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EFFECTS OF TEA LEAF-BASED DIETS ON THE GROWTH AND REPRODUCTION IN TWO TROPICAL EARTHWORM SPECIES (*Pontoscolex corethrurus* and *Drawida assamensis*)

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ABSTRACT

A laboratory-based experiment was conducted to study the effect of tea leaf-based diets on the growth and reproduction in two dominant earthworm species (*Pontoscolex corethrurus* and *Drawida assamensis*) of tea plantations in Tripura (India). Among the four different diets [soil (S); soil-tea leaf (ST); soil-cow dung (SC) and soil-tea leaf-cow dung (STC)], *P. corethrurus* maintained significant higher growth rate than native species, *D. assamensis* in each diet. Growth rate was highest in SC for both *P. corethrurus* (11.35 mg worm^{-1} day^{-1}) and *D. assamensis* (10.70 mg worm^{-1} day^{-1}). Cocoon appeared in all the experimental culture pots, except in ST with *D. assamensis*. In both the species, growth rate was lowest in S and ST. Among all the experimental diets, both the earthworm species produced highest number of cocoons in SC diet for the whole experimental period (150 days). The rate of juvenile production in *P. corethrurus* (0.82 juvenile worm^{-1} week^{-1}) was significantly higher than those in *D. assamensis* (0.28 juvenile worm^{-1} week^{-1}). The experiment clearly indicates that for vermiculture of these two geophagous earthworm species, cow dung is preferred to tea leaf-based diets probably because of rich content of polyphenols in latter.

Keywords: Cocoon, cow dung, growth, *P. corethrurus*, *D. assamensis*, reproduction, tea leaf

INTRODUCTION

The earthworms by their burrowing, feeding and casting activities modify the soil structure and change the soil physicochemical properties, thereby play a major role in ecosystem services. Inoculation of appropriate species of earthworms to promote soil health has recently been regarded as an important step towards restoration of degraded soils (Butt *et al*., 1995; Tapia-Coral *et al*., 2006). Based on laboratory-based experiment, Garcia and Fragoso (2002) concluded that *Pontoscolex corethrurus* and *Amynthas corticis* had the potential for rehabilitation of Mexican soils degraded by cement industry. Production of sustainable field population of earthworms may be done through mass rearing of the required species under controlled conditions.

Tea (*Camellia sinensis*) is economically an important cash crop in India. Exotic earthworm, *Pontoscolex corethrurus* dominates the human managed tea plantations in Tripura (Jamatia and Chaudhuri, 2017), as well as in Tamil Nadu (Senapati *et al*., 2005). In tea plantation soils of Tripura, native earthworm *Drawida assamensis* represents the second highest relative density (23.9%) following exotic earthworm *Pontoscolex corethrurus* (36.22%) (Jamatia and Chaudhuri, 2017). Tea leaf litter has high polyphenol contents (Harbowy and Balentine, 1997). According to Edwards and Bohlen (1996), litter palatability to earthworms has an inverse relationship with polyphenol contents of leaves.

Food source influences not only the size of an earthworm population but also their growth and reproduction rates (Dominguez *et al.*, 2001). Cow dung is considered an ideal food additive for all earthworm species (Lowe and Butt, 2005), while tea leaves have high content of polyphenol - a less palatable substance for earthworms. So, it is expected that cow dung mixed with tea leaves would be a better food choice than only tea leaves provided to earthworms. On the above context, the present paper deals with the growth and reproduction in *Pontoscolex corethrurus* and *Drawida assamensis* on tea plantation soils with food additive as cow dung or tea leaves or a combination of both under laboratory conditions.

MATERIALS AND METHODS

Geophagous earthworms, *Pontoscolex corethrurus* and *Drawida assamensis*, collected in June, 2017 from the soils of Durgabari Tea Plantation, West Tripura (India), were acclimatized in habitat soils under laboratory conditions (26-28^{0}C) for seven days. Freshly plucked tea leaves, collected from Durgabari Tea Plantation, were air-dried in a shaded place for 30 days. Fresh cow dung collected from neighbouring cowshed of Suryamaninagar was air-dried. Air-dried leaves, cow dung and soils were ground separately and sieved (2 mm).

Tea plantation soils (loamy sand; sand 76.3%, clay 14.1%, silt 9.1%, organic carbon 1.71% and total nitrogen 0.228%) were mixed with feed additives such as tea leaves [N 2.6%, P 0.2%, K 1.2%, C/N 17.62 (Fan *et al*., 2017), total polyphenol 0.495 mg GAE g^{-1}, total flavonoid 1.33 mg QEE g^{-1}] and cow dung [N 1.5%, P 0.9%, C/N 18 (Karmegam and Daniel, 2009)] in different combinations (w/w) to have four treatments *viz*., 1) soil alone (S), 2) soil-tea leaf (in 30:2 ratio, ST), 3) soil-cow dung (in 30:2 ratio, SC) and 4) soil-tea leaf-cow dung (in 30:1:1 ratio, STC). The experiment was conducted in a completely randomized design with four replications for each treatment. Since both the earthworm species were endogenic, all the experimental diets included mineral soil as the main substrate. The laboratory-based experiment was started on 15th June 2017 with the introduction of one pair of non-clitellate earthworms (450-550 mg) of each species in 2.5 L earthen pot containing 1600 g substrate. Water was sprinkled by hand sprayer every two days interval to maintain the moisture level of substrate at 22-25%. The casts produced during experiment in each experimental pot were retained. Food was limited i.e. no additional food was supplied during the entire experimental period. Depending upon the decrease in body weight of earthworms in different treatments, the culture of *P. corethrurus* in SC and STC was terminated on 150th day, S on 135th day and ST on 120th day. Similarly, the culture of *D. assamensis* was terminated in ST, S and STC pots on 105th, 120th and 135th day, respectively, while in SC pot the study was terminated on 150th day.

The biomass (live worm weight with gut content, mg worm^{-1}) of individual worm, number of cocoon and the number of juvenile worm^{-1} was recorded every fortnightly. The rate of growth (mg weight gained worm^{-1} day^{-1}) and the rate of reproduction in terms of cocoon reproduction (number of worms week^{-1}) and juvenile production (juvenile adults week^{-1}) were calculated following Nath and Chaudhuri (2014). Student's t-test was applied to determine the difference in significant increase in earthworm body weight (compared to the initial weight) in different diets. Maximum weight, growth rates and fecundity were compared among treatments by one-way ANOVA followed by Tukey's post-hoc test (Tukey, 1953).

RESULTS AND DISCUSSION

The major physicochemical characteristics of soils mixed with selected organic residues is given in Table 1. Among different experimental diets, C/N ratio was highest in S treatment and lowest in SC treatment. The total N (%) and organic C (%) was highest in SC and lowest in S experimental pot.

Table 1: Initial chemical characteristics of the experimental diets used

Parameters	Worm-soil	Worm-soil-tea leaf	Worm-cow dung	Worm-soil-tea leaf-cow dung
pH	4.5 ± 0.02a	4.43 ± 0.01b	4.96 ± 0.02c	4.23 ± 0.005d
Organic carbon (%)	2.21 ± 0.04b	2.87 ± 0.04c	2.77 ± 0.02d	1.87 ± 0.05a
Total nitrogen (%)	0.178 ± 0.008a	0.213 ± 0.003b	0.315 ± 0.008c	0.262 ± 0.002d
C/N ratio	10.44 ± 0.16d	10.78 ± 0.39a	10.46 ± 0.23b	8.91 ± 0.15c

Values are mean ± SE of 3 replicates; Different letters (a, b, c, d) in a group denote significantly different values at $p<0.05$.

Growth

Initially (first 15 days) the body weight of *P. corethrurus* declined in S and STC and that in *D. assamensis* declined in ST culture among the four experimental diets (Fig. 1a,b). After 15 days, both earthworm species had positive growth phase, the duration of which differed amongst the two species (Fig. 1a,b). Positive growth phase was followed by negative growth. In *P. corethrurus*, positive growth phase was much higher in SC (105 days) than STC (90 days) and S (75 days) compared to that in *D. assamensis* in SC (90 days), STC (75 days) and S (60 days). Individual weight gain for *P. corethrurus* and *D. assamensis* was highest in SC (1192.5 and 963.75 mg worm^{-1}, respectively), lowest in ST (218.75 and 88.75 mg worm^{-1}, respectively) and intermediate in STC (756.25 and 465.56 mg worm^{-1}, respectively) [Table 2]. Individual highest weight, weight gain and growth rate in each diet were significantly higher ($p<0.05$) in *P. corethrurus* than in *D. assamensis*. In different treatments, the rate of weight loss was significantly higher ($p<0.05$) in *D. assamensis* than in *P. corethrurus*.

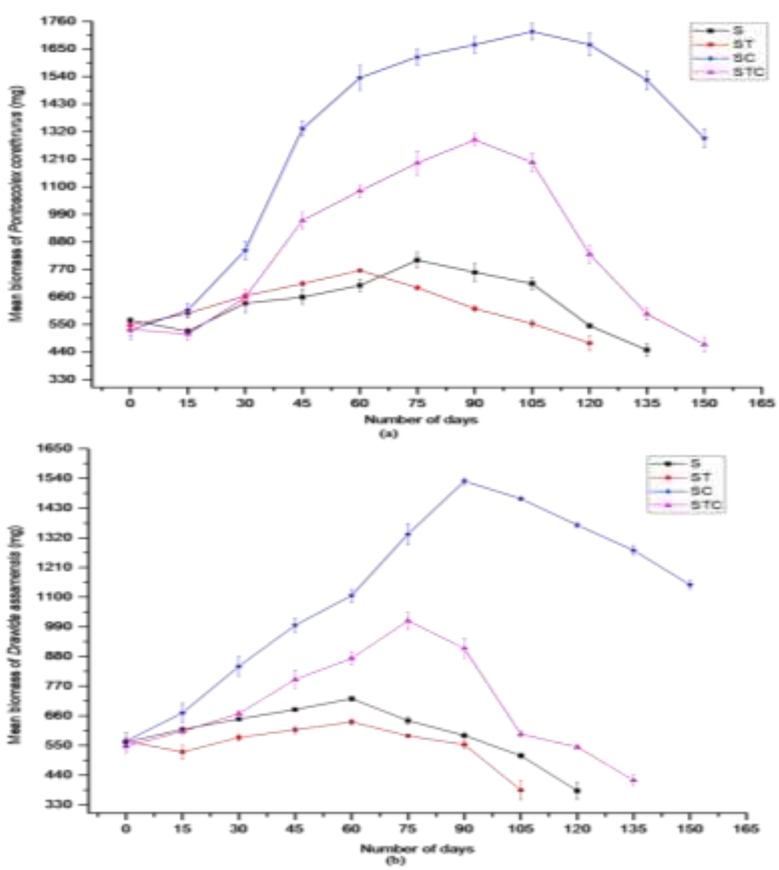

Fig. 1: Effect of different food substrates (S = worm + soil, ST = worm + soil + tea leaf, SC = worm + soil + cow dung, STC = worm + soil + tea leaf + cow dung) in the biomass of a) *Pontoscolex corethrurus* and b) *Drawida assamensis*

Table 2: Effect of different food substrates on growth and reproduction of *P. corethrurus* and *D. assamensis*

Parameters	Worm-soil		Worm-soil-tea leaf		Worm-cow dung		Worm-soil-tea leaf-cow dung	
	Pontoscolex	*Drawida*	*Pontoscolex*	*Drawida*	*Pontoscolex*	*Drawida*	*Pontoscolex*	*Drawida*
Initial mean body wt. (mg $worm^{-1}$)	567.5±9.3a	562.5±10.5a	547.5±23.2a	566.25±33.1b	527.5±23.2a	566.5±23.2b	531.25±40.1a	547.5±28.0b
Highest individual wt. (mg $worm^{-1}$)	807.5±31.2a	723.75±8.0b	766.25±6.6a	637.5±11.6b	1720±32.5a	1530±14.0b	1287±93.2a	1012±32.0b
No. of days to attain maximum body wt.	75	60	60	60	105	90	90	75
Weight gain (mg $worm^{-1}$)	240±24.1a	161.25±13.9b	218.75±21.1a	88.75±29.7b	1192.50±47.7a	963.75±22.9b	756.25±13.0a	465±56.5b
Growth rate (mg $worm^{-1}$ day^{-1})	3.2±0.32a	2.68±0.23b	3.64±0.35a	1.47±0.49b	11.35±0.45a	10.70±0.25b	8.40±1.45a	6.20±0.75b
Weight loss (mg $worm^{-1}$)	358.7±42.9a	341.2±36.4b	290±33.5a	252.5±28.9b	426.2±40.3a	383.7±11.1b	816.2±83.0a	591.2±34.5b
Rate of body wt. loss (mg $worm^{-1}$ day^{-1})	3.25±0.72a	5.41±0.18b	4.58±0.94a	5.41±0.51b	3.16±0.96a	4.33±0.62b	6.01±2.40a	6.83±0.70b
Total cocoon produced (No. $worm^{-1}$)	13.5	6	5.5	0	111	30.5	43	11
Total No. of days with cocoon production	75	60	45	0	120	105	105	75
Rate of cocoon production (No. $worm^{-1}$ $week^{-1}$)	0.28±0.49a	0.14±0.02b	0.14±0.03	0	1.47±0.02a	0.42±0.04b	0.66±0.12a	0.21±0.04b
Hatching success (%)	77.77	30.76	63.63	0	86.93	36.06	77.91	31.81
Rate of reproduction (juvenile $adult^{-1}$ $week^{-1}$)	0.16±0.02a	0.13±0.01b	0.15±0.01	0	0.82±0.09a	0.28±0.02b	0.36±0.04a	0.17±0.02b

Values are mean ± standard error of 3 replicates; Different letters (a,b) in a group denote significant differences ($p<0.05$)

Moreover, weight loss was lower in SC and higher in tea leaf-based diets. Significantly highest growth rate (11.35 mg $worm^{-1}$) for *P. corethrurus* and *D. assamensis* (10.7 mg $worm^{-1}$) were achieved in SC experimental diet. Significantly high ($p < 0.05$) N and organic carbon contents in SC diet is responsible for highest growth rate. General growth rate on different diets followed the order: *P. corethrurus* SC > STC > ST > S; *D. assamensis* SC > STC > S > ST.

The type and amount of food available to the earthworm species greatly influence their growth rate and fecundity (Edwards and Bohlen, 1996). Comparatively more sustained growth of *P. corethrurus* than *D. assamensis* was noticed in all the experimental diets, except in ST (soils with tea leaves) wherein both the species had limited periods (up to two months) of positive growth. Polyphenols which has an inverse relationship with litter palatability (Edwards and Bohlen, 1996) is present in very high concentration (18-34%) in dry tea leaves (Fan *et al.*, 2017). Lowest growth rate of both *P. corethrurus* (3.64 mg $worm^{-1}$ day^{-1}) and *D. assamensis* (1.47 mg $worm^{-1}$ day^{-1}) in ST and highest growth rate of *P. corethrurus* (11.35 mg $worm^{-1}$ day^{-1}) and *D. assamensis* (10.7 mg $worm^{-1}$ day^{-1}) in SC indicated the nutritional superiority of cow dung over ST and other experimental diets. Intermediate growth rate of *P. corethrurus* (8.4 mg $worm^{-1}$ day^{-1}) and *D. assamensis* (6.2 mg $worm^{-1}$ day^{-1}) recorded in STC (soil mixed with tea leaves and cow dung) indicated the role of cow dung in improving the palatability and quality of diet for earthworms. The nutritional superiority of cow dung diets over all other diets for earthworms has earlier been reported (Lowe and Butt, 2005). In spite of low palatability of polyphenol rich tea leaves, significantly higher ($p < 0.05$) growth rate of body weight gain and significantly lower ($p < 0.05$) rate of body weight loss in *P. corethrurus* than in *D. assamensis* in ST indicated that the tea leaf litter is comparatively more acceptable to *P. corethrurus* than to *D. assamensis*. Thus, Jamatia and Chaudhuri (2017) reported comparatively much higher biomass, density and frequency in *P. corethrurus* than *D. assamensis* in tea plantation soils of Tripura. Similarity in the growth rate of *P. corethrurus* and *D. assamensis* in S and ST diets indicated that addition of organic matter in the form of tea leaves to the soil did not increase the rate of growth in them because of high polyphenol contents in tea leaves.

Growth rate of *P. corethrurus* in soils mixed with cow dung and tea leaf litter (8.4 mg worm^{-1} day^{-1}) is very similar to the growth rate of *P. corethrurus* (8.37 mg worm^{-1} day^{-1}) and *Eutyphoeus comillahnus* (8.51 mg worm^{-1} day^{-1}) but much less than that of *Metaphire houlleti* (12.42 mg worm^{-1} day^{-1}) in the soils mixed with cow dung and rubber leaf litter (Nath and Chaudhuri, 2012). On the other hand, growth rate of *D. assamensis* (6.2 mg worm^{-1} day^{-1}) in the soils mixed with tea leaf litter and cow dung, was much higher than the growth rate of *D. assamensis* (3.55 mg worm^{-1} day^{-1}) and *D. papillifer papillifer* (4.4 mg worm^{-1} day^{-1}) reared in soils mixed with rubber leaf and cow dung (Nath and Chaudhuri, 2012).

Reproduction

Appearance of clitellum: Sexual maturity of *P. corethrurus* was attained on 15th day with the development of clitellum in SC and STC diets. But in S and ST clitellum appeared on and after 30 days in this species. In *D. assamensis*, clitellum appeared on 15th day in SC and on 30th day in S and STC. According to Bohlen (2002) a nutritionally superior feed substrate shortens the time of sexual maturity in animals. Thus, in SC (soil with cow dung additive), clitellum (sign of sexual maturity in earthworm) appeared earlier i.e. on 15th day in *P. corethrurus* and *D. assamensis* as compared to S and ST substrates which had lesser nutrients.

Cocoon production: After attainment of certain amount of biomass (i.e. after initiation of growth phase), cocoon laying started within 30th to 45th day. Generally, when cow dung was present (SC and STC), cocoon production started earlier i.e. on 30th day and continued up to 135th day in *P. corethrurus* and in *D. assamensis* up to 120th day, thus cocoon production phase is much longer (15 days more) in exotic worm, *P. corethrurus* than in native worm, *D. assamensis*. In S culture pot, *P. corethrurus* and *D. assamensis* laid cocoons up to 105th and 75th days, respectively (Fig. 2a,b). Peak days of cocoon production in *P. corethrurus* and *D. assamensis* in different food substrates are shown in Table 3. Interestingly, peak of cocoon production and peak of biomass production were on the same day for each of the earthworm species but peak days differed amongst the species (*P. corethruus:* SC - 105th day, STC - 90th day, S - 75th day, ST - 60th day; *D. assamensis*: SC - 90th day, STC - 75th day, S - 60th day) [Fig. 1, 2]. A maximum of 111 cocoons worm^{-1} and 30.5 cocoons worm^{-1} were produced in SC by *P. corethrurus* and *D. assamensis*, respectively, during the entire experimental period (Table 2). When tea leaves were mixed with soils (ST), *D. assamensis* produced no cocoons and *P. corethrurus* produced only a few cocoons (5.5 cocoons worm^{-1}). However, when both cow dung and

Table 3: Peak day of cocoon production and juvenile production in *P. corethrurus* and *D. assamensis* in different food substrates

Diets	Peak day of cocoon production		Peak day of juvenile production	
	P. corethrurus	*D. assamensis*	*P. corethrurus*	*D. assamensis*
Soil (S)	75	60	105	105
Soil-tea leaf (ST)	60	Nil	105	Nil
Soil-cow dung (SC)	105	90	135	120
Soil-tea leaf-cow dung (STC)	90	60	120	105

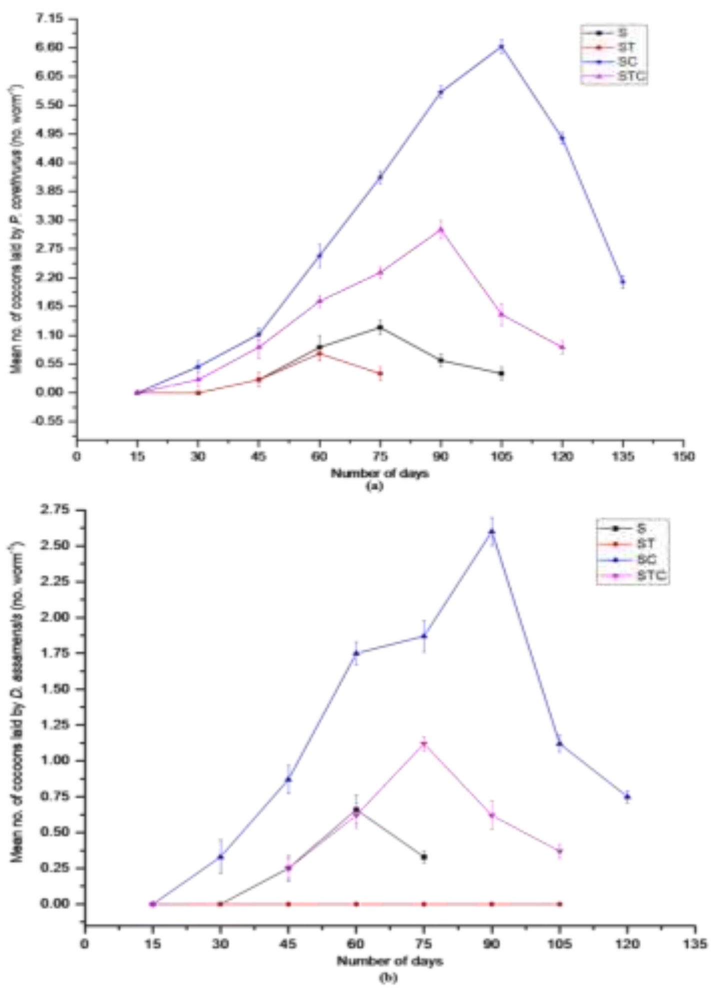

Fig. 2: Cocoon production of two earthworms: a) *Pontoscolex corethrurus* and b) *Drawida assamensis* in different food substrates (S = worm + soil, ST = worm + soil + tea leaf, SC = worm + soil + cow dung, STC = worm + soil + tea leaf + cow dung)

tea leaves were present in diet, there was > 2 times increase in cocoon production by *P. corethrurus* and *D. assamensis* in STC as compared to their cocoon production in only soil diet (S). Rate of cocoon production was highest in SC for both *P. corethrurus* (1.47 cocoons worm^{-1} week^{-1}) and *D. assamensis* (0.42 cocoons worm^{-1} week^{-1}) due to significantly higher ($p < 0.05$) contents of N and organic C in SC diet. Rate of cocoon production was significantly higher in *P. corethrurus* than *D. assamensis* in each experimental diet (Table 2). Cocoons of *P. corethrurus*, *D. assamensis* and developmental stages and hatching of cocoon of *P. corethrurus* are shown in Fig. 3.

Absence of reproduction in ST diet was probably due to high polyphenol contents in tea leaves which decreased the rate of food intake in *D. assamensis*. Nath and Chaudhuri (2012) reported that native earthworm species such as *D. assamensis*, *D. papillifer papillifer* and *Eutyphoeus comillahnus* failed to produce any cocoon in habitat soils when mixed with rubber leaf litter which also contains high polyphenol contents.

The addition of organic matter in the form of cow dung or a combination of cow dung and tea leaves to soils increased the rate and peak of cocoon productions in earthworms. Sustained cocoon production with appearance of larger peak in *P. corethrurus* as compared to *D. assamensis* in experimental diets, especially in SC and STC, indicates that the rate of cocoon production by *P. corethrurus* was more than that of *D. assamensis*. Highest rate of cocoon generation, hatching success and juvenile production in SC among different diets for both *P. corethrurus* and *D. assamensis*

Fig. 3: Cocoons of (a) *P. corethrurus* and (b) *D. assamensis*, (c) Developmental stages of cocoon of *P. corethrurus*, (d) Hatching of cocoon of *P. corethrurus*

indicated that nutrient rich diets are essential for higher reproduction. *D. assamensis* which did not produce any cocoon in ST culture, however laid cocoons with subsequent production of juveniles in STC which indicated that the tea leaves were inhibitory to cocoon generation while addition of cow dung to it stimulated cocoon generation in this species. Earlier, Nath and Chaudhuri (2012) also reported cocoon production by exotic species, *P. corethrurus* and *M. houlleti* in polyphenol rich rubber leaf litter diet where the native species such as *D. assamensis, D. papillifer papillifer* and *Eutyphoeus comillahnus* did not produce any cocoon. Hatching success of cocoon in *P. corethrurus* and *D. assamensis* in SC (87 and 36%, respectively) is very much similar to the hatching success of these earthworm species as reported by Chaudhuri and Bhattacharjee (2011).

Juvenile emergence and production: Earthworm cocoons after a definite period of incubation, hatched out to produce juveniles. Rate of juvenile production in earthworm species depends upon their cocoon production and hatching success. Hatching success varied in both *P. corethrurus* and *D. assamensis* depending on the rearing media of the species concerned. Thus, hatching success was highest for both the species reared in SC diet (87% for *P. corethrurus* and 36% for *D. assamensis*). In each of S and STC diets, hatching success of both *P. corethrurus* and *D. assamensis* was 78 and 31-32%, respectively (Table 2). In absence of cocoon laying by *D. assamensis* in ST, no juveniles were observed in it. Cocoon development time in *P. corethrurus* and *D. assamensis* varied from 30 to 35 days. While in S, both *P. corethrurus* and *D. assamensis* had juvenile peak on 105th day, in SC and STC, *P. corethrurus* had juvenile peak 15 days later than *D. assamensis* (Table 3).

The number of juveniles at 15 days interval and rate of reproduction in terms of juvenile production in *P. corethrurus* and *D. assamensis* in different food media are provided in Table 4 and 5, respectively. In all the treatments, rate of juvenile production was significantly higher ($p < 0.05$) in *P. corethrurus* than that of *D. assamensis*. Highest rate of juvenile production for both the earthworm species (*P. corethrurus* 0.82 juvenile adult^{-1} week^{-1}; *D. assamensis* 0.28 juvenile adult^{-1} week^{-1}) was

Table 4: Number of adults and juveniles and reproduction rate of *P. corethrurus* in different food substrates

Days	Stages	Diets			
		Worm-soil	Worm-soil-tea leaf	Worm-soil-cow dung	Worm-soil-tea leaf-cow dung
0-45	Adult	2	2	2	2
	Juvenile	0	0	0	0
60	Adult	2	2	2	2
	Juvenile	0	0	0.32 ± 0.07	0.24 ± 0.05
75	Adult	2	2	2	2
	Juvenile	0.23 ± 0.03	0.24 ± 0.04	0.51 ± 0.04	0.53 ± 0.08
90	Adult	2	2	2	2
	Juvenile	0.40 ± 0.10	0.35 ± 0.07	1.22 ± 0.19	0.80 ± 0.23
105	Adult	2	2	2	2
	Juvenile	0.56 ± 0.07*	0.36 ± 0.11*	1.90 ± 0.23	1.08 ± 0.33
120	Adult	2	-	2	2
	Juvenile	0.28 ± 0.08	-	2.65 ± 0.14	1.40 ± 0.18*
135	Adult	2	-	2	2
	Juvenile	0.23 ± 0.08	-	3.06 ± 0.16*	0.69 ± 0.10
150	Adult	-	-	2	2
	Juvenile	-	-	2.25 ± 0.10	0.40 ± 0.05
Rate of reproduction (No. of juvenile adult^{-1} week^{-1})	-	0.16 ± 0.02a	0.15 ± 0.01a	0.82 ± 0.09b	0.36 ± 0.04c

*Juvenile peak

Values are mean ± standard error of 3 replicates; Different letters (a, b, c) denotes significantly different values ($p < 0.05$)

Table 5: Number of adults and juveniles and reproduction rate of *P. corethrurus* in different food substrates

Days	Stages	Diets			
		Worm-soil	Worm-soil-tea leaf	Worm-soil-cow dung	Worm-soil-tea leaf-cow-dung
0-45	Adult	2	2	2	2
	Juvenile	0	0	0	0
60	Adult	2	2	2	2
	Juvenile	0	0	0.25 ± 0.04	0
75	Adult	2	2	2	2
	Juvenile	0.25 ± 0.04	0	0.43 ± 0.09	0.25 ± 0.08
90	Adult	2	2	2	2
	Juvenile	0.31 ± 0.05	0	0.56 ± 0.11	0.31 ± 0.06
105	Adult	2	2	2	2
	Juvenile	0.34 ± 0.06*	0	0.74 ± 0.11	0.58 ± 0.11
120	Adult	2	-	2	2
	Juvenile	0.25 ± 0.04		1.16 ± 0.07*	0.31 ± 0.06
135	Adult	-	-	2	2
	Juvenile			0.53 ± 0.05	0.25 ± 0.07
150	Adult	-	-	2	-
	Juvenile				0.38 ± 0.07
Rate of reproduction (No. of juvenile adult^{-1} week^{-1})	-	0.13 ± 0.01a	No juveniles	0.28 ± 0.02b	0.17 ± 0.02c

*Juvenile peak

Values are mean ± standard error of 3 replicates; Different letters (a, b, c) denotes significantly different values ($p<0.05$)

recorded in SC diet. When tea leaves were added, STC culture showed declined rate of juvenile production by *P. corethrurus* (0.36 juvenile adult^{-1} week^{-1}) and *D. assamensis* (0.17 juvenile adult^{-1} week^{-1}). The rate of reproduction in terms of both cocoon generation and juvenile production of these two earthworm species in different diets were as follows: *P. corethrurus* SC > STC > S > ST; *D. assamensis* SC > STC > S (no cocoon was produced in ST).

Reproductive success of an earthworm species depends not only upon the number of cocoons produced by it in a given period of time, but also on its hatching success. The rate of juvenile production is always lesser than the rate of cocoon production. This is because some of the earthworm cocoons always fail to hatch which often depends upon the type of species and also the incubation media. Thus, hatching success was highest for both *P. corethrurus* and *D. assamensis* in SC culture. Hatching success of *P. corethrurus* was lowest in ST where *D. assamensis* did not produce any cocoon. Significantly higher ($p < 0.05$) rate of juvenile production in *P. corethrurus* than that in *D. assamensis* in each experimental diet was due to the higher rate of cocoon production and higher hatching success in the former. In ST culture, where *P. corethrurus* had lowest rate of juvenile production (0.15 juvenile worms^{-1} week^{-1}) and *D. assamensis* produced no juveniles and in STC, *P. corethrurus* and *D. assamensis* produced 0.36 juvenile worms^{-1} week^{-1} and 0.17 juvenile worms^{-1} week^{-1}, respectively. The rate of reproduction in *P. corethrurus* in soils with cow dung-tea leaf (0.36 juvenile worms^{-1} week^{-1}) was far lower than that of soil-cow dung-rubber leaf (0.487 juvenile worms^{-1} week^{-1}) and soil-cow dung-bamboo leaf litter (0.94 juvenile worms^{-1} week^{-1}) [Nath and Chaudhuri, 2014].

Conclusion: The present study clearly indicates that cow dung is the best food of choice for growth and reproduction in *P. corethrurus* and *D. assamensis*. Polyphenol rich tea leaf should first be made palatable by mixing it with cow dung before its successful use in vermiculture. Among the two studied earthworm species, only *Pontoscolex corethrurus* had sustainable growth and reproduction which is probably linked with its adaptive strategies like efficient assimilation capacity for wide range of diets, continuous breeding with high fecundity, short incubation period and high hatching success (Chaudhuri and Bhattacharjee, 2011). So, vermiculture of *Pontoscolex corethrurus* becomes essential in order to maintain its successful field population for its systematic use in land restoration (Nath and Chaudhuri, 2012) and as a source of protein (vermitin) in poultry, fishery and piggery (Debnath *et al.*, 2017).

Author's Contribution Corresponding author, Prof. P.S. Chaudhuri prepared the manuscript and supervised the overall work, while Dr. S.K.S. Jamatia, a Ph. D. student carried out the study.

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