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Life cycle of *Lampito mauritii* (Kinberg) in comparison with *Eudrilus eugeniae* (Kinberg) cultured on different substrates

K. Parthasarathi*
***kpsarathi_2000@rediffmail.com**

Department of Zoology, Division of Vermibiotechnology, Annamalai University, Annamalainagar-608 002, India

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Abstract: *Growth (length, biomass and mean growth rate) and reproduction (total duration, clitellum appearance, clitellum completion, cocoon commencement, rate of cocoon production, incubation period, hatching success and mean number of hatching per cocoon) of indigenous Lampito mauritii (Kinberg) in comparison with exotic Eudrilus eugeniae (Kinberg) cultured on three feed substrates-clay loam soil, cowdung and pressmud (filter cake) have been studied over a period of 360 days under laboratory conditions (30 ± 2°C, 60-65% moisture). There is a positive relationship between length and biomass of both worms cultured on three feed substrates throughout the period of study. The decrease of worm length and biomass observed slightly on 63-70th days in Lampito mauritii and 42-49th days in Eudrilus eugeniae cultured on three fed substrates are the results of the onset of cocoon production. After 270 days both worms in all these fed substrates show decreasing trends of length and biomass which are due to continued reproduction and aging. Among the three fed substrates, pressmud supports significantly maximum worm length and biomass (between 90-130 days in Eudrilus eugeniae and 110-170 days in Lampito mauritii), earlier attainment of sexual maturity (between 51-76 days in Limpito mauritii and 27-37 days in Eudrilus eugeniae), earlier commencement of cocoon production (37.7 ± 0.0 days in Eudrilus eugeniae and 76.4 ± 0.10 days in Limpito mauritii), shorter incubation periods (16.3 ± 0.28 days in Eudrilus eugeniae and 26.7 ± 0.81 days in Limpito mauritii), more hatching success (98% in Limpito mauritii and 86% in Eudrilus eugeniae), more mean number of hatchling per cocoon (3.2 ± 0.03 in Limpito mauritii and 2.6 ± 0.06 in Eudrilus eugeniae) and shorter duration of life cycle (108.8 ± 0.07 days in Limpito mauritii and 60.2 ± 0.09 days inEudrilus eugeniae) than cowdung and clay loam soil.*

Key words*: Pressmud, Lampito mauritii, Eudrilus eugeniae, Growth, Reproduction, Vermiculture, Vermicomposting*
PDF file of full length paper is available with author

Introduction

Earthworm's growth, maturation, cocoon production and reproductive potential are not only influenced by environmental conditions alone but are also strongly affected by the quality and availability of food. Growth, reproduction, life cycle and environmental requirements of earthworms were studied by Neuhauser *et al.* (1979) using sludge and horse manure; Loehr *et al.* (1985) using a mixture of animal and vegetable waste materials, Bano and Kale (1988) and Viljoen and Reinecke (1992) using cowdung; Elvira *et al.* (1998) using sludges from paper and pulp industries; Amoji *et al.* (1998) using agricultural organic waste; Manivannan *et al.* (2004) using sugar industrial wastes and Loh *et al.* (2005) using cattle and goat manures. Further, the trends of reproduction, the characteristics of cocoons, incubation period, hatching success and fecundity were studied in *E.eugeniae* (Reinecke and Viljoen, 1993), and in Indian earthworms *Perionyx excavatus*, *Lampito mauritii, Polypheretima elongata, Pontoscolex corethrurus, Eutyphoeus gammiei, Dichogaster modiglianii* and *Drawida nepalensis* (Bhattacharjee and Chaudhuri, 2002; Tripathi and Bhardwaj, 2005). Furthermore, relative population growth in time and space (Kale and Bano, 1991); moisture requirement and reproduction (Hallatt *et al*., 1992; Dominguez and Edwards, 1997) and temperature relations and their effect on survival growth, maturation and cocoon production (Viljoen and Reinecke, 1992; Biradar *et al*., 1999) were also well documented. Though earthworm biology reared on various organic wastes have been studied (Reinecke and Viljoen, 1993; Edwards and Bohlen, 1996; Elvira *et al.,* 1998; Bhattacharjee and Chaudhuri, 2002; Manivannan *et al.* 2004; Loh *et al*., 2005; Parthasarathi, 2006), till-to-date earthworm biology on their natural habitat has been poorly understood.

The studies on growth, reproduction and life cycle of the wide spread Indian megascolicid worm, *Lampito mauritii* – one among the four endemic species - are very scanty. It can withstand wide range of temperature, soil moisture and various other physical factors (Kale, 1988) and with wide choice of habitats and food preferences it has the highest frequency of distribution (Kale and Bano, 1992). Only cocoon morphology, hatching and emergence pattern in this worm have been studied by Bhattacharjee and Chaudhuri (2002). A thorough understanding of the reproductive biology and growth of a worm is a pre-requisite before subjecting the worm to any experimentation in the laboratory and more particularly in the agro-industrial practices. This paper describes the growth pattern and reproduction of *Lampito mauritii* when cultured on different substrates – clay loam soil, cowdung and pressmud, in comparison with *Eudrilus eugeniae*.

Materials and Methods

Preparation of feed substrates: Clay loam soil (S) and cowdung (C) collected from the agricultural experimental farm of Annamalai

University, and two months old, cured and dried pressmud (P) obtained from E.I.D. Parry Sugar Mill at Nellikuppam, Tamil Nadu were used as feed substrates. Press mud, a by product of sugar industry, is rich in OM (53%), OC (31%), protein (12-16%), sugar (10-14%), micro and macro nutrients, enzymes and microbes (Parthasarathi and Ranganathan, 1998, 1999; Parthasarathi, 2004; Parthasarathi *et al.,* 2006). They have shown P to be an ideal medium for vermiculture and is vermicomposted into good organic manure. The feed substrates (S, C and P) were powdered and passed through a 1 mm mesh sieve to obtain 1 mm particle sized substrates. 1000 g of each substrate at 65-70% moisture were provided to support the growth of 10 worms for 15 days. The substrates were left over for 48 hr to stabilize before the experimental animals were introduced into them.

Cocoon incubation, hatching inoculation and worm culture maintenance Cocoon of *L. mauritii* and *E. eugeniae* were collected from the stock culture and incubated at room temperature (28±1°C) in petridishes containing sun dried, fine particled cattle dung and moistened by sprinkling required quantity of water. Newly emerged hatchings were carefully removed using a fine painting brush and placed in petridish containing distilled water. One kg of 48 hr stabilized substrates were taken in plastic tray (32 x 27 x 7 cm). The sides and bottom of the tray were perforated to facilitate free aeration and to avoid water logging in the tray. Ten one day old hatchings of *L. mauritii* and *E. eugenia* were inoculated into separate trays. The trays were covered with nylon mesh and maintained in the vermiculture house at room temperature (30 ± 2°C) with 60-65% moisture for 360 days. Stabilized substrates were changed every fortnight from the commencement of experiments.

Growth parameters: The biomass (wet weight), body length (cm) and mean worm growth rate (mg/w/d) of each hatchlings were determined before both animals were introduced into each substrates and thereafter every seven days upto the age of 70 days, subsequently once in every 20 days upto 210 days and thereafter at the interval of 30 days upto 360 days during November 2003-2004. The worm body length (cm) was determined at the resting stage of worms, by leaving the worm on a graduated and laminated graph sheet. The biomass (wet weight) of worms were weighed in an electronic balance and the worm's growth rate (mg/worm/day) for specific periods was calculated using the formula

$$\frac{W_2 - W_1}{t_1 - t_2}$$

where W_1 and W_2 are the body weight at the beginning and end, respectively and t_1 and t_2 are the age of the worms at the beginning and end of the specific period of study.

Reproductive parameters: Worms were examined daily from the day 40 onwards in the case of *L. mauritii* and day 20 onwards in the case of *E. eugeniae* to trace the degree of differentiation and development of clitellum. In order to determine the onset of cocoon production, the substrates were examined daily. The rate of cocoon production were recorded once in 3 days for a period of first 30 days from the day of initiation of cocoon laying by all the 10 worms (as evidenced by the well developed and bulged clitellum). The rate of cocoon production was observed between 90 and 120 days for *L. mauritii* and between 50 and 80 days for *E. eugeniae*.

The cocoons were counted by hand sorting, biomassed / weighed and length and diameter of the cocoons were measured using slide calliper. Before weighing, the cocoons were washed lightly in distilled water. Fifty cocoons of both worms were selected and each one was placed in a separate petridishe (9 cm diameter) filled with 60-65% moistened substrates and at 27±1°C. Emergence of hatching were observed daily, counted by hand sorting and removed daily using a fine painting brush in order to determine the total number of hatchlings emerged from a single cocoon. The viability of the cocoon and its incubation period were also determined.

Statistical analysis: A three way ANOVA (Analysis of Variance – Software package Statistica, Version 5.0) and Duncan Multiple Range Test was used to evaluate the interaction effects between substrates (S, C and P), animals (earthworms) and periods (days). The level of significance was fixed at 0.05.

Results and Discussion

Data on growth – length, biomass and mean growth rate, and life cycle parameters – duration, clitellum appearance, clitellum completion, cocoon commencement, rate of cocoon production, incubation period, hatching success and mean number of hatchlings / cocoon of *L. mauritii* in comparison with *E. eugeniae* cultured on three different feed substrates – Clay loam soil(S), cowdung(C) and pressmud(P) are given in Fig. 1-4(a-h). A schematic diagrams of life cycle of both worms are depicted in Fig. 5 (i-vi).

The mean body length (Fig. 1) of newly hatched (one day old) *L. mauritii* and *E. eugeniae* cultured in S, C and P were 0.32 and 0.34 cm, 0.43 and 0.48 cm, and 0.51 and 0.55 cm, respectively. Both worms in all the cultural media exhibited slow growth during the first (or initial) 14 days, followed by an accelerated growth and attained the body lengths of 8.61 cm (S), 14.8 cm (C) and 15.34 cm (P) in *L. mauritii* after 63 days, and 10.40 cm (S), 17.56 cm (C) and 18.35 cm (P) in *E. eugeniae* after 42 days. Thereafter the growth rates were gradual and after 170 to 240 days in *L. mauritii* and 130 to 270 days in *E. eugeniae*, decreasing trends in growth rates were discermible, followed by an increase thereafter. After 270 days, both worms showed decling trends in their body lengths.

From the analysis of results on growth parameters – body length, biomass and mean growth rate of *L. mauritii* and *E. eugeniae* cultured in S, C and P, it is evident that both worms showed slow growth rates initially after hatching. Similar observations were made previously for *E. eugeniae* (Neuhauser *et al*., 1979) and *Perionyx excavatus* and *Eisenia andrei* (Neuhauser *et al*., 1980) cultured in different substrates (decaying animal matter, horse manure, activated sewage sludges, urine free cattle droppings, fresh solid pig manure *etc.*). The present study showed a positive relationship between length and biomass in *L. mauritii* and *E. eugeniae* cultured on three

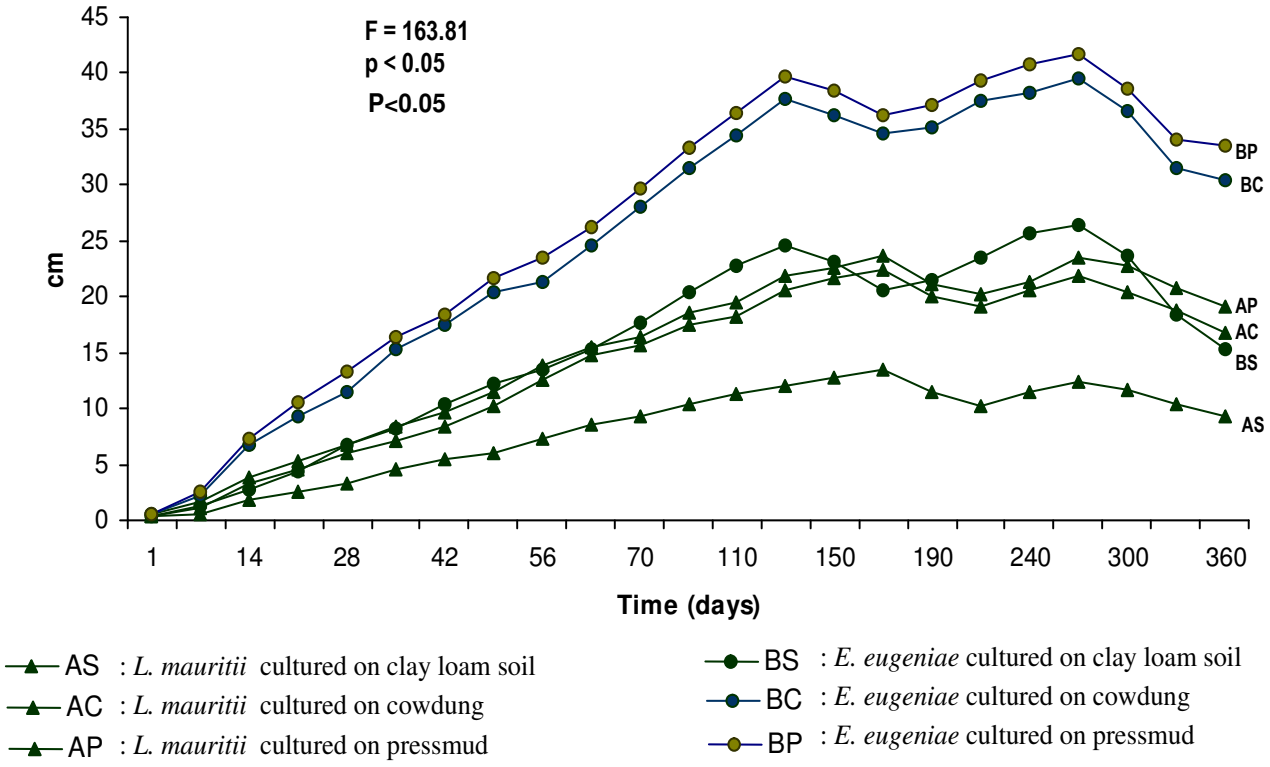

Fig. 1: Growth (length) of *L.mauritii* and *E. eugeniae* cultured on different substrates for a period of 360 days

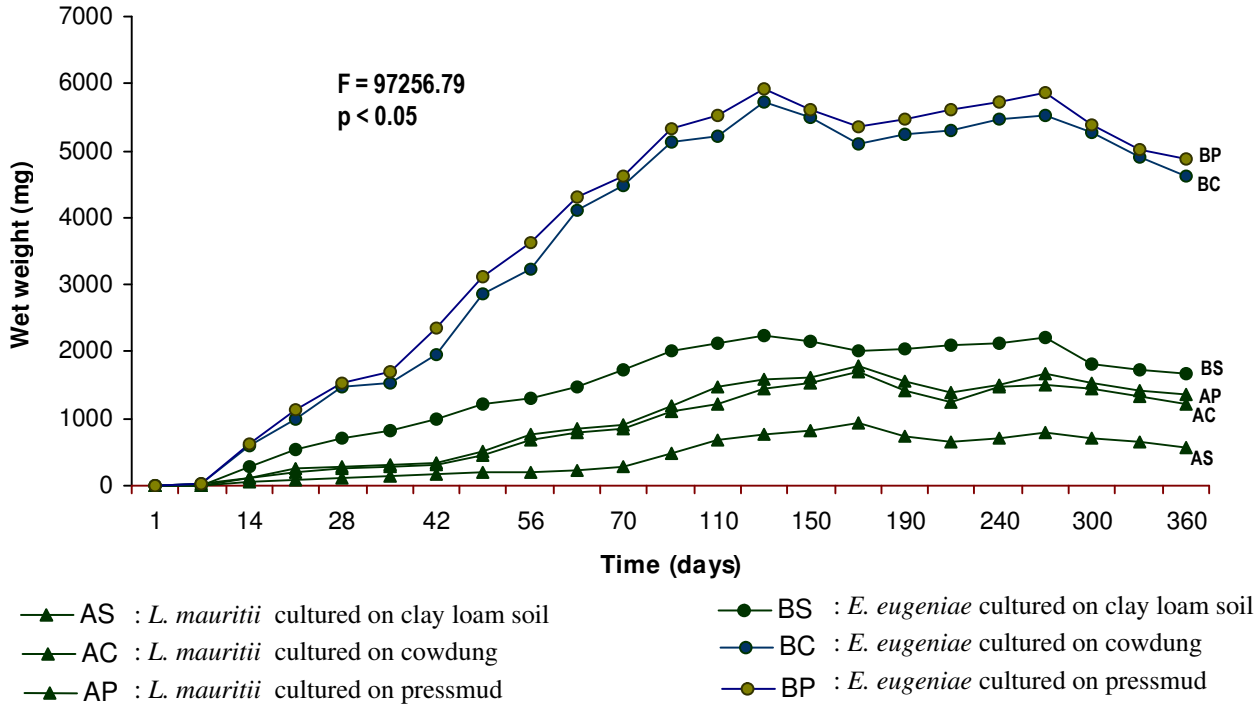

Fig. 2: Growth (biomass) of *L.mauritii* and *E. eugeniae* cultured on different substrates for a period of 360 days

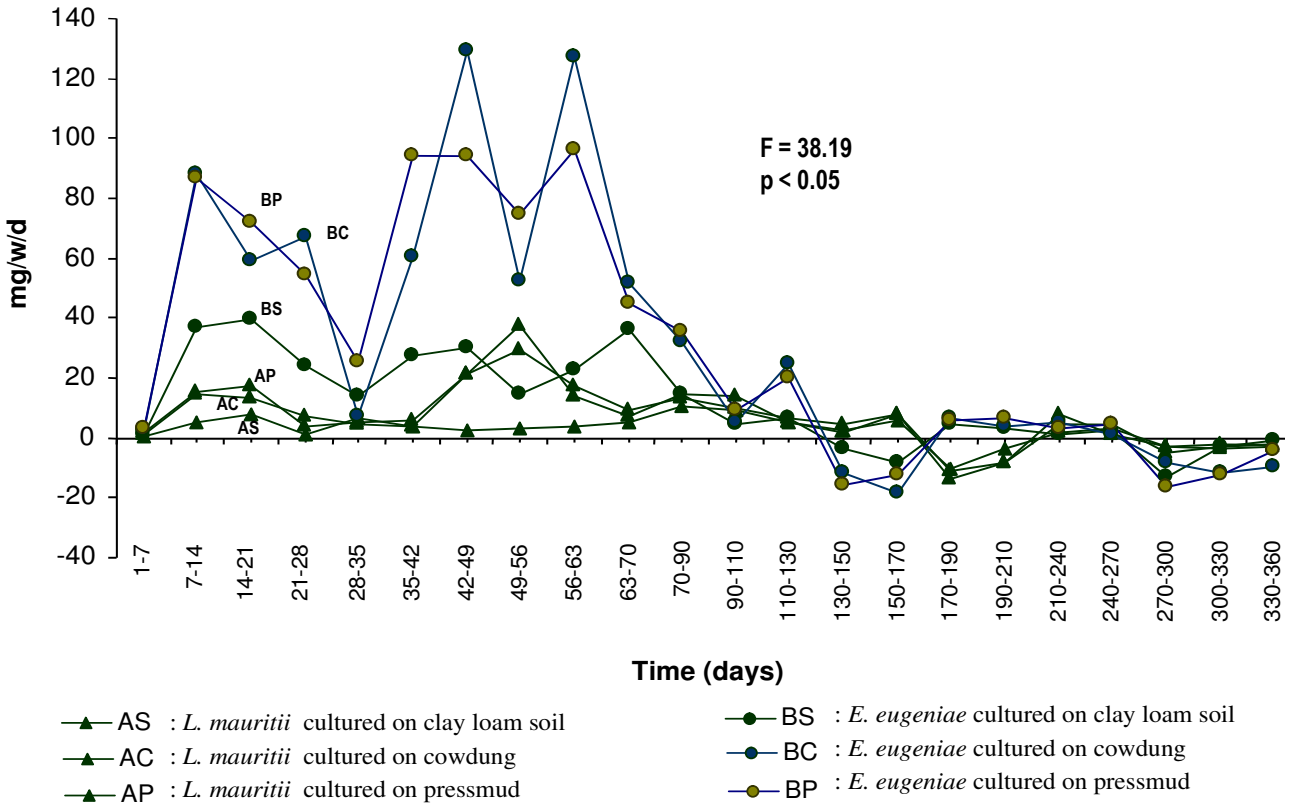

Fig. 3: Mean growth rate of *L.mauritii* and *E. eugeniae* cultured on different substrates for a period of 360 days

different fed substrates. Many researchers have established such a relationship among earthworms in general (Edwards, 1967; Patra and Dash, 1973; Mishra and Dash, 1980).

A generalized growth pattern was evident in *L. mauritii* and *E. eugeniae* cultured in S, C and P from 14 to 360 days: (a) an increase in growth rate upto pre-reproductive period of 63 days in *L. mauritii* and 42 days in *E. eugeniae,* (b) a decrease of the same (even though mean biomass continued to increase) during the active reproductive period upto 190 days in *L. mauritii* and 150 days in *E. eugeniae* and (c) very slow growth rates and regression of biomass during post – reproductive period. These findings agree with the conclusions arrived by Michon (1957), Avel (1959) and Nowak (1975) on different species of earthworms. They reported three phases of growth: (a) a rapid increase of growth rate during pre-productive phase followed by (b) a phase of steady decrease after attainment of sexual maturity and (c) a post-productive phase of very slow growth and decrease in body weight in senescent worms. The present findings of accelerated growth rate during the pre-reproductive phase of *L. mauritii* and *E. eugeniae* cultured in three fed substrates were more or less similar with the findings of Mba (1983) and Viljoen and Reinecke (1989b) who observed an accelerated growth in *E. eugeniae* upto 50 days from hatching.

The mean biomass of one day old (newly hatched) *L. mauritii* and *E. eugeniae* cultured in S, C and P were 3.2 and 3.3 mg, 4.4 and 4.6 mg, and 5.1 and 5.2 mg, respectively. Similar to mean body length, both worms in all the cultural media exhibited slow growth (body biomass) during the first 14 days, followed by an accelerated biomass and attained the biomass of 236.4 mg (S), 792.4 mg (C) and 864.2 mg (P) with a mean growth rate of 3.74 mg/d/w(S), 17.01 mg/w/d (C) and 14 mg/w/d (P) in *L. mauritii* after 63 days, and biomass of 1002.8 mg (S), 1948.7mg (C) and 2353.6 mg (P) with a mean growth rate of 27.08 mg/d/w(S), 60.4 mg/d/w (C) and 90.4 mg/d/w (P) in *E. eugeniae* after 42 days (Fig. 2 and 3). Thereafter the rate of biomass and mean growth rate were gradual, and after 170 days to 240 days in *L. mauritii* and 130 to 270 days in *E. eugeniae*, decreasing trends in rate of biomass and mean growth rate were discermible, followed by an increase thereafter. After 270 days, both worms showed decling trends in their biomass and mean growth rate.

The decrease of worm biomass observed during 63-70 day in *L. mauritii* and 42-49 day in *E. eugeniae* in three fed substrates were the result of the onset of cocoon production. This is in accordance with the findings of Graff (1981), Mba (1983) and Viljoen and Reinecke (1994) in *E. eugeniae*, who reported the decrease in worm biomass due to the requirement of large amount of energy for cocoon production and also for copulation and the last phase of decrease in worm biomass due to continued reproduction and aging.

Viljoen and Reinecke (1988, 1989 a and b, 1994) reported a decrease in biomass in 55 day old *E. eugeniae* cultured on cattle manure. But in the present study, similar decrease was observed

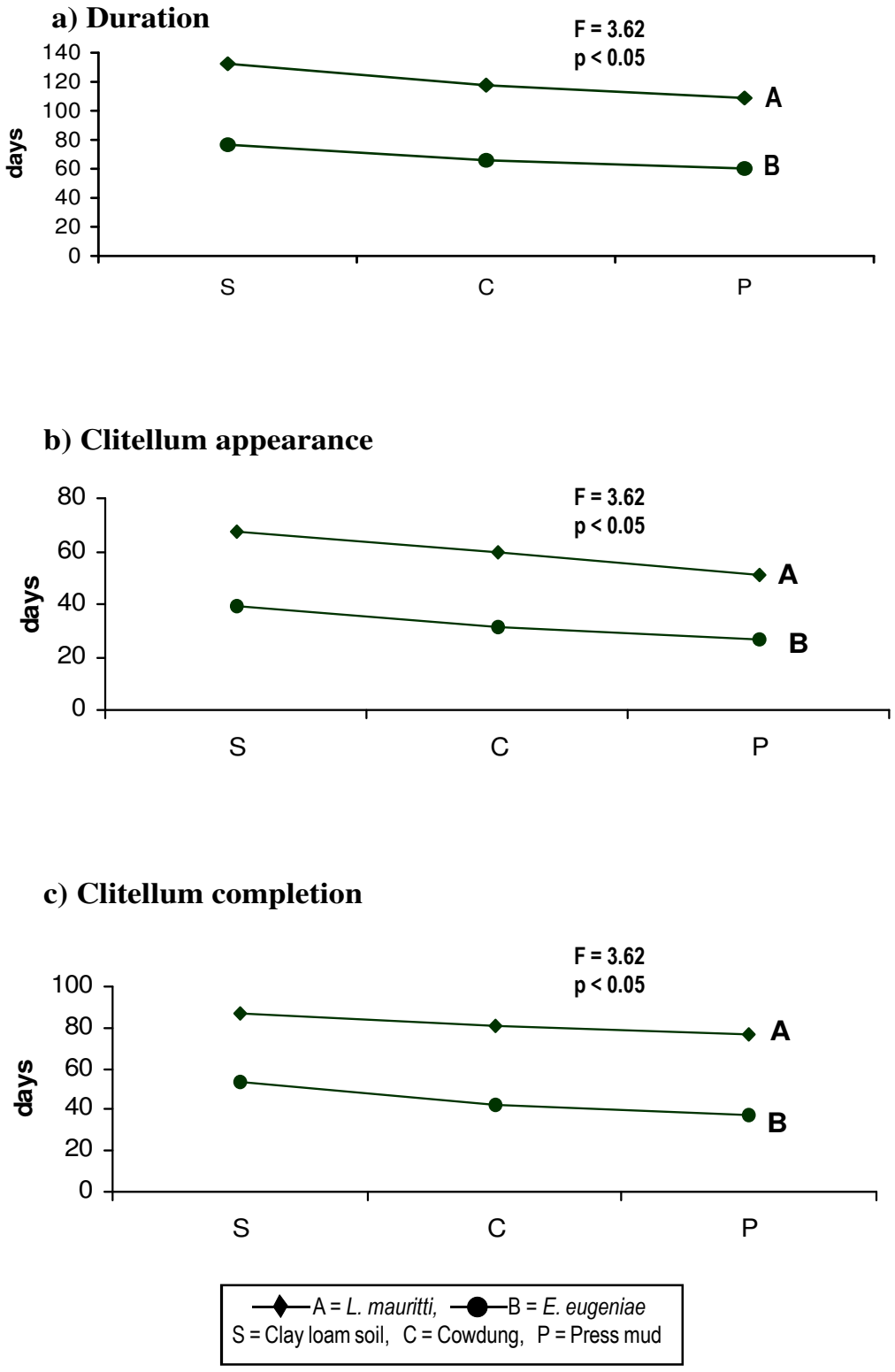

Fig. 4(a-c): Reproductive parameters of *L.mauritii* and *E. eugeniae* cultured on different substrates for a period of 360 days

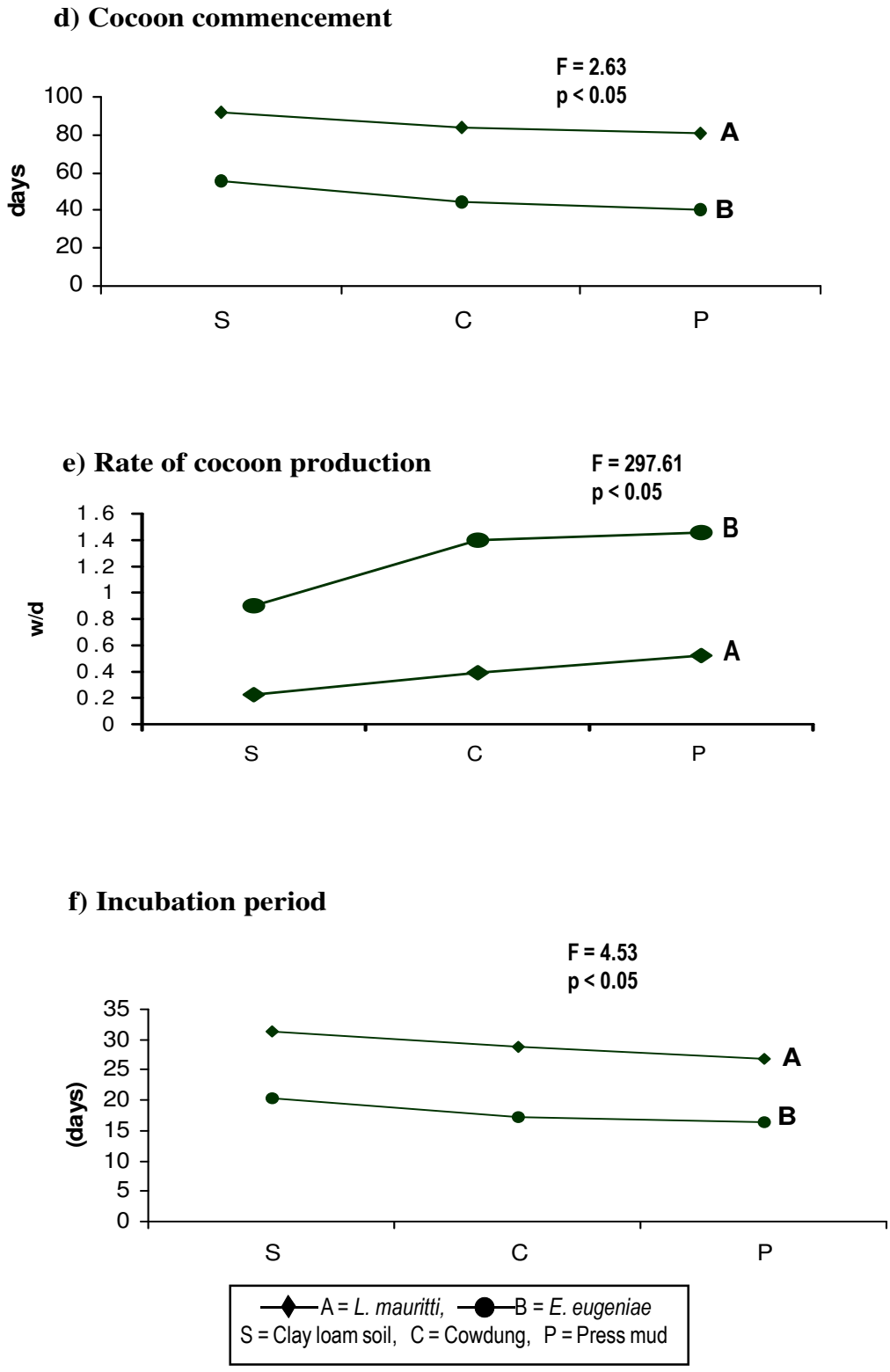

Fig. 4(d-f): Reproductive parameters of *L.mauritii* and *E. eugeniae* cultured on different substrates for a period of 360 days

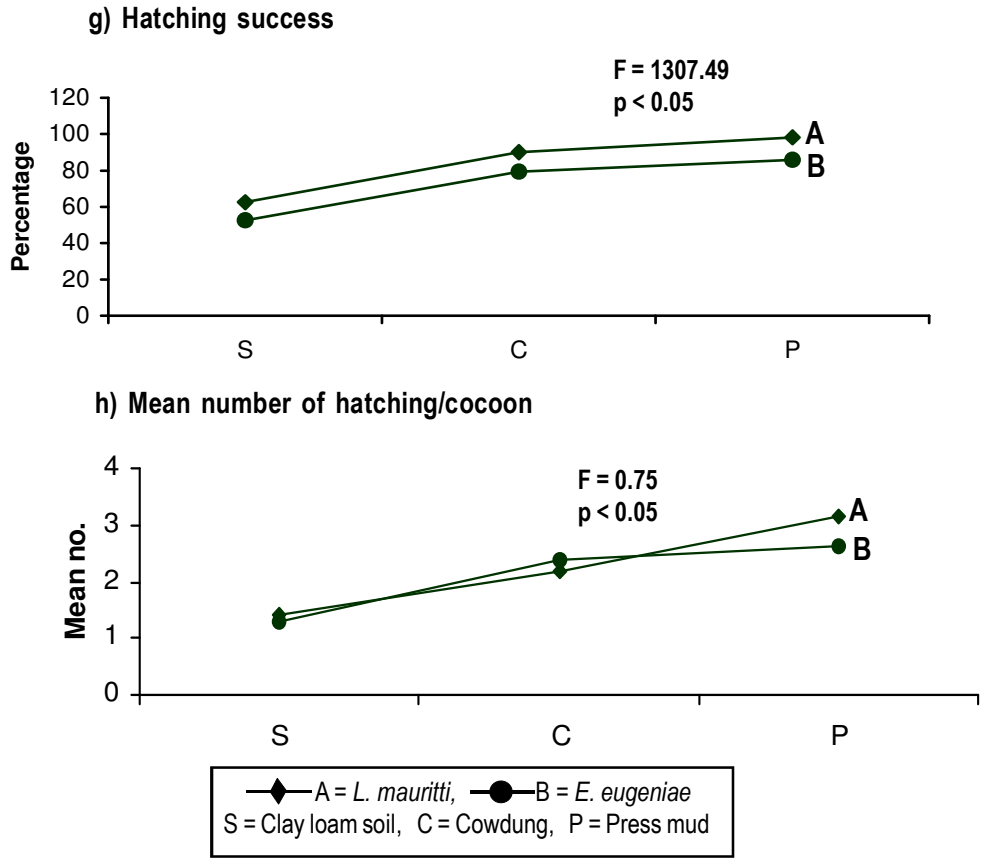

Fig. 4(g-h): Reproductive parameters of *L.mauritii* and *E. eugeniae* cultured on different substrates for a period of 360 days

slightly earlier for *E. eugeniae* and for *L. mauritii* cultured in three substrates. The earlier commencement of cocoon production in *E. eugeniae* and *L. mauritii* cultured in P followed by C and S reveals the stimulatory effect of nutrient rich pressmud on growth and reproduction of both worms.

Chaudhuri (2002), stated that the maximum size and development of an animal are determined by heredity and nutrition. In the present study, the maximum length and biomass of *L. mauritii* and *E. eugeniae* are found on 170 and 130 days cultured on P followed by C and S. The same species of earthworms when cultured on different fed substrates show variation in their growth (length and biomass) which refelcts the nutrient superiority of the feed substrates (Kale and Bano, 1992; Viljoen and Reinecke, 1994; Dominguez *et al.,* 2001). On the otherhand, *E. eugeniae* cultured on cattle dung attained a maximum biomass of 5600 mg (Viljoen and Reinecke, 1994) and *L. mauritii* 1500 mg (Kale and Bano, 1992). In the present study the maximum length and biomass of *L. mauritii* and *E. eugeniae* cultured in P were 23.680 and 41.630 cm and 1772.30 and 5923.50 mg, respectively. Ranganathan and Parthasarathi (1999) and Parthasarathi and Ranganathan (2000 a, b) reported that worm cultured in P show earlier differentiation of lobules in the ovary, earlier maturation of oocytes with increased number, earlier cocoon production with more hatchlings, earlier differentiation of clitellum and increased secretory activity of brain neurosecretory cells.

The duration of life cycle of *L. mauritii* and *E. eugeniae* cultured in S, C and P were 132.6 and 76.6 days, 117.6 and 65.7 days and 108.8 and 60.2 days, respectively. *L. mauritii* when compared to *E. eugeniae* showed a longer duration of life cycle cultured on three cultural media. Among the three substrates, P support significantly a shorter duration of life cycle in both worms [Fig. 4a, 5(i-vi)].

The development of clitellum in *E. eugeniae* cultured in S,C and P appeared on 39, 31 and 27 days, respectively. This was manifested by a slight change in colour in the clitellar region from reddish to yellow and afterwords, the clitellar region swelled progressively to a fully developed state of 53, 42 and 38 days, respectively during which periods the worms become clitellated and reproductively active. Unlike *E. eugeniae*, *L. mauritii* exhibited days for the development of clitellum were 67.5 (S), 59.6(C) and 51.3(P), respectively. This was manifested by a change in colour from brown to pale white in the clitellar area, and subsequently the clitellum swelled progressively to a fully developed state on 86.7, 81.2 and 76.2 days, respectively. A sexual mature *L. mauritii* showed pale – brown clitellum (Fig. 4b, c).

The formation of first cocoon in *E. eugeniae* and *L. mauritii* cultured in S, C and P were 53 and 86.7 days, 42 and 81 days and 37.7 and 76 days, respectively and continued upto 300 days in *E. eugeniae* and 360 days in *L. mauritii*. The mean cocoon production

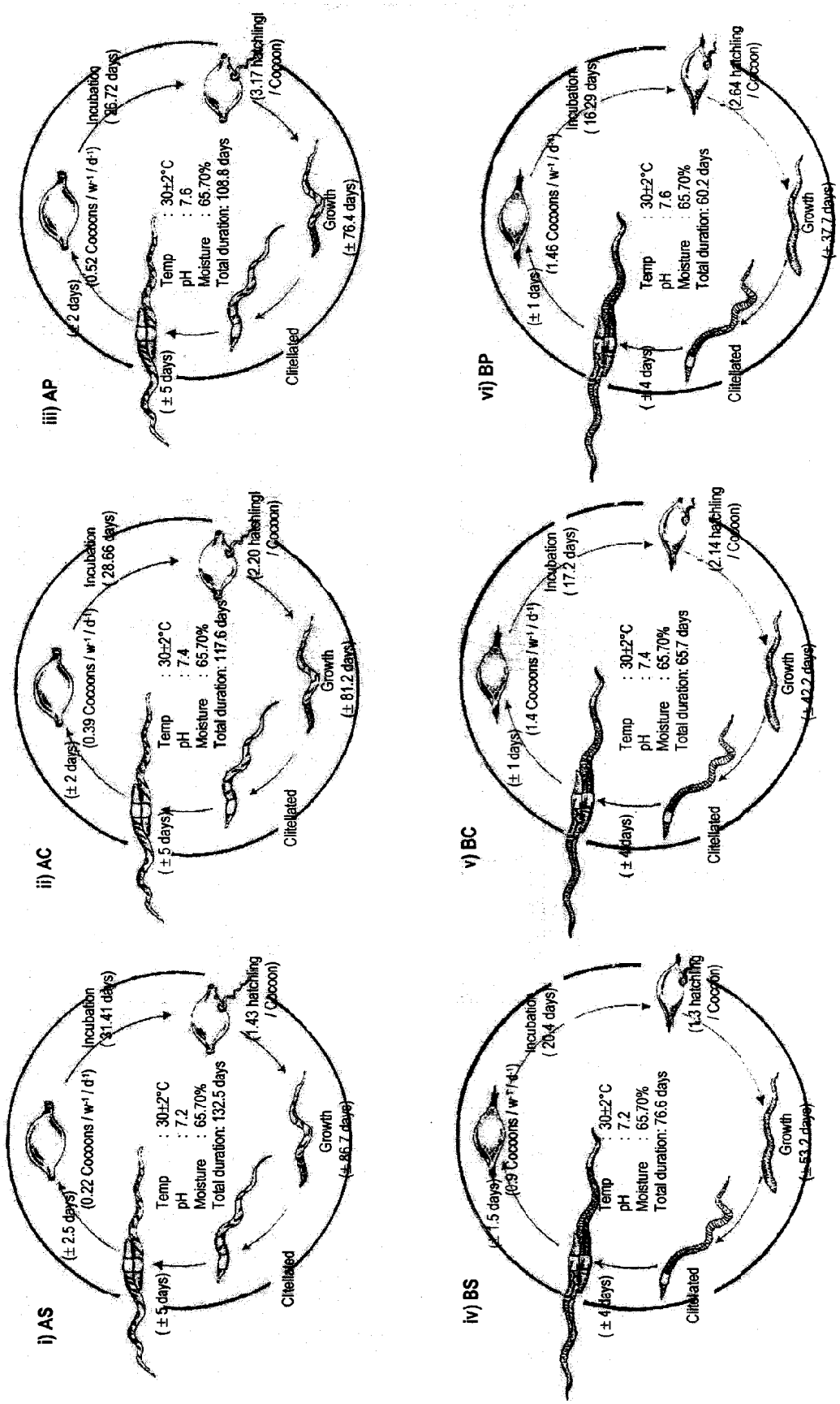

Fig. 5: Life cycle of Lampito mauritii (A) and Eudrilus engeniae (B) Cultured on different substrates

AS: L. *mauritii* cultured on clay loam soil
AC: *L. mauritii* cultured on cowdung
AC: *L. mauritii* cultured on pressmud
BS: *E. eugeniae* cultured on clay loam soil
BS: *E. eugeniae* cultured on cowdung
BS: *E. eugeniae* cultured on pressmud

of *E. eugeniae* and *L. mauritii* cultured in S,C and P were 0.9 and 0.22, 1.4 and 0.39 and 1.5 and 0.52 cocoon/w/d, respectively between 39-53 and 68-87, 31-42 and 60-81 and 27-28 and 51-76 days, respectively (Fig. 4d, e).

The mean incubation period of cocoon of *L. mauritii* and *E. eugeniae* cultured in S, C and P were 31 and 20, 28 and 17 and 27 and 16 days, respectively (Fig. 4f) and showed their hatching success were 63 and 53, 90 and 79 and 98 and 86 percent, respectively (Fig. 4g). The mean number of hatchlings per cocoon in both worms cultured in S, C and P were 1.4 and 1.3, 2.2 and 2.4 and 3.2 and 2.6, respectively (Fig. 4h). *L. mauritii* showed significantly longer incubation period of cocoon, hatchling success and more mean number of hatchling / cocoon than *E. eugeniae.* Among the three cultural media, P exhibited significantly short incubation period, more hatching success and more mean number of hatchling / cocoon in both worms (Fig. 4f-h).

The nutritive superiority of feed substrate stimulate the time of sexual maturity in earthworms (Bohlen, 2002). The time of attainment of sexual maturity varied in different species of earthworms as well as in the same species when cultured on different substrates. The attainment of sexual maturity in *E. eugeniae* was reported between 35-45 days (Viljoen and Reinecke, 1989b, 1994) and between 35-49 days (Neuhauser *et al.*, 1979) cultured on cattle manure and after 56 days on sludges and cattle manure (Graff, 1981). In the present study, *L. mauritii* attained sexual maturity between 67-87 days in S, 60-81 days in C and 51-76 days in P and *E. eugeniae* attained sexual maturity between 39-53 days in S, 31-42 days in C and 27-38 days in P. *E. fetida* cultured in cattle manure attained sexual maturity after 60 days (Venter and Reinecke, 1988), 7-8 weeks in farm waste (Edwards *et al.*, 1985) and 10 weeks in sludge (Neuhauser *et al.*, 1979). The earlier sexual maturity of *L. mauritii* and *E. eugeniae* cultured in P than in C and S and also the earlier maturity of *E. eugeniae* cultured in P when compared to the value of the same species cultured on other substrates such as cattle manure, farm waste, sludge *etc.,* confirm the nutritional superiority of P and indicate a possible positive stimulatory effect of P on sexual maturity of both species of worms.

The commencement of cocoon production was earlier and more in number in *E. eugeniae* cultured on P than in *L. mauritii* cultured on the same substrate. Reinecke *et al.* (1992) observed production of cocoons in *E. eugeniae* at the age of 46 days at 25°C in fresh urine-free cattle manure. The observed commencement of cocoon production in *E. eugeniae* on C and P in the present study was earlier than *E. fetida* cultured on cattle manure where cocoon production started only at 70 days after hatching (Venter and Reinecke, 1988). The cocoon production of *E. eugeniae* in C and P agree with the earlier findings on the mean cocoon production of 1.3 cocoons/w/d in the same species cultured on cattle dung (Viljoen and Reinecke, 1994). The present results also support the findings of Elvira *et al.* (1998), Dominguez *et al.* (2001) and Loh *et al.* (2005) who reported an earlier commencement of cocoon production and more mean number of cocoon production in *E. andrei* and *E. fetida* with vermiprocessing of sludges from paper-dairy industrial wastes and cattle - goat manure.

The P exhibited shorter incubation period of cocoon in *E. eugeniae* (16.2 days) followed by *L. mauritti* (26.7 days) This observation is comparable with the values reported for *E.eugeniae* cultured in cattle dung by Viljoen and Reinecke (1994) and Dominguez *et al.* (2001). However, the incubation period of *E. eugeniae* found in the present study was shorter than the periods reported for *E. fetida* and *P. excavatus* cultured on urine free cattle manure (Reinecke *et al.*, 1992). In *L. mauritti,* Dash and Senapati (1985), reported an incubation period of 28-30 days in cocoons cultured in house hold garbage. Similarly, P supports more hatching success for *L. mauritii* (98.2%) followed by *E. eugeniae* (86%) when compared with the worm cultured in C (90.10% and 79.4%) and S (62.5% and 52.5%). Reinecke and Viljoen (1988) reported cocoon hatching success of 84% in *E. eugeniae* cultured on cattle manure. The present study revealed for the first time a 100% cocoon hatching success for *L. mauritii* cultured on P followed by C (90%) and S (63%). More mean number of hatchlings per cocoon was discermible in the present study for *L. mauritii* (3.2) than for *E. eugeniae* (2.6) cultured on P, followed by C (2.4 for *L. mauritii* and 2.2 for *E. eugeniae*) and S (1.4 for *L. mauritii* and 1.3 for *E. eugeniae*). Such mean numbers of hatchling per cocoon obtained from P was found to be higher than other species of earthworms : *E. fetida* with 2.7 cultured on cattle manure (Venter and Reinecke, 1987, 1988), *P. excavatus* with 1.1 and *P. hawayana* with 1.2 on liquid municipal sludges (Loehr *et al.*, 1985) and *E. eugeniae* with 2.6 on cattle dung (Viljoen and Reinecke, 1994).

The total duration and the different stages of life cycle of *E.eugeniae* cultured on P are shorter than other fed substrates like C and S. In the present study *L. mauritii* when compared to *E. eugeniae* showed a longer duration of life cycle.

Finally, it is concluded that *E. eugeniae* is a suitable species for vermiculture and vermicomposting due to its rapid growth rate, larger size, quick attainment of sexual maturity, high rate of cocoon production, shorter incubation period and short life cycle. On the other hand, *L.mauritii* due to its higher rate of hatching success, high number of hatchlings production and tolerance to a wide range of environmental conditions (personal observation) could be preferred as an alternative species for vermiculture and vermicomposting.

Acknowledgments

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