Vermicomposting of Spent Mushroom Compost Using *Perionyxexkavatus* and Artificial Nutrient Compound

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***Abstract*—** *Vermicomposting is a cost effective technology for processing or treatment of a gricultural wastes. This simple yet effective technology was easily applicable in developing countries. In Vietnam, vermicomposting is not new but has few publications. Implementing vermicomposting with spent mushroom compost (SMC) as culture medium is one research approach not only in Vietnam but also in the world. With its poor nutrition nature, in order to be used as culture medium, SMC needs well treated and supplementing with artificial nutritional compound (ANC). This study reported valuation of moisture and light on the growth of Perionyx exkavatus. Results showed that at 80% moisture, earthworm has the most growth rate and gain 100% clitellum development after 30 days. In natural light, growth rate and manure rate reached maximum with 5.61 mg.worm-1.day-1 and 235 mg.worm-1. ANC supplementing showed strong effects on earthworms' growth rate. Earthworms gained maximum growth rate at 20ml ANC added in three kg of substrates with over double biomass after 60 days. However, due to ANC's high acidity, with supplement volume over 25ml, there was a down trend of earthworm growth rate. ANC supplement did not show noticeable affect to manure rate of earthworms.*

Keywords*— *Vermicompost, Perionyxexcavatus, spent mushroom compost, artificial nutrient compound

I. INTRODUCTION

Vermicomposting is a cheap and effective technology for processing or treatment of organic wastes in many countries [1-5]. Many investigations have established the viability of using earthworms in treatment of agricultural wastes such as cattle dung, pig manure, crop residues [4,5]. The temperate earthworms *Esenniafetida* and *Esenniaandrei*[6 – 8] or tropical *Perionyxexkavatus* species [7-10] have been using almost all vermicomposting systems but other earthworm species are also used e.g. *Dendrobaenaveneta*[11], *Eudrriluseugenidae, Lampitomauritii,Drawwidawillsi*[12]. Physico-chemical properties of the substrate will be modified by microbial and enzyme activities in earthworm in test in track and energy was recovered as earthworm biomass and compost [13]. Usually, vermicomposting system is set up in cooperation with other processes and it is part in a larger chain of farms [14]. The vermicomposting system has been improved from small-scale (in laboratory or family level only) to large-scale (in farm or factory level) [14]. However, earthworm biomass yield obtained from plant waste is lower than from animal one [15].

Commercial mushroom industry production has been grown since the early of 20^{th} century all over the world. In Vietnam, the mushroom industry has been spreading sine 1980s, and more than 500.000 tons of spent mushroom compost (SMC) are spurned in the mushroom farm every year (Hoang-Dung Tran, personal data). SMC is an organic and fungal biomass rich complex which poses pollution to mushroom farm itself if they were left untreated but it can serve in a number of applications. While Kakkar *et al*. [16] used SMC as animal feed, Shojaosadati *et at.*[1999)[17] mixed SMC with grounded snail shell (GSS) (shell: compost, 1:5) in a pilot-scale biofilter for removing hydrogen sulfied (H_2S) from leather industry wastewater. Eggen (1999) [18]used SMC for degradation of polycyclic aromatic hydrocarbons in creosote contaminated soil i.e. *Pleurotusostreatus* (oyster mushroom), *Lentinulaedodes*(shiitake mushroom). Some farmers in Vietnam re-used SPM from cultivation of oyster mushroom to grow *Volvariellavolvacea* straw mushroom. Until now, there is not paper report the using SMC as the substrate for growing earthworm in vermicomposting process in Vietnam

In our work, we applied the vermicomposting system to recycle SMC from Pleurotusostreatus cultivation. We used pre-treated SMC by Effective Microorganisms (EM) [19] as the main food source to earthworm. The effects of physiological factors including moisture, light and supplementation with artificial nutrient compound (ANC) on growth rare and maturation of earthworm were determined.

II. MATERIAL AND METHOD

Two series of experiments were done, the first for determining effects of moisture and light conditions to growth rare and maturation of earthworm. And the second is for amount of ANC added into the pre-treated SMC to support earthworm growth.

2.1 First Series Of Experiment

Epilegic *Perionyxexkavatus* is the tropical manure worm which distribute extensively in many tropical Asian areas and have been used in the Philippine, India, Australia for vermicomposting system because of its high growth rate and reproduction. They were harvested by hand-sorting from cow farms near the mushroom farm in suburb Hochiminh City and kept in urea-free cow dung. Urea-free cow dung container then water is added. The mixed was kept in dark for 48h before excess water was removed (water content was about 80%).

SMC was obtained from Center for Applied Biotechnology, Hochiminh City and pre-treated in 90 days by EM [19] was supported by Center for Biological Sciences and Biotechnolgy, School of Nature Sciences, Vietnam National University Hochiminh City, Vietnam (VNU). Three kg of that material with 60% moisture content was distributed into 6 lit circular plastid container ($0.3m^2$ in surface area) ten days before introducing 120 non fully-developed clitellum earthworms with 25g total biomass (the ratio of earthworm/food is 1/24).

We set up experiments of variants of moisture and light levels and consider these effects on the total biomass (g), mean maximum weight achieved ($mg.worm^{-1}$), growth rate ($mg.worm^{-1}.day^{-1}$), mature rate of earthworm (percentage of fully clitellum developed earthworm) [20].

The water content was controlled at 70, 80 and 90% to consider the effect of moisture content to these biological indexes of earthworm. These containers were kept in normal level of light condition.

To evaluate the impacting of light conditions to earthworm, we put containers in three light conditions: fully dark (0lux during 24/24h), fully light (2400lux in 24/24h) and normal light (0lux in 12/24h and 2400lux in another hours). The water content in each container was remained at 80% during the experiment time.

All of them were placed at room temperature (25- 27 ^oC) and no new food was added all experiment long. Every 15 days, earthworms were removed by hand-sorting for counting the number of fully developed clitellum earthworms in population and weighting total biomass. Every experiment was repeated five times.

2.2 Second Series Of Experiment

We chose cheap materials and common in Vietnam that could be found easily as the component in artificial nutrient complex (ANC) following introduction of *Szymanski*[19]. The ANC complex was fermented two times as our modifying.

ANC was added to five constructed experiments with the volume at 0, 5, 10, 15, 20 and 25 ml. Urea-free cow dung was used as the control food. The experiment had 5 replicates.

Containers were kept at room temperature with normal level of light condition, 80% moisture content and no new food was added during growth.

Every 15 days, these parameters of earthworm were calculated in the same way as the above experiment. The content of nutrient was analyzed at the Lab of Biochemistry, School of Natural Sciences, VNU.

Data were analyzed follow STATA software.

III. RESULTS AND DISCUSSION

3.1 Effects of Moisture on Earthworm Biomass

Earthworms require adequate moisture for growth and survival. Beds should be crumbly moist, not soggy wet. Thus, the moisture of around environment plays an importance role in earthworm's life. The moisture of food is one ò the main factors impacting to production of the vermicomposting technology. Fig. 1 show the effect of moisture to the total biomass of *Perionyxexkavatus*

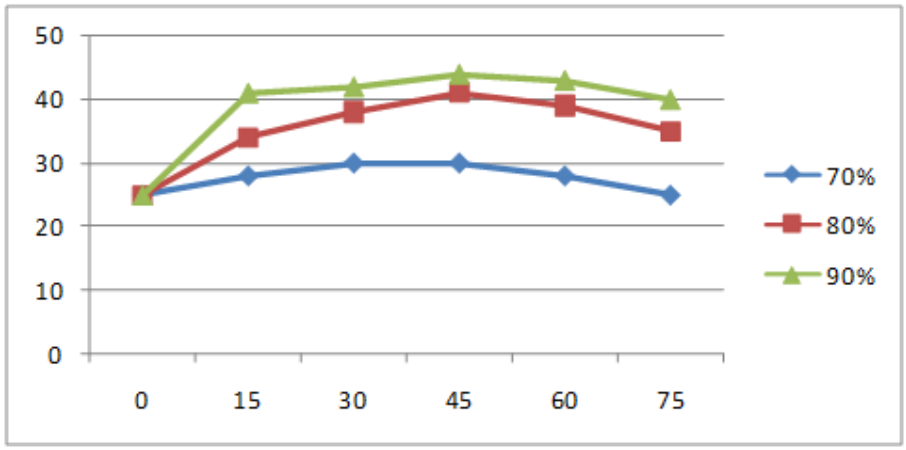

FIG 1: EFFECT OF MOISTURE TO TOTAL BIOMASS OF *PERIONYXEXKAVATUS*

In the food hold 90% water, earthworms had remarkably increased their total biomass only after 15 days of cultivation in comparison with 80% and 70% water (total biomass at this time point is 41.57 and 34.30 and 27.92, respectively). There is a relationship strongly between earthworm and moisture content of around environment [11, 21, 22]. Earthworms living in the low degree moisture of habitat don't often accumulate the water in their body fully. Muyima*et al.* (1994) [11] showed that earthworms moved from the low moisture environment to the higher would increase their weight up 15% after 5-7 days. After that, these earthworms would return their original mass if they were put to old habitat. That flexibility was due to water absorbability and removability of earthworm living in the moisture or/and dryness habitat. The earthworms got enough water in their body will have strongly physiological actions. Haltt *et al.* (1992)[21] reported that the optimal moisture for the cycle life of *Perionyxexkavatus* is 75.2-83.2%. Dominguez and Edwards (1997)[20] reviewed some data about effect of moisture content on the growth and maturation of earthworm using in vermicomposting in which the optimal moisture for earthworm to survival in range between 50-90% and they grow more rapidly between 80-90%. Growth rare (GR) and mean maximum weight achieved (Mw) effected by moisture is given in the table 01.

TABLE 1
THE EFFECT OF MOISTURE (%) TO GR AND MW OF *PERIONYXEXKAVATUS*

Biomass / Moisture	GR (mg.worm^{-1}.day^{-1})	Mw (mg.worm^{-1})
70	1.03	203.67
80	2.9	271.07
90	3.57	295.2

These data showed that GR and Mw of earthworm increased fast in the high degree moisture. The effects of moisture on earthworm's maturation were showed in table 02 and Fig.2.

TABLE 2
PERCENTAGE (%) OF FULLY CLITELLUM DEVELOPED *PERIONYXEXKAVATUS* UNDER VARIANTS OF MOISTURE LEVELS (%)

Maturation / Độẩm	0	15	30	45	60
70	0	23	52	98	100
80	0	49	85	100	-
90	0	46	86	100	-

(-) *Stop counting*

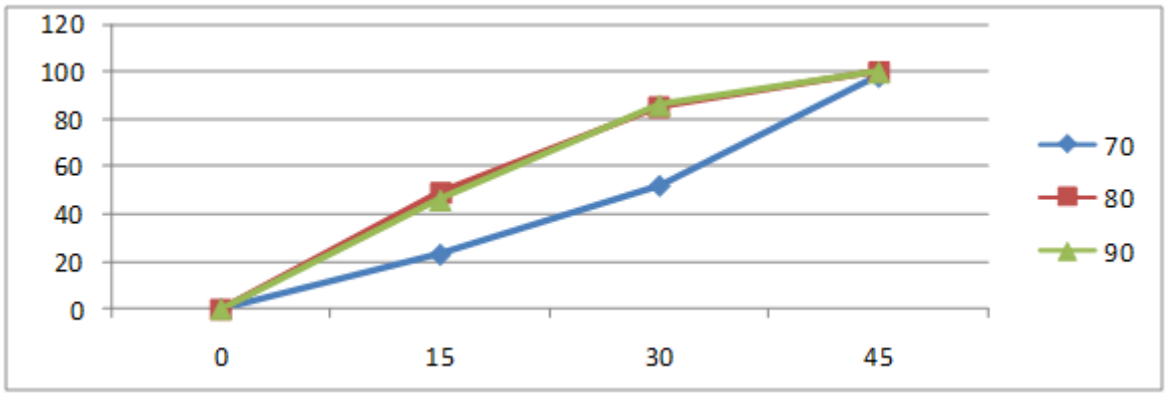

FIG. 2: PERCENTAGE OF FULLY OF CLITELLUM DEVELOPED EARTHWORM UNDER EFFECT OF MOISTURE CONTENT

The interrelation of moisture (X) and the time of cultivation (Z) and percentage of fully developed clitellum earthworm (Y) is given in equation I

$$Y = 0.49166*X + 1.3466*Z - 22 \qquad (R^2 = 0.8135) \qquad (1)$$

Thus, maturation of earthworm is enhanced if they are living in high moisture. After 15 days of cultivation, 50% earthworm has developed their clitellum in moisture 80% and 90%, and almost all of them had developed fully their clitellum after 30 days. There was no young earthworm at moisture 90% during 60 first days of feeding. Some research [11, 21-23] reported that optimal moisture in which epigeic earthworm species produce cocoons and their cocoons could hatch is 73.1-79.9%.

3.2 Effect of light to earthworm

Most earthworms in this experiment obtained the maximum total biomass after 15 days of cultivation (table 03 and fig.03). However, only the earthworms under normal light level can remain in this value after longer time. Total biomass of the earthworms living in fully light level reduced fast in the end of experiment duration. And we didn't see clearly the stationary phase in the curve biomass of earthworm feeding in the fully dark level. Similarly, the GR and Mw of earthworm in the fully dark level is the lowermost, the detail data is given in the table 04.

TABLE 3
THE EFFECT OF LIGHT CONDITIONS TO TOTAL BIOMASS (G) OF (*PERIONYXEXKAVATUS*)

Day / Light	0	15	30	45	60	75
Fully Light Level	25	33.49	32.19	29.27	27.49	27.14
Fully Dark level	25	30.06	30.76	32.51	29.71	26.90
Normal level	25	35.17	35.33	35.10	32.19	27.14

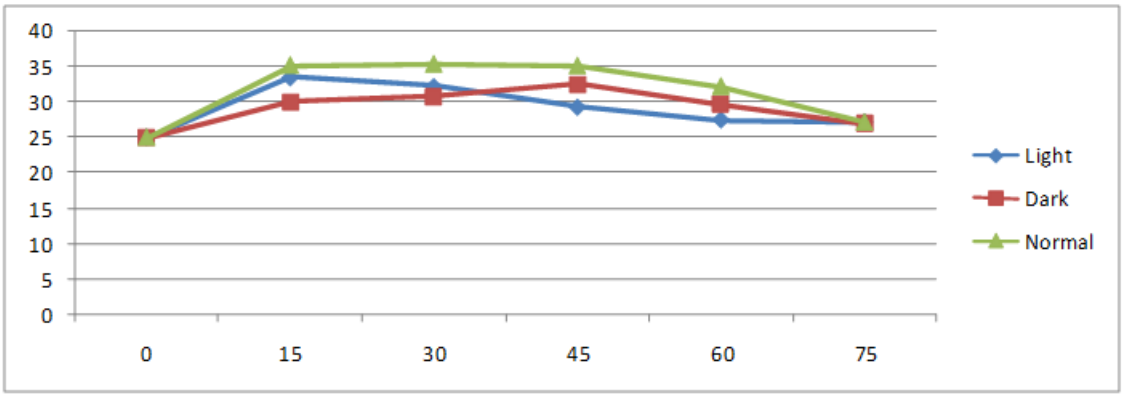

FIG. 3: THE CURVE BIOMASS OF *PERIONYXEXKAVATUS* UNDER EFFECT OF LIGHT LEVEL

TABLE 4
THE GR AND MW OF A *PERIONYXEXKAVATUS* IN THE VARIANTS OF LIGHT CONDITIONS

Biomass / Light condition	GR ($mg.worm^{-1}.day^{-1}$)	Mw ($mg.worm^{-1}$)
Fully light level	4.71	223
Fully dark level	2.81	217
Normal level	5.61	235

Considering the effect of variants of light condition in the ability to mature, we have the fig. 04

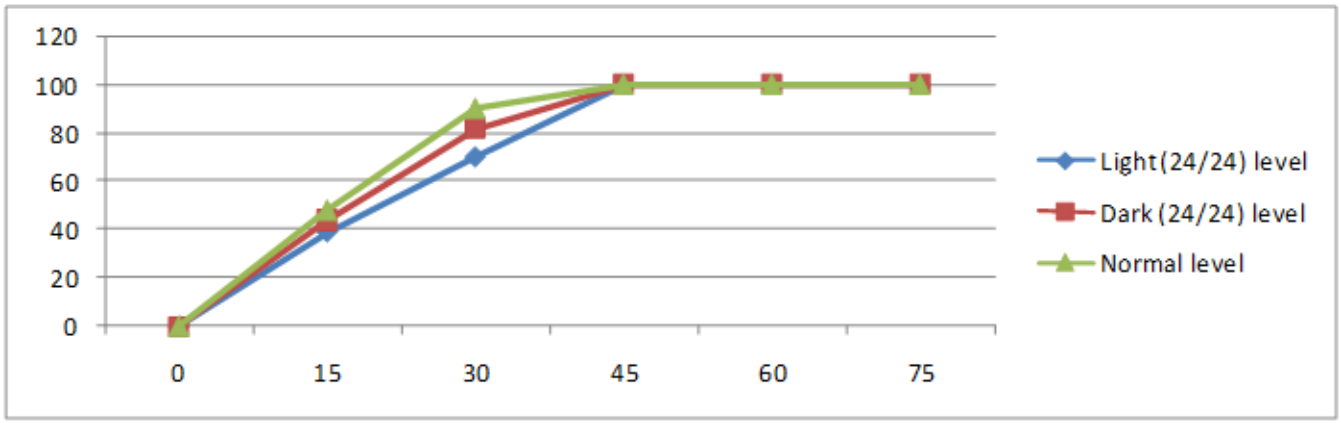

FIG. 4: PERCENTAGE (%) OF THE FULLY CLITELLUM DEVELOPED *PERIONYXEXKAVATUS* UNDER VARIANT OF LIGHT CONDITIONS

Clearly, light conditions have effect not only on the biomass and growth rare but also to mature rate of earthworm. In normal light level, earthworms became mature faster than the other light levels. 50% fully clitellum developed earthworm appeared after 15 days in normal and fully dark level. And we only get that value for the earthworms living in the fully light level after 60 days. Earthworm is the non-eye animal, however, they can sense light and dark by lenses-like cells found mainly on in the epidemic and mesoderm layers of the anterior body [22). Maybe, that ability of earthworm keeps their balance in their actions daily.

Our result show that beside the traditional source of food for *Perionyxexkavatus* such as cow dung, pig manure etc., we can use SMC treated by EM to feed earthworm.

3.3 Effect of ANC (artificial nutrient complex) to earthworm

TABLE 5
CONTENT OF ANC

Content	Percent
Water (%)	70
Crude Protein(%)	45 – 55
Crude Fat (%)	1.5 – 2.3
Roughage (%)	5 – 7
Cancil (%)	0.5 – 0.8
Phospho (%)	1.3 – 1.8
Total microorganisms	8.5×10^{10}
pH	4 – 5

TABLE 6
SOME ANALYZED INDEXES (%) OF MATERIAL USING OR/AND OBTAINING IN THAT EXPERIMENT

	Urea free cow dung	Mushroom compost	SMC	Pre-treated SMC	Worm-cats
Water	85 – 90	35 – 40	50 – 55	75 – 80	65 – 70
Total Nitrogen	0.75 – 1.2	0.12 – 0.20	0.036 – 0.400	0.05 – 1.00	0.75 – 0.12
Total cacbone	32.00 – 38.45	40.50 – 46.80	32.35 – 35.00	33.50 – 37.80	27.65 – 28.95
Cellulose	12.09 – 14.59	40.10 – 42.70	23.15 – 28.75	12.50 – 15.85	2.10 – 5.65

SMC is rather poor in the content of nutrition for both microbiology and earthworm. The main component of SMC is cellulose (table 06), hemicellulose, lignin… They are complex carbohydrate compounds and probably earthworm cannot digest them directly. The pre-treatment process of SMC by EM helped to transform that material to 0.01 – 1 mm in size. However, nutrition in the pre-treatment SMC was improved but still not high enough to support earthworm (table 06). On the other hand, on the organic rich control medium (urea-free cow dung), earthworm doubled biomass after 30 days of cultivation in the medium (Fig 05). Manna*et al.*(1997) [21] has reported the *Perionyxexkavatus* increased from 300 mg to 600 mg after 28 – 30 days. We decided to supplement pre-treated SMC with ANC to improve nutrient value (table 05) since total nitrogen is important for growth and reproduction of earthworm, especially the manure earthworm such as *Perionyxexkavatus*[14]. As expected, earthworm in that experiment had grown up over 2 times when they was supported the ANC at the 10, 15, 20 and 25ml in volume after over 60, 30, 15 and over 45 days of cultivation, respectively (table 07 and fig 05).

TABLE 7
THE EFFECT OF ANC (ML ADDED) TO THE BIOMASS (MG) OF EARTHWORM *PERIONYXEXKAVATUS*

Day / Volume of ANC	0	15	30	45	60	75
0	25	33.32	38.0	41.62	39.01	33.90
5	25	35.19	38.92	34.85	33.28	28.02
10	25	43.08	46.41	52.55	49.45	44.87
15	25	43.14	48.12	48.74	45.94	40.55
20	25	49.81	50.95	54.04	52.28	51.08
25	25	33.04	47.54	53.84	49.47	48.40
Control	25	40.06	53.76	55.51	39.71	36.90

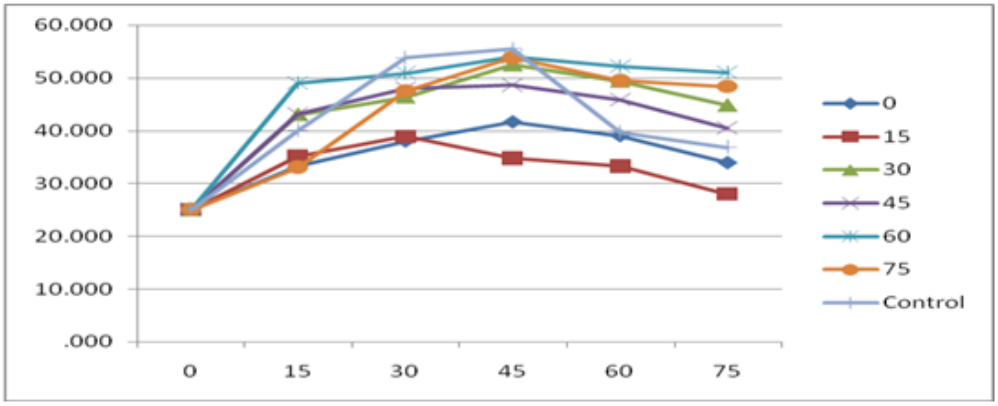

FIG 5: THE EFFECT OF ANC TO TOTAL BIOMASS OF EARTHWORM *PERIONYXEXKAVATUS*

Corresponding, GR and Mw index had been affected, the date given in the table 088. Clearly, the ANC have an influent strongly to GR and Mw. However, considering the relationship between the volume of added ANC (X) and GR index (Y), we have the equation II

$$Y = -0{,}01117x^2 + 0{,}03861x + 1{,}45 \ (R^2 = 0{,}8159) \quad (2)$$

TABLE 8
THE EFFECT OF ANC TO GR AND WM OF *PERIONYXEXKAVATUS*

Biomass / Volume of ANC	GR (mg.worm^{-1} day^{-1})	Mw (mg^{-1}.worm^{-1})
0	2.85	276.9
5	3.09	266.1
10	4.08	350.3
15	5.14	324.9
20	4.27	358.9
25	4.27	358.9
Control	6.39	370.1

Although earthworms can survive in the pH arrangement from 4.5 – 8, the prefer the neutral pH (6.8-7) [14]. Acidity of the ANC had a negative effect to earthworm when added volume is over 20 ml (fig 06).

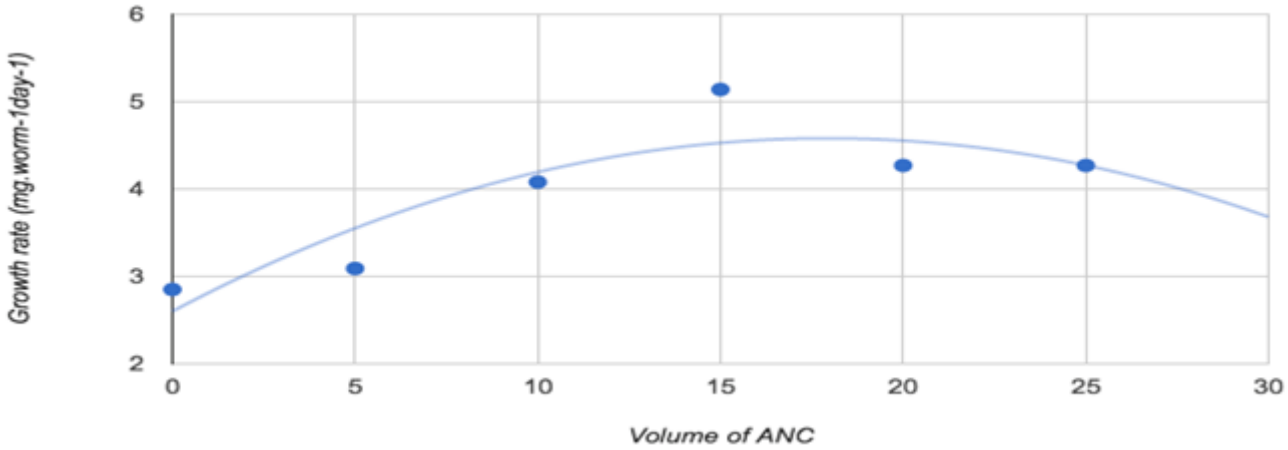

FIG 6: THE RELATIONSHIP BETWEEN THE VOLUME OF ADDED ANC AND GR OF EARTHWORM *PERIONYXEXKAVATUS*

Maturation of earthworm is one of the main indexes to estimate productivity of vermicomposting process [13, 14]. The earthworm *Perionyxexkavatus* is prolifically animal; they can produce 1-2 cocoons per week [21]. And that index is affected by many factors such as temperature, moisture or quality and quantity of food source [21, 22]. The effects of added ANC on maturation of earthworm were source [22]. There was no clear effect of ANC on maturity (table 09).

The equation III show the relationship between volumes of added ANC (X), the time of cultivation (Z) and the percentage of appearance of fully clitellum developed earthworm (Y).

$$Y = 0{,}252*X + 1{,}6626*Z + 13{,}26 \ (R^2 = 0{,}8532) \ (III)$$

Thus, the effect of the volume levels of added ANC to earthworm depended on the physiological action phase of earthworm the time of cultivation. To help the earthworm obtain the best biological indexes (growth rate and maturation), the ANC will be supplied at the low level (10 ml or 15 ml) at the initial phase of action. And this work will be repeated when earthworms need more nutrients to develop their clitellum in the stationary phase.

TABLE 9
PERCENTAGE (%) OF APPEARANCE OF FULLY-CLITELLUM DEVELOPED EARTHWORM UNDER EFFECT OF ADDED ANC

Day / Volume of ANC	0	15	30	45
0	0	46	83	100
5	0	48	67	100
10	0	52	83	100
15	0	54	86	100
20	0	48	90	100
25	0	51	94	100
Control	0	56	98	100

IV. CONCLUSION

This study is the paper in Vietnam show that spent mushroom compost can be used as the substrate for vermicomposting with *Perionyx exkavatus*. The data showed that moisture and light have affected positively on the growth of *Perionyx exkavatus*. The substrate with 80% moisture made earthworm archived the most growth rate and gain 100% clitellum development after 30 days. In natural light, growth rate and manure rate reached maximum with 5.61 mg.worm-1.day-1 and 235 mg.worm-1. Artificial nutritional compound supplementing showed strong effects on earthworms' growth rate. If ANC was added with 20 ml into 3 kg of spent mushroom compost, it made earthworms gained maximum growth rate and over double biomass after 60 days. But over 25 ml of artificial nutritional compound will effect negatively to earthworm growth rate.

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