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Optimal Stocking Density for Culturing Tropical Soil-dwelling Earthworm, *Pontoscolex corethrurus*

(Kepadatan Optimum untuk Pengkulturan Cacing Tanah Tropika, *Pontoscolex corethrurus*)

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ABSTRACT

The present study was carried out to determine the optimal stocking density for culturing tropical soil dwelling earthworm, Pontoscolex corethrurus. *F1 generation earthworms were cultured in four different stocking densities of 1, 4, 7 and 10 worms per vessel, corresponding to field densities of 50, 200, 350 and 500 individuals per* m^2*. Earthworms were kept under laboratory conditions (25±2°C and 25% moisture) for the 14 weeks study period. The results showed that at higher earthworm densities (>350 individuals per* m^2*), the earthworm growth was slower and sexual maturation was delayed as compared with their counterparts in lower stocking density. With the high survival rate and parthenogenetic reproduction mode,* P. corethrurus *could potentially be used as tropical soil rehabilitation agent.*

Keywords: Cocoon; culture; earthworm; stocking density

ABSTRAK

Kajian ini telah dijalankan untuk menentukan kepadatan optimum pengkulturan cacing tanah tropika Pontoscolex corethrurus. *Generasi pertama (F1) cacing tanah dikultur dalam empat kepadatan berbeza, iaitu 1, 4, 7 dan 10 individu setiap bekas kultur, bersamaan dengan kepadatan lapangan 50, 200, 350 dan 500 individu setiap* m^2*. Cacing tanah dikultur dalam keadaan makmal (25±2°C dan 25% kelembapan) selama 14 minggu. Hasil kajian menunjukkan bahawa dalam kepadatan yang tinggi (>350 individu setiap* m^2*), pertumbuhan* P. corethrurus *menjadi perlahan dan kematangan seksual lebih lewat berbanding dalam kepadatan yang lebih rendah. Dengan kadar kemandirian yang tinggi dan bersifat partenogenetik,* P. corethrurus *berpotensi digunakan sebagai agen pemulihan tanah tropika.*

Kata kunci: Cacing tanah; kepadatan; kokon; pengkulturan

INTRODUCTION

Nowadays, growing demand for food has led to intensification of farming, which contributed to degradation of agricultural soil. Greater amount of chemical fertilizers is used each year to maintain soil fertility. However, the application of chemical fertilizers only temporarily restores soil condition and the status may deteriorate after a very short period of time. Butt et al. (1995) reckoned that the absence of beneficial soil fauna, especially earthworm could cause the restored soil deteriorates even sooner. Earthworms are well known as the most important soil engineers which modify soil structures, regulate soil organic matter and build organo-mineral structures in the form of casts with specific physical, chemical and microbiological properties (Lavelle et al. 1997).

Generally, based on their ecological behaviors earthworms can be classified into three different groups; epigeic, endogeic and anecic (Lavelle & Spain 2001). Epigeic worms are litter dwellers and feed on litter on the soil surface (García & Fragoso 2002). Anecic and endogeic worms are soil dwelling earthworms which create a burrow system in the soil. Anecic worms feed on litter on the soil surface, while endogeic worms feed on soil particles. As a soil inhabitant, endogeic worms play a major role in soil restoration (Laffan & Kingston 1997; Lavelle et al. 1997). Inoculation of soil dwelling earthworms into soil has been done in some countries for many years in order to improve soil conditions. In Holland, earthworms have been used to accelerate the process of soil maturation in reclaimed polders (Van Rhee 1977). Whilst, the inoculation of earthworms into pasture land in New Zealand has significantly improved soil structure and increased the yield (Stockdill 1982).

Pontoscolex corethrurus is a tropical endogeic worm and one of the most widely distributed worms in Malaysia (Sabrina et al. 2009). The burrowing activity of *P. corethrurus* can lead to the formation of an extensive pore system in the soil. A number of studies showed that the cast of endogeic earthworms contained higher mineral phosphorous and nitrogen than surrounding soil resulting positive effect on plant growth (Lavelle et al. 1992; Lopez-Hernandez et al. 1993; Sabrina et al. 2009; Teng et al. 2012).

Most of the soil earthworm inoculation practices depend on field-collected earthworms, which is very laborious and expensive. In addition, the earthworm availability is strongly affected by seasonal changes. For example, earthworms may become inactive or migrate into deeper soil during drought season (Lavelle 1988) and soil moisture and temperature fluctuations in the nature could cause spatial and temporal heterogeneity of earthworm populations. Thus, it may cause difficulty in getting enough number of earthworms for soil inoculation purpose. Therefore, indoor culturing earthworms are considered the most practical way to obtain a large number and consistent supply of earthworms (Butt et al. 1992).

Stocking density is one of the important factors in establishing earthworm culture. The effect of stocking density on growth, sexual maturity and reproduction has been well documented for epigeic worms, which are used in vermicomposting, e.g. *Eisenia andrei* (Domínguez & Edwards 1997), *Lumbricus rubellus* (Klok 2007) and *Eisenia feotida* (Garg et al. 2008). Unfortunately, the information available on endogeic worms is very scarce. Therefore, the objective of the present study was to determine the optimal stocking density for culturing tropical endogeic earthworm *P. corethrurus*.

Materials and Methods

FIELD SAMPLING

Clitellate adult worms of *P. corethrurus* were collected from the oil palm plantation in Universiti Putra Malaysia by formalin expellant method. Sub-samples of soil (0-20 cm depth) which categorized as Serdang series soil were taken around the earthworm sampling site and used for worm culture purpose. Partial decomposed cow dung was collected from a cow-shed in Universiti Putra Malaysia. The soil sample and decomposed cow dung were air-dried, grounded and passed through a 2 mm mesh prior to worm culture.

SET UP FOR OFFSPRING PRODUCTION

Adults *P. corethrurus* (parental generation earthworms) were kept in a vessel filled with sieved soil and cow dung (9:1, on dry weight basis) and a moisture content of 25% (v/w). Cocoons sampling was done in two week intervals for two months. As recommended by Aziz et al. (1999), the collected cocoons were washed with tap water and kept in Petri dishes containing distilled water and incubated at room temperature. The hatchlings were then used for the subsequent stocking density study.

GROWTH OF THE FIRST GENERATION (F1) EARTHWORMS

The hatchlings (3±1 days aged) were cultured at four different stocking densities: 1, 4, 7 and 10 worms per vessel (3.5 L vessel with 16 cm diameter; 5 replicates), corresponding to field densities of 50, 200, 350 and 500 worms per m^2, respectively. The culture vessel was filled with 500 g of sieved soil and cow dung (9:1, w/w) and the medium moisture content was maintained at 25% (v/w). The experiment was conducted under laboratory conditions (25±2°C) with 12 h of light, 12 h of dark and in 52% humidity. A new set of culture medium was replaced monthly as recommended by Lowe and Butt (2005). Earthworm survival, biomass and sexual maturity were determined on weekly basis for 14 weeks. Cocoons were separated from the culture media by hand sorting. The number of cocoons produced in each treatment was also recorded.

DATA ANALYSIS

The data obtained was analyzed using SPSS (Statistical Package for Social Sciences), 17.0. The data underwent normality test and tested for homogeneity of variance using Levene's test. Earthworm survival, biomass, sexual maturity and reproduction in the four stocking densities were analyzed using One-way analysis of variance (ANOVA) with post-hoc Duncan test. A significant level of $p<0.05$ was considered throughout the analysis.

Results and Discussion

The present study recorded that the mean percentage of survivors ranged between 83±5 and 100%, in which the lowest stocking density (1 worm per vessel) has 100% survivors and the stocking density of 7 worms per vessel recorded 83±5% survivors. One-way ANOVA showed that the percentage of survivors for *P. corethrurus* for the four different stocking densities were not significantly different from initial to the end of the experiment. The percentage of earthworm survivors in all the stocking densities is presented in Table 1.

The initial individual earthworm biomass in all treatments were not significantly different, however, at the end of the experiment, *P. corethrurus* cultured at the highest stocking densities (10 worms per vessel) recorded the lowest biomass ($p<0.05$) compared with their counterpart at lower stocking densities (1, 4 and 7 worms per vessel) (Figure 1). The results showed that the net biomass gained by the earthworms decrease as stocking density increase. Increasing earthworm stocking density might subject to intra-specific competition for food and living space and thus decreasing earthworm biomass. Furthermore, earthworms grow much slower at higher stocking densities. Earthworms cultured at relatively lower stocking density spent less time to achieve sexual maturity (Table 2). At 12^{th} week, all earthworms in stocking density of 1 and 4 worms per vessel became sexually mature, whereas most of the earthworms cultured at higher stocking densities (7 and 10 worms per vessel) remained as the sub-adult stage until week 14. This clearly suggested that earthworms cultured at stocking density of >7 worms per vessel take longer time for sexual maturation. Slower earthworm growth at higher stocking densities could delay sexual maturation. Similar observations have been reported in other earthworm species include *Lumbricus terrestris* (Butt et al. 1994) and *Lumbricus rubellus* (Klok 2007). Earthworms cultured at high density grew slower and

TABLE 1. The percentage (mean ± standard error) of survivors for 14-weeks study period

Weeks	Survival (%)			
	1 worm	4 worms	7 worms	10 worms
0	100±0[a]	100±0[a]	100±0[a]	100±0[a]
1	100±0[a]	95±5[a]	94±4[a]	94±3[a]
2	100±0[a]	95±5[a]	94±4[a]	94±3[a]
3	100±0[a]	95±5[a]	94±4[a]	94±3[a]
4	100±0[a]	95±5[a]	94±4[a]	92±4[a]
5	100±0[a]	95±5[a]	91±6[a]	92±4[a]
6	100±0[a]	95±5[a]	91±6[a]	88±6[a]
7	100±0[a]	95±5[a]	91±6[a]	88±6[a]
8	100±0[a]	95±5[a]	89±5[a]	88±6[a]
9	100±0[a]	90±6[a]	86±5[a]	88±6[a]
10	100±0[a]	90±6[a]	83±5[a]	86±5[a]
11	100±0[a]	90±6[a]	83±5[a]	86±5[a]
12	100±0[a]	90±6[a]	83±5[a]	86±5[a]
13	100±0[a]	90±6[a]	83±5[a]	86±5[a]
14	100±0[a]	90±6[a]	83±5[a]	86±5[a]

Similar 'superscript' letter (horizontal) showed no significant difference ($p \geq 0.05$), $n=5$

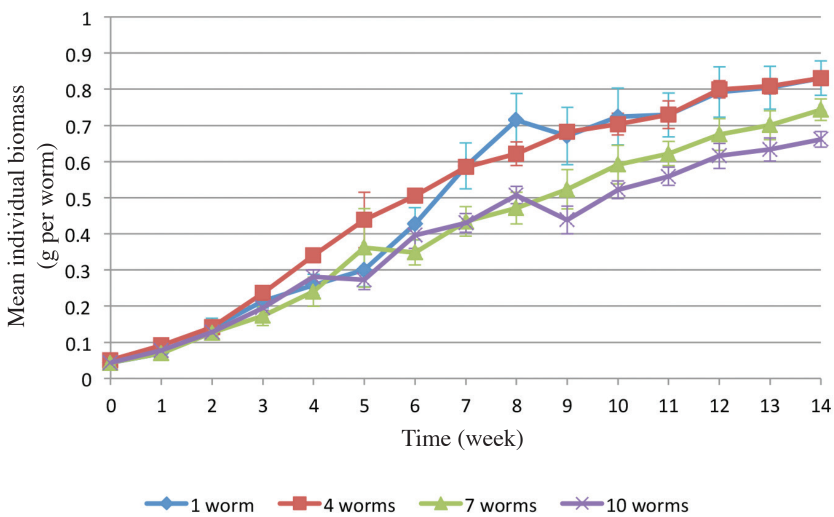

FIGURE 1. Individual biomass (mean ± standard error) of *Pontoscolex corethrurus* at different time intervals for 14-weeks study period

recorded lower biomass than those cultured in lower density which leads to increase maturation time.

The rate of *P. corethrurus* cocoon production ranged from 0.33 to 0.46 cocoons per adult worm per week (Table 3). The present study showed that stocking density has no significant effect on number of cocoons produced per adult worm per week. The result was similar to other findings as *Lumbricus rubellus* (Klok 2007) and *Perionyx ceylanensis* (Karmegam & Daniel 2009). The present study also found that the single culturing *P. corethrurus* could produce cocoons following maturation, which strongly suggested that this worm is a parthenogenetic species.

The parthenogenetic reproduction mode and high survival rate suggested that *P. corethrurus* could potentially be used as tropical soil restoration agent. Both survivorship characters indicated that *P. corethrurus* might have an outstanding ability to colonize new environment. With its high colonization capacity, distribution (Sabrina et al. 2009) and great ecological plasticity (García & Fragoso 2002), *P. corethrurus* would be well adapted into soil ecosystem. Their burrowing and casting activities could help in improving soil structure, nutrient availability and microbial population in soil. Therefore, the inoculation of *P. corethrurus* into high value commodities such as rubber

TABLE 2. The percentage (mean ± standard error) of adult *Pontoscolex corethrurus* at different time intervals for 14-weeks study period

Weeks	Adult worm (%)			
	1 worm	4 worms	7 worms	10 worms
0	-	-	-	-
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	-	-	-	-
7	-	-	-	-
8	20.0±20.0	25.0±12.3	17.5±1.1	16.7±4.9
9	40.0±24.5	41.6±10.2	35.5±5.9	23.6±1.5
10	40.0±24.5	80.0±9.4	41.7±4.0	42.1±2.7
11	80.0±20.0	80.0±9.4	56.6±6.5	51.1±3.6
12	100±0	100±0	75.6±4.5	67.5±1.5
13	100±0	100±0	83.6±4.6	84.2±3.6
14	100±0	100±0	100±0	100±0

TABLE 3. The number of cocoons per adult worm per week (mean ± standard error) for the four different stocking densities

Stocking density	Cocoons/Adult worm/Week*
1 worm	0.33±0.09[a]
4 worms	0.46±0.07[a]
7 worms	0.45±0.08[a]
10 worms	0.41±0.06[a]

Similar 'superscript' letter (vertical) showed no significant difference ($p \geq 0.05$), $n=5$
* Refer to time after sexual maturation achieved

and oil palm plantation in Malaysia might help to improve soil condition, which would then increase the crop yield.

CONCLUSION

P. corethrurus biomass and sexual maturation were significantly affected by earthworm culture stocking density. The optimal stocking density for culturing this worm is 4 worms per vessel, corresponding to 200 individuals per m^2. This worm has favorable characteristics to be used as a tropical soil rehabilitation agent.

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