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Artificial Diets and Rearing Techniques of *Helicoverpa armigera* (Hubner)

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SUMMARY

Helicoverpa armigera is a polyphagous lepidopteran pest which completes its life cycle in 4-6 weeks which causes extensive losses. Artificial diet and mass rearing are prerequisites for carrying out studies on insecticide bioassays, evaluation of germplasms and studying various mechanisms and inheritance of resistance to insects. For successful multiplication of *Helicoverpa armigera*, there is necessity for standardizing a diet that supports survival and development of the insects for many generations. The major ingredients used in artificial diet are chickpea flour, wheat germ, sorbic acid, methyl p-hydroxy benzoate, ascorbic acid, casein, streptomycin sulphate, cholestrol, multivitamin solution, formaldehyde, vitamin e, yeast, agar-agar, tapioca granules, water, alcohol and their proportions vary depending on the type of diet being prepared. The facilities, materials required, diet preparation and rearing and handling procedures of the life stages are discussed.

INTRODUCTION

Helicoverpa armigera is a species belonging to order Lepidoptera in the family Noctuidae. It is a polyphagous pest reported to infest 60 cultivated and 67 wild host plants in India. The most prevalent hosts are tomato, cotton, chickpea, pigeon pea, sorghum and cowpea etc. Itis a pest with an immense ability to thrive adverse climatic conditions, with extensive dispersal ability, distinctive survival mechanisms serving its offseason carryover and the ability to resist high doses of recurrent insecticidal exposures. Depending on the crop, it can cause a damage of 50 to 90 per cent yield loss thereby causing a huge monetary loss.

Identification

Eggs-single, creamy white in colour which are spherical in shape
Larva-appears greenish to brown in colour and body possessing dark brown grey lines with white lines laterally and also has dark band.
Pupa-brownish in colour and pupates in soil, pods, leaves and crop debris
Adult-female moth is pale brownish yellow stout moth and male is pale green in colour.

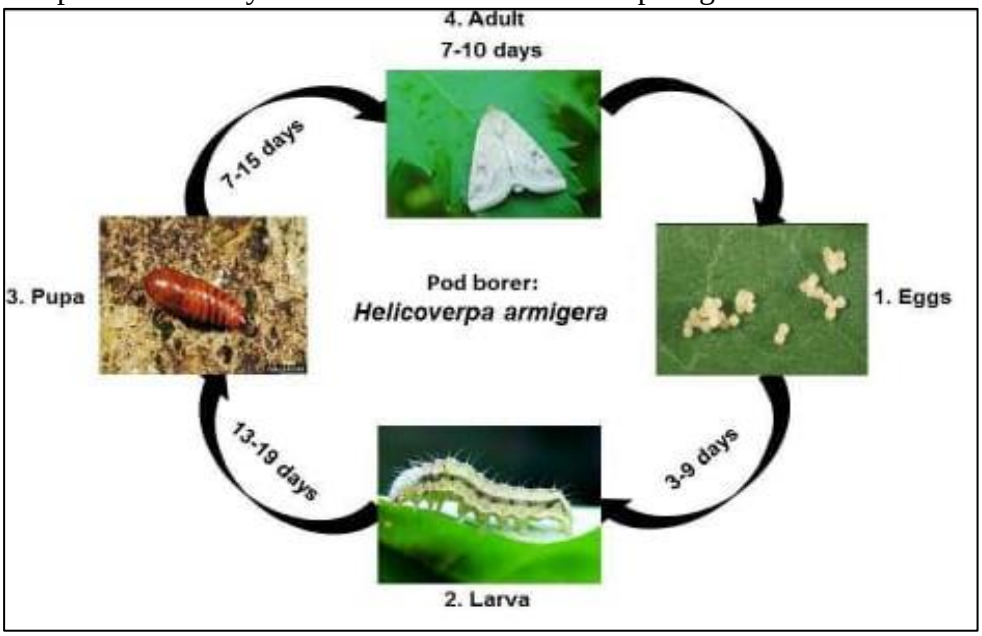

Fig 1. Life cycle of *Helicoverpa armigera*

Rearing of *H. armigera* on artificial rather than on their host plants is advantageous for undertaking studies related to insecticide bioassays, evaluation of germplasms and studying various mechanisms and inheritance of resistance to insects.

Artificial diet for rearing *H.armigera*

Artificial diet is the diet that is not the natural food of the insects. The ingredients of the diets used for rearing *H. armigera* in laboratory conditions are presented in Table 1.

- Boil agar-agar and water and thereafter cool it down to 60˚C.
- Add the agar in to the mixture of other ingredients which should be blended into a homogenous mixture.
- The hot liquid should be dispensed in to multi cavity rearing trays.
- After solidification, the trays should be kept in sterilized rearing unit.

Procedure for mass multiplication of *Helicoverpa armigera*(Devi *et al*., 2020)

Materials Required

- Black cotton cloth as ovipositional substrate.
- Culture trays.
- Semi-synthetic diet, honey, and vitamin syrup.
- Plastic culture vials, plastic bowls with mesh lid.
- Plastic jar for oviposition (3–5 L) and petriplates.
- 0 or 1 numbered camel hair brush, blunt forceps.
- Labels and markers.
- Sodium hypo chlorite solution (0.25 and 1%). 0.25% Sodium hypochlorite foregg sterilization and 1% Sodium hypochlorite for pupal sterilization. Label andstore at room temperature for not more than 1 month.
- 70 percent alcohol for hand and surface sterilization
- RO/ distilled water
- Measuring cylinder (10-1000 ml) and beaker (50-500 ml)
- Plastic trays, filter paper.

Field collection

Collect grown up *H. armigera* larvae from fields during peak occurrence of the pest on its host crops.

Selection of healthy population

The population collected from the fields should be examined and the larvae infected with microsporidia, bacteria and NPV were discarded in the laboratory.

Methodology

Eggs

- Select eggs from screened/disease-free colonies.
- Soak the egg substratum/ cloth containing 1- or 2-day-old eggs in a tray containingsodium hypochlorite solution (0.25%) for about 3 min.After gentle agitation,remove the ovipositional substratum from sodium hypochlorite solution.
- Keep the egg cloth/egg substratum in a box with mesh lid and rinse under runningtap water for 15–20 min.
- Use filter lined with cloth to collect detached eggs.
- Drain the water and place eggs over filter paper for drying (2–3 h).
- After drying, put the egg cloths/papers in a plastic jar and place it in incubator at 25 ± 2 °C until complete hatching.
- Label the jars indicating date of egg collected.

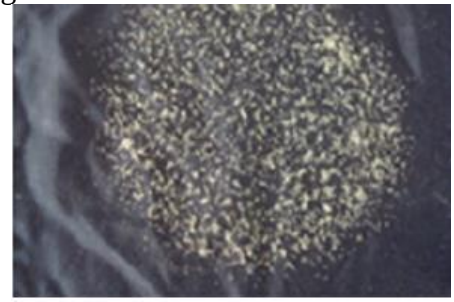

Fig 2. Eggs on black cloth (Devi *et al*., 2020)

Larval Rearing

- Pour semi-synthetic diet into culture trays and culture tubesusing beaker.
- Before transferring the neonates, allow the diet to cool for at least 30 min,.
- Transfer the neonates into each well with sterile camel hair brushesand cover properly.
- Label the trays indicating date of transferring and expecting date of larvae reachingto later instars (fifth instar) and incubate in environmental chamber (25 ± 2 °Cand RH 65 ± 5%).
- Check trays periodically for change in growth and development of larvae, record anychanges in diet, larval mortality, etc.
- Handling laterinstars: After eighth day, transfer active grown up instar larvaeinto culture trays/tubes containing 4–5 mL of semi-synthetic diet and plug tightlyto avoid larval escape.
- Check culture tubes/trays every day for diet consumption by larvae and changeto fresh diet if required until pupation.
- Examine dead larvae for pathogen infection whenever the proportion of larvalmortality exceeds 5%.
- Collect 1-day-old healthy and fully formed pupae from the culture tubes/traysand keep them separately in plastic bowls and cover with mesh lid, keep in isolatedchamber until sterilization.

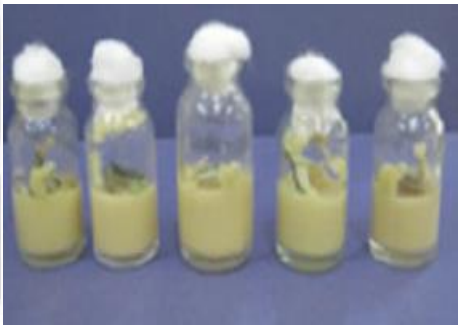

Fig 3. Larvae in rearing tray(Devi *et al*., 2020)**Fig 4.** Larvae in vials (Devi *et al*., 2020)

Pupae Handling

- Select the 4 or 5 days old healthy and fully formed pupae.
- Soak in a bowl containing 1% sodium hypochlorite solution for3–5 min.
- Collect floating pupae and discard.
- Keep selected pupae in a plastic bowl and cover with mesh lid. Rinse under runningwaterfor 15–20 min to remove the hypochlorite residue.
- Drain the water and place pupae over filter paper and dry.
- After complete drying, place in sterilized and labelled pupal bowls for mothemergence (8–10 days).
- If required, sex the pupae according to standard protocol and keep in separatejars for moth emergence.

Fig 5. Pupae (Devi et al., 2020)

Moth Handling

- Select a black cotton cloth as egg substratum and earthen pot as an oviposition jar for *H.armigera.*
- Place moth food inside the oviposition jar in a plastic lid.

- Preparation of moth food—Soak the absorbent cotton in the 10% honeysolution similarly, dip absorbent cotton in RO/distilledwater and place it on another plastic lid. Keep one each of the plastic lid inthe oviposition jars and replace every day till moths exhaust.
- Select female and male moths from pupal bowls (vigorous adults that emergeearly are to be used for continuation of culture).
- Release 10–15 pairs of moths/ovipositional jar. The males are plain greenish andthe females(chocolate brown).
- Cover the mouth of the oviposition jar with black cotton cloth and secured withrubber bands.
- Collect eggs from second day and end on fifth day from each jar and estimatenumber of eggs.
- Keep selected egg sheets in plastic containers with date of collection. Sterilize1-day-old eggs and keep again in the plastic containers till they hatch.
- Examine malformed adults for pathogen infection whenever the proportion ofmalformation exceeds 5%.

Fig 6. Oviposition chamber (Devi *et al*., 2020)

Safety

Follow appropriate personal protective equipment (PPE), Safety glasses, laboratory coats, toe-covered shoes, gloves, face mask, head cover, etc.

Table 1. The ingredients of the diets used for rearing *H. armigera* in laboratory conditions.

Ingredients	Wheat germ diet[2]	Chikpea flour diet[2]	Tapioca granules diet[2]	Tapioca granules agar agar diet [2]	Diet[3]	Chickpea flour modified diet[2]
Chickpea flour	100 g	100 g	100 g	100 g	100g	100 g
Wheat germ	13 g	-	-	-	-	-
Sorbic acid	1.2 g	1.0 g	1.0 g	1.0 g	1.0 g	1.5 g
Methyl p-hydroxy benzoate	2 g	1.5g	1.5 g	1.5 g	2 g	3 g
Ascorbic acid	6 g	1.67 g	1.67 g	1.67 g	3g	4.7 g
Casein	15 g	-	-	-	-	-
Streptomycin sulphate	0.2 g	-	-	-	0.25 g	0.2 g
Cholestrol	0.6 g	-	-	-	-	-
Multivitamin solution (ABDEC drops)	1.5 ml	3.0 ml	3 ml	3 ml	4.0 ml	1.0 ml
Formaldehyde 1 %	1 ml	-	-	-	2.0 ml	10 ml
Vitamin E (400 mg)	1	-	-	-	-	1
Yeast	16 g	16 g	16 g	48 g	30 g	-
Agar-agar	15 g	5.8	-	3.0g	13 g	14 g
Tapioca granules	-	-	40	30	-	-
Water	825 ml	400 ml	400 ml	400 ml	800 ml	700 ml
Alcohol	-	-	-	-	2.0 ml	-
Bavistin	-	-	-	-	2.0 g	-
Wessons salt	-	-	-	-	7g	-

CONCLUSION

Laboratory rearing of insects has made it possible to study not only their nutrition but also their biochemistry, behaviour, and other biological processes.Mass production of insects has provided a large supply for studies in methods of control of pests, release of parasites, predators, and viruses, release of sexually sterile insects andidentification of attractants, hormones, and other biologically active compounds that occur in such trace amounts that large numbers of insects are needed for study.

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