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Unveiling the Efficiency of Psychrophillic *Aporrectodea caliginosa* in Deciphering the Nutrients from Dalweed and Cow Manure with Bio-Optimization of Coprolites

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Abstract: There is an immense demand for vermicomposting employing psychrophilic vermiculture (*Aporrectodea caliginosa*) for management of wastes under the Himalayan ecosystem. Dalweed (weeds from the world-famous urban Dal Lake) and cow manure (CM) are cheaply and abundantly available bio resources in Kashmir valley. Dalweed (DW), disposed of in the heart of the city, ascribes unpleasant effects on tourism and the natural ecosystem. Initial substrate mixtures of DW and CM with different ratios (CM_{100}, DW_{100}, CM_{80}:DW_{20}, CM_{60}:DW_{40}, CM_{40}:DW_{60} and CM_{20}:DW_{80}) and castings harvested were analyzed for the following parameters: pH, TOC, TN, NO_3^- P, K, Fe, Zn, C:N, C:P, and C:S ratio. The results of a 56day study revealed in consistency and disparity towards the bio-optimization of coprolites depending upon the type of waste residue and mixture ratio used. Treatments with medium to low dalweed residues (CM_{60}:DW_{40} followed by CM_{80}:DW_{20}) were found to be optimum and significantly primed chemical properties of castings using *A. caligenosa*. C:N, C:P, and C:S ratios showed a non-linear response with maximum decrease in C:N ratio by 35%, C:P ratio by 38% in CM_{100}, and C:S ratio by 67% in DW_{100}. Humification ratio, humification index, and percent humic acids were changed across all the treatments with the highest respective values of 21.33 ± 1.05, 11.33 ± 0.76, and 47.83 ± 0.76 for CM_{60}:DW_{40}. Results also showed that the earthworm population and biomass significantly increased with the highest respective increments of 57.53% and 74.88% in CM_{60}:DW_{40} over initial values. Moreover, the highest number of cocoons (95.67 ± 1.17) were recorded within CM_{60}:DW_{40} and the lowest in the control (43.33 ± 1.53). Dehydrogenase and fluorescein diacetate activities were inconsistent with the highest in CM_{40}:DW_{60} (64.64%) and CM_{20}:DW_{80} (63.54%) respectively over the initial substrates, while highest urease activity (74.40%) was observed from CM_{100}. The results highlight the role of *A. caliginosa* in sustainable transformation of CM and DW with insightful, beneficial, and priming impacts on castings for its agronomic value.

Keywords: *Aporrectodea caliginosa*; dalweed; cow manure; cold tolerant; nutrient recovery; reproductive performance

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1. Introduction

Kashmir, a Himalayan region and world tourist destination owing to its scenic beauty, eco-climate, urban (Dal Lake), and rural (Wular, Manasbal) lakes, is the backbone of local economy and ecotourism. Dal Lake is situated in the heart of Srinagar city (latitude 34°18′ N longitude 74°91′ E) of the state of Jammu and Kashmir (J&K). Dalweed (DW), or Dal lake weeds, are a diverse group of aquatic weed flora comprising *Nelumbo nucifera*, *Lemna minor, Azolla sp., Salvinia cuculata, Trapa natans, Potamogetton crispus, Potamogetton-lucens, Pistia sp. Hydrilla verticillata, Ceratophyllum demersum, Myriophyllum spicatum, and Myriophyllum verticillatum* [1]. Rapid urbanization, traditional tourism, and commerce have increased the rate and magnitude of these weed species and led to several fold increases which exacerbated the detriment to the native biodiversity and ecosystem of the world-famous Dal Lake of Kashmir. The death of aquatic weeds (*Nelumbo nucifera, Salvinia cuculata)* brings out the accumulation of debris at the bottom and threatens the aquatic ecosystem by creating shallow water, diminishing dissolved oxygen, and serving as a vector for human diseases [2]. DW proliferates abundantly and is difficult to manage due to its rapid underwater multiplication. Mechanical eradication is a year-round process leaving an abundance of weed biomass on roadsides in the city. Landfilling of weed biomass is hazardous because it produces gas emissions and leachates which cause substantial damage to ecosystems and biodiversity. Moreover, lifting the DW from the Dal Lake raises the cost of disposal to cover thewide distance of the landfill at Shalimar. Annually, a hundred thousand cubic meters of mixed fresh weed biomass are removed from the Dal Lake by de-weeding harvesters [3]. Aquatic weeds (*Nelumbo nucifera, Azolla, Salviniacuculata*) produce a number of important secondary metabolites like alkaloids, flavonoids, steroids, triterpenoids, glycosides, and polyphenols [4] and are rich in lipids, carbohydrates, proteins, and minerals [5,6]. Earthworms kept in media *Nelumbo* leaves for 30 days showed increased body weight and produced relatively more cocoons [7]. Recycling of aquatic weeds can solve disposal issues [8]), and enhances their agronomic value with the building up of earthworms (biomass, hatchlings, and cocoons) [9]. Traditionally, cow manure is regarded as an important resource for sustainable rural agriculture [10,11] and a primary substrate for vermicomposting [12,13]. There has been a rapid increase in cattle farming as a pivotal source of livelihood among Himalayan farmers. Evoked cattle population has resulted in abundant generation of cattle manure among Himalayan agriculture communities. Mishandling and improper disposal of cattle dung among rural areas generates serious environmental pollution and human health hazards [14], with nutrient (N) losses of 800 kg ha^{-1} per year through different routes [15] Cow manure is rich in carbon and nutrients, and mismanagement may lead to greenhouse gas emissions (GHGs) and hypertrophication [16–18] (Nevertheless, cattle manure occupies an irreplaceable position in compost production with other organic wastes [10]. CM-based vermicomposting enhances growth and reproduction of earthworms and microbials leading to bio-stabilized nutrient rich vermifertilizer [13,14,19]. Vermicomposting, a bioecologically sustainable technology [20]), has been regarded as an ecofriendly alternative for solid waste recycling [21]. Vermitechnology causes environmental sustainability by minimizing the pollution risks associated with bio wastes and generating castings which act as a conditioner for soil and plant growth [14]). *Aporrectodea caliginosa* (endogeic) strongly influence the degradation of organic residue and nutrient cycling [22] with logical evidence of cold tolerance [23]. The present work can contribute to our understanding about the potential of CM and DW towards dynamics and recovery of nutrients by employing indigenous psychrophilic *A. caligenosa*. In addition, results can guide waste managers, agronomists, environmentalists, and policy makers with emissions and nutrients-recycling options for a better environment. Therefore, the study was carried out with the following objectives: (i) to investigate the influence of variable mixed combinations of CM and DW focusing on bio-optimization of the end product, and (ii) to evaluate the effect of waste mixtures on reproductive performance of *A. caligenosa* (biomass, cocoon production).

2. Material and Methods

2.1. Earthworms and Raw Material

The experiment was conducted under protected conditions at Mountain Extension and Research Institute, Izmarg-Guraz (74.73° E, 34.64° N) of District Bandipora of Jammu and Kashmir, India. The annual average temperature and precipitation of the location are 15.6 °C and 2200 mm, respectively. Geophagous earthworms, *Aporrectodea caliginosa* (endogeic), were obtained from the biowaste conversion unit at the farmer's field in Chorwan, Guraz, where it had been cultured for the last 3 years. Initially, this species was explored from different cold habitats of the Guraz valley under the project National Mission on Himalayan Research supported by the Ministry of Environment, Government of India, New Delhi. Fresh samples of DW were collected from the dumping site at Shalimar, while CM was taken from the livestock dairy unit (Bakerwal cows) located near the experimental station.

2.2. Experimental Set Up

CM was mixed with DW at different ratios on w/w basis and air dried to a moisture content of 25%. After blending the raw material thoroughly, they were placed into the plastic troughs (polyvinylchloride–PVC) with the dimensions 45 cm × 30 cm × 10 cm. Different substrate mixtures were allowed pre-decomposition for 2 weeks with optimum moisture to soften the materials and to eliminate the volatile toxic gases and uric acid of cow manure [24]. Treatment substrates (2 kg) of CM and DW in triplicates were formulated in different ratios with corresponding control as shown in Table 1 and their C:N ratio was maintained to 25–30 for initiation of the composting process (Table 2). The earthworms were adapted to the experimental conditions by placing them in the troughs filled with feeding material for 24 h. Afterwards, the earthworms were brought out and washed properly with water to remove adhesive residue and mucus before being inoculated into the experimental troughs. In the present study, 80 earthworms with an average body weight of 0.5 g each were allocated to each trough for the composting process. The troughs were placed under ambient light, with an average air temperature and relative humidity of 16.8 ± 1.5 °C and 67 ± 4%, respectively, to ensure uniform microclimatic conditions during the course of the experiment. Surface vermicast samples of 10 g were collected from each trough and placed into petri dishes with moistened filter paper for further analysis.

Table 1. Treatment combinations of cow manure and dalweed.

S.No.	Treatments	Composition
1.	CM_{100}	Cow manure = 2.0 kg (control)
2.	DW_{100}	Dal weed = 2.0 kg (control)
3.	CM_{80}:DW_{20}	CM_{80} = Cow Manure 1.60 kg + DW_{20} = Dal weed 0.40 kg
4.	CM_{60}:DW_{40}	CM_{60} = Cow Manure 1.20 kg + DW_{40} = Dal weed 0.80 kg
5.	CM_{40}:DW_{60}	CM_{80} = Cow Manure 0.80 kg + DW_{20} = Dal weed 1.20 kg
6.	CM_{20}:DW_{80}	CM_{80} = Cow Manure 0.40 kg + DW_{20} = Dal weed 1.60 kg

Table 2. Chemical properties of castings as influenced by various substrate ratios of CM and DW.

Treatments	pH	TOC (%)	TP (%)	NO_3^- (%)	TN (%)	TK (%)	Fe (%)	Zn (%)
CM_{100}(Control)	7.000 ^{a}	21.667 ^{a}	0.710 ^{ab}	0.213 ^{b}	3.167 ^{a}	1.333 ^{a}	0.097 ^{a}	0.063 ^{a}
DW_{100} (Control)	7.400 ^{a}	14.067 ^{d}	0.510 ^{b}	0.190 ^{b}	1.900 ^{c}	0.467 ^{d}	0.063 ^{bc}	0.028 ^{b}
CM_{80}:DW_{20}	6.867 ^{a}	19.533 ^{ab}	0.747 ^{ab}	0.297 ^{a}	3.200 ^{a}	1.060 ^{ab}	0.087 ^{ab}	0.051 ^{ab}
CM_{60}:DW_{40}	7.133 ^{a}	18.200 ^{bc}	0.847 ^{a}	0.273 ^{a}	2.867 ^{ab}	0.927 ^{bc}	0.098 ^{a}	0.051 ^{ab}
CM_{40}:DW_{60}	7.433 ^{a}	16.500 ^{cd}	0.613 ^{ab}	0.187 ^{b}	2.503 ^{abc}	0.860 ^{bc}	0.060 ^{c}	0.046 ^{ab}
CM_{20}:DW_{80}	7.300 ^{a}	16.267 ^{cd}	0.510 ^{b}	0.173 ^{b}	2.283 ^{bc}	0.687 ^{cd}	0.060 ^{c}	0.036 ^{b}

Means with different superscripts differ significantly ($p < 0.05$).

2.3. Chemical Properties of Coprolites

Vermicast samples collected in triplicates at the end of the experiment were analyzed for pH (determined in 1:5, sample: water ratio) by a glass electrode pH meter; C, N, and S content were determined by the CHNS analyzer (FLASH EA 1112 series); total N [25]; NO_3^- (cadmium reduction method). Phosphorus and potassium were quantified by the procedure of [26] through flam photometer-128 (Systronics) after assimilating samples in diacid suspension ($HClO_4$:HNO_3 in the ratio of 4:1). The micronutrients were analyzed by an atomic absorption spectrophotometer. Table 2 lists the initial chemical properties and nutrient status of substrates mixtures. Percent increase–decrease among chemical parameters relative to respective control substrates (CM and DW) was calculated [27].

2.4. Coprolite Enzyme Analysis

Samples of vermicast were also subjected to differential analysis for micro flora using the method as described by [28]. Comparative and diversity enumeration of isolates were determined. Microbial activity was evaluated by determining dehydrogenase (DH) activity using the triphenyl tetrazolium chloride (TTCM) method [29]. Fluorescein diacetate activity (FDA) was determined bythe method of [30]. Urease activity was assayed by determining NH_4^+ release from the organic wastes based on the method described by [31].

2.5. Humification Indices

Analysis of humic substance (humus ratio, humic index, percent humus) in vermicast was carried out by the method as described by [32]. A 0.1 mol L^{-1} NaOH solution was used in the ratio of 2:4 (*w/v*) and left on a mechanical shaker for 5 h. Afterwards, samples were centrifuged (4000 rpm for 20 min) and supernatant was divided into two fractions, where one half was acidified to pH 2 by gradually adding concentrated H_2SO_4 which developed into a precipitate for humic acid fractions. Non-precipitated parts of samples were analyzed for the fulvic acid form of carbon (C_{FA}). The leftover half fraction of the supernatant was analyzed for the total extractable carbon fraction (C_{tEx}). The concentration of fractional humic acid (C_{HA}) was calculated as the difference between C_{tEx} and C_{FA}. The indices of humification *viz*, humification ratio (HR), humification index (HI), and percent humic acid (Pha) were calculated by the Equations (1)–(3), respectively [32].

$$HR = \frac{C_{tEX}}{C} \times 100 \quad (1)$$

$$HI = \frac{C_{HA}}{C} \times 100 \quad (2)$$

$$Pha = \frac{C_{HA}}{C_{tEX}} \times 100 \quad (3)$$

2.6. Reproductive Performance of A. caliginosa

Growth and reproduction performances of the earthworms were assessed from the individual troughs after the composting process. The growth rate and reproduction of *A. caligenosa* were determined in terms of population and cocoon production during the experimentation. The adult and baby worms were manually isolated from each trough, and weighted and counted separately. Worms from each trough were sorted, washed with water, blotted by filter paper (Whatman No.1), and weighed in a digital analog balance. After recording data, cocoons were placed under incubation for future studies. The progressive increments in worm population during the study were calculated as per the standard method.Influence of various substrate combinations of CM and DW on biomass of *A. caliginosa* was determined as per the formula [33] given below:

$$Biomas\ (g) = \frac{W_2 - W_1}{t_2 - t_1}$$

2.7. Statistical Analysis

The data were statistically analyzed with Origin 2021. One-way analysis of variance (ANOVA) and post hoc analysis were performed to compare mean values of the samples ($p < 0.05$). Mean differentiation for humic indices, chemical parameters of substrates, and reproductive performance in the experiment were done through Student's *t*-test.

3. Results and Discussion

3.1. Chemical Properties of Coprolites

The evaluation of the effect of different substrate combinations of CM and DW was based on determining the changes in physicochemical characteristics like pH, TOC, TN, NO_3^-, TP, TK, Fe, and Zn. After 56 days of vermicomposting, the pH of the controls CM and DW were slightly acidic (6.33 ± 0.15) and alkaline (7.80 ± 0.61), respectively. Meanwhile, all other treatments showed slight alkaline values which differed from the values of the controls, with the treatment CM_{40}:DW_{60} showing the highest pH of 7.43. However, the pH of all treatments did not show any significant ($p < 0.05$) difference. The earthworms promoted the stabilization of the pH of all feed stocks to slightly alkaline (Table 2). Mixing of increased amounts of DW progressively neutralized the pH of the end product. The pH in all treatments increased with the onset of composting relative to respective control, which is attributed to rapid degradation of acidic compounds, secondary metabolites, and ammonification which isatypical pattern of vermicomposting [34,35]. The data (Table 2) revealed that after 56 days of vermicomposting, the control treatment DW_{100} showed significantly ($p < 0.05$) lower (14.067%) TOC in the end product in comparison to other treatments. In addition, the control treatment DW_{100} showed significantly ($p < 0.05$) higher TOC (21.667%) in the end product. The other substrate mixtures (CM_{80}:DW_{20}, CM_{60}:DW_{40}, CM_{40}:$DW_{60,}$ and CM_{20}:DW_{80}) showed a nonsignificant ($p < 0.05$) difference in TOC between each other, and the values ranged between 16.26% and19.53% with maximum carbon decline in CM_{60}:DW_{40} (19.53 ± 0.93), followed by treatment CM_{40}:DW_{60} (18.20 ± 0.61). The reason for reduction in organic carbon could be due to production and evolution of CO2 during the process [36] With respect to TP content in the end product of different treatments after 56 days of vermicomposting, it was found that the treatment CM60:DW40 showed the highest TP content of 0.85% which was significantly ($p < 0.05$) different from the treatments CM20:DW80 and DW100 (control) with a TP content of 0.51%. However, the TP value of the treatment CM_{60}:DW_{40} was non significant ($p < 0.05$) with TP values of the treatments CM_{100} (control), CM_{80}:DW_{20}, and CM_{40}:DW_{60}. It was also observed that there was increase in TP in all treatments from the initial values of different substrate mixtures. The increase in total phosphorus observed during the vermicomposting of different substrate combinations is due to the mineralization of organic phosphorus fractions carried by secretion of phosphatase by the earthworms [37] (The varied substrate combinations influenced the total nitrogen (TN) in the coprolites with an increase in all the treatments observed between 1.90% and 3.16%. The increasing trend followed the following order: CM_{100} (3.16%) > CM_{80}:DW_{20} (3.20%) > CM_{60}:DW_{40} (2.86%) > CM_{40}:DW_{60} (2.50%) > CM_{20}:DW_{80} (2.28%) > DW_{100} (1.90%). It was observed that the control treatment CM_{100} showed the highest TN (3.16%) in the final end product, however it was non significant ($p < 0.05$) with other treatments except for the treatment DW_{100} (control). The addition of many metabolic components like excretory products, mucus and body fluids by worms, decomposition of organic matter, respiratory activity ofmicrobes, and earthworms causing loss in TOC during the vermicomposting process led to improvements in nitrogen content in the final product [36,38,39]. The other factors that can improve the nitrogen content of the vermicompost include nitrogen-fixing bacteria and dead tissues of earthworms [40,41]. Decrease in pH is an important factor for stabilization and retention of N by preventing its loss from volatile ammonia. Previous studies suggested the final N content depends on the initial N content in wastes and extant of decomposition [42,43]. Analysis also resulted in high consistent concentration of nitrates following the order DW_{100} (7.02%) > CM_{20}:DW_{80} (7.69%) > CM_{40}:DW_{60} (16.07%) CM_{100} (18.75%) > CM_{80}:$DW2_{0}$

(20.22%) > CM_{60}:DW_{40} (24.39%). The increase in NO_3^- during the vermicomposting process is due to the enhanced aeration and nitrification process by the activities of the earthworms [44]. The K difference between treatments was significant, while its content was found to be high, relative to initial content (Table 2). The K concentration evidenced a steady increase, and the concentration in the final product followed the order CM_{100} (1.33%) > CM_{80}:DW_{20} (1.06%) > CM_{60}:DW_{40} (0.93%) > CM_{40}:DW_{60} (0.86%) > CM_{20}:DW_{80} (0.68%) > DW_{100} (0.47%). Interestingly, the analyzed results showed significantly ($p < 0.05$) higher TK in the control treatment of cow manure CM_{100} (1.33 ± 0.15%) followed by CM_{60}:DW_{40} (0.93 ± 0.05%) that was non significant with respect to other treatments. In a similar study, the vermicomposting of different treatment combinations of cow dung and duckweed resulted in increased K content in the final product that was proportional to the concentration of cow dung [36]. The increase in K content in the vermicompost could be due to respiratory loss of organic matter, hydrolysis of organic substrate by the microbial and earthworm gut enzymes, chelation process by humic acids, and release of K [45]. Data pertaining to micronutrient content indicated that Zn and Fe showed a similar recovery trend. The mean content of Fe in the final product varied between 0.10 ± 0.01 and 0.06 ± 0.01 with the highest recovery percentage in DW_{100} (21.05%) followed by CM_{40}:DW_{60} (18.64%). Data pertaining to micronutrient content indicated that Zn and Fe showed a similar recovery trend. The mean content of Fe in the final product varied between 0.10 ± 0.01 and 0.06 ± 0.01with the highest recovery percentage in DW_{100} (21.05%) followed by CM_{40}:DW_{60} (18.64%). Irrespective of substrate combinations, the Zn recovery percent considerably increased with the highest recorded in CM_{60}:DW_{40} (23.68%) followed by CM_{40}:DW_{60} (21.58%). Degradation of organic matter by *A. caligenosa* and subsequent mineralization of mineral elements increased the content of Fe and Zn in the coprolites.

3.2. Microbial Activity and Enzyme Property

The data analysis revealed that microbial activity showed rapid reduction in all treatments during the incubation of 56 days. Higher dehydrogenase activity was recorded during the first 45 days. A comparative analysis of initial and final dehydrogenase activity recorded (Figure 1) interesting values in the following order: DW_{100} (62.66%) > CM_{20}:DW_{80} (64.64%) > CM_{100} (58.78%)> CM_{60}:DW_{40} (56.21%). CM_{40}:DW_{60} (42.39%) > CM_{40}:DW_{60} (40.06%). Such findings may be due to the rapid enzymatic activity by distinguished microbes from the beginning of the process; however, due to the quick loss of organic matter causing nonsignificant effects in DHA across the treatments, this is in line with previous studies on vermicomposting [46]. Earthworms strongly escalate the microbial enzymatic activities such as DHA for conversion of volatile suspended solids [47]. Vermicomposting was also directly related to fluorescein diacetate activity present in the gut of earthworms and bacteria. The activity of fluorescein hydolysing enzyme increased the mineralization and stabilization of organ matter during the vermicomposting. The higher value of fluorescein diacetate hydrolysis enzyme (FDHE) activity was recorded and followed and the order CM_{100} (57.23%) > DW_{100} (59.91%) > CM_{60}:DW_{40} (63.32%) > CM_{40}:DW_{60} (63.54%) > CM_{20}:DW_{80} (70.66%). A balanced mixture of CM and DW residues showed a higher FDHE activity with change over the initial status as depicted in Figure 1. Substrates having higher content of DW residues reflected higher FHE activity (Figure 1), which is attributed to higher aerobic conditions due to higher lignocellulosic content in DW compared to CM. Our results are supported by the findings of [48] who reported higher FDHE activity in top soil (aerated), with limited activity in lower layers under the same conditions. Urease is closely linked with the decomposition of biowastes by hydrolyzing NH_4^+ into CO_2 [49]. In this study, the initial and final values were influenced by the substrate mixtures with urease activity following the order DW_{100} (48.52%) > CM_{20}:DW_{80} (55.83%) > CM_{40}:DW_{60} (58.72%) > CM_{60}:DW_{40} (62.21%) > CM_{80}:DW_{20} (63.32%) > CM_{100} (74.40%) (Figure 2). The increased DW residue in the substrates showed a linear decrease in urease activity and can be attributed to diminished initial content of NH4+ in the substrate mixture with high weed residue. The change over the initialstatusin urease activity (Figure 1) during the composting process is associated with the decrease in readily available organic matter which resulted in decreased microbial activity. Previous findings also reported decreased urease activity towardstheterminal phase of vermicomposting [50,51]. The final values of the urease activity across various treatments, regardless of the considerable difference in initial N contents and enzyme activities, implies that urease activity is a promising indicator of maturity. Similar results reported that measuring enzymatic activity and organic matter decomposition can provide information about compost maturity [52].

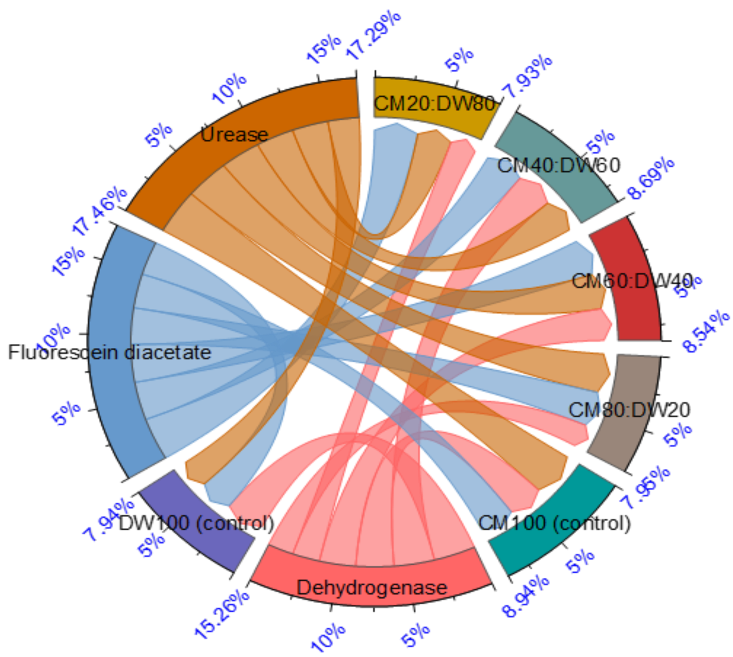

Figure 1. Change over initial (%) in enzymatic activity compared to final vermicatings from different mixtures of CM and DW.

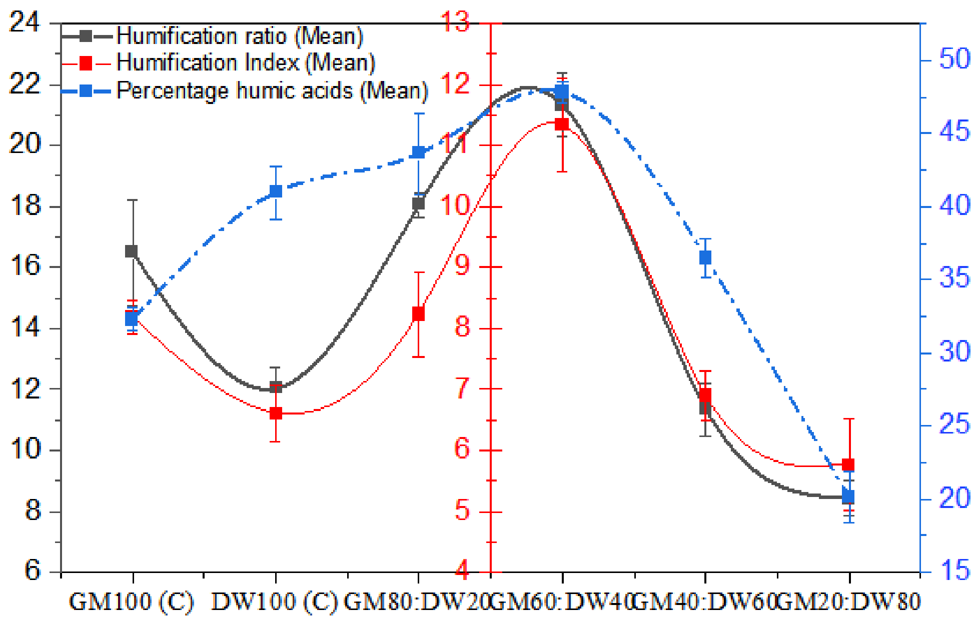

Figure 2. Humification indices with variable combinations of CM and DW after 8 weeks of vermicomposting. (Bars are means ± SD)

3.3. C:N, C:P, and C:S Ratio

Mixtures of cow manure and dalweed influenced the bio-gradation process. Diminished changes were recorded in C:N, C:P, and C:S ratios during partial and final decomposition relative to the initial status (Table 3). The C:N ratio of different substrate mixtures varied initially, decreased gradually during the vermicomposting process of 56 days, and ranged from 14.83 ± 1.03 to 23.67 ± 1.33 in the final product. The highest decrease in C:N ratio was evidenced in control treatment CM_{100} (23.67 ± 1.33) followed by control treatment DW_{100} (22.33 ± 1.41) which were decreased by 34.87% and 34.33%, respectively, in the final product. However, the lowest values of C:N ratios were observed in the treatments CM_{20}:DW_{80} (14.83 ± 1.03) and CM_{40}:DW_{60} (15.83 ± 0.29). The carbon loss due to respiration of earthworm-associated microbes could be the reason for the decreased C:N ratio. Previous studies also suggested the transformation of C to CO_2 through microbial respiration and an increase in N content due to mineralization [53,54] The recycling of sludge through vermitechnology revealed a decreased C:Nratio (Srivastava et al., 2020) of dry grass and rice straw [55]. Scientific studies revealed the value of the C:N ratio as an indicator of compost maturity with a ratio less than 20 indicating advanced degree of stabilization [56] and <15 desirable for crop production [57]. The C:P ratio of waste substrates ranged from 20.17 ± 1.04 to 52.03 ± 1.03 and 18.33 ± 0.58 to 42.17 ± 1.04 in varied substrates after partial decomposition and final vermicomposting, respectively. The maximum decrease (38%) in the final product was recorded in the control (CM_{100}) treatment followed by GM_{60}:DW_{40} (36%) (Table 3). C:S ratio showed a similar decreasing trend with a maximum decrease in DW_{100} (67%) followed by GM_{40}:DW_{100} (64%). Rapid degradation of organic matter and a high degree of stabilization was achieved in all the treatments. This demonstrates the role of earthworms in degradation and mineralization of mixed substrates giving a stabilized output. Mounted evidence also reported a decrease in C:P ratio during vermicomposting from kitchen waste, farm yard manure and temple wastes [58,59]. Previous research work also suggested the decrease in C:P ratio during vermicomposting of urban wastes [60].

Table 3. Mean values (±standard error) of C:N, C:P, and C:S ratios at initial, partial, and final stages of CM and DW mixtures.

Treatments	CM_{100}(Control)	DW_{100}(Control)	CM_{80}:DW_{20}	CM_{60}:DW_{40}	CM_{40}:DW_{60}	CM_{20}:DW_{80}
			Initial			
C:N ratio	36.33 ± 1.53	28.33 ± 0.58	29.67 ± 0.58	27.67 ± 0.57	23.33 ± 1.53	17.67 ± 2.08
C:P ratio	48.00 ± 1.00	38.00 ± 1.00	53.33 ± 1.51	34.67 ± 1.53	31.00 ± 1.00	27.33 ± 1.48
C:S ratio	168.33 ± 1.53	73.67 ± 1.15	154.67 ± 2.08	128.00 ± 3.00	111.33 ± 2.08	92.33 ± 3.06
			Partial			
C:N ratio	25.67 ± 1.48	16.67 ± 2.08	24.67 ± 1.95	22.67 ± 1.53	18.33 ± 1.15	15.67 ± 1.12
C:P ratio	52.03 ± 1.03	33.00 ± 1.00	45.33 ± 1.21	29.00 ± 1.11	26.00 ± 1.02	20.17 ± 1.04
C:S ratio	153.33 ± 1.42	56.67 ± 1.33	142.67 ± 1.14	118.33 ± 1.53	95.67 ± 2.08	73.33 ± 1.21
			Final			
C:N ratio	23.67 ± 1.33	22.33 ± 1.41	20.67 ± 2.02	18.17 ± 1.04	15.83 ± 0.29	14.83 ± 1.03
C:P ratio	42.17 ± 1.04	25.00 ± 1.00	37.67 ± 1.53	22.33 ± 1.14	21.33 ± 1.38	18.33 ± 0.54
C:S ratio	72.67 ± 1.41	24.00 ± 1.05	55.33 ± 1.23	63.33 ± 0.97	40.33 ± 1.15	35.67 ± 1.53

3.4. Humification

Humification reflects the formation of humic substances of waste material. The study observed an inconsistent change in humification ratio (HR) and humification index (HI) during the analysis (Figure 2). HR showed a significant difference ($p < 0.05$) across treatments. For HR, the increasing trend followed the order CM_{20}:DW_{80} (8.43 ± 0.6) > CM_{40}:DW_{60} (11.34 ± 0.87) > DW_{100} (12.03 ± 0.68) > CM_{100} (16.50 ± 1.74) > CM_{80}:DW_{20} (18.07 ± 0.40)

> CM_{60}:DW_{40} (21.33 ± 1.05). The higher input quantity of CM positively influenced the humification process and led to positive interactions among biochemical parameters of substrate mixtures. Vermicomposting increased both the HR and HI which is corroborated with the findings of [61]. The HR above 7 is considered an indicator of maturity as reported by [62,63]. Our study evidenced HR higher than 7, which signifiesthatthe positive influence of *A. caligenosa* and associated microbes caused enrichment in the vermicastings. The increase in both HR and HI in the vermicastings is due to the combined action of earthworm-microbes and is also based on the initial chemical composition of different mixture substrates. A recent study also confirmed the positive influence of vemicomposting on humification of cow and pig manure blended with paper wastes [64,65], and other scientific reports projected the highest humification degree in recycling of municipal solid waste blended with cow dung [66]. The high HI during the vermicomposting could also have contributed to the improvement in mineralization and dissolution of organic matter which reflected an increased percentage of humic acid (Pha) in the coprolites. The Pha was found to be highest in CM_{60}:DW_{40} (47.83 ± 0.76) and lowest in GM_{20}:DW_{80} (20.12 ± 1.70). The increased percent of humic acids demonstrated more maturity in the end product.

3.5. Reproductive Performance of A. caliginosa

The reproductive performance of an earthworm is considered a good indicator of substrate preference, which leads to an effective composting process. The reproductive performance in the present study was assessed in terms of population increase, biomass gain, and cocoon production at the end of the study. The initial number of *A. caliginosa* inoculated was 80 individuals per trough subjected to study for 8 weeks. Development of clitellum in *A. caliginosa* started from day 25 of worm inoculation. The varied substrate combinations of CM and DW significantly ($p < 0.05$) influenced the reproductive indices of earthworms during the study. The increased trend in the population followed the order DW_{100} (31.43%) > CM_{20}:DW_{80} (31.62%) > CM_{100} (42.86%) > CM_{40}:DW_{60} (43.40%) > CM_{80}:DW_{20} (44.44%) > CM_{60}:DW_{40} (57.52%). The treatment combinations with a DW residue proportion of 40% (CM_{60}:$DW_{40)}$ and 20% (CM_{80}:$DW_{20)}$ was reflected in the highest population of 188.33 ± 2.08 and 143.67 ± 0.58, respectively. Theresults (Figure 3) revealed that sole dalweed (control) substrates showed significantly ($p < 0.05$) poorer results indicating least preference as a food substrate because of the significantly ($p < 0.05$) higher pH and wide C:N ratio ofsole DW_{100} compared to other combinations. Lower pH, carbon, and nitrogen are important factors of suitable food for *Esenia fetida* [67]. The weight gain by earthworms is considered a universal comparative index to compare the biomass of earthworms. The biomass values of *A. caligenosa* at the end of the experiment differed significantly ($p < 0.05$). Notching the values of biomass, the increasing order follows DW_{100} (55.24%) CM_{20}:DW_{80} (56.73%) > CM_{80}:DW_{20} (67.04%) > CM_{40}:DW_{60} (67.78%) > CM_{100} (69.63%) > CM_{60}:DW_{40} (74.88%). The substrate combinations having higher input content of CM positively influenced the earthworm biomass, with the highest value recorded in CM_{60}:DW_{40} (282.67 ± 2.52) and minimum in DW_{100} (79.33 ± 1.53).

Research evidence favored our findings by observing the consistent higher live weight of *P. sansibaricus* with cow dung during 13 weeks of study [68] and the weight reduction may be attributed to quick degradation of cow manure [13]. Number of cocoons recovered after week 8 of composting registered more or less similar trends with the highest number of cocoons found in CM_{60}:DW_{40} (95.67 ± 1.15), and the minimum in DW_{100} (43.33 ± 1.53). The study also revealed that the control treatment (DW_{100}) with sole dalweed residues imparted stress to earthworms by lower biological parameters which could be attributed to lower multiplication rates, simultaneously resulting in low cocoon production. The blending mixture of cow manure and dalweed (CM_{60}:DW_{40}) was evidenced as an optimum substrate combination with a higher positive influence on the biomass and reproductive performance of *A. caliginosa.*

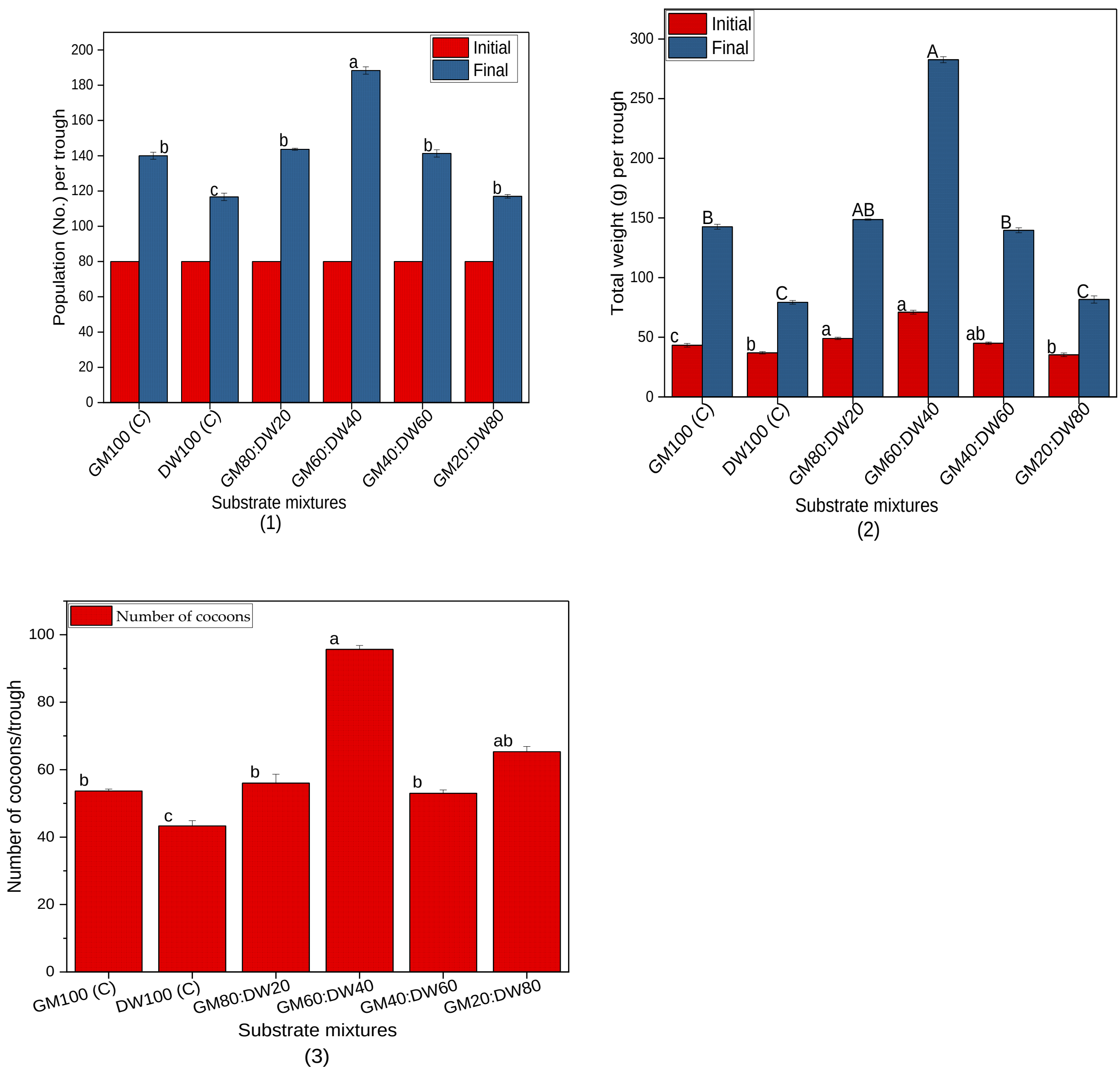

Figure 3. Biological parameters of *A. caliginosa*: (**1**) population increase, (**2**) body weight gain, and (**3**) total cocoon production during the experiment. Error bars indicate SD. For population and weight, the significant differences ($p < 0.05$) are indicated with different capital and lower-case letters for initial and final, respectively. (Means with different superscripts differ significantly ($p < 0.05$)).

4. Conclusions

Experimental findings affirmed that vermicastings from CM and DW with a blending mixture of CM_{60}:DW_{40} ratio were found optimum compared to either of these as sole treatments. CM, a potential bio resource hosting a wide variety of micro flora, promotes the bio-optimization of vermicastings and contributes significantly by adding value to DW in definite proportion. Humification indices also favor the CD_{60}:DW_{40} for maximum deciphering of nutrients (OC, N, P, K, Fe, and Zn) and stability (pH, C:N) of the end product. Dehydrogenase and fluorescein diacetate activities as maturity indicators were highest in CD_{60}:DW_{40} (64.64%) and CD_{40}:DW_{60} (63.54%), respectively, while highest urease activity (74.40%) was from CD_{100}. The study revealed that the 40% DW residues blended with 60% CM is an optimum combination with sufficient essential nutrients for growth body synthesis and multiplication of *A. caliginosa*. *A. caliginous* promotes the growth of microorganisms, which is otherwise largely limited due to severe winter and low ambient temperature, and hence is a suitable candidate for the recycling of CM and DW under temperate Himalayan climatic conditions. Further large scale trials are required in the future under variable substrates and microclimates (temperatures) of the Himalayas.

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