

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(5): 741-745
www.biochemjournal.com
Received: 01-03-2026
Accepted: 04-04-2026

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Preparation and evaluation of artificial diets for mass rearing of girdle beetle of soybean [*Glycine max* (L.) Merrill]

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i5i.8520>

Abstract

The present experiment entitled “Preparation and evaluation of artificial diets for mass rearing of girdle beetle of soybean [*Glycine max* (L.) Merrill].” was carried out at the laboratory of the Department of Entomology, ICAR-National Soybean Research Institute, Indore, Madhya Pradesh (India) during Kharif season of 2023-24. The evaluation of twenty artificial diets of girdle beetle, *Obereopsis brevis* (Gahan, 1894) were based on four biological parameters viz. larval instars, larval period, larval weight and survival percent. These criteria have an important bearing in selecting suitable artificial diets for the mass rearing of girdle beetle, *Obereopsis brevis* as much as the effect of replacement of principal base ingredients is expected to reflect significantly on these aspects of the life cycle. *Obereopsis brevis* reared on different artificial diets revealed that, larvae completed all five instars in the shortest amount of time (4.83 ± 0.76 , 5.08 ± 0.87 , 4.91 ± 0.62 , 5.16 ± 1.04 , and 5.25 ± 0.90 days) in Ist, IInd, IIIrd, IVth and Vth larval instars, respectively were recorded in diet-1. This was followed by diet-17, diet-2, diet-20, diet-11 and these were superior over rest of the diets. The greatest larval time of (60.66 ± 2.08 days) was obtained in diet-1. While maximum larval weight of (0.012 ± 0.003 , 0.016 ± 0.001 , 0.019 ± 0.001 , 0.023 ± 0.002 and 0.026 ± 0.003 gm) in Ist, IInd, IIIrd, IVth and Vth larval instars, respectively recorded in diet-2. The maximum larval survival percent (66.66) was recorded in diet-1, which was on par with diet-2, diet-17, diet-20, diet-11 and these were superior over rest of the diets.

Keywords: Artificial diet, diet evaluation, diet preparation, mass rearing, *Obereopsis brevis*

Introduction

Soybean [*Glycine max* (L.) Merrill] belongs to the family Fabaceae, one of the most important economic oilseed crops and recognized as the most important grain legume in the world, in terms of total production and international trade. It is considered “Wonder Crop” and “Golden bean” of the 20th century and “Miracle Crop” of the 21st century owing to its good quality vegetable protein and edible oil. It is an excellent source of protein (40%) and edible oil (20%), carbohydrates (30%) and fibre (5%). Soybean oil contains about lecithin (0.5-1.0%) which is essential for building up of human nerve tissues. Due to high protein content, soybean is known as “poor man’s meat”. It is a native of China and was introduced in India subcontinent about 1000 AD (Hymowitz and Kaizuma, 1981) [5]. The global soybean production reached 385.524 million metric tonnes in the year (2021-22), with Brazil leading (144 MMT), followed by the USA (119.88 MMT), Argentina (52 MMT), China (19 MMT) and India (11.2 MMT), (Anonymous, 2024). In the 2023-24, India cultivated soybean in 13.26 million hectares area, with a productivity of 985 kg/ha and a total production of 13.06 million tonnes. Madhya Pradesh is known as “Soya State”-and contributes significantly to soybean production in the country with 5.749 MMT productions during 2022-23 from 5.97 Mha (Khound *et al.*, 2024) [6]. In short period of time soybean become a, major Kharif crop in Madhya Pradesh, Maharashtra, Rajasthan, North Karnataka, etc.

Over the past few years, biotic stresses posed by, insects such as girdle beetle (stem borer), has become a major challenge for soybean production in India. Girdle beetle, is a specific pest of soybean that can reduce yield by (14-42%). It attacks soybean crop in the early stages of development which is vulnerable to damage and plant population can be reduced, during the rainy season, from August to September.

It completes two generations on soybean crops in one season. The female girdle beetle builds two girdles, which can be on stems, branches, or petioles. The egg is laid into the centre hole, and 5% of plants that are affected by the girdle beetle in one meter row length are considered to be ETL level.

Insect rearing is a very important for making and testing of artificial diet for suitability and mass multiplication purpose of insects, as in entomology any kind of experiment needs a lot of insects. Raising insects is essential for many entomological study topics that result in effective pest control. Because plant material must be changed frequently, rearing insects on their natural hosts takes time and requires more laborers. In addition to wasting a great deal of plant material, handling the culture frequently causes a high insect fatality rate. Because of these disadvantages, artificial diets have evolved. Given the extensive bibliography on artificial diets for insects developed in most parts of the world, including India (Singh, 1977) ^[10] and the book "Hand book of insect rearing, " edited by Singh and Moore (1985) ^[11], it is now established fact that insects can be raised on artificial diets.

There are three types of artificial diets based on the substances required: Diets that have chemical formulas for their constituents are referred as chemically defined diets (Vanderzant, 1966) ^[12] or HOLIDIC diets (Dougherty, 1959); Diets made up of crude material which are intended to mimic natural food and presumed to have all the necessary nutrients with indigestible inert material; or Diets containing one or more unrefined plant or animal substance (plant tissue, liver power or extracts, yeast, wheat germ) are referred to as MERIDIC DIETS (Dougherty, 1959) ^[4]. Known as OLIGIDIC DIETS, this diet is used to rear insects in large quantities (Vanderzant, 1974) ^[13].

The main objective of using an artificial diet is to inspect the survivability of an insect beyond the natural diet, under the regular monitoring by providing regular food material for the insect, resulting the insect can complete its life cycle on timely by which the pest management of that concerned insects can be done successfully.

Materials and Methods

Studies on the "Preparation and evaluation of artificial diets for mass rearing of girdle beetle of soybean [*Glycine max* (L.) Merrill]" was carried out in the Entomology Lab, ICAR-National Soybean Research Institute, Indore, (MP), during kharif-2023. The normal temperature and relative humidity was maintained during the course of investigation.

Maintenance of stock culture

Stock culture of the girdle beetle was initiated by collecting the larvae of first instar from the field and rearing them in the glass vials. Twenty artificial diets poured in these glass vials, and larvae released singly in each glass vials. Rearing procedure completed in the laboratory of ICAR-National Soybean Research Institute, Indore. Diet was changed within 3-4 days interval and recorded all concerned observations related to biological parameters of girdle beetle.

Details of Experiment

The present experiment was carried out with 20 treatments (artificial diets) in three replications (R1, R2 and R3) for

each treatment. All ingredients used for preparation of artificial diets are from HIMEDIA Laboratories Pvt. Ltd. and each glass vial contained 0.5 gm of artificial diet. The replication no., type of instars and date of release (larvae), mentioned on glass vials. After release of larvae of girdle beetle in glass vials, those were plugged it with cotton wool. Later diets were changed within 3-4 days. All work was done at Entomology Lab, ICAR-NSRI, Indore. The statistical design used in the experiment was Completely Randomized Design.

Diet preparation

The preparation of artificial diets is an essential prerequisite for mass rearing of the girdle beetle. This has assumed greater significance in view of the shift in emphasis on integrated pest management. Twenty artificial diets were prepared for mass rearing of girdle beetle, using the ingredients like, soybean meal, casein and yeast as protein sources, Sucrose as carbon source, ascorbic acid and formaldehyde as preservatives-whereas methyl-p-hydroxy benzoate served for free microbial medium and agar-agar used as solidifying agent. All ingredients used for preparation of artificial diets were purchased from HIMEDIA Laboratories Pvt. Ltd. All twenty diets were stored at 5-10 °C in freezer.

Diet 1: For preparing the diet ingredients of fraction A [Soybean meal, dried yeast powder], were added in 250 mL of distilled water, heated to boiling point then cooled, stirred well and transferred to blender(mixer) for mix well. Fraction B, containing agar was boiled in 350 mL of distilled water then added to fraction A in blender and mixed finally ingredients of fraction C [Ascorbic acid, thiamine hydrochloride, streptomycin sulphate, formaldehyde, casein, sucrose, folic acid, cholesterol, riboflavin, choline chloride, methyl-p-hydroxyl benzoate], were added to the contents in the blender(mixer) for mixed well. The diet was poured into petri-dish then diet was stored at 5-10 °C in freezer.

Diet 2: For preparing the diet ingredients of fraction A [Soybean meal, dried yeast powder], were added in 200 ml of distilled water, heated to boiling point then cooled, stirred well and transferred to blender(mixer) for mixed well. Fraction B, containing agar was boiled in 250 mL of distilled water then added to fraction A in blender and mixed finally ingredients of fraction C [Pyridoxine, nicotinamide, ascorbic acid, streptomycin sulphate, formaldehyde, casein, fructose, folic acid, cholesterol, riboflavin, choline chloride, sorbitol liquid], were added to the contents in the blender(mixer) for mixed well.

Diet 3: For the preparation of diet, the required amount of casein and cholesterol dissolved in acetone solution. Other ingredients [Casein, sucrose, cholesterol, methyl-p-hydroxy benzoate and agar], except vitamins [Thiamine, riboflavin, nicotinic acid, pyridoxine and folic acid], were mixed thoroughly with pestle and mortar. Agar was boiled in 60 mL of distilled water in saucepan and the ingredient were slowly added, after vitamins were added with required amount of 10% potassium hydroxide. The viscous diet was poured into dry flask, flask plugged with cotton wool wrapped. Diet was again shaken to maintain homogeneity of medium.

Diet 4: Primarily agar was boiled in 800 mL of distilled water in a saucepan. The ingredients [Yeast, casein, methyl-p-hydroxy benzoate, soybean meal, folic acid, sorbitol liquid and sucrose] were mixed thoroughly with pestle and mortar then added the mixture in agar solution then continuous stirring. The viscous diet mixture was poured in well dried flask. Diet was kept in freezer for set properly.

Diet 5: Primarily cholesterol dissolved in acetone. It was mixed properly then added other ingredients [Casein, sucrose, cholesterol, ribonucleic acid and ascorbic acid] of diet and mixed thoroughly with pestle and mortar then vitamin solution [Thiamine, riboflavin, nicotinic acid, pyridoxine, choline chloride, inositol and folic acid], was added and again diet mixed properly then prepared diet poured in Petri dishes. The diet with flowing consistency was obtained which was then stored in freezer at 5-10 °C.

Diet 6: Agar powder was added in 200 mL of distilled water and boiled it, then rest of ingredients [Soybean meal, dried yeast powder, agar-agar, propionic acid, sucrose, fructose], except propionic acid were added in 400 mL of distilled water and it boiled for 5 minute then cooked agar mixture was added to boiling mixture and further boiled for 5 minute with continued stirring then add propionic acid again boil for 3 minute, finally medium poured in equal quantity in sterilized Petri dish.

Diet 7: Agar was boiled in 15 mL of distilled water then added all remaining ingredients [Casein, sucrose, methyl-p-hydroxyl benzoate, cholesterol, thiamine, riboflavin, nicotinic acid, pyridoxine, inositol, choline chloride and folic acid], in 5 mL of distilled water then it poured in agar solution, diet shaken continue with the help of blender(mixer) then diet poured in sterilized Petri dish.

Diet 8: Agar was boiled in 20 mL of distilled water in a saucepan then all the remaining ingredients [Soybean meal, casein, sucrose, fructose, methyl-p-hydroxyl benzoate, cholesterol, yeast powder, thiamine, riboflavin, nicotinic acid, pyridoxine, inositol, choline chloride and folic acid] were homogenized with 15 mL of distilled water and poured in to the saucepan having agar-agar solution. The saucepan was shaken until it attained beading consistency. The diet was poured in to sterilized Petri dishes.

Diet 9: Agar was boiled in 100 mL of distilled water in a saucepan then other ingredients [Yeast powder, casein, Methyl-p-hydroxy benzoate, soybean meal, methionine, histidine, arginine, tryptophan, leusine, threonine, lysine and cysteine] dissolved in 50 ml of distilled water then it added into boiled agar solution, thoroughly continue stirring and diet mixture stirred with the help of conical flask then diet mixture poured in well sterilized Petri plate.

Diet 10: Agar was boiled in 100 mL of distilled water in a saucepan then other ingredients[Yeast powder, casein, Methyl-p-hydroxy benzoate, soybean meal, methionine, histidine, arginine, tryptophan, leusine, threonine, lysine, cystine, thiamine, folic acid, riboflavin, nicotinic acid and pyridoxine] dissolved in 50 mL of distilled water then it added into boiled agar solution, thoroughly continue stirring and diet mixture stirred with the help of conical flask then diet mixture poured in well sterilized petri-dish.

Diet 11: Agar was boiled in 100 mL of distilled water at boiling point then added amino acids[Alanine, arginine, aspartic acid, cystine, glutamic acid, histidine, leucine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine] and vitamins[Choline chloride, folic acid, inositol, p-amino benzoic acid, nicotinic acid, pyridoxine, riboflavin, thiamine], in boiled agar solution then stirred continuously with the help of blender(mixer) and other ingredients[Agar, cholesterol, Methyl-p-hydroxy benzoate and sucrose] were mixed in solution and continue stirring to homogenous solution then poured into sterilized petri-dish.

Diet 12: Primarily casein and cholesterol dissolved in acetone solution then other ingredients [Sucrose, ribonucleic acid, Methyl-p-hydroxy benzoate, agar and potassium hydroxide], except vitamins and amino acids were added and mixed thoroughly with pestle and mortar. Agar was boiled in 50 mL of distilled water then ingredients of diet added slowly after that vitamins [Thiamine, riboflavin, nicotinic acid, pyridoxine, choline chloride, folic acid] and amino acids [Alanine, arginine, aspartic acid, cystine, glutamic acid, histidine, leucine, methionine, phenylalanine, proline, serine, threonine and tryptophan] were mixed with 10% potassium hydroxide, all ingredients mixed together with the help of blender (mixer) then diet poured into Petri-dish.

Diet 13: Agar was boiled in 15 mL of distilled water then all remaining ingredients [Casein, sucrose, Methyl-p-hydroxy benzoate, cholesterol, thiamine, riboflavin, nicotinic acid, pyridoxine, inositol, folic acid, alanine, arginine, aspartic acid, cystine, histidine, leucine, methionine, proline, tryptophan and tyrosine], were homogenized with 5 mL of distilled water and poured into flask having agar solution flask was shaken until it attained beading consistency. Diet poured into sterilized petri-dish.

Diet 14: For preparing the diet ingredients of fraction A [Soybean meal, dried yeast powder], were added in 25 mL of distilled water, heated to boiling point then cooled, stirred well and transferred to blender(mixer) for mixed well then fraction B, containing agar was boiled in 15 mL of distilled water then added to fraction A in blender and mixed finally ingredients of fraction C[thiamine hydrochloride, folic acid, riboflavin, nicotinic acid, pyridoxine, alanine, arginine, aspartic acid, cystine, histidine, methionine], were added to the contents in the blender(mixer) for mixed well. The diet was poured into Petri dish.

Diet 15: Agar was firstly dissolved in 80 mL of distilled water at boiling point and other ingredients [Yeast powder, soybean meal, ascorbic acid, Methyl-p-hydroxy benzoate, sorbic acid], except formaldehyde dissolved in 30 mL of distilled water the hot agar solution was slowly poured into the blender finally formaldehyde was added and the entire mixture was again blended and thoroughly mixed in blender then poured into petri-dishes.

Diet 16: All dry ingredients [Casein, glucose, salt mixture, yeast, choline chloride, agar, Methyl-p-hydroxy benzoate], thoroughly mixed with the help of pestle and mortar then cholesterol dissolved in acetone solvent and added in mixture to its uniform distribution then mixture was poured

in 25 mL of distilled water, continue stirring for the homogenization of the solution.

Diet 17: Agar was boiled in 80 mL of distilled water and other ingredients [Yeast powder, soybean meal, ascorbic acid, Methyl-p-hydroxy benzoate, sorbic acid, thiamine, riboflavin, nicotinic acid, folic acid, pyridoxine, alanine, arginine, aspartic acid, cystine, histidine, methionine], except formaldehyde dissolved in 30 mL of distilled water and blended in blender then added formaldehyde and thoroughly mixed in blender then poured into petri-dish.

Diet 18: All dry ingredients [Casein, glucose, salt mixture, yeast, choline chloride, agar, Methyl-p-hydroxy benzoate, thiamine, riboflavin, nicotinic acid, folic acid, pyridoxine, alanine, arginine, aspartic acid, cystine, histidine, methionine], thoroughly mixed with the help of pestle and mortar then cholesterol dissolved in acetone solvent and added in mixture to its uniform distribution then mixture was poured in 25 mL of distilled water, continue stirring for the homogenization of the solution.

Diet 19: Primarily agar was boiled in 20 mL of distilled water then remaining ingredients [Soybean meal, yeast powder, ascorbic acid, sorbic acid, Methyl-p-hydroxy benzoate and formaldehyde], mixed in blender then cooked agar solution was added in blender mixed thoroughly, cooled, and then prepared diet poured in petri-dish.

Diet 20: Initially agar was boiled in 300 mL of distilled water then mixed in its soybean meal there after other ingredients [Yeast powder, ascorbic acid, formaldehyde, sorbic acid, streptomycin sulphate, Wesson salt mixture and cholesterol], mixed in prepared solution and mixed thoroughly well with the help of blender (mixer), then prepared diet cool for some time and poured in petri-dish.

Diet evaluation

All the twenty diets were evaluated for girdle beetle with the biological parameters viz. Larval instars, larval period,

larval weight and survival percent then biological observations was recorded for the following parameters visually with the help of continuous monitoring and on the bases of these data evaluated the twenty artificial diets and resulting the artificial diet performed better in terms of all the biological parameters of girdle beetle.

Statistical analysis

Completely randomized design with twenty artificial diets with each at three replications was used for "Preparation and evaluation of artificial diets for mass rearing of girdle beetle of soybean. The data was analyzed among the twenty artificial diets and values were expressed as (Mean $\pm$ S.D.).

Results and Discussion

The biological characteristics of the girdle beetle, *Obereopsis brevis*, reared on twenty artificial diets and related observations are provided in table-1 and 2.

Comparative analysis of the growth characteristics:

Obereopsis brevis reared on artificial diets revealed that, larvae completed all five instars in the shortest amount of time (4.83 ± 0.76 , 5.08 ± 0.87 , 4.91 ± 0.62 , 5.16 ± 1.04 , and 5.25 ± 0.90 days) in Ist, IInd, IIIrd, IVth and Vth larval instars, respectively were recorded in diet-1. This was followed by diet-17, diet-2, diet-20 and diet-11. These were superior over rest of the diets. The greatest larval time of (60.66 ± 2.08 days) was obtained in diet-1. The present findings are in agreements with the reports of Patil (2023) [8], who reported that larval period of *O. brevis* about (32-62 days) and Mishra *et al.* (2023) [7], who reported that larval period of *Obereopsis brevis* about (32-65 days), these findings were closely to our present research. While maximum larval weight of (0.012 ± 0.003 , 0.016 ± 0.001 , 0.019 ± 0.001 , 0.023 ± 0.002 and 0.026 ± 0.003 gm) in Ist, IInd, IIIrd, IVth and Vth larval instars, respectively recorded in diet-2. The maximum larval survival percent was (66.66) recorded in diet-1, which was on par with diet-2, diet-17, diet-20 and diet-11 and these were superior over rest of the diets.

Table 1: Growth and survival parameters (Mean $\pm$ SD) of soybean girdle beetle, *Obereopsis brevis* under different diets.

Biological parameters	Diet-1	Diet-2	Diet-3	Diet-4	Diet-5	Diet-6	Diet-7	Diet-8	Diet-9	Diet-10
Larval instars (days)										
1st instar	4.83 ± 0.76	5.58 ± 0.38	6.25 ± 0.25	6.58 ± 0.38	6.25 ± 0.25	6.33 ± 0.76	5.91 ± 0.38	7.25 ± 0.25	6.58 ± 0.38	6.00 ± 0.50
2nd instar	5.08 ± 0.87	5.66 ± 1.04	6.50 ± 1.25	7.25 ± 0.25	6.58 ± 0.38	7.25 ± 0.25	6.00 ± 0.66	6.66 ± 0.76	7.25 ± 0.25	6.83 ± 0.28
3rd instar	4.91 ± 0.62	5.91 ± 0.62	6.08 ± 0.52	6.91 ± 0.38	6.25 ± 0.25	6.58 ± 0.94	6.25 ± 0.25	7.08 ± 0.52	6.83 ± 0.52	6.50 ± 0.50
4th instar	5.16 ± 1.04	5.08 ± 0.52	6.58 ± 0.80	6.75 ± 0.66	6.50 ± 0.50	6.50 ± 1.00	5.58 ± 0.38	6.75 ± 0.66	6.75 ± 0.66	6.16 ± 0.76
5th instar	5.25 ± 0.90	5.41 ± 1.01	6.41 ± 1.01	7.08 ± 0.52	6.50 ± 0.50	6.75 ± 1.08	5.91 ± 0.62	6.58 ± 0.62	7.00 ± 0.66	6.66 ± 0.57
Larval period (days)	60.66 ± 2.08	50.33 ± 2.52	35.66 ± 5.03	34.33 ± 5.13	29.33 ± 1.53	37.66 ± 5.51	34.33 ± 0.58	37.33 ± 2.52	33.66 ± 2.52	37.33 ± 2.08
Larval weight (gm)										
1st instar	0.011 ± 0.0005	0.012 ± 0.003	0.012 ± 0.001	0.012 ± 0.002	0.012 ± 0.001	0.012 ± 0.002	0.011 ± 0.001	0.013 ± 0.0005	0.012 ± 0.0005	0.013 ± 0.001
2nd instar	0.014 ± 0.001	0.016 ± 0.001	0.014 ± 0.002	0.013 ± 0.0005	0.015 ± 0.001	0.014 ± 0.0005	0.014 ± 0.002	0.015 ± 0.0005	0.014 ± 0.0005	0.015 ± 0.002
3rd instar	0.015 ± 0.001	0.019 ± 0.001	0.015 ± 0.0005	0.016 ± 0.002	0.016 ± 0.0005	0.017 ± 0.0005	0.015 ± 0.001	0.017 ± 0.002	0.016 ± 0.0005	0.017 ± 0.001
4th instar	0.018 ± 0.001	0.023 ± 0.002	0.017 ± 0.0005	0.017 ± 0.001	0.018 ± 0.0005	0.018 ± 0.001	0.017 ± 0.0005	0.018 ± 0.002	0.018 ± 0.002	0.018 ± 0.002
5th instar	0.022 ± 0.001	0.026 ± 0.003	0.021 ± 0.002	0.021 ± 0.003	0.022 ± 0.0005	0.022 ± 0.003	0.021 ± 0.0005	0.021 ± 0.001	0.021 ± 0.002	0.022 ± 0.0005
Survival percent	66.66 $\pm$	66.66 $\pm$	33.33 $\pm$	33.33 $\pm$	33.33 $\pm$	33.33 $\pm$	33.33 $\pm$	33.33 $\pm$	33.33 $\pm$	33.33 $\pm$

*(Survival percent = Twenty treatments with each at three replication and single larvae reared in each replication for proper growth and development therefore 66.66% indicates survival of two replication out of three and 33.33% indicates survival of one replication out of three.)

Table 2: Growth and survival parameters (Mean $\pm$ SD) of soybean girdle beetle, *Obereopsis brevis* under different diets.

Biological parameters	Diet-11	Diet-12	Diet-13	Diet-14	Diet-15	Diet-16	Diet-17	Diet-18	Diet-19	Diet-20
Larval instars (days)										
1st instar	6.25 $\pm$ 0.25	7.08 $\pm$ 0.14	6.50 $\pm$ 0.50	7.25 $\pm$ 0.25	8.08 $\pm$ 0.14	6.50 $\pm$ 0.50	5.50 $\pm$ 0.50	7.16 $\pm$ 0.28	7.00 $\pm$ 0.57	5.60 $\pm$ 0.50
2nd instar	6.33 $\pm$ 0.76	7.50 $\pm$ 0.50	6.83 $\pm$ 0.28	7.25 $\pm$ 0.90	7.50 $\pm$ 0.50	7.25 $\pm$ 0.25	5.58 $\pm$ 0.94	7.50 $\pm$ 0.50	7.25 $\pm$ 0.25	6.25 $\pm$ 0.25
3rd instar	6.08 $\pm$ 0.52	7.25 $\pm$ 0.25	6.50 $\pm$ 0.50	7.58 $\pm$ 0.38	7.91 $\pm$ 0.38	6.91 $\pm$ 0.38	5.91 $\pm$ 0.38	7.33 $\pm$ 0.28	6.83 $\pm$ 0.76	5.75 $\pm$ 0.66
4th instar	6.41 $\pm$ 0.52	7.41 $\pm$ 0.52	6.50 $\pm$ 0.50	6.83 $\pm$ 0.52	7.75 $\pm$ 0.66	6.66 $\pm$ 0.76	5.00 $\pm$ 0.50	7.50 $\pm$ 0.50	7.08 $\pm$ 1.01	5.66 $\pm$ 0.76
5th instar	6.25 $\pm$ 0.75	7.58 $\pm$ 0.38	6.50 $\pm$ 0.50	7.16 $\pm$ 0.87	7.58 $\pm$ 0.62	7.08 $\pm$ 0.52	5.41 $\pm$ 0.87	7.66 $\pm$ 0.28	6.91 $\pm$ 0.80	5.91 $\pm$ 0.80
Larval period (days)	47.66 $\pm$ 1.53	38.33 $\pm$ 2.52	30.66 $\pm$ 2.08	37.33 $\pm$ 2.52	39.33 $\pm$ 2.08	37.33 $\pm$ 4.04	53.66 $\pm$ 1.53	37.33 $\pm$ 3.21	38.66 $\pm$ 1.53	49.33 $\pm$ 0.58
Larval weight (gm)										
1st instar	0.011 $\pm$ 0.0005	0.011 $\pm$ 0.0005	0.011 $\pm$ 0.002	0.013 $\pm$ 0.0005	0.012 $\pm$ 0.002	0.011 $\pm$ 0.0005	0.011 $\pm$ 0.0005	0.013 $\pm$ 0.001	0.011 $\pm$ 0.001	0.011 $\pm$ 0.003
2nd instar	0.014 $\pm$ 0.001	0.015 $\pm$ 0.001	0.013 $\pm$ 0.0005	0.015 $\pm$ 0.001	0.014 $\pm$ 0.0005	0.013 $\pm$ 0.0005	0.013 $\pm$ 0.0005	0.014 $\pm$ 0.001	0.014 $\pm$ 0.0005	0.015 $\pm$ 0.002
3rd instar	0.015 $\pm$ 0.0005	0.015 $\pm$ 0.0005	0.015 $\pm$ 0.0005	0.017 $\pm$ 0.002	0.016 $\pm$ 0.001	0.016 $\pm$ 0.001	0.015 $\pm$ 0.0005	0.017 $\pm$ 0.002	0.016 $\pm$ 0.001	0.018 $\pm$ 0.003
4th instar	0.018 $\pm$ 0.001	0.018 $\pm$ 0.0005	0.017 $\pm$ 0.002	0.018 $\pm$ 0.002	0.018 $\pm$ 0.0005	0.018 $\pm$ 0.001	0.017 $\pm$ 0.0005	0.018 $\pm$ 0.0005	0.018 $\pm$ 0.0005	0.021 $\pm$ 0.002
5th instar	0.022 $\pm$ 0.002	0.020 $\pm$ 0.0005	0.021 $\pm$ 0.001	0.020 $\pm$ 0.003	0.021 $\pm$ 0.0005	0.021 $\pm$ 0.003	0.023 $\pm$ 0.002	0.022 $\pm$ 0.003	0.021 $\pm$ 0.003	0.024 $\pm$ 0.001
Survival percent	66.66 $\pm$	33.33 $\pm$	33.33 $\pm$	33.33 $\pm$	33.33 $\pm$	33.33 $\pm$	66.66 $\pm$	33.33 $\pm$	33.33 $\pm$	66.66 $\pm$

In conclusion, we can say that the artificial diet that we prepared in our lab could be utilized to raise *Obereopsis brevis* in large quantities of uniform insects. In-depth research to enhance the current diet and evaluate the respond of larvae under lab environment becomes essential for maintaining field performance comparable to that of a population using this artificial diet. In terms of larval instars, larval period, larval weight, and survival percentage the artificial diet had superior results for the girdle beetle. It was evident from these results that the artificial diet is more effective in raising these insects in huge quantities in a lab environment.

Acknowledgment

"The author expresses sincere gratitude to the Director, ICAR-National Soybean Research Institute, Indore (MP), for providing the Entomology Lab to conduct the experiment, and for their indispensable assistance and support in the successful culmination of this research endeavor."

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