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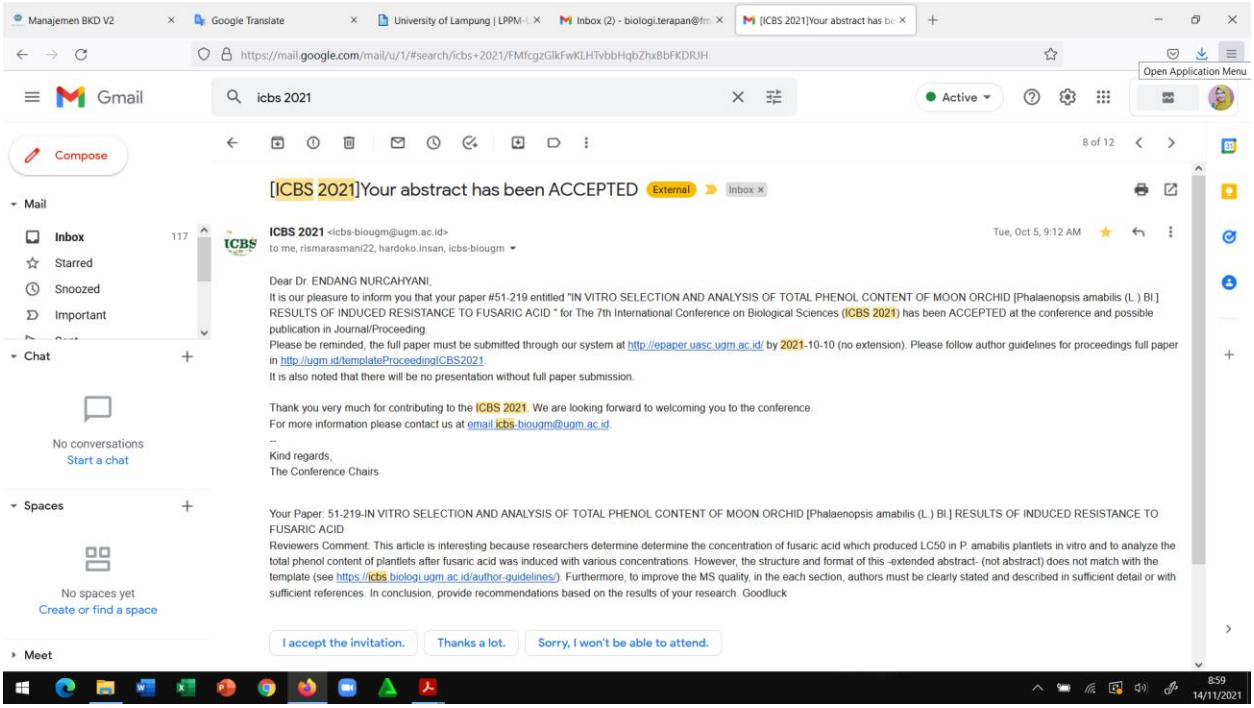
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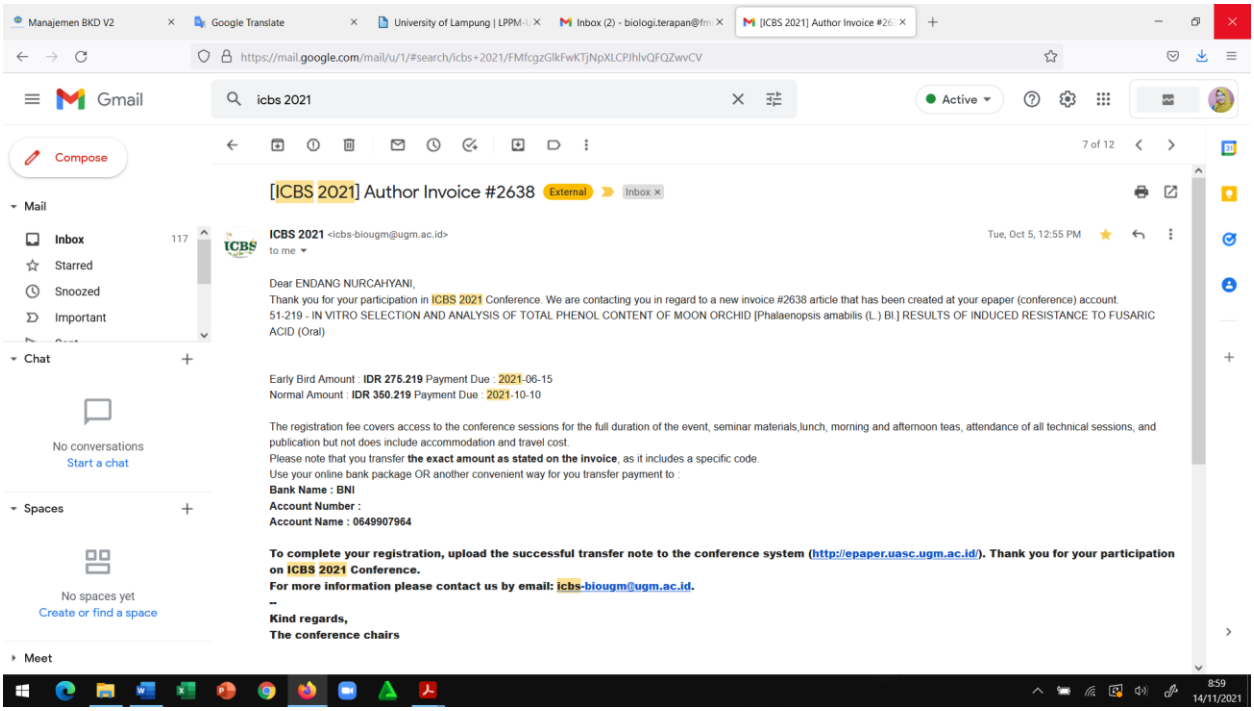
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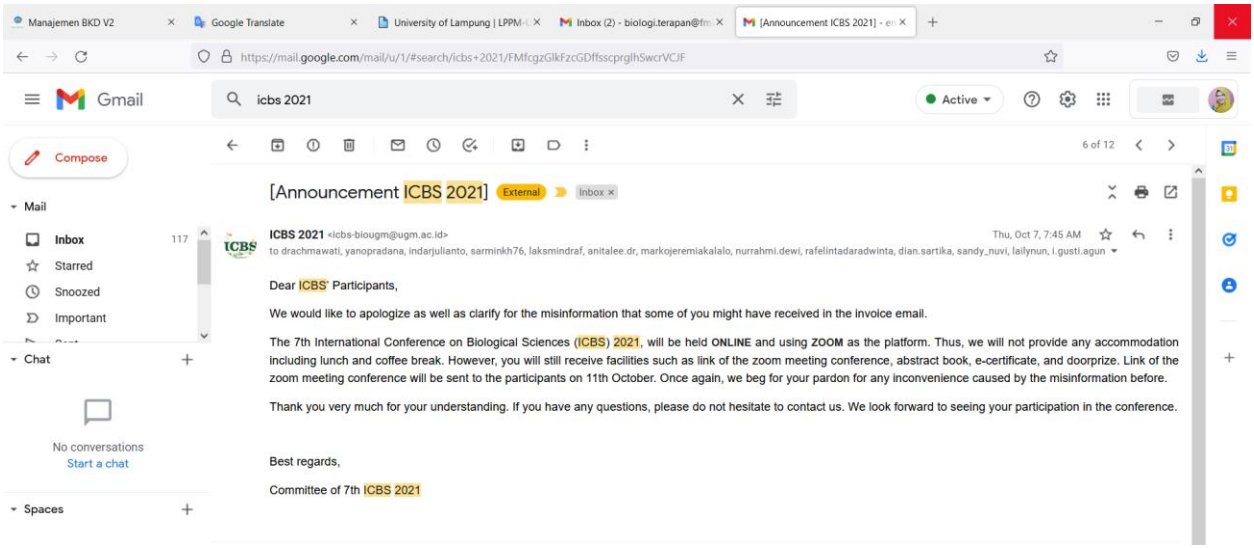
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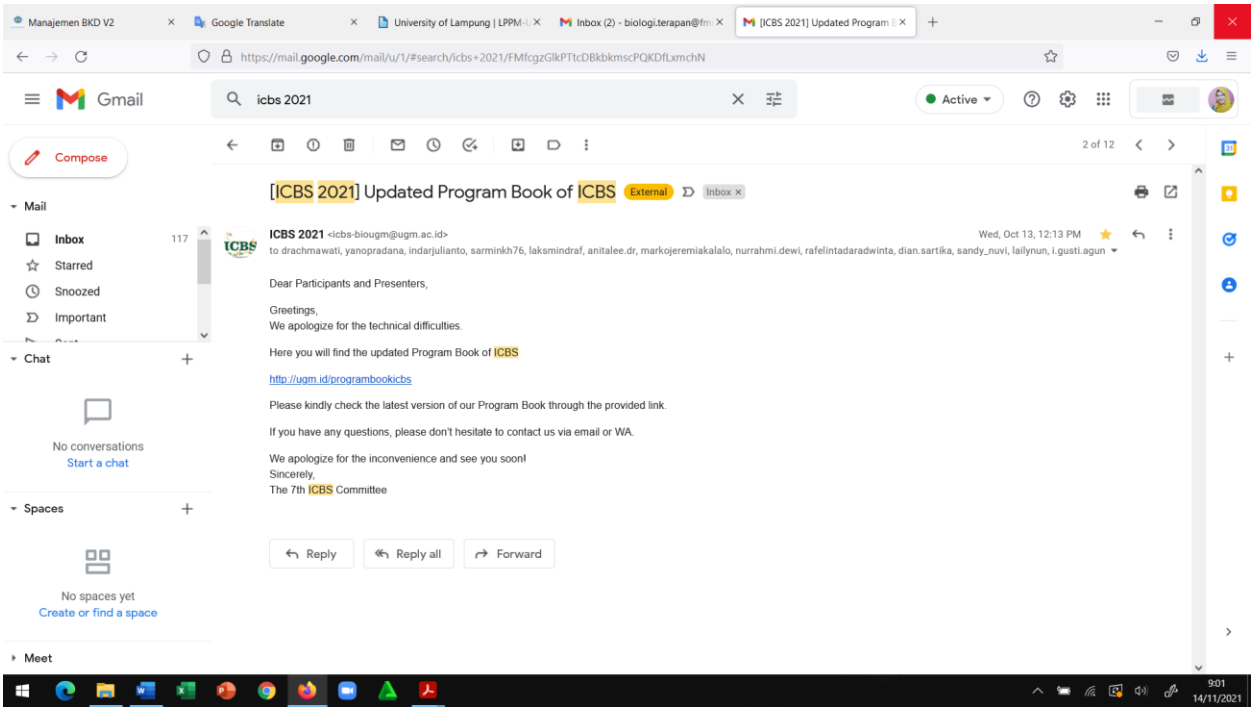
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Advances in Biological Sciences Research, volume 22
7th International Conference on Biological Science (ICBS 2021)

In Vitro Selection and Total Phenol Analysis of Moon Orchid [*Phalaenopsis amabilis* (L.) Bl.] Results of Induced With Fusaric Acid

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ABSTRACT
Phalaenopsis amabilis (L.) Bl. is an orchid that is included in the list of endangered plant species. *The Fusarium* wilt disease on *P. amabiliviral*a vital disease caused by *Fusarium oxysporum*, which of one constraint and up to now days is not well yet managed. Disease control that does not cause negative impacts can be done by using superior cultivars that are resistant to *Fusarium oxysporum* infection, through in vitro selection on medium with the addition of selective concentration of fusaric acid. The purpose of this study was to determine the concentration of fusaric acid that was tolerant to *P. amabilis* plantlets and to analyze the total phenol content of plantlets after fusaric acid was induced with various concentrations. This study used a completely randomized design with one factor, with 5 levels of fusaric acid concentration, namely 0 ppm, 30 ppm, 60 ppm, 90 ppm, and 120 ppm. Data analysis used Analysis of Variance with a significance level of 5% and further test with Tukey's test at a significance level of 5%. The results showed that: the concentration of fusaric acid that was tolerant to *P. amabilis* plantlets was 120 ppm; there was an increase in total phenol content along with an increase in fusaric acid concentration, an increase in total phenol content from 1.54 mg/g at a concentration of 0 ppm (control) increased to 2.0 mg/g at a concentration of 30 ppm, followed by 2.64 mg /g at a concentration of 60 ppm, 3.48 mg/g at 90 ppm and 4.27 mg/g at a concentration of 120 ppm. This proves that the higher the concentration of fusaric acid, the higher the total phenol content produced.

***Keywords**: Fusaric acid, In vitro, Phalaenopsis amabilis, Total phenol*

1. INTRODUCTION

Indonesia is a country that has a diversity of orchids scattered on trees in the wilderness [1,2]. *Phalaenopsis* is included in the Orchidaceae family which has 800 genera and 25,000 species in the world. Phalaenopsis amabilis or commonly known as moon orchid is one of the most popular orchid species. [2,3]. The moon orchid is one of Indonesia's national flowers. Indonesia has three national flowers that have been determined by Presidential Decree No. 4/1993, including the jasmine flower (*Jasminum sambac* L.) as the nation's puspa, the giant lotus flower (*Rafflesia arnoldii* R.Br.) as a rare pup, and the moon orchid. (*Phalaenopsis amabilis*) as a charm puspa [6]. Promising economic value makes moon orchids hunted in nature which threatens their sustainability so that the conservation status of moon orchids can be threatened with extinction [7,8,9].

In its growth, orchid plants get quite serious disturbances such as the emergence of diseases from pathogenic fungi, bacteria, or viruses that attack parts of the orchid plant body. Some of the pathogenic fungi that often attack orchid leaves are *Fusarium* sp. [10,11,12] Fusarium wilt disease causes huge losses, mostly

attacking *Phalaenopsis*, *Cattleya,* and *Oncidium*. In the United States, this disease has caused the death of ornamental plants including orchids, resulting in yield losses of more than 50% [13,14]. There are various genera of orchids such as the genera *Phalaenopsis, Dendrobium, Cymbidium*, and *Cattleya* based on research that has been shown to show results that are susceptible to infection from *Fusarium* [4,5].

Disease control against *Fusarium oxysporum* in orchids has been carried out using synthetic chemical pesticides.[10] One way to control Fusarium wilt that is safe for the environment is to use varieties that are resistant or resistant to *Fusarium oxysporum* infection through in vitro selection in the medium with the addition of fusaric acid. [15,16,17,18,19,20]. Fusaric acid is a metabolite produced by several species of fungi of the genus *Fusarium*. Chemically Fusaric acid is called 5-n-butylpicolinic acid. This acid can be toxic (concentration more than 10^{-5} M) which plays a role in inhibiting cytokinin oxidation and respiration processes in mitochondria, reducing Adenosine Tri Phosphate (ATP) in plasma membranes, and reducing polyphenol activity so that it inhibits growth and culture regeneration [30; 31], but at non-toxic concentrations (below 10^{-6} M) it helps induce the synthesis of phytoalexins, a form of plant response to inhibit pathogen activity [31]. The use of fusaric acid as a selection agent in vitro selection can produce mutant cells or tissues that are insensitive to fusaric acid so that after being regenerated into plants they can produce strains that are resistant to pathogen infection [32]. Phenol compounds are the result of plant metabolism that are formed with one function as a plant chemical resistance system that can prevent the growth and development of pathogens [29]. The purpose of this study was to determine the concentration of fusaric acid that was tolerant to *P. amabilis* plantlets and to analyze the total phenol content of plantlets after fusaric acid was induced with various concentrations.

2. MATERIAL AND METHOD

2.1. Plant Material

This research was conducted from June to October 2020 at the In Vitro Culture Laboratory, Faculty of Mathematics and Natural Sciences, University of Lampung. The research material was moon orchid plantlet [*Phalaenopsis amabilis* (L.) Bl.] which was added with Fusaric Acid.

2.2. Preparation for Planting Medium and Selection

The medium used was Vacin and Went (VW) which was sterilized using an autoclave at a pressure of 17.5 psi, 121 for 15 minutes. Next, the medium was added with fusaric acid with concentrations of 0 ppm (Control), 30 ppm, 60 ppm, 90 ppm, and 120 ppm. The fusaric acid used is pure fusaric acid produced by Sigma chemical Co. [Fusaric acid (5-butylpicolinic acid) from *Giberella fujikuroi*]. Before use, fusaric acid which had been dissolved with distilled water at a certain concentration was filtered using a syringe filter having a diameter of 0.45 µm twice, followed by a filter with a diameter of 0.22 µm once. The gradual filtration is carried out in a sterile room inside the Laminar Air Flow Cabinet. Furthermore, fusaric acid was added to the VW medium which had been sterile. Before use, this medium was incubated for 7 days at room temperature (25) to ensure that the fusaric acid was well filtered.

2.3. Planting of Plantlets in Fusaric Acid Selection Medium

The explants used were sterile *P. amabilis* plantlets. The plantlets from the culture bottles were removed with a sterile scalpel knife and placed one by one on a 10 cm diameter petri dish, then the plantlets were planted in each culture bottle containing the specified treatment medium. Each concentration was repeated 5 times and each replication consisted of one *P. amabilis* plantlet in each culture bottle.

2.4. Percentage of Number of Living Plantlets and Plantlets Visualization

Includes the color of plantlet after being given Fusaric Acid treatment with the following classification: green, green-brown, and brown.

2.5. Total Phenol Analysis

Materials for the analysis of phenolic compounds using fusaric acid-influenced plantlets were compared with controls. Analysis of total phenol compounds using the Singleton & Rossi method [33] with the following steps.

2.5.1. Preparation of standard calibration curve of phenol compounds

Gallic acid was used as a standard solution. The concentrations of pure gallic acid used were 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 ppm in distilled water. Then 0.5 mL of each concentration was taken and put into a 100 mL volumetric flask, then 2.5 mL of Folin-Ciocalteau reagent and 2 mL of 7.5% sodium carbonate were added. After being mixed, put it in a cuvette and

see the absorption value at a wavelength of 765 nm, as a control, distilled water was used. After knowing the absorption value, then a standard curve is made, and the regression equation is seen between the concentration of gallic acid and the absorption value.

2.5.2 Sample plantlet preparation

Plantlet extracts were prepared according to the method of [34]. Planet weighing 2 g was crushed using a glass mortar and dissolved in 25 mL of 80% ethanol. The solution was then centrifuged at 13,000 rpm for 15 minutes. After centrifugation, the supernatant was taken.

2.5.3 Measurement of total phenol

The supernatant was taken as much as 0.5 mL and put into a 100 mL volumetric flask, then 250 L of Folin-Ciocalteu reagent was added, after being allowed to stand for 5 minutes, then 1 mL of Na_2CO_3 was added. After thoroughly mixed, it was put into a cuvette with a volume of 5 mL and the absorption value at a wavelength of 765 nm was observed using a spectrophotometer (Beckman DU-65), as a control, distilled water was used as a control. From the absorption value, the content of phenolic compounds was determined based on the gallic acid regression equation, namely the relationship between the plantlet extract absorption value and the gallic acid concentration series.

2.6. Data Analysis

This study used a completely randomized design with one factor, namely the addition of fusaric acid concentration which was divided into 5 levels, namely 0 ppm, 30 ppm, 60 ppm, 90 ppm, and 120 ppm. Data analysis used Analysis of Variance with 5% significance level and further test with Tukey's test at 5% significance level. Data obtained from the growth of *P. amabilis* plantlets during selection with Fusaric Acid in the form of qualitative data and quantitative data. Qualitative data are presented inthe form of comparative descriptive and supported by photographs. Quantitative data were tabulated with different concentration factors with 5 replications for each treatment.

3. RESULTS AND DISCUSSION

Observation of the selection of *P. amabilis* plantlets grown on VW medium with theaddition of fusaric acid treatment in five concentration levels, namely 0 ppm (control), 30 ppm, 60 ppm, 90 ppm, and 120 ppm.

3.1. Percentage of the Number of Living Plantlets Selected with Fusaric Acid

Observations of the selection results of *P. amabilis* plantlets grown on VW medium with the addition of Fusaric Acid in five concentration levels, namely 0 ppm (control), 30 ppm, 60 ppm, 90 ppm, and 120 ppm is presented in Figure 1. Based on Figure 1. shows that at week 1 to week 2, the percentage of the number of surviving P. amabilis plantlets reached with 100% in all fusaric acid treatments. In the 3rd week, plantlets treated with 120 ppm fusaric acid experienced 20% mortality. In the 4th week, plantlets in control and those treated with fusaric acid with a concentration of 30 ppm did not die, but at a concentration of 60 ppm they experienced 20% death, while at a concentration of 90 ppm, plantlets died 40% and at a concentration of 120 ppm, plantlets experienced death 50% mortality. Death that occurs is indicated by the brown roots and leaves. The live plantlets were indicated by the increase in plantlets height, leaves and the emergence of new shoots on the

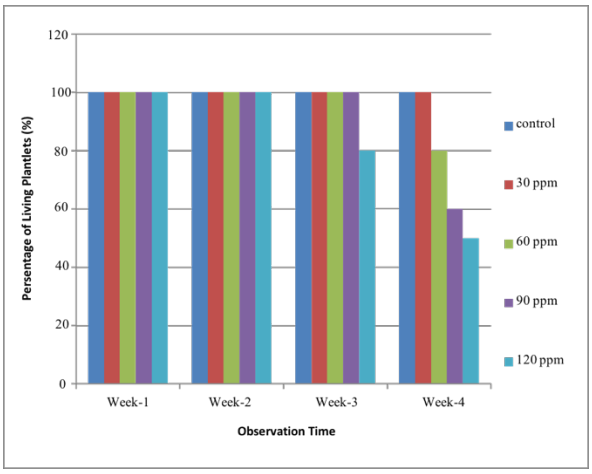

Figure 1. Percentage of viable *P. amabilis* plantlets after being induced by Fusaric Acid

plantlets.

3.2. Plantlet Visualization

Plantlet visualization observations were carried out for four weeks (30 days) after planting. During the treatment period, observation of plantlet visualization was carried out in the form of observing plantlet color (green, green-brown, and brown) [15,22]. Visualization of Plantlet Selection Results with Fusaric Acid is presented in Table 1.

Based on Table 1, it can be seen that the observations of the 1st and 2nd week of *P. amabilis* plantlets were green. The treatment began to give effect at week 3, at a concentration of 60 ppm visualization of

plantlets green, and green-brown. At a concentration of 90 ppm, plantlet visualization was green, brown-green and at a concentration of 120 ppm, plantlet visualization was green, green-brown, and brown. Observations in the 4th week with a concentration of 60 ppm visualization of plantlets green, green-brown, brown (dead). At a concentration of 90 ppm and 120 ppm, plantlet visualization was green, green-brown, brown (dead) [34] stated that fusaric acid can affect the elimination of O_2^- through the reduction of quinone which induces brown color, and is thought to be related to the transformation of phenol. The result of the phenol oxidation reaction is brown so the plantlets undergoing phenol oxidation appear brown. If this oxidation reaction takes place continuously, the brown color will spread and diffuse into the medium and affect the growth of other plantlets cultured with the browning plantlets.

Table 1. Visualization of Plantlet Selection Results

Fusaric Acid Concentration (ppm)	Visualization of Plantlet Selection Results with FusaricAcid			
	I	II	III	IV
0	H	H	H	H
30	H	H	H	H
60	H	H	H H	H HC C
90	H	H	H HC	H HC C
120	H	H	H HC C	H HC C

Description: H = Green, HC = Green-Brown, C = Brown

Table 2. Total Phenol Content of Moon Orchid Plantlets Induced with Fusaric Acid for30 Days

Treatment (ppm)	Total Phenol Content (mg/100g)
0	1.54 ± 0.048[a]
30	2.0 ± 0.082[b]
60	2.64 ± 0.047[c]
90	3.48 ± 0.046[d]
120	4.27 ± 0.042[e]

Description: Numbers followed by different letters indicate that there is a significant difference between treatments.

3.3. Total Phenol Content

P. amabilis plantlets that have been treated with fusaric acid can be characterized, among others, by the content of total phenol compounds. Measurement of total phenol content in P. amabilis plantlets that had been treated with fusaric acid at various concentrations using the Folin-Ciocalteu method. Before observing the phenolic compounds in *P. amabilis* plantlets, measurements were made using a standard gallic acid curve, to estimate the total phenol quality by linear regression.[23] The total phenol content of moon orchid plantlets induced by fusaric acid is presented in Table 2.

Based on Table 2. the total phenol content of *P. amabilis* plantlets showed that there was an increase in the total phenolic compound content along with the increasing concentration of fusaric acid. The total phenol content increased from 1.54 mg/g at a concentration of 0 ppm (control), increased to 2.0 mg/g at a concentration of 30 ppm, followed by 2.64 mg/g at 60 ppm, 3.48 mg/g at 90 ppm and 4.27 mg/g at a concentration of 120 ppm. This proves that the higher the concentration of fusaric acid, the higher the total phenol content produced. Phenolic compounds are one of the compounds that are distributed in plant parts, especially in the leaves [24,25,26]. Phenol is an aromatic compound that has a chemical structure derived from benzene and phenolic compounds tend to dissolve in water, generally bind to sugars as glycosides, present in water. cell vacuole [27].

The effect of fusaric acid induction on the formation of phenolic compounds is the resultof plant metabolism which is formed with one function, namely as a plant

chemical resistance system that can prevent the growth and development of pathogens. The increase in total phenol in orchid plants was caused by fusaric acid in plant tissues. The mechanism that plants go through includes the induction of fusaric acid in the medium which is then absorbed by the plant and the plant will give a chemical response, one of which is the production of phenolic compounds. [15] The results of this study are similar to the results of research conducted by [35] on Chickpea plants infected with *Fusarium oxysporum* f.sp. *ciceros*. From this research, it is known that there is an increase in total phenol of around 16-17%. Total phenol levels were also reported by [28], on patchouli infected by endophytic bacteria and nematodes, which showed that there was an indication of an increase in phenol content by *Achromobacter xylosoxidans.*

The increase in total phenolic compounds in *P. amabilis* plantlets induced by fusaric acid is another evidence of increased plant resistance in restraining the rate of *Fusarium oxysporum* infection. This is by the opinion of [29], which states that one of the parameters for increasing plant resistance to pathogens is the increase in phenolic compounds. According to [31], fusaric acid at non-toxic concentrations (10^{-7}) stimulates the formation of H_2O_2 which is closely related to the peroxidase enzyme. Furthermore, this enzyme will oxidize phenol compounds.

The results showed that: the concentration of fusaric acid that was tolerant to *P. amabilis* plantlets was 120 ppm; there was an increase in total phenol content along with an increase in fusaric acid concentration, an increase in total phenol content from 1.54 mg/g at a concentration of 0 ppm (control) increased to 2.0 mg/g at a concentration of 30 ppm, followed by 2.64 mg /g at a concentration of 60 ppm, 3.48 mg/g at 90 ppm and 4.27 mg/g at a concentration of 120 ppm. This proves that the higher the concentration of fusaric acid, the higher the total phenol content produced.

AUTHORS' CONTRIBUTIONS

Endang Nurcahyani: Participated in all experiments, coordinated the data analysis, and contributed to the writing of the manuscript. **Risma Rasmani**: Analyze the total phenol content data and in vitro selection. **Hardoko Insan Qudus**: Coordinated the data analysis and translation of the manuscript.

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