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Possibility for laboratory mass rearing of the potato tuber moth *Phthorimaea operculella* (Lepidoptera: Gelechiidae) through simplified steps and procedures

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Abstract:

Insects, laboratory rearing is a prerequisite for any works related to fundamental or applied entomology, the number of needed individuals either for small experiment or for mass production of a biological control agent, is one of the main aspects to be considered. The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera*:* Gelechiidae), represent one of the major threads for potato production worldwide and generally laboratory reared in many labs worldwide, using the same concept and principals. In this work, a validated simplified system for PTM mass production under laboratory conditions is presented. The presented fabricated rearing unit aims to maximize production of germ free PTM stages with optimal use of available infrastructure, equipment, labors, and at the same time, minimizing risks of potato pathogens spread among PTM rearing containers or boxes.

Introduction

Insect laboratory rearing is essential for securing testing, insect materials needed for either ecological and/or biological studies, developing and mass production of biological control agents including natural enemy or production of sterile insect for release programs. A lack of proper planning and well-designed equipment in an insect rearing and harvesting program can result in wasted money, labor, and time, leading to failure of a research or control program (Hsin and Wayne, 1988 and Rameswor and Chuleui, 2011).

The potato tuber moth (PTM) or tobacco split worm, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is an indigenous pest that originated in tropical mountains of South America (Hristina *et al.*, 2016). It attacks and accompanying potato and other solanaceous plants , that grown and found in over 90 countries and distributed in temperate and subtropical climates regions, such as tomatoes, eggplants (*Solanum melongena* L.), peppers (*Capsicum* spp.), tobacco, and wild solanaceous plants like jimson weed or datura (*Datura stramonium* L.) (Alvarez *et al.*, 2005). Most economic damage that occurs to potato tubers under storage conditions are attributed to PTM larval feeding and their boring tunnels and deposit excreta in tubers, resulting in the unfit of tuber for either local consumption and/or for exportation (Malakar and Tingey 2006 and Rondon, 2010).

Among semi-artificial diets described in insect laboratory rearing, few diets were reported for PTM and

request quite well defined and precisely weighing chemicals, and mixing of a large number of components that surely present high costs (Badegana and Ngameni,2000; Gleave *et al.*, 1998 ; Sharaby and Saleh,1985 and Singh and Charles (1977) however, Kashyap *et al.* (2008) stated that testing of all these published diets gave negligible to very low rates of larval survival.

The applied conventional PTM laboratory rearing approach worldwide is essentially relay on potato tubers as a main source for larval feeding and both PTM mature and immature stages are kept in closed ventilated containers or boxes that regularly supplied with potato tubers and kept under controlled weather conditions. Application of these conventional rearing procedures for the insect mass rearing may require much space, precautions and effective measures of temperature and relative humidity to suppress disease outbreaks and surely are labor-intensive therefore, the objective of this work was the design and development of a practical laboratory potato tuber moth rearing unit (PTMRU) that permit easier observation, dealing with different PTM stage, and a practical harvest of a particular PTM stage, when needed for further purposes. During the course of this work, we examined the proposed simplified PTMRU to ensure minimal handling intervention and consequently, reducing risks of disease spread, if occur, labor cost, and duration and efforts needed for the regular daily work.

Materials and methods

1. Origin and maintenance of *Phthorimaea operculella* colony:

The PTM moths used in this study were derived from the laboratory colony of *P. operculella* maintained at the Plant Protection Research Institute which is affiliated with the Egyptian Agricultural Research Center. At the beginning, the PTM individuals were conventionally reared according to procedures described by Jordao *et al.* (2010), under controlled conditions (25 ± 1°C and 14hrs photoperiod). To facilitate pupae harvesting the technique was slightly modified so the pupation was formed in the provided corrugated carton strips of about 1.2 cm in width, which were prepared by longitudinally cutting of corrugated carton boxes, sheets, which traditionally used for packaging, then the strips were placed among and on potato tubers in part (B) of the designed and fabricated PTMRU as it will be explained later. For transferring PTM pupae to adults' box, carton strips were longitudinally opened (Tear apart) as shown in (Figure 1 C and D) and liberated pupae were surface disinfected in 0.3% sodium hypochlorite solution for 1 minute, rinsed in distilled water, dried on filter paper and finally sexed.

For egg production, sexed disinfected pupae were transferred to adult box in 1:1 ratio (Male: female) then the adult box was covered by a circular piece of white paper (Or filter paper), sitting on top of a black fine mesh net fitted tightly over the opening with an elastic band. To ensure contact of the used white paper with the fine black mesh, a piece of cork and slight weight were used as shown in Figure 2 (A, B and C). The PTM moths were provided with 10% honey solution as a source for adult feeding through a rolled tissue paper that is placed at the internal extremity of U-shape drinking straw and soaked via capillarity with the honey solution.

Papers containing collected PTM produced eggs, as shown in Figure (3),were surface disinfected in 0.3% sodium hypochlorite solution for 1 minute, rinsed in distilled water, dried on filter paper, then kept in plastic ventilated cylinder boxes for further use under the same controlled conditions.

Finally, cut papers with counted PTM eggs with black head capsules were transferred to the potato tubers surface-drilled at the rate of two grams potato per one PTM larva and new formed pupae were used for re-initiation of the new PTM rears cycle regularly.

2. The potato tuber moth rearing unit (PTMRU) description:

The whole unit was made from available house hold commercial plastic tools for the kitchen. As shown in Figure (4), it consists of two parts A and B. The part (A) is designed for capturing PTM adults (Moths) and their source for feeding, and even egg production if desired. This part is composed of two plastic cylinders. The 1st plastic cylinder (ca) contains one inlet hole and is freely placed, inside the 2nd plastic cylinder (cb) that contains number of holes equivalent to a number of sectors in part (B) of the PTMRU. The cylinder (ca) is placed inside the cylinder (cb) to create a form- fit seal with it to open or close the inlet holes and to prevent moths from exiting the cylinder (ca) and backing to any sector of part (B). The formed moths coming from different sectors of part (B) can be captured either selectively (from one selected sector) by simply turning the cylinder (ca) around its axe and adjusting its inlet holes to the chosen sector outlet open, or collectively (from all sectors at the same time) by pulling cylinder (ca) up so all the inlet holes of the cylinder (cb) is allowed to function at the same time, and a common recipient for PTM moths coming from all sectors is formed as shown in Figure (5).

All inlet holes in the two cylinders of part (A) are precisely aligned with each other and with outlets of all potato tubers sectors located in part (B). The part (B) is designed to receive clean, disinfected and surface, drilled potato tubers for infestation with newly hatched PTM neonates regularly. This part (B) is divided into sectors (S) or parts using plastic sheets and each sector is provided with an external lateral hole that is closed with a cover containing a window of fine muslin (0.1m) for ventilation, and used to facilitate regular observation, manipulation and daily works, and reduce risks of possible spread of diseases among used potato tuber, if occur, all over the rearing unit Figure 4 (B, C and D).

Results and discussion

In the presented work, we designed, fabricated and examined a simplified PTM rearing unit (PTMRU) that aims to maximize production of different PTM individuals needed for further laboratory studies and/or for production of particular biological control agents or pathogen, through a simple approach based on arranging potato tubers in separate parts (Sectors) to ensure reduction of manual interference, spread of potato diseases, financial cost for infrastructures and labor, and time required for mass production of a certain germ free PTM stage.

In the conventional rearing procedures described by many authors worldwide, the PTM laboratory rearing is essentially based on almost the same concept of preparing a main rearing box, made of suitable material and covered by fine mesh gauze with zips at different positions to facilitate manipulation, and a common system for receiving formed pupae in either sand or sawdust layers by placing an additional separated recipient under the main rearing box. Normally these labs should be supported with extra facilities such as CO_2 containers or freezer to subdue or sedate moths .

In the present work, we report a simple PTMRU that shows practical and useful. The unit minimizes manual interfering leading to prevention of physical damage to any of the insect

stages, at the same time it allows rapid observation and manipulation of PTM rearing cycle, including, capturing of adult moth by orienting them to egg laying box (Adult box), harvest of pupae, if needed for further investigations or for egg production, by simply collecting corrugated carton strips placed in part (B). In this respect, it worth to mention that even larval individuals that preferred to build their cocoons and to pupate inter (Among) tubers or elsewhere within its rearing sector, are not considered wasted because potato tuber sector located in part (B) are directly opened on part (A) which is designed for capturing the PTM moths and getting benefit from most formed PTM moths.

Advances of the proposed model:

The Part (A) that is designed for capturing PTM adults (moths) either selectively or collectively. Presence of the two cylinders (ca and cb) of the part (A) with their precisely aligned inlet holes permitted a faster and controlled capturing of PTM moths. Beside its function of capturing PTM moths, the part (A) can be directly used for PTM egg production by simply attaching an adult box that provided with black fine mesh net and a feeding source as shown in Figure (2D). Concerning the design of the prepared adult box either for direct capturing of emerging PTM moths from the PTMRU or for receiving formed PTM pupae in the corrugated carton stripes and production of PTM elsewhere, in both cases, the bottom of this box can be easily opened or closed for either introducing new formed PTM pupae or for cleaning and discarding of dead moths and pupae cadavers, so, different steps, efforts and time required for transferring PTM moths from the PTMRU to another adult box are considerably minimized.

Regarding source of adult feeding, the fact that used honey solution for feeding moths is supplied externally by using a U shape drinking straw as shown in Figure 2 (A, B and C), allowed PTM moths to be fed through the soaked rolled tissue paper placed at the internal extremity of the same U shape straw, with minimum PTM moths escape, handling and time waste.

The fact that (B) part is divided into sectors(Sections) using plastic sheets as previously shown in Figures (4 and 5), and each sector is provided with a window for ventilation, it helps on one hand, in minimizing risks for disease spread among the whole amount of the used tubers due to raised humidity or infected ones,and also facilitate manual manipulation and /or interference, when needed, for each sector separately, and replacement of consuming or expired tubers with new ones without affecting the running rearing cycle in other sectors. Furthermore, dividing tubers into sectors permits, managing the production rate of different PTM stages easily through controlling and managing time intervals for tuber infestation per sector (Section) with PTM neonates accordingly, number of producing pupae or adults or even eggs is continuously controlled. The cover of each sector lateral hole with ventilated windows was designed for two purposes; firstly, allowing continuous ventilation and air exchange all over the sector, secondly, it permits manual interference when needed.

Regarding pupae formation, the use of corrugated carton strips placed among potato tubers in the part (B) of the PTMRU, considerably facilitate and reduced required manual interference and at the same time, minimized sources for contamination and additional required materials for pupae formation, such as sterilized sand or saw dust, sieves and separated recipients for pupae formation as

applied in conventional PTM rearing methods elsewhere worldwide. In our work, the formed pupae can be easily detected by a simple observation of cocoons filaments that plug the corrugated carton stripes' holes as shown in Figure 1 (A and B), then the PTM pupae can be easily liberated from their cocoons by longitudinal tearing the carton strips apart as shown in Figure 1 (C and D).

The dimensions, in terms of height and diameter, of part (A) and part (B) of our PTMRU described here are modifiable depending on one hand, on purpose and volume of PTM production, and on the other hand space availability for units' arrangement. In this regard, the PTMRUs can be arranged either vertically or horizontally according to available place and equipment. In case of arrangement of several units vertically, the part (A), can be modified by using a long cylinder with the same described system for inlet holes to serve as a common recipient for adult capturing from each level (Part B), when a large number of adults are needed as for example in the sterile insect release program (Figure 6).

Finally, the PTMRU's two main parts and their subparts could be easily disassembled, cleaned and disinfected with no need for intensive labor or extra time and the whole unit is made from available house hold commercial plastic tools for the kitchen and in case of need, it could be manufactured from durable autoclaveable materials and the same concept (Model) of this work can be adopted and/or inspired for rearing other insect species that yet hard to find a practical rearing method for them.

Figure (1):Harvested potato tuber moth (PTM) pupae formed in the provided corrugated carton strips (A and B), and liberation of formed PTM pupae by tearing apart the carton strips (C and D). Arrows indicate formed cocoon filaments (cf).

A B C D

Figure (2): (A) Shows adult box for potato tuber moth (PTM) egg production. The plastic box is covered by a circular piece of white paper (w) sitting on top of a black fine mesh net (b) that fitted tightly over the box's opening with an elastic band (e), then a piece of cork (c) and slight weight(r) were used to ensure contact between the placed white paper and the fine black mesh.
(B and C) Show feeding of PTM moths on nutritive solution 10% honey solution (hs) via a rolled tissue paper (tp) placed at the internal extremity of U-shape drinking straw (u).
(D) A simplified figure for adult box designed to be attached to part (A) of the potato tuber moth rearing unit (PTMRU) and used for either capturing and transferring of PTM moths for further work, or for direct production of PTM egg as well. The upper opening (uo) can be easily closed with the screw led or cap, or simply covered with black fine mesh net for egg production as previously described. The down opening (bo), can be easily opened for either introducing new formed PTM pupae or for cleaning and discarding of the dead moths and pupae cadavers.

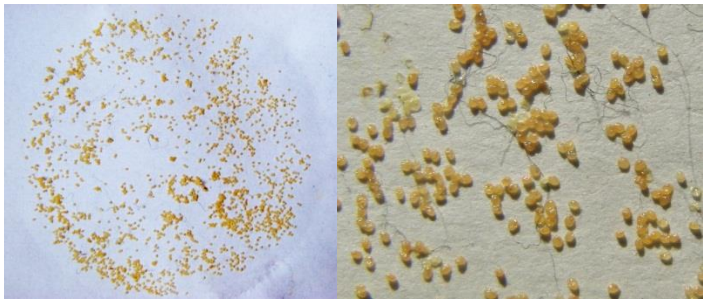


Figure (3): Potato tuber moth eggs produced on the provided white paper.

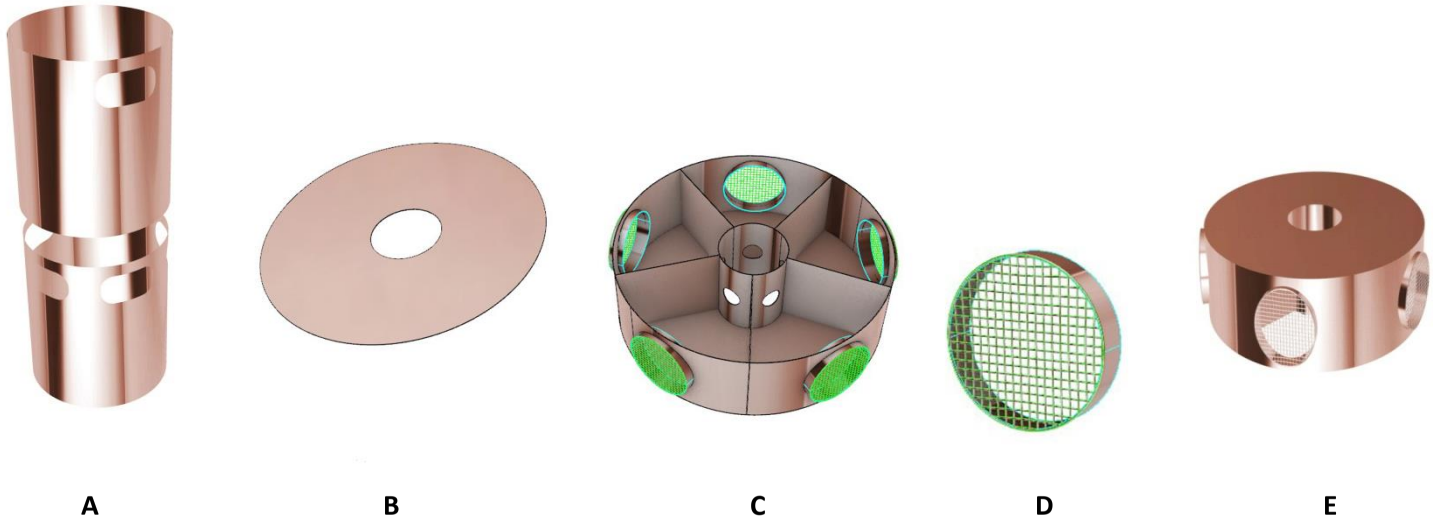


Figure (4): 3 D modeling of the PTMRU's parts: (A) Shows part (A) with its two plastic cylinders and their respective inlet holes. (B, C and D) Shows the upper cover or lid, a view for the internal sectors, and circular cover with fine muslin that is used to close lateral sector hole, respectively. (E) Assembled part (B) of PTMRU. (Refer to text for details of assembly).

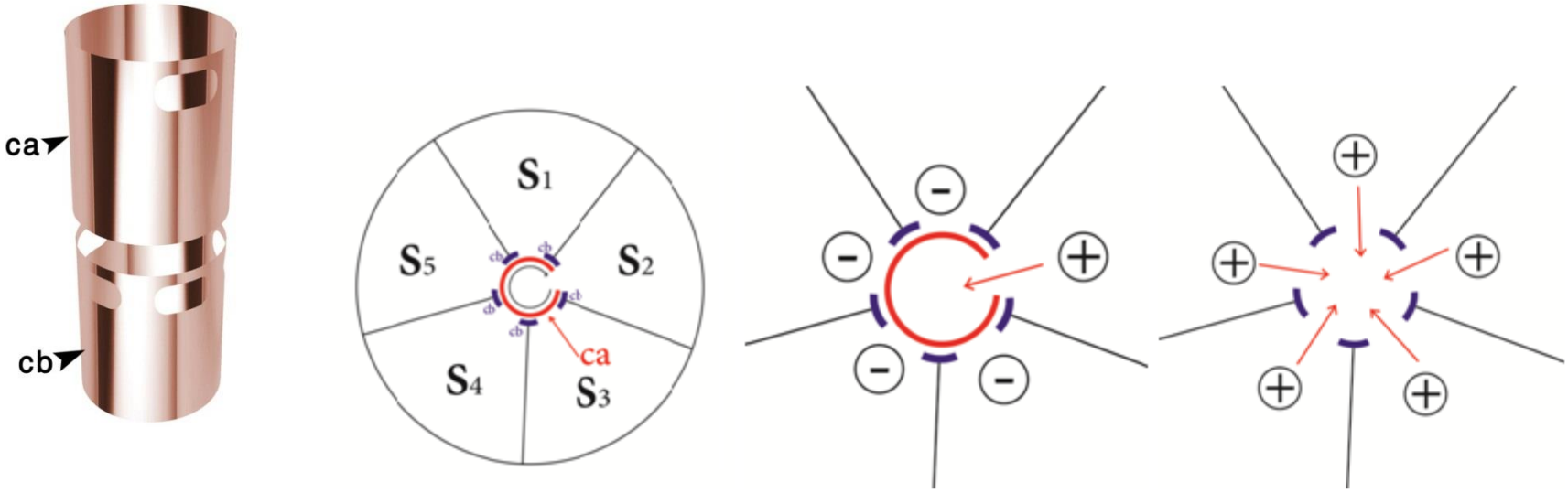
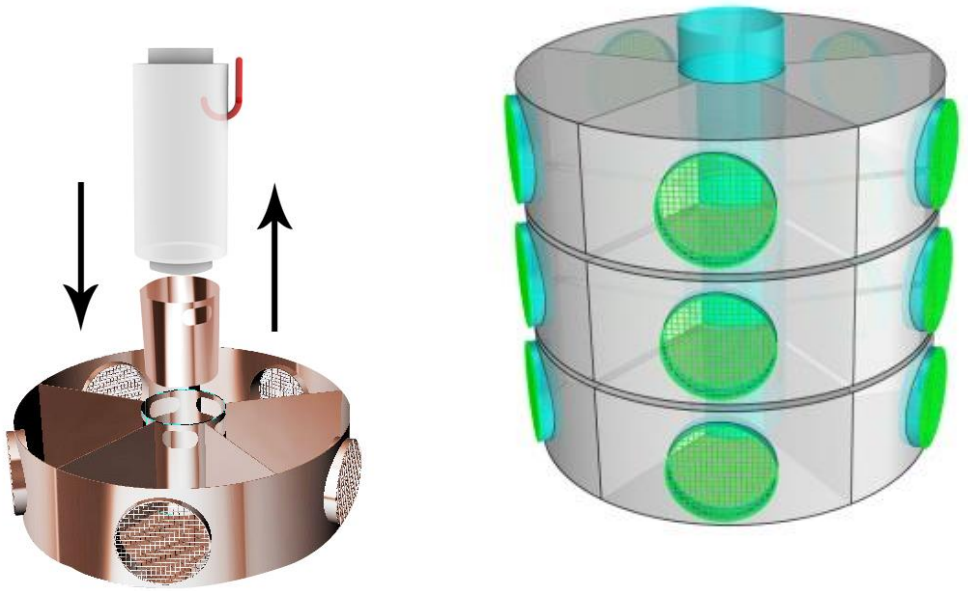


Figure (5): 3D modeling of the potato tuber moth rearing unit (PTMRU) part (A) and its subparts (cylinder a (ca) that placed inside cylinder b (cb) and their respective inlet holes), and illustration of different positions for the two cylinders inlet holes depending on desired potato tuber moth (PTM) capturing mode.
(A) The two cylinders (ca) and (cb) with their respective inlet holes.
(B) Illustration of positioning of (ca) and (cb) cylinders among different sectors (S) of PTMRU's part (B).
(C) Positions of (ca) and (cb) cylinders inlet holes when PTM moths are captured selectively (From one sector). Note that the signs (+) and (-) denote allowed or not allowed access respectively, for PTM moths from sector(S) to the part A cylinders.
(D) Positions of (ca) and (cb) cylinders inlet holes when PTM moths are captured collectively (From all sectors at the same time). Note that, when the collectively capturing mode is desired, the (ca) is pulled up so it doesn't appear in the illustration).



A B

Figure (6): (A) 3D modeling for disassembled parts and subparts of PTMRU (Arrows indicate directions of assembly or disassembly of the rearing unit). (B) Vertical arrangement of several PTMRUs with an elongated cylinder that serves as a common part (A) for capturing PTM moths.

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