STUDY ON THE BIOLOGICAL CHARACTERISTICS OF WAXWORM AND THE APPLICATION OF WAXWORM TO NYLON PE WASTE TREATMENT IN INTERNATIONAL SCHOOL - THAI NGUYEN UNIVERSITY

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ABSTRACT

Globally, the situation of plastic waste is causing several negative impacts on the environment and human health. Besides being a food for birds and reptiles, waxworms are known for their ability to eat plastic waste. The research aimed to study biological characteristics of waxworms (Galleria mellonella), then apply them to solve the nylon waste problem in International School - Thai Nguyen University. The study was conducted on 3 experiments with main food sources as PE nylon and honey, under the appropriate temperature and humidity conditions at the laboratory of the International School - Thai Nguyen University for 3 months: August, September and October in 2020. The research results showed that experiencing 4 stages of development, waxworms completed their whole life in a period of 35 - 40 days. Nylon PE is assimilated only at larval stage of waxworms. With appropriate living conditions of 26-33^{o}C, 80-83% humidity, the amount of nylon that 100 waxworms could handle in the larval stage when the feed contained only nylon was 1517.7 ± 8.9 mg, this figure reached 2200.2 ± 8.7 mg when the feed was added 5g honey/tray and achieved 2120.3 ± 7.9 mg when the feed was mixed 10 g honey/tray. The result showed that waxworms are capable of handling nylon PE in the temperature and humidity conditions at International School - Thai Nguyen University.

NGHIÊN CỨU ĐẶC TÍNH SINH HỌC CỦA SÂU SÁP VÀ ỨNG DỤNG SÂU SÁP VÀO XỬ LÍ RÁC THẢI NILON PE TẠI KHOA QUỐC TẾ - ĐẠI HỌC THÁI NGUYÊN

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TỪ KHÓA

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Đặc tính sinh học
Xử lý nilon
PE

TÓM TẮT

Tình trạng rác thải nhựa đang gây ra nhiều tác động tiêu cực đến môi trường và sức khỏe con người. Sâu sáp được biết đến là thức ăn cho các loài chim, bò sát, và khả năng ăn nilon. Mục tiêu của bài báo nhằm nghiên cứu đặc tính sinh học của sâu sáp (Galleria mellonella), và ứng dụng chúng để giải quyết rác thải nilon ở Khoa Quốc tế - Đại học Thái Nguyên. Nghiên cứu được thực hiện trên 3 công thức thí nghiệm với nguồn thức ăn là nilon PE và mật ong, dưới điều kiện nhiệt độ và độ ẩm thích hợp tại phòng thí nghiệm của Khoa Quốc tế - Đại học Thái Nguyên trong tháng 8, 9, 10 năm 2020. Kết quả nghiên cứu cho thấy, qua 4 giai đoạn phát triển, sâu sáp đã hoàn thành vòng đời trong khoảng thời gian 35 - 40 ngày. Nilon PE chỉ được đồng hóa ở giai đoạn ấu trùng của sâu sáp. Với nhiệt độ 26-33^{o}C, độ ẩm 80-83%, 100 con sâu sáp có thể xử lý 1517,7 ± 8,9mg khi thức ăn chỉ chứa nilon, 2200,2 ± 8,7mg khi thức ăn được bổ sung 5g mật ong/khay và khi thức ăn được trộn thêm 10g mật ong/khay là 2120,3 ± 7,9mg. Nghiên cứu cho thấy sâu sáp có khả năng xử lý nilon PE trong điều kiện nhiệt độ và độ ẩm tại Khoa Quốc tế - Đại học Thái Nguyên.

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1. Introduction

Waxworm is caterpillar with a white body, many bites and a small black head. Naturally, they parasitize in bee nets, consume cocoons, pollen, bee stalks and beeswax [1]. They have been noted to be able to assimilate nylon. The worms' ability to assimilate nylon demonstrates that the enzyme inside them is able to break down chemical bonds in the plastic. Currently, there are studies on the ability to consume nylon of waxworms, such as "This bug can eat plastic. But can it clean up our environment?" by Carrie Arnold [2], and "Plastivores: Remarkable waxworms devour plastic waste in BU study" by Brandon University [3].

Nylon is mainly produced from petroleum with the trade name as polyethylene (PE). According to the statistics of the World Environment Organization, the annual global PE output is about 80 million tons, which is mainly used to produce the packaging serving the daily needs of people [4]. PE is one of the non-biodegradable and hard to be degraded and this is the main source of environmental pollution [5]. Garbage in general, especially nylon waste discharged into the environment from human daily activities is a very serious problem globally, especially in developing country, which brings negative impacts on human health and the environment as well. Therefore, the disposal of nylon waste is an important issue and needs to be addressed.

Until now, the nylon waste treatment methods have certain disadvantages. Normally, plastic takes up to 1.000 years to decompose in landfills and nylon also takes 500 to 1.000 years [6], which causes pollution of soil and water. If the nylon waste is burned by local people or heat treatment process in substandard factories, it will release a extreme toxic gas as dioxin and furan, that makes people feel shortness of breath, disturbs digestion, and increases the chances of getting cancer directly affecting people living near that area. The treatment of nylon waste is an urgent issue and the application of waxworm in treating nylon waste is an extremely environmentally friendly biological method, so we have focused on conducting research on the biological characteristics of the waxworm in PE nylon waste treatment process at International School - Thai Nguyen University.

2. Materials and methods

According to the report written by Vitthalrao Bhimasha Khyade [1], there are two types of waxworm as Achroia grisella and Galleria mellonella, both of them can eat plastic. However, Galleria mellonella moth is known for better ability to process plastic than Achroia grisella. Hence, we used Galleria mellonella for this study.

2.1. Research on biological characteristics

- The research was carried at International School - Thai Nguyen University.
- The number of waxworms used in the study was 900 waxworms/ 9 trays.
- The main biological characteristics of waxworms were studied in each growth stage.
- The appropriate temperature and humidity conditions for each growth stage in the whole life of waxworms were determined.
- The daily growth and development as color, length, width, weight, number of internodes and shape of waxworms were observed and monitored.
- Instruments used for studying the biological characteristics of waxworms were BAS 31-plus analytical balance, Optika SZM-1 Microscope, glass petri dishes, and ruler.

2.2 Research on the PE treatment process

2.2.1 Experiment design

– The research was conducted under the temperature and humidity conditions at International School - Thai Nguyen University, Thai Nguyen province. The temperature of Thai Nguyen is about 26-35^{o}C in summer, and approximately 17-25^{o}C in winter. In the winter, the temperature is lower

than waxworm request, so it is necessary to adjust that higher to make the development process of waxworms faster and more efficient. The humidity of Thai Nguyen province is quite high, average above 80%. The study was carried in the period from August to October in 2020, with the temperature ranging from 28 to 32^{o}C, and the humidity from 79 to 82%.

- Waxworm (Galleria mellonella) was used in the experiment.
- The research was carried out in the tray of 600cm^2 with 100 waxworms for each tray. To study the influence of feed ration on consuming PE of waxworm, the experiments was set up as follows: feeding only nylon PE (F_1), feeding PE with 5 g honey (F_2), feeding PE with 10 g honey (F_3). There were 03 replications for each experiment, corresponding to 9 trays. The waxworms selected in the study were healthy, uniform in weight and size, with no signs of diseases or dying.
- Temperature was monitored and adjusted to suit the growing conditions of waxworms.
- Dried PE nylon waste was weighted and divided evenly into 09 trays; the worms' nylon processing speed was observed during their whole life.

2.2.2 Data processing method

The data were processed using Excel software and analyzed by the method of analysis of variance (Anova).

3. Results and discussion

3.1. Biological characteristics

Waxworms are the caterpillars of insects, recognized generally as wax moths. Taxonomically, they are belonging to Class: Insecta; Order: Lepidoptera; Superfamily: Pyraloidea and Family: Pyralidae (snout moths). Two closely related species are commercially bred – the lesser wax moth (Achroia grisella) and the greater wax moth (Galleria mellonella). They belong to the tribe Galleriini in the snout moth subfamily Galleriinae [1].

Waxworms go through 4 stages in their whole life, which are eggs, larvae, pupal and butterflies. Accordingly, with the weather conditions of Thai Nguyen, the waxworms existed the main stages of their life cycle in just 5-6 weeks at 26-33^{o}C, 80-83% humidity. For room temperature or in poorly ventilated areas, their growth is slowed down. The average incubation time of waxworms was 1.0 ± 0.2 days. After hatching into larvae, it took about 19.5 ± 1.0 days to complete the development of this stage. From the cocoon stage, the larvae rested and pupated with the time of 9 ± 1.5 days and the life expectancy of the butterfly stage was 8.0 ± 1.2 days. Total development stages of waxworms were 35-40 days.

* Stage of eggs: With temperatures of 26-33^{o}C, humidity 80-83%, females laid from 100-250 eggs. The eggs were oval in shape and stick together in plaques; before hatching, they had cream-white in the first cycle and turn white in the final cycle. The average weight of 100 eggs was 0.002g.

* Larval stage: Larva's body was creamy white from hatching. During further development, it turned light brown, and then its body began to thicken, become large and firm in the end of its growth. Their body had an average of 11-13 internodes (head, chest, abdomen), and no change in number of internodes from baby to maturing. They had three pairs of legs on the chest and four pairs of legs on the abdomen.

During the larval stage, the size and weight of waxworms changed markedly from hatching to the preparation of the cocoon (Table 1) and there was a lot of hair on the body. Dead larvae were identified by their inactivity and a change in color from creamy white to gray to black.

The main purpose of this research was to apply waxworms into PE nylon treatment, but waxworms could only handle PE nylon at the larval stage, so the study mainly focused on this stage.

Table 1. *Growing time, size and weight development of waxworms in the larval stage*

No.	Day	Length (mm)	Width (mm)	Weight (mg)
1	1-3	1.13 ± 0.7	0.11 ± 0.05	10.56 ± 1.25
2	3-5	1.43 ± 0.3	0.23 ± 0.09	12.33 ± 1.03
3	5-7	2.87 ± 0.9	0.52 ± 0.22	20.84 ± 1.17
4	7-9	4.98 ± 0.2	1.33 ± 0.7	38.42 ± 2.01
5	9-11	10.3 ± 0.6	2.76 ± 0.92	70.56 ± 1.98
6	11-13	11.68 ± 0.5	3.34 ± 0.5	80.37 ± 2.63
7	13-15	15.5 ± 1.2	4.03 ± 0.17	148.8 ± 1.55
8	15-17	19.2 ± 1.3	4.68 ± 0.66	168.7 ± 3.67
9	17-19	20.9 ± 0.4	5.14 ± 0.74	174.2 ± 2.31
10	19-21	21.8 ± 0.1	5.47 ± 0.32	180.6 ± 1.33
	P < 0.05	< 0.005	< 0.05	< 0.05
	CV (%)	19.5	17.1	22.1

Results in table 1 showed that from 1-5 days old, the length, width and weight of larvae had not change much. The fastest growing stage was between the day 5-11 and 13-17. On the period of 17-21 day, their growth slowed down, in which the variation in width was harder to witness than in the length and weight.

Period 5-11 days old, larvae increased about 4 times both in size and weight compared to hatched stage. In the last days of the larval stage, worms increased in length and weight twice as much as the thirteenth-day period.

* The pupal stage: Pupal is the stage that the larvae is in white chamber. The color of the pupal changes with age from white (right after the larval process is finished) through yellow and brown to dark brown. The corresponding mean size of the pupal was 11.5-19.5 mm in length and 5.5 ± 0.9 mm in width.

* Butterfly stage: At the end of the pupation cycle, the waxworms turned into the butterfly stage, also known as a moth. Moths had an average body length of 12-20 mm and a wingspan of 28-30 mm. The wings were gray in varying density, the front 2/3 was darker pigmented than the rear 1/3. After their wings were fully spread and strong, the male and the female would find each other to mate and lay eggs. Butterflies would die after they lay eggs.

The results on biological characteristics of waxworms showed that they could grow well with temperature from 26-33^{o}C, humidity from 80 to 83%. In conditions of low average temperature (below 26^{o}C), the growth and development of waxworms was slow, time through stages of the life cycle is prolonged. The average temperature was high (above 38^{o}C) could lead to a high rate of worm death due to their body lacks water. Therefore, it is necessary to pay special attention to the factors of daily temperature and humidity to adjust the growth and development of waxworms.

In summer in Thai Nguyen, the average temperature is quite suitable for the development of waxworms. In winter, the average temperature is quite low (17-25^{o}C), it is necessary to adjust to make the development of waxworms faster and more efficient. August, September, and October are the best times for worms to grow and develop.

With the ambient temperature of 26-33^{o}C, whole life of waxworm was completed in about 35-40 days, this result was quite similar to previous studies by some scientists such as Iwona Wojda and colleagues [7], and Kamilu Ayo Fasasi and Malaka SLO [8].

3.2 .Waxworms' ability to treat PE nylon

In their whole life, worms feed only in the larval stage, which is the time they store up nutrients to feed their bodies during the rest of their growth. With this feature, this is the stage that they treat PE nylon the best.

To determine the exact amount of PE nylon being consumed, the larval stage was divided into 5 smaller stages, the number of waxworms was 100 (Table 2).

Table 2. *Data of nylon PE waste treatment of waxworms in the larval stage (calculated on 100 waxworms)*

Day	F_1 (mg)	F_2 (mg)	F_3 (mg)
1-5	18.2 ± 0.7	19.5 ± 0.8	18.9± 0.5
6-9	78.7 ± 0.4	86.8 ± 0.6	83.2 ± 0.9
10-13	345.2 ± 3.2	397.2 ± 1.4	386.8 ± 1.8
14-19	1056 ± 2.7	1320 ± 3.6	1267.2 ± 2.9
20-21	19.6 ± 1.9	21.2 ± 2.3	20.8 ± 1.8
Whole life	1517.7 ± 8.9	2200.2 ± 8.7	2120.3 ± 7.9
P < 0.05	< 0.05	< 0.05	< 0.05
CV(%)	20.3	18.9	21.5

The results from table 2 show that the PE nylon weight, which 100 waxworms larve consumed through each development stage was different. The ability to assimilate nylon PE at the period 1-5 days of age through the experiments was only about 18.2 to 19.5 mg. At the next stage of age, the ability to handle nylon PE of waxworms increased, from 78.7 to 86.8 mg. The period of 10-13 was the waxworms stage in the maturing process, they began to eat more, the amount of nylon assimilated about 345.2 - 397.2 mg. The stage they could handle the most PE nylon was 14-19 days old, from 1056 to 1320 mg. In the last days of larvae, the ability to handle PE nylon of worms decreased significantly. They reduced their food intake to wrap the chamber in preparation for the next growth stage.

For the whole life, in F_1, waxworms consumed 1517.7 ± 8.9 mg PE. In F_3, if mixed 10g honey into the nylon they could solve 2120.3 ± 7.9 mg PE. Waxworms assimilated highest at F_2 was 2200.2 ± 8.7 mg PE when added 5 g honey to the feed, 45.1% higher than F_1 and 1.1% higher than F_3. With all experiments, the ability to treat nylon at $F_2$was the highest, it might due to their food characteristics and amount of honey adequate with their demand. In the nature, the characteristic of waxworms that parasite in the hive, when honey is added to feed, it is more consistent with their inherent biological characteristics and they could consume more nylon PE. The ability of PE nylon treatment was stimulated by adding 5g of honey at F_2. If a multiplicity of honey as F_3, waxworms mainly eat honey without focusing on nylon PE treatment. Therefore, F_2 increased about 45.1% nylon consumption and F_3 was about 39.7% compared to F_1 with 95% of significant ($P < 0.05$).

4. Conclusion and recommendation

- Research results on biological properties of waxworms at International School - Thai Nguyen University with temperature conditions 26-33^{o}C, humidity 80-83% showed the growth and development time of waxworms were fast, whole life completed after 35-40 days. Larvae size from hatching to the last days of the stage was about 1.13 ± 0.7 mm to 21.8 ± 0.1 mm in length, 0.11 ± 0.05 mm to 5.47 ± 0.32mm in width and from 10.56 ± 1.25mg to 180.6 ± 1.33mg in weight.

- The PE nylon treatment ability of waxworms on 3 different experiments showed that waxworms' feeding ration of added 5g of honey gave the highest PE consuming at 2200.2 ± 8.7 mg. Compared with the other feeding ration, the PE nylon treatment of waxworms was less effective. Therefore, only 5g of honey/tray/ 100 waxworms larvae should be added.

- From the above research results, it is necessary to have more study on this worm with other type of plastic. This is a study in the laboratory, so it is important to deploy waxworms into farming to contribute to reduce amount of PE in the environment and research application in other localities.

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