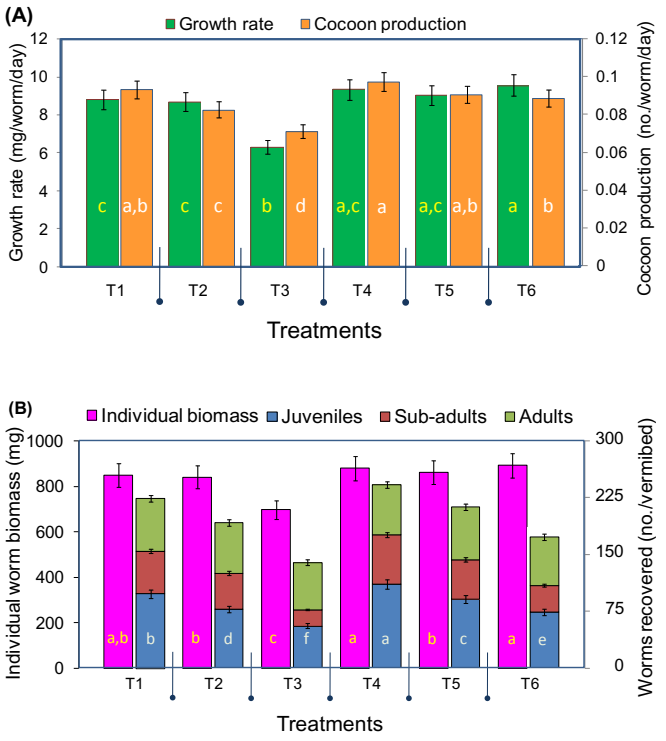

Fig. 4. (A) Growth and cocoon production rate, and (B) Individual biomass and number of *E. fetida* in different treatments (60 days). Values are mean of three replicates. Error bars indicate ± SEM. The difference in mean values between the treatments followed by the same letter are not significant at 5% level ($P < 0.05$) by Tukey's honestly significant difference (HSD) multiple comparison test.

reported by Hussain et al. (2016) and Negi and Suthar (2018). The growth and reproduction of *E. fetida* is dependent on the type of organic substrates provided and their chemical/nutrient contents. Importantly, the nature of bulking materials like CD plays a major role in growth and reproduction of the earthworms (Negi and Suthar, 2013; Sharma and Garg, 2018b).

The individual biomass and number of worms recovered also revealed prominently that the addition of CD along with GLS and TEP (T4, T5 and T6) have a significant impact. A maximum individual biomass was found in T6 (892.21 mg/worm) followed by T4 (879.38 mg/worm), T5 (860.44 mg/worm) and T1 (848.47 mg/worm). Total number of worms recovered from each treatment after 60 days recorded a maximum in T4 followed by T1 and T5. The growth rate of the worms, individual biomass and number of worms recovered in T3 showed very low values when compared with other treatments, T1, T2, T4, T5 and T6, and this could have been due to the support rendered by CD in these treatments, for the reproductive activity of earthworms. The addition of CD in vermiconversion of PMS in combination with GLS and/or TEP is required when considering the reproduction of the worms.

3.6. Seed germination test of vermicomposts

The seed germination test is normally employed to assess the degree of maturity and phytotoxicity of composts for agricultural use. The GI is the most appropriate measurement which provides seed germination percentage along with the speed of germination (Kader, 2005). In the present study, the GP and GI respectively ranged from 86 to 98% and 279–360 for maize and 84–98% and 285–372 for cowpea. The vermicompost extracts of the treatments, T1, T2, T4, T5 and T6 showed the GP and GI on par with distilled water treatment ($P > 0.05$) whereas, T3 recorded significantly lower

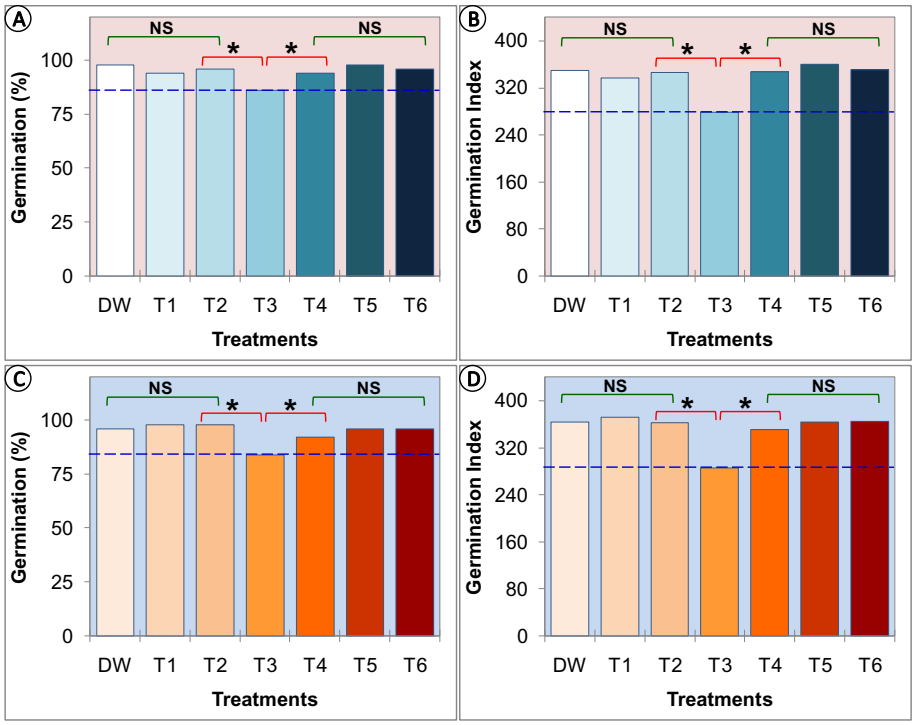

Fig. 5. Germination percentage and germination index of maize (A, B) and cowpea (C, D) in VC extracts. DW – double distilled water; * and NS - the difference between treatments is significant and not significant, respectively at $P < 0.05$ by ANOVA; The vertical line (–) shows the treatments with minimum values.

GP and GI ($P < 0.05$) (Fig. 5). The higher GI for the vermicomposts of PMS in combination with CD and green manure plants indicate that the vermicomposts are mature and less phytotoxic. Parallel seed germination studies have also been used to assess the maturity and phytotoxicity of vermicomposts of banana stem (Khatua et al., 2018).

4. Conclusion

The increase in major plant nutrients, TKN, N–NO_3^-, TP and TK, decrease in TOC, C/N, C/P and OMC related to that of enzymatic and microbiological activities in vermicompost of PMS, CD and TEP/GLS combinations in this study indicates that green manure plants, TEP and GLS amendment positively supported the enhanced bioconversion of PMS into vermicompost using *E. fetida*. The worm growth and reproductive activity in PMS, CD and TEP/GLS treatments are in support of the encouraged vermiconversion process. The PMS generated from paper industries in combination with CD and green manure plants TEP/GLS (50% PMS + 25% CD + 25% TEP/GLS, 2:1:1 ratio) can be used for enriched vermicompost production with *E. fetida,* which in turn helps sustainable utilization of PMS. The maturity of the vermicompost as indicated by the activities of the enzymes, DEH, UA, SPA and ALP corroborated with the seed germination assay using cowpea and maize highlights that the vermicompost is suitable for agricultural use. The results of the present study signify that the industry generated organic wastes like PMS and organic materials with poor nutrient contents can be utilized for vermicomposting by amending the green manure plants for 'nutrient rich' vermicompost production. Further studies with the amendment of green manure plants in vermiconversion systems may provide possible utilization of different organic materials for nutrient recovery in an eco-friendly manner. However, the changes in the heavy metal contents in PMS amended with CD and green manure plants need to be established.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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