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Zophobas Morio Semi Industrial Cultivation Peculiarities

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ABSTRACT

In order to develop various Zophobas morio cultivation ways, the experiments were conducted to find out the optimal storage and reproduction conditions. Based on the acquired data, the Zophobas morio cultivation technology was developed. The dependencies were found between different fodder substances and the specimens' growth and reproduction rates. The optimal cultivation fodder substance was also found.

Keywords: Zophobas Morio, Insect, Larvae, Cultivation, Imago, Population.

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INTRODUCTION

The development and the implementation of the new efficient fodder additives has become the leading tendency in the feeding technology improvement and the preventive veterinary measures of the modern livestock and poultry farming [1, 2]. Most of the insects' species are extremely fertile, namely hundreds times more fertile than any subject of the modern livestock breeding [3, 4]. Insect cultivation and insect-based fodder additives development have been very prospective and highly feasible fields. Lately, the chitin-based compounds, have been considered as highly promising in terms of livestock breeding applications. Chitin is the main substance of the insect cuticle [5]. The chitosan, chitin's de-acetylated form is the most biologically active of all its (chitin's) derivatives. Those are natural polymers, they are characterized by a high sorption capacity, immune system stimulation, anti-tumoral, bacteriostatic, and wound healing properties [6]. They are also compatible with the animal tissues. Antibacterial, antifungal, and antiviral chitosan's properties are widely used in the biomedicine [7, 8]. The insects' bioconversion ability of various substances justifies the interest to the topic, and making them the object of research. Thus, there are certain insect species, i.e. *Zophobas Morio*, which are capable to convert cellulose fodder into their own protein mass [9, 10]. As of now, there is a certain technology deficit, in terms of producing the needed assortment of biologically active compounds, based on cheap and available raw materials of animal or plant origin. That is why a need to develop new technologies has arisen, the technologies that enable the researchers to produce and acquire highly efficient broad-spectrum agents. While the biggest market share has belonged to expensive foreign products, it (the market) suffers from the lack of affordable Russian-made compounds. That is why the researches aimed at fodder production issues resolution, including the fodder base improvement, and new fodder additives development is par-

ticularly actual. Thus, *Zophobas morio* semi-industrial cultivation peculiarities were studied in this article.

METHODS AND MATERIALS

To develop an insects cultivation method, 40 kg *Zophobas morio* larvae, which was equivalent to 40 000 units from "Zoofond" LLC (from Russian ООО "Зоофонд") (Moscow region, Solnechnogorsk district) was acquired. Insect cultivation has been conducted in terrariums, 1.2 kg larvae each. The terrariums volume was equal to 400 liters; they have been made of sheet glass. The larvae differed in size and weight (ranging from 0.6 to 1 g). With an average weight of 0.8 g, there were about 1,500 larvae per terrarium. The container was covered with a 20 cm thick layer of loose soil with a high content of rotten leaves. The rotten logs were put in this fodder substance for the larvae to pupate. The storage temperature and humidity corresponded to the habitat environmental conditions (t = 28-30 °C, W = 80-85%).

The *Zophobas morio* larvae population density was determined by sampling, considering the population size. The optimal sample size was selected in order to ensure the minimum results dispersion, and to minimize the labor and time costs. The following formula was used to calculate the number of larvae:

$$P=вн2Tмп2t \quad (1)$$

where P is the optimal size of a unit, вн2– dispersion within the sample, мп2– dispersion between the samples, T is the time required for a transition from one unit (sample) to another, t – time to analyze a unit.

There was a kind of special case here, as the logs were also considered, taken from the cultivation terrarium, i.e. there was a population density dispersion within the soil and between the logs.

To determine the number of samples that allowed to draw a conclusion concerning the population size with a certain accuracy, the following formula was used:

$$n=t\partial\varepsilon m2 \quad (2)$$

where n is the required number of samples, t is the Student's test (with an accuracy of 0.8 (error 0.2) is equal to 1.5), δ is the dispersion, obtained during the preliminary study, m is the average number of specimens per sample according to the results of the preliminary study, ε is the value of the error in fractions relative to the average (10-20%, i.e. 0.1 or 0.2).

The adult bug population density was determined by keeping the record of the linear routes (route recording), as these specimens live mainly on the surface. The total insect population density on the surface can be determined by the following formula:

$$P=NSR \quad (3)$$

where P is the population density per $1m^2$, N is the total number of insects found on the surveyed surface along the route (only on one side: to the left or to the right of the observer), S is the length of the route, R is the width of the surveyed surface, in this case 1.11*0.66 m.

The insect survival analysis at different development stages was determined by compiling survival tables by the D. K. Varley, D. R. Gradwell and M. P. Hassel method [11].

The qualitative and quantitative of various fodder substances' influence on the larvae development rate and mass growth were studied. The experiment has been conducted in plastic containers of *150x200x100* mm size. We used several fodder substances: coconut flakes (test group 1); vermiculite (test group 2); saw dust (test group 3); oat flakes (test group 4). To create a control group (test group 5), we put larvae in containers without any fodder substance. The temperature and humidity were stable during this experiment: t =28°C, W = 80%.

RESULTS AND DISCUSSION

While developing the *Zophobas morio* potential cultivation methods, the experiments were conducted to find out the optimal conditions for storage and reproduction.

First of all, in order to get a reference position, the natural conditions in a 400 liters terrarium, glued of glass sheet where the researchers placed 1.2 kg *Zophobas morio* larvae, were recreated. The larvae differed in size and weight (ranging from 0.6 to 1 g). With an average

weight of 0.8 g, there were about 1,500 larvae per terrarium.

The container was covered with a 20 cm thick layer of loose soil with a high content of rotten leaves. Rotten logs were put in this fodder substance for the larvae to pupate. The storage temperature and humidity corresponded to the habitat environmental conditions (t = 28-30 °C, W = 80-85%).

The main purpose of creating an experimental terrarium was to study the behavioral characteristics, larvae maturation and pupation timing in natural conditions. Figure 1 shows the *Zophobas morio* population density dynamics graph in the constructed terrarium.

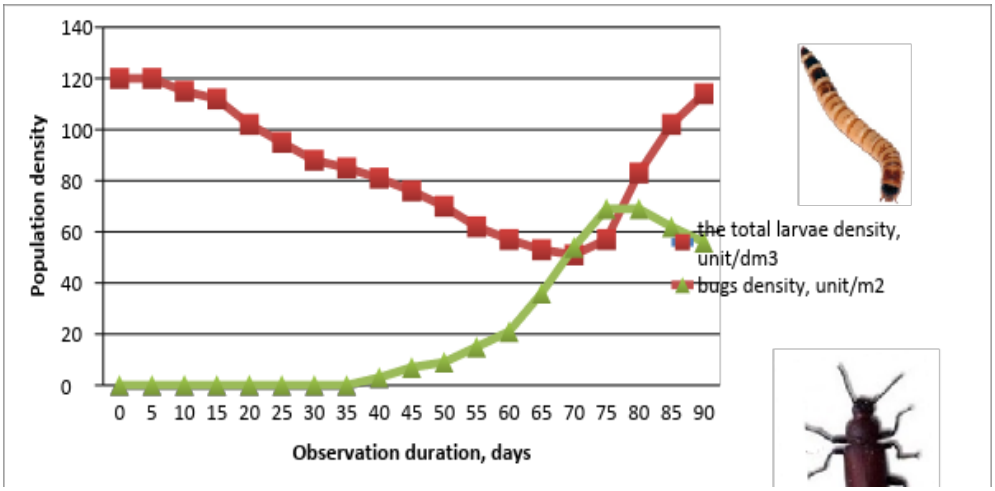

Fig. 1: *Zophobas morio* population density dynamics change in the experimental terrarium.

According to the graph, the larvae density during the placement to the terrarium was equal to 120 units / dm^3. Over time, the number of units per sample began to decrease, which might be connected with the mature larvae isolation and preparation for pupation, or with the survival struggle within the population. It was needed to conduct the population mortality analysis to draw more accurate conclusions.

The first bugs appeared in the terrarium on 35-40 observation days, then their number increased up to 69 units during the next month. However, the further period was characterized by the static growth of the number of adult specimens, and then the fall of their number.

Thus, in conditions close to the natural condition, only a certain larvae quota was able to pupate, and the rest, apparently, did not have enough "snug places".

The adult bugs' number decreased by the end of the experiment which was most likely connected to the struggle within the population. At the same time, since the 70th observation day, there was a spike in the larval population growth. The larvae, however, were much smaller, which indicated the adult bugs' reproduction cycle completion.

Yet, according to the larvae population density change, the growth has reached only 12% of the expected results. Presumably, there was a high competition for the food, females and the best conditions in the terrarium, thus cannibalism arose, especially for the case of the adult beetles and larvae eating the young ones. During the experiment, only less than 5% of specimens have pupated. The specimens' survival rate was also relatively low (table 1).

Table 1: *Zophobas morio* population survival table in the terrarium during the 90-day observation.

Indicators	a_x	m_x	d_x	$\log a_x$	k_x
0-30 days					
Imago	Zero	Zero	–	–	–
Mature larvae	88	32	0.27	2.07	0.13
- infested with parasites	–	6	0.05	–	–
- cannibalism victims	–	26	0.22	–	–
Pupae		9	0.43	1.32	0.24
- cannibalism victims	12	6	0.29	–	–
– anomalies		3	0.14	–	–
30-60days					
Imago	21	Zero	Zero	–	–
Mature larvae	57	31	0.35	1.94	0.19
- infested with parasites		9	0.10	–	–

- cannibalism victims		22	0.25	–	–
Pupae		17	0.31	1.74	0.17
- cannibalism victims	38	6	0.11	–	–
– anomalies		11	0.20	–	–
60-90 days					
Imago	56	13	0.23	1.32	-0.43
Mature larvae	114	34	0.23	2.07	0.36
- infested with parasites		8	0.05	–	–
- cannibalism victims		26	0.18	–	–
Pupae		15	0.41	1.32	0.24
- cannibalism victims	22	8	0.22	–	–
– anomalies		7	0.19	–	–

a_x - the number of survived specimens

m_x- the number of dead specimens at this stage

d_x - the mortality (the percentage of specimens, died at this stage)

k_x - mortality rate ($loga_x - logb_x$).

$loga_x$ - logarithm of the units number at the beginning of the development stage

$logb_x$- logarithm of the units number at the end of the development stage

Thus, dividing the three-month observation period into three equal segments, the larvae, bugs and pupae mortality rates were analyzed. It is worth to mention, that the larvae and pupae mortality was determined by cannibalism (mostly) and by infestation with parasites. Certain deviations, discovered during pupae research were generalized and indicated as the anomalies in the table. Anomalies caused irregular-shaped bugs appearance. In some cases, the pupa could not completely shed its cover (figure 2, A), which was associated with the low humidity and the chitinous cover adhesion to the newly appearing bug tissues. As a result, the adult specimen was unable to reproduce, and the life span was reduced to a month.

A B C

Fig. 2: fixed anomalies detected during the transition of the pupa to the imago.

It was also found that young bugs, which did not yet have the chitin cover, had not come out until they became stronger; and during this period, they could eat pupae. But, they ate only a small area of the pupae. As a result, crippled bugs appeared (figure 2, B). Such bugs lived for a long time, but were not perceived as full-fledged specimens in the population. The third anomaly type was caused by the pupa mechanical damage. As a result, a bug with no natural symmetry appeared (figure 2, C). This type of beetle was also unable to reproduce.

According to the calculations in table 1, the larvae and pupae mortality rate was stable and quite high, throughout the whole period, and the mortality rate was peaked by the third reporting period. The natural mortality was also observed in the terrarium, and most likely it was caused by the achievement of the maximum age of the larva without the possibility of pupation, which was exactly 3 months. Bugs' mortality was caused by the males struggle within the population and mature specimens cannibalism against the young bugs with weak chitin cover. Thus, the population which was initially placed, had the density of 1 500 individuals per 200 liters, significantly decreased in quantity and were stabilized at the density value (51 unit/dm^3 larvae and 69 unit/m^2 beetles). A further spike in the population was caused by a new cycle. The demographic balance in the population indicated the ineffectiveness of the natural *Zophobas morio* cultivation in the static boxes without human intervention. Thus, the cultivation of *Zophobas morio* larvae in terrariums with conditions close to natural, was not attractive from the industrial perspective.

According to the scientific literature, *Zophobas morio* are omnivores, as they are able to recombine fodder mass and synthesize all amino acids. They are also capable of feeding on depleted homogeneous fodder substances with no time

limitation. Such a range of capabilities is caused by *Zophobas morio* symbiosis with the nitrogen-fixing bacteria, due to which, the larvae have acquired the bioconversion ability. The dependency between the fodder type and the *Zophobas morio* development speed and mass gaining has become of an utmost scientific and applied interest.

Therefore, 4 experimental larvae groups have been given different fodder. Since the project's task was the low-cost fodder acquisition, and it was aimed to feed the insects with the cheapest material, so the food production waste was chosen.

The meat-processing waste was used as the fodder substance for the first group; fruit and vegetable processing waste - for the second; grain processing waste - for the third; and the sunflower waste - for the fourth. In the course of studies, the larvae development rate (in days) and the mass change (in milligrams) were recorded.

Table 2 presents the results of studying the dependency between the fodder substance type and the *Zophobas morio* development rate. The development cycle has been divided into 5 stages: I – larva; II – nymph (pre-pupal); III – pupa; IV – subadult (bug without chitinous cover); V – adult (imago).

Table 2: results of studying the dependency between the fodder substance type and the *Zophobas morio* development rate.

Group	Stage duration, days				
					
1	44±3	9±2	15±3	5±1	90+
2	51±3	7±2	16±2	7±2	90+
3	62±5	9±1	21±4	4±1	90+
4	53±5	6±1	14±1	6±1	90+

It was found out that the fastest larva-bug cycle for the *Zophobas morio* larvae (73 days) was possible when the animal origin fodder substance was used. It happened as the amino acids digestion made it possible not to synthesize them via recombination. The fruit and vegetables group was developed for 81 days and the grain group - for 79, which was correspondingly 8% and 10% longer than the meat group. As for the sunflower waste group, the development cycle was 96 days long.

Thus, it was concluded that the preferred fodder substance for the *Zophobas morio* was the meat waste.

Yet, the most cost-effective solution remained the fruit-and-vegetables and the grain waste, since it was incomparably cheaper. It was confirmed in the larvae test groups biomass change dynamics analysis (Table 3).

Table 3: *Zophobas morio* biomass change dynamics analysis research results

Experiment duration, days	Average larvea mass, g			
	Group 1	Group 2	Group 3	Group 4
Zero	0.55	0.59	0.51	0.63
10	0.64	0.66	0.59	0.71
20	0.76	0.77	0.68	0.78
30	0.88	0.85	0.79	0.83
40	0.97	0.91	0.87	0.88
50	1.12	0.98	0.96	0.93
60	1.17	1.09	1.12	1.01
70	1.23	1.15	1.16	1.08
80	1.29	1.22	1.22	1.12
90	1.34	1.28	1.29	1.18

According to the results, the fastest larvae biomass increase (60% increase during the first month, 144% increase during 3 months) occurred while using meat-based fodder substance. When using the grain-based waste, the biomass increase amounted up to 142% (during 3 months); for fruit-and-vegetables waste - 117% increase. As for the 90 days period, the biomass increase for the grain-based waste fodder equaled to 87%. Thus, the data acquired from studying the dependency between the

fodder substance type and the larvae biomass change went along with *Zophobas morio* development rate study in the corresponding groups.

CONCLUSION

The experement revealed that the *Zophobas morio* larvae utilized the dry feed ineffectively, with complications. That is why there was a need to moisten it artificially. However, the increased humidity often caused fodder substance infection (malignant bacterium, fungus, pest insects such as treehopper or mites), which has been a big issue, as it could cause lethal effects. To prevent the potential hazard, a technical solution was needed, i.e., something to sterilize fodder substance before using it for the cultivation.

The observation showed that to intensify the pupation process and ensure the specimen's individualization, it was vital to place it to a solitary chamber of a certain size. The chamber size was determined by the adult larvae geometrical size and the self-gnawed in the log pupation chambers measurements. At the same time, the scientific and practical interest implied finding the optimal fodder substance for the best larvae survival rate and the least larvae pupation time.

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