

Enrichment of organic complex  
compounds of coconut water  
and mungbean extract in  
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(*Chrysanthemum morfolium* L.)  
tissue culture media

*by* Fachirah Ulfa

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## Enrichment of organic complex compounds of coconut water and mungbean extract in chrysanthemum (*Chrysanthemum morfolium* L.) tissue culture media

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**Abstract.** A study was conducted aimed to determine the most effective concentration of mungbean sprouts extract and coconut water in chrysanthemum tissue culture to promote growth of chrysanthemum plantlet. The trial was conducted at the Tissue Culture Laboratory, Horticulture Seed Center, Bonto-Bonto District, Gowa Regency, South Sulawesi Province from August to December 2017 using a Randomized Block Design. Seven combination of Murashige and Skooge (MS) culture media added with mungbean sprouts extract and coconut water were used as treatments consisted of the addition of 1 ppm BAP synthetic hormone, coconut water (50, 75, and 100 ml/L, respectively) and mungbean sprout extract (20, 40, and 60 ml/L, respectively). The results show that the addition of coconut water and mungbean sprout extracts in the culture media had a significant effect on the parameters of time of shoots emergence, plantlet height, number of leaves, number of roots and root length. The addition of coconut water as much as 75 ml/L showed the best results based on the parameters of the time of shoots emergence. While the addition of mungbean sprout extract with a concentration of 40 ml/L showed the best results based on the parameters of plantlet height, number of leaves and root length.

### 1. Introduction

One of the ornamental plants that is in demand by the public is chrysanthemum. Chrysanthemum (*Chrysanthemum morfolium* L.) has a variety of shapes and colors as well as unique and attractive so that it is in demand by the public. High market demand for chrysanthemum led to a need for growing the plants both now and in the future. However, the higher demand for chrysanthemum plants are not comparable to the parent plant preparations. There is a lack of parent plants for the propagation of chrysanthemum seedlings. In addition, quality of seeds produced decreased with the increase of the age of the parent plant. This condition happened in conventional propagation of the chrysanthemum plants which propagated by shoot or shoot cuttings.

Propagation of chrysanthemum plants can also use a modern propagation such as tissue culture. Propagation of chrysanthemum plants carried out by tissue culture is expected to produce superior and uniform quality of chrysanthemum seeds, resistant to disease, high production levels and a relatively faster growing time compared to the conventional propagation. One of the factors that influence the



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1

success in the tissue culture is the composition of the media used. Culture media should not only contains macro and micro elements, but also carbon or other organic material sources. Growth regulator is a media component that is needed for growth and differentiation. Without growth regulators growing in the medium, explant growth is very inhibited growth may not occur at all.

The application of synthetic hormones in media in tissue culture is one of the factors causing high production costs. This is because the price of synthetic hormones is quite expensive and not always ready in stock. Therefore, it is necessary to look for alternative sources for hormones that can be used to replace the role of synthetic hormones (cytokinins or auxins). Natural hormones can be obtained from a variety of fruits, vegetables, fresh coconut water, and bean sprout extracts. Young coconut water is known to contain kinetin (cytokinin) of 273.62 mg/L and zeatin 290.47 mg/L, while the IAA content (auxin) is 198.55 mg/L [1]. In addition to plant growth regulator (PGR), the coconut water also contain vitamin that will act as addition to the vitamin content in the MS media. The use of MS media coupled with 10% coconut water on patchouli propagated in vitro showed the best response with an average percentage of live shoots of 100%, shoot number of 3 shoots and 9.10 leaves and shoot height of 1.61 cm [2]. Enrichment of mungbean sprout extract can also affect the success of plant propagation by tissue culture. Bean sprout extract contains essential amino acids such as tryptophan which is a precursor in the biosynthetic process of IAA (Indole Acetic Acid). Auxin can promote root growth which in turn will help the plantlet in absorbing water and nutrient from the media as a result from better root growth. Addition of the bean sprout extract in the media stimulated the root growth of moon orchid (*Phalaenopsis amabilis* L.) compared to control without the use of bean sprout extract [3].

Based on the description above, a research was conducted to study the effect of enrichment of the tissue culture media with organic compound of coconut water and mungbean sprout extract for chrysanthemum (*Chrysanthemum morfolium* L.).

## 2. Methodology

This research was carried out at the Tissue Culture Laboratory Horticulture Seed Center, Bonto-Bonto District, Gowa Regency, South Sulawesi Province. The trial was conducted from August to December 2017 using a randomized block design (RBD). Seven treatments tested in the study were the addition of 1 ppm BAP synthetic hormone, coconut water (50, 75, and 100 ml/L, respectively) and mungbean sprout extract (20, 40, and 60 ml/L, respectively) into MS culture media (Murashige and Skoog). Each treatment consisted of 3 replications, each treatment consisted of 3 units resulted in a total culture bottles used in this study of 63 bottles.

### 2.1. Preparation of the media

Prior to extraction, mungbean sprouts were prepared by germinating the mungbean seeds with a hypocotyl length of  $\pm 1$  cm and separated from the skin. Mungbean seeds were soaked for 24 hours, then drained and moistened. Moisture was maintained by sprinkling water to the mungbean seeds then placed in a dark place for 2-3 days. The mungbean sprout then extracted by dissolving 100 grams of mashed sprouts with 500 ml of distilled water then filtered.

MS control media (M0) was prepared by adding 1 ppm BAP to the MS solution. Other treatment media are prepared by adding 50 ml (M1), 75 ml (M2), and 100 ml (M3) of the coconut water to 500 ml of MS media solution in the measuring flask before the volume was added to 1 liter. Similarly, for the mungbean extract treatment, mungbean sprout extracts of 20 ml (M4), 40 ml (M5) and 60 ml (M6), respectively were added into the MS media. Media solution was then heated using a hot plate while stirring. Subsequently, the pH of the media was set to a range of 5.8 to 6.0 then the solution was poured into a sterile culture bottle of 20 ml per bottle. The bottle was tightly closed with plastic and rubber and then labeled before sterilized in an autoclave with a temperature of 121°C for 15 minutes.

## 2.2. Explant preparation and sterilization

Explants used in this study was two months old chrysanthemum parent plant of Aiko variety that are still in vegetative phase. The explants were obtained from the Horticultural Seed Center, Bonto-Bonto, Gowa Regency. The part of the plant taken for explants was the stem. Chrysanthemum stems were cleaned using running water then removed from the outer leaves during washing. Subsequently, sterilization of explants was performed by washing and removing dirty parts of former soil and small animals under running water for about 15 minutes. The cleaned explants material were placed in an Erlenmeyer that contains a solution of soap or fine detergent and then shaken for 30 minutes and rinsed three times using sterile water. Sterilization was continued by soaking explants using two drops of twin 20 + clorox 10% for 15 minutes then rinsed with sterile distilled water 5 times each for 5 minutes then drained.

## 2.3. Planting

Planting was carried out in Laminar Air Flow. A sterile explant was placed on a sterile petridish dish. Before planting, the stem was cut off by leaving a segment of 1.5 cm long. The stem pieces were then planted directly on media that has been prepared with 2 explants in one bottle. Following the planting, the bottle was closed using plastic wrapping then stored in a storage room.

## 2.4. Data analysis

Data were analyzed using analysis of variance to test the significance of the treatment given. If there is a significant effect then proceed with further tests using the contrast test.

## 5 Results

The results show that the addition of coconut water and mungbean sprout extracts in the tissue culture media had a significant effect on the parameters of time of shoots emergence, plantlet height, number of leaves, number of roots and root length of the Chrysanthemum plantlet. Average values of these parameter and the contrast test results are shown in Table 1.

Table 1 show that C3 contrast test ie. M2-3 treatments were significantly different and resulted in earlier shoots emergence compared to M1 treatments with an average of 2.67 days after planting (DAP) in M2 and M3 treatments and 3.50 DAP in M1 treatment. The treatments of M0, M4, M5, and M6 treatments were not significantly different from the various observational parameters. This shows that coconut water treatment can accelerate the time of shoot formation in chrysanthemum explants.

In plantlet height parameter, three contrast test pairs resulted in significant to highly significant differences. The contrast test of C3 ie M2-3 treatments is significantly different and showed higher plantlet compared to M1 treatment with an average plantlet height of 5.13 cm in M2-3 treatments and 4.50 cm in M1. The contrast test of C5 (M5-6 vs M4), the M5-6 treatment was significantly different than the M4 treatment with an average of plantlet height in M5-6 treatments was 5.21 cm while in the M4 treatment, average of plantlet height was 4.25 cm. The contrast test of C6 (M5 vs M6), the M5 treatment was significantly different and showed higher plantlet (5.50 cm) compared to the M6 treatment (4.92 cm). These contrast tests show that the addition of the organic compound of coconut water and mungbean sprout extract can affect the height of chrysanthemum plantlets. Similarly, the addition of coconut water and mungbean sprouts extract in to the MS media affected the parameter of plantlet leaves number of the chrysanthemum plantlet. The C2 contrast test results in Table 1 show M4-6 treatments were significantly different and showed more leaves compared to M1-3 treatments. Average of leaves number of the plantlets in M4-6 treatments was 11.36 leaves while M1-3 treatments showed 10.94 leaves. In the contrast test of C5 (M5-6 vs M4), higher plantlet leaves number was shown by the M5-6 treatments (11.92 leaves) compared to M4 treatment (10.25).

Table 1 also shows that number of roots of the chrysanthemum plantlet was affected by the addition of coconut water and mungbean sprouts extract in the media. There was a significant different between control or MS media with 1 ppm BAP with other treatments added with the organic compounds. Plantlet grown in the culture media added with coconut water and mungbean sprout

extract resulted in higher number of root (11.19) compared to control (9.67). Addition of mungbean extract into the media seems to increase the number of root more than the use of coconut water. Addition of 60 ml/L mungbean sprouts extract showed the highest number of root (13.33). In the coconut water treatments, the highest number of root of the plantlet obtained from the addition of 100 ml/L of coconut water in MS media.

**Table 1.** Effect of enrichment of organic complex compound of coconut water and mungbean sprouts extract in the tissue culture media on the growth of Chrysanthemum plantlet.

Contrast Table	Mean value		F-cal	Sig.	F-table	
					0.05	0.01
<i>Time of shoots emergence (DAP)</i>						
C1 : M <sub>0</sub> vs M <sub>1-6</sub>	2.67	24	3.11	3.28	ns	4.75 9.33
C2 : M <sub>1-3</sub> vs M <sub>4-6</sub>	2.94	vs 3.28	3.28	3.23	ns	
C3 : M <sub>1</sub> vs M <sub>2,3</sub>	3.50	vs 2.67	8.97		*	
C4 : M <sub>2</sub> vs M <sub>3</sub>	2.67	vs 2.67	0.00		ns	
C5 : M <sub>4</sub> vs M <sub>5,6</sub>	3.00	vs 3.42	2.24		ns	
C6 : M <sub>5</sub> vs M <sub>6</sub>	3.50	vs 3.33	0.27		ns	
CV						12.91%
<i>Planlet heights (cm)</i>						
C1 : M <sub>0</sub> vs M <sub>1-6</sub>	4.83	vs 4.90	0.15		ns	4.75 9.33
C2 : M <sub>1-3</sub> vs M <sub>4-6</sub>	4.92	vs 4.89	0.04		ns	
C3 : M <sub>1</sub> vs M <sub>2,3</sub>	4.50	vs 5.13	9.49		**	
C4 : M <sub>2</sub> vs M <sub>3</sub>	5.08	vs 5.17	0.13		ns	
C5 : M <sub>4</sub> vs M <sub>5,6</sub>	4.25	vs 5.21	22.31		**	
C6 : M <sub>5</sub> vs M <sub>6</sub>	5.50	vs 4.92	6.20		*	
CV						5.86%
<i>Number of leaves</i>						
C1 : M <sub>0</sub> vs M <sub>1-6</sub>	11.00	vs 11.15	0.38		ns	4.75 9.33
C2 : M <sub>1-3</sub> vs M <sub>4-6</sub>	10.94	vs 11.36	4.89		*	
C3 : M <sub>1</sub> vs M <sub>2,3</sub>	11.00	vs 10.92	0.09		ns	
C4 : M <sub>2</sub> vs M <sub>3</sub>	10.83	vs 11.00	0.26		ns	
C5 : M <sub>4</sub> vs M <sub>5,6</sub>	10.25	vs 11.92	34.78		**	
C6 : M <sub>5</sub> vs M <sub>6</sub>	12.00	vs 11.83	0.26		ns	
CV						7.12%
<i>Number of root</i>						
C1 : M <sub>0</sub> vs M <sub>1-6</sub>	9.67	vs 11.19	9.82		**	4.75 9.33
C2 : M <sub>1-3</sub> vs M <sub>4-6</sub>	10.61	vs 11.78	10.02		**	
C3 : M <sub>1</sub> vs M <sub>2,3</sub>	9.50	vs 11.17	9.09		*	
C4 : M <sub>2</sub> vs M <sub>3</sub>	10.33	vs 12.00	6.82		*	
C5 : M <sub>4</sub> vs M <sub>5,6</sub>	10.00	vs 12.67	23.27		**	
C6 : M <sub>5</sub> vs M <sub>6</sub>	12.00	vs 13.33	4.36		ns	
CV						3.59%
<i>Root length (cm)</i>						
C1 : M <sub>0</sub> vs M <sub>1-6</sub>	8.24	vs 8.73	3.05		ns	4.75 9.33
C2 : M <sub>1-3</sub> vs M <sub>4-6</sub>	8.47	vs 8.98	5.89		*	
C3 : M <sub>1</sub> vs M <sub>2,3</sub>	7.75	vs 8.83	12.01		**	
C4 : M <sub>2</sub> vs M <sub>3</sub>	9.17	vs 8.50	3.41		ns	
C5 : M <sub>4</sub> vs M <sub>5,6</sub>	8.60	vs 9.17	3.29		ns	

C6 : M <sub>5</sub> vs M <sub>6</sub>	9.50	vs	8.83	3.41	ns	
CV						5.11%

ns = not significant ; \* = significant; \*\* = highly significant. DAP = days after planting.

M0 = Murashige and Skoog (MS) + BAP 1 ppm; M1 = MS media + coconut water 50 ml/L; M2 = MS media + coconut water 75 ml/L; M3 = MS media + coconut water 100 ml/L; M4 = MS media + Mungbean sprouts extract 20 ml/L; M5 = MS media + Mungbean sprouts extract 40 ml/L; M6 = MS media + Mungbean sprouts extract 60 ml/L.

In the parameter of plantlet root length, significant difference was found between the addition of coconut water treatment and mungbean sprouts extract indicated by contrast test of C2 (table 1). Chrysanthemum plantlets planted in media with mungbean extract were slightly longer than plantlets planted in media with coconut water. Despite this, no significant differences found between the mungbean extract treatments (C5 and C6). Longest root was observed in the plantlet planted in the media MS added with 40 ml/L mungbean sprouts extract (9.50 cm).

#### 4. Discussion

The results showed that some treatments had a significant effect on almost all observational parameters (time of shoot emergence, plantlet height, number of plantlet leaves, the number of roots and root length of the plantlets). The results indicate that the treatment given had an influential role so that it can produce better growth in chrysanthemum plantlets.

Based on the observation of the parameters, the 40 ml/L of mungbean sprout extract treatment was the treatment that responded better to almost all parameters such as plantlet height (cm), number of leaves and root length (Figure 1). This is because bean sprouts contain auxin hormones and compounds that can stimulate plant growth. The mungbean sprouts contain macro nutrients, micro nutrients, vitamins, amino acids, and sugar which is needed for plant growth [4]. The significant essential amino acids contained in bean sprouts include tryptophan, threonine, phenylalanine, methionine, lysine, glutamic, isoleucine, and valine [3]. Bean sprout extract contains the amino acid tryptophan which is the most important organic substance in auxin biosynthesis, also has mineral contents such as calcium, iron, magnesium, phosphorus and zinc. Abidin [5] suggested that magnesium is a constituent of chlorophyll which acts as a food reserve for plant growth such as stems, leaves and roots. A previous study showed that the mungbean sprouts extract used as a substitute for synthetic growth regulators was very influential on the growth of moon orchids *in vitro* on the parameter of plant height, length and number of leaves, and length and number of roots [6].



**Figure 1.** Chrysanthemum plantlets 30 days after planting (DAP) on different organic compound in Murashige and Skoog (MS) media.

M0 = Murashige and Skoog (MS) + BAP 1 ppm; M1 = MS media + coconut water 50 ml/L; M2 = MS media + coconut water 75 ml/L; M3 = MS media + coconut water 100 ml/L; M4 = MS media + Mungbean sprouts extract 20 ml/L; M5 = MS

media + Mungbean sprouts extract 40 ml/L; M6 = MS media + Mungbean sprouts extract 60 ml/L.

<sup>4</sup> In the treatment of coconut water, the concentration <sup>6</sup> of 75 ml/L is the concentration that responded well to the parameter of shoot emergence although it was not significantly different from the control media <sup>22</sup> treatment (MS + 1 ppm BAP). This is related to the function of cytokines in coconut water which plays a role in the process of cell division, especially in the growth of the shoots. Cytokinins and auxins in coconut water both can provide interaction effects on tissue differentiation. Addition of auxin at relatively high levels affected the tissue differentiation that will promote root formation. While the cytokines at relatively high levels in the media will promote tissue differentiation for the <sup>23</sup> formation of stems or shoots [7].

Based on the results in the recent study, the use of BAP in MS media was less effective to affect the growth of chrysanthemum plantlets. It is suspected that the media and explants used are capable of producing endogenous cytokinins that can stimulate cell division, so the addition of exogenous cytokinins causes excess cytokinin content which can also cause retarded plant growth. Similarly, slower growth of plantlet roots observed in this treatment is presumably longer time needed for the root growth compared with other treatments. The use of BAP in low concentrations was likely more efficient enough to produce leaves [8]. It is known that cytokinin and auxin hormones work together to stimulate cell division and affect cell differentiation [9].

## <sup>6</sup> 5. Conclusions

Based on the results obtained, it can be concluded that:

- a) The treatment of <sup>5</sup> mungbean sprout extract 40 ml/L gave a better response to almost all parameters (plantlet height, number of leaves, and number of root formed and root length).
- b) Addition of 75 ml/L coconut water in MS media gave a better response to parameter of the time of shoot emergence.

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