ejpmr, 2022,9(7), 233-239

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

A REVIEW ON INSECT COLLECTION AND PRESERVATION TECHNIQUES

Manoj Kumar*, Rakesh Ranjan, M. P. Sinha, Anjali Dhan, Fahreen Naaz, Gulfishan Khanum, Kumari Anita Rani, Sejal Sharma and B. S. Raipat

Department of Zoology (Entomology specialization), St. Xavier's College, Ranchi – 834001, Jharkhand, India.

***Corresponding Author: Dr. Manoj Kumar**

Department of Zoology (Entomology specialization), St. Xavier's College, Ranchi – 834001, Jharkhand, India.

Article Received on 13/05/2022 **Article Revised on 03/06/2022** **Article Accepted on 23/06/2022**

ABSTRACT

Insects are found in every terrestrial environment on the planet and play a crucial role in the evolution and preservation of biotic communities. They are important pollinators of blooming plants, consumers and recyclers of decaying organic materials, and vital components of vertebrate and invertebrate food webs. Insects are collected for various purposes. The final purpose of the samples obtained determines the insect collection methods. Some individuals collect insects as a pastime for their beauty and diversity, while others collect them for their courses in biology and related subjects. The insects are also collected by experts for research works. Whatever the purpose may be, the insect collection and preservation need expert inputs so that the samples are preserved in their native appearance as far as practicable.

KEYWORDS: Insect collection, Killing jar, Aspirators, Preservation.

INTRODUCTION

The arthropoda consists of insects, spiders, mites etc. Arthropoda is one of the most successful organisms on the earth. The insects alone make up around 55% of all the species known till date (Kumar and Saksena, 2021; Barrowclough, 1992). For any study related to insect, one of the primary tasks is collection of insects followed by their preservation for later studies.

Insects, spiders, mites and other arachnids live in every terrestrial ecosystem of the world and play an important role in the evolution and preservation of biotic communities. They are key pollinators of flowering plants, important consumers and recycles of decaying organic matter, and vital components of a variety of food webs.

For the reasons stated above, as well as many others, the study of insects and their relatives is important as society faces increased challenges to preserve and improve environmental quality, reduce pesticide usage, increase crop production, control food costs, and increase trade in the global community (Schauff, 2001).

The insect behaviour may be seen most closely in their native habitats. However insects must occasionally be gathered and carefully maintained before they can be identified and classified. Since accurate identification on the spot is sometimes not possible, the specimens must be preserved in the best possible condition for any later references (Schauff, 2001).

Insect collection may be done for diverse of causes. Novice entomologists often begin collecting insects to get aware with subject and diversity of insects. University and research students may start collecting insects as a part of fulfilment of their course of studies, while ecologists, taxonomists maintain a collection of the insects they are studying, or even for reference purposes. There are many who collect insects, just to satisfy their aesthetic needs (Murray and Mantle, 2010).

This review focuses on insect collection and preservation procedures, however much of what is covered here will also apply to other related groups.

1. Equipment required.

1.1. Basic equipment

Small samples are generally sufficient for students and hobby insect collectors. However for identifying major pest insects and mites, they should be gathered in series if at all possible. If more than the needed number of specimens are gathered, the excess specimens should be kept or swapped with other collectors, as it is not always possible to collect and get the same specimen repeatedly.

While picking up insects by hand is simple and sometimes efficient, their size, movement, and the risk of being bitten typically need the use of various types of equipment and methods for collecting insects. Forceps, vials holding alcohols, killing bottles, small boxes for keeping specimens after removal from killing bottles, aspirators, cotton, a notepad and a hand held magnifying glass are essential tools needed for insect collections.

The list may be customized to meet specific collection requirements.

1.2. Insect net

Insect nets may be easily made at home. The necessary parts are a handle, hoop or ring attached to it and a cloth bag hung from the ring. The handle should be strong and fairly light. The ring is made up of steel wire, which if bent by rough usage springs back to its round shape and will stand a great deal of wear and tear (Figure 1).

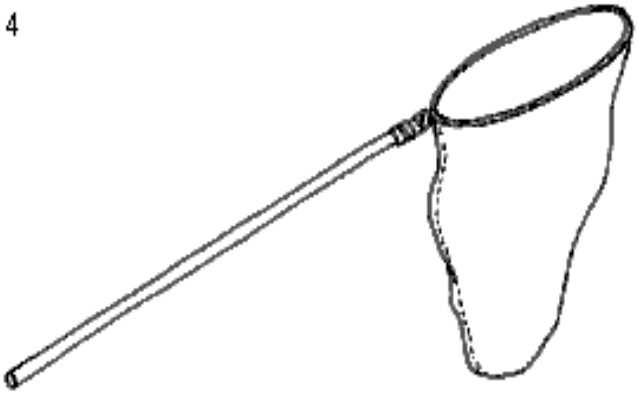

Figure 1: Insect net construction.

1.3. Aquatic dip net

The aquatic insects are collected using a dip net. It's like an aerial net, but considerably shallower (no deeper than the diameter of the rim) and much stronger. The handle should be substantial, and the rim should be formed of metal rod that is tightly connected to the handle. Dip net rims are made into the shape of the letter D. Dip nets are useful for catching free-swimming insects, insects on plants, and insects digging in the sand (Singh, 1981).

1.4. Killing jar

Effective collecting of insects and related groups usually requires that the specimens be killed so that they may be properly mounted and studied. The most widely employed method for killing of specimens is the killing jar (or bottle).

Killing bottle are prepared with killing agents, which kill the insects immediately without affecting their colours or unduly hardening them. Although potassium cyanide is a widely used killing agent, but owing to its extremely poisonous nature, ethyl acetate is most commonly used killing agent in killing jar. There are many other killing agents used, which are described in upcoming sections.

An empty plastic bottle with lid is used. Cotton or plaster of paris is used as absorbent for liquid killing agent (such as ethyl acetate). Once an insect is trapped it is transferred to the killing jar, with cotton containing ethyl acetate (on any other killing agent) inside the jar. At the end of each day, take out the insects collected in the bottle for preservation. The bottle may be charged with killing agent for each day of collection. The killing bottle should be labelled as POISON and kept out of reach of others, specially children. The killing bottle should not be overcrowded with specimens, overcrowding bottles with tough and fragile as well as small and large insects may result in damaging of all of them.

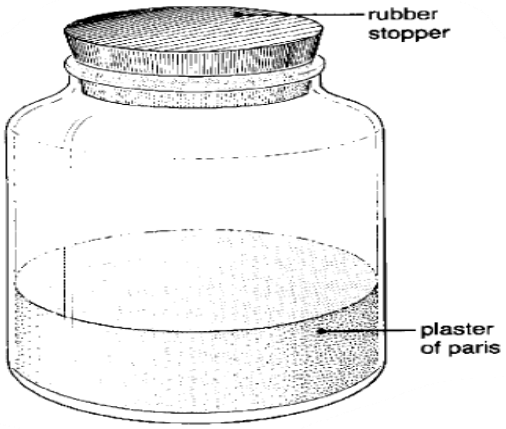

***Figure 2: Killing jar (*Schauff, 2001).**

1.5. Killing agents

On the basis of types of killing agents, different types of jars are employed.

1.5.1. Liquid killing agents

Jars for use with liquid killing agents are prepared by pouring about 2.5 cm of plaster of paris mixed with water into the bottom of the jar and allow the plaster of paris dry. Enough of killing agent is then added to saturate the plaster; any excess of killing agent should be poured off.

The liquid killing agents used in the killing jars are ethyl acetate, ether, chloroform, ammonia water.

Ethyl acetate is most widely used. Its fumes are less toxic to humans than those of the other agents. Although it usually stuns insects quickly, it kills insects slowly. Thus specimens that appear dead may revive after removal from the killing jar containing ethyl acetate as killing agent. One of the crucial advantage of using ethyl acetate is that it delays hardening of insects. All these chemicals are extremely volatile and flammable and should never be used near fire. Non-supervised use of these chemicals is not advisable.

1.5.2. Solid killing agents

The most prevalent solid killing agents used in the killing jars are potassium, sodium or calcium cyanides. Because cyanides are toxic, fast-acting poisons with no antidotes, they should be handled with great caution.

If even a single grain comes into touch with the skin, it should be rinsed immediately with water. Cyanides should be stored and disposed of in a safe way (Ghoneim, 2021; Banks, 1981; Pennington, 1967)

1.6. Aspirator

An aspirator is a highly handy tool for catching small insects while they are still alive. Various types of aspirators have been developed, however the vial type of aspirator is one of the simplest and easiest to device and use. Sucking via the mouthpiece draws small insects into the vial, and placing a cloth over the inner end of the mouthpiece tube prevents the insects from being pulled into the mouth.

For serial collection, a series of vials may be fitted to the cork with aspirator arrangement, and then an insect-filled vial can be removed and replaced with an empty one (Singh, 1981).

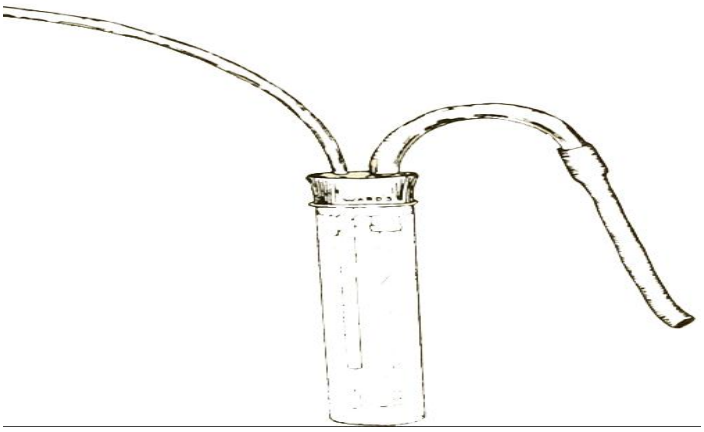

Figure 3: Aspirator.

1.7. Beating sheet

A beating sheet should be made of robust fabric, preferably white, and secured to a 1 metre square frame with two pieces of doweling or other light wood crossed and fitted into pockets at each corner of the cloth. A standard light-colored umbrella can be used as a pounding sheet as well. Place the beating sheet or umbrella under a tree or shrub and violently beat the branches or leaves with a club or stick. Specimens will fall onto the sheet and can be retrieved from the light-colored material by hand or with forceps, a moist brush, or an aspirator.

Finding insect specimens on the sheet may be troublesome sometimes because of leaves or other undesired things falling onto the sheet. Looking for movement will help with distinguishing the specimens, tilting the sheet helps in dispersal or even falling off of the detritus, leaving the insects and mites gripping to the linen (Schauff, 2001).

1.8. Pitfall traps

The main agent in pitfall traps is the odour of the specific type of food or pheromones (sex attractants). Overripe fruit, pieces of meat or fish, and anything else that attracts insects are examples of attractants. These attractants can be strategically put in areas where insects congregate. One of the most basic is the pit fall trap, which consists of a jar with an attractant that is put below the soil surface to collect crawling insects.

Pitfall traps are often plastic containers that might be coloured, clear, or even white. Depending on the needs, the container's diameter ranges from 0.25 cm to 50 cm and its height extends from 5.2 cm to 65 cm.

(Hohbein and Conway, 2018; Singh, 1981).

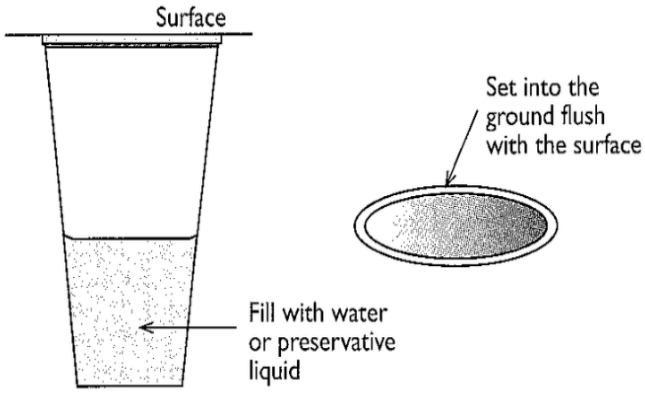

Figure 4: Pit fall trap **(Bonnett *et al.*, 2009).**

1.9. Light traps

An artificial light (kerosene lamp, gas light, led lamp), if placed adjacent to a white muslin cloth in field attracts a number of insects like crickets, grasshoppers, moths, mantids, beetles, etc. most of the insects attracted to light would rest on the white cloth from where they may easily be picked up by hand or aspirator.

Light traps (figure 5) can be constructed easily from materials generally available at home (Singh, 1981; Schauff 2001).

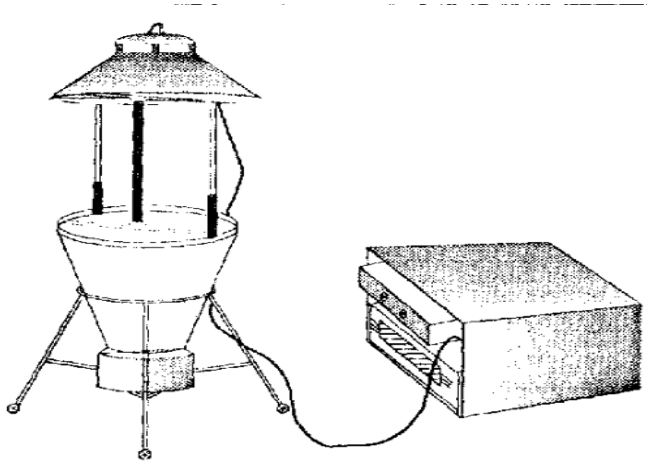

Figure 5: Light trap **(Mathew and Rahamathulla, 1995).**

2. Specimen preservation

Insect specimens must be killed and preserved after successful collection.

2.1. Liquid agents for Killing and Preserving

All types of insects and mites can be killed and kept in liquid agents, however it's necessary to examine if a liquid killing agent is preferable to a dry gaseous death agent. Ethanol coupled with water is the best overall killing and preservation agent (75 percent). Other preservatives, as well as higher or lower alcohol concentrations, may be appropriate for specific insects and mites. Isopropyl alcohol can be used in place of ethanol. Isopropyl alcohol does not have the same tendency to harden the specimens as ethanol (Schauf, 2001). Formalin (of any grade) should not be used since it excessively hardens the tissues and makes the specimen difficult to handle. In order to fix their protein and keep the specimen from turning black, insect larvae must be caught in alcohol and then fixed.

2.2. Temporary storage of specimens

Collectors may not always have enough time after collecting specimens to permanently keep them. There are several alternatives for maintaining them in good condition until they can be mounted and conserved properly.

2.2.1. Refrigeration and Freezing

Medium to large specimens can be stored in tightly closed bottles in the refrigerator for many days and still be appropriate for pinning, as can microscopic specimens if left overnight. When freezing, some humidity must be present in the container to prevent the specimens from freeze-drying.

As described in the preceding section, the insects can be kept in alcohol and conserved for several years before being pinned or otherwise treated. Little insects can decay when kept in alcohol for a lengthy amount of time; this can be avoided by freezing them.

2.3. Dry preservation
Many types of insects (Excluding soft-bodied insects) are stored dry in tiny boxes, paper envelopes, and other containers.

2.4. Liquid preservation
Ethanol and isopropanol mixed with water are the most often used preservation fluids. The most frequent alcohol proportion used is 75% (V/V). Because alcohol and water do not mix well, the water used to combine with the alcohol should be distilled water, and the solutions should be thoroughly mixed (Schauf, 2001).

2.5. Spreading insects
When most insects are pinned, the position of their legs or wings is inconsequential as long as all of the body parts can be seen and studied; however, the wings of moths, butterflies, and maybe other insects should be spread before the insect is placed in the collection box.

A spreading board is used to spread the dorsal side of an insect's wings. A spread insect's wings have a set of regular locations. In butterflies, moths, and mayflies, the rear borders of the forewings should be straight across, at right angles to the body, and the hindwings should be far enough forward so there is no gap at the back.

The wings are kept in place by strips of paper or other material affixed to the board, as seen in figure 6, while the antennae and other structures are aligned and held in place by pins. Once the wings are in position, crossed pins may be used to secure the antennas (Singh, 1981).

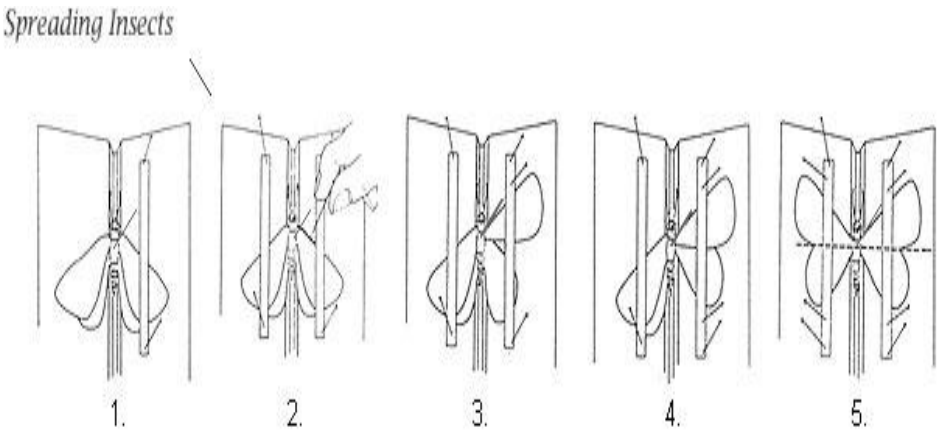

Figure 6: Spreading of insects **(Johan, 2017).**

2.6. Mounting and Preserving specimens
Once a collection has been made, it must be preserved in a method that allows for both identification and study of the specimens as well as long-term storage with proper care.

Insects can be maintained in a number of different ways. The bulk of the specimens are pinned, and once dried, they will last indefinitely. Specimens that are too tiny to pin might be put on microscopical slides or minuscule pin points. In a variety of glass-topped exhibit cases, large, spectacular insects such as butterflies, moths, grasshoppers, dragonflies, and damselflies may be seen.

Midges, caddisflies, mayflies, and stone flies with soft bodies, such as nymphs, larvae, and adults, should be maintained in 70% alcohol.

2.6.1. Preparing dry specimens for mounting
Dried insects need to be relaxed before they are pinned, i.e. moistened again to soften their muscles so that the specimen does not break when the pin is inserted. Before pinning, the wings of insects such as those of lepidopterans must be spread. Small insects must be kept in relaxing chambers for at least 8 hours, whereas larger insects can be kept in relaxing chambers for at least 24 hours (Lane 1965, Schauf, 2001).

2.6.2. Preparing liquid-preserved specimens
The majority of liquid-preserved specimens should be withdrawn from the liquid in which they were stored to ensure that they dry with little deformation and matte. Before drying, samples that have been in the preservative solution for a long period should be cleaned with a clean solution.
Specimens having a firm exoskeleton, such as beetles and insects, can be pinned directly after being taken from the liquid preservative.

2.6.3. Direct pinning
The insertion of a normal insect pin directly through the body of an insect is known as direct pinning. It is important to highlight that only entomological pins should be utilised. Ordinary pins are overly short and thick, as well as having other flaws. Pins with a diameter of no. 2 (0.46 mm) are the most useful. In the case of larger insects, larger pins can be used if desirable and practical. (Peterson *et al.*, 1961; Landry and Landry, 1994).

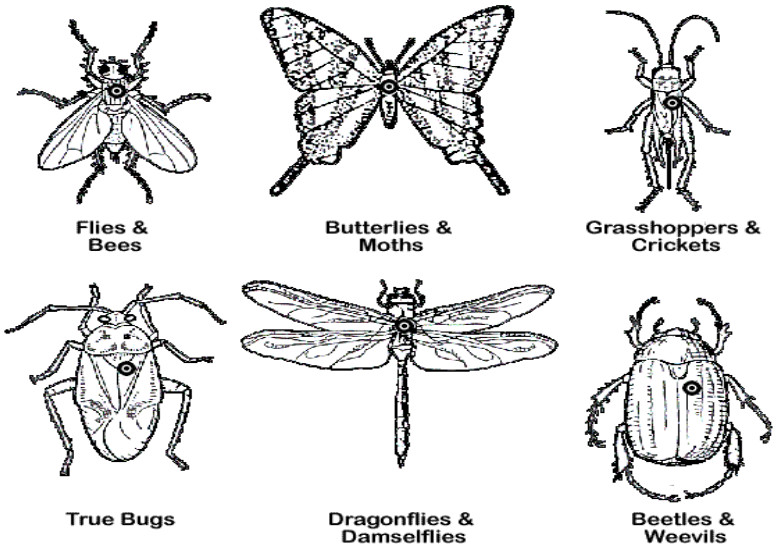

Figure 7: Most preferable pinning style for insects of different orders.

When the insects are dry, a drop of glue may be needed to keep them attached to the pin; otherwise, the specimen will become loose and revolve around the pin. In the event of recently dead insects, glue may not be required. Insects of different orders should be pinned in different ways, as indicated in figure 7, so that no insect body characteristics are concealed. The following are some standard methods for pinning some of the more common sorts of insects

- **Orthoptera**

In orthopterans, the pin should preferably pass through right of mid-dorsal line. For display of wings, one pair of wings may be spread (as shown in figure 7).

- **Heteroptera**

In heteropterans the pin should preferably pass through triangular scutellum to right of mid-dorsal line as shown in figure 7. Wings may not be spread.

- **Hymenoptera**

In hymenopterans the pin should preferably pass through thorax between or a little behind the base of forewings and to right of mid-dorsal line (figure 7).

- **Coleoptera**

In coleopterans the pin should preferably pass through right wing cover near base such that pin exits through the metathorax between the hind legs. Wings may be spread if required.

- **Lepidoptera**

In leipdopterans the pin should preferably pass through middle of thorax at the thickest point (figure 7). Wing may be spread so that venation is clearly visible. Same can be followed in case of odonatans.

2.6.4. Double mounts

As certain insects are too small to be placed directly on a pin, they must be fastened twice. As seen in figure 8, the bug is mounted on a minute or card point, which is then mounted or fastened to a pin.

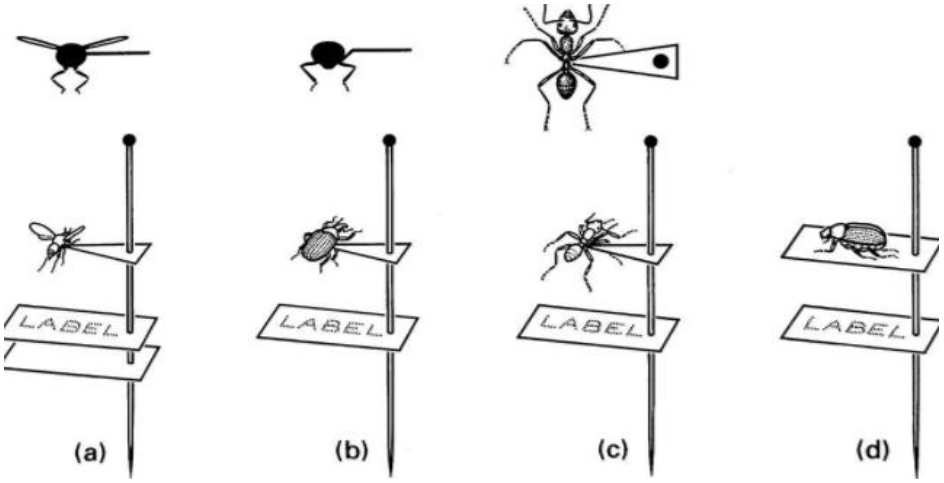

Figure 8: Double mounting.

CONCLUSION

As mentioned in the text, the insects are collected for different purposes, they can be collected either only for hobby purpose, or by students during their academics in schools and colleges as part of fulfilment of the degree/course, or they can be collected for research and study purposes. Whatever is the purpose of collection of insects, their collection and preservation needs to be done in a manner that will lead to preservation of their native form for as longer period as possible. Thus keeping in consideration all these factors, this review article was written to review and present the techniques involved in collection and preservation of insects in a simple and comprehensive language with diagrams wherever possible.

REFERENCES

1. Kumar R, and Saksena S. Insect Collection and Preservation Techniques. In Advances in agricultural entomology. Ed. Karem Ghoneim. Akinik Publications, New Delhi, 2021.
2. Barrowclough GF. Systematics, biodiversity and conservation biology, 1992; 121-143.
3. Schauff ME. Collecting and preserving insects and mites. Systematic Entomology Laboratory, USDA National Museum of History. Washington, DC, 2001
4. Murray S. and Mantle B. L. Methods for collecting, preserving, and studying insects and other terrestrial arthropods. Miscellaneous Publication Fifth Edition, 2010; 3. The Australian Entomological Society.
5. Singh R. Elementary knowledge of collection and preservation of insects, 18-28. In Elements of Entomology, 2nd Edition. Rastogi Publications, Meerut, India, 1981; ISBN 978-93-5078-098-5.
6. Ghoneim K. Advances in Agricultural Entomology. Akinik Publications, New Delhi, India, 2021; 15.
7. Banks W. A. Techniques for collecting, rearing and handling imported fire ants. U. S. Department of Agriculture., Agr. Res. Serv, 1981; AAT-S-21, 9.
8. Pennington N. E. Comparison of DDVP and cyanide as killing agents in mosquito light traps. J. Med. Entomol, 1967; 4: 518.
9. Hohbein R. and Conway C. A review of methods for estimating arthropod abundance: Pitfall traps: Estimating arthropod abundance. Wildlife Society Bulletin, 2018; 42(4).
10. Bonnett, SAF, Ross S, Linstead C, and Maltby E. A review of techniques for monitoring the success of peatland restoration. Natural England Commissioned Report, 2009; NECR086.
11. Mathew G, and Rahamathulla VK. Biodiversity in the Western Ghats – A study with reference to moths (Lepidoptera: Heterocera) in the Silent Valley National Park, India. Entomon, 1995; 20(2): 25-33.
12. Johan J. I. Le Nobel (2017). Spreading Insects, in Extreme Macro. url: http://extreme-macro.co.uk/spreading-insects/ Visited on 17-06-2022; 10:25 IST.
13. Lane J. The preservation and mounting of insects of medical importance. WHO/Vector control, 1965; 152.65, 27 pp. Mimeographed.
14. Peterson B. V., McWade J. W. and Bond E. F. A simple method of preparing uniform minute pin double mounts. Bull. Broklyn Entomol. Soc, 1961; 56: 19-21.
15. Landry J. F. and Landry B. A technique for setting and mounting microlepidoptera. J. Lepid. Soc, 1994; 48: 205 – 227.