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THE GROWTH AND REPRODUCTION OF *EISENIA FETIDA* (OLIGOCHAETA) IN COW MANURE AND FIELD SOIL

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Development, growth and reproduction of *Eisenia fetida* in cow manure and field soil were studied under laboratory conditions for 11 months. The study revealed that copulation is not a prerequisite for production of viable cocoons. A higher growth rate in cow manure (0.74 mg and 0.79 mg worm^{-1} day^{-1} raised singly and in batches, respectively) than in field soil (0.18 and 0.69 mg worm^{-1} day^{-1} raised singly and in batches, respectively) and production of more cocoons in cow manure than in field soil indicate a slightly better suitability of cow manure than field soil for the development of *E. fetida.* After an incubation period of 27.5 days, 100% of the cocoons hatched with a mean of 2.4 hatchings per cocoon.

The potential of using earthworms in the digestion of raw organic wastes has been established in small and large projects[1-3]. Many factors such as temperature, soil moisture, worm density have been studied to understand earthworm ecology. Although the physico-chemical properties of different organic wastes that influence the life-cycle are well known for *Eisenia fetida* elsewhere[4-6] but such studies are lacking in Kumaun Himalaya. For the same reason it is important to determine the life cycle of E. fetida under laboratory conditions. The present study was undertaken to determine: (1) growth and maturation of *Eisenia fetida*, (2) quantitative production of cocoons, (3) hatching success and the incubation time of earthworms within the project "Conservation and sustainable management of belowground Biodiversity" in Kumaun Himalaya.

MATERIAL AND METHODS

Adults of *E. fetida* used during the present study were collected from cropland of Ghorakhal 15 km from Nainital and cultured in the laboratory at 20-25^{0}C. The worms were kept in soil with a pH of 7 and moisture content of 70-80%. The soil used in the experiment was brought from the study site and the water content was maintained at a uniform level by regular watering. The worms were fed with cow manure every 15 days.

The cocoons were incubated in petri dishes (15 cm diameter) using cow manure without urine or straw and fine-particle field soil as a substrate to maintain the hatchlings and growing worms. This nutritive medium was prepared by air-drying, grinding and then sieving through a 2 mm sieve.

For the growth study, newly hatched specimens were collected from the petri dishes. The hatchlings were weighed individually after they had been rinsed in distilled water and gently dried on filter paper. The hatchlings were placed in petri dishes (15 cm diameter) singly and in batches of two in each type of substrate and covered with plastic mesh (1 mm) for ventilation. Fresh cow manure and soil were added to each container on a regular basis according to the number of worms in the container. This was repeated every 15 days till the completion of experiment. The worms were weighed every 15 days.

With each weighing, the worms were examined to determine the extent of maturation as indicated by clitellum development. In order to determine the onset of cocoon production, the substrates were examined every day under a magnifying glass as soon as clitellate worms are observed.

The cocoons were counted and weighed. Before weighing, the cocoons were washed lightly in distilled water and most of the debris and organic particles adhering to the sticky hull were removed with fine brush. The cocoons were randomly divided into five batches and incubated in petri dishes. Five media were used for incubation: moist, fine-particle cow manure; field soil; litter; distilled water (replaced with clean water every 7 days); and moist filter paper (kept moist by adding distilled

water periodically). The cocoons were observed every 24 h and hatchling were removed, weighed and maintained for stock culture. The hatching success of the cocoons in different media and over the incubation period was determined together with the number and weight of hatchlings per cocoon.

RESULTS AND DISCUSSION

Food: The composition of food given to *E. fetida* showed that amount of C is higher in cow manure than field soil (Table 1). We also observed difference in pH and C:N ratio.

Growth rate and maturation: The mean weight of newly hatched worms was 1.89 ± 0.85 mg (kept singly) and 1.02 ± 0.46 mg (kept in batches) in cow manure; and 2.49 ± 1.11 mg (kept singly) and 1.17 ± 0.52 mg (kept in batches) in field soil. The mean growth rate was 0.74 mg worm^{-1} day^{-1}, reaching a mean weight of 324.0 ± 145.0 gm worm^{-1} (kept singly) and 0.79 mg worm^{-1} day^{-1}, reaching mean weight of 344.0 ± 154.0 mg worm^{-1} (kept in batches) in cow manure (Fig. 1a); and 0.18 mg worm^{-1} day^{-1}, reaching mean weight of 61.8 ± 27.6 mg worm-1 (kept singly) and 0.64 mg worm-^{1} day^{-1}, reaching mean weight of 106.0 ± 47.5 mg worm^{-1} (kept in batches) in field soil after the completion of the experiment (Fig. 1b)

The worms showed a decline in growth rates after attaining the maximum biomass in both substrates (Tables 2, 3).

According to growth curve, growth rates were better in cow manure than on field soil. Significant differences were observed in growth rates for two substrates ($r = 0.922$; $P < 0.01$ raised singly and $r = 0.676$; $P < 0.01$ raised in batches) in cow manure; and ($r = 0.782$; $P < 0.01$ raised singly and $r = 0.976$; $P < 0.01$ raised in batches) in field soil.

The first indication of clitellum development appeared between days 34 and 36 after hatching with almost all worms being clitellate between days 43 and 47 (Fig. 2).

Our results of growth rates for *E. fetida* fed on cow manure and field soil (0.18 to 0.79 mg worm^{-1} day^{-1}) are low compared to published results for *Eisenia andrei* and *Eisenia fetida* fed on various types of wastes; e.g. 7 to 19 mg worm^{-1} day^{-1} for *Eisenia fetida* fed on horse manure;5 14 mg worm^{-1} day^{-1} fed on activated sludge;7 4.5 mg worm^{-1} day^{-1} in batch culture for immature *E. andrei* fed on horse manure and peat;8 7 mg worm^{-1} day^{-1} for *E. fetida* fed on cattle manure;9 5.1 to 17.5 mg worm^{-1} day^{-1} for *E. andrei* fed on fresh green material10. Growth rates for *E. fetida* in the present study were better on cow manure (C:N = 111.2) than on field soil (C:N = 13.7), and are similar to those for *Dendrobaena veneta* showing higher biomass production and growth rate when fed on paper sludge (C:N = 45.5) than on horse droppings (C:N = 25.4)11.

Cocoon production: Mating does not seem to be a prerequisite for cocoon production in *E. fetida* as worms kept singly in two substrates produced cocoons, which hatched successfully.

The cocoons of *E. fetida* have and oval shape. There are two sticky fibrous spines at both ends of the cocoons to which particles can adhere. The cocoons are soft and light brownish directly after their formation, harden rapidly, colour changing for reddish brown, and turning transparent before hatching. The mean length of the cocoons produced by worm was 3.37 ± 0.27 (2.5-5.1 mm; n=10) and the diameter 2.15 ± 0.19 mm (ranged 1.2-3.2 mm; n=10).

In our study, *E. fetida* needed 75 to 90 days to produce cocoons (Tables 2-3). Production of cocoons was higher in worms reared in batches than those reared singly in both substrates. Production of cocoons stopped after 285 days in field soil and 330 days in cow manure when the worms became older and then died.

The mean cocoon production rate was 0.03 ± 0.004 cocoons worm^{-1} day^{-1} and 0.02 ± 0.002 worm^{-1} day^{-1} for worms kept singly and in batches, respectively in cow manure; 0.02 ± 0.002 kept both singly and in batches in field soil.

Cocoon mass in uncorrelated with the mass of the parent adult when reared singly ($r = 0.028$) and in batches ($r = 0.163$) in cow manure (Fig. 3a); and in field soil reared singly ($r = 0.002$) but positively correlated in worms raised in batches ($r = 0.453$; $P < 0.5$) (Fig. 3b).

Cocoon mass and mass of hatchlings emerging from this cocoons is positively correlated in worms reared singly ($r = 0.998$; $P < 0.01$) and in batches ($r = 0.909$; $P < 0.01$) in cow manure (Fig. 4a); and in worms reared singly ($r = 0.756$; $P < 0.01$) and in batches ($r = 0.899$; $P < 0.01$) in field soil (Fig. 4b).

With regard to reproduction rate, *E. fetida* in the present study commence cocoon production later than *E. andrei*[11] and *E. fetida*[12] and total number of cocoons produced by *E. fetida* in the present study was more in cow manure than that in field soil. However, mean cocoon production rates were not very

different in two substrates. The overall mean cocoon production rates of *E. fetida* recorded in the present study (0.02 to 0.03 cocoons worm^{-1} day^{-1}) are very low than those reported for *E. fetida*; 0.1 to 0.5 cocoons worm^{-1} day^{-1}.13,14 and 0.17 to 0.27 cocoons worm^{-1} day^{-1} for *E. andrei*[8, 10,15,16].

Hatching success of cocoons and fecundity: 100% hatching success was observed in cocoons placed in different media (Table 4). In the present study of 50 cocoons, 2.4 hatchlings emerged per cocoon hatched with figures with all media combined.

Table 1. Composition of food substrates given to *Eisenia fetida*

Material	pH	Lignin	Ligno-cellulose	Ash (%)	Total C (%)	N (%)	C:N (%)
Cow Manure	8.5	15.2	3.9	0.89	12.9	0.116	111.2
Field soil	6.69	N.A.	-	-	2.72	0.198	13.7

Table 2. Relationship between worm biomass and the mass of the cocoons and hatchlings produced in cow manure by *Eisenia fetida* (± SE)

Age of worms (Days)	Singly				In batches			
	Worm biomass (mg)	No. of Cocoons (mg)	Mass of cocoons (mg)	Mass of hatchling (mg)	Worm biomass (mg)	No. of Cocoons (mg)	Mass of cocoons (mg)	Mass of hatchling (mg)
75	84.2 ± 37.7	2	19.0 ± 1.4	14.0 ± 4.2	102.0 ± 45.8	2	11.5 ± 0.4	9.2 ± 0.8
90	82.3 ± 36.8	5	18.0 ± 2.6	11.7 ± 1.0	123.3 ± 10.4	3	14.5 ± 0.4	7.5 ± 0.4
105	84.3 ± 37.7	3	16.0 ± 1.7	12.3 ± 1.7	122.6 ± 10.1	4	12.3 ± 1.4	10.2 ± 1.7
120	90.8 ± 40.6	12	17.2 ± 0.9	13.0 ± 0.5	216.0 ± 96.9	4	14.0 ± 2.2	10.0 ± 2.2
135	78.6 ± 35.2	4	15.0 ± 0.4	11.3 ± 2.3	112.0 ± 50.4	7	16.0 ± 0.3	12.5 ± 0.5
150	67.2 ± 30.0	3	17.6 ± 1.2	12.6 ± 1.8	143.0 ± 64.0	10	18.4 ± 1.1	15.4 ± 1.2
165	71.9 ± 32.2	8	12.9 ± 0.5	9.6 ± 0.5	109.0 ± 48.9	6	12.0 ± 0.08	9.0 ± 0.06
180	79.6 ± 35.6	2	14.0 ± 4.2	11.5 ± 3.9	129.0 ± 57.7	8	11.2 ± 2.6	8.2 ± 0.9
195	77.9 ± 34.8	3	17.2 ± 1.9	12.5 ± 1.9	127.0 ± 57.1	12	18.1 ± 1.6	14.8 ± 1.7
210	78.1 ± 34.9	2	18.0 ± 0.9	14.1 ± 1.1	124.0 ± 55.9	5	15.5 ± 1.3	11.4 ± 2.0
225	94.2 ± 42.1	2	16.0 ± 0.5	10.2 ± 2.1	186.0 ± 83.0	9	12.6 ± 3.7	8.3 ± 2.9
240	77.4 ± 34.6	3	17.2 ± 1.5	13.9 ± 1.3	174.0 ± 78.0	3	14.0 ± 0.6	11.2 ± 1.4
255	77.1 ± 34.5	4	13.9 ± 1.3	7.2 ± 0.05	184.0 ± 82.0	2	18.2 ± 1.3	14.0 ± 1.5
270	172.0 ± 77.3	3	12.5 ± 0.7	9.1 ± 2.3	193.0 ± 86.0	3	17.0 ± 0.5	14.5 ± 1.2
285	174.0 ± 78.1	2	19.1 ± 1.5	15.3 ± 1.1	190.0 ± 85.0	6	12.6 ± 0.7	9.7 ± 3.2
300	177.0 ± 79.4	1	17.2 ± 0.0	12.3 ± 0.0	159.0 ± 71.0	2	14.0 ± 1.2	10.1 ± 3.6
315					118.0 ± 53.0	2	18.6 ± 0.8	16.0 ± 2.5
330					100.0 ± 44.0	4	14.6 ± 0.5	10.8 ± 1.9

Correlation coefficients (r) between worm biomass and mass of cocoons:

r = 0.028 (singly) **r = 0.163 (In batches)**

Correlation coefficients (r) between mass of cocoons and mass of hatchlings:

r = 0.998; P < 0.01 (singly) **r = 0.909; P < 0.01 (In batches)**

Table 3. Relationship between worm biomass and the mass of the cocoons and hatchings produced in field soil by *Eisenia fetida* (± SE).

Age of worms (Days)	Singly				In batches			
	Worm biomass (mg)	No. of Cocoons (mg)	Mass of cocoons (mg)	Mass of hatchling (mg)	Worm biomass (mg)	No. of Cocoons (mg)	Mass of cocoons (mg)	Mass of hatchling (mg)
75	41.0 ± 18.3	-	-	-	58.0 ± 25.9	4	13.0 ± 0.8	10.8 ± 2.1
90	30.0 ± 13.4	5	17.5 ± 2.4	12.3 ± 1.1	69.0 ± 30.9	9	16.2 ± 0.5	13.6 ± 1.8
105	32.0 ± 14.3	5	15.2 ± 0.3	10.8 ± 1.9	67.0 ± 30.0	13	15.7 ± 0.6	12.1 ± 3.5
120	39.0 ± 17.4	3	18.3 ± 1.1	14.0 ± 2.8	61.0 ± 27.3	7	16.4 ± 1.5	12.0 ± 1.7
135	39.0 ± 17.4	2	17.6 ± 0.9	13.0 ± 0.5	76.0 ± 34.0	6	16.0 ± 0.3	12.2 ± 0.8
150	54.0 ± 24.2	3	18.5 ± 3.9	10.5 ± 0.4	95.0 ± 6.7	7	18.6 ± 0.7	14.3 ± 2.2
165	48.0 ± 21.7	1	11.6 ± 0.0	9.0 ± 0.0	106.0 ± 47.5	5	11.9 ± 0.2	8.4 ± 2.9
180	48.0 ± 21.5	4	18.2 ± 1.1	14.8 ± 0.9	63.0 ± 28.4	2	18.2 ± 1.6	14.2 ± 0.5
195	43.4 ± 19.4	2	16.5 ± 2.5	14.0 ± 1.4	63.48 ± 28.5	3	17.8 ± 0.6	13.0 ± 0.7
210	38.4 ± 17.2	2	12.2 ± 0.4	10.2 ± 2.2	62.1 ± 27.8	2	13.6 ± 3.7	9.6 ± 2.8
225	39.2 ± 17.5	3	14.7 ± 0.5	10.3 ± 0.7	20.2 ± 9.0	1	16.2 ± 0.0	14.0 ± 0.0
240	41.1 ± 18.4	1	15.0 ± 0.0	12.0 ± 0.0	22.9 ± 10.2	4	18.2 ± 1.2	12.6 ± 0.6
255	39.9 ± 17.9	3	17.1 ± 2.1	14.0 ± 0.8	38.2 ± 17.1	3	14.8 ± 0.7	11.2 ± 2.8
270	42.7 ± 19.1	2	15.0 ± 1.0	12.8 ± 0.9	52.7 ± 23.6	5	17.4 ± 2.2	12.8 ± 1.4
285	44.1 ± 19.7	3	12.3 ± 1.9	9.0 ± 1.7	46.6 ± 20.9	1	12.1 ± 0.1	7.2 ± 2.2

Correlation coefficients (r) between worm biomass and mass of cocoons:

r = 0.002 (singly) **r = - 0.453; P < 0.05 (In batches)**

Correlation coefficients (r) between mass of cocoons and mass of hatchlings:

r = 0.756; P < 0.01 (singly) **r = 0.899; P < 0.01 (In batches)**

Table 4. Number of hatchlings cocoon^{-1} and hatchlings success of cocoons of *Eisenia fetida* in different substrates

Medium	No. of cocoons used	No. of cocoons and % of total no. of cocoons that hatched										No. of cocoons hatched	No. of hatchlings (mean ± SE)
		1 hatchling		2 hatchling		3 hatchlings		4 hatchlings		5 hatchlings			
		No.	%	No.	%	No.	%	No.	%	No.	%		
Cow manure	10	2	20.0	2	20.0	3	30.0	2	20.0	1	10.0	10	2.9 ± 0.46
Field Soil	10	2	20.0	4	40.0	3	30.0	1	10.0	-	-	10	2.3 ± 0.28
Moist filter paper	10	3	30.0	5	50.0	2	20.0	-	-	-	-	10	1.9 ± 0.22
Distilled water	10	6	60.0	3	30.0	1	10.0	-	-	-	-	10	1.5 ± 0.21
Litter	10	1	10.0	3	30.0	2	20.0	2	20.0	2	20.0	10	3.3 ± 0.51
Total	**50**	**14**	**28.0**	**17**	**34.0**	**11**	**22.0**	**5**	**10.0**	**3**	**6.0**	**50**	**2.38 ± 0.19**

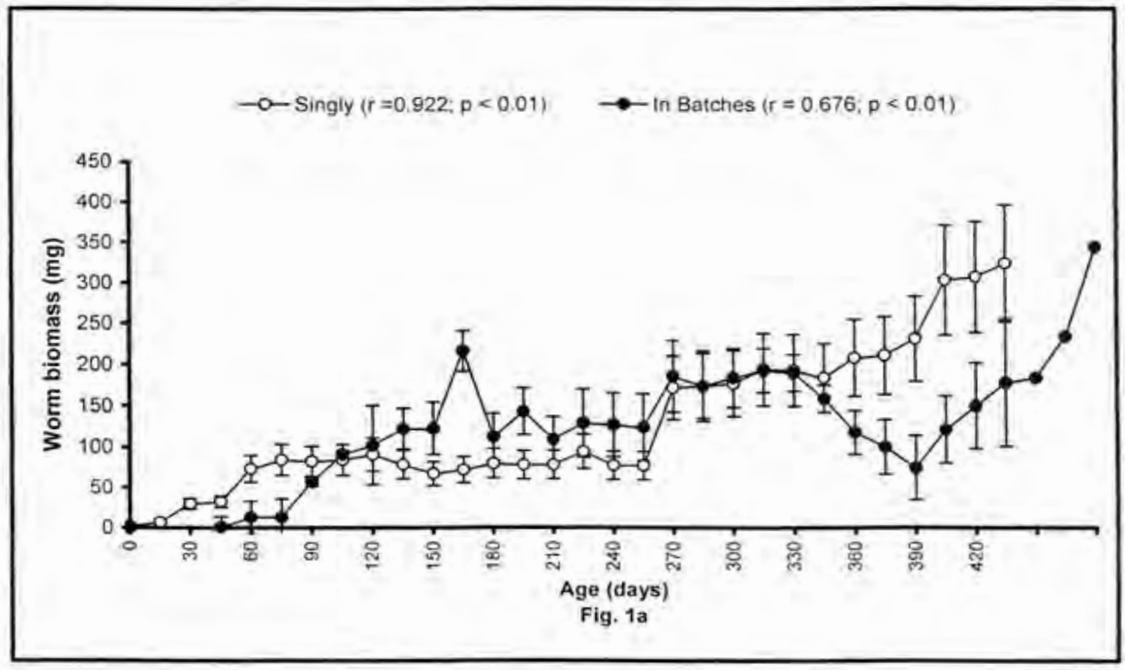

Fig. 1a

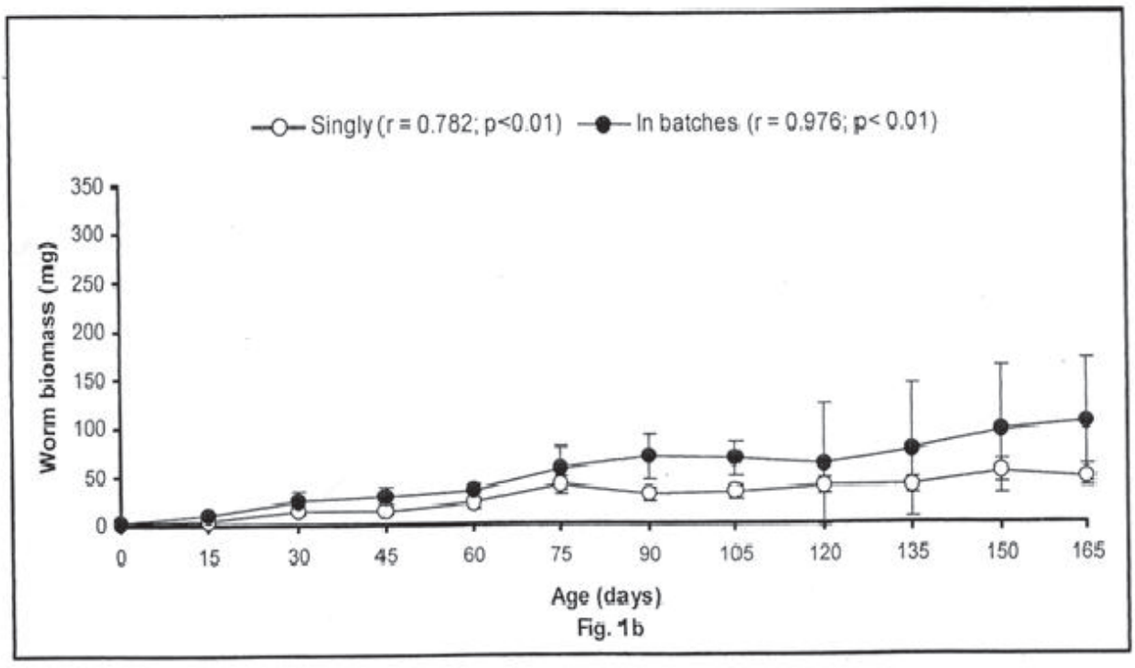

Fig. 1b

Fig. 1a-b. Growth rates of Eisenia fetida raised singly and in batches in urine-free cow manure (a) and in field soil (b).

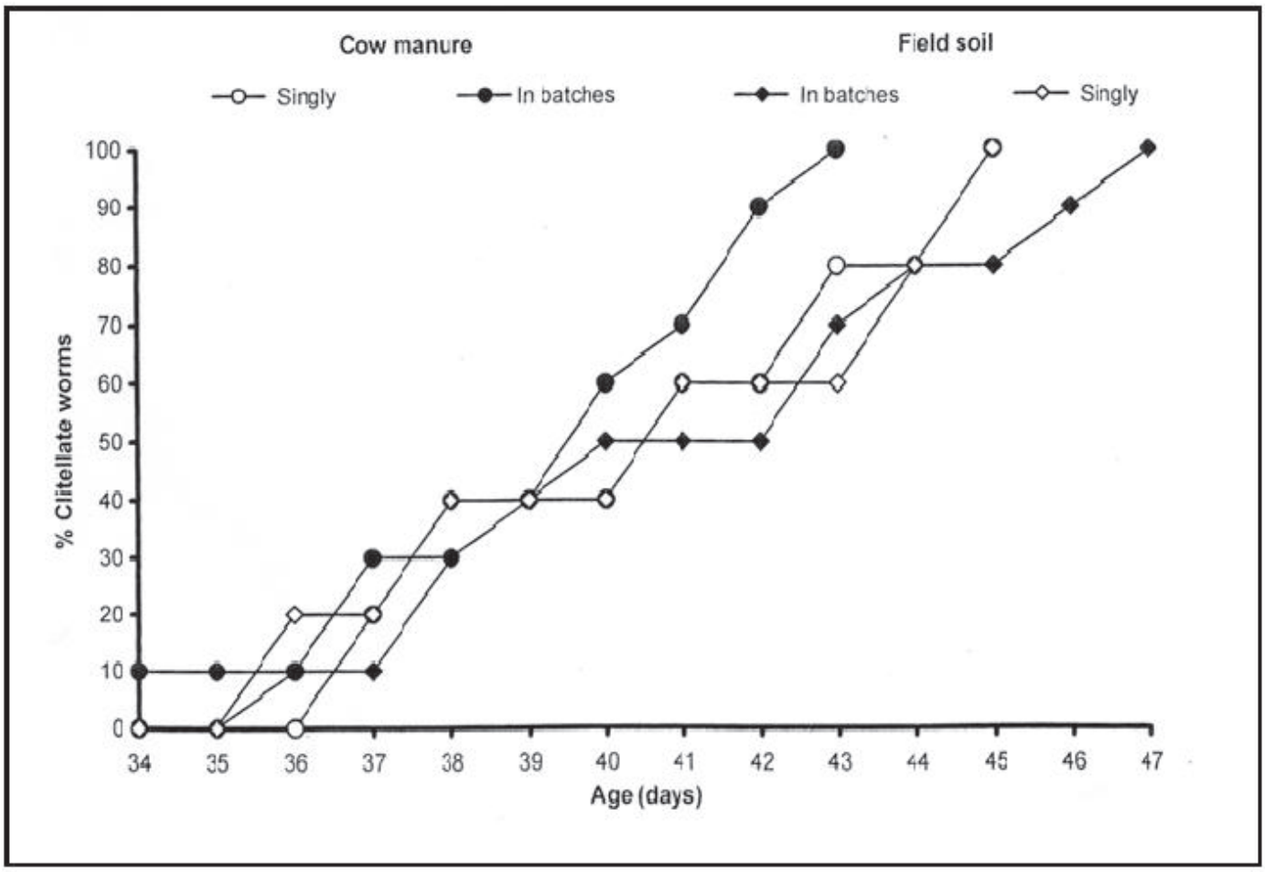

Fig. 2. Percentage of clitellated individuals of *Eisenia fetida* kept singly and in batches in cow manure and in field soil.

The incubation period of cocoons hatched in 5 media were; 26.0 ± 0.85 days in cow manure, 27.2 ± 0.86 days in field soil, 28.0 ± 1.1 days in moist filter paper, 2.9 ± 0.9 days in distilled water, and 26.5 ± 0.82 days in litter.

A hatching success of 100% for cocoons in the present study is similar to that reported for *Dendrobaena rubida* and *Lumbricus rubellus*[17] and *Drawida nepalensis*[18].

Our figures of 2.4 hatchlings obtained for *E. fetida* are similar to other reports of 2.7 hatchlings for *E. fetida*;14 2.12 hatchlings per cocoon;6 2.77 hatchlings for *Eudrilus eugeniae*;19 2. 47 to 3.45 hatchlings for *E. andrei*[8,16].

Our data demonstrated that food can greatly influences the growth rate and cocoon production in *E. fetida*. Further studies at different densities and on easily degradable organic matter such as household refuse are needed for breeding this speciesn Kumaun Himalaya.

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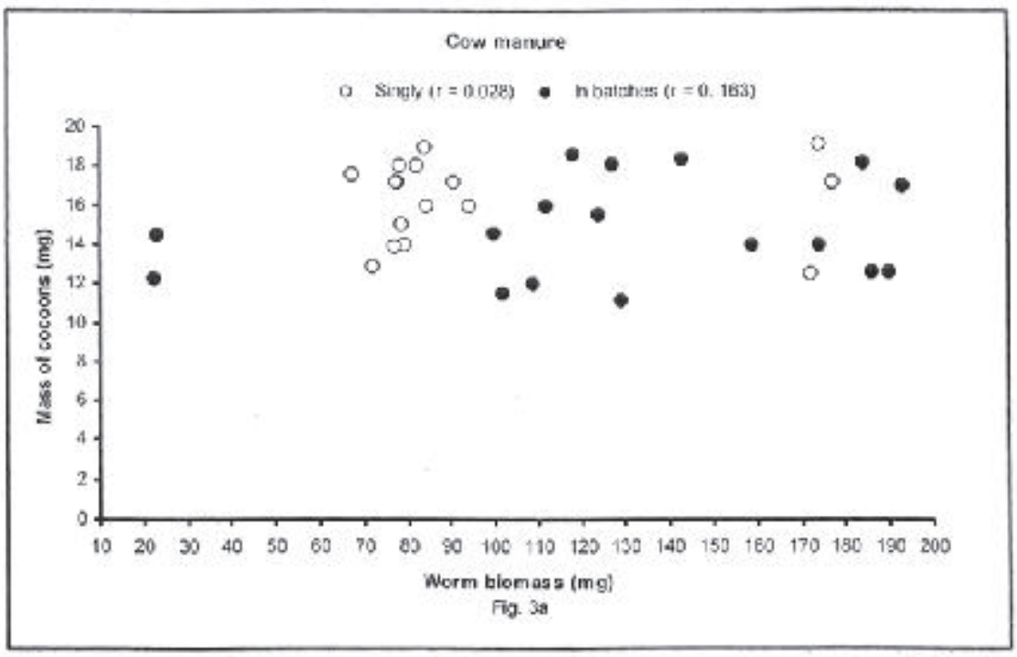

Fig. 3a

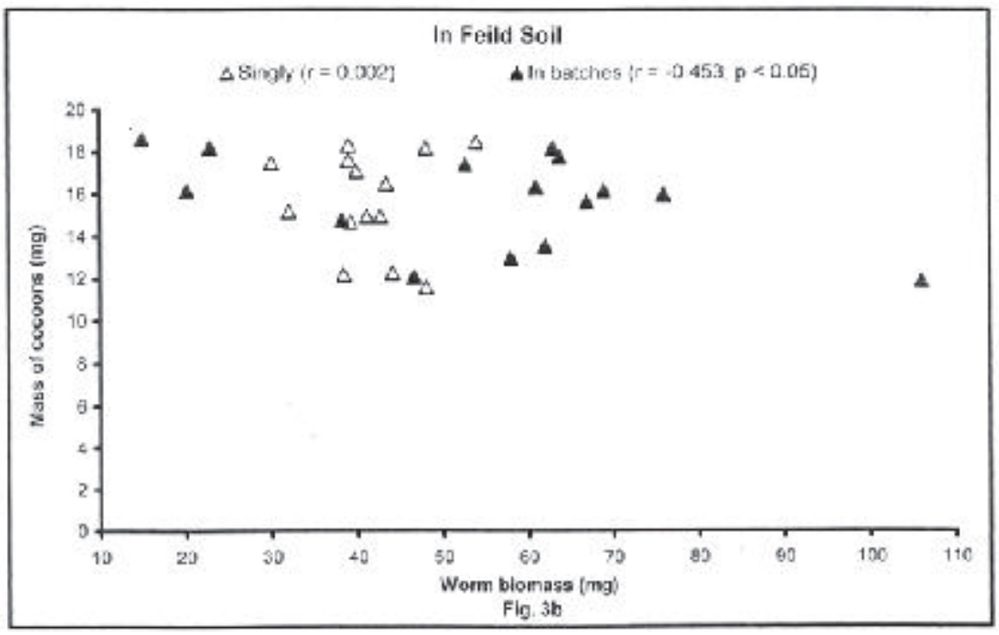

Fig. 3b

Fig. 3a-b. Relationship between worm biomass and cocoon mass for *Eisenia fetida* kept singly and in batches in cow manure (a) and in field soil (b).

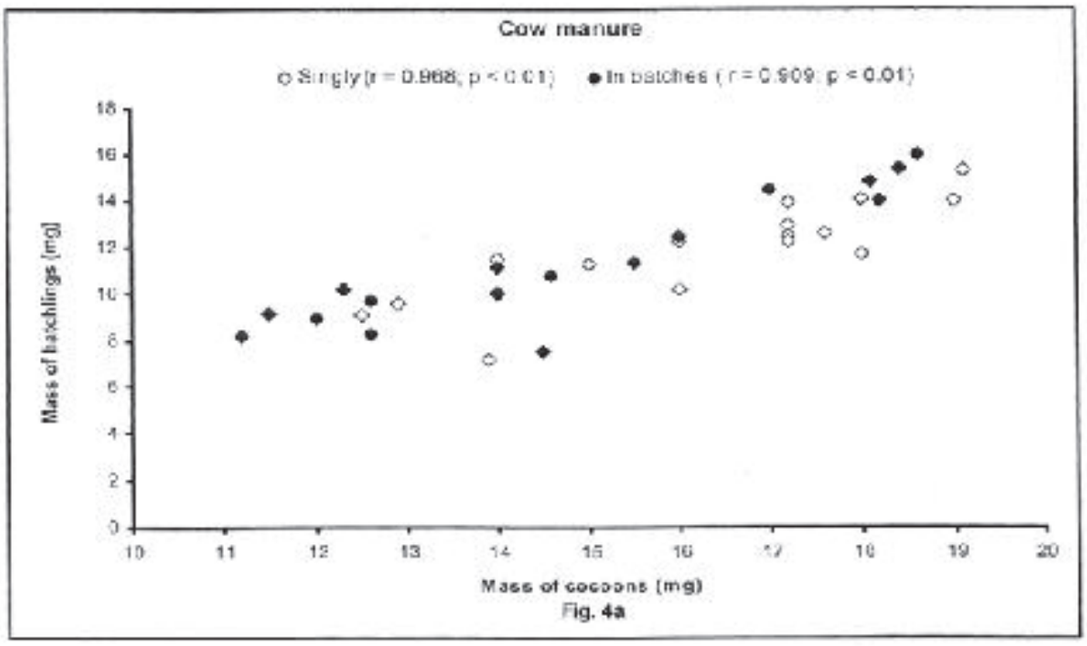

Fig. 4a

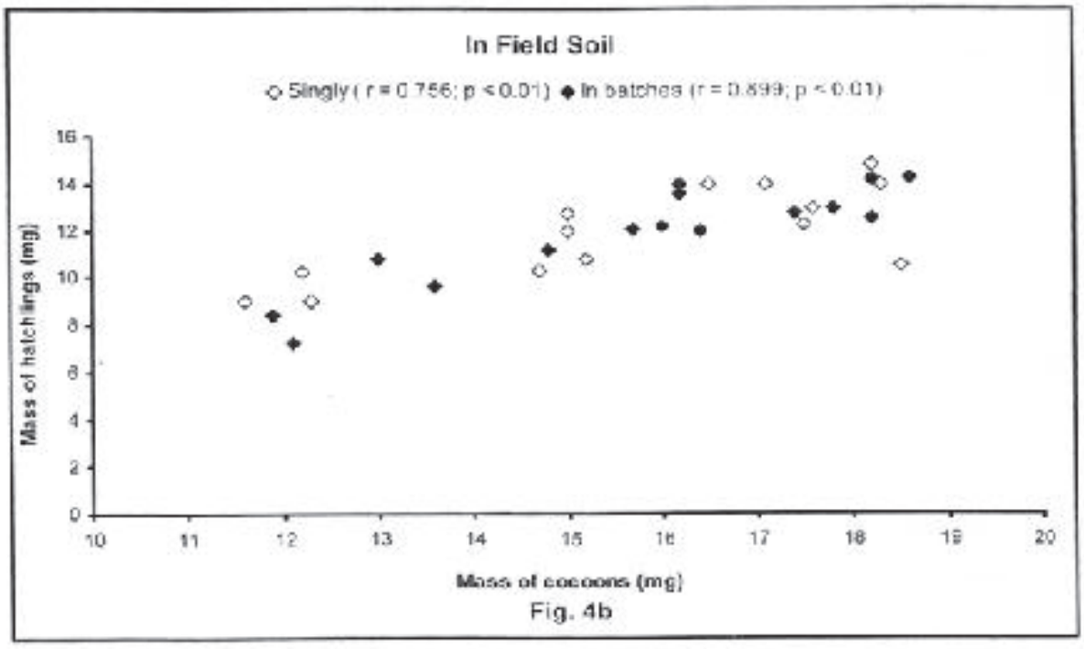

Fig. 4b

Fig. 4a-b. Relationship between the biomass of hatchlings of *Eisenia fetida* and the mass of cocoons from which they emerged after incubation, kept singly and in batches in cow manure (a) and in field soil (b).

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