

**Effect of different concentrations of selected liquid fertilizers on acclimatization of weakly grown *in vitro* plantlets of *Anthurium andraeanum* L.var. 'Lalani'****Kumarasinghe, P G S A<sup>1</sup>, Srikrishnah, S<sup>1</sup>, Anjali, Y M U<sup>2</sup> and Sutharsan, S<sup>1</sup>**<sup>1</sup>*Department of Crop Science, Faculty of Agriculture, Eastern University, Sri Lanka*<sup>2</sup>*Department of National Botanical Gardens, Peradeniya, Sri Lanka***Abstract**

An experiment was conducted to evaluate the effects of different concentrations of selected liquid fertilizers on the acclimatization of weakly grown *in vitro* *Anthurium* plantlets at the Royal Botanic Gardens, Peradeniya from June to August 2018. Treatments were defined as T1 (1.11g/L Albert's solution applied as 1<sup>st</sup> application and 0.55g/L Albert's solution applied as 2<sup>nd</sup> application), T2 (1.11g/L Albert's solution applied in both application), T3 (0.55g/L Albert's solution applied in both application), T4 (0.625g/L N:P:K (30:10:10) solution applied as 1<sup>st</sup> application and 0.3125g/L N:P:K (30:10:10) solution applied as 2<sup>nd</sup> application), T5 (0.625g/L N:P:K (30:10:10) solution applied in both application), T6 (0.3125g/L N:P:K (30:10:10) solution applied in both application), T7 (1.333g/L N:P:K (20:20:20) solution applied as 1<sup>st</sup> application and 0.666g/L N:P:K (20:20:20) solution applied as 2<sup>nd</sup> application), T8 (1.333g/L N:P:K (20:20:20) solution applied in both application), T9 (0.666g/L N:P:K (20:20:20) solution applied in both application), T10 – Sterile water (control). First application was done at the time of transplanting and second application was done two weeks after transplanting. Experimental design was Completely Randomized Design with ten replicates for each treatment. Other agronomic practices were followed uniformly for all the treatments. Plant height, Number of roots, Number of leaves and Length of petiole were measured at four weeks after transplanting. Analysis of Variance was performed to determine significant difference among treatments ( $p < 0.05$ ). Results revealed that better growth performances viz. increase in plant height, leaf number and roots number and length of petiole were observed in plantlets grown exposed to T2. This may be due to optimum amount of nutrients received by the plantlets at T2. Thus it may be concluded that application of Albert's solution (1.11g/L in two applications at two weeks interval) provide optimum amount of nutrients for acclimatization of weak *in vitro* *Anthurium* plantlets.

**Key words:** Acclimatization, Albert's solution, *In vitro* plantlets, Liquid fertilizers**1. Introduction**

*Anthurium* is an imperative tropical cut flower that belongs to the family Araceae. Conventionally *Anthurium* was mainly propagated through seeds. *Anthuriums* also reproduce vegetatively through suckers, which arise around the base of the stem. Micropropagation is an

effective alternative to conventional propagation of plants and the culture of

somatic cells, tissues and organs of plants under laboratory conditions is a suitable way to produce a large number of plants which are genetically identical to the stock plant in a short time (Chugh *et al.*, 2009). Features of micropropagation viz.

multiplicative capacity in a relatively short time and healthy and disease-free production capacity (Atak and Celik, 2012) are conducive for commercial production of plants

However there are some constraints in micropropagation of Anthurium plantlets such as, time consume for regeneration from ex plants, diseases, slow growth of plantlets and problems during acclimatization process (Gerszberg, 2018). Anthuriums are epiphytic plants (Tatte, 2016). Quality of growing medium and nutrient management has a significant influence on growth and flowering of Anthurium plants (Atak and Celik, 2012). Nutrient requirements of Anthurium can be met through various sources. One of the main sources of plant nutrients are chemical fertilizers. Application of fertilizers in small quantity with frequent intervals has been found beneficial to increase growth and flower production (Thangam *et al.*, 2013).

Foliar sprays are used to increase the growth of *in vitro* Anthurium plantlets. The quality, quantity and frequency of foliar applications also influence growth and quality of plants. Albert's solution, N: P: K (30:10:10) and N: P: K (20:20:20) liquid fertilizers could increase the growth rate of weakly grown *in vitro* Anthurium plantlets. However, optimum concentration and frequency of application of these solutions to the *in vitro* plantlets has not yet been identified. Hence the present study was conducted with the objectives of;

1.To investigate the effects of different concentrations of selected liquid fertilizers on the growth of *in vitro* Anthurium plantlets.

2.To identify the optimum concentration of the suitable liquid fertilizers to increase the growth of *in vitro* Anthurium plantlets.

## 2. Materials and Methods

### 2.1 Experimental Site

A pot experiment was carried out in the Glasshouse of the Anthurium section, Royal Botanical Garden, Peradeniya from June to August 2018. The Experimental site was located in the mid country wet zone (MW<sub>2</sub>) of Sri Lanka.

### 2.2 Experimental design

The experiment was arranged in a completely randomized design (CRD) with ten treatments. Each treatment was replicated 10 times with 10 plants per replicate. An experimental unit consisted of one plant. Treatments were defined as follows:

- T1 (1.11g/L Albert's solution applied as 1<sup>st</sup> application and 0.55g/L Albert's solution applied as 2<sup>nd</sup> application)
- T2 (1.11g/L Albert's solution applied in both application)
- T3 (0.55g/L Albert's solution applied in both application)
- T4 (0.625g/L N:P:K (30:10:10) solution applied as 1<sup>st</sup> application and 0.3125g/L N:P:K (30:10:10) solution applied as 2<sup>nd</sup> application)
- T5 (0.625g/L N:P:K (30:10:10) solution applied in both application)
- T6 (0.3125g/L N:P:K (30:10:10) solution applied in both application)
- T7 (1.333g/L N:P:K (20:20:20) solution applied as 1<sup>st</sup> application and 0.666g/L N:P:K (20:20:20) solution applied as 2<sup>nd</sup> application)
- T8 (1.333g/L N:P:K (20:20:20) solution applied in both application)
- T9 (0.666g/L N:P:K (20:20:20) solution applied in both application)
- T10 - Sterile water (control)



**Plate 2.1. Experimental arrangement of plantlets inside the glass house**

### 2.3 Preparation of pots & potting media

Plastic pots were used for the experiment. The diameter and height of the plastic pots were 5.2cm and 11cm respectively. Pots were disinfected with a 1g/L captan solution. Tile pieces 1 cm× 1cm in size were placed at the bottom of each pot for improved drainage. Tile pieces were covered with a single dried leaf to prevent clogging of tile pieces by media. The composition of the potting media was coir dust and sand in a ratio of 1:1 (volume basis). Media was sterilized and filled in pots.

### 2.4 Planting materials

Uniform, nine to ten months old *in vitro* plantlets were used as planting material for the experiment. Plantlets were transplanted in the prepared pots after disinfection.

### 2.5 Application of treatments

First application of treatments was carried out immediately after transplanting. Liquid fertilizers (10ml respectively) were applied as a foliar

spray to each replicate as per treatment. After application of the foliar spray, each pot was covered with 300 gauge transparent polythene and kept inside the glass house. Second application was done two weeks after transplanting. Other agronomic practices were followed uniformly for all treatments as recommended by the Royal Botanic Garden, Sri Lanka.

### 2.6 Measurements

Plant height, Number of roots, Number of leaves and Average length of petioles were measured. Initial measurements were taken at the time of transplanting and final measurements were taken four weeks after the transplanting. Sampling method was non-destructive. Sampling interval was four weeks. All the measurements were taken from ten replicates from each treatment.

### 2.7 Statistical Analysis

Analysis of Variance was carried out using SAS Version 9.1 to determine significant differences among treatments

( $p < 0.05$ ). Treatment means were compared using Duncan Multiple range test (DMRT) test at the 0.05 probability level.

### 3. Results and Discussion

#### 3.1 Plant Height

It was found that there were significant

( $p < 0.05$ ) differences in plant height of Anthurium var. 'Lalani' plantlets grown under different treatments at 4 weeks after transplanting (WAT) (Fig.3.1). Highest increase in plant height (1.78 cm) was recorded in T2 (1.11g/L Albert's solution applied in both application) compared to other treatments at 4 WAT.

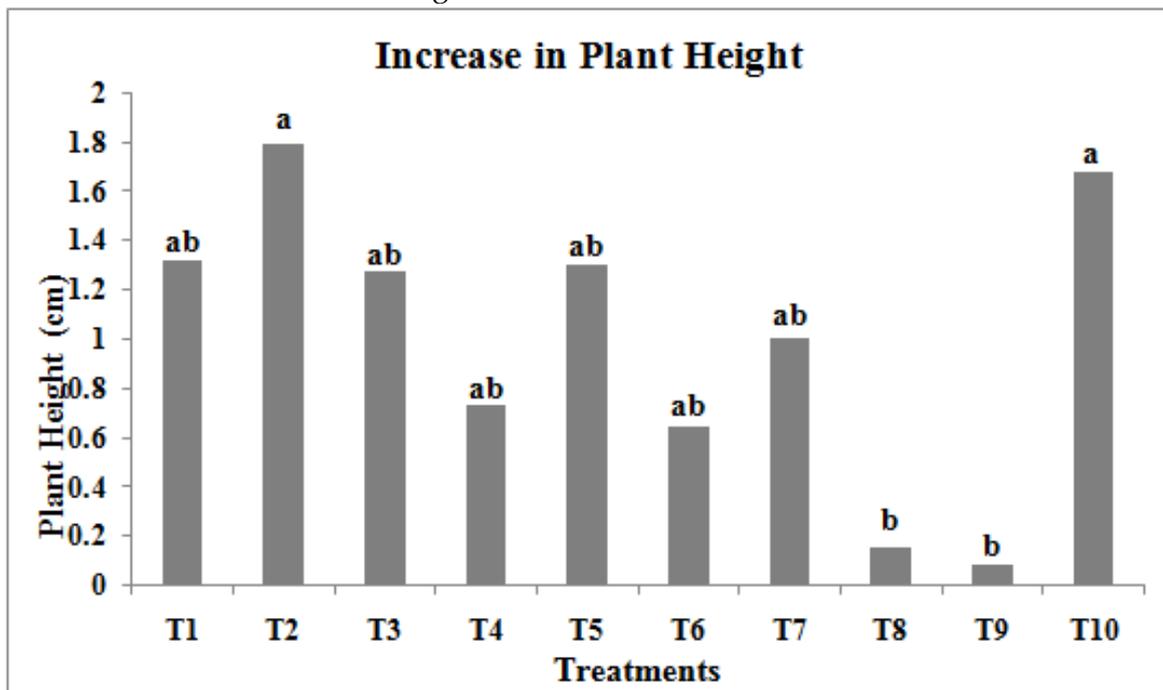


Fig. 3.1. Effect of different concentrations of selected liquid fertilizers on plant height of Anthurium (*Anthurium andraeanum*) var. 'Lalani' at 4 weeks after transplanting. Bars on the graph with same letter are not significantly different according to the Duncan's multiple range test at 5% probability. (n=10)

Plantlets grown at T9 had lowest increase in plant height (0.087cm). Fertilizer application is widely used to improve plant growth and productivity (Shen *et al.*, 2010). Liquid fertilizers have the potential to increase plant growth and development (Liu *et al.*, 2014). Plant height is an important agronomic trait (Zhang *et al.*, 2017) and an important morphological character that acts as a potential indicator for availability of growth resources in its vicinity (Rao *et al.*, 2016).

Increase in height is a response and indicator to the nutrient status of a plant. Plant height is a function of combined

effect of genetic characteristic, nutrient status, plant vigour and environmental conditions under which it was grown (Farooq *et al.*, 2009). Increase in plant height is an indicator for plant nutrient status. According to Frahdian *et al.* (2018) plant height is influenced by availability of nutrients and nutrient uptake by roots. Plantlets grown at T2 would have received required amount of nutrients compared to plants exposed to other treatments. Therefore, they had shown highest increase in plant height.

An essential nutrient has an obvious physiological role and its absence suppresses plant growth (Taiz and Zeiger, 1998). Presently 17 elements are

considered essential for most plants (Salisbury and Ross, 1992). Albert's solution contains most of these essential macro and micronutrients, which are required for plant growth.

In this experiment, maximum plant height was obtained in T2. It could be due to optimum amount of micro and macronutrients would have received by the plantlets. Iqram and Seran (2016) reported that, in tomato maximum plant height was obtained when 2 g/L of Albert's was solution applied four times. Optimum amount of nutrients are required for maximum plant growth. Optimum growth is attained when nutrients are available in adequate amount (Frahadian *et al.*, 2018).

Several researchers reported that, foliar application of micronutrient promotes plant growth. Husain *et al.* (1989) reported that foliar application of iron in the form of ferrous ammonium citrate along with Zn and B increased the height of chillis. Hooda and Pandita (1982) found that foliar application of Zn, Mn, Fe and Cu at the rate of 1 kg per ha to potato also increased plant height. Tamilselvi *et al.* (2002) found that, foliar application of micronutrients increased plant height of tomato.

Plantlets grown at T2 would receive optimum amount of micro and macronutrients as foliar sprays. Application of micronutrients along with macronutrients as foliar spray has the capacity to increase the plant height. Application of nutrient as foliar spray has been found beneficial to improve the growth and flower production of Anthurium (Valsalakumari *et al.*, 2001). Tatte (2016) reported that, aerial sprays of nutrient solution give quicker growth in Anthurium. Therefore application of Albert's solution at the rate of 1.11g/L in two times as foliar spray could be the optimum dosage to increase the plant height of *in vitro* Anthurium plantlets.

In T1 (1.11g/L and 0.55g/L Albert's solution) and T3 (0.55 g/L Albert's solution applied in both times) plantlets would have received sub optimum amount of nutrients. It could be the reason for lowest increase in the plant height. Frahdian *et al.* (2018) opined that, if the nutrients available in the medium are low, the plants exhibit reduction in growth. Baloch *et al.* (2008) stated that the reduction of HiGrow (commercial foliar fertilizer consisting of micro and macronutrients) concentration to 5 ml/L and 4 ml/L water caused significant negative effect on all the growth and yield components of chilies.

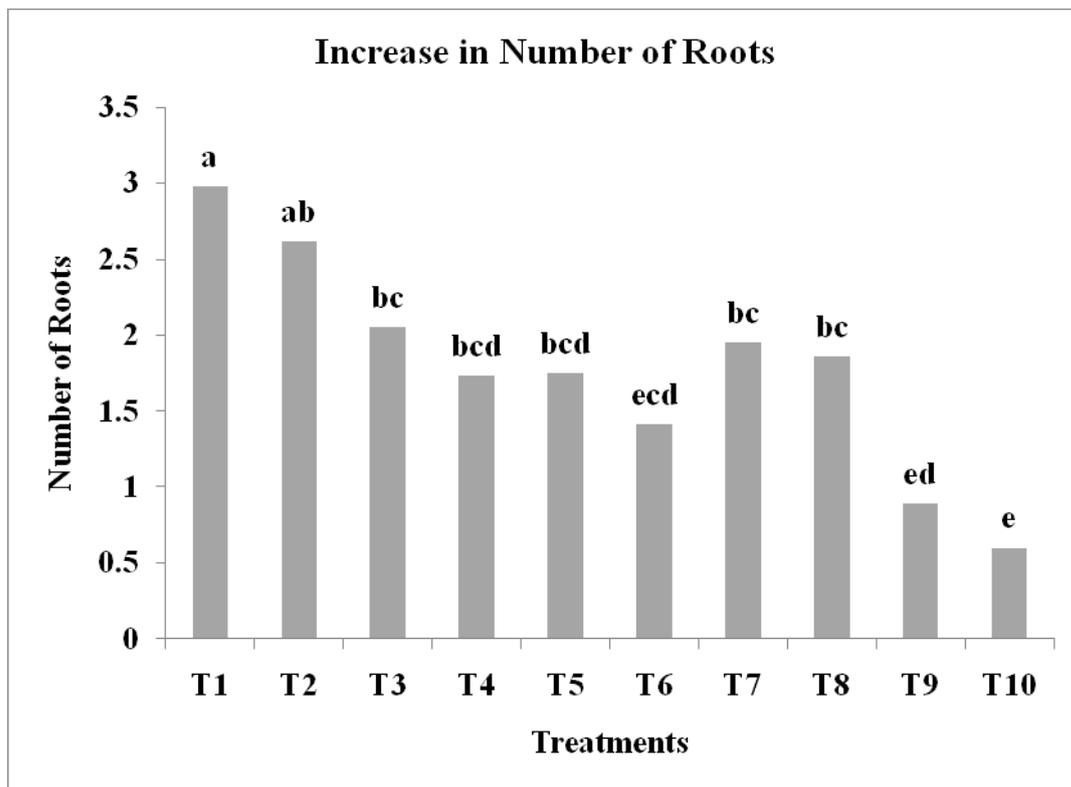
Lowest increase in plant height was also observed in treatment T4 to T9. In these treatments, experimental plantlets received only macronutrients (N, P and K). It might be the reason for lowest increase in plant height. Plant growth is affected by deficient in micronutrients (Alamdari and Mobasser, 2014). Younis *et al.* (2013) reported that lowest plant height was observed in treatment that only included nitrogen, phosphorus and potassium for *Rosa* hybrid. Thus findings of this study and previous reports by various scientists support the conclusion that both micro and macronutrients directly affect height of plants.

### 3.2 Number of Roots

It was found that there were significant ( $p < 0.05$ ) differences in increase in root number of Anthurium var. 'Lalani' plantlets belong to different treatments at 4 weeks after transplanting (WAT) (Fig.3.2). Highest increase in roots number was recorded in T1 (1.11g/L applied in 1<sup>st</sup> application and 0.55g/L of Albert's solution applied in 2<sup>nd</sup> application) compared to other treatments at 4 WAT. However there were no significant differences between T1 and T2 for increase in root number.

Application of liquid fertilizers increased root numbers of experimental plants over the control. At 4 WAT, highest increase in number of roots was observed in T1. However, there were no significant differences between T1 and T2. Plantlets grown at T10 showed lowest increase in root number. Roots are important organs

(Merrill *et al.*, 2002) and they absorb nutrients and water from the soil and translocate them within plants (Sainju *et al.*, 2005). Roots also produce hormones that affect many physiological and biochemical processes associated with growth and development (Zobel, 1991).



**Fig. 3.2. Effect of different concentrations of selected liquid fertilizers on root number of Anthurium (*Anthurium andraeanum* L) var. ‘Lalani’ at 4 weeks after transplanting. Bars on the graph with same letter are not significantly different according to the Duncan’s multiple range test at 5% probability. (n=10)**

Several studies reported that, application of foliar sprays of nutrients promote root development. Forde and Lorenzo (2001) reported that foliar application of nitrogen, phosphorus, potassium and ferrous affect developmental processes such as root branching, root hair production, root diameter, root growth angle and nodulation. Plants treated with foliar application of micronutrients along

with NPK showed significant increase in root number and dry weight of rose (Younis *et al.*, 2013). Yadegari (2016) suggested that foliar application of  $Mn^{2+}$  and  $H_2BO_3^-$  along with phosphorus and nitrogen in the form of phosphate and nitrate would increase the root number and root length in citrus.

Highest increase in root number was observed in T1 (1.11g/L Albert's solution in 1<sup>st</sup> application and 0.55g/L of Albert's solution in 2<sup>nd</sup> application) and T2 (1.11g/L Albert's solution in two applications). It might be due to optimum amounts of nutrients received by plants exposed to these treatments. Therefore, they had shown highest increase in number of roots. Adequate availability of nutrients enhances the optimum root growth (Sathiyavani *et al.*, 2017).

Root development is an indication of plant nutrient status. Kohli *et al.* (2012) stated that, Ca and P were essential nutrients for root-growth. Albert's solution contains macronutrients (N, P and K) and micronutrients (Ca, Mg, B, Cu, Fe, Mn, Zn and Mo) (Iqram and Seran, 2016). Foliar application of nutrients could enhance the root growth (Younis *et al.*, 2013). Foliar spraying of nutrients enhances the growth and quality of orchids (Ali *et al.*, 2013). Anthurium is also an epiphytes and prefers foliar application of nutrients as Orchid. Therefore application Albert's solution as foliar spray increased the root development of plants at T1 and T2 as it contains optimum amount of essential nutrients for root development.

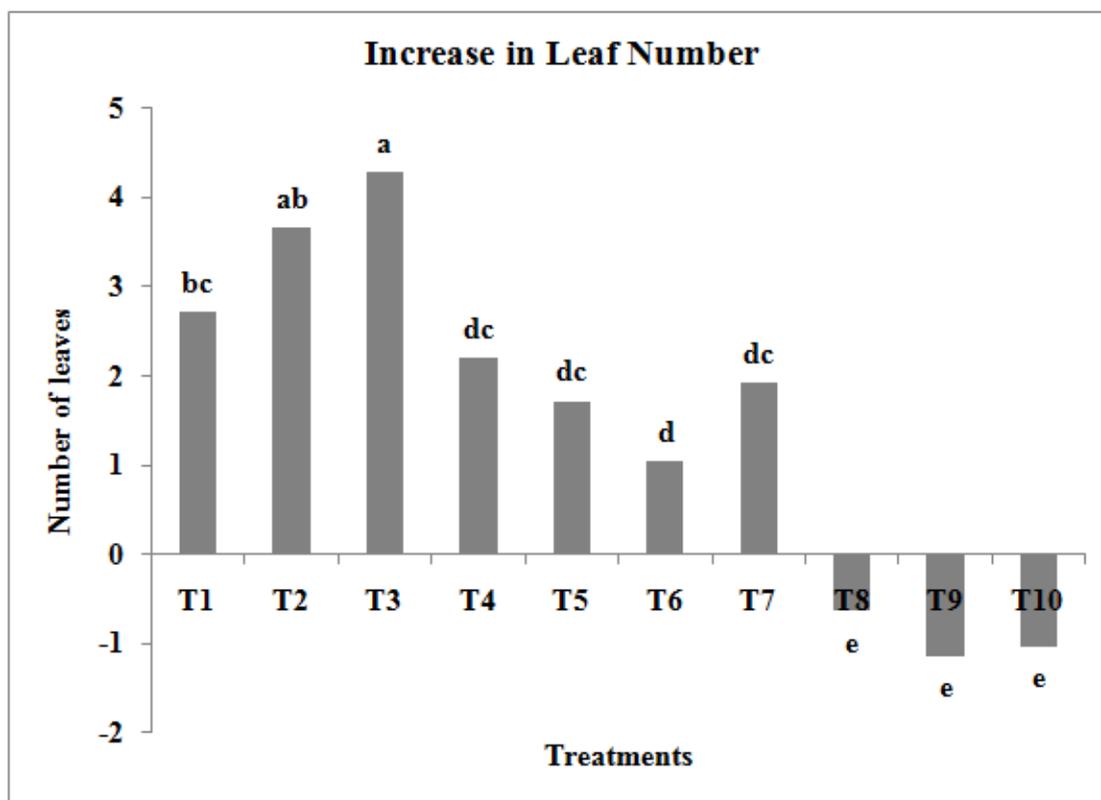
Plantlets grown at T3 would have received sub optimum amount of nutrients. It could be the reason for lowest growth of roots at this treatment. Among the seventeen essential nutrients, some of the nutrients are antagonistic in relation to root growth and some of the

nutrients have a synergistic relation to the root growth and developments. A less than adequate supply of any one of these essential elements will lead to metabolic disruptions, including changes in activities of enzymes, rate of metabolic reactions, and concentration of metabolites. This can cause retarded growth of plants (Sathiyavani *et al.*, 2017).

Plants grown in T4 to T9 only received macronutrients (N, P and K). Calcium is an essential nutrient for root growth (Kohli *et al.*, 2012). However, plants grown at these treatments did not receive exogenous calcium for root growth. It could be the reason for lowest increase in root number. Weerasinghe *et al.* (2014) noted that treatments containing micro and micronutrient, significantly increased most of the growth parameters due to the application of all essential macro and micronutrients. Higher application N and P also had a growth retardant effect (Omotoso and Shittu, 2007).

### 3.3 Number of leaves

It was found that there were significant ( $p < 0.05$ ) differences in leaf number of Anthurium var. 'Lalani' plantlets belong to different treatments at 4 weeks after transplanting (WAT) (Fig.3.3). Highest increase in leaf number was observed in T3 (0.55g/l Albert's solution in two applications) compared to other treatments at 4 WAT. However there were no significant differences between T3 and T2 in increase in leaf number.



**Fig. 3.3.** Effect of different concentrations of selected liquid fertilizers on leaf number of *Anthurium (Anthurium andraeanum)* var. 'Lalani' at 4 weeks after transplanting. Bars on the graph with same letter are not significantly different according to the Duncan's multiple range test at 5% probability. (n=10)

Application of different liquid fertilizers influenced leaf number of experimental plantlets over the control. At 4 WAT, highest increase in number of leaves was observed in T3. However, there were no significant differences between T3 and T2. Plantlets grown at T8 to T10 showed decrease in leaf number. Leaves are the most important morphological organ in the plants (Waldhoff and Parolin, 2010). Leaves directly affect photosynthesis, gas exchange and other important biological activities of plants. Number of leaves in the plants affect to the rate of photosynthesis, which is a main metabolic activity that affects growth and development (Suárez, 2010).

Numerous researches revealed that foliar application of nutrients increase leaf number. Application of macronutrients

and micronutrients as foliar application enhances number of leaves of tomato (Mohsin *et al.*, 2011). Khosa *et al.* (2011) reported that, vegetative growth such as number of leaves, length of stalk and flowering of gerbera was increased through application of liquid fertilizers in the form of foliar application. Ji *et al.* (2017) found that foliar application of liquid organic fertilizers significantly promoted the leaf growth in Chrysanthemum. Liquid fertilizers can increase plant metabolic activities and thus led to increase growth of plants by increasing number of leaves, plant height, dry matter content etc.

Plantlets grown at T3 and T2 showed highest increase in leaf number. It could be due to optimum amount of nutrients received by these plantlets at optimum

frequency. Optimum amount of nutrients are important for maximum leaf growth. Weerasinghe *et al.* (2014) reported that, mint (*Menthaspicata*) plants achieved highest leaf number and height and leaf area in 50% concentrated Albert's solution. Albert's solution contains all the essential nutrients required for plant growth. Application of Albert's solution at optimum amount and appropriate frequency would have improved leaf growth in plants exposed to T3 and T2.

Multi nutrient foliar fertilization could be a practical method to provide balanced plant nutrition in horticulture (Fageria *et al.*, 2009). Albert's solution is also a multi nutrient foliar fertilizer, that consists of macro and micronutrients. Application of Albert's solution at the optimum amount would have increased the leaf number in the plantlets at T3 and T2. Hatwar *et al.* (2003) opined that improvement in growth characters such as leaf area by the application of nutrients could be due to enhanced photosynthetic and other metabolic activity which leads to an increase in various plant metabolites responsible for cell division and elongation.

Lowest increase in leaf number was observed in T4 to T7 and reduction in leaf

number was observed in T8 and T9. Plants exposed to T4 to T9 received only macronutrients as foliar spray. Bashir *et al.* (2013) reported that micronutrients such as Zn and Mo increase the number of leaves. Most researchers concluded that micronutrients enhance the number of leaves and branches. Khosa *et al.* (2011) found that NPK along with Zn, B, Fe, Mn attain highest number of leaves. Sawan *et al.* (2001) reported that foliar spray of micronutrients produce good quality healthy leaves and it enhances the fruit quality in sweet orange. Javaid *et al.* (2005) revealed, highest number of leaves can be obtained by application of macro and micronutrients solution. Therefore lack of availability of micronutrients could be the reason for reduced number of leaves in T4 to T9.

### 3.4 Length of Petiole

It was found that there were significant ( $p < 0.05$ ) differences in the average length of leaf petioles of Anthurium var. 'Lalani' plantlets exposed to different treatments at 4 weeks after transplanting (WAT) (Fig 3.4). Highest increase in petiole length (6.93 mm) was recorded in T7 compared to other treatments at 4 WAT. However there were no significant differences between T7 and T2 in increase of petiole length.

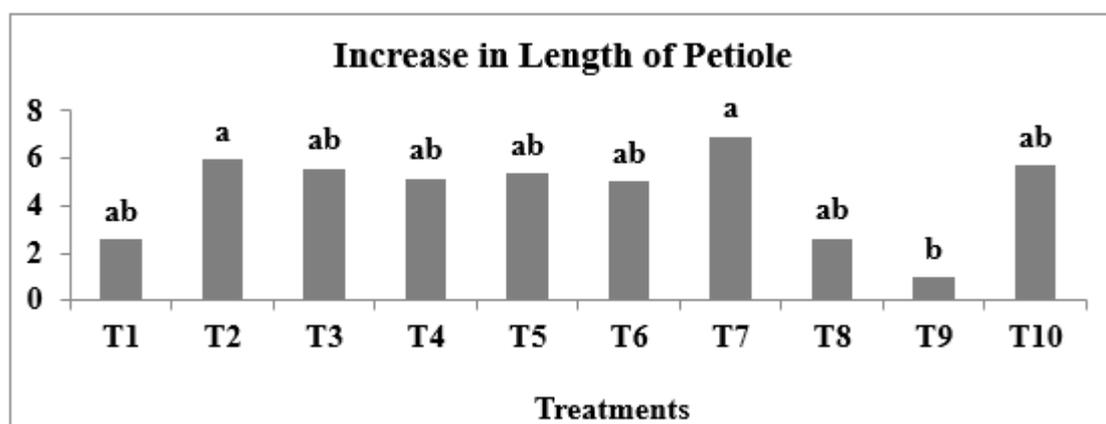


Fig. 3.4. Effect of different concentrations of selected liquid fertilizers on petiole length of Anthurium (*Anthurium andraeanum*) var. 'Lalani' at 4 weeks after transplanting. Bars on the graph with same letter are not significantly different according to the Duncan's multiple range test at 5% probability. (n=10)

Application of liquid fertilizers influenced the length of petiole of experimental plantlets. Plantlets grown at T9 (0.98mm) showed lowest increase in petiole length. Petiole plays an important role in plants. It helps to transport nutrients and water that are absorbed by the roots and passed up through the xylem, to the leaf (Pasini and Mirjalili, 2006). Foliar application of nutrients (N, P and K) significantly influenced the petiole length of Anthurium (Tatte, 2016). Kumar *et al.* (2017) reported that application of macronutrients together with micronutrients enhances the length of petiole in strawberry plants.

Application of foliar spray of nutrients promoted growth of the petiole length. Nutrient viz. Nitrogen (N), Phosphorus (P) and Potassium (K) mainly affect the vegetative growth of plants. Therefore optimum amount of N, P and K could enhance the petiole length. Application of macronutrients together with micronutrient increases the petiole length and girth of petiole (Mullangi, 2013). Lahijie (2012) reported that FeSO<sub>4</sub> and ZnSO<sub>4</sub> could also increase the length of petiole. Choi *et al.* (2013) reported that length of petiole increased in strawberry treated with phosphorus solution. Tatte (2016) found that Anthurium plants treated with foliar spray of 0.2% of N: P: K: (30:10:10) at weekly interval produced maximum length of leaf petiole.

Highest increasing in petiole length was observed in T7 (1.333g/L in first application and 0.666g/L in second application of N: P: K (20:20:20) solution at two weeks interval) and T2 (1.11g/L Albert's solution in two applications). It could be due to optimum amounts of nutrients received by plantlets exposed to both treatments. Therefore, they had shown highest increase in length of petiole. Tatte (2016) revealed that, aerial sprays of nutrient solution enhance the length of petiole in Anthurium plants.

Plantlets grown at other treatments showed reduced rate of increase in petiole length. It could be due to plantlets grown at these treatments not receiving optimum amount of nutrients. Optimum concentrations of nutrients influence petiole length of Anthurium plantlets. Low and higher concentrations of liquid fertilizers could suppress the growth of plants by changing enzymatic activities and metabolic reactions (Crawford, 1995).

#### 4. Conclusion

Highest increase in plant height was observed in T2 at 4 weeks after transplanting (WAT). Higher increase in root number was recorded in T. However there were no significant differences between T1 and T2 in increase in root number at 4 WAT. At 4 WAT, highest increase in number of leaves was observed in T3. Conversely, there were no significant differences between T3 and T2 in increase in leaf number. Highest increase in petiole length was observed in T7. However, there were no significant differences between T7 and T2 in increase in petiole length at 4 WAT. Therefore Albert's solution at the rate of 1.11g/L in two times (T2) at two weeks interval as a foliar spray could be the optimum amount and frequency to maximize the growth of *in vitro* Anthurium (var. 'Lalani') plantlets.

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