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RESEARCHING GANODERMA RESIDUES APPLYING IN EARTHWORM CULTIVATION PROCESS (*PERIONYX EXCAVATUS*)

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ABSTRACT

The research of applying Ganoderma residue in earthworm *Perionyx excavatus* cultivation have promised in reducing environmental pollutions from mushroom farm and brought high economic efficiency. A 9 m square with 9 experimental formulas were applied to investigate the effect of Ganoderma residue to earthworm growth. The results showed that *Perionyx excavatus* increased mass in all formulas added cultivated Ganoderma residues, in which the largest increased in formula of 75 % cultivated Ganoderma residues treated with Trichoderma, from 0.59 kg/m^2 initial to 2.62 kg/m^2. In addition, *Perionyx excavatus* growth and development completely was seen on the medium with 100 % cultivated ganoderma residues, reached 1.89 kg/m^2. Although the growth rate of the *Perionyx excavatus* cultivated with Ganoderma residues was slower as compared to farming entirely by cow manure (2.94 kg/m^2), in the long run, this process will bring farmers higher profits (56.500 VN dongs /m^2 of culturing). This cultivation process might be promising to be applied in regions with mushrooms farms for economic and environmental reasons.

Keywords: Ganoderma residues, *Perionyx excavatus*, cow dung.

1. INTRODUCTION

Perionyx excavatus have high protein content, rich in essential amino acids, they are often used as food for fish [1] and chicken [2]. Therefore, in many countries such as the Philippines, Canada, Japan, China, Italy, *Perionyx excavatus* are cultivated in large scale to produce rich protein powder foods for animal, cattle and poultry [3]. Previously, the *Perionyx excavatus* is raised mainly by cattle manure [4] and in recent years people are gradually added organic substrate with low cost to food source for *Perionyx excavatus* such as sewage sludge [5], kitchen waste [6], crop residues [7], water hyacinth [8], straw, bamboo leaves [9] and duckweed [10].

With current practices in Viet Nam, huge residue resources after planting mushrooms has not been used in a reasonable manner is causing environmental pollution, especially the Linhzhi residue with component are mainly sawdust that very persistent. Therefore, the application of Ganoderma residue to raise *Perionyx excavatus* biomass are studied with a desire to contribute to solve the problem of environmental pollution, while promoting *Perionyx excavatus* farming model with low cost, highly economical effective, to take part in improving the live conditions of the people.

2. MATERIALS AND METHODS

2.1. Material

Manure was taken from cow farm treat with Trichoderma or none. Earthworms (*Perionyx excavatus*) and cultivated Ganoderma residue (GR) was bought from farmer in Cu Chi District, Ho Chi Minh City.

2.2. Experimental design

Cow manure was mixed with cultivated Ganoderma residue in different ratios (Table 1). The experimental containers were prepared as per method described by Suthar [11].

Main ingredient of residue after cultivating Ganoderma is sawdust of rubber, made to complement and lime ($CaCO_3$), divided into two samples, keep moisture from 60-70% then conduct composting. The first samples have composted with preparations of Trichoderma (1 kg preparations of Trichoderma per 1ton of Ganoderma residue). The remaining samples did not conduct additional preparations Trichoderma. After composting for 1.5 months, conducting mixed with cow manure and soak for 10 days to make food for earthworm *Perionyx excavatus*.

Table 1. Composition of treatments.

Substrate	Composted GR added Trichoderma				Composted GR without Trichoderma				Control
Treatment	A1	A2	A3	A4	A5	A6	A7	A8	A9
Ratio of the GR	100% GR	75% GR: 25% Cow manure	50% GR: 50% Cow manure	25% GR: 75% Cow manure	100% GR	75% GR: 25% Cow manure	50% GR: 50% Cow manure	25% GR: 75% Cow manure	100% Cow manure

GR*: cultivated Ganoderma residue*

Perionyx excavatus was raised in 36 plots corresponding to 9 treatments and each treatment was repeated four times. The size of each plot was 50 cm × 50 cm × 40 cm. Initial *Perionyx excavatus* biomass (including worm and substrate) was 5 kg per plot. Moisture content of the treatment was maintained at about 60–70 % by spraying the surface with fresh water every two days (30–60 ml per plot) according to Parthasarathi [12]. The plots were incubated in dark and humid place at room temperature (28.5 ± 0.5 °C). A 0.6 kg substrate/plot was supplied every day. After 40 days feeding, the worm was harvested for analysis. Experimental layout completely

randomized according to a block using the software Minitab 16 (Figure 1). The collected data was analyzed using Excel 2007 software and Minitab 16.

Figure 1. Arrangement of block experiments.

Earthworm growth rate was calculated based on the amount of worm (g)/ kg of applied biomass after 40 days of growing.

Benefit calculation for culturing earthworm using optimal ratio of waste Ganoderma residue was based on actual market prices of input cost and output benefit.

- Input costs included cost of GR treatment (GR, chemicals), cost of cow manure, cost of initial earthworm biomass, general labor.
- Output benefit was calculated from yield of earthworm (kg) and yield of earthworm manure (kg).

3. RESULTS AND DISCUSSION

3.1. Effect of Ganoderma residue to worm growth

After 40 days of experiment, earthworms were separated from the residual substrate. Both worms and substrate were weighed and analyzed. The results showed that the *Perionyx excavatus* increased in amount in A1 and A5 samples with 100% Ganoderma residue, which was added Trichoderma and without Trichoderma (Table 2). The result indicated that *Perionyx excavatus* had fully adapted to and grew well on Ganoderma residue with or without the help of Trichoderma. However, the growth rate of worm was much lower in Ganoderma residue than in 100% of cow manure (A9).

Table 2. Effect of Ganoderma residue with or without additional Trichoderma on weight of *Perionyx excavatus* (g/kg biomass).

	100 % Ganoderma residue added Trichoderma	100 % Ganoderma residue without Trichoderma	100 % Cow manure	P-value
	A1	A5	A9	
Initial weight	29.7	29.7	29.7	
Final weight (Mean ± StDev)	56.1 ± 1.84^{b}	39.97 ± 1.45^{c}	82.55 ± 2.03^{a}	0.000

^{abc} Mean value followed by different letters is statistically different at $P<0.05$

3.2. Effect of mixing ratios of Ganoderma residue and cow manure to worm growth

Four mixing ratios of Ganoderma residue and cow manure treated with Trichoderma were feed to earthworm including A1 (100 % GR), A2 (75 % GR: 25 % Cow manure), A3 (50 % GR: 50 % Cow manure), and A4 (25 % GR: 75 % Cow manure) (Table 1).

When mixing Ganoderma residue with cow manure, with the help of Trichoderma, the highest growth rate of worm was found in A2 (75 % GR: 25 % Cow manure) followed by A4 (25 % GR: 75 % Cow manure), and A3 (50 % GR: 50 % Cow manure) as compared to the control A1 (100 % GR). The result was similar to previous studies of Suthar *et al*., (2008) [13, 14] that earthworm could have good growth on medium with 20-60 % cow manure. It was concluded that the mixing of cow manure to GR increased the growth rate of worm, possibly due to more availability of growth retarding substances (e.g. pH, C:N and microorganisms) so that earthworms could digest the feed better .

Table 3. Effect of GR with additional Trichoderma on the growth of *Perionyx excavatus* (g/kg biomass) after 40 days of culture.

	Varying mixing ratios of cow manure and Ganoderma residue				P1-value
	A1	A2	A3	A4	
Initial weight	29.7	29.7	29.7	29.7	
Final weight (Mean ± StDev)	56.1 ± 1.84^{c}	72.84 ± 2.75^{a}	68.14 ± 1.45^{b}	71.16 ± 1.52^{ab}	0.000

^{abc} Mean value followed by different letters is statistically different at $P<0.05$

In the mixing ratios not treated with Trichoderma, there were also changes in worm weight on every treatment compared to initial weight. The highest growth rate was recorded in A8 plot (25 % GR: 75 % Cow manure) with 68.7 ± 1.36 g worm /kg biomass, followed by A7 (62.1 ± 4.77 g/ kg), A6 (55.25 ± 3.77 g/ kg) and A5 (39.97 ± 1.21g/ kg). When increasing the ratios of cow manure in the mixture, worm growth rate increased as seen in the experiment treat with Trichoderma (Table 3, Table 4).

Table 4. Effect of GR without additional Trichoderma on the growth of *Perionyx excavatus* (g worm/ kg biomass) after 40 days of culture.

	Cow manure with GR				P-value
	A5	A6	A7	A8	
Initial weight	29.7	29.7	29.7	29.7	
Final weight (Mean ± StDev)	39.97 ± 1.21^{d}	55.25 ± 3.77^{c}	62.10 ± 4.77^{b}	68.70 ± 1.36^{a}	0.000

^{abc} Mean value followed by different letters is statistically different at $p < 0.05$.

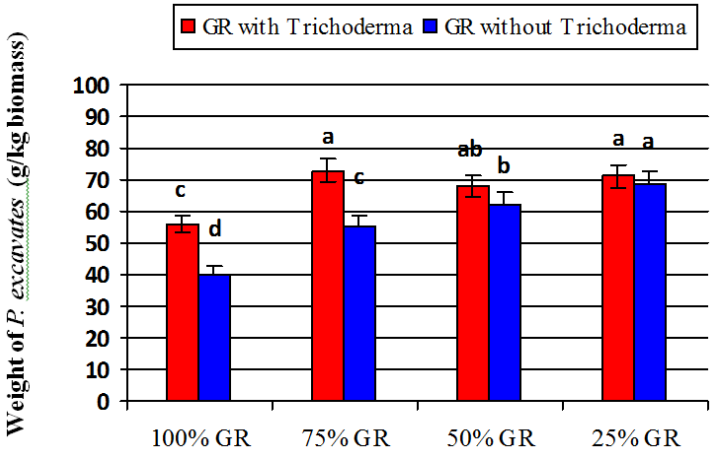

Figure 2. Effect of processing conditions and rate of GR supplements to *Perionyx excavatus* weight (g/kg biomass).

According to Suthar, 2007, the C:N ratio of the substrate related to the mineralization of organic waste and the stabilization of composting process. The lowest C: N ratio in compost indicates that earthworm enhances the organic matter mineralization more efficiently than not composting [15]. As compared in Figure 2, *Perionyx excavatus* was found to completely adapt to substrate with 100 % GR. In particular, the growth rate of earthworms was better on the GR treated with Trichoderma than the GR not treated. The reason might be that components of GR was mainly hard digest compound remained after growing mushroom, such as cellulose, crude protein and mushroom filaments, which needed to be treated with Trichoderma to enhance the decomposing process. It was proved previously that adding fresh manure with available microorganisms should increase the decomposed of GR without additional Trichoderma [16].

According to earlies studies, one of the most important factors that control the establishment and continuity of earthworm populations were food quality and quantity. Higher nitrogen ratios help in faster growth and greater production of cocoons. Fresh green matter is not easily fed upon. Decomposition by microbial activity is essential before earthworm can feed on fresh manure. The C: N ratio is the critical factor that limits earthworm populations. When the C: N ratio of the feed material increase, it become difficult to extract enough nitrogen for tissue production [15, 17]. When cultivated Ganoderma residue was not decomposed by Trichoderma, it was difficult for earthworm to feed on. Weight of *Perionyx excavatus* was increased better on all GR treatments with Trichoderma (Table 3) than not added Trichoderma (Table 4). The highest growth rate of worm was found in plot A2 (75 % GR: 25 % Cow manure) treated with Trichoderma.

3.3. Benefit calculation for culturing earthworm feeding on mixture of Ganoderma residue and cow manure

In Table 5, The plot A9 with 100 % cow manure got largest amount of *Perionyx excavatus*

(2.94 kg/m^2) after 40 days of culture. Other 8 treatments additional GR, the largest mass was in treatments of 75 % additional GR, concretely increased more than four times compared to the initial value (0.59 kg/m^2 to 2.62 kg/m^2). Although, the total weight harvested after 40 days of culture earthworm by 100 % cow manure higher fed additional GR treated, but in terms of long-term, the *Perionyx excavatus* feed by additional GR should bring greater economic efficiency to the next harvest, due to GR was much cheaper (370 thousands VN dong) than cow manure (800 thousands VN dong) as shown in Table 6. Especially in mushroom farm, farmer can reused waste mushroom residue to produce earthworm biomass and manure which will lower the input cost and bring more benefit.

Table 5. Amount *Perionyx excavatus* (kg/m^2) after 40 days of culturing.

	Cow manure added GR with Trichoderma				Control	P-value
	A1	A2	A3	A4	A9	
Initial weight	0.59	0.59	0.59	0.59	0.59	
Total Final weight (Mean± StDev)	1.89 ± 0.05^{d}	2.62 ± 0.1^{b}	2.46 ± 0.05^{b}	2.56 ± 0.06^{c}	2.97 ± 0.08^{a}	0.000
	Cow manure added GR without Trichoderma				Control	P-value
	A5	A6	A7	A8	A9	
Initial weight	0.59	0.59	0.59	0.59	0.59	
Total Final weight (Mean± StDev)	1.44 ± 0.04^{c}	1.99 ± 0.13^{d}	2.24 ± 0.16^{c}	2.47 ± 0.05^{b}	2.97 ± 0.08^{a}	0.000

The ratio A2 with highest yield of earthworm per square meter was chosen to be used for $9m^2$ area earthworm culturing and benefit calculation.

Cost for A2 earthworm culturing was calculated from worm feed, worm biomass and labour as about 1.212.500 VN dong, which was lower than culturing by cow manure (Table 6). (1)

Earthworms and manure collected from A2 culturing were including 30.5 kg of worm and 400 kg of manure. Hence benefit from A2 earthworm culturing came from earthworm biomass (42.000 VN dongs/ kg) and worm manure (1.100 VN dongs/ kg). (2). So benefit per $9m^2$ of A2 earthworm culturing = Benefit from A2 earthworm culturing (1) – Costs of A2 culturing (2) equal to 508.500 VN dongs for 40 days of growing.

Table 6. Comparison of cost for GR treatment and cow manure.

Components	Cost per ton (VN dong)			
	Ganoderma residues	Cow manure	Cost for A2 earthworm culturing	Cost for cow manure earthworm culturing
Ganoderma residues	37.000	-	-	-
Cow manure	-	800.000	-	800.000
A2 (75 GR : 25 Cow manure)	-	-	470.000	-
Input earthworm	-	-	405.000	405.000
Chemical, Trichoderma	80.000	-	-	
Labor	200.000	-	337.500	337.500
Total	**370.000**	**800.000**	**1.212.500**	**1.542.500**

In conclusion, with an area of 9 m^2 after 40 days of A2 culturing, famers might gain 508.500 VN dongs or 56.500 VN dongs /m^2. This interesting result not only leads to a promising solution for solving the solid waste pollution from mushroom farms but also gain benefit for farmers.

4. CONCLUSION

In the process of raising the *Perionyx excavatus* based on waste Ganoderma residue, the rate of 75% GR treated Trichoderma and 25 % cow manure was shown to be most suitable. *Perionyx excavatus* growed well and produce manure when feeding with mixture of GR treated with Trichoderma and cow manure. The application of this process could bring benefits to farmers in worm biomass and converting a large amount of mushroom farm waste into earthworm compost, which could reduce the environmental pollution from mushroom farms.

REFERENCES

1. Phan Thi Bich Tram, Tram P. T. Q, Toan H. T and Hong P. T. A. - Look at the factors affecting the performance of the self-protein amino resolution of earthworms (*Perionyx excavatus)*, Journal of Agriculture and Rural Development **4** (2008) 53-63.
2. Rodríguez L., Salazar P. and Arango M. F. - Lombriz roja californiana y azolla-anabaena como sustituto de la proteina convencional en dietas para pollos de engorde, Livest Res Rural Develop. **7**(3) (1995) 145-149.
3. Thai Tran Bai. - The value of *Perionyx excavatus* practices, Journal of Biology **11**(1) (1989) 39-43.
4. Garg V. K., Chand S., Chhillar A. and Yadav A. - Growth and reproduction of *Eisenia foetida* in various animal wastes during vermicomposting, Appl. ecol. environ. res. **3**(2) (2005) 51-59.
5. Benitez E., Nogales R., Elvira C., Masciandaro G. and Ceccanti B. - Enzyme activities as indicators of the stabilization of sewage sludges composting with *Eisenia foetida*, Bioresour. Technol. **67**(3) (1999) 297-303.
6. Chaudhuri P. S., Pal K. T., Bhattacharjee G. and Dey S. K. - Chemical changes during vermicomposting (*Perionyx excavatus*) of kitchen wastes, Tropical Ecology. **41**(1) (2000) 107-110.
7. Bansal, Sudha, and Kapoor K. K. - Vermicomposting of crop residues and cattle dung with *Eisenia foetida*, Bioresour. Technol. **73** (2) (2000) 95-98.
8. Gajalakshmi S., Ramasamy E. V. and Abbasi S. A. - Assessment of sustainable vermiconversion of water hyacinth at different reactor efficiencies employing Eudrilus eugeniae Kinberg, Bioresour. Technol. **80**(2) (2001) 131-135.
9. Chaudhuri P. S. and Bhattacharjee G. - Capacity of various experimental diets to support biomass and reproduction of *Perionyx excavatus*, Bioresour. Technol. **82**(2) (2002) 147-150.
10. Kostecka J. and Kaniuczak J. - Vermicomposting of duckweed [Lemna minor L.] biomass by *Eisenia fetida* [Sav.] earthworm, Journal of Elementology **13**(4) (2008) 571-579.

11. Suthar S. - Production of vermifertilizer from guar gum industrial wastes by using composting earthworm *Perionyx sansibaricus* (Perrier), The Environmentalist **27**(3) (2007) 329-335.

12. Parthasarathi K. - Influence of moisture onthe activity of *Perionyx excavatus* (Perrier) and microbial–nutrient dynamics of pressmud vermicompost, Iran. J. Environ. Health. Sci. Eng. **4**(3) (2007) 147-156.

13. Suthar S. and Singh S. - Feasibility of vermicomposting in biostabilization of sludge from a distillery industry, Science of the total environment **394**(2) (2008) 237-243.

14. Suthar S. and Singh S. - Vermicomposting of domestic waste by using two epigeic earthworms (*Perionyx excavatus* and *Perionyx sansibaricus*), International Journal of Environmental Science & Technology **5**(1) (2008) 99-106.

15. Suthar S. - Nutrient changes and biodynamics of epigeic earthworm *Perionyx excavatus* (Perrier) during recycling of some agriculture wastes, Bioresource technology **98**(8) (2007) 1608-1614.

16. Sherman R. - Raising earthworms successfully, North Carolina Extension Service, North Carolina State University, USA, 2003, pp.5 -10.

17. Gajalakshmi S. and Abbasi S. A. - Earthworms and vermicomposting, Indian journal of biotechnology **3**(10) (2004) 486-494.