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The Effect of Concentration and Immersion Time Disinfectant on Sterilization *Aglaonema* Hybrid (Pink Katrina) Leaves

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Abstract

***Aglaonema sp.* is well known as an ornamental plant in Indonesian society. It has leaf with various beautiful patterns. *Aglaonema* pink katrina is one of the imported hybrids *Aglaonema*. A method to increase plant yield quickly is tissue culture. The crucial step in plant tissue culture is sterilization. This study aimed to find the best sterilization method by looking at the effect of concentration and immersion time of sodium hypochlorite (NaOCl) as disinfectant to reduce contaminants explant *Aglaonema* Pink Katrina leaves. In this study, there were 11 groups consisting of positive control, negative control, and immersion with 2,5; 5%; and 10% NaOCl for 1 minute and 3 minutes. Parameters observed included the time of first contamination, percentage of bacterial and fungal contamination, and appearance of explants for 28 days. The DMRT test showed significant differences in all treatments of disinfectant concentration and soaking time.The results show contamination first time occurred in code S0a (negative control), the highest percentage of sterile explants was in code A5, and explants that were still green/fresh were found in codes A5 and A8 after observation.**

Keywords: *Aglaonema* Pink Katrina, contamination, disinfectant, explants sterilization, tissue culture

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Introduction

Ornamental plant is a popular agricultural business sector. Ornamental plant can be used to beautify rooms, provide serenity, and are needed for traditional events. In 2018, Indonesia produced 853,544 pots of *Aglaonema* plants (Central Bureau of Statistics, 2018). The agricultural sector, especially ornamental plant has increased demand, because of the gardening trend carried out by people during the pandemic. This increase affects the amount of production farmers (Akbar, 2021).

One of the ornamental plants that is well known in Indonesia is *Aglaonema sp.* This plant is a leaf ornamental plant that can live in tropical climates such as Indonesia. There are about 30 types of *Aglaonema sp.* in Indonesia (Akbar, 2021). One of the variants resulting from crossing by Greg Hambali is the Pride of Sumatra which won second place in the category of indoor ornamental plants at Floriade event in the Netherlands. This plant is characterized by a variety of beautiful leaf shapes and colors (Muhammad & Wibowo, 2021). Therefore, this plant is a popular ornamental plant among traders and nurseries (Simamora *et al*., 2017). High demand affects the cultivation production of *Aglaonema*, thus making it possible to use *Aglaonema* plants more widely. Cultivation of *Aglaonema* plants is relatively easy to do generatively by using seeds and vegetatively by cutting stems, separating tillers, grafting and tissue culture (Widyastuti, 2018).

One of the important stages in tissue culture is sterilization. Explants must be in aseptic conditions, free from contaminants so it

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can grow optimally. The type of explant also determines the success of tissue culture (Maisarah & Isda, 2022). The selection of sterilization method for tissue culture is based on disinfectant and immersion time. The difficulty of tissue culture is killing or eliminating contamination without killing the sterilized plant (explants) (Nida *et al*., 2021). Disinfectants are chemicals used to prevent contamination of surfaces by killing or reducing microorganisms, such as bacteria, fungi and viruses (Nurjannah *et al*., 2021). Sterilant or disinfectant has toxic properties againts tissues, so it is important to note the amount of concentration used. Various methods of sterilization have been carried out that are expected to be effective to remove the source of contaminants. The concentration of disinfectant and immersion time applied are factors that determine a success sterilization process. There are various disinfectant chemicals needed for explants sterilization, such as sodium hypochlorite (NaOCl), mercury chloride ($HgCl_2$), detergent, and 70% alcohol (Ma *et al*., 2018). However, disinfectant and immersion time has different effects on each plant species (Shofiyani & Hajoeningtijas, 2010). Based on previous research on the sterilization of candlenut (*Aleurites moluccana* (L). Willd) leaves with 1% NaOCl treatment with 2.5 minutes of immersion time, it was proven to be able to reduce contamination up to 0% (Lutfiyani, 2018). Therefore, in this study sterilization was carried out using 2.5%, 5% and 10% NaOCl on *Aglaonema* Pink Katrina leaves explants with immersion time of 1 and 3 minutes to get the best sterilization method that can reduce contamination.

Methods

Preparation of plant material

Aglaonema Pink Katrina plant is maintained in the Laboratory of Biotechnology, BRIN (Puspiptek, Serpong) for two months as a source of explants. The newest leaf was used for the plant section explants.

Culture medium

The growth media used consisted of modified Murashige and Skoog (MS – Caisson), adjusted with 1 N NaOH and 1 N HCl into solution until pH 5,7 and addedd with 7 gr of agar, then stirred with a magnetic stirrer and heat until boiling. The solution was poured into a test tube @ 5 mL, then autoclaved at 121 psi pressure for 30 minutes (Setiani *et al*., 2018).

Explants sterilization and analysis data

This study used a completely randomized design (CRD) with concentration and immersion time disinfectant as treatment factors. As stated in Table 1, the treatment was split into 9 groups, each of which has 10 replications and 3 experimental units (Setiani *et al*., 2018).

The first step of the sterilization process is to thoroughly wash the explants under running water for around 30 minutes. This step aims to eliminate any dust or impurities that may have accumulated on the surface before rinsing it with detergent for 10 minutes. After that, it was shaken with bactericidal and fungicidal (at 2 gr/L each) added with tween 80 (3 drops/100 mL). Then, it was rinsed with water and immersed according to the single or combination NaOCl used as shown in Table 1. Explants are sterilized in LAF (Laminar Air Flow). LAF was cleaned with 70% alcohol, exposed to a UV lamp for 1 hour, and allowed to air for 30 minutes. The explant planting stage was carried out aseptically. Leaf explants were cut into squares of 1 cm^2. Each tube contains 1 explant (Hutabarat *et al*., 2022).

Variables observed included the first time contamination appeared, the percentage of contaminants (bacterial/fungal) and the appearance of explants for 28 days (green/brown). The most effective sterilization method was found by observing the level of contamination that appears based on different sterilization treatments. Quantitative data was analyzed statistically using Analysis of Variance (ANOVA). If the difference is significant, a further test is carried out using the DMRT Test (Duncan's Multiple Range Test) with a significance level of 95%.

Table 1. Factor Treatment

Code	Immersion Time Disinfectant (minute)		
	NaOCl 2,5 %	**NaOCl 5 %**	**NaOCl 10 %**
S0a (negative control)	Media control sterilized; explants are not sterilized		
S0b (positive control)	Media control sterilized, no explants		
A1	3	1	0
A2	3	0	1
A3	0	3	1
A4	3	0	3
A5	0	3	0
A6	0	0	3
A7	1	0	0
A8	0	1	0
A9	0	0	1

$$Percentage\ of\ contamination(\%) = \frac{\text{Total number of contaminated explants}}{\text{Total number of explants}} \times 100\%$$

$$Color\ of\ the\ explants = \frac{Total\ number\ of\ green\ or\ brown\ explants}{Total\ number\ of\ explants} \times 100\%$$

Results and Discussion

Contamination time

Therefore, before sterilization explants were sprayed with bactericidal and fungicidal once a week for 2 months. Table 2 shows the effect of sterilization variation on contamination time in explants for 28 days of culture. Based on these results, the contamination time appeared in various explants. On non-sterilized explants (control negative) indicates the time of contamination *Aglaonema* explants progressed the fastest i.e. 3-8 DAI (days after inoculation) and on media without explants (control positive) showed no contamination during testing. In single sterilant treatment with immersion duration of 1 or 3 minutes (code A4-A9) indicates the time of appearance contamination i.e. 5-28 DAI. In combination sterilant treatment with immersion time explants 1 and 3 minutes (code A1-A3) indicates the time of appearance of contamination i.e. 7-25 DAI. The development of contamination time can be seen in Figure 1. The difference in the time of this contamination is caused by internal or external contaminants. Internal contamination comes from contaminants carried from within explants plant tissue, while external contaminants originate from the explants surface (Pratiwi *et al*., 2021). External contamination or common plant surface contamination grows after 2 DAI while internal contamination or contamination sourced from plant explants show up for longer than 7 days even up to one month (Anggoro *et al*., 2022; Handayani *et al*., 2021). Some sterilants can be toxic for explants tissue if the concentration and inappropriate immersion duration. Besides the kind of explants used also affects the effectiveness of sterilization, different types of explants will give a different response (Hutabarat *et al*., 2022).

Effect of concentration and immersion time disinfectant on contamination

The addition of disinfectant and immersion time can indeed reduce level of contamination and can also affect appearance of explants as shown in Figure 2 and Figure 3. The level of explants contamination is influenced by many factors such as the growing medium, work environment, tools, and the origin of the explants, especially those from the field. Based on Figure 1 without the use of sterilizing materials, the explants will be contaminated so the in vitro propagation of *Aglaonema* Pink Katrina will not be successful (Setiani *et al*., 2018). One of the observations can be seen in Figure 2, where the sterile explants in 2.a and explants contaminated bacteria and fungi in 2.b.

Table 2. Contamination time

Code	Time of First Contamination	
	Bacterial	**Fungal**
S0a (negative control)	3 DAI	8 DAI
S0b (positive control)	Sterile	Sterile
A1	25 DAI	7 DAI
A2	15 DAI	Sterile
A3	14 DAI	23 DAI
A4	10 DAI	28 DAI
A5	22 DAI	Sterile
A6	15 DAI	21 DAI
A7	18 DAI	Sterile
A8	5 DAI	18 DAI
A9	Sterile	7 DAI

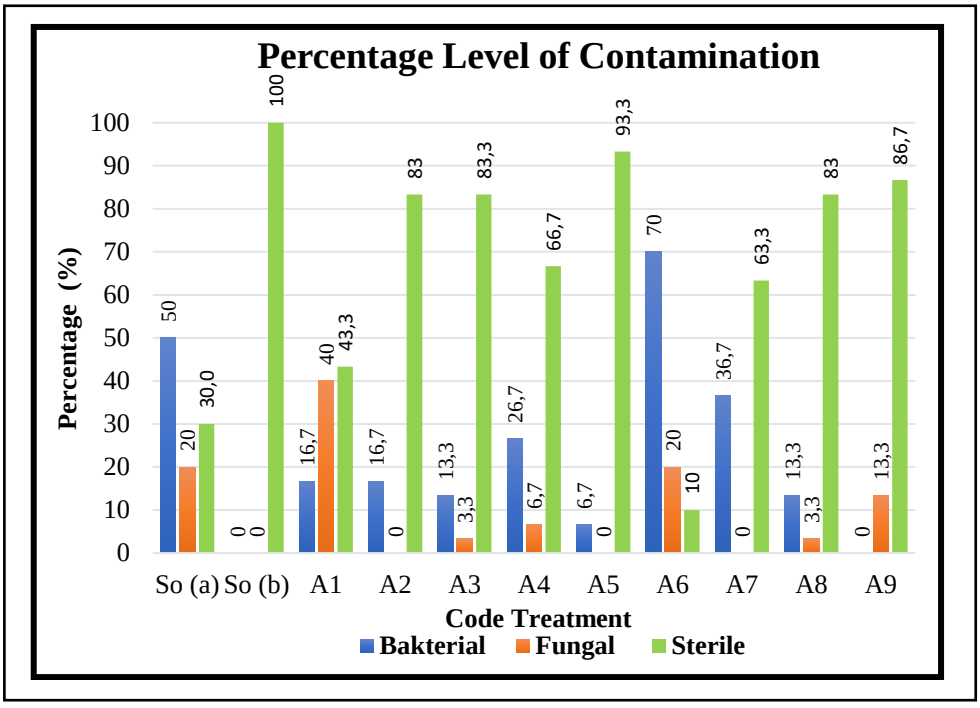

Figure 1. Percentage Level of Contamination on Explants

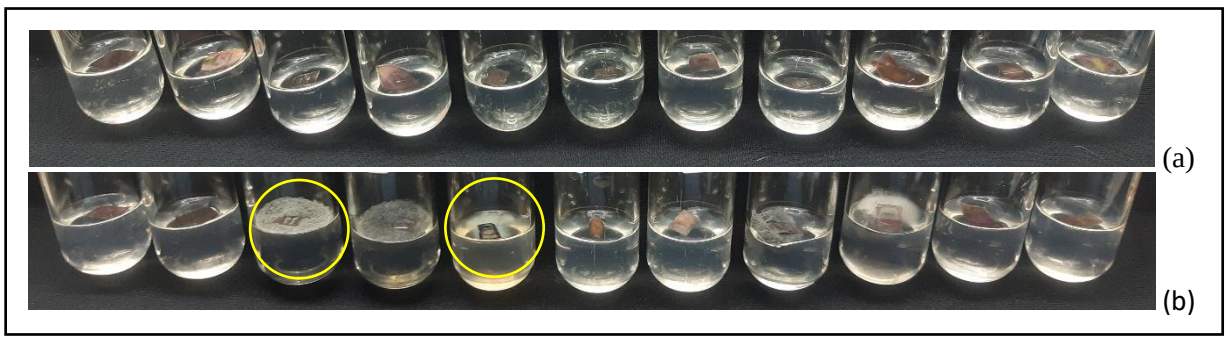

Figure 2. The result of observation from explant steril (a) and contaminated (b)

Table 3. DMRT Test Results of Contamination on Explants

Code	Percentage Level of Contamination on Explants
A5	.07[a]
A2	.17 [ab]
A9	.17 [ab]
A3	.20 [ab]
A8	.20 [ab]
A7	.37 [ab]
A4	.40 [b]
S0	.87 [c]
A1	.97 [c]
A6	1.10 [c]

Note: *) different notations (a, b, c) indicate that there is a significant difference between one treatment and another other treatment with a level of 0.05 based on the DMRT Test

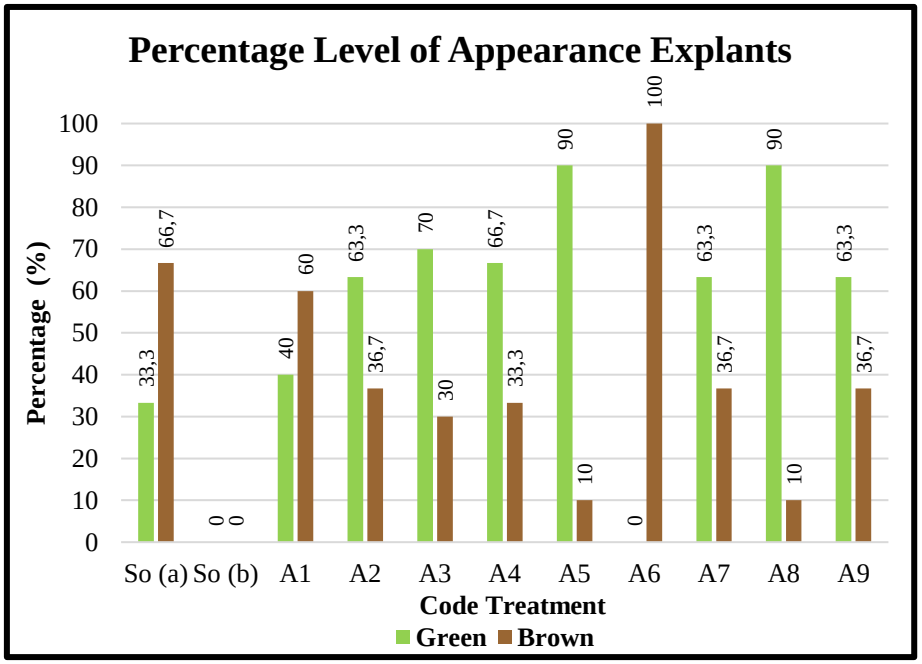

Figure 3. Percentage Level of Appearance on Explants

Based on results of Figure 1, the data were analyzed using a one-way statistical test (ANOVA). The DMRT test results in Table 3 show significant differences in all treatments of disinfectant concentration and soaking time. The best treatment was A5 using 5% NaOCl for 3 minutes.

Sterilization using NaOCl 5% for 3 minutes (A5) showed 6.7% contamination, while immersion with NaOCl 10% for 3 minutes (A6) showed the highest contamination with a total of 90% contamination. Contaminants such as fungi and bacteria compete to get nutrients in the media and become the parasites for explants because contaminants hinder the growth of explants until the explants die (Imanudin, 2016). Sodium hypochlorite is widely used because very effective to killing bacteria by damaging cell membranes. Hypochlorite compounds can clean microorganisms that participate in plant materials, remove soil particles, dust and others. If this compound is given in low concentrations and short immersion time it is not very effective in controlling contamination of explants (Farooq *et al.*, 2002). The shorter immersion time with sodium hypochlorite can make explants more pathogens. However, the longer immersion of sodium hypochlorite can inhibit the development of explant tissue which is characterized by browning (brownish color) on the explants (Rismayanti & Hamzah, 2010).

The concentration of sodium hypochlorite has also an effect because the higher it is, the more it will penetrate the tissue to clean organisms, but as a result the tissue will also be damaged, and the explants will rot quickly so that fungi and bacteria will grow. These results indicate that the type of disinfectant and immersion time affects the level of contamination.

Effect of concentration and immersion time disinfectant on explants appearance

Treatment of various disinfectants and immersion time can indeed reduce contamination, but it can also affect the appearance of explants as shown in Figure 3. Figure 3 shows the explants of samples A5 and A8 which were both treated with NaOCl 5 % still fresh green. The absence of sterilizing agents also changed the color of the explants, this was possible because the condition of the explants damaged tissue due to contamination. Whereas the use of NaOCl 10% for 3 minutes A6 also resulted in brown explants. This shows that the disinfectant affects the color of the explants (Hardarani & Nisa, 2022). The browning process is oxidation of phenolic compounds into quinones originating from explants (Fitriani *et al*., 2019). Explants with 3 minutes of immersion had a browner color than those with 1 minute of immersion. This is due to characterize sodium hypochlorite which can change the structure and texture of explants. Explants with longer immersion caused surface to bruise or brown. The longer the explants were soaked in high concentrations of sodium hypochlorite, the greater the surface area of the explants that experienced browning (Rismayanti & Hamzah, 2010). This is also supported by a statement from Farooq *et al*. (2002) that exposure of explants to sterile materials over a long time will cause the explants to brown (browning) and can even cause death of the explants. Based on the research, immersion treatment using NaOCl 5% for 3 minutes (A5) showed the best results, namely it suppressed the appearance of contamination and the duration of immersion of the explants did not change the color of the explants.

Conclusion

The difference in concentration and immersion disinfectant has an impact on contamination and changes the appearance of explants. The results of ANOVA test showed that concentration and immersion disinfectant had a significant effect on explant contamination. Contamination first time occurred in code S0a (negative control), the highest percentage of sterile explants was in code A5 using NaOCl 5% and immersion for 3 minutes, and explants that were still green/fresh were found in code A5 and A8 after observation for 28 days.

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