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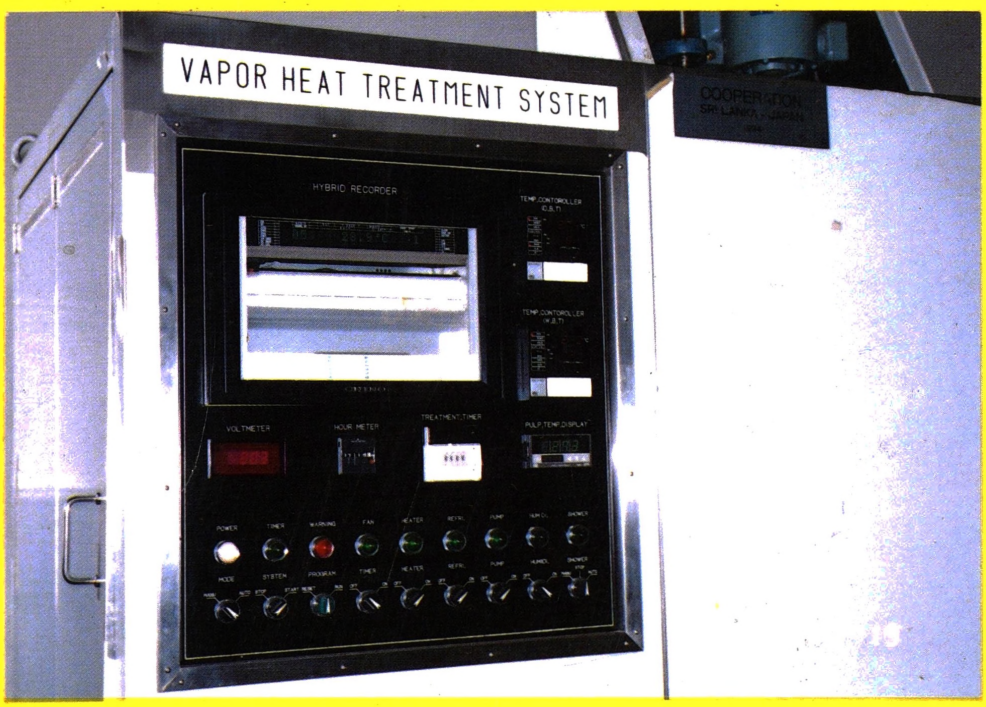

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INTRODUCTION

There can be considerable economic loss in several fruit and vegetable crops due to fruit fly infestation. Most of the other countries do not permit to import such fruit fly infested fruits and vegetables. Therefore, to export fruit and vegetable to foreign countries, we have to establish treatment methods such as vapor heat treatment or low temperature treatment to achieve complete control of fruit fly in crops.

Therefore, it has become necessary to investigate and develop the standarized treatment method for the fruit fly control and eradication. Measures also have to be sought that do not harm or deplete the freshness of the crops without any loss in its nutritive qualities in situ. In order to achieve this objective a number of studies have to be carried out on species infesting these crops, to establish the standardized treatment method. Different species and stages of life cycle from egg to larval stages of fruit flies have different susceptibilities to heat.

Artificially bred fruit flies are needed to carry out these tests.

The tests have to be performed on artificially inoculated fruits with fruit fly (eggs, 1st, 2nd or 3rd instar larvae) under laboratory conditions to assess the heat susceptibility.

So, it is very important to understand and master the handling techniques of fruit fly very well.

The handling methods of fruit fly eggs and larvae in such tests are described as follows.

HARVESTING EGGS

The eggs are gathered by oviposition tube. Usually a cylindrical plastic or polyethylene vessel with a lid is used as the oviposition tube. Small pinholes (4 ~ 5 mm in diameter) are drilled at about 2 cm intervals on all parts of side wall except lower part. Host fruit or vegetable juice are introduced in the vessel and the juice can be coated on the side wall by rotating the vessel. The tube is left for 2 ~ 3 hours in adult rearing cage. So, many eggs are oviposited through pinholes to the inside of vessel wall (Fig. 1). Then the tube is taken out and inside of it is washed with water onto a piece of cloth (black, 100 ~ 150 mesh) to filter the fluid that contained eggs. Then the remaining eggs on the black cloth are transferred to an appropriate medium for rearing.

If necessary, the collected eggs are transferred to a measuring cylinder by washing down through a glass funnel. When the eggs sink to the bottom of the measuring cylinder they form an egg mass. Now the volume of egg mass has to be recorded to enable calculation of the number of eggs in it. As one m*l* of egg mass contains about 10,000 eggs

Fig. 1 Oviposition tube kept inside adult rearing cage for egg collection

Fig. 2 Measurement of volume of egg mass

(little variation in number from species to species), can calculate the approximate number of collected eggs in the egg mass (Fig. 2).

REARING METHOD

1. Old Egg and 1st Instar Larvae

Collected eggs to be used for the test as old egg or 1st instar larvae are kept on wet black filter paper in petri dish and kept at constant room temperature of about 25°C (Fig. 3). It is very important to remove excessive water on the filter paper and not to dry up the filter paper during incubation.

The incubated eggs begin to hatch after 24 hours. Thus the old eggs can be collected at 24 hours after incubation and 1st instar larvae can be collected at 48 hours after incubation for tests. But the exact time to get the old eggs and the 1st instar larvae varies a little according to rearing temperature and species of fruit flies.

2. 2nd and 3rd Instar Larvae

A petri dish or a plastic container is filled with the artificial medium and toilet paper is laid over the medium. Then collected eggs are seeded on this paper. This layer of toilet paper results in low mortality, as the eggs are not soaked in the water that has got accumulated in the depression of the medium. A fine brush is very useful for the egg transferring work. Then this medium is kept at constant room temperature (about 25°C) until the larvae grow up to 2nd or 3rd instar larval stage (Fig. 3).

In general, you can get the 2nd instar larvae in 3 days after incubation and the 3rd instar larvae in 5 days after incubation: but these times differ according to rearing temperature and species of fruit flies.

Constituents of Artificial Medium (Larval Diet):

* Wheat bran	175.0 g
* Sugar	50.0 g
* Dried brewer yeast	35.0 g
* Conc. HCl	about 20 m*l*
* Methyl hydroxy benzoate	0.75 g
* Sodium benzoate	0.75 g
* Toilet paper	25.0 g
* Water	650 m*l* (only to saturate but without excess water)
* pH	4.5 ~ 4.9 pH can be adjusted to 4.5 ~ 4.9 with 35% HCl. A pH meter is used for its determination.

Fig. 3 Inoculation of test insects
Left: Eggs seeded on wet black filter paper, Right: Artificial medium

ARTIFICIAL INOCULATION PROCEDURE

When the eggs incubated by above methods grew up to expected stage, they are then inoculated with the relevant fruits or vegetables and treated accordingly.

1. Separation of Test Insects from the Medium

At first, the test insects of expected stage are separated from the medium and divided into groups of equal number depending on the number of test fruits to be inoculated.

The separating methods of each stage of insects are as follows:

(A) *Egg* : Pour the water on the wet filter paper containing eggs. Then, the eggs sink in the water one by one, and it is easy to pick up and transfer the eggs to another container by using a micro pipette with a rubber cap. You should pick up eggs very carefully to avoid mixing with 1st instar larvae, as some eggs would have already hatched and grown up to 1st instar larvae.

The eggs floating on the surface of water cannot be used for test, as the hatchability of these floating eggs is very low.

(B) *1st instar larvae* : 1st instar larvae on wet filter paper are usually mixed with unhatched eggs and it is not easy to separate each other. At first the mixed population of eggs and 1st instar larvae is transferred on to a piece of black cloth of 80 ~ 100 mesh kept on a petri dish with water. Adding a little more water on them cause many 1st instar larvae to move into water from the black cloth, leaving eggs on it (Fig. 4). Then remove the black cloth and collect 1st instar larvae using a micro pipette from water in the petri dish. Separate and pick up 1st instar larvae with great attention so as not to contaminate with unhatched eggs.

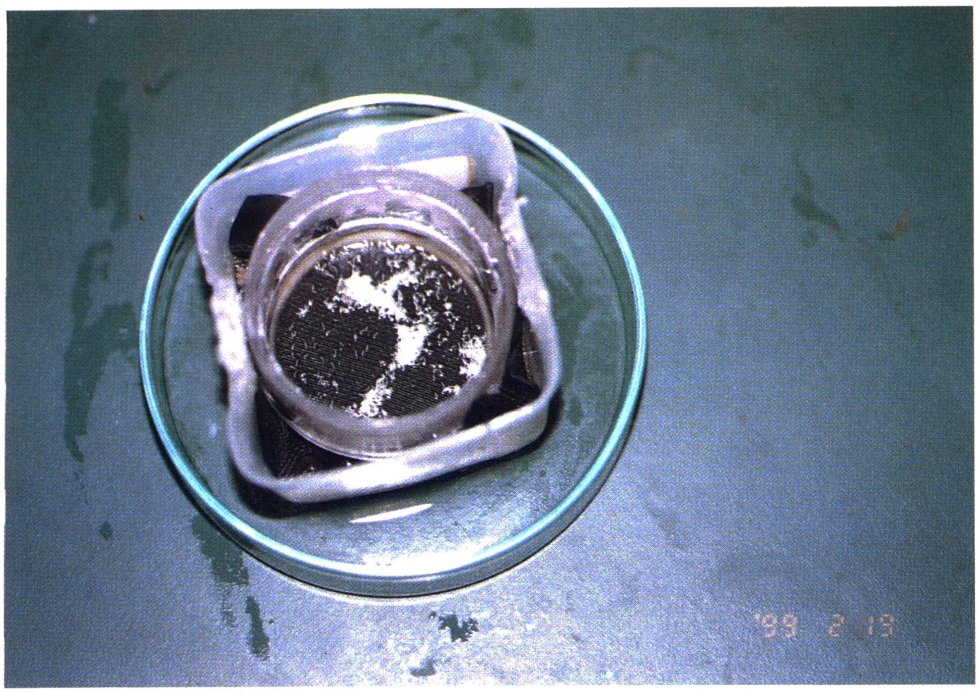


Fig. 4 Separation of 1st instar larvae from eggs (Apparatus is a trial product by Entomology Division)

(C) *2nd instar larvae* : Place a black filter paper containing juice of the host fruit or vegetable on the rearing medium and keep it until larvae come out onto the paper from the medium. Therefore 2nd instar larvae can be collected easily out of this paper with the use of a fine brush.

(D) *3rd instar larvae* : It is not necessary to separate 3rd instar larvae from the rearing medium, as they can be collected easily from the medium with or without dilution with water.

Great attention should be paid not to mix another instar of larvae during collection of the test insects. Sometimes the size of larvae is not in absolute scale of 1 ~ 3 instar larvae. therefore it is necessary to understand the morphological differences among 1, 2 and 3rd instar larvae.

Fig. 5 indicates the simple methods to distinguish 1st, 2nd and 3rd instar larvae with the use of their morphological differences.

2. Transferring Method of Separated Test Insects

Expected number of eggs or 1st ~ 3rd instar larvae are transferred into a watch glass containing water. A micro pipette with a rubber cap can be used to transfer the eggs and 1st instar larvae (Fig. 6). A fine brush can be used to transfer 2nd instar larvae and forceps can be used to transfer 3rd instar larvae (Fig. 7). The insects can be counted using a tally counter.

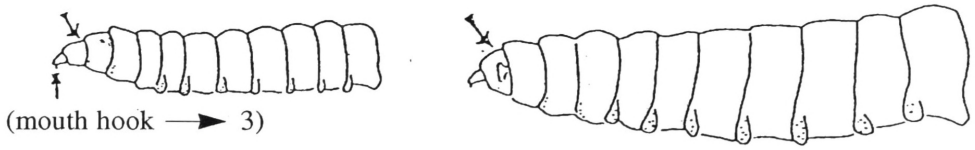
3. Inoculation of Test Insect to Test Fruit

Fruit to be inoculated with fruit fly eggs or larvae have to be cut in a specified manner depending on the type of fruit (Fig. 8). As an example, the fruit skin of a mango fruit has

1 Size of larvae

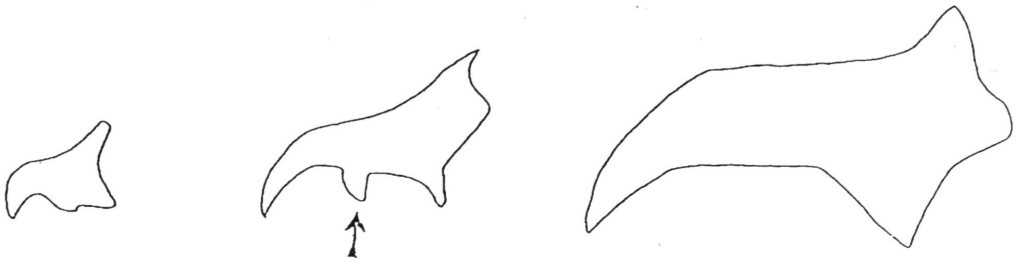
1st instar larvae ≦ 2nd instar larvae ≦ 3rd instar larvae

2 Presence of anterior spiracle



1st instar larvae (not present) 2nd & 3rd instar larvae (present)

3 Shape of mouth hook



1st instar larvae 2nd instar larvae 3rd instar larvae

Fig. 5 Morphological differences among 1st, 2nd and 3rd instar larvae of *Bactrocera dorsalis*

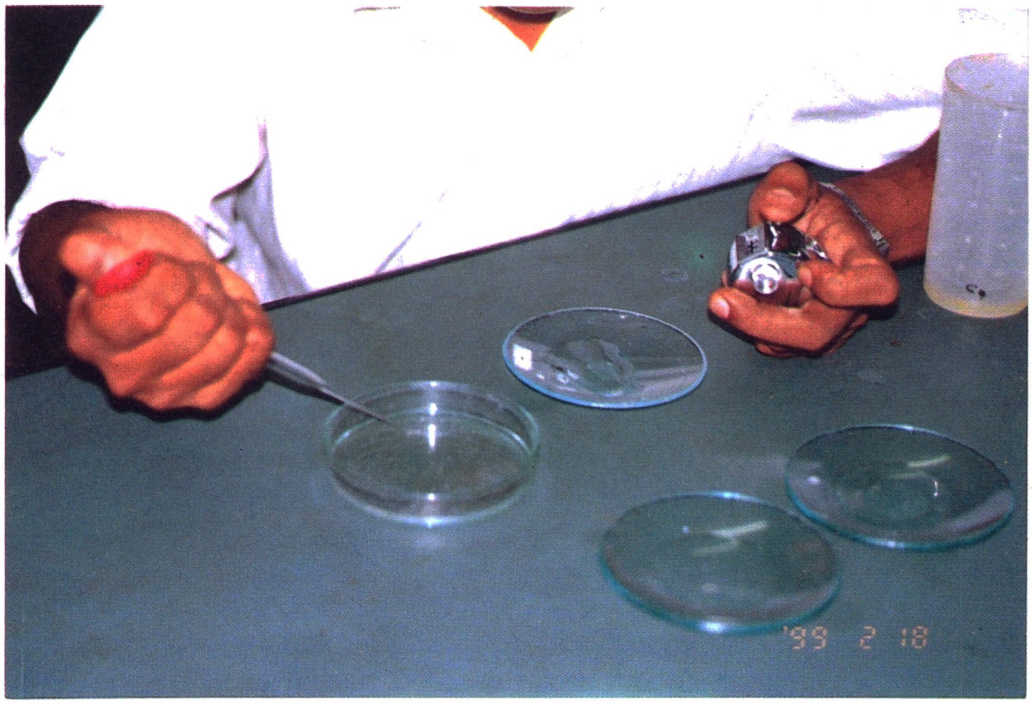


Fig. 6 Picking up and transferring of eggs

Fig. 7 picking up and transferring of 3rd instar larvae

Fig. 8 Preparation of various fruits (mango, papaya, mandarin) for inoculation of fruit flies

to be cut on three sides along a small rectangle leaving one side of it (Fig. 9). Then this piece of skin should be cut open (window). Thin layer of pulp under this skin should be scraped off. Then the pulp underneath is cut into fine ditches crosswise to facilitate settlement of insects. In the middle of this small piece of fruit skin, a small hole (4 ~ 5 mm in diameter) is drilled by using a cork borer (Fig. 10). Then each group of the eggs, 1st, 2nd or 3rd instar larvae kept in separate watch glasses (See Artificial inoculation procedure - 2) is inoculated

Fig. 9 Cutting of mango for inoculation

Fig. 10 A mango fruit prepared for inoculation with opened window

into each window of fruit. Before transferring the test insects to fruit, the excessive water in the watch glass is removed by a micro pipette. Then, the test insects are transferred into the window with the use of a brush (Fig. 11). During this process of transferring, it is necessary to take great care not to loose any test insects from the watch glass.

Fig. 11 Inoculation of larvae to mango

Fig. 12 Mangoes sealed with Cellotape after inoculation

After inoculation of relevant stage of insect the window is sealed up with cellotape (Fig. 12).

It is better to keep these inoculated fruits for about half a day under a favourable condition, for the larvae to get accustomed to the fruit before the heat treatment.

Then the inoculated fruits should be treated by high or low temperature treatment to investigate the heat or cold tolerance of insects.

STORAGE OF TREATED FRUITS

The treated fruits with eggs and larvae upto 3rd instar are cooled down quickly using electric fan and stored in separate containers to avoid reinfestation of fruit flies at about 25°C (Fig. 13). Untreated (control) fruits inoculated with the test insects are also kept in the same manner. These fruits in the containers are kept in the constant temperature room (about 25°C) until the inoculated eggs or larvae grow up to 3rd instar larvae. The fruits treated with larvae at 3rd instar stage are kept in the same manner for 2 days before investigation for mortality of insects.

Fig. 13 Mango kept in containers separately after heat treatment

INVESTIGATION OF INSECT MORTALITY

When eggs or larvae in control (untreated) fruits become 3rd instar larvae, investigation of mortality of insects is carried out. Each fruit to be investigated is placed on a plate and the fruit skin is peeled off. Then the fruit is dissected with great care to look for larvae (survivors) (Fig. 14). Then the larvae are transferred into a petri dish containing water with the use of a pair of forceps. Thereafter, the total survived number of larvae in the petri dish is counted with the use of a tally counter.

Perhaps at the time of investigation, some larvae do not grow up enough, and they may be still small in size due to individual variation or effect of treatment. Thus it is necessary to take great care not to miss the small larvae during investigation.

Also the full grown 3rd instar larvae sometimes try to hop out and escape. Therefore great care should be taken to prevent loss of larvae or to avoid introduction of larvae from other plots.

Tally counter can help the counting of larvae.

Fig. 14 Dissection of treated mango for mortality investigation

HANDLING TECHNIQUES OF FRUIT FLIES IN HOT WATER DIPPING TEST

The handling techniques of fruit flies in hot water dipping test are almost similar to those of the techniques described before. But in hot water dipping test, naked eggs and larvae kept in glass tubes are used instead of the test insects inoculated into fruit.

Only the different techniques needed for the handling of naked test insects (eggs, 1st, 2nd or 3rd instar larvae) are described as follows.

(1) One end of the glass tube (open at both sides, about 25 mm in diameter, about 100 mm in length) is left open and a black cloth (100 ~ 200 mesh) is attached at one end by a rubber band. The required number of these tubes are marked to indicate the species, stage of fruit flies and exposure time to hot water etc. Each of them is placed in a beaker containing a little volume of water.

(2) At first, the test insects are counted and transferred into a watch glass containing water as described before (See Artificial inoculation procedure – 2). They are then transferred into the glass tube by washing down with water (Fig. 15). A glass funnel can be used to transfer the eggs or larvae into such a tube. Cool water is poured over the inside wall of the tube to wash down all of the test insects into water in the tube.

(3) These tubes containing the test insects are taken out from each beaker, and placed in a test tube rack before treatment, to facilitate immersion of all the tubes into hot water bath at the same time.

At the time of immersion of tubes into the water bath, it is necessary to take care about the hot water level in it, to avoid complete sinking of tubes and to prevent any loss of insects into hot water. Therefore, the hot water level should be kept below 2 ~ 3 cm from top of a tube. The test insects may float on the surface of hot water in the tube during the immersion. Then hot water from the water bath should be poured over the floating insects with the use of a pipette to push them down into water.

Fig. 15 Transferring of test insects to tube

(4) At the end of the hot water dipping treatment, the tubes containing test insects are taken out from hot water quickly and transferred into beakers containing cool water for quick cooling. And the test insects lying on upper part of the inside wall of tubes are washed down into water with more cool water. After cooling down, the black cloth is removed very carefully from the end of tube and all of the treated insects remaining both in the tube and on the black cloth are transferred as follows.

a) *1st, 2nd or 3rd instar larvae*

Each of 1st, 2nd or 3rd instar larvae on the black cloth and inside the tube is transferred onto the artificial medium in a petri dish using a fine brush or forceps.

b) *Eggs*

Eggs in each tube and on the black cloth are placed on a wet black filter paper using a fine brush. The eggs should be arranged regularly on the paper to facilitate counting during the following investigation of insect mortality.

As some test insects may get lost during hot water dipping treatment, the counting has to be repeated at the time of this transferring process.

(5) Mortality investigation of eggs is conducted by counting the number of unhatched eggs on the wet black filter paper under stereo microscope, at 2 days after treatment. The difference in number between treated eggs and unhatched eggs is recorded as hatched eggs.

The other mortality investigations of 1st, 2nd and 3rd instar larvae are conducted by collecting survivors using forceps from the medium directly or after dilution with water, when larvae in corresponding control plot grew up to the 3rd instar larvae.

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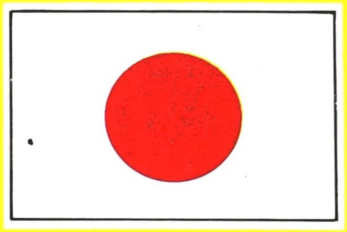

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