STERILIZATION OF PLANT TISSUE CULTURE EQUIPMENT

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Abstract

This practicum aims to learn the methods and principles of sterilization of tools and materials in tissue culture techniques. The function of the sterilized tools is to avoid the presence of microorganisms that are still carried by the tools that will be used, because the presence of microorganisms causes contamination and can even grow bacteria that are not completely sterile. Tissue culture tools were carried out on December 8, 2019 at the Yahdi Tissue Culture Laboratory, Jl. Ambung, Tanah Enam Ratus, Medan Marelan, Medan City, North Sumatra.The materials used in the Tissue Culture Tool Sterilization practicum include: detergent/soap and clean water. The tools used in the tissue culture tool sterilization practicum include: culture bottles. The conclusion of this practicum is that the Sterilization tools are carried out in two stages, namely Stage I Sterilization, the bottles are sterilized or cleaned with detergent and soaked for 10-15 with bleach water (pr°C lin), while in Stage II Sterilization, the tools are sterilized using an autoclave at a temperature of 1210 C for 1 hour 20 minutes.

INTRODUCTION

Tissue culture is one way of vegetative plant propagation, namely a plant propagation technique by isolating plant parts such as leaves, buds, protoplasm, cells, groups of cells, tissues or organs; and growing these parts aseptically in artificial media rich in nutrients and growth regulators in a closed container that is translucent so that the plant parts can grow.many selves and regenerate into complete plants (Sany 2007: 1).

Equipment used in a research or practicum must be sterilized first to free all materials and equipment from all forms of life. Sterilization is a process to kill all organisms contained in an object. The sterilization process can be divided into 3 types, namely, the use of heat, filtration, and the use of chemicals (ethylene oxide, acid, peracetate, formaldehyde and alkaline gluturaldehyde) (Hadioetomo, 1993).

Sterilization is a process to kill all organisms found on or in an object. Wet sterilization can be used to sterilize any material that can be penetrated by water vapor and is not damaged when heated at temperatures ranging from 110°C -121°C. Sterilization in every process, whether physical, chemical or mechanical, that kills all forms of life, especially microorganisms or efforts tofree tools and materials from all forms of life, especially microbes (Fardiaz 1992: 4).

According to Hamdan (2012: 11), sterilization in every process that is commonly done can be: (a) Physical sterilization (heating, use of short-wave rays that can be done as long as the chemical compound to be sterilized will not change or decompose due to high temperature or pressure). With hot air, a "hot vessel/chamber" tool is used (an oven with a temperature of 170°C - 180°C and a time of 2 hours which is generally for glassware); (b). Chemical sterilization, for example by using disinfectants, alcohol solutions, formalin solutions; (c). Mechanical sterilization, used for several materials that will change due to high heating or high pressure, for example with a filter. The filter's working system, like other filters, is to select the particles that pass through (in this case microbes).

Sterilization is closely related to the creation of isolation media and pure microorganism cultivation. However, it should be noted that materials or tools that have gone through the sterilization process will not be completely free from microorganisms. Sterilization only minimizes interference by unwanted microorganisms (contaminants), while minimizing interference due to the sterilization process itself (Sany 2007: 1). In addition to the medium equipment and plant materials used are attempted to be sterile, everyone who wants to plant with tissue culture techniques and their hands must be relatively aseptic while working (Zulkarnain, 2009).

Sterilization in all tissue culture activities must be carried out in a sterile place, namely in laminar flow and using sterile tools. Sterilization is also carried out on equipment, namely using ethanol which is sprayed evenly on the equipment used. Technicians who carry out tissue culture must also be insterile conditions, the room to be used must also be in aseptic conditions. This is intended so that the room and culture equipment are protected from contamination by microorganisms in the equipment or in the free air around the room. This treatment must be carried out, especially in the sowing room or the place used for planting explants (Fardiaz 1992: 2).

It is important to understand that not all laboratory equipment is resistant to the sterilization process with high heating and pressure, especially equipment made of plastic such as polypropylene, polymethylpentene which can be autoclaved repeatedly (Suryowinoto, 1996). Heating at 160°C for 1-2 days according to Despatch Oven Company is

included with sterilization in an autoclave at a temperature of 121°C or 132°C with a pressure of 15psi (Hartmann and Kester, 1975).

Sterile workbench is an important solid in tissue culture activities. Sterile workbench is placed in the planting room (transfer room) or (planting room). It is called a sterile workbench because the working principle of this tool relies on the presence of sterile air flow produced by a very small filter wall (mesh 0.22 - 0.24 microns), so that it can withstand bacteria and fungi in the sterile workspace (Nugroho and Sugito, 2000). Sterilization of glass and metal tools can be done through an oven to be free from bacteria (Hartmann et al., 2002).

It should be emphasized here that serious efforts to overcome organism contamination are very important in tissue culture because all media, culture bottles, and tools used to handle tissue must be kept as sterile as the plant material used (Santoso and Nursandi, 2004).

Sterilization of glassware such as Erlenmeyer flasks, test tubes, petri dishes is sterilized by autoclave. Before use, the equipment is washed and brushed with detergent then rinsed with fresh water, wait until dry, then tightly closed with aluminum foil and plastic. After that, it is neatly arranged in the autoclave, the autoclave is tightly closed and operated at a temperature of 121 ° C with a pressure of 1 atm, for 30 minutes (Sari and Abdul, 2012).

Explant sterilization is done using alcohol, clorox, dithane, and betadine because these chemicals can create aseptic conditions. A plant material, especially its outer surface that is in direct contact. This is in accordance with the opinion of Doreswany (1983) who stated that surface sterilization creates aseptic conditions for a plant material. The chemicals needed include alcohol, Ca(Cl)2), NaCl which are marketed in the form of clorox, bayclean, and antibiotics such as betadine.

METHODS

Plant Tissue Culture Practicum Sterilization of Tissue Culture Equipment was carried out on December 8, 2019 at the Yahdi Tissue Culture Laboratory, Jl. Ambung, Tanah Enam Ratus, Medan Marelan, Medan City, North Sumatra. The materials used in the Tissue Culture Tool Sterilization practicum include: detergent/sunlight, proclin and clean water. The tools used in the tissue culture tool sterilization practicum include: culture bottles. The sterilization procedure is carried out after direction from the supervisor. This process is carried out starting from cleaning the tools, and of course for sterilization an autoclave is used and then cleaning the room.

RESULTS AND DISCUSSION

Results

Based on the results of the practicum conducted, the tools that were sterilized during the practicum with an autoclave were culture bottles, the tools were successfully sterilized and stored. And can be used for plant tissue culture.

Figure 1. Culture bottle

Figure 2. Autoclave

Sterilization of tissue culture tools is divided into two stages. The following are the results of the two stages of the practicum. Stage I Sterilization: 1) Select 21 bottles. The bottles and tools that will be used in the practicum are washed using detergent. This activity is carried out in detail; 2) Scrub all parts of the bottles and tools until they are completely clean with soap containing detergent. This activity is also carried out in detail; 3) Rinse the bottles and tools that have been washed with running water. Then rinse with water added with proclin; 4) Drain and wait until dry. The steps for stage I sterilization are shown in Figure 3.

Stage II Sterilization (sterilization with autoclave): 1) Fill the outer pot of the autoclave with water, using distilled water to avoid Ca precipitation which is usually found in tap water; 2) Sterilize the media bottles, put them into the autoclave in an inverted position; 3) The bottles are arranged up to 2/3 of the surface of the autoclave pot; 4) The position of the pot is adjusted by paying attention to the groove where the steam channel is located in the inner pot, as well as the arrow on the lid and the circle on the surface of the outer pot; 5) Close tightly, tighten the lock without using tools. Locking is done face to face so that the locking is balanced and water vapor does not come out during heating; 8) Close the steam outlet valve; 9) Heat the autoclave until the water in the autoclave boils and steam starts to come out of the steam outlet valve. Let it reach a temperature of 121°Cor 17.5 Psi pressure. Maintain this position for 1 hour 20 minutes; 9) During sterilization, the size of the combustion source is adjusted manually so that the

temperature is not too high; 10) After the sterilization time is complete, turn off the stove. And wait until the pressure drops, and never open the valve and let the steam out at once because this condition damages the steam and water outlet valve to bubble up; 11) After the pressure drops to line 0, the autoclave lock is opened and the tools are removed from the autoclave, then stored; 12) Tissue culture bottles are ready to use (Figure 4).

(a) (b) (c) (d) (e)

Figure 3. Stage I Sterilization: (a) Selecting bottles; (b) Scrubbing tools; (c) Washing tools; (d) Soaking bottles in proclin water; (e) Drying tools

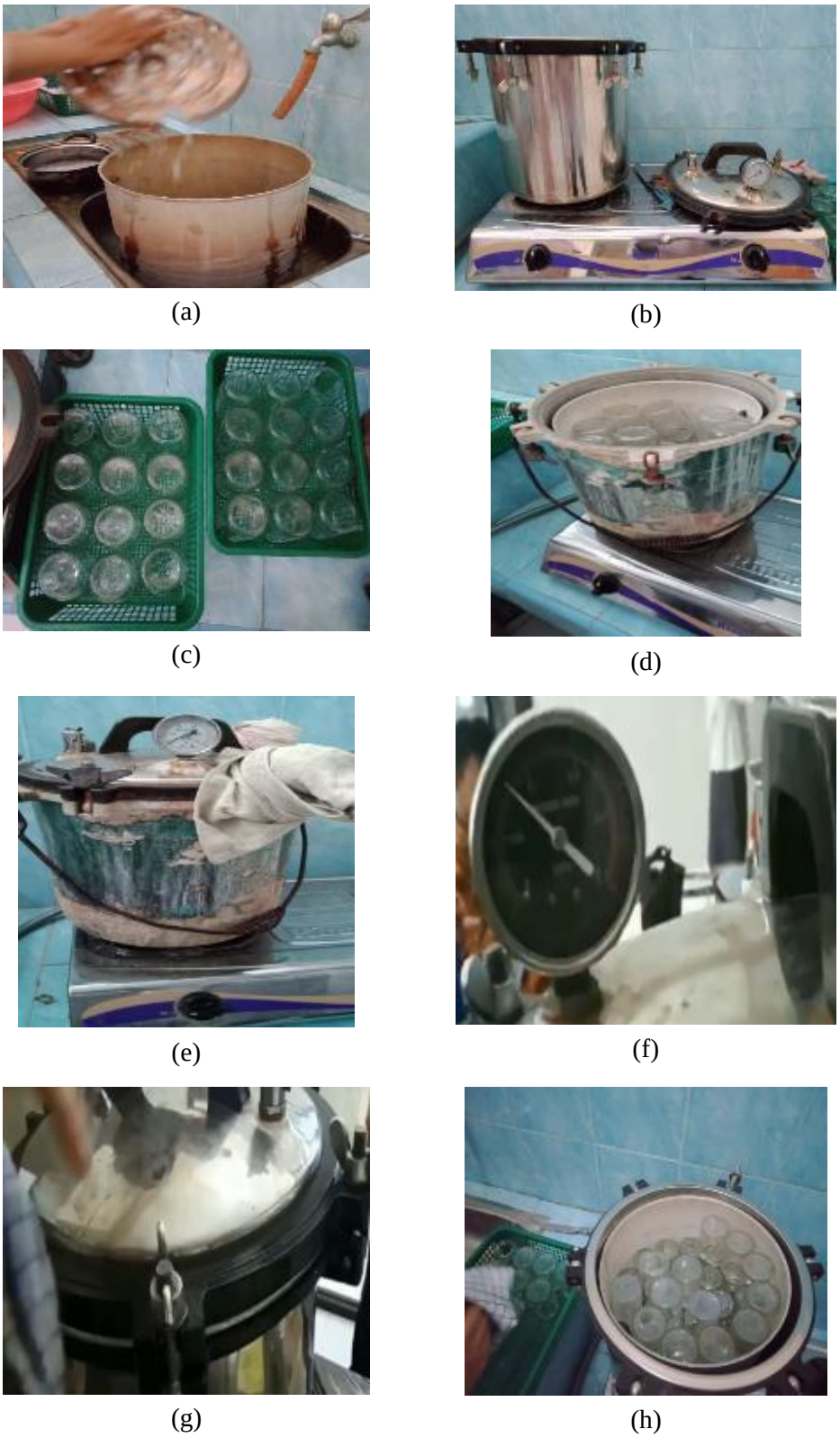

Figure 4. Stage II Sterilization (Sterilization with Autoclave): (a) Washing the inside of the autoclave; (b) Inserting the steamer into the autoclave; (c) Inserting water into the autoclave; (d) Laboratory bottles; (d) Arranging laboratory equipment into the autoclave; (e) Turning on the stove to heat the autoclave; (f) Setting the temperature to 121°Cor 17.5 Psi pressure in the autoclave; (g) Opening the autoclave valve using a cloth; (h) Lifting laboratory equipment from the autoclave; (i) Arranging and storing sterile culture bottles.

Discussion

Based on the results of the practicum, it is known that culture bottles and others. From the tools that have different functions and methods of use, but are sterilized together using an autoclave. Sterilization is all activities in tissue culture that are very important and must be carried out in a sterile place, namely in laminar flow. As expressed by Wetherell (1976: 1), that an aseptic environment as one of the main requirements for the success of tissue culture activities needs to be implemented seriously. For this reason, it is necessary to make efforts to sterilize the equipment and media that will be used in the culture process so that they are free from microbes.

Sterilization generally consists of physical sterilization, chemical sterilization and modified sterilization, which is a combination of physical and chemical sterilization. According to Yuan (2012: 2), there are several methods of sterilization of tools and plant materials that are carried out in several ways, namely by burning, dry heating, wet heating, filtering or chemically. Sterilization of tools and plant materials is also carried out sterilization in tissue culture activities must be carried out in a sterile place, and using tools that are also sterile, namely sterilization of the work environment, sterilization of tools and media and sterilization of planting materials.

Autoclaveis a tool for sterilizing various kinds of tools and plant tissue culture media using a pressure of 15 psi (1.02 atm) and a temperature of 121°C. Sterilization with an autoclave is one of the sterilization methods with water vapor under pressure. The high temperature and pressure given to the tools and plant tissue culture media being sterilized provide greater power to kill cells compared to hot air. Usually, to sterilize the media, a temperature of 121°C and a pressure of 15 lb/in² (SI = 103.4 Kpa) are used for 15 minutes (Torres, 1989).

If the objects to be sterilized are quite thick or numerous, the heat transfer inside the autoclave will be slowed down, so that the total heating time is extended to ensure that all objects are at 121°C for 10-15 minutes. Extension of time is also needed when large volumes of liquids are to be autoclaved because large volumes require a longer time to reach the sterilization temperature.

The function of sterilized tools is to avoid microorganisms that are still carried by the tools to be used, because the presence of microorganisms causes contamination and can even grow bacteria that are not completely sterile. A tool or material is said to be sterile if the tool or material is free from microbes, either in vegetative or spore form. An object or substance can only be said to be sterile or not sterile, it will never be possible to be half sterile or almost sterile. For sterilization of tools and media, sterilization is also used using a tool called an autoclave. Sterilization is carried out to kill bacteria and fungi that are attached to explants or to tools and materials used in planting explants (Fardiaz 1992: 4).

CONCLUSION

The conclusion obtained after conducting the tissue culture tool sterilization practicum is that: 1) the tools that are sterilized by autoclave are culture bottles, 2) Sterilization is carried out in two stages, namely Sterilization stage I, the bottles are sterilized or cleaned with detergent and soaked for 10-15 with bleach water (proclin), while in Sterilization Stage II, the tools are sterilized using an autoclave at a temperature of 121°C for 1 hour 20 minutes; 3)The function of sterilization is to avoid the presence of microorganisms that are still carried by the tools that will be used, because the presence of microorganisms causes contamination and can even grow bacteria that are not completely sterile; 4) All equipment used in tissue culture must be sterile through the sterilization process.

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