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Development of culture protocols for the production of *zophobas morio* (fabricius, 1776) (Coleoptera: Tenebrionidae), an alternate source of protein for ornamental fishes under laboratory conditions

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Abstract
The ongoing interest of the public in pets has increased the need for animal protein to rise in the coming years. The majority of the expenditure in fish farming was occupied by food. Aquarists are trying different types of feed to meet their fish protein requirements at a low price. They are spending more than they are worth to reach their protein requirements in the feed, and not even getting a good protein food according to the requirements of the fish. In this context, insects could play an essential role in the establishment of a sustainable food system because they can convert low-value organic waste into nutritious food with minimal environmental impact. They also serve pesticide-free, chemical-free food. *Zophobas morio* is such an insect which has high quantities of protein, minerals and vitamins. The aim of the present work is to standardize the culture practices of the super worm *Z. morio* (Fabricius 1776) under captive conditions so that these protocols can be popularized among ornamental fish farmers for its culture and commercial production. *Z. morio* comes under the family Tenebrionidae of the order Coleoptera of class Insecta. In the present study, 200 larvae of super worm were purchased from market. They were reared in the laboratory and isolated to pupate, and developed into adult beetle. These beetles were made to breed under captivity. The eggs hatched out into larvae and these larvae were reared in the same conditions and further developed into beetle thus completing the life cycle. The larvae were harvested for feeding ornamental fishes. Thus, the culture protocols of super worm under captive conditions were standardized. This insect can be quickly multiplied in large numbers in a short period of time with relatively few culture procedures. It can replace the major packet feed of high price. This protein-rich feed plays a major role in the rate of hatching especially in the larger breeds like Oscar, Arowana and Flower horn. Following this standardized procedure, the commercial production of super worm can be developed into a low cost industry by housewives and unemployed youth, thus earning an additional source of income to the family.

Keywords: *Zophobas morio,* fish feed, captive breeding

1. Introduction
Insects could play an essential role in the establishment of a sustainable food system because they can convert low-value organic waste into nutritious food with minimal environmental impact [2, 3]. They also serve pesticide-free, chemical-free food [19]. As the global population grows, the demand for protein in food also increases; however, animal protein alone will not be able to meet the demands of the global population in the coming years, so including insect protein as an alternative to animal protein may be a better solution to meet the protein needs of both humans and animals. These insects can be quickly multiplied in big numbers in a short period of time with relatively few culture procedures.
Zophobas morio is such a species of darkling beetle whose larvae are known by the common name “super worm”. The super worms have the capacity to even fulfil the protein requirements of humans. *Z. morio* has high quantities of protein, and these are gradually turning into an alternative protein source in a fish feed with a cheaper price for the larger fish that can be fed in different ways, such as in live condition, frozen condition, or by drying them.

They can also be made into a fine powder and given to the fry of the fish, or they can be chopped according to the mouth size of the fish [4].

The easy culture methods in a short space and low cost of feeding the larvae produce a larger number in a short period, making the super worms more recommended to the aquarists who use them as the life feed for ornamental fish. The soft exoskeleton and fat, which can be easily digestible, are added advantages of the super worm that make it suitable to feed in live condition to fish or reptiles.

In the case of brooders, the protein-rich feed plays a major role in the rate of hatching in the larger breeds like Oscar, arowana and Flower horn because of the protein requirements in the feed for their fish. The aquarists are spending more than they are worth to reach their protein requirements in the feed, which are sometimes more difficult, and not even getting a good protein according to the requirements of the fish's body. Hence, this insect can remove all these difficulties because of their nutritional value and low maintenance. The majority of the expenditure in fish breeding was occupied by food, and aquarists are also trying different types of feed to meet their fish protein requirements at a low price and struggling to meet their goals [6-8]. Knowing the culture techniques of super worms will nourish the aquarists to not only feed their fish but also obtain a good income by selling the super worms because the super worms are not only fed to the fish but are also very much liked by the reptiles and birds.

With less manpower and less time, a housewife or children alone can handle the culture and rearing the super worms is one of the major advantages of the culture's practises. The super worms can be fed with vegetables like potatoes, tomatoes, carrots, etc. that have water content; they can also be fed with leafy vegetables. They can be transported to any place easily and can stand without food for many days; hence, exporting is made easy by being in a life condition that requires feeding during travels or very little compared to others in the stage of rearing larvae, which can leave a sufficient amount of food in a single day or until it is completed.

The work is mainly aimed to standardize the culture practises of super worms under captive conditions and to transfer the technology to the ornamental fish farmers for their utilization.

2. Materials and methods

2.1 The taxonomic status of super worm is as follows:

Kingdom	**Animalia**
Phylum	Arthropoda
Class	Insecta
Order	Coleoptera
Family	Tenebrionidae
Genus	*Zophobas* Fabricius1776
Species	*Z. morio*

2.1.1 Nutritional composition (%) and energy content (Kcal/100g), [16] based on dry matter

Protein	43.13-46.79
Fat	40.80-42.04
Fiber	9.26-13.00
NlFE(Carbohydrates)	2.61
Energy content (Kcal/100g)	575.53

2.1.2 Vitamin composition based on dry matter

VitaminA	29.16mcg
VitaminE	18.29-32IU/kg
Vitamin B2	1.78 mg
Vitamin C	2.85 mg
Vitamin B3	7.67 mg
Vitamin B5	4.61 mg
Vitamin B7	83.14 mg
VitaminB9	0.16 mg
Vitamin B1	0.14 mg

Mineral composition
Calcium-42-120
Potassium-750.59
Magnesium-118.29
Phosphorus-562.95
Sodium-112.83
Iron-3.92-5.03
Zinc-7.29-8.75
Manganese-0.15-1.02
Copper-0.86
Selenium-0.03
Amino acid content (mg/g protein):Valine-52.3
Arginine-48.7
Histidine-30
Serine-46.7
Proline-54.8
Alanine-72.6
Glycine-48.2
Glutamic Acid-122.8
Met+Cys-18.3
Isoleucine-47
Leucine-97
Lysine-52.3
Phenylalanine-34.5
Methionine-10.7
Cysteine-7.6
Phenylalanine+Tyrosine-31Threonine-39.6
Tryptophan-9.1

2.2 Materials required for breeding

1. Oats.
2. Rectangular container (transparent plastic) withlid24×18×8inchesforlarvae.
3. Small cup (optional to maintain humidity)
4. Gloves for handling or picking dead Larvae or unwanted materials.
5. Card board egg trays for beetles as hides.
6. Wooden fork to mix or to check the bottom.
7. Corrugated sheets for egg deposit.
8. Rubber bands for making corrugated sheet bundles.
9. Roundsmallplasticcontainer1.5×1.5×1.5(L×W×H)for isolation.
10. Rectangular container (transparent plastic) with lid 24×18×8 inches for beetles.
11. Round small plastic container 1.5×1.5×1.5 (L×W×H) for keeping pupa.
12. Knife to chop vegetable.
13. Racks to place the containers to reduce the occupancy of place.
14. Dust pan for winnowing.
15. Paper to place high water content food.
16. Four extra empty rectangular containers for future use

2.3 Methods

2.3.1 Procurement of larvae

200 larvae of live *Z. Morio* were procured from market. They consisted of both males and females. They were brought to the laboratory of Aquaculture Department. They were kept in beds of oatmeal contained in rectangular, round or square shaped plastic containers. Oat bed with a height of 1.5inch is provided so larvae are kept hidden from others. After setting the bed, the containers are placed in an area that has proper ventilation, temperature, and light. Optimum temperature of the room was 25-28°C. They were fed with tomato's and potato's once a day. The left-over feed was removed to prevent the entry of any mites or parasites. Temperature ranging between 25 and 28°C, with an average relative humidity of 70% was usually employed for *Z.morio* cultivation. The number and duration of larval stage depend on whether larvae are maintained under isolated or grouped conditions. During this stage they can be harvested for feeding fishes. They will further develop to a pupa stage only if they are allowed to become isolated. If the larvae are kept in crowded conditions, they fail to pupate.

The worms that are placed in the rectangular container were monitored for their cannibalism behaviour. The dead worms, if any, were removed from the container. Adequate feed and moisture were provided to prevent cannibalism. Feed like tomato that has a high water content may make the oat bed wet which leads to the formation of mould. This causes early spoilage of the bed which is harmful to the larvae. Hence the feed is placed on a sheet of paper so the excess water is absorbed and the bed is protected from the wet spoilage. Air passage is allowed through air holes of the top covering so that the bed is kept dry.

2.3.2 Prepupation and pupation

To continue the cycle for further development, the mature larvae are allowed to pupate. They are selected according to size or weight. Two inch sized larvae having a weight of 1gm are ideal for pupation. At the eighth day larvae becomes a C-shaped curve, and that is the indication that the larvae are going to become a pupa. The larvae are isolated and kept in a separate container. The isolation container is 1½" height and 1½"width with perforated top for air passage so that they are allowed to get enough oxygen for breathing. During this time they are not given any food. These containers are kept in dark place to avoid any disturbance until the larvae become pupa. It takes about 6 to 8 days for the larvae to form C-shaped body indicating that the larva is about to pupate. The containers are checked every day for any death of larvae. Dead larva, if any, is removed immediately. No feed is given during this stage. The duration of the pupa stage is 16-18 days at 28°C.After 18 days the pupa is completely turned into the beetle.

3. Results and discussion

Z. morio could be successfully bred under captive conditions of the hatchery. The results of its life cycle is described as below. During breeding, eggs were deposited in the corrugated sheet in a row. Ideal ambient temperature for laying eggs and hatching of eggs is 26°C-28°C. Each female beetle laid about 500-600 eggs during its lifespan. The eggs hatched out after four days. Rectangular box of size 18×4×8cm (L×H×W) is used for the rearing. Oat meal bed of 1.5inch thickness is used for the rearing of larvae. Sliced potato, tomato, maize, and carrot are given as feed during this time. After emerging from the egg, the larvae differed in size and weight (ranging from 0.250 to 0.430 g). During the period of 45 days. With an average weight of 0.3 gm the container was covered with a 5 cm thick layer of oatmeal. The storage temperature and humidity corresponded to the habitat environmental conditions, no mortality could be noted. After the 45th to 80th day, the larvae showed rapid growth in the container that had a low density of larvae. It also had a high yield of 90% after 70 days. The larvae attained a weight of 0.750-0.800 mg whereas in the container having high density exhibited very little weight gain. They only attained 0.500 mg. 5% of the worm remained the same, without much increase in weight [18]. Soon after the puapa stage the larva developed into the beetle stage [1]. The different stages are given in table 1 and the photos are given in Plate 1.

On the first day of hatching the beetle looked white. On the second day, the beetle slowly turned to light brown. On the third day, the beetle became dark brown. On the fourth day, the beetle was completely turned to black. Within the eighth day, the beetle is ready for breeding.

Many studies have suggested that super worms are omnivorous and will consume whatever feed supplied, but in the experiment, the super worms did not accept the feeds with low water content. Brinjal was not well received by *Z. morio*, who preferred potatoes over tomatoes among vegetables. They consumed grapes more often than other fruits; however, there was food waste when giving grapes. The super worms are more inclined to ingest fresh sweet corn. Beetles exhibited defence mechanisms by secreting white, milk-like secretions that have a pungent or acidic smell [10]. The culture methods that were followed gave similar results as previous works. The culture and production method done in the hatchery can be utilized for the commercial production of the worm by the farmers. Based on the above, the utilization of *Z. morio* as an alternative nutrient and protein source holds promises for the future.

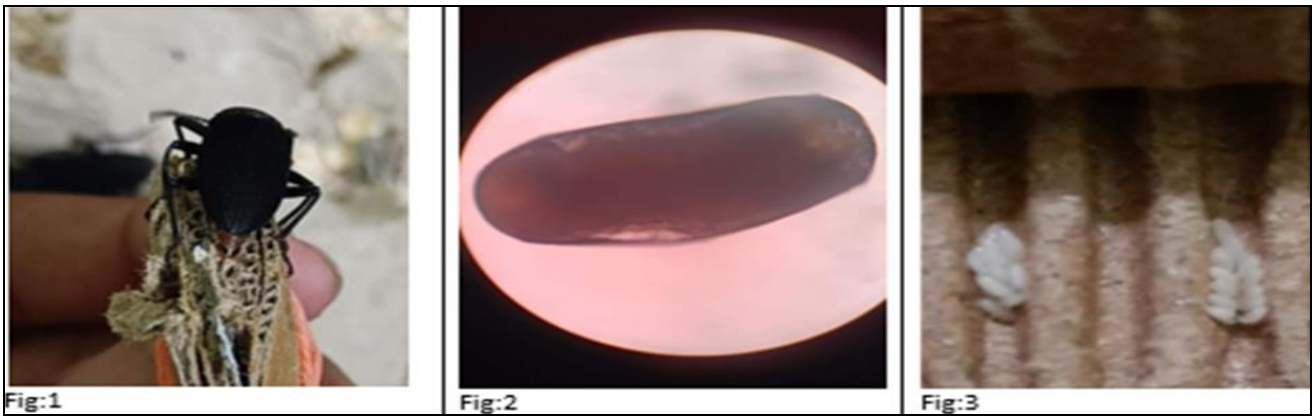

Fig:1 Fig:2 Fig:3


Fig:4


Fig:5


Fig:6


Fig:7


Fig:8

Fig:9


Fig:10


Fig:11

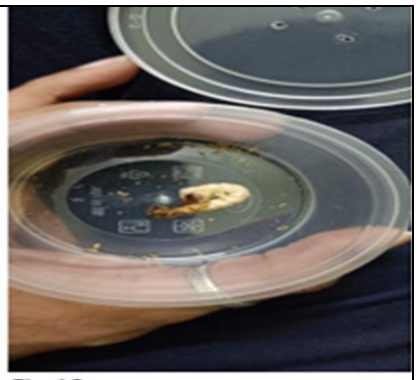
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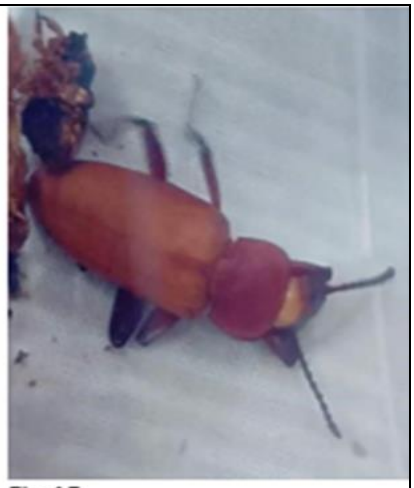
Fig:13


Fig:14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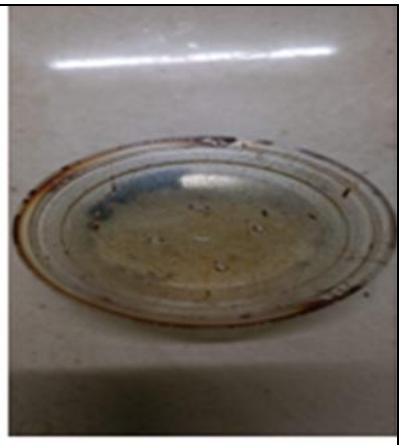

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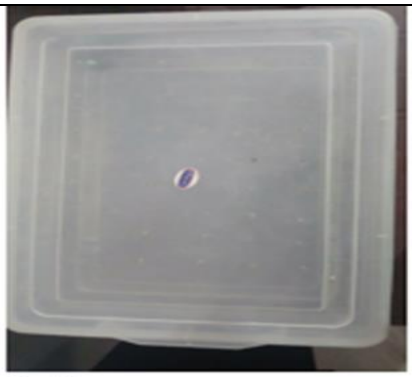

Fig:26

Fig:27

SL. No.	Figure Explanation
1.	A female beetle lays eggs on a corrugated sheet
2.	Egg view under a simple microscope
3.	A row of eggs on a corrugated sheet
4.	Four day old larva under a simple microscope
5.	Five days old larva moving on a corrugated sheet
6.	Larva showing a brown exoskeleton on 8th day
7.	Shedding of exoskeleton after 24 hours
8.	Mature larva 110 years old
9.	Weight of larva on a digital balance
10.	Isolated larva ready to convert into 'C' shaped larva on 4th day
11.	'C' shaped larva ready to pupate on 8th day of isolation
12.	The pupa sheds its exoskeleton on 15th day of isolation
13.	Pupa stage is completed on 16th day of isolation
14.	Just hatched out beetle on day 1, after 20 days of isolating the larva
15.	Semi adult Beetle day 2
16.	Mature adult day 3
17.	Ongoing breeding process.
18.	Irregular shaped bugs due to less humidity
19.	Dead worms in the process of pupation due to less humidity or underweight
20.	A completely spoiled worm
21.	Maggots obtained from a spoiled worm
22.	Mites view under simple microscope
23.	Wood husk bed for larval rearing
24.	Oatmeal bed for beetle rearing
25.	Round plastic container with closed lid
26.	Rectangular plastic container for larvae or beetles
27.	Superworms – larvae for feeding fishes.

Plate 1: Life cycle of *Zophobas morio*

4. Conclusion

Majority of the expenditure in fish breeding and rearing was occupied by food. Aquarists are also trying different types of feed to meet their fish protein requirements at a low price and struggling to meet their goals. Knowledge about the culture techniques of super worms will nourish the aquarists to not only feed their fish but also obtain a good income by selling the super worms because the super worms are not only fed to the fish but are also very much liked by the reptiles and birds.

The culture technique of *Z. morio* under captive conditions of the laboratory is standardized. The larvae can be commercially produced using this method. The major advantages of the super worm culture are less manpower and less time consumption. A housewife or children in the home can alone handle the culture. The super worms can be fed with vegetables like potatoes, tomatoes, carrots, etc. that have water content; they can also be fed with leafy vegetables. They can be transported to any place easily and can stand without food for many days; hence, exporting is made easy by being in a life condition that requires feeding during travels or very little compared to others in the stage of rearing larvae, which can leave a sufficient amount of food in a single day or until it is completed

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