The influence of different breeding conditions of Galleria Mellonella larvae on the possibility of recycling polyethylene from the natural environment.

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Jastrzębie-Zdrój, June 2021

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Introduction

Recently, the problem of environmental pollution and ecological actions intrigued us and made us aware of the seriousness of the situation related to the destruction of the natural environment. We came across articles on the possibility of utilizing the ubiquitous polyethylene and polypropylene by the larvae of Galleria Mellonella.

We considered how this organism functions, what it is capable of, and whether we can contribute to expanding our knowledge of the functioning of this species and its positive impact on changes in the natural environment. We decided to start research and for the last period we have cultivated and observed how larvae behave and develop under different thermal conditions, with different access to food, its quantity, and most of all, we wondered about the influence of these factors on disposal of polyethylene.

We hope that our research will help in the future to use the potential of larvae to protect the natural environment, which is after all so polluted.

Galleria Mellonella larvae

Galleria Mellonella is a popular pest of the honeybee. The moth lays its eggs in the slots inside the hive. After hatching, the larvae feed on the core of the wax comb, brood dead skins and small amounts of propolis and honey. They can also eat organic waste and dried fruit. The development of one generation of this species is estimated at about 8 weeks. The larval state lasts from 26 days to 6 months depending on temperature and other environmental conditions. They spin silk, from which in the last larval stage they form a cocoon and begin the process of pupation.

The most optimal conditions for the development of larvae are temperature of around 29 - 33°C and a humidity level of around 30%. Average temperatures above 45 ° C lead to death, and even brief exposures to temperatures below 0°C kill larvae and pupated individuals. Temperatures below 23°C slow development between stages and can only contribute to ending part of the life cycle. With little food, cases of cannibalism have been observed and a diet deprived of nutrients makes the larvae more susceptible to yeast contamination.

According to the already confirmed research of the larvae Galleria Mellonella, they are capable of biodegradation of polyethylene. After leaving the polyethylene foil in direct contact with the larvae, holes appear in the foil after 40-50 minutes. A population of 100 larvae in contact with a polyethylene shopping bag for 12 hours resulted in a weight loss of about 90 mg of polyethylene. The studies have also shown that the larvae convert polyethylene into ethylene glycol.

Our research methodology

The influence of temperature on larvae and disposal of polyethylene

1. Terrarium No 1 – the highest temperature:

- temperature, 24° C

- 10 larvae

- a piece of PE foil, 34x26 cm, mass 0,535 g

10 medium-sized larvae were placed in a jar together with a bag made of polyethylene foil. The holes of the appropriate diameter were made in the covers so that the larvae could not get out and had access to air. The terrarium prepared in this way was placed in an open box to prevent sunlight. The larvae were kept in a safe place in the room and their behavior and temperature were checked in the terrarium.

2. Terrarium No 2 – the medium temperature:

- temperature, 20 – 21° C

- 10 larvae

- a piece of PE foil, 34x26 cm, mass 0,535 g

10 medium-sized larvae were placed in a jar together with a bag made of polyethylene foil. The holes of the appropriate diameter were made in the covers so that the larvae could not get out and had access to air. The terrarium prepared in this way was placed in an open box to prevent sunlight. The larvae were kept in a safe place in a room with a lower air temperature and their behavior and temperature in the terrarium were checked.

3. Terrarium No 3 – the lowest temperature:

- temperature, 18° C

- 10 larvae

- a piece of PE foil, 34x26 cm, mass 0,535 g

10 medium-sized larvae were placed in a jar together with a bag made of polyethylene foil. The holes of the appropriate diameter were made in the covers so that the larvae could not get out and had access to air. The terrarium prepared in this way was placed in an open box to prevent sunlight. The larvae were kept in a safe, cool place and their behavior and temperature were checked in the terrarium.

The influence of different dosages of beeswax and polyethylene on the viability of the larvae and the amount of used polyethylene

1. Research sample No 1 – beeswax : PE 1:1

- room temperature

- 9 larvae

- a piece of PE foil, 18x6 cm, mass 0,065 g

9 medium-sized larvae were placed in a jar together with a bag made of polyethylene foil and beeswax (ratio 1: 1). The holes of the appropriate diameter were made in the covers so that the larvae could not get out and had access to air. The terrarium prepared in this way was placed in an open box to prevent sunlight. The larvae were kept in a safe place in the room and their behavior was checked in the terrarium.

2. Research sample No 2 – beeswax : PE 2:1

- room temperature

- 9 larvae

- a piece of PE foil, 18x6 cm, mass 0,065 g

9 medium-sized larvae were placed in a jar together with a bag made of polyethylene foil and beeswax (ratio 1: 2). The holes of the appropriate diameter were made in the covers so that the larvae could not get out and had access to air. The terrarium prepared in this way was placed in an open box to prevent sunlight. The larvae were kept in a safe place in the room and their behavior was checked in the terrarium.

3. Research sample No 3– the amount of beeswax is unlimited

- room temperature

- 9 larvae

9 medium-sized larvae were placed in the jar along with the beeswax. The holes of the appropriate diameter were made in the covers so that the larvae could not get out and had access to air. The terrarium prepared in this way was placed in an open box to prevent sunlight. The larvae were kept in a safe place in the room and their behavior was checked in the terrarium.

OBTAINED TEST RESULTS

THE INFLUENCE OF TEMPERATURE ON LARVAE AND DISPOSAL OF POLYETHYLENE

TERRARIUM No 1 - THE HIGHEST TEMPERATURE
TEMPERATURE, 24^{O}C
THE AMOUNT OF LARVAE: 10
THE MASS OF PE: **0,535 g**

DAY	Temp.	L.K.	L.M.	L.A.	L.N.	M.P.	Observations
12h	20°C	0	0	10	0	0,535	After 12 hours, the first major cavities are visible
1	23°C 22,7°C	0	0	10	0		After 24 hours, losses of similar volume are visible in each of the samples.
2	23°C 24°C	2	0	8	2		The first larvae in cocoons.
3	24°C 25°C	3	0	7	3		No changes
4	24,5°C 24°C	4	0	6	4		No changes
5	24°C 24,3°C	5	0	5	5		One of the larvae forms a thick cocoon, the other four form thin cocoons in which they can move.
6	25°C 23,5°C	6	0	4	6		No changes
7	24,6°C 25,1°C	5	1	4	6		Small larvae from cocoon was eaten
8	25°C 23,7°C	5	1	4	6		No changes
9	25,2°C 24,8°C	4	2	4	6		Another larvae from cocoon was eaten by the rest.
10	25,2°C 25,5°C	3	3	4	6		Another larvae from cocoon is dead
11	25°C 23,5°C	3	4	3	7		Another larvae from cocoon is dead. A new cocoon.
12	23,5°C 22,5°C	3	5	2	8		Another larvae is dead.
13	23,5°C 24,2°C	1	6	3	7		Another larvae is dead.
14	24,8°C 24,8°C	2	6	2	8		No changes
15	25°C 25,4°C	2	6	2	8		No changes
16	25,8°C 23°C	2	6	2	8		No changes
17	22°C 24,1°C	3	6	1	9		No changes
18	25,6°C 24,9°C	3	6	1	9		No changes
19	24°C 24,3°C	3	6	1	9		No changes
20	24,7°C 24°C	3	6	1	9		No changes
21	**Average** **24,3°C**	2	6	1	9	0,515	A larvae was pupated on day 21. Two larvae remained in the cocoons. One was left without a cocoon alive. In total, 6 out of 10 larvae are dead.

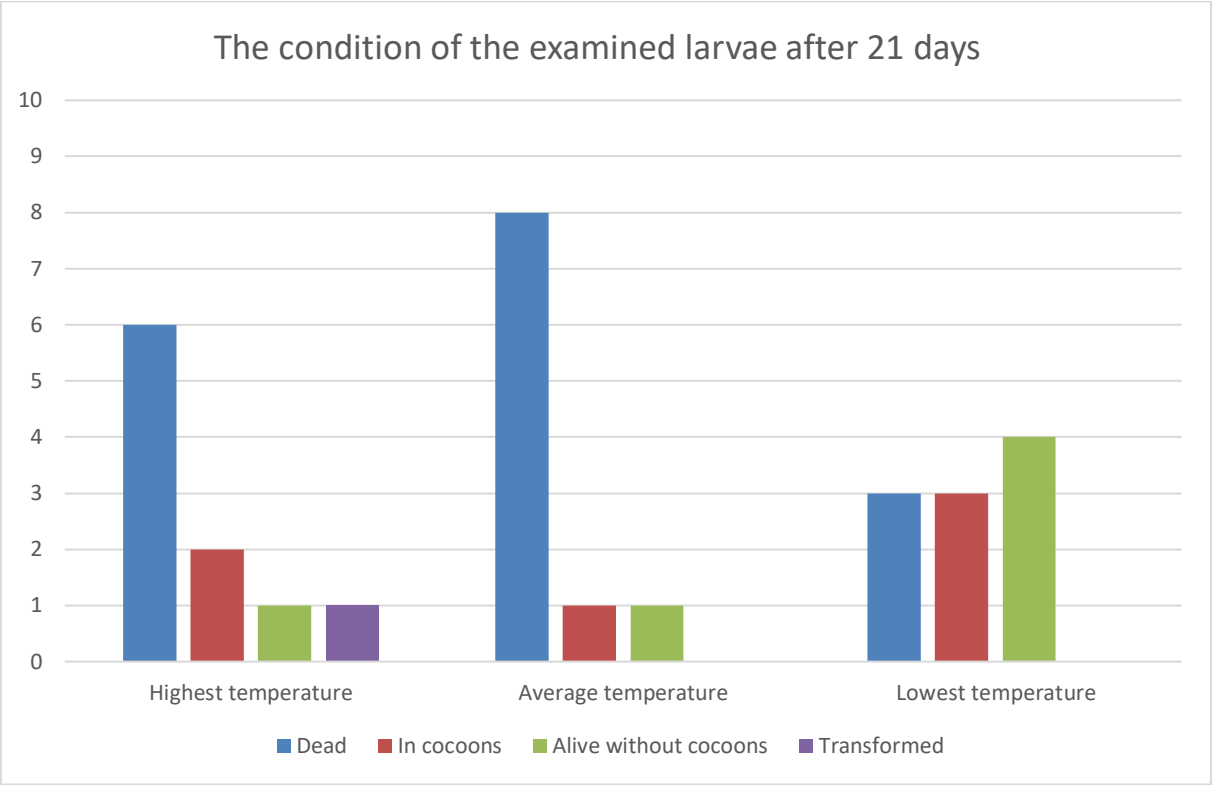
L.K. – The amount of larvae in cocoons, **L.M.** – The amount of dead larvae, **L.A.** – The amount of active larvae, **L.N.** – The amount of inactive larvae, **M.P.** – the mass of PE [g]

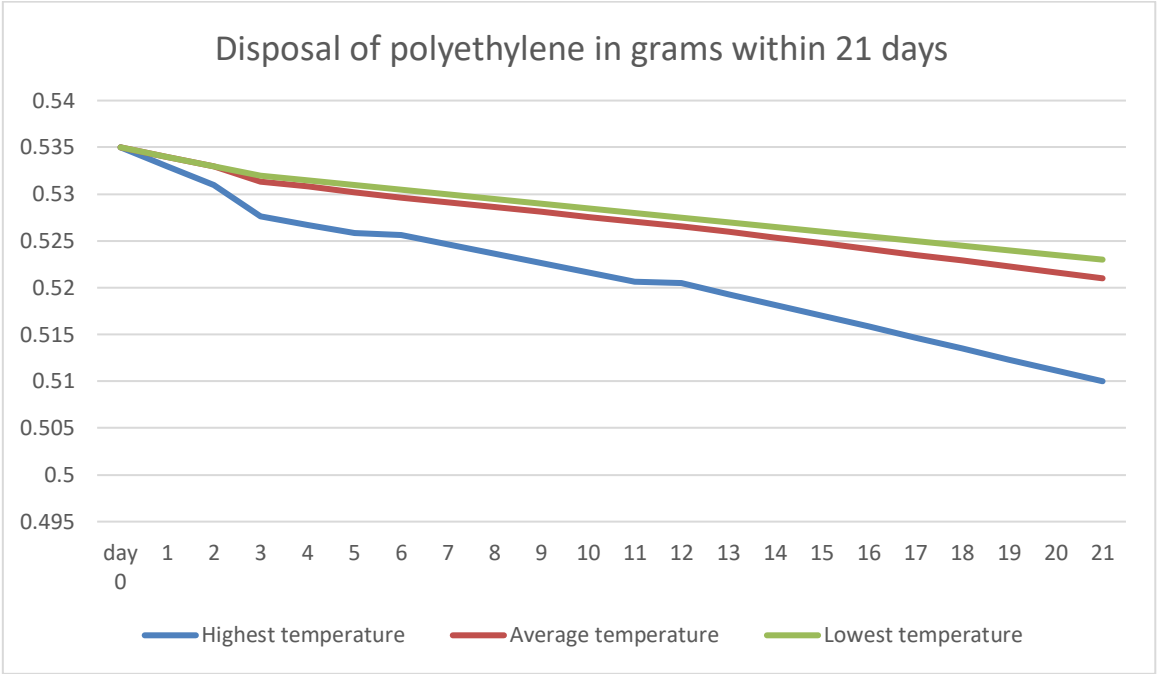
TERRARIUM No 2 – THE MEDIUM TEMPERATURE
TEMPERATURE, 20 – 21°C
THE AMOUNT OF LARVAE: 10
THE MASS OF PE: 0,535 g

Day	Temp.	L.K.	L.M.	L.A.	L.N.	M.P.	Observations
12h	20°C	0	0	10	0	0,535	The first cavities are visible after 12 hours.
1	18,6°C 19,5°C	0	0	10	0		After 24 hours, losses of a similar amount are visible in each trial.
2	21°C 18,2°C	1	0	9	1		A larva formed a cocoon.
3	19,3°C 19°C	1	0	9	1		No changes
4	18,7°C 20,8°C	1	0	9	1		No changes
5	20°C 20,1°C	1	0	9	1		The larvae begin to form thin cocoon-like threads at the bottom of the jar.
6	21°C 21,1°C	1	0	9	1		No changes
7	20,2°C 20,7°C	1	0	8	2		No changes
8	21°C 21,8°C	2	0	8	2		No changes
9	20,5°C 20,2°C	2	0	8	2		Two thin cocoons at the bottom.
10	20,1°C 21,3°C	2	0	7	3		No changes
11	21°C 20°C	2	0	6	4		No changes
12	20,5°C 21°C	2	1	6	4		One small larva without a cocoon is dead.
13	22°C 20,4°C	3	2	5	5		Another larva is dead.
14	21,4°C 21°C	3	2	5	5		No changes
15	20,6°C 20,8°C	3	2	4	6		One cocoon is clearly thicker and full,
16	19°C 19°C	3	2	3	7		No changes
17	20,4°C 20,3°C	2	4	3	7		Four larvae are dead, including one in the cocoon, they are dry.
18	20,5°C 20,4°C	1	6	2	8		Six larvae are dead.
19	21°C 21°C	1	6	1	9		No changes
20	19,5°C 20,4°C	1	8	1	9		No changes
21	**Average** **20,3°C**	1	8	1	9	0,522	One larva remained in a thick cocoon. Only one has no cocoon and is alive. In total, 8 larvae are dead. They died much later than those of the first attempt.

TERRARIUM No 3 – THE LOWEST TEMPERATURE
TEMPERATURE, 18°C
THE AMOUNT OF LARVAE: 10
THE MASS OF PE 0,535 g

Day	Temp.	L.K.	L.M.	L.A.	L.N.	M.P.	Observations
12h	20°C	0	0	10	0	0,535	After 12 hours, small cavities are visible.
1	17,6°C 18,5°C	0	0	9	1		After 24 hours, losses of a similar amount are visible in each trial. The larvae are not very mobile, they stay mainly at the bottom of the jar.
2	16,5°C 17°C	0	0	9	1		The larvae are the least mobile of all the trials. They produce less strands than the rest of the larvae.
3	17,4°C 17,3°C	0	0	9	1		No changes
4	18,1°C 18,2°C	0	0	9	1		No changes
5	18°C 17,9°C	0	0	9	1		The larvae are slowed down, staying at the bottom of the jar
6	17,7°C 17,5°C	0	0	8	2		No changes
7	18,6°C 18,7°C	1	0	7	3		One larvae darkened and formed a thin cocoon at the bottom.
8	18,6°C 18,7°C	2	1	6	4		The smallest larva is dead. The body is dry. The two larvae formed thin cocoons at the bottom.
9	18,9°C 19°C	2	1	5	5		No changes
10	18,4°C 19,5°C	3	1	5	5		The third larva formed a cocoon in the cleft of the foil.
11	18,2°C 18,6°C	4	1	4	6		The four larvae are in thin cocoons.
12	18,5°C 18,4°C	4	1	3	7		No changes
13	18,4°C 18,5°C	4	1	3	7		No changes
14	18,6°C 18,7°C	4	1	3	7		No changes
15	18,6°C 18,7°C	4	1	3	7		No changes
16	18,7°C 18,8°C	3	1	4	6		The larva left the cocoon.
17	18,5°C 18,5°C	3	1	4	6		No changes
18	18,3°C 17,8°C	4	1	5	5		Another cocoon in the foil gap.
19	17,6°C 17,8°C	4	2	4	6		No changes
20	18°C 18,1°C	4	2	4	6		No changes
21	**Average** **18,2°C**	**3**	**3**	4	6	0,528	Three larvae remained in the cocoons. Three of the larvae are dead. The four larvae are alive and have no cocoons.


The condition of the examined larvae after 21 days
10
9
8
7
6
5
4
3
2
1
0
Highest temperature
Average temperature
Lowest temperature
Dead
In cocoons
Alive without cocoons
Transformed


Disposal of polyethylene in grams within 21 days
0.54
0.535
0.53
0.525
0.52
0.515
0.51
0.505
0.5
0.495
day 0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
Highest temperature
Average temperature
Lowest temperature

After 24 hours		
Temperature 24,3°C	Temperature 20,3 °C	Temperature 18,2 °C
		

After 21 days		
Temperature 24,3 °C	Temperature 20,3 °C	Temperature 18,2 °C
		

The influence of different dosages of beeswax and polyethylene on the survival rate of larvae and the amount of POLYETHYLENE consumed

Research sample No 1 – beeswax : PE 1:1
TEMPERATURE, 22°C,
THE AMOUNT OF LARVAE: 9
THE MASS OF PE: 0,065 g

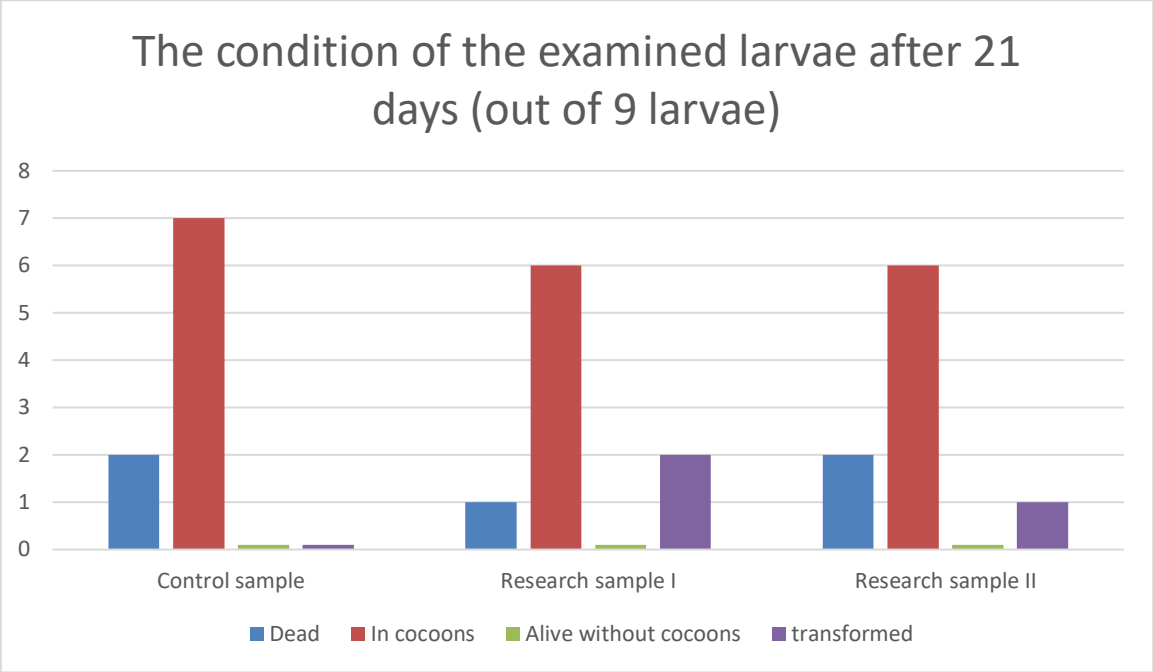
DAYS	The amount of active larvae	The amount of inactive larvae	The mass of PE [g]	Observations
1	9	0	0,065	The larvae are rather lethargic, not very mobile, hidden in the wax
2	9	0	no measurement	The larvae are not very mobile, there is a visible loss of wax mass
3	9	0	no measurement	3/9 larvae showing mobility, 6/9 larvae rather lethargic, visible loss of wax mass.
4	9	0	0,064	The larvae are moving, wax weight loss, the first holes in the polyethylene bag.
5	9	0	no measurement	the larvae are calm
6	9	0	no measurement	No changes
7	9	0	no measurement	No changes
8	9	0	0,063	Visible holes in the polyethylene bag
9	8	1	no measurement	1 larva in a cocoon
10	8	1	no measurement	No changes
11	7	2	no measurement	2 larvae in a cocoon
12	6	3	no measurement	3 larvae in a cocoon
13	6	3	no measurement	No changes
14	6	3	no measurement	No changes
15	5	4	0,062	4 larvae in a cocoon
16	4	5	no measurement	1 larvae is dead
17	4	5	no measurement	No changes
18	3	6	no measurement	1 new moth
19	2	7	no measurement	1 new moth
20	2	7	no measurement	No changes
21	1	8	0,0611	1 new moth

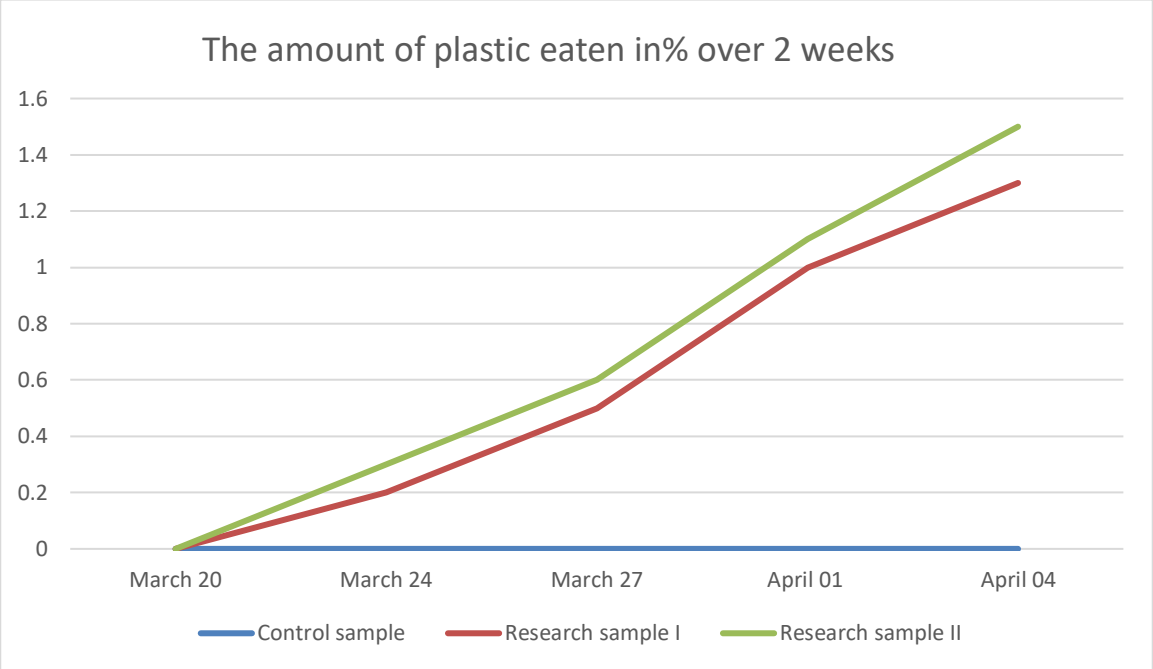
Research sample No 2 – beeswax : PE 2:1
TEMPERATURE, 22°C,
THE AMOUNT OF LARVAE: 9
THE MASS OF PE: 0,065 g

DAYS	The amount of active larvae	The amount of inactive larvae	The mass of PE [g]	Observations
1	9	0	0,065	The larvae are rather lethargic, not very mobile, hidden in the wax
2	9	0	no measurement	Larvae still sluggish, hidden in wax.
3	9	0	no measurement	4/9 motile larvae, 5/9 calm larvae hiding in wax.
4	9	0	no measurement	Calm larvae, visible wax weight loss
5	9	0	0,0645	Calm larvae, first holes in a polyethylene bag
6	9	0	no measurement	No changes
7	9	0	no measurement	No changes
8	9	0	0,0640	Visible holes in the polyethylene bag
9	7	2	no measurement	2 larvae in a cocoon
10	7	2	no measurement	No changes
11	6	3	no measurement	3 larvae in a cocoon
12	5	4	0,063	4 larvae in a cocoon
13	5	4	no measurement	No changes
14	4	5	no measurement	1 larvae is dead
15	4	5	no measurement	No changes
16	3	6	no measurement	5 larvae in a cocoon
17	1	8	no measurement	6 larvae in a cocoon, 1 new is dead
18	1	8	no measurement	No changes
19	0	9	no measurement	1 moth
20	0	9	no measurement	No changes
21	0	9	0,0624	No changes

Research sample No 3– the amount of beeswax is unlimited:
TEMPERATURE, 22°C,
The amount of larvae: 9
The mass of PE: not applicable

DAYS	The amount of active larvae	The amount of inactive larvae	The mass of PE [g]	Observations
1	9	0	not applicable	Mobile larvae, slight loss of wax
2	9	0	not applicable	Mobile larvae eat a lot of wax
3	9	0	not applicable	Calm larvae
4	9	0	not applicable	Full larvae, loss in wax mass
5	9	0	not applicable	Calm, fed larvae gained significantly weight, the amount of wax decreased
6	8	1	not applicable	1 larvae is dead
7	8	1	not applicable	No changes
8	8	1	not applicable	Calm larvae
9	6	3	not applicable	2 larvae in cocoons
10	6	3	not applicable	No changes
11	5	4	not applicable	3 larvae in cocoons
12	5	4	not applicable	No changes
13	4	5	not applicable	4 larvae in cocoons
14	3	6	not applicable	2 larvae are dead
15	2	7	not applicable	5 larvae in cocoons
16	1	8	not applicable	6 larvae in cocoons
17	0	9	not applicable	7 larvae in cocoons
18	0	9	not applicable	No changes
19	0	9	not applicable	No changes
20	0	9	not applicable	No changes
21	0	9	not applicable	1 moth


The condition of the examined larvae after 21 days (out of 9 larvae)
8
7
6
5
4
3
2
1
0
Control sample
Research sample I
Research sample II
Dead
In cocoons
Alive without cocoons
transformed


The amount of plastic eaten in% over 2 weeks
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
March 20
March 24
March 27
April 01
April 04
Control sample
Research sample I
Research sample II

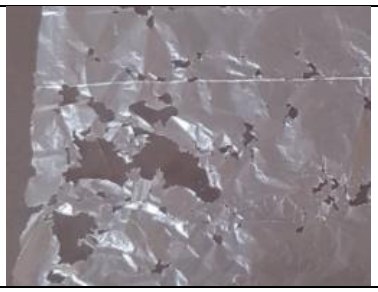
PHOTOGRAPHIC DOCUMENTATION

THE INFLUENCE OF TEMPERATURE ON LARVAE AND DISPOSAL OF POLYETHYLENE

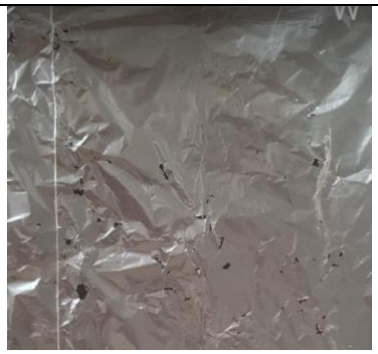
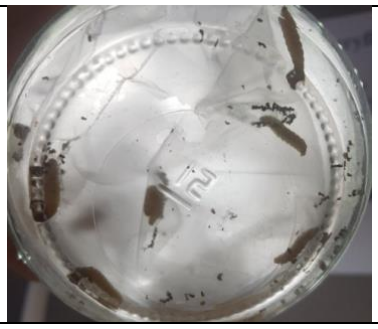
Terrarium No 1 – the highest temperature

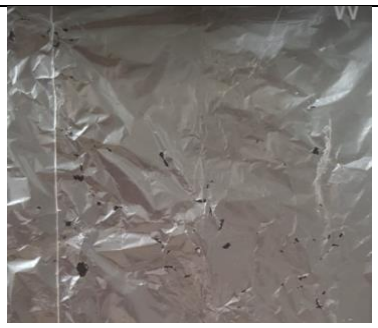
Day	L.K.	L.M.	Photos	Photos	Photos
1					
2	2				
3	3				
4	4				
5	5				
6	6				
7	5	1			

8	5	1			
9	4	2			
10	3	3			
11	3	4			
12	3	5			
13	1	6			
14	2	6			
15	2	6			
16	2	6			

17	3	6			
18	3	6			
19	3	6			
20	3	6			
21	2	6			
					

Terrarium No 2 – the medium temperature

Day	L.K.	L.M.	Photos	Photos	Photos
1					
2	1				
3	1				
4	1				
5	1				
6	1				
7	1				
8	2				

9	2				
10	2				
11	2				
12	2	1			
13	3	2			
14	3	2			
15	3	2			
16	3	2			

17	2	4			
18	1	6			
19	1	6			
20	1	8			
21	1	8			

Terrarium No 3 – the lowest temperature

Day	L.K.	L.M.	Photos	Photos	Photos
1					
2					
3					
4					
5					
6					
7	1				
8	2	1			

9	2	1			
10	3	1			
11	4	1			
12	4	1			
13	4	1			
14	4	1			
15	4	1			
16	3	1			

17	3	1			
18	4	1			
19	4	2			
20	4	2			
21	**3**	**3**			

RESEARCH SAMPLE NO 2

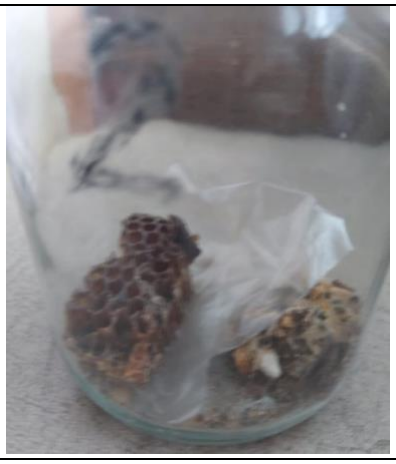
THE INFLUENCE OF DIFFERENT DOSAGE OF BEE WAX AND POLYETHYLENE ON THE LARVAL SURVIVABILITY

1. Research sample No 1 – beeswax : PE 1:1

the beginning of the study		
PE i BEESWAX 1:1	**PE i BEESWAX 1:2**	**BEESWAX**
		

Polyethylene foil after the end of the experiment.

2. Research sample No 2 – beeswax : PE 1:2

The end of the study		
PE i BEESWAX 1:1	**PE i BEESWAX 1:2**	**BEESWAX**
		

Polyethylene foil after the end of the experiment.

CONCLUSIONS FROM THE CONDUCTED RESEARCH

RESEARCH SAMPLE NO 1

THE INFLUENCE OF TEMPERATURE ON LARVAE AND DISPOSAL OF POLYETHYLENE

The larvae from the first terrarium which were bred at 24 ° C processed the most polyethylene foil than in the other two research trials. They were also more mobile, recycling polyethylene faster than others. The larvae showed little survival rate, cocooned and metamorphosed into adults more quickly.

The larvae from the second terrarium, which were bred at 20 ° C processed less polyethylene foil than individuals living at higher temperatures. They were reluctant to eat polyethylene. The larvae showed the lowest survival, but lived longer than those in terrarium number 1.

The larvae from the third terrarium which were bred at 18 ° C processed the least polyethylene film of all research trials. They were the least mobile, most of the time they were still at the bottom of the vessel and stayed in certain places, although they did not immediately form cocoons. They survived the longest of all individuals and formed the fewest cocoons.

Summary:

The conducted research showed that Galleria Mellonella larvae at low temperatures, i.e. 18 ° C, are more lethargic, have a slower life cycle, which means that they live longer than at higher temperatures, although this does not translated into a greater amount of recycled polyethylene. The Larvae bred at 24 ° C showed greater consumption of polyethylene film than those kept at lower temperatures, despite their lower survival and faster cocoon formation.

RESEARCH SAMPLE NO 2

The influence of different dosages of beeswax and polyethylene on the survival rate of larvae and the amount of PE used

In the first terrarium with one wax cube and one piece of polyethylene foil (1: 1), the larvae developed at a similar rate as in the other terrariums. They processed more polyethylene than the larvae that were fed in a 2: 1 ratio (two wax cubes and one piece of polyethylene foil).

The second study allowed us to prove that larvae fed with basic food (beeswax) survive in 80%. The greater weight loss of the polyethylene foil was observed in the terrarium where the ratio of beeswax to polyethylene foil was 1: 1 (the larvae were more active).

If the ratio of beeswax and polyethylene foil supplied was 2: 1, less consumption of polyethylene foil was observed, and less larvae pupated into moths.

Summary:

It is puzzling that the greater consumption of polyethylene was in the terrarium where the greater amount of beeswax was prepared (2: 1). The larvae could eat their preferred food because there was more of it, but they consumed more polyethylene foil compared to the other terrarium.

Bibliography

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