Earthworm mediated nutrient recovery and sustainable management of rice weed Barnyard grass (Echinochloa crus-galli)

Renuka Gupta
JC Bose University of Science and Technology
Monika Mago
JC Bose University of Science and Technology
Dr. Vinod Kumar Garg (vinodkgarg@yahoo.com)
Central University of Punjab

Research Article

Keywords: Echinochloa crus-galli, vermicomposting, earthworm, respiration rate, feed stock, weed mass.

Posted Date: October 10th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2074716/v1

Abstract

Present research is an attempt to manage Barnyard grass *(Echinochloa crus-galli)*, common rice weed, sustainably by vermicomposting technology. *E. crus-galli* (EC) feed stocks were blended with cow dung in combination: 20:80, 40:60, 50:50, 60:40 and 80:20 with two controls 100% CD and 100% EC for 63 days. Earthworms could not grow well in higher percentage of EC. Total kjeldhal nitrogen, total available phosphorus and total potassium in final vermicomposts were in range of 13.6-21.5g/kg, 11.8-15.9g/kg and 20.1-27.6g/kg, respectively, after 63 days vermicomposting. Respiration rate (42–98 $mgCO_2\ kg^{-1}$ VC $48h^{-1}$) affirms vermicompost maturity and falls within the recommended limits (< 120 $mgCO_2\ kg^{-1}$ VC $48h^{-1}$). Vermicomposting also reduced the weed mass by 2.2–3.03 folds depicting effective feed decomposition. It was inferred that rice weed can be converted into nutrient embedded manure employing vermicomposting and can prove to be instrumental in facilitating management of *E .crus-galli* in a cost effective and environmentally modest manner.

Novelty Statement

Weeds constitute large quantities of phytobiomass which is considered as a waste and disposed in unsustainable manner. In this study Barnyard grass (a weed) biomass has been converted into value added manure using vermicomposting technology.

1. Introduction

Echinochloa crus-galli, commonly known as Barnyard grass, cock spur grass or water grass, is a semi-aquatic weed that belongs to family Poaceae and genus *Echinochloa* with 250 known species all over the world. Native to Europe and India, it is one of the world's ruinous weeds which have invaded grasslands, coastal forest areas, agricultural fields and roadsides of Asia, Australia and America (FAO, 2014). It was initially inducted for fodder in these regions. *E. crus-galli* weed is a persistent competitor of rice crop due to fast growth, rapid germinating ability, imitation of rice characteristics particularly during early growth and great plausible allelopathy (Zhang et al., 2017). In addition, it is considered among the top 15 herbicide resistant weeds, responsible for reducing rice yields (Yang et al., 2017) along with other crops like maize, soybean, lucerne, root crops, orchards (Holm et al, 1991). This annual grass about 1.5 cm in height has smooth, hairless and thickened culms with lateral branches, tufted stem and fibrous root system. With high invasiveness, super capacity to compete with rice, significant allelopathy and strong herbicide resistance, this weed is one of an important environmental invading species in India too.

While competing with rice, *E.crus-galli* produces 5000–7000 seeds that are capable of dispersion too far off places due to their more buoyancy than rice seeds (Barret and Wilson,1983), another aspect is that these seeds have different time of germination which relies on suitable environmental conditions. Therefore, its control becomes troublesome for agriculturalists and scientists. Besides using several control methods of clean cultivation, hand weeding, use of pathogens and use of allelopathy sound biological strategies are required to manage these weeds on site where they are produced. Earlier several researchers have employed different methods for managing aquatic, semi-aquatic and terrestrial weeds, viz., biogas production from *Ipomoea, Eicchornia* (Jain and Kalamdhad, 2018a), biopolymer production from *Parthenium* and *Eicchornia* (Pradhan et al., 2017), etc. Trending innovative alternative methods include use of weed biomass as feed material in composting and vermicomposting thus converting them to a valuable resource. Many vermicomposting studies have been carried out for some invasive weeds including water hyacinth (Gupta et al., 2007), *Parthenium hysterophorus* (Hussain et al., 2016) *Pistia stratiotes* (Suthar et al., 2017), *Hydrilla verticillata* (Jain and Kalamdhad, 2018b), *Lantana camara* (Devi and Khwairakpam, 2020a), *Ageratum conyzoides* (Gusain and Suthar, 2020a; Devi and Khwairakpam, 2020b), duck weed/*Spirodela* (Gusain and Suthar, 2020b), *Ipomoea* (Balachandran et al, 2020), etc. Vermicomposting is a bio-degradative process performed by earthworms jointly with microorganisms to convert waste materials into a stabilized vermicompost. Feedstock quality and earthworm species employed in vermi-conversion has a great impact on amount and quality of manure formed. Earthworm *Eisenia fetida* does a marvelous job of organic waste degradation due to its palatability for large variety of wastes, high fecundity, wide temperature tolerance and faster vermi-conversion. Incorporation of blending material in form of animal dung or any other substrate is pre-requisite for getting vermicompost of good manurial quality as well as to sustain vermicomposting. As per the available literature, no single study has been done on harnessing the potential of *E. crus-galli* weed in vermicomposting to provide a cost-effective and eco-friendly solution for its management. This perilous rice weed has been found in peri-urban areas (agricultural fields) of Faridabad city (Haryana, India) and despite control by burning, manual hoeing and herbicide use, its management is still a nuisance for local farmers. In the light of aforestated information, authors have made a small attempt to divert the attention of scientists and researchers towards sustainable management of rice weed Barnyard grass by earthworm mediated process – vermicomposting. Present investigation is probably the first ever study to vermi-transform *E. crus-galli* and recovering nutrients engaging earthworm *Eisenia fetida*. Several feed mix ratios were tested for suitability of weed *E. crus-galli* for vermi-conversion, increment in nutrient levels, effect on fecundity and earthworm growth, assessing maturity of vermicompost through evaluation of ash content, C: N & C: P ratios and respiration rate. This comprehensive study can lay a foundation for vermi-conversion of pernicious rice weed *E. crus-galli* and henceforth its subsequent management.

2. Materials And Methodology:

2.1. *Collection of earthworms, cow dung and weed E. crus-galli*

Earthworm *Eisenia fetida* was maintained as stock culture in lab conditions for use in the vermicomposting experiment. Fresh cow dung (urine free) was procured from a local dairy farm. Weed *E. crus-galli* (EC) was collected from agricultural fields, Faridabad city, Haryana (India). EC and CD were analyzed individually (Table 1) and in combination for their physicochemical characteristics - pH, electrical conductivity, organic matter (OM), ash content, total organic carbon (TOC), macronutrients - total kjeldahl nitrogen (TKN), total available phosphorus (TAP), total potassium (TK) and micronutrients (Total Fe, Mn, Zn, Cu & Pb).

Table 1
Initial Physico-chemical characteristics of Cow dung and *Echinochloa crus-galli*

Parameter	CD	EC
pH	8.14	6.76
EC (ds /m)	1.84	2.86
Ash content(g/kg)	228	272
TOC (g/kg)	447.7	422.2
OM (g/kg)	772	728
TKN (g/kg)	8.8	9.1
TP (g/kg)	7.3	5.32
TK (g/kg)	7.9	7.34
C:N ratio	50.8	46.3
C:P ratio	61.3	79.3
Moisture content	85	73
Fe(mg/kg)	1204	1372
Mn (mg/kg)	77.3	134
Zn (mg/kg)	91.2	19.7
Cu (mg/kg)	22.8	30.5
Pb(mg/kg)	31.6	28.4

2.2. *Experimental design and vermicomposting*:

Seven vermi-experiment set ups in combination of 1/4, 2/3, 1/1, 3/2 and 4/1 with two controls 100% CD and 100% EC were established in triplicates (Table 2). Each vermi-experiment contained one kg feedstock on dry weight basis. After three weeks of pre-decomposition, 20 earthworms (individual weighing ≈ 500–575 mg) from stock culture were added to each vermi-experiment maintained at an ambient temperature of 20–25°C. Experiment was carried out for 63 days with regular turning for maintaining aerobic conditions while regulating 60–70% moisture. Samples from each vermi-experiment was drawn, dried in air, finely grounded and placed in air tight vials for further evaluation. Physico-chemical parameters (on dry weight basis) and biological parameters (on fresh weight basis) of vermicomposts prepared from *E.crus-galli* and cow dung were analyzed.

Table 2
Composition and ratio of feed mix (Cow dung and *Echinochloa crus-galli*) in different vermi-experiments

Vermi Experiment (VE)	Nomenclature	Composition (EC%+CD%)	Ratio of feed mix
VE1	CD_{100}	100%	0/1
VE2	EC_{20}	20% +80%	1/4
VE3	EC_{40}	40% +60%	2/3
VE4	EC_{50}	50%+50%	1/1
VE5	EC_{60}	60% +40%	3/2
VE6	EC_{80}	80%+20%	4/1
VE7	EC_{100}	100%	1/0

2.3. *Physico-chemical analysis and vermicompost maturity indices*:

E. crus-galli (EC) and cow dung (CD) as well as feed mixtures (EC + CD) were evaluated for pH, electrical conductivity, organic matter, ash content, TOC, TKN, TAP, TK and micronutrients ((Total Fe, Mn, Zn, Cu & Pb) (Table 3). An extract of 5g feed waste sample with 50 ml distilled water (1w:10v) was prepared and filtrate obtained was analyzed for pH and electrical conductivity. Nelson and Sommers (1983) dry combustion method was adopted to evaluate ash content, organic matter and TOC content. Quantification of TKN, TAP and TK was done by Micro Kjeldahl method (Bremmer and Mulvaney, 1982), Ammonium Molybdate method (using UV-Vis spectrophotometer) and Flame photometric method, respectively. Micronutrient estimation was performed in diacid (9 nitric acid:1 perchloric acid) digested samples using ICP-MS (Thermo Scientific Model (iCAPQc). Ash content, C: N ratio, C: P ratio and soil respiration assessment were implied as indicators to assess maturity of vermicompost produced from weed *E. crus-galli* and cow dung mixture. TOC, TKN and TAP levels were used to find C: N and C: P ratios. Respiration rate reflects the rate of microbial decomposition indicative of vermicompost maturity. Vermicompost sample (10 g) was introduced into a closed gas jar having NaOH (10 ml) and kept in incubation for 48 hrs. Taking phenolphthalein as an indicator, NaOH solution was back titrated with $BaCl_2$ for estimation of CO_2 dissolved and measured as mg CO_2kg^{-1}VC48hr $^{-1}$ (Meng et al., 2017).

Table 3
Physico-chemical characteristics of initial feed mix (Day 0) and final vermicompost (Day 63) produced in different vermi-experiments

Vermi Experiment	VE 1 (CD_{100})		VE2 (EC_{20})		VE3 (EC_{40})		VE4 (EC_{50})		VE5 (EC_{60})		VE6 (EC_{80})	
Parameters	**Day 0**	**Day 63**	**Day 0**	**Day 63**	**Day 0**	**Day 63**	**Day 0**	**Day 63**	**Day 0**	**Day 63**	**Day 0**	**Day 63**
pH	8.0 ± 0.04e	7.5 ± 0.03d	7.7 ± 0.03c	7.2 ± 0.02b	7.8 ± 0.04d	7.21 ± 0.01b	7.9 ± 0.02f	7.31 ± 0.03c	7.6 ± 0.02b	7.05 ± 0.01a	7.3 ± 0.01a	6.9 ± 0.02a
Electrical Conductivity	1.87 ± 0.02c	3.17 ± 0.04b	1.54 ± 0.01a	3.02 ± 0.02a	1.72 ± 0.02b	3.26 ± 0.03c	2.12 ± 0.04d	3.8 ± 0.05d	2.23 ± 0.02e	3.84 ± 0.03d	2.58 ± 0.05f	3.96 ± 0.02d
Ash content	236 ± 2.8a	531.6 ± 3.7a	241 ± 2.1b	601.3 ± 3.8d	245 ± 2.02c	86.4 ± 4.2c	252 ± 1.6d	531.4 ± 2.1a	258 ± 1.8e	528.9 ± 2.3a	267 ± 3.2f	537.8 ± 2.6b
TOC (g/kg)	443 ± 5.2c	271.7 ± 3.4c	440 ± 4.5c	231.3 ± 2.7a	437.9 ± 3.8c	239.9 ± 2.9b	433.8 ± 2.6b	271.8 ± 1.8c	430.3 ± 1.9b	273.3 ± 1.6c	425.1 ± 1.65a	268.1 ± 1.2c
OM (g/kg)	764 ± 3.1e	468.4 ± 2.5d	758.5 ± 4.0d	398.7 ± 2.3a	755 ± 2.3d	413.6 ± 0.4b	748 ± 2.4c	468.6 ± 1.8d	742 ± 3.02b	471 ± 2.43e	733 ± 2.35a	462.2 ± 1.9c
TKN (g/kg)	8.6 ± 1.40a	13.6 ± 1.42a	12.8 ± 1.25c	21.5 ± 2.28f	11.2 ± 1.04 b	18.4 ± 2.21e	11.4 ± 1.87b	17.9 ± 2.10d	10.8 ± 1.02b	16.5 ± 2.03c	10.7 ± 0.09 b	15.9 ± 2.3b
TAP (g/kg)	7.5 ± 1.4a	12.8 ± 1.42a	7.8 ± 0.87a	15.2 ± 1.08c	8.1 ± 0.78a	14.8 ± 0.98b	8.8 ± 1.11a	15.9 ± 2.31c	8.9 ± 0.80 a	13.5 ± 1.23a	9.0 ± 0.56a	11.8 ± 0.98a
TK (g/kg)	7.9 ± 0.89a	21.3 ± 2.8 a	8.2 ± 0.88a	25.2 ± 2.14b	8.4 ± 1.04 a	27.6 ± 2.67b	8.6 ± 0.68a	23.3 ± 1.45a	8.8 ± 1.02a	22.2 ± 2.3a	8.9 ± 0.98 a	20.1 ± 1.45a
Electrical Conductivity (ds/m); Ash content (g/kg)												

2.4. *Earthworm growth parameters*:

Vermicompost samples were analyzed for biological characteristics as population buildup, live earthworm biomass, biomass gain, number of cocoons/worms, growth rate/worm and fecundity rate. Small heaps of wet samples were made, earthworm move towards the center from where these were counted by removing upper parts of heap. Worms were hand sorted into hatchlings and cocoons, washed in water and finally weighed on digital balance.

2.5. *Statistical analysis of data*:

IBM-SPSS 21 tool was used for statistical analysis of research data. All the parameters were analyzed using ANOVA and results were depicted as mean of triplicates and standard error of mean (mean ± SEM). Tukey's HSD was used as post-hoc tool for determining statistical significance of means among different vermi-experiments ($p < 0.05$).

3. Results And Discussion

Earthy brown, homogeneous and nutrient enriched vermicompost was prepared from all the feed mixes of EC + CD. Out of seven vermi-experiments, VE7 (EC_{100}) was unable to sustain earthworm population and resulted in mortality of worms; therefore, this setup was abandoned in the study. In present investigation, significant mass reduction in the range of 2.2–3.03 folds was recorded in all the feed mixes of weed *E.crus-galli* and cow dung with highest drop in mass levels of VE3 (EC_{40}).

3.1. *Changes in pH, Electrical conductivity and TOC*:

pH is an important parameter to decide the fate of organic waste degradation in vermicomposting process as it affects earthworm and microbial action. In the experiment, initially pH was within the range of 7.32–8.06 which tend to decrease in all the experiments near the completion of vermicomposting process. pH in final vermicomposts was within the range of 6.98–7.5 (Table 3). Difference among various vermi-experiments for pH change was statistically different for CD_{100}, EC_{20}, EC_{40} and EC_{50} (ANOVA; Tukey's HSD test, $p < 0.05$) whereas no significant difference was found in EC_{20} & EC_{30} and EC_{60} & EC_{80}. This shifting of pH may be attributed to conversion of ammonical nitrogen to nitrites and nitrates together with organic acids production as a result of combined action of earthworms and microbes (Ananthavalli et al., 2019). Ideal pH for mineralization and feed mix decomposition is in range of 7–8. Maximum percentage decrease (8.26%) was recorded in EC_{40} whereas least decrease (4.64%) in EC_{80} was observed (Fig. 1).

Electrical conductivity is reflective of vermicompost suitability and manure quality in plant application. A continuous increasing trend in electrical conductivity was found in all the vermi- experiments in the present study. The increase was from initial values of 1.54–2.58 dS/m to 3.02–3.96 dS/m in final vermicompost presented in Table 3 and percentage increase have been given in Fig. 1. The maximum allowable limits for electrical conductivity are considered as < 4 dS/m which is non-toxic for agronomic use and is one of the recommended organic compost quality standard (Yuvaraj, 2018). An increase in electrical conductivity can be owed to release of bound nutrients into more available forms along with soluble salts, production of ammonium and inorganic ions during the degradation of initial feed mix (Gupta and Garg, 2011; Awasthi et al., 2014). Decomposition of organic matter leads to production of ammonium, phosphate, and potassium ions thereby increasing electrical conductivity. Significant difference was observed among CD_{100}, EC_{20} & EC_{40} (ANOVA; Tukey's HSD test, $p < 0.05$) and EC_{50} & EC_{60}, but latter are statistically indifferent from each other ($p > 0.05$). Increase in electrical conductivity has been also reported in earlier literature studies.

TOC reduction marks the decomposition of organic matter in vermicomposting process as organic carbon is consumed by microbes and earthworms as a part of their respiratory processes. Yuvaraj et al (2018) attributed reduction of TOC in vermicompost to combined action of microbes and earthworms, organic carbon assimilation into biomass of earthworms and CO_2 evolution in the decomposition process. In the present study, TOC decreased from 425.1- 443.3 g/kg to 231.3- 273.8 g/kg in final vermicomposts (Table 3). There was 36.4–47.4% reduction in different vermi-experiments towards the completion of process. TOC reduction was also reported in earlier studies and in present study it was found to be more than 24.34–38.25% (Gusain and Suthar, 2020b), 20.7–30.7% (Devi and Khwairakpam, 2020a), 27.3–35.3% (Devi and Khwairakpam, 2020b) and lower than 69.8–78.5% (Boruah et al., 2019). Difference between VE1(CD_{100}), VE2 (EC_{20}) and VE3 (EC_{40}) was statistically significant (ANOVA; Tukey's HSD test, $p < 0.05$) whereas difference in VE4 (EC_{50}), VE5 (EC_{60}) and VE6 (EC_{80}) was not significant for TOC reduction.

3.2. *TKN, TAP and TK profile*:

TKN values enhanced in all the vermi-experiments from initial levels of 8.6–12.8 g/kg to 13.6–21.5 g/kg in final vermicompost at the end of experiment (Table 3). Difference among different vermi-experiments was statistically significant for TKN increase (ANOVA; Tukey's HSD test, $p < 0.05$). Profound increase of 48.5–67.9% recorded in vermi-experiments is indicative of high nitrogen mineralization in feed mixes of EC + CD. Highest increment was observed in VE2 (EC_{20}) and least ascend was found in VE7 (EC_{80}) irrespective of initial contents. It followed the trend EC_{20} (67.9%) > EC_{40} (64.2%) > CD_{100} (58.1%) > EC_{60} (57.0%) > EC_{50} (52.7%) > EC_{80} (48.5%). Increment in TKN can be due to organic matter mineralization and addition of mucus and nitrogenous excretory substances in vermicompost and depends upon initial nitrogen content in feed mix along with decomposition rate. Gusain and Suthar (2020a) documented 59.6–69.9% increase in nitrogen content for weed *Ageratum* combined with cow dung. Increase in nitrogen was also reported by other researchers previously (Jain and Kalamdhad, 2019)

An increase in TAP was recorded in all vermi-experiments up to different extents. Initially, TAP values varied from 7.5- 9.0 g/kg and enhanced to 11.8–15.9 g/kg towards the completion of process (Table 3). Augmentation in TAP values can be attributed to combined activity of microbes and earthworms along with phosphatase enzyme and phosphate solubilizing bacteria thus helping in solubilization of organically bound phosphorus into soluble forms (Ananthavalli et al., 2019). An increase of 31.1–94.8% was recorded in CD + EC combinations. Decomposition of organic matter converts phosphorus in its soluble form via action of organic acids formed in initial feed mix. The variation in different vermi-experiments can be owed to initial phosphorus content in feed mix as well as different earthworm activities. Difference among VE1 (CD_{100}), VE2 (EC_{20}) & VE3 (EC_{40}) was statistically significant (ANOVA; Tukey's HSD test, $p < 0.05$) whereas VE4 (EC_{50}), VE5 (EC_{60}) & VE6 (EC_{80}) were insignificantly different from each other with respect to phosphorus content.

TK content enhanced significantly in all the vermi-experiments and found higher in vermicomposts (20.1–27.6 g/kg) prepared from different combinations of EC + CD when compared with initial values (7.9–8.9 g/kg) (Table 3). A 2.25–3.28 fold increase was recorded in TK in the end products which may be attributed to efficient joint activity of microbes and earthworms that resulted in effective waste degradation. Organic matter reduction and release of potassium enhanced levels of potassium in final vermicompost. Difference in vermi-experiments for potassium increase can be due to variation in organic matter mineralization rates and loss of organic matter via CO_2 respiration (Zhang and Sun, 2014). Potassium values were not statistically different from each other ($p > 0.05$) although VE2 (EC_{20}) and VE3 (EC_{40}) (statistically indifferent from each other) were significantly different from rest of the vermi-experiments.

3.3. *Changes in micronutrient contents*:

Micro-nutrients (Fe, Mn, Zn, Cu, Pb) are essential for plant growth at low levels and can hinder fertility of soil affecting the plant growth at high levels preceded by bioaccumulation paving their way into food chain. Thereby, it becomes important to substantiate the concentration of these micro-nutrients/trace metals before agricultural application. Vermicomposting may increase or decrease the level of trace metals. It can be either due to mass / volume reduction (Malinska et al., 2016), organic matter degradation (Rai et al., 2021), liberation of free metal ions from organically bound state (Song et al., 2014), adherence/chelation of metals to humic acids limiting their reduction (Zhu et al., 2014) or concentration of trace metals by earthworms (Liu et al., 2012).

Previous studies have reported increase as well as decrease in micronutrients for above mentioned reasons. In the present study, EC + CD vermicomposts have shown an increment in micronutrients (Fe, Mn, Zn, Cu, Pb) in comparison to initial feed mix in all the vermi-experiments in spite of foremost values and feed mix composition (Table 4). Fe content in the feed mix was in the range of 1212–1287 mg/kg and escalated to 1342–1520 mg/kg in final vermicomposts. There was 9.58–12.25% increase in Fe content in vermicomposts obtained from different compositions. There was significant variation among different vermi-experiments for increase in Fe content (ANOVA; Tukey's HSD, $p < 0.05$). Total Mn levels raised from initial values of 80.2–141 mg/kg to final values of 275.2- 343.2 mg/kg and showed an increase of 123.5- 243.1%. No statistical significant difference was recorded for increase in Mn levels among different vermi-experiments ($p > 0.05$). Zn showed an increment over initial content (21.7–93.5 mg/kg) and was found in the range of 214.9–291.4 mg/kg in final vermicomposts. An increase of 96.2- 234.3% in Cu content was recorded for all the combinations of EC + CD. Both Zn and Cu levels showed significant difference in final vermicomposts (ANOVA; Tukey's HSD, $p < 0.05$). Initially Pb was in the range of 30.2–33.5 mg/kg and was found to be enhanced (51.7–67.2 mg/kg) in final vermicomposts with no significant difference among the vermi-experiments ($p > 0.05$). Although there was escalation in all the micronutrients at the end of the process, vermicomposts produced from *E.crus-galli* and cow dung mix were within the acceptable limits for composts (Brinton, 2000) and is stipulated for use in agronomic and horticultural purposes .

Table 4
Micronutrient contents in initial feed mix and final vermicompost in different vermi-experiments

Vermi Experiment	**VE 1 (CD_{100})**		**VE2 (EC_{20})**		**VE3 (EC_{40})**		**VE4 (EC_{50})**		**VE5 (EC_{60})**		**VE6 (EC_{80})**	
Micro-nutrients	**Day 0**	**Day 63**	**Day 0**	**Day 63**	**Day 0**	**Day 63**	**Day 0**	**Day 63**	**Day 0**	**Day 63**	**Day 0**	**Day 63**
Fe (mg/kg)	1212 ± 8.5a	1342 ± 6.4a	1245 ± 6.8b	1395 ± 7.2b	1287 ± 7.8c	1440 ± 5.9c	1322 ± 5.7d	1484 ± 4.4d	1356 ± 6.8e	1512 ± 6.1e	1387 ± 5.6f	1520 ± 4.8f
Mn (mg/kg)	80.2 ± 4.2a	275.2 ± 5.4a	92.4 ± 4.8b	316.4 ± 5.8b	109.0 ± 5.1c	323.5 ± 6.4c	118.2 ± 4.8d	343.2 ± 3.1d	130.4 ± 4.1e	334.4 ± 5.6c	141 ± 3.89f	315.2 ± 4.2 b
Zn (mg/kg)	93.5 ± 2.56f	220.5 ± 3.0b	79.9 ± 4.1 e	214.9 ± 4.2a	65.4 ± 3.4d	291.4 ± 3.8f	44.8 ± 2.65c	242.8 ± 2.1c	31.0 ± 1.01 b	251.0 ± 1.8d	21.7 ± 1.02a	262.7 ± 3.2e
Cu (mg/kg)	23.9 ± 2.1a	46.9 ± 2.2a	25.8 ± 1.7a	56.8 ± 2.2b	26.9 ± 1.9a	72.9 ± 2.3c	28.8 ± 1.15a	88.8 ± 2.6d	30.6 ± 1.3b	102.3 ± 2.5e	32.4 ± 1.02b	91.0 ± 2.04d
Pb (mg/kg)	33.5 ± 1.03a	51.7 ± 1.7a	32.9 ± 0.8a	52.3 ± 0.16a	32.1 ± 1.1a	55.8 ± 2.0ab	31.7 ± 0.9a	57.4 ± 1.0b	30.8 ± 1.10a	63.7 ± 2.1c	30.2 ± 0.8a	67.2 ± 0.6d

3.4. *Vermicompost stability and maturity indices*:

3.4.1. *Ash content*:

Ash content enhanced after 63 days of vermicomposting in vermi-experiments from their initial levels. Incremental change in ash content is suggestive of vermicompost maturity due to effective degradation and mineralization of feed mixture and conversion of organic materials into inorganic forms. Initially ash contents varied between 236–267 g/kg and raised to 528.9-601.3 g/kg as the process was completed (Table 3). Percent increase in ash content was highest in EC_{20} (149.5%) and least in EC_{80} (101.4%). Order of percent increase was: EC_{20} (149.5%) > EC_{40} (139.3%) > CD_{100} (125.5%) > EC_{50} (110.8%) > EC_{60} (105%) > EC_{80} (101.4%).

More is the rate of organic matter decomposition due to surplus amount of feed mix available, higher the increase in ash levels at the completion of vermicomposting. Remarkable increase in ash content was also recorded by earlier researchers (Jain et al, 2018) but lower than present findings. About 57.49–67.33% increase was recorded in *Ageratum* and CD mixtures (Gusain and Suthar, 2020a) that is lower than EC + CD mix in present investigation. Statistically significant difference for ash content was observed in VE1(CD_{100}), VE2 (EC_{20}) and VE3 (EC_{40}) (ANOVA; Tukey's HSD test, $p < 0.05$), whereas VE4 (EC_{50}), VE5 (EC_{60}), and VE6 (EC_{80}) were not statistically different from each other.

3.4.2. *C: N and C: P ratio*

C: N and C: P ratios are important index of vermicompost stability/ maturity and establish its use for agronomic purposes. The C: N ratio decreased in all the vermi-experiments at completion of vermicompost process. C: N decrease may be attributed to increased nitrogen levels and reduced organic matter content. Microbial respiration results in loss of carbon due to combined degradative action of microbes and earthworms as well as greater stabilization and mineralization of feed mix (Esmaeili et al., 2020). The C: N ratio of the final vermicompost is also influenced by nitrogen and carbon content of initial feed mix. It decreased from initial range of 34.3–51.5 to 10.7–19.9 in all vermi-experiments after 63 days cycle of vermicomposting (Fig. 2a). The vermi-experiments VE2 (EC_{20}), VE3 (EC_{40}) and VE4 (EC_{50}) had final C: N ratio below 15 whereas C: N ratio < 20 was observed for VE1(CD_{100}), VE5 (EC_{60}) and VE6 (EC_{80}) suggesting the use of *E. crus-galli* as feed mix for earthworms to have mature vermicompost. C: N values varied significantly among different vermi-experiments (ANOVA; Tukey's HSD test, $p < 0.05$). Present findings can be correlated with the previous studies (Devi and Khwairakpam, 2020a) which recorded 62% decrease in C:N value. C:N ratio for *Ipomoea* weed varied between 16.5–23.6 in final vermicomposts (Balachandar et al., 2020); 9.35–18.92 for

Ageratum and cowdung mix (Gusain and Suthar, 2020a) and 9.90–23.84 for *duck weed* (Gusain and Suthar, 2020b). Percent decrease in C:N ratio from their initial levels varied between 57.5–68.8% in final vermicompost. The C:N ratio in the final product must be ≤ 20 for agronomic application and reflects vermicompost stability (CPHEEO, 2016) whereas mature compost with C:N < 15 is considered most preferable (Boruah et al., 2019).

C:P ratio showed decreasing trend in all vermi-experiments due to decline in TOC that can be owed to combined action of microbes and earthworms as a part of their respiratory and assimilatory activities. Initially, C:P ratios ranged from 47.2–59.0 in different vermi-experiments that varied between 15.2–22.7 towards the completion of vermicomposting process (Fig. 2b). Decrease in C:P might be due to loss of carbon and organic matter stabilization in different feed mixes of EC + CD and continuous release of available phosphorus from binded forms to mobile forms (Biruntha et al., 2019). There was 51.8- 73.03% decrease in C: P levels in final vermicompost. Significant difference was observed between VE2 (EC_{20}), VE3 (EC_{40}) and VE4 (EC_{50}) ($P < 0.05$) but VE1 (CD_{100}), VE5 (EC_{60}) and VE6 (EC_{80}) did not differ significantly ($P > 0.05$) for overall decrease in C: P ratio.

3.4.3. *Respiration rate assessment ($mgCO_2\ kg^{-1}$ VC $48h^{-1}$)*

Respiration rate is reflective of high microbial activity during vermicomposting period, marks the completion of decomposition process and is taken as an important index of vermicompost maturity. Respiration rate is measured as $mgCO_2\ kg^{-1}$ VC $48h^{-1}$. Earthworms fasten the growth and activity of microorganisms in feed mix that enhances the rate of respiration as the process of degradation initiates. The respiration rate instigated in all the vermi-experiments due to readily available organic materials for microbial action at the onset. Highest respiration rate ($mgCO_2\ kg^{-1}$ VC $48h^{-1}$) was recorded on 36th day: 587 (EC_{20}); 545 (EC_{40}); 513 (CD_{100}); 486 (EC_{50}), 410 (EC_{60}) and 317 (EC_{80}) (Fig. 2c). Vast carbon pool in the feed mix acts as a decent source of energy to activate microbial respiration, hence accelerates rate of respiration. As soon as the carbon source depletes due to consumption of feed materials by population of microbes, respiration rate declines towards the end. Final respiration rates after 63 days were in the permissible levels of 120 $mgCO_2\ kg^{-1}$VC $48h^{-1}$ (Hue and Liu, 1995) and ranged from 42–98 $mgCO_2\ kg^{-1}$ VC $48h^{-1}$ which marked vermicompost maturity in the finished products. Significant variation was observed in all the vermi-experiments for respiration rates (ANOVA, Tukey's HSD test, $p < 0.05$) Present results corroborates with earlier findings. Gusain and Suthar (2020a) had reported respiration rates of 34-70.1 $mgCO_2\ kg^{-1}$ VC $48h^{-1}$ in weed Ageratum and 61–100 $mgCO_2\ kg^{-1}$ VC $48h^{-1}$ in duckweed rich vermicomposts (Gusain and Suthar, 2020b)

3.5. *Changes in Earthworm growth and fecundity in EC + CD mix*

Earthworm population indicates potentiality of feed mix used in vermicomposting and is affected by feed acclimatization, earthworm growth in feed environment and fecundity. Final earthworm population at the completion showed the following trend: EC_{40} (126) > EC_{20} (118) > CD_{100} (108) > EC_{50} (98) > EC_{60} (74) > EC_{80} (50) and found to be significantly different from each other. Initial live earthworm biomass was in the range of 545–575 mg/worm and increased to 1136–1336 mg/worm after 63 days of vermicomposting (Table 5). It was inferred that blending of weed *E. crus-galli* with cow dung enhanced the digestibility of feed mix by the earthworms and thus boosted earthworm biomass up to different extents. Highest live biomass among different feed mixes followed the order: EC_{40} (1336) > EC_{20} (1299 mg) > CD_{100} (1288mg) > EC_{50} (1258 mg) > EC_{60} (1202mg) > EC_{80} (1136mg). Maximum increase of 2.40 folds was recorded in EC_{40} followed by EC_{20} and CD_{100} with 2.36 folds increase in biomass. Difference between vermi-experiments was statistically significant for earthworm biomass gain ($p < 0.05$). No. of cocoons produced in different vermi-experiments followed the order: EC_{40} (435 ± 0.52) > EC_{20} (400 ± 0.42) > CD_{100} (336 ± 0.41) > EC_{50} (256 ± 0.34) > EC_{60} (100 ± 0.26) > EC_{80} (63 ± 0.11). The values in all the vermi-experiments were significantly different from each other ($p < 0.05$). Presence of nutrients and organic matter in EC + CD mix has supported good earthworm growth and cocoon production. The earthworm growth is suggestive of utilization of weed *E. crus-galli* as feed mix for vermicompost production from different proportions. Fecundity rate (cocoon/worm) was highest (3.45 ± 0.05) in 40% EC than other combinations and least in 80% EC (Table 5). Fecundity of earthworms in cow dung and *E.crus-galli* feed mix were significantly different from each other ($p < 0.05$). Earthworm growth rate was found between 11.44–18.57 mg/worm/day in the present study. The earthworm growth and fecundity suggested that *E.crus-galli* can be efficiently utilized as feed material for earthworms when amended in suitable proportions.

Table 5
Biological characteristics of initial feed mix and final vermicompost in different vermi-experiments

Vermi Experiment / Biological Parameters	VE 1 (CD_{100})	VE2 (EC_{20})	VE3 (EC_{40})	VE4 (EC_{50})	VE5 (EC_{60})	VE6 (EC_{80})
Earthworm population (Initial feed mix)	20	20	20	20	20	20
Earthworm population (Final vermicompost)	108 ± 1.32d	118 ± 1.72e	126 ± 1.56f	98 ± 1.45c	74 ± 1.32b	50 ± 1.23a
Live earthworm biomass (mg) (Initial feed mix)	545 ± 2.56a	549 ± 2.87b	556 ± 3.12c	565 ± 3.52d	572 ± 3.75e	575 ± 3.91e
Maximum Live earthworm biomass (mg) (Final vermicompost)	1288 ± 3.97d	1299 ± 4.11e	1336 ± 4.02f	1258 ± 3.92c	1202 ± 3.45b	1136 ± 3.65a
Net Biomass gain (mg)	743 ± 3.14d	750 ± 3.21e	780 ± 3.6f	693 ± 2.91c	630 ± 2.88b	561 ± 2.68a
No. of cocoons	336 ± 0.41d	400 ± 0.42e	435 ± 0.52f	256 ± 0.34c	100 ± 0.26b	63 ± 0.11a
Fecundity(cocoon/worm)	3.11 ± 0.04d	3.38 ± 0.03e	3.45 ± 0.05f	2.61 ± 0.03c	1.35 ± 0.01b	1.31 ± 0.01a
Maximum Biomass achieved (days)	42	42	42	42	49	49
Growth rate/worm/day (mg)	17.69 ± 0.03d	17.85 ± 0.04e	18.57 ± 0.04f	16.50 ± 0.03c	12.85 ± 0.02b	11.44 ± 0.02a

4. Conclusions

Rice weed *E.crus-galli* was found to be a potential substrate for earthworms. Present findings imply that vermicompost prepared from 20–80% of weed blended with cow dung has shown a significant increment in TKN, TAP, TK and micronutrients in all the vermi-experiments with exception of 100% EC which showed earthworm mortality. The most efficient proportions were 20–50% EC with highest fecundity and earthworm growth in 40% weed mass. All the mixing ratios have performed very well and met compost quality standards i.e. pH: 6.5–7.5, EC < 4ds/m, TKN > 0.8%, TAP > 0.6%, TK > 0.4%, C:N ratio < 20 (CPHEEO, 2016) for agronomic use. Respiration rate was found to be 42–98 $mgCO_2\ kg^{-1}$ VC $48h^{-1}$ that was well within the recommended limits. Thus vermicomposting has demonstrated its likeliness for entire usage and on site management of *E.crus-galli* in a sustainable way.

Declarations

Ethical approval: No prior ethical approval was necessary for the study.

Informed consent: No human subjects included in the study. Consent not required.

Declaration of Potential conflicts of Interests: The authors declare no competing interests.

Funding: No funding available.

Availability of data: The authors have included all the relevant data and the source of freely available data in the manuscript.

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Figures

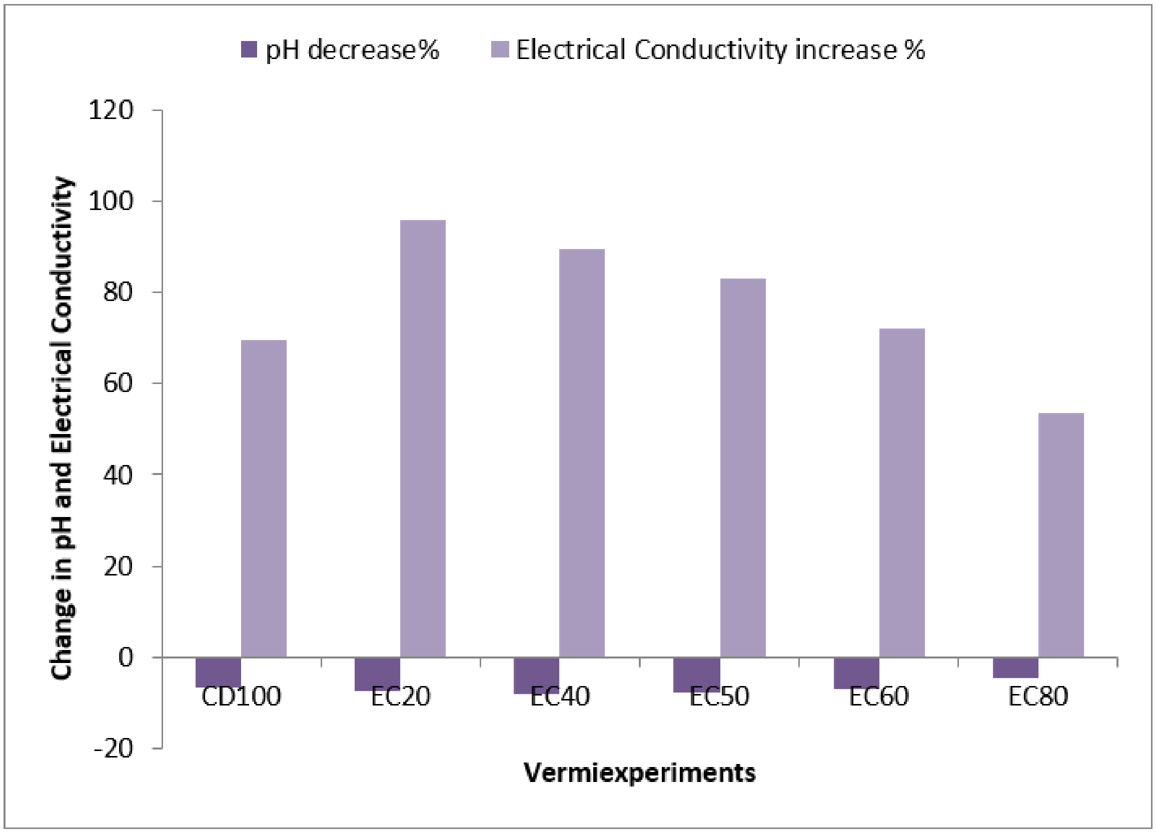
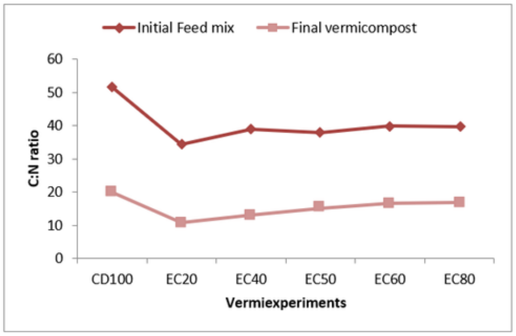
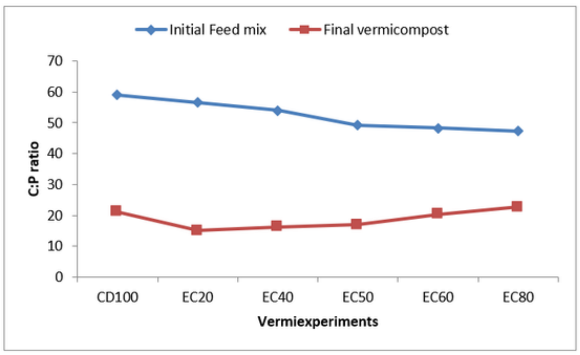


Figure 1

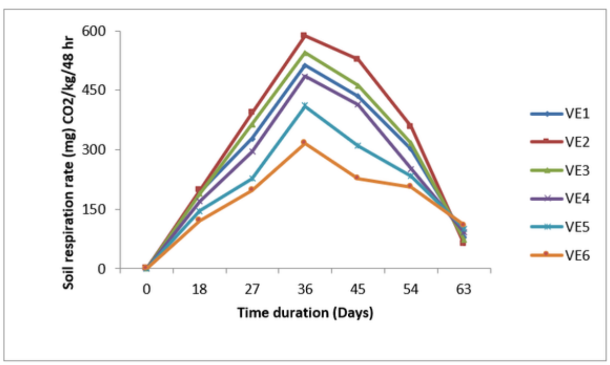
Changes in pH and electrical conductivity in different vermi-experiments



(a)



(b)



(c)

Figure 2

(a) C: N ratio (b) C: P ratio in initial feed mix and final vermicomposts (c) Changes in Respiration rates (mgCO_2 kg^{-1} VC $48h^{-1}$) with time