

Artificial Diets and Rearing Techniques of Mealybugs

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SUMMARY

This chapter describes the protocols for large scale rearing of mealybug species with respect to its varied substrates viz., potato sprouts, ripe pumpkin and butternut etc. including its different host plants under the laboratory conditions. Some successful examples of chemically defined artificial diets for the pseudococcids *Planococcus citri*, *Phenacoccus manihoti* and *Phenacoccus herreni* are also presented with its nutritional composition and rearing system units to suit the rearing under indoor conditions for their multipurpose use in different studies. The major ingredients among the defined diet of mealybug include several amino acids especially for its phagostimulatory and nutritive role.

INTRODUCTION

Mealybugs are scales of the Pseudococcidae family and members of the Sternorrhyncha (formerly Homoptera) order. They are named due to the thin-to thick mealy or cottony wax secretion covering them. The main motto linked to organized mass – rearing of insect is to ensure the reliable sources of high quality insects for their multiple purposes by understanding and providing species-specific requirements for temperature, humidity, amounts of space, light characteristics, photoperiod, population size, food, oviposition stimulants and substrates, and other environmental conditions. Most of the times insect rearing operations are performed in synchronized manner, so that they can be interfaced at the most appropriate times (Leppa 2009). Enhancement of insect rearing can be made by understanding their natural history viz., their ecology, behaviour and systematics. Artificial diets have been developed with the concern to simplify and improve the rearing of insects. The advent of artificial diets has provided a sound knowledge about the nutritional requirements of insects.

Artificial diets for mealybugs

The first report regarding the artificial diet of mealybugs was for the pseudococcid *Planococcus citri* given by Gothilf and Beck (1966). This chemically defined (holidic) dietary media for the rearing of mealybugs contained the ingredients (Table 1) that have been used successfully in rearing mealy bugs from eclosion to reproduction.

The procedure for preparation of chemically defined diet by Gothilf and Beck (1966).

- The dietary ingredients (Table 1) were stirred for 3 hours (pH maintained at 6.9-7.0 by means of KOH).
- The diet was sterilized by using the bacterial filters and then introduced into the rearing units (Fig. 3) with the help of sterile pipettes.
- The larvae were introduced to the assembled rearing units under aseptic conditions in an isolation chamber.

However, the use of the *Planococcus citri* defined diet and rearing unit was unsuccessful for rearing the cassava mealybug (*Phenacoccus manihoti*) from newly hatched larvae to adults (Calatayud *et al.* 1998). So, the diet used (called Ap2), was diet A5 of Febvay *et al.* (1988), except for some alterations like sucrose which was adjusted to 200 g/l after several trials, the deficiency in tyrosine amino acid was compensated for by addition of beta-alanyltyrosine at 109 mg/100 ml and the rearing technique involves the enclosure of the dietary liquid medium in a sterile Para film sachet as previously described by Srivastava & Auclair (1971). Calatayud and co-workers (2002A) observed for the first time in a Pseudococcid species that amino acids viz., aspartic acid, glutamic acid, valine and alanine play a major phagostimulatory role while lysine, ornithine, asparagine, methionine and histidine have a nutritive role in *Phenacoccus herreni*. Other constituents of the artificial diet necessary for mealybug growth and development include vitamins, cholesteryl benzoate and oligoelements.

Rearing techniques of mealybugs

In this regard several mealybug species have been reared in laboratory by using various suitable substrates, among which the potato sprouts and ripe pumpkin are mostly employed in different countries (Mani and Shivaraju 2016).

Potato sprouts as an insectary host for mealybug rearing

This was first discovered by H. S. Smith and E. J. Branigan (1916) and latter modified by Smith and Armitage (1920). The methodology for rearing mealybugs (*Planococcus citrii*) on the potato sprouts is described through the flow chart (Fig. 1a and 1b) as given by Fisher (1963).

Ripe pumpkin as an insectary host for mealybug rearing

The rearing of mealybugs on cucurbits was first reported by Sysoev (1953). The steps involved in propagation of citrus mealybug (*Planococcus citrii*) on ripe pumpkin is described through the flow chart (Fig. 2) as outlined by Chacko *et al.* (1978) and Singh (1978).

Alternate host plant evaluations for mass – rearing of mealybugs

Sometimes mass culturing of mealybugs is also done on host plants. In this regard, several alternative host plants have been selected and studied for the preferences by different mealybugs for mass rearing in laboratory. Accordingly, Helen *et al.* 2013 suggested that rearing of papaya mealybugs (*Paracoccus marginatus*) on *Hibiscus cannabinus* plants is easy, economical and suitable for tropical conditions than the potato sprouts which is difficult to maintain in high temperature areas owing to the spoilage of potatoes. *Hibiscus cannabinus* plants with a height of 40 cm or 20 to 25 days old seedlings is the suitable stage for infestation with papaya mealybug for effective mass multiplication purposes. Johnson and Giliomee (2011) investigated the suitability of various substrates viz., sprouting potatoes, butternuts and citrus for the mass rearing of oleander mealybug (*Paracoccus burnerae*) and they concluded that there was incomplete development on butternut, and prolonged development on citrus when compared to sprouting potatoes, therefore despite the shorter shelf life, sprouting potatoes are the preferred host for mass rearing of mealybugs. Nguyen Tuan *et al.* (2020) examined the suitability of taro *Colocasia esculenta* for rearing the cassava mealybug *Phenacoccus manihoti* in the laboratory alternative to cassava seedlings. And the results suggested that taro is suitable for mass rearing of *P. manihoti* under laboratory conditions.

Mealy bug rearing units (Fig. 3) for artificial diet feeding by Gothilf and Beck (1966) and later modified by Srivastava and Auclair (1971).

- The rearing unit was constructed with 2 pieces of soft glass tubing.
- The smaller piece (diet chamber) with the dimensions 9 mm OD and 30 mm long served to contain the liquid diet.
- The larger piece (larval chamber) with the dimensions 12 mm OD and 25 mm long housed the mealy bug larvae.
- One end of the diet chamber was covered with Para film that had been stretched to about one-third of its original thickness (because mealybug larvae are strongly thigmotactic and few, if any, would remain on a membrane that provided no folds or crevices).
- The feeding-membrane end of the diet chamber was then inserted to a depth of about 3 mm into the larval chamber.
- Both the chambers are then sealed together by small strips of Para film.
- Diet chamber was then filled with dietary medium, and then sealed with a Para film.
- The units were incubated in a horizontal position, so that the membrane through which the mealy bugs fed was vertically oriented.

Fig. (1a) Mass production of mealybugs on potato sprouts Source: Fisher (1963); Mani and Shivaraju (2016)

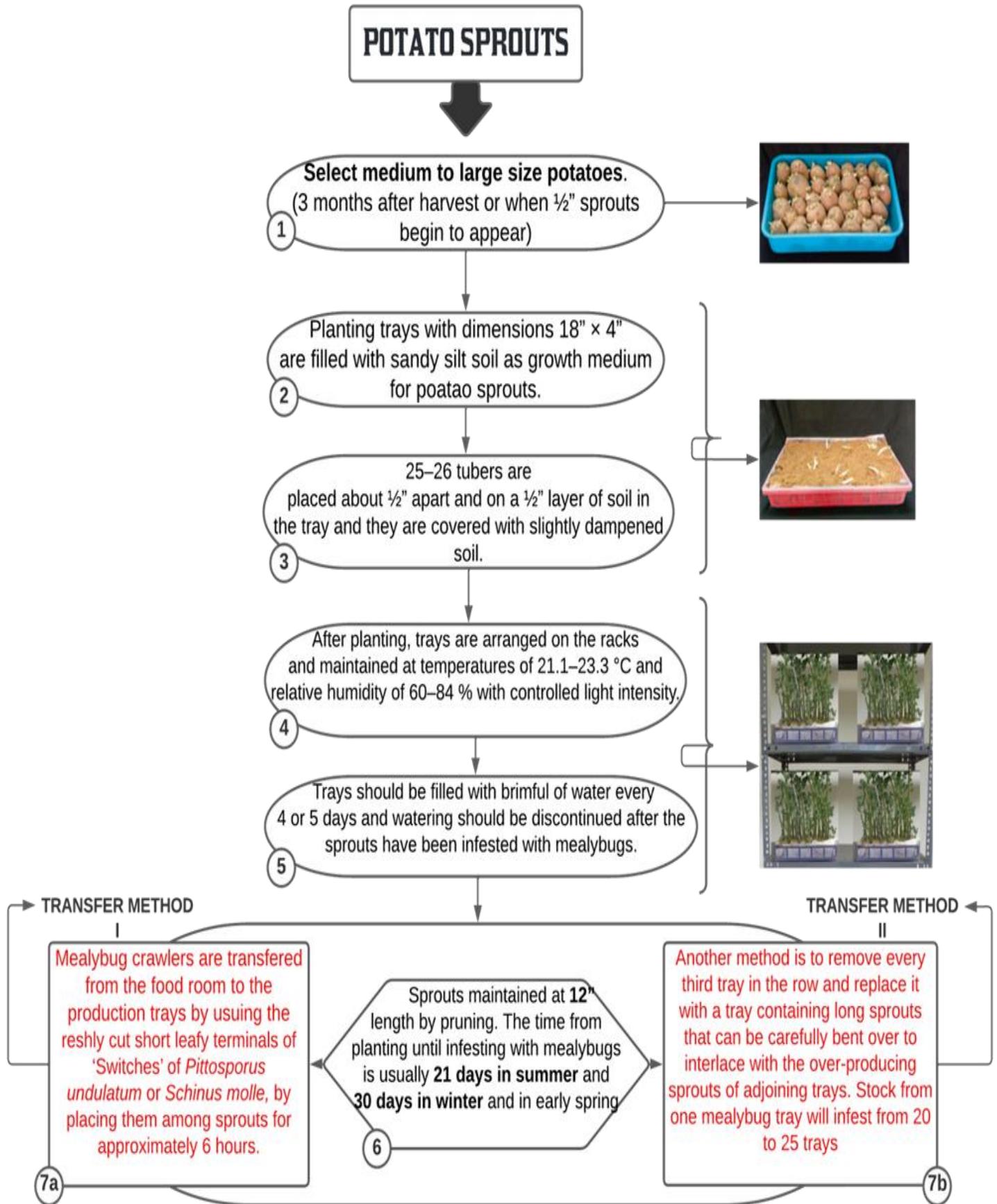


Fig. (1b). Under Indian conditions. Source: Fisher (1963); Mani and Shivaraju (2016).

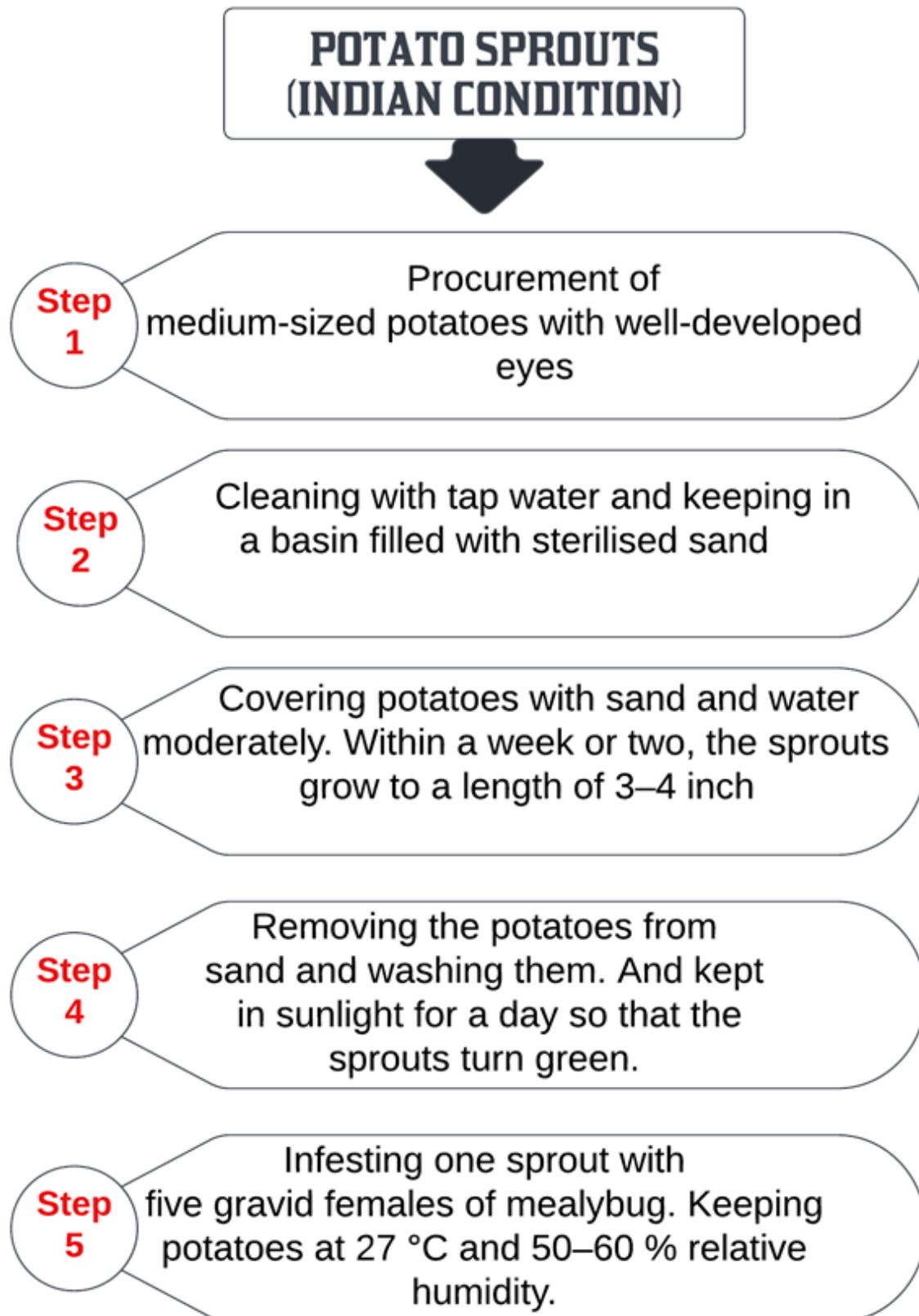


Fig. 2 Rearing of mealybugs on pumpkins. Source: Chacko *et al.* (1978) and Singh (1978).

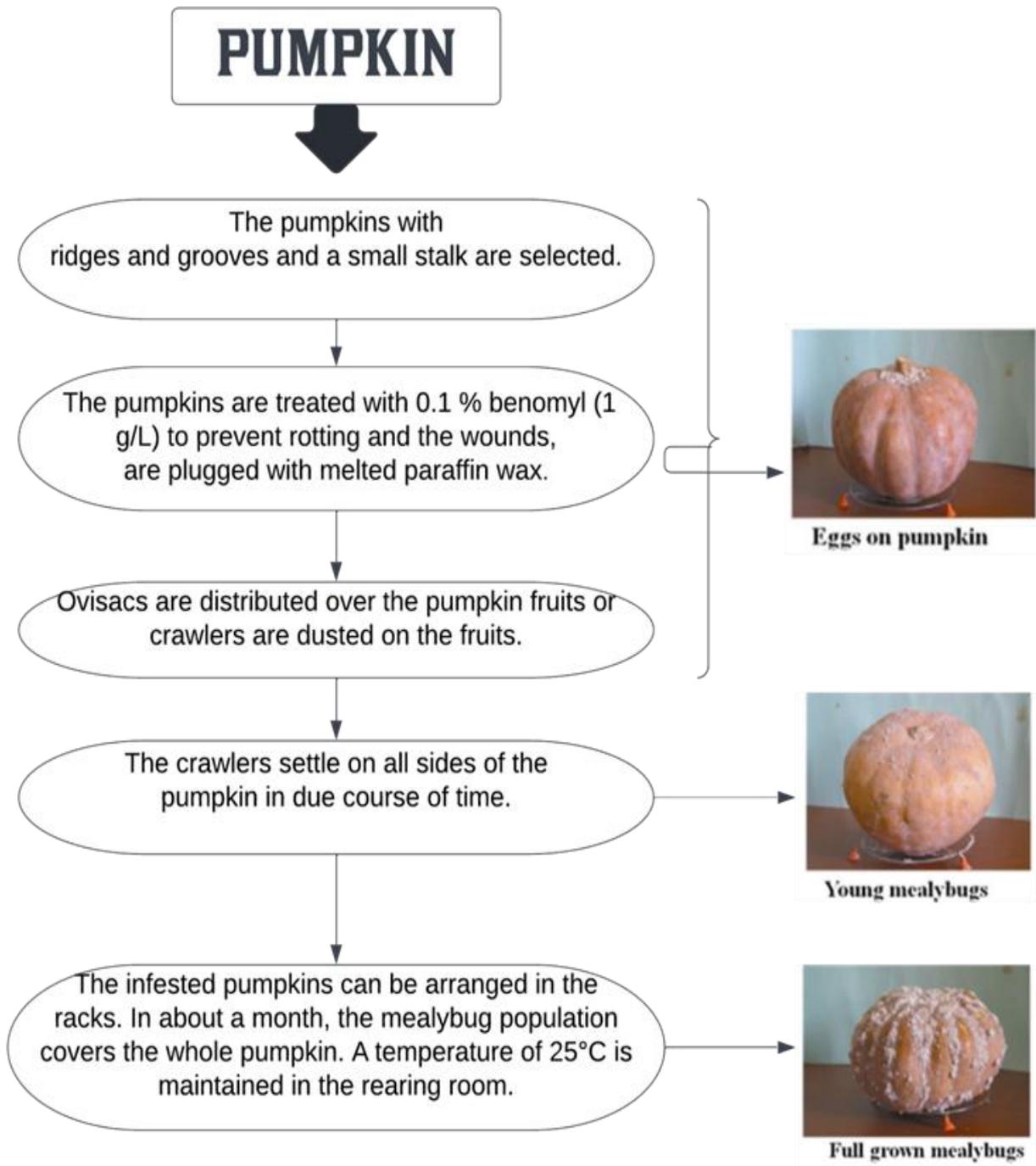
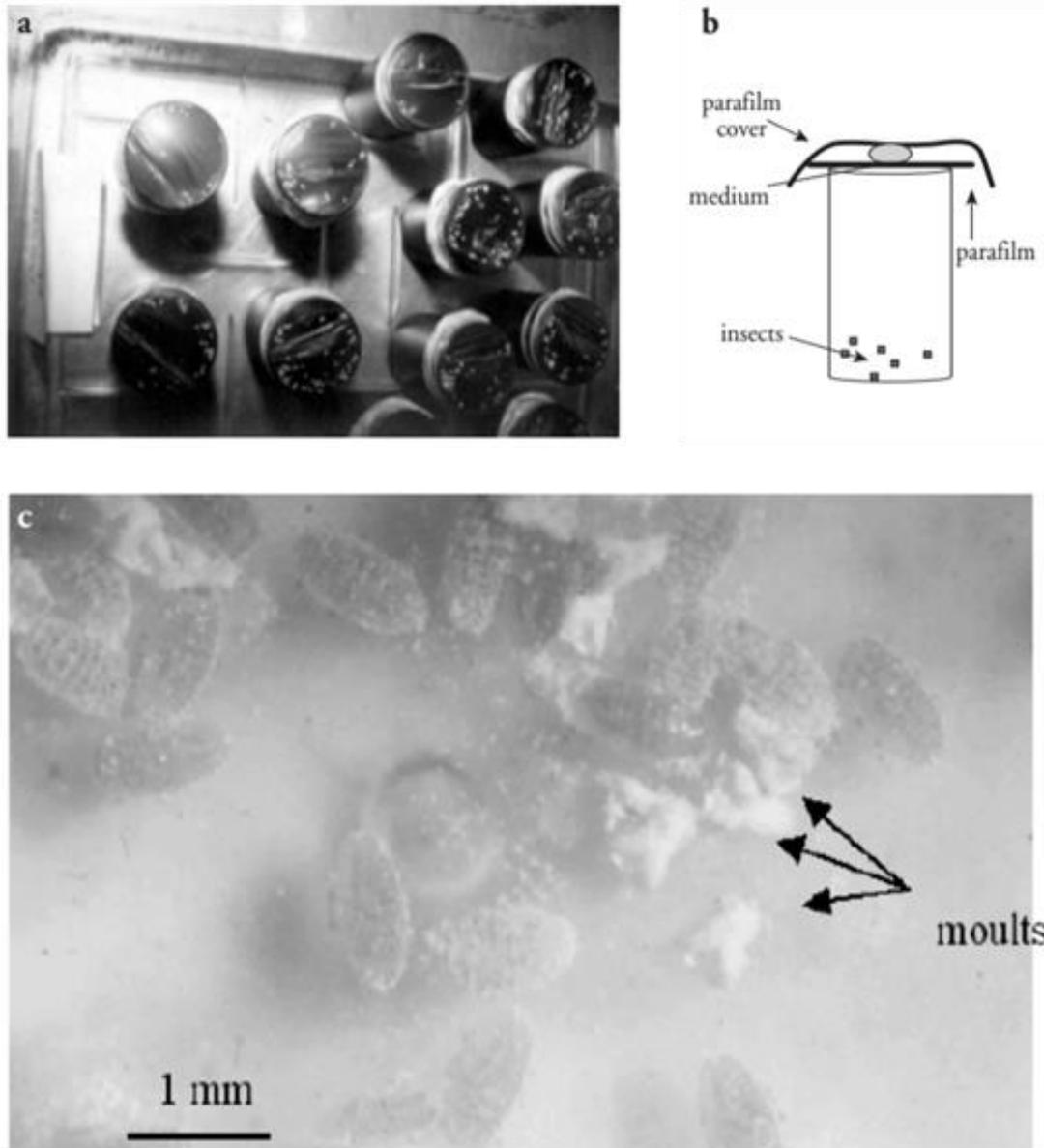


Fig. 3 Rearing units to rear mealybugs on holidic diet Source: Calatayud *et al.*1998; 2002

Rearing units used to rear mealybugs on a holidic diet (according to Calatayud, 2000 and Calatayud *et al.*,1998; 2002a).



(a) Consists of an enclosed liquid medium sandwiched between two pieces of Parafilm.

(b) which is then mounted on the top of a black standard film cannister.

(c) *P. manihoti* larvae grow under artificial conditions.

L – AMINO ACIDS (mg)		VITAMINS (mg)		OTHER NUTRIENTS	
Glutamine	300	Ascorbic acid	150	Sucrose	30 g
Asparagine	250	Choline chloride	20	Cholesterol (acetate)	20 mg
Glutamic acid	200	Inositol	20	Potassium phosphate (tribasic)	500 mg
Aspartic acid	200	Nicotinic acid	6	Magnesium chloride	200 mg
Arginine	140	Ca pantothenate	1.5	USP Salt #2	5 mg
Gamma amino Butyric acid	140	Thiamine HCL	0.6	Water to 100 ml	
Valine	100	Riboflavin	0.3		
Threonine	100	Pyridoxine	0.3		
Lysine	80	Folic acid	0.1		
Serine	50	Biotin	0.01		
Alanine	50				
Phenylalanine	50				
Methionine	50				
Histidine	50				
Proline	40				
Leucine	40				
Isoleucine	40				
Tyrosine	40				
Tryptophane	40				
Glycine	20				
Cysteine	10				
Cystine	10				

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