

Optimizing mass rearing of *Periplaneta americana* (Linnaeus, 1758): Enhancing collection devices and rearing unit management for homogeneous and heterogeneous populations

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Abstract

The American cockroach, *Periplaneta americana* which is a domiciliary pest and mechanical vector of various kinds of disease and micro-organisms requires specialized sanitation procedures and optimized rearing techniques in laboratory condition. The odoriferous smell associated with cockroach various predators, hence dilution of odour and reconstructing rearing tanks with minimal changes enhancing ventilation create ideal condition for handling the insects during conducting experiment. Standard operating procedures (SOP) and Personal Protection Equipment's (PPE) such as goggles, double layered mask, silicon hand gloves, hair net, sanitizers are preliminary requisite while handling with cockroach population. Monitoring and inspection play a major role in checking the blowfly population, dead cockroaches, microbial contamination and predation effect. A handmade plastic box for collection of cockroaches from manhole supports optimal population next to sewage collectors compared to various other sources which consumes more energy and time.

Keywords: American cockroach, odour reduction, ventilation, aluminium mesh, personal protective equipment, sanitization, predation

Introduction

The American Cockroach, *Periplaneta americana* (Linnaeus) which is a widely distributed urban pest, known for its ability to reproduce and resilience (Addy *et al.*, 2021) ^[1]. They were found in homes and shops (Lee and Lee, 2000) ^[11]. American Cockroach is a major mechanical vector transmitting *Salmonella*, *Shigella* and *Cryptosporidium parvum* bacteria causing diarrhoea, nosocomial infections in hospitals and cause allergenic symptoms (Majumdar *et al.*, 2016) ^[12]. They were the most notorious and important medical pest found in and around homes (Akbari *et al.*, 2015) ^[2]. They can hold and spread bacteria, fungi, parasite and protozoic pathogens (Roh *et al.*, 2007 and Kinfu and Erko, 2008) ^[10, 18]. They are omnivorous scavengers which survive in warm place with high moisture and thrive in dirty places like drainage and sewage manholes (Jaramillo-Ramirez, 2010) ^[8]. They were major nuisance in Indian homes which were relatively larger in size compared to German cockroaches. They passively transport microorganisms, through their body surface, faecal matter and contaminating food through while feeding. They spend most of their time in sewage which contain high density of pathogens and were known to spread innumerable pathogens through their cuticle and exuviae by morphological and biological ways (Basseri *et al.*, 2016, Pai *et al.*, 2005, Allen, 1987 and Mpuchane *et al.*, 2006 a and b) ^[3, 6, 13, 14, 16]. The vertical transmission of pathogen from female cockroach to progeny take place (Jennifer, 2008) ^[9]. Besides cockroaches also secrete a mixture of Xanthurenic acid, kiturenic acid and 8 hydroxyquinoline acid, which are tryptophan derivatives that have mutagenic and carcinogenic properties (Mullins and Coachan, 1973) ^[15]. It triggers allergic response to people who were sensitive to cockroach. Hence it is ideal to maintain proper sanitation and safety protocols while handling cockroach populations.

The experimental setup was designed in such a way that it never affects the development and behavioural activity of homogenous population. The personal protection equipments required while handling was listed and the sanitation procedures, food selection, changes in rearing unit and handmade plastic boxes for collection from manhole were essential which supports adequate populations and safe level while handling. Monitoring and inspection during rearing and during experimentation were ideal for subsequent maintenance and handling were detailed below.

Materials and Methods

1. Collection of Ootheca of American Cockroach

The American cockroach ootheca's were collected from kitchen cabinets for mass rearing of American cockroach. The majority of ootheca were from buildings which were not maintained in a sanitized condition. The adults of American cockroach were traced back in the night to explore ootheca availability. The gutter pit filled with soil has the most ootheca availability and were collected to establish the population.

2. Mass rearing of American cockroach, *Periplaneta americana*

A separate fish tank of size 45 x 30 cm was used to rear the American cockroaches. The cockroach population were provisioned with egg cartons for sheltering. The cockroaches were provided with dog food (Tunaz *et al.*, 2009, Gaire *et al.*, 2017) ^[7, 19] as it contains necessary nutrients and on the other hand dog food was far more comfortable in handling compared to other foods and biscuits which absorbs moisture over the surrounding atmosphere which leads to microbial contamination. A 25 ml sauce cup were used for provision of water, where cotton balls wrapped in tissue paper were placed to avoid drowning

of nymphs and to aid drinking. The foods were replaced within week and contaminated foods were removed immediately and disinfected to check the cockroach population. The fish tank was sanitized using disinfectant and sun dried in a weekly interval. The insects were anesthetized using ethyl ether. The ethyl ether was taken in cotton squeezed and placed inside the fish tank and closed with glass top to anesthetize the cockroach populations for a minute.

3. Collection of heterogenous population of American cockroach from manhole

A handmade plastic box was devised to collect American cockroach from drainage manhole in Annamalai Nagar, Chidambaram, Tamil Nadu. A hole was made on the sides of plastic box. The stick was attached to the plastic box aside the hole, where the stick length equals the diameter of the manhole and was rigid enough to touch with the edges of the manhole where it facilitates entry of cockroach through the stick and enters the plastic box. The plastic box was placed with sugars, butter and vegetables which aids as a food attractant. The setup was left undisturbed overnight to collect cockroach. The plastic box was smeared with Vaseline (Wang *et al.*, 2021^[20] and Baldwin and Koehler, 2007)^[5] to prevent cockroach population from escaping. The collected cockroaches were transferred to separate glass containers and provisioned with dog foods to acclimatize into lab condition.

4. Laboratory protocols and personal protective equipment's

Since, American Cockroaches were carrier of various microorganisms, it has to be held with utmost care while handling and conducting experiments. Sanitation works were carried out to make a conducive environment for caring out experiments. Personal protective experiments and standard operating procedure were carried out. Personal protective equipment's such as goggles, silicon hand gloves, sanitizers, hair net while handling cockroach population. The treatment boxes and fish tanks which were used for rearing containers were washed in disinfectant in a weekly interval. The lab was well aerated to minimize the odoriferous smell due to rusty odour and pheromone such as periplanone B (Persoons *et al.*, 1990)^[17] associated with cockroach. A biosafety symbol was placed in front of the lab and the rearing containers. The lab safety protocols were placed in the entrance and the potential risk associated with it. Some of the essential protocols were wearing mask while entering the lab and sanitizing hands after leaving the lab.

Results and Discussion

The mechanism behind bulk collection of cockroaches is that when the water levels over the manhole raises, so that the cockroaches cannot escape and comes outside where manual collection using sewage workers can hold more collection of cockroaches compared to various designs. But considering the ill effect to sewage collectors and associated sludge, this low-cost design using plastic boxes for collecting is designed in such a way that one which enters cannot crawl out of the box as it was smeared with Vaseline (a petroleum jelly). This structure supports collection of required cockroach population for conducting study. Majority of the cockroach collection is from sewage manholes, considerably from kitchen wastes, pipelines and

populations were found to be less in wooden structures, water absorbed cement buildings and crevices, wooden barks. Large collection of cockroaches is possible only from manhole and gutter pit and all other possible resource where difficult as it found to compress its body inside crevices. The American cockroach population were identified using taxonomic keys (Al-Hussain, 1962)^[4].

Acclimatization of heterogenous population

Once the cockroach populations were collected, they were transferred to the required rearing containers. The cockroach often gets dead due to overcrowding and suffocation. Prolonged time leads to more unpleasant and associated odours of food and dead cockroaches while handling. They were transferred to the rearing containers which was smeared with Vaseline to avoid cockroach populations from escaping. The Vaseline were smeared in such a way, leaving less space so the insect can be anesthetized with solvent.

Maintenance between heterogenous and homogenous American cockroach populations

Unlike homogenous populations heterogenous populations are more prone to microbial contamination and shows sensibility to moisture, overcrowding which results in death of population. Whereas homogenous population are more convenient to maintain sanitation. The homogenous population were sanitized once in a week and were in good health condition compared to heterogenous population which requires daily monitoring of inspecting healthy adult and nymph. It is much essential to remove the dead cockroaches earlier using forceps as it may cause blowfly population to scale up and to avoid unpleasant odour.

Abiotic factors during maintenance of American cockroach population

The initial setup was maintained in a laboratory condition but eventually overclouding of odours were pronounced upon increase in population. In heterogenous population, apart from specific odours, the unpleasant odour from sewage is bounded hence it is necessary to minimize the odour residue while handling cockroach population. Hence the entire setup was well ventilated using fans and windows were kept open during day so that it would be easier to handle insects while handling. Wearing two layered mask and N95 mask cannot resist the odours associated with cockroach, thereby the only way to reduce the accumulated odour by ventilating during the day time.

Predation threats associated with culturing American Cockroach populations

Although culture can be kept open as Vaseline, mineral oil and any other petroleum jelly prevents escape of cockroach, but the ensign wasp, were found to invade the cockroach population and parasitize ootheca of cockroach. Lizards, frogs were found to reside near the containers to the odour effect of cockroach populations. Ants on the other hand destroys the colonies and ootheca during the initial days of rearing cockroach. The predations were completely avoided by using chalk and naphthalene to avoid ants entering the fish tank. Plastic cups with water placed in foot of the table to avoid ant entry to the respective rearing fish tank. The chalk was lined all over the tank to avoid enters entering out and also prevent escape of cockroach population. The frogs were completely removed and kept in check as it may

welcome other reptiles inside the laboratory. The lab was completely inspected daily to monitor the windows in closed condition and any alarming threats to cockroach population. The room freshener was used around the corners to subsidize the odours which reduced predation pressures imposed for cockroach populations.

Fish tank design for rearing cockroach populations

Initially the fish tank was covered with plastic sheets and pin holes were made for ventilation. Although the cockroach population were found to growth without any change in development and behavioural activity, the accumulated odour and warm humid nature was not ideal to handle the culture for treatments. Hence, after odour regulation the setup was mounted with aluminium mesh which gives more ventilation and also checks odour regulation and convenient to handle. The aluminium mesh sheet requires one-time investment unlike plastic sheet which is a weekly replacement which require continuous plastic wrapper and tapes consuming more energy, cost, time, inconvenience while handling. The dog food is convenient and completely devoured by cockroach unlike other biscuits and foods which absorbs moisture and harbour microorganism culture and odours. The moisture absorbing foods and degrading foods is where cockroach deposits ootheca for food availability for the young nymphs as they come out of ootheca. The dog food has not been noticed with ootheca deposition and microorganism accumulation. The ideal food for laboratory rearing of cockroach is dog food. The aluminium mesh was placed over the fish tank during day and it can be alternated with glass top with minimal space for air circulation during night.

Conclusion

The rearing and maintenance of domiciliary pest, American Cockroach, *Periplaneta americana* requires more sanitation procedures and safety protocols for the working individuals as all other pest consumes less space, shorter life cycle and size. Insect rearing techniques has it own challenges and differs according to life cycle, morphology, physiology and developmental activity of pest and its own constraints to be grown in a laboratory condition. The developmental period of adult American cockroach is higher to other cockroach population which requires proper sanitation procedures especially during nymph to adult stage. The rearing techniques using odour subsidization checks the ideal growth and behavioural condition of cockroach populations. The selection of food most preferably relying on dry foods helps in reducing the sanitation intervals. Monitoring and inspection frequently avoid predation, dead cockroaches, checks blowfly population and threats during rearing of cockroach populations. Future aspects of designing cages for segregation of different instar, ventilation and faecal matter removal could be ideal in maintaining population in a good condition.

Conflicts of interest

The authors declare no conflict of interest.

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