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In Vitro Application of Kinetin and Tomato Extract on Orchid Growth of *Dendrobium stratiotes*

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ABSTRACT

Orchid is an ornamental plant which is an essential commodity in the international trade market because of the beauty of its flowers. This study aimed at examining the effect of kinetin and tomato extract on orchid growth and obtain the best concentration for developing *Dendrobium stratiotes* orchids *in vitro* on $^{1}/_{2}$ MS media. This research was conducted at the Laboratory of Plant Physiology and Biotechnology, Universitas Sebelas Maret Surakarta, from July to December 2022. The method used was a factorial, completely randomized design (CRD) with two factors, the first factor was the concentration of kinetin (0, 0.5, 1.0, 1.5, and 2.0 ppm) and the second factor was the concentration of tomato extract (0, 50, 100, 150 and 200 g/l). Each treatment was repeated in five replications. Data analysis was carried out by analysis of variance (ANOVA). The Duncan Multiple Range Test (DMRT) was used at the 5% for regression test. The results showed that the interaction of kinetin and tomato extract treatments significantly affected plant height, number of roots, number of leaves and fresh weight. Applying kinetin at two ppm significantly affected plant height, number of lateral shoots and fresh weight. The application of tomato extract 200 g/l considerably affected plant height and fresh weight.

Key words: Cultured plantlet, hormone, micro-propagation, ornamental plant, plant growth promotors

INTRODUCTION

Indonesia is known as a country with a diversity of fauna and flora. The abundant flora consists of various types of plants, including ornamental plants. BPS (2021) shows that orchid cultivation in Indonesia reached 3,999,203 trees. Kumar *et al.* (2018) state that orchids have multiple colours, shapes, sizes and long-lasting floral freshness. Generative propagation of orchids is done by growing seeds *in vitro* or tissue culture. Tissue culture is important in mass and rapid plant production (Lal and Singh, 2020).

Propagation of *Dendrobium* sp requires tissue culture techniques to increase the success of seedling propagation. The success of tissue culture techniques is determined by the planting material used, the growing medium and sterile conditions. Kumara *et al.* (2022) stated that the commonly used planting medium in orchid tissue culture was Murashige and Skoog (MS). MS media contains ammonium, potassium and nitrate. ½ MS media contains macro and micronutrients from full MS media. MS media is necessary for plants because it has all the necessary elements.

Growth regulators commonly added to tissue culture media are auxins and cytokinins. Auxin has a role in cell growth and elongation and can induce cell division and differentiation (Kang *et al.*, 2020). Cytokinins function to stimulate protein synthesis and cell division (Zahrotunnisa *et al.*, 2022). High cytokinin concentrations can stimulate shoot growth, while relatively high auxin concentrations stimulate root growth (Chen *et al.*, 2019). The use of auxin can be obtained from natural ingredients, one of which is tomato extract. The research results by Mose *et al.* (2020) stated that using tomato extract at a dose of 150 g/l produced the best height growth in *Phalaenopsis amabilis*. Research by Lawrie *et*

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al. (2021) giving 100 ml/l tomato extract increased germination in *Dendrobium capra*. One type of synthetic cytokinin is kinetin. Kinetin is a class of cytokinins that can spur cell division and organ formation (Harahap *et al*., 2020). Kinetin can affect the process of plant development at the right concentration. Maharjan *et al*. (2020) stated that using $^{1}/_{2}$ MS media with the addition of 2 ppm kinetin could increase the number of buds in *Dendrobium chryseum*. The use of suitable media to produce orchid seedlings is essential. This study examined the application of tissue culture to orchid growth response by adding kinetin and tomato extract.

MATERIALS AND METHODS

The research was carried out from July to December 2022 at the Laboratory of Plant Physiology and Biotechnology, Faculty of Agriculture, Universitas Sebelas Maret. The study began with the sterile germination of *Dendrobium stratiotes* seeds, followed by medium preparation, subculture and observations. Plantlets were prepared by germinating *D. stratiotes* seeds in a sterile environment. The seeds were planted in a medium and germinated in an aseptic environment. The plantlets of the germinated seeds were used as an explant in this study. Plantlets of *D. stratiotes* were ready to be used after the age of six months.

Sterilization was also carried out on equipment such as culture bottles, dissection equipment (big tweezers and scalpel knives) and petridishes. The tools were sterilized using an autoclave for 30 min at 121°C and 1 atm pressure, then stored in the oven till use. Subculture was done in a Laminar Air Flow (LAF). The sterilized plantlets were planted in a culture bottle containing the medium by two plantlets for each bottle. The culture medium was produced by adding varying kinetin and tomato extract concentrations to ½ Murashige and Skoog (MS) based medium.

This study used a completely randomized factorial design consisting of two treatment components and 25 treatment combinations. The first factor was the growth regulator kinetin (0, 0.5, 1.0, 1.5, and 2.0 ppm), and the second factor was the growth regulator from tomato extract (0, 50, 100, 150 and 200 g/l). The variables measured in the study were the plantlet height, number of lateral shoots, number of roots, emergence time of root, root length, number of leaves and fresh weight. The collected data were examined using analysis of variance with a 5% level test. If there was a significant difference, the Duncan Multiple Range Test (DMRT) at a 5% level was used as a regression test.

RESULTS AND DISCUSSION

Plant height increased due to vegetative growth activity. Vegetative growth started from the beginning of plant growth until flowering. Plantlet height was observed by measuring the height from the base of the stem to the tip of the leaf that was cupped using a ruler (cm). The results of ANOVA analysis and DMRT test at 5% level showed that the treatment producing the best plantlet height of 16.60 cm differed significantly from the treatment given a single tomato extract (Table 1). The single tomato extract treatment with a concentration of 200 g/l produced a plantlet height of 12 cm. This happened because adding auxin from tomato extract stimulated protein synthesis in plantlet tissues, which caused an increase in plant height. The increased plantlet height was influenced by the given growth regulator. The concentration of cytokinin was too high; which inhibited the growth of plantlet height and caused browning (Diengdoh *et al*., 2023). Adding tomato extract to media supplied some nutrients needed for plant growth (Dwiyani *et al*., 2022).

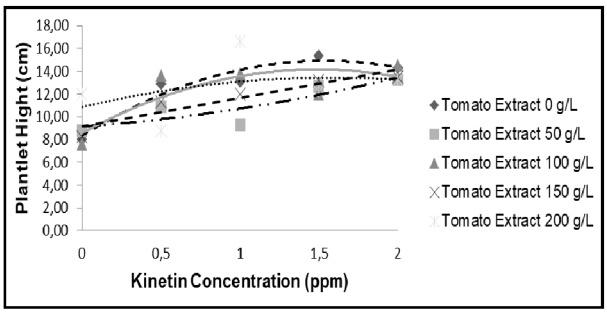
Table 1. The result of the interaction of kinetin and tomato extract on the height of *D. stratiotes* at 16 WAP

Kinetin (ppm)	Tomato extract (g/l)				
	0	50	100	150	200
0.0	8.02^{ab}	8.80^{abc}	7.58^{a}	8.26^{ab}	12.00^{defg}
0.5	12.90^{efgh}	10.90^{bcde}	13.60^{efghi}	11.30^{cdef}	8.80^{abc}
1.0	13.10^{efgh}	9.30^{abcd}	13.72^{efghi}	12.00^{efgh}	16.60^{i}
1.5	15.32^{hi}	12.70^{efgh}	12.02^{defg}	13.20^{efgh}	12.20^{defgh}
2.0	14.30^{fghi}	13.30^{efgh}	14.56^{ghi}	13.50^{efgh}	13.30^{efgh}

The same superscripts in the same column are not significantly different at 5%.

The single kinetin treatment that produced the best plant height was at a concentration of 1.5 ppm, with a plantlet height of 15.32 cm. Giving kinetin to tissue culture media affected cell division. The provision of cytokinins can

affect the activeness of cells to divide, so cell division occurs, and cells become more numerous (Kriswanto *et al.*, 2020). The increase in plantlet height was due to the process of cell division and elongation that occured in the apical meristem. Hamad *et al.* (2023) stated that giving kinetin stimulated the development of apical meristems of stem and root shoots, which caused an increase in stem and root length. Giving cytokinin and auxin spured cell division and cell elongation. The regression graph showed that tomato extract concentrations of 0, 50, 100 and 200 g/l produced a quadratic pattern, while the 150 g/l extract had a linear pattern (Fig. 1). Tomato extract at a concentration 150 g/l with the addition of kinetin at a concentration of 0 to 2 ppm increased the height of the plantlets (Fig. 1). The coefficient of determination showed the high and low effects of kinetin concentration and tomato extract on plantlet height. Based on the regression test, the graph showed that providing kinetin with a concentration of 0 to 2 ppm without tomato extract affected the height of orchid plantlets *D. stratiotes* by 92%. Hartati *et al.* (2022) stated that the balance and interaction between endogenous and exogenous growth regulators (absorbed from the media) could control plant growth *in vitro*.



Where:

Y (Tomato extract 0 g/l) = 8.3349 + 8.5846x – 2.7943x^2 R^2 = 0.9286

Y (Tomato extract 50 g/l) = 9.1257 + 1.0171x + 0.5714x^2 R^2 = 0.7506

Y (Tomato extract 100 g/l) = 8.5631 + 7.5274x – 2.5257x^2 R^2 =0.6689

Y (Tomato extract 150 g/l) = 9.176 + 2.476x R^2 = 0.8727

Y (Tomato extract 200 g/l) = 10.866 + 3.2571x – 1.0286x^2 R^2 = 0.1439

Fig. 1. Graph of the interaction of kinetin and tomato extract on the height of *D. stratiotes* plantlets at 16 WAP.

The number of shoots was influenced by the given growth regulators. Lateral shoots grow beside the main plant. The increasing number of shoots indicated that the given growth regulators were appropriate. Shoot growth was influenced by the given cytokinin hormone. The analysis showed that kinetin gave significantly different results on the number of lateral shoots. Giving kinetin increased the number of lateral shoots. It was because kinetin was a synthetic cytokinin growth regulator that could cause inhibited shoot growth if the concentration was too high.

Kinetin significantly affected the number of lateral shoots (Table 2). A concentration of 2 ppm of kinetin produced the most lateral shoots 1.64. Kinetin treatment of 0.5 ppm had fewer shoots than 1.32. Based on Maharjan *et al.* (2019), giving 2 ppm kinetin in $^1/_2$ MS media produced the best shoot growth. The control treatment had lateral shoots of 1.34, which was not significantly different from the kinetin treatment with a concentration of 1 ppm, producing shoots of 1.38. Kinetin with a concentration of 0 ppm produced more shoots than kinetin with a concentration of 0.5 ppm because in plants there were endogenous cytokinin hormones that control plant growth. The growth of *Cattleya xanthina* with MS media produced the best shoot growth in providing higher cytokinin than auxin (Juras *et al.*, 2019). The growth of shoots was influenced by the given cytokinins. Giving cytokinin from kinetin with higher concentration produced the highest number of shoots (Gantait *et al.*, 2020).

Table 2. Effect of kinetin on the number of lateral shoots in *D. stratiotes*

Kinetin (ppm)	No. of lateral shoots
0.0	1.34^a
0.5	1.32^a
1.0	1.38^{ab}
1.5	1.45^b
2.0	1.64^c

The same superscripts in the same column are not significantly different at 5%.

Roots absorb nutrients, both macronutrient and macronutrient, from the growing media used by plants for their growth. The interaction of kinetin 0.5 ppm with 100 g/l tomato extract produced the best number of roots, namely, 14.60 (Table 3). Giving higher auxin concentration than cytokinins in MS media caused tissue morphogenesis, leading to root formation in *Coelogyne xanthina* (Juras *et al.*, 2019). Cytokinin can stimulate adventitious

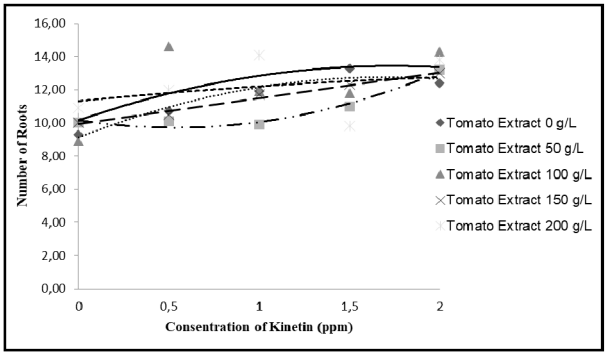
Table 3. The result of the interaction of kinetin and tomato extract on the number of roots of *D. stratiotes* at 16 WAP

Kinetin (ppm)	Tomato extract (g/l)				
	0	50	100	150	200
0.0	9.30^{ab}	10.00^{abcd}	8.90^{a}	10.00^{abcd}	10.90^{abcdef}
0.5	10.70^{abcde}	10.10^{abcd}	14.60^{g}	10.50^{abcde}	12.00^{bcdefg}
1.0	11.90^{abcdefg}	9.90^{abc}	11.90^{abcdefg}	11.70^{abcdef}	14.10^{g}
1.5	13.30^{efg}	11.00^{abcdef}	11.80^{abcdefg}	12.30^{bcdefg}	9.80^{abc}
2.0	12.40^{cdefg}	13.20^{efg}	14.30^{g}	13.00^{defg}	13.80^{fg}

The same superscripts in the same column are not significantly different at 5%.

root formation by synthesizing plant parts injured by cutting explant. However, giving high concentrations of cytokinin reduce root growth. Nowakowska *et al.* (2022) stated that adding cytokinin to the media reduced the percentage of root growth.

Tomato extract contained auxin, which played a role in root formation. According to Jing and Strader (2019) auxin had a role in activating enzymes that manufacture cell components so that cell division occurs. The concentration of 150 g/l tomato extract affected the number of roots of orchid plantlets by 98% (Fig. 2). Bhowmik and Rahman (2020) gave low concentration of auxin, which induced root growth and caused roots to grow and develop well, then giving auxin at a high concentration. Root emergence time determines the fulfill ment of nutrients from the plantlet; so the faster the emergence of roots, the faster the nutrients will be fulfilled. Kinetin at 2 ppm gave a faster root emergence time than 0.5 ppm kinetin treatment (Table 4), which gave rise to roots at 11.08 days after planting (DAP). This was probably due to the low cytokinin content in the plantlets. Hence, it is necessary to increase the cytokinin concentration to induce root emergence in plantlets. Arafa *et al.* (2021) stated that adding kinetin to treatment media did not affect the root formation time but the number of roots formed.



Where:

Y (Tomato extract 0 g/l) = 9.1314 + 4.2743x – 1.2571x^2 R^2 = 0.9421

Y (Tomato extract 50 g/l) = 10.166 – 1.6829x + 1.5714x^2 R^2 = 0.9687

Y (Tomato extract 100 g/l) = 10.157 + 3.7714x – 1.0857x^2 R^2 = 0.3495

Y (Tomato extract 150 g/l) = 9.94 + 1.56x R^2= 0.9845

Y (Tomato extract 200 g/l) = 11.314 + 1.0629x – 0.1714x^2 R^2 = 0.097

Fig. 2. Graph of the interaction of kinetin and tomato extract on the number of roots of *D. stratiotes* plantlets at 16 WAP.

Table 4. Effect of kinetin on root emergence time in *D. stratiotes*

Kinetin (ppm)	Root emergence time (DAP)
0.0	11.52^{b}
0.5	11.08^{b}
1.0	11.44^{b}
1.5	11.32^{b}
2.0	8.92^{a}

The same superscripts in the same column are not significantly different at 5%.

Tomato extract with a concentration of 200 g/l produced the fastest root emergence time 9.72 DAP (Table 5). The treatment of 100 g/l tomato extract was not significantly different from the treatment of 0, 50 and 100 g/l tomato extract, which produced the fastest root emergence time after the treatment of 200 g/l tomato extract. The 0 g/l tomato extract treatment delivered the longest root emergence time 11.48 DAP. This was because

Table 5. Effect of tomato extract on root emergence time in *D. stratiotes*

Tomato extract (g/l)	Root emergence time (DAP)
0	11.48^{b}
50	11.20^{b}
100	10.72^{b}
150	11.16^{b}
200	9.72^{a}

The same letters in the same column are not significantly different at 5%.

the endogenous auxin content had not reached the balance point for the plant growth process. Thus, adding exogenous auxin which stimulates root formation is necessary. Relatively high auxin concentrations strongly influenced plantlet root growth (Konstantinova *et al.*, 2021).

The length of the root plant affects the absorption field of nutrients; the wider the absorption field, the better nutrients are taken from the growing media. Nutrients in the growing media cause root growth. The elongation of the tip meristem cell influenced root length. One ppm kinetin treatment produced the best root length of 4.61 cm significantly different from the treatment without kinetin which only had a root length of 3.93 cm (Table 6). High concentrations of cytokinin inhibited root growth. Kasem and Helaly (2021) showed that kinetin at low concentration produced significant root length compared to kinetin at high concentration, which inhibited root elongation.

Table 6. Effect of kinetin on root length in *D. stratiotes*

Kinetin (ppm)	Root length (cm)
0.0	3.93[a]
0.5	4.54[ab]
1.0	4.61[b]
1.5	4.36[ab]
2.0	4.46[ab]

The same superscripts in the same column are not significantly different at 5%.

Single tomato extract had no significant effect on root length. Tomato extract concentration of 50 g/l produced the best root length of 4.65 cm (Table 7). Root elongation was strongly influenced by auxin and the optimal amount of endogenous auxin to increase division in the root meristem. Tomato extract contains auxin in the cell vacuole so that the osmotic pressure increases and decreases the pH causing the cell wall arrangement to be organized and elastic (Utami and Hariyanto, 2020). Increasing the concentration of tomato extract did not affect root length because the concentration of auxin had reached the optimal concentration, as the need for auxin was fulfilled endogenously or exogenously. Exogenous auxin had a role in root formation and elongation. Hossen *et al.* (2021) stated that adding auxin with higher concentration and lower cytokinins was perfect for root growth as it provided many roots.

Table 7. Effect of tomato extract on root length in *D. stratiotes*

Tomato extract (g/l)	Root length (cm)
0	4.35[a]
50	4.65[a]
100	4.35[a]
150	4.10[a]
200	4.45[a]

The same superscripts in the same column are not significantly different at 5%.

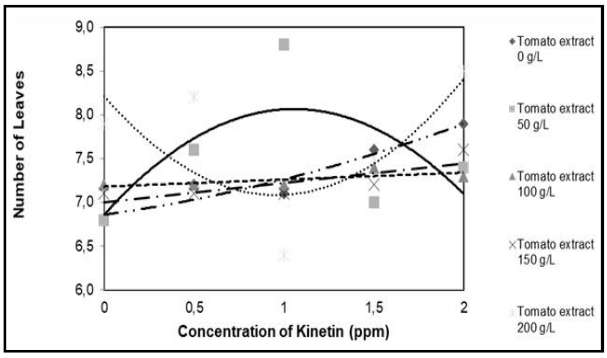
The number of leaves in the kinetin treatment with a concentration of 1 ppm and 50 g/l tomato extract had the highest number of leaves 8.80 (Table 8). Leaf formation cannot be separated from the influence of cytokinin and auxin hormones. Longchar and Deb (2022) stated that kinetin gave the highest number of leaves in *D. heterocarpum*. The interaction effect of 1 ppm kinetin and 200 g/l tomato extract showed the least number of leaves 6.40. This treatment significantly differed from kinetin 2 ppm without tomato extract which produced several leaves 7.90. The provision of low exogenous auxin made a high number of leaves because the endogenous auxin in the plantlets balanced the cytokinin concentration. The proportion of concentration and interaction between plant growth promotors used in culture media and endogenous hormones determined the growth of a plant (Kasutjianingati *et al.*, 2022). PGR with a higher concentration than the optimum concentration can disrupt plant metabolic processes. Nelson *et al.* (2023) stated that providing auxin and cytokinin with balanced concentrations positively affected the growth of orchid leaves. An unbalanced concentration of growth regulators caused non-optimal growth.

Table 8. The result of the interaction of kinetin and tomato extract on the number of leaves of *D. stratiotes* at 16 weeks

Kinetin (ppm)	Tomato extract (g/l)				
	0	50	100	150	200
0.0	6.80[ab]	6.80[ab]	7.20[abcd]	7.10[abc]	7.90[bcde]
0.5	7.20[abcd]	7.60[abcde]	7.20[abcd]	7.10[abc]	8.20[cde]
1.0	7.10[abc]	8.80[e]	7.20[abcd]	7.10[abc]	6.40[a]
1.5	7.60[abcde]	7.00[abc]	7.40[abcd]	7.20[abcd]	7.50[abcd]
2.0	7.90[bcde]	7.40[abcd]	7.30[abcd]	7.60[abcde]	8.50[de]

The same superscripts in the same column are not significantly different at 5%.

The interaction effect of kinetin and tomato extract (Fig. 3) obtanied the coefficient of determination R^2 = 0.919 with the equation 6.8571 + 0.2914x + 0.1143x^2. Giving kinetin concentration of 0 to 2 ppm without tomato extract had a 91% effect on the number of leaves of *D. stratiotes*. High concentrations of auxin caused the leaf differentiation process to be inhibited because the ability of meristem cells to divide was higher than the differentiation process into shoots or leaves. Auxin played a role in the process of cell division, so that during the process of cell differentiation into leaf tissue cytokinin affected the process (Wu *et al.*, 2021).



Where:

Y (Tomato extract 0 g/l) = 6.8571 + 0.2914x + 0.1143x^2 R^2 = 0.919

Y (Tomato extract 50 g/l) = 6.8571 + 2.2914x – 1.0857x^2 R^2 = 0.436

Y (Tomato extract 100 g/l) = 7.18 + 0.08x – 7E-15x^2 R^2 = 0.5

Y (Tomato extract 150 g/l) = 7 + 0.22x R^2 = 0.6436

Y (Tomato extract 200 g/l) = 8.2143 – 2.3571x + 1.2286x^2 R^2= 0.5059

Fig. 3. Graph of the effect of the interaction of kinetin and tomato extract on a number of leaves of *D. stratiotes* at 16 WAP.

The rise in the number of roots, leaves, root length and plantlet height increased the plantlet weight. Fresh weight was carried out using digital scales (g). The interaction of kinetin and tomato extract significantly affected the fresh weight of plantlets (Table 9). The treatment of 1 ppm kinetin and 200 g/l tomato extract produced the best plantlet fresh weight of 2.95 g, though it was not significantly different from the treatment of 1.5 ppm kinetin and 200 g/l. which increased the growth of plant tissue and caused an increase in plantlet fresh weight. The interaction of organic matter with synthetic growth regulators produced more suitable orchid growth than a single treatment of organic matter without the addition of synthetic growth regulators (Aung *et al.*, 2022). The hormone auxin increased the diffusion of water into cells (Band, 2021). Nutrients contained in the media were absorbed along with water through the diffusion process. Exogenous growth regulators balanced endogenous hormones to influence physiological responses as a driver of cell division.

Table 9. The result of the interaction of kinetin and tomato extract on the fresh weight of *D. stratiotes* at 16 WAP

Kinetin (ppm)	Tomato extract (g/l)				
	0	50	100	150	200
0.0	1.56^{abcd}	1.14^{ab}	1.26^{abc}	1.55^{abcd}	1.85^{bcd}
0.5	1.83^{bcd}	1.94^{cd}	1.88^{bcd}	1.89^{cd}	1.81^{bcd}
1.0	1.62^{abcd}	0.97^{a}	1.80^{bcd}	1.76^{bcd}	2.95^{e}
1.5	1.86^{bcd}	1.44^{abcd}	1.24^{abc}	1.57^{abcd}	2.88^{e}
2.0	2.08^{d}	1.81^{bcd}	1.86^{bcd}	1.59^{abcd}	2.14^{d}

The same superscripts in the same column are not significantly different at 5%.

Kinetin without tomato extract increased plantlets' fresh weight (Fig. 4). This treatment had a regression equation Y = 1.626 + 0.014x + 0.1x^2 with a coefficient of determination R^2 = 0.7148; which meant that an increase in kinetin concentration without the provision of tomato extract affected 71%. Adding kinetin to the equilibrium point increased the fresh weight of the plant. Growth regulators play an important role in regulating the direction of growth. Puri *et al.* (2022) stated that giving kinetin was essential to increase the fresh weight of plantlets because kinetin played a role in the physiological processes in the roots and shoots; this physiological process resulted in the growth and increase of plant organs.

CONCLUSION

The interaction treatment of kinetin and tomato extract increased the growth of *D. stratiotes* orchid plantlets in plantlet height, number of roots, number of leaves and fresh weight. The concentration of kinetin 2 ppm gave the highest number of lateral shoots and accelerated the time of root emergence in orchid *D. stratiotes*. A tomato extract concentration of 200 g/l accelerated the time of root emergence, and a tomato extract concentration of 50 g/l increased root length.

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