The Effect of plasma radiation with leaf fertilizer combination on vegetative growth of orchid planlets *Dendobrium* sp. at the acclimatization stage

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Abstract. Plasma discharge produces ozone for decontamination; when it is combined with leaf fertilizer, it is expected to support orchid growth. The purpose of this study is to determine the effect of plasma discharge and leaf fertilizer on the vegetative growth of orchid plants and to reduce contamination. The study used factorial Completely Randomized Design 3X3. The first factor is the duration of plasma discharge, including D0 (0 seconds), D1 (60 seconds), and D2 (120 seconds). The second factor is the level of leaf fertilizer which includes G0 (0 g/l), G1 (3 g/l), and G2 (5 g/l) given at two weeks after planting. The research parameters included the percentage of life, plant height, number of leaves, root length, wet basis, and chlorophyll content. Data are analyzed by using ANOVA (Analysis of variance) followed by DMRT Test (Duncan's Multiple Range Test). The results showed that 60 second irradiation treatment produced the best percentage of life with low contamination. The results of the combination of plasma irradiation and leaf fertilizer treatment in 60 seconds irradiation and 3 g/l leaf fertilizer on parameters of plant height, root length, plant weight and the number of leaves.

1. Introduction

*Dendrobium* sp. is an ornamental plant that is in great demand by the public [1] so that it has the potential to be developed in the horticulture sector. The high demand for orchids cannot be fulfilled because conventional propagation of orchids is very slow [2]. The results of orchid production have continued to decline since 2012 [3]. Tissue culture, on the other hand, provides alternative solutions for producing orchids in large quantities, uniform results, and in a short time [4]. The stages in tissue culture are initiation, sterilization, multiplication, and acclimatization. At the acclimatization stage, plantlets produced by tissue culture are not able to defend themselves against pathogens caused by microbes or against biotic and abiotic pressures [5]. Planlets must maintain a sterile condition to be semi-sterile when acclimatized.

Plasma corona is one type of non-thermic plasma and is a source of ions, electrons and free radicals [6]. Corona plasma technology can be an alternative to sterilization of plantlets before being acclimatized. Plasma can be used as an ozone generator with a source of gas in the form of free air or oxygen. Ozone as a disinfectant can kill germs, bacteria, viruses, fungi, spores, mosses, and other organic substances [7]. In previous studies, plasma corona could reduce the number of E. coli milk contaminants [8].

Plasma technology, in addition to producing ozone, it also can produce nitrogen ions that are needed for growth. The amount of composition of nitrogen in free air which up to 80% causes the plasma discharge in free air to produce N+ ions which can be used as a source of nitrogen for plants.
The use of plasma corona is carried out in the plant breeding business which is to accelerate plant growth [10].

Plants in the acclimatization stage require sufficient availability of nutrients because at this stage there is a change in the nature of the plant from heterotroph to autotroph. Addition of nitrogen through plasma technology is not enough to provide the nutrients needed because the administration is only at the beginning before acclimatization. Fertilizers are needed for growth and development. Proper application of fertilizer to orchid plants is through the leaves [11]. The advantage of leaf fertilizer compared to root fertilizer is the absorption of nutrients through the mouth of the leaf (stomata) runs fast. Besides, the nutrients given through the leaves can almost be absorbed by the plant so that it does not cause soil damage [12].

Plasma ray radiation in orchids *Dendrobium* sp. expected to reduce the contamination by bacteria, viruses, and fungi that can interfere with the growth of orchids and together with the leaf fertilizers, it can provide additional nitrogen and other nutrients so as to meet nutrient requirements for growth. The purpose of this research is to see the results of the combination of plasma ray radiation treatment and leaf fertilizer addition which is expected to affect the vegetative growth of orchid plantlets *Dendrobium* sp. optimally and minimize contamination at the acclimatization stage.

2. Material and Methods

The materials used are the *Dendrobium neonteik-hongplanlet* Orchid which is a cross of *Dendrobium streblocceras* and *Dendrobium linsale* "Blue" that obtained from the Indonesian Orchid Temple located on No. 1 Jolotundo Street. No. 16 Semarang., the planting media is in the form of a mixture of wood charcoal, husk charcoal, and sphagnum moss, while the materials for testing chlorophyll content including aquadest, 80% acetone, tissue, filter paper, alum, 10% fungicide, leaf fertilizer that is gandasil. The tools used in this research include digital scales, trays, stoves, pans, wok, trowel, UV-Vis spectrophotometers, mortar, ose, brushes, basins, plasma reactors, Erlenmeyer, beker glass, test tubes, rulers, and pot with 10 cm in diameter.

The research method which is used is a completely randomized 3X3 factorial design with three replications. The first factor is the duration of plasma irradiation, including D0 (duration 0 seconds), D1 (duration 60 seconds), and D2 (duration 120 seconds). The second factor is the level of leaf fertilizer which includes G0 (leaf fertilizer 0g / l), G1 (leaf fertilizer 3 g / l), and G2 (leaf fertilizer 5 g / l) given at two weeks after planting. The research parameters included the percentage of life and contamination, plant height, number of leaves, root length, wet basis, and chlorophyll content. Data are analyzed by ANOVA (Analysis of variance), and DMRT (Duncan Multiple Range Test) tests if the results were significantly different.

3. Result and Discussion

Life percentage of orchid plants *Dendrobium* sp. since acclimatization until one week after treatment (MSP) with corona plasma irradiation treatment shows a high percentage of life that is 100%, while at the end of 2 MSP a decrease in the percent of life starts to be seen. This percentage is calculated before the *Dendrobium* sp. get the second treatment, namely the addition of Gandasil leaf fertilizer. Growth percentage results are shown in (Table 4.1)

| Duration of corona plasma irradiation | Growth percentage (%) |
|--------------------------------------|------------------------|
|                                       | 1st week | 2nd week |
| (D0) 0 seconds                        | 100      | 60       |
| (D1) 60 seconds                       | 100      | 73       |
| (D2) 120 seconds                      | 100      | 67       |
Based on Table 4.1 it can be seen that the treatment of corona plasma irradiation with a duration of 60 seconds (D1) produces a percentage of growth which is better than the duration of 0 seconds (D0) and 120 seconds (D2). At the stage after being removed from the bottle, the seedlings are very susceptible so that they need protection from predator attacks or pathogens [13]. The acclimatization stage which is not carried out properly can cause a low percentage of orchid plants. Warseno et al. [21] in their study stated that the percentage of acclimatization success was 44.4%. Corona flare plasma can also be used as a sterilizer. Microorganisms irradiated by plasma corona, the plasma membrane will rupture in a short time followed by cytoplasmic leak [14]. Plasma can produce ultraviolet light, active oxygen molecules and some active radical species that allow decontamination [22].

Table 2 Analysis results of growth parameters and total chlorophyll content of Dendrobium sp. orchid with the treatment of the corona plasma irradiation and Gandasil leaf fertilizer.

| Treatment | Parameter | The increase of plant height (cm) | Number of leaves (strands) | The increase of plant roots length (cm) | The increase in plant weight (gr) | Total chlorophyll content (gr/mg) |
|-----------|-----------|-----------------------------------|---------------------------|---------------------------------------|-------------------------------|----------------------------------|
| D0G0      |           | 0.73                              | 3.00                      | 0.37                                  | 0.077                         | 3.41\textsuperscript{b}         |
| D0G1      |           | 0.60                              | 3.33                      | 0.37                                  | 0.080                         | 2.93\textsuperscript{cd}        |
| D0G2      |           | 0.83                              | 3.00                      | 0.33                                  | 0.073                         | 3.67\textsuperscript{a}         |
| D1G0      |           | 0.90                              | 3.33                      | 0.30                                  | 0.087                         | 3.09\textsuperscript{bcd}      |
| D1G1      |           | 1.17                              | 3.00                      | 0.43                                  | 0.110                         | 2.85\textsuperscript{d}        |
| D1G2      |           | 0.87                              | 2.67                      | 0.47                                  | 0.050                         | 3.11\textsuperscript{bcd}      |
| D2G0      |           | 0.43                              | 2.33                      | 0.37                                  | 0.053                         | 2.99\textsuperscript{d}        |
| D2G1      |           | 0.80                              | 3.00                      | 0.27                                  | 0.060                         | 3.31\textsuperscript{abc}      |
| D2G2      |           | 0.70                              | 2.67                      | 0.27                                  | 0.047                         | 3.2\textsuperscript{bcd}       |

Remarks: Numbers followed by the same letter in the same column show an effect that is not significantly different based on Duncan’s test 95% confidence level.

D0: 0 seconds of plasma corona irradiation
D1: 60 seconds of plasma corona irradiation
D2: 120 seconds of plasma corona irradiation
G0: the application of Gandasil 0 g/1 leaf fertilizer
G1: the application of Gandasil 3 g/1 leaf fertilizer
G2: the application of Gandasil 5 g/1 leaf fertilizer

3.1 Plant Height increase

![Figure 1. Dendrobium sp. Orchid Plant Height Increase Histogram after treatment of corona incandescent plasma irradiation and Gandasil leaf fertilizer.](image-url)
The results of the plant height increase (Figure 1.) show that the D2 treatment has a higher increase compared to the treatment D0 and D1 because orchids had an optimal limit of nitrogen that could be accepted by their bodies. Too much nitrogen can cause growth to be not optimal. The provision of nitrogen nutrients that are too high will result in a decrease in the quality of the plant due to a decrease in carbohydrate content and accumulation of nitrates in plants [15].

The addition of N elements from irradiation and fertilization can result in interactions that affect the growth of *Dendrobium* sp. This can be seen in (Figure 1.) that D1G1 treatment (60 second corona plasma irradiation treatment and 3 g / l Gandasil leaf fertilizer treatment) is better than D1G0 (60 second corona plasma irradiation treatment and 0 g / l Gandasil leaf fertilizer treatment) which were not given additional Gandasil leaf fertilizer.

### 3.2 Number of Leaves

![Figure 2](image2.png)

**Figure 2.** The Average Number of Leaves of *Dendrobium* sp. Orchid Plant Histogram after treatment of corona incandescent plasma irradiation and Gandasil leaf fertilizer.

The number of leaves of *Dendrobium* sp. orchid plants based on the treatment group, the duration of corona plasma irradiation time, D0 treatment has better results compared with the treatment of D1 and D2. Plasma corona irradiation results in the addition of nitrogen received by *Dendrobium* sp. which is thought to cause the leaves of orchid plants to fall more easily. The excess of N can reduce plant resistance to disease as a result of plants that receive more N nutrients will be more susceptible to disease attacks which cause leaves to fade, rot and eventually die resulting in a decrease in the number of leaves [12].

### 3.3 Root Length Increase

![Figure 3](image3.png)

**Figure 3.** Average of root length increase of *Dendrobium* sp. orchid plants after treatment of corona incandescent plasma irradiation and Gandasil leaf fertilizer.
The average of root length increase of *Dendrobium* sp. orchid plants based on the treatment group, the duration of corona plasma irradiation time shows that the highest increase in root length is in D1 treatment compared to D0 and D2 treatment (Figure 4.3). In orchid plantlets that have just been acclimatized, nutrients are used for plant growth. Roots grow elongated through cell division in the apical meristem (the area of the root tip). Optimal nitrogen administration can increase plant growth, protein synthesis, and root shoot ratio [16]. The long growth of the roots of orchid plants can be disrupted by the media's ability to store water. Media that has the ability to store large amounts of water results in lower evaporation and results in a moister media [17].

3.4 Wet Basis Gain

(Figure 4.) Shows that among the three groups of differences in duration of corona plasma irradiation, D1 treatment produced the most weight gain compared to treatment D0 and D2. Plant height and root length that increase directly will increase changes in plant mass. If there is an increase in plant height and root length but is not followed by an increase in wet basis, there are other factors that influence the event. There are several factors that influence the decrease in wet basis, among others, the loss of leaves or innate roots, wilting leaves, shrinking roots, and reduced water content in the plant body due to high evaporation [18].

The wet basis of orchid plants is also influenced by the temperature of the environment. A decrease in wet basis can be caused by high transpiration and respiration. The weight loss is caused by the loss of carbon during the respiration process. In the process of respiration, carbon compounds contained in sugar (carbohydrates) will bind and react with oxygen and produce simple volatile compounds, namely carbon dioxide, and water vapor, so that the orchid plants of *Dendrobium* sp. will lose its weight [19].

3.5 Total chlorophyll content
Figure 5. Histogram of total chlorophyll average of Dendrobium sp. after treatment of corona incandescent plasma irradiation and Gandasil leaf fertilizer.

The total chlorophyll content of the corona plasma irradiation treatment at the duration of 0 seconds and the 5 g/l gandasil leaf fertilizer (D0G2) produced the highest total chlorophyll content (Figure 4.5). The lowest total chlorophyll content is in the 60 second corona plasma irradiation treatment and 3 g/l gandasil leaf fertilizer (D1G1). Chlorophyll content in plant leaves is closely related to the presence of nitrogen in plants. Chlorophyll levels in leaves will decrease with increasing doses of nutrients given. This can be explained that giving a high fertilizer dose will provide sufficient nutrients for plant growth so that plants will do more primary metabolism to produce biomass than produce secondary metabolites [20].

4. Conclusion
Plasma reactors can produce ozone and nitrogen ions. Ozone produced from plasma discharge can be used as decontamination while the nitrogen ions which is produced are used as a support for vegetative growth of orchid plants together with leaf fertilizer. The best combination to produce the most optimal vegetative growth of Dendrobium sp. orchid plant and the least contamination at the acclimatization stage is the 60 seconds irradiation of plasma and the dose of leaf fertilizer 3 g/l.

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