Modified diet assay for studying feeding RNAi in cotton leafhopper, Amrasca biguttula biguttula

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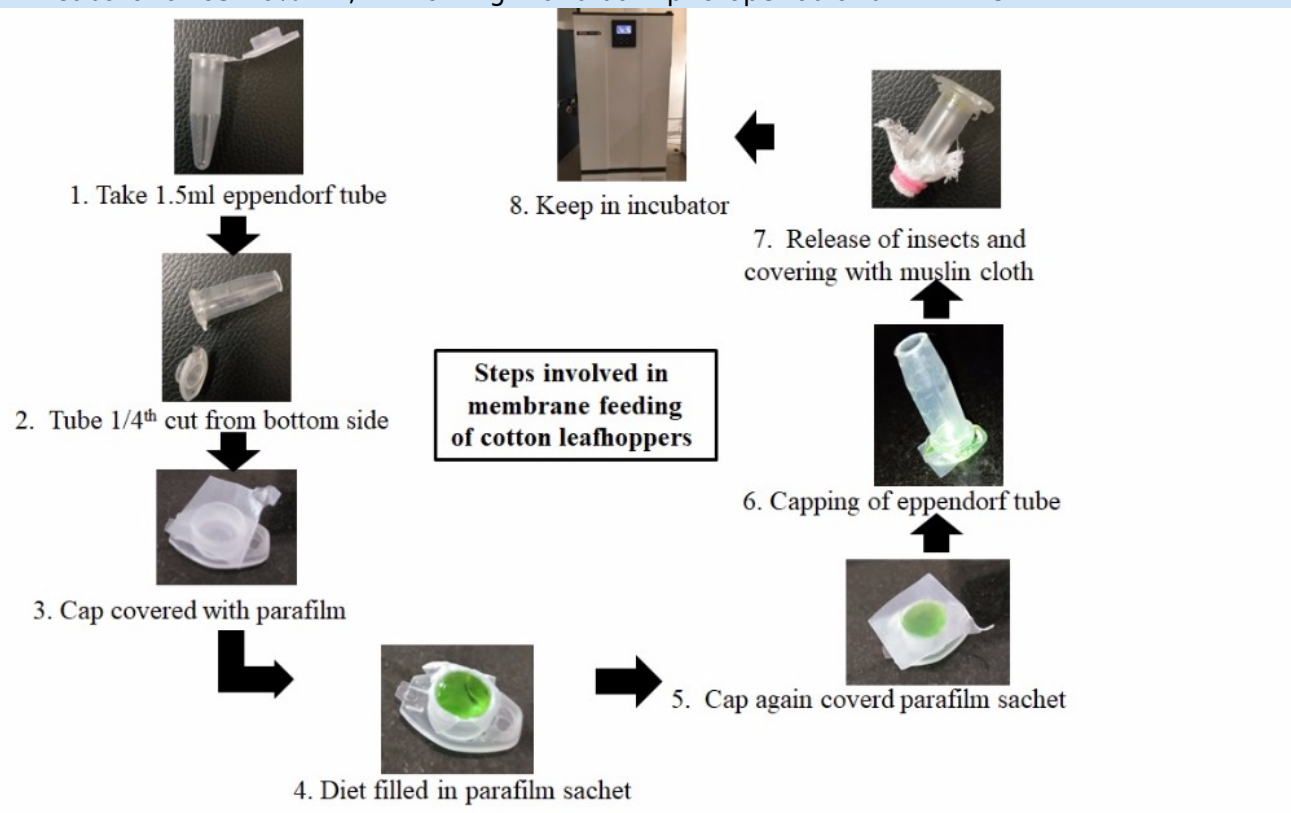
Abstract

Amrasca biguttula biguttula (Ishida) commonly known as cotton leafhopper is a serious pest of cotton and okra. Nutritional requirements or growth physiology for this insect has not been much known till date. We developed artificial membrane feeding method for leafhopper which can be further used for RNA interference studies. Main components of this diet were L-cysteine (5.0 mg), glycine (2.0 mg), nicotinic acid (1.0 mg), sucrose (500 mg), K_2HPO_4(50 mg), $ZnCl_2$ (0.04 mg), Thiamine HCl (0.25 mg), Vitamin B6 (0.25 mg), Becosules capsule powder (Pfizer Limited, USA) (2.0 mg), Green food dye (GanpatiSyn Food Colour, India) (2.0 mg) per 10ml of autoclaved double distilled water. To make diet accessible to the cotton leafhoppers the 1.5 ml of Microcentrifuge tube's (MCT) cap (Tarsons Products Pvt. Ltd. India) was removed and diet was filled in 2 layers of parafilm sachet (diet was poured on first layer of parafilm on cap and covered with another layer of stretched parafilm) into cap. The MCT's was cut 1/4th from bottom end, which was covered with muslin cloth (40 count mesh size). This MCTs with leafhopper was kept in incubator at 65-70% RH, 14:10 h light and dark photoperiod and 27± 2°C.



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Guidelines

1. Carefully dispense the diet into the parafilm sachet so that there should not be any bubble formation.
2. Always use sterile knife to cut eppendorf tube.
3. Muslin cloth of optimum size is cut to cover the opening of MCT so that it does not form any fold.
4. Upright position of eppendorf tube with diet side facing upwards must be considered.
5. Diet should be sterilized using filter sterilization (0.22 µm MCE hydrophilic membrane HIMEDIA) method to remove any bacterial contamination.
6. The diet after filter sterilization, should be kept at 70°C for 15 minutes for dsRNAses deactivation,
7. Do not release more than six leafhoppers in one tube to avoid congestion.
8. The diet should be regularly checked for any bacterial/fungal contamination before use.

Before start

1. All apparatus (parafilm, knife, brush, forceps, scalpel, scissors, gloves) should be treated with DEPC solution prior autoclaving.
2. All apparatus (parafilm, knife, brush, forceps, scalpel, scissors, gloves) should be autoclaved before use.
3. Swab work bench with 75% ethanol

Protocol

Warnings

1. Use sharp devices (Knife, scalpel, scissors, etc) carefully to avoid injury