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Rearing Protocol of Whitefly, *Bemisia tabaci Gennadius* (*Hemiptera: Aleyrodidae*)

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SUMMARY

Bemisia tabaci is a plant sap-sucking insect in the family Aleyrodidae. *B. tabaci* is a species complex that cannot be distinguished morphologically. As increasing damage to crops *by B. tabaci* and control of this pest by use of any systematic insecticide leads to resurgence and resistance along with a severe threat to non-target organisms. So there is a need for biological control of *B tabaci* which interns require a natural enemy to control. So this is very inevitable to have a proper standard mass rearing technique. As of now, there is no such specified artificial diet to mass-produce *B. tabaci*, instead, we can go for the rearing of whitefly on its naturally preferred hosts like brinjal, tomato, etc in a laboratory under controlled conditions. Thus the right choice of the host plant, proper abiotic conditions, with adequate whitefly population has an inoculum we can mass rare whitefly easily. Upon establishment of whitefly populations, natural enemies can be mass-produced and hence can be applied for natural control of the pest.

INTRODUCTION

Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae) is ranked 14th among the world's 100 worst invasive species. *B. tabaci*, one of the 1556 whitefly species classified into 161 genera, is the most important for agriculture since it may infest over 1000 plant species, including food, fiber, and ornamental crops. Whitefly infestations generate chlorotic leaf patches and the growth of sooty mould due to honeydew excretion. It can transmit over 300 plant pathogenic viruses in addition to causing direct damage to crops. Begomovirus, Carlavirus, Crinivirus, Ipomovirus, and Torradovirus are the most common plant viruses spread by whiteflies. Adult females lay oval to ellipsoidal whitish eggs on the adaxial surface of the leaf, which is anchored by a pedicel. As the eggs mature, they turn brown. Depending on the host species, temperature, and humidity, the egg hatches in 5-9 days. The crawler is a first-instar nymph that is round, flat, and mobile. Crawlers seek the bottom leaf surface for a suitable eating spot. The crawler loses its legs and becomes sessile once it has settled for feeding. Each of the first three instars lasts 2-4 days. The fourth nymphal stage, commonly known as 'puparium,' lasts around 6 days and is affected by temperature. Copulation begins 12-20 hours after the adult emerges through a "T"-shaped tear in the puparium's skin. Females can live up to 60 days, but males have a shorter lifespan. Within a year, *B. tabaci* generates eleven to fifteen generations. In *B. tabaci*, both sexual and asexual reproduction is observed. Arrhenotoky, on the other hand, appears to produce all males parthenogenetically. The labrum, labium, and stylets make up the mouthparts of adult *B. tabaci*. Two maxillary stylets and two mandibular stylets make up the stylet bundle. By putting its stylets into the phloem sap, the whitefly feeds.

Protocol for the rearing of *B. tabaci*:

All research involving whitefly-transmitted plant viruses needs the rearing of whiteflies. The majority of these viruses are difficult to spread through other means. Although agroinoculation has been demonstrated to be effective in the transmission of numerous begomoviruses, the process necessitates the development of infectious constructs as well as excellent laboratory abilities. As a result, whitefly rearing is critical for maintaining virus cultures and producing virus-infected plant materials. The methodology outlined below demonstrates how to maintain and propagate a whitefly population under controlled conditions.

Rearing of *B. tabaci* in the rearing chamber

Setting up of *B. tabaci* rearing chamber

A whitefly colony should be kept in a climate-controlled environment. Relative humidity, temperature, and photoperiod are all important elements in the formation of a whitefly population. A temperature of 282°C, 50% relative humidity, 8 hours of darkness, and 22,200 lux of illumination enable a good whitefly population and host plant proliferation.

Maintenance of host plants

The selection of a host plant is an important consideration when starting a whitefly colony.

- Without collapsing, the plant should be able to maintain a large insect population. Cotton, cucumber, and brinjal are excellent host plants for whiteflies.
- These plants should be grown in an insect-free environment with ideal temperature, light, and relative humidity.
- The use of plants contaminated with whiteflies, thrips, or mites before they join the colony can lead to the colony's collapse. Infested plants may also be infected with insect-borne viruses, which will make it difficult to create a virus-free *B. tabaci* population.

Fig: Life cycle of *B tabaci* (Ghosh *et al.*, 2019)

Generating a *B. tabaci* population in rearing cages

- Any research laboratory or environment can provide the first virus-free, clonal whitefly population. If the population was collected in the field, it should be reared for at least two generations on plants that do not plant virus hosts, and the lack of plant viruses should be confirmed by PCR.
- Depending on the number and height of the plants, a translucent acrylic sheet can be used to make rearing cages. An insect-proof net or fabric is draped over the top. Eight cotton plants in 8 cm x 8 cm x 8 cm plastic pots can be kept in an acrylic cage measuring 40 cm x 25 cm x 60 cm. The cage's bottom side should be left open. To gather or release whitefly, a small sliding window should be built in the front. All of the plants should be placed in a 45 cm × 30 cm plastic tray with an acrylic cage covering them. To keep the plants alive, the plant-containing tray is watered regularly.
- Release a single *B. tabaci* into the rearing cage and keep it in the whitefly chamber at 28±2 °C, 30-50% relative humidity, 8 hours of darkness, and 22,200 lux of illumination. Keep these cages undisturbed for a few days, until a significant number of adults in the following generation can be seen.

Releasing clonal *B. tabaci* in the rearing chamber

- As indicated above, grow a healthy host plant in plastic containers. Transfer the healthy host plants to the whitefly rearing chamber in a plastic tray.
- Inside the whitefly raising chamber, open the rearing cages containing the clonal *B. tabaci* population.
- Place all of the healthy host plants near the *B. tabaci* population so that the bacteria can readily spread to all of them.
- All whiteflies will eventually find a new host plant to feed and breed on.
- Irrigate the plant with water from the tray. When necessary, replace the old plants with new ones.

Regular maintenance of *B. tabaci* population

Once the pure whitefly population has been established, the following care should be taken:

- Regularly check relative humidity, temperature, photoperiod, and light intensity.
- Fresh host plants should be substituted for dry and declining plants as soon as possible.
- Irrigate your plants regularly.
- Infested plant entry should be closely controlled.
- After spraying with insecticide outside the whitefly rearing chamber, the old and dried plants should be destroyed promptly

Artificial feeding of whitefly

In distilled water, nymphal and adult diets will be prepared with 15% sucrose and 5% Difco yeast extract (nymphs) or 15% sucrose and 10% FreAmine III (adults). Sugars (10 percent final concentration) are added individually to diets to preserve the sucrose and yeast or FreAmine III concentrations found in the diet. the diets were sterilized using a 0.22-micron filter.

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