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Use of B-Sugar complex in increasing the Anthurium cut flower's vase-life.

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Abstract— *Anthurium is one of the important economic cut flowers all over the world. One of the most important problems in the production and sale of cut flowers is their vase life after their separation from the plant. A good solution for holding cut flowers must contain antimicrobial materials and nutrients. B is an element that is not in fungi and bacteria, therefore, its high levels are toxic to them. Sucrose can also be a source of energy for cut flowers. Our aim is to use Sucrose and B properties to increase the longevity of this cut flowers. According to the characteristics that B-sucrose complex has, the effect of this complex on increasing the longevity of cut flowers in terms of pollution reduction and energy supply was tested. Analysis of data showed that this B-Sucrose complex reduces rotting of the flower stem end by reducing the pollution. Since the rotting of stem end reduces the absorption of water and nutrients through the stem, this reduction in pollution could increase the vase life. Sucrose is also a source of energy and B can enhance the absorption of sucrose by the plant. Therefore, the B-Sucrose complex could significantly affect the vase-life and increased it.*

Keywords— Water absorption, B-Sucrose complex, pollution reduction, energy supply.

Abbreviation

According to the data of this research and available information about these two elements, the sucrose-B complex can increase the vase life of cut flowers such as Anthurium that have economic importance. Also, use of this compound has economic profits in two ways: 1) Increasing the vase life and being inexpensive. 2) Sucrose is accessible anywhere. So, this holding solution can be used instead of many other holding solutions in markets.

Highlight:

1- Sucrose-B complex can increase the vase life of cut flowers.
2- Sucrose-B complex is inexpensive and is accessible anywhere.

I. INTRODUCTION

Anthurium belongs to the Araceae family. These beautiful flowers are produced and sold as ornamental flowers throughout the world (Croat, 1988).The decline of this flower takes place by the appearance of blue spots on the margins of the spathe, spathewilting and spathe or spadix darkening (Croat, 1988).

Different factors affect the vase-life of cut flowers; chemical and physiological factors: such as the content of stored foods of flower, humidity, light, and temperature of the place that flowers are kept. Factors affecting water uptake such as air embolism and duration of vascular occlusion contribute to cut-flower senescence in Anthurium. Vascular occlusion is a mechanism for and as a result of water stress that induces senescence in Anthurium (Elibox & Umaharan, 2010).

Wilting is more important than senescence in the termination of the vase life of cut flowers. The end of their life is usually the result of their inability to draw water from the vase solution. Also, this blocking can take place by clogging of the vascular tissue in the stem by phloem-produced material (Blevins & Lukaszewski, 1998). Another factor affecting vase-life of cut flowers is content of stored foods. Cut flowers are forced to continue living with reserved carbohydrates, proteins and fat for their longevity. Adding sucrose can provide some energy for the flower until the end of its life. Also, some other chemicals can help prolong vase life and the marketability and quality of flowers(Sudagar, Sankarnarayannan, & Aruna, 2009).

Chemical factors: Senescence can be affected by many molecules and hormones. They can be ethylene, ABA, auxin, cytokinin, inositol trisphosphate, diacylglycerol, calcium, polyamines, jasmonic acid and NO(Ya'acov, Wills, & Ku, 1998; Rubinstein, 2000; Rogers, 2006).

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Biotic factors:Role of fungi and bacteria in vase-life of cut flowers: Sometimes, wilting occurs by fungi and bacteria, they cause wilting by blocking stem vessels. A large number of fungi, such as some species of *Fusarium*, and bacterial agents such as*Ralstonia* and *Xanthomonas*, can attack the flower through the end of the cut stem(Mansfield, et al., 2012) and block the vessels of the stem. Therefore, water can't reach the upper parts of the stem and the flower will wilt. The shelf life of Anthurium cut flower is relatively good but vascular obstruction by such fungi and bacteria can reduce this good shelf life (van Doorn, 1997).

Globally, much research has been done to reduce the shelf life of ornamental flowers and researchers have achieved much success. In 2007, Agampodi and Jayawardena showed that a 50% Coconut water with 0.23% NaOCl has the potential to be used as a preservative medium for Anthurium cut flowers. The vascular tissue of the stem can be blocked by phloem-produced material. By adding silver nitrate to distilled water, this blockage can be reduced(Blevins & Lukaszewski, 1998). Ram and Rao(Ram & Rao, 1977)proved that the shelf life of *Lupinushartwegii*could be increased by aluminum sulfate and Citric acid.

Therefore, there are many elements and components that can be used to increase the shelf life of cut flowers. One of these is B element.

One of the roles of B in delaying wilting is limiting the growth of fungi and bacteria. B itselfcan also be toxic to some fungi like *Neurosporacrassa* as Bowen and Gauch(Bowen & Gauch, 1966) reported and some decay fungi (Freitag & Morrell, 2005; Kartal, Yoshimura, & Imamura, 2004; Lesar, Kralj, & Humar, 2009). In another research, Bowen and Gauch(Bowen & Gauch, 1966) indicated that toxicity level of B prevents the use of carbohydrate in *S. cerevisiae*. In this case, these levels of B reduced the aldolase activity; therefore, the fungi were unable to utilize a sufficient rate of carbohydrate.So B inhibits the activities of glycerol phosphate dehydrogenase and aldolase in fungi(Bowen & Gauch, 1966; Misawa, Kaneshima, & Akagi, 1966; Kaneshima, Kitsutaka, & Akagi, 1968). It forms a complex with fungi substances which have cis-hydroxyl groups in their molecules (Lee & Aronoff, 1967). It is also toxic to bacteria(Ahmed & Fujiwara, 2010).

Benefits of sucrose in increasing the vase life of cut flowers:Sugar has an important role in the longevity of flowers, especially cut flowers, because after harvest they receive no nutritional and hormonal support from the mother plant(Van Staden, 1995). Sugar supplies substrates for respiration, which is a structural material and osmoticum and can also suppress the biosynthesis of ethylene (Dilley & Carpenter, 1973). So, it can prevent the sensitivity of cut flowers to ethylene(Mayak & Dilley, 1976). The role of sugar as a structural material is in cell wall synthesis of plant organs(Ichimura, 1998). Also, its role in water uptake could be due to the increase in the osmotic concentration of the cut flower(Pun & Ichimura, 2003).

In the experiment, which we carried out on tomato seedlings previously, B and sucrose were autoclaved together to form a B-sucrose complex. The tomato seedlings in the sucrose-Bcomplex containing solution showed a better longevity than other seedlings and were juicier and their roots were also healthier. Hence, we decided to study the effect of this complex on the cut flowers that here is Anthurium.

II. MATERIALS AND METHODS

Fig.1: Anthurium var. Alexi.

In this experiment, in April 2016, Anthurium var. Alexi cut flowers from 4 years-old bushes were prepared from Nochin Sepahan hydroponic greenhouse located in the city of Isfahan in Iran. Flowers were harvested in the morning and with 1/4 of theirspadices opened. They were immediately placed in distilled water with a 4.3 pH and a temperature of 10 degrees Celsius to prevent transport stress on flowers. Then, we transferred them to ten-liter buckets containing 500 mL of solution. The treatments included a series of solutions containing 1) 0%B+0%sucrose (as control), 2) 0% B+0%sucrose, 3) 0.4% B+0%sucrose, 4) 0.4 % B+2%sucrose, 5) 0.8% B+0%sucrose, 6) 0.8% B+2%sucrose, 7) 1.2% B+0%sucrose, 8) 1.2% B+2%sucrose, 9) 1.6% B+0%sucrose, 10) 1.6% B+2%sucrose, 11) 2% B+0%sucrose, 12) 2% B+2%sucrose. The experiment was done in twelve repeats, with 5 cut flowers per replicate. Buckets containing cut flowers were placed in a condition with a temperature of 20 degrees Celsius and humidity of 50% in indirect sunlight. To measure the vase life of flowers, they were inspected individually on a daily basis. We continued daily observing of the flowers until all of them experienced senescence. The number of faded flowers was counted during a 25 day period. Criteria considered for the faded flower were withered spathe, an

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appearance of blue spots on the margins of flowers and darkening of the tip of the spadix.

2-1 Isolation and culture of Fungi and bacteria from the stem end:

10 ml vase solution of each bucket was removed and the number of spores and bacterial cells was counted by Neobar lam. To isolate fungi and bacteria associated with rotten stem end, 2 cm of the rotten stem end was cut and cultured on Petri dishes respectively containing modified PDA and Nutrient Agar mediums.

2-2 Investigate the effect of B on isolated fungi and bacteria:

To investigate the effect of different concentrations of B on isolated fungi and bacteria we added five different concentrations of B(0.4%, 0.8%, 1.2%, 1.6% and 2%) in each Petri dish containing the desired fungi and bacteria. Their growth rates were measured and compared with the control. To measure the growth rates of bacteria, we used "the spread plate method".

2-3 Vase solution uptake rate:

Weights of vases containing vase solutions without the cutspikes were recorded in theseventh day.

The total weight of absorbed water: W_T

The weight of the water on the first day: W_0

The weight of the water on the seventh day: W_1

$W_T = W_1 - W_0$

2-4 Dry weight of the fungus/ Biomass production

The fungi were cultured in 100 cc of liquid culture media containing different B concentration+ 2%sucrose and kept at 25 ° C inside the incubator. After 7 days, the fungus weight was calculated using the following formula. The fungal mycelium was harvested after 7 days, separated from the culture liquid by filtration through a Whatman No. 2 filter paper. The mycelial pellet dried at 65°C overnight. The dry weight wascalculated by using the following formula:

Dry weight = (weight of filter paper + mycelium) - (weight of filter paper)

2-5 Statistical analysis:

The results were interpreted using R 3.3.1 software and Excel 2013.

III. RESULTS

3-1 Cut flower vase-life:

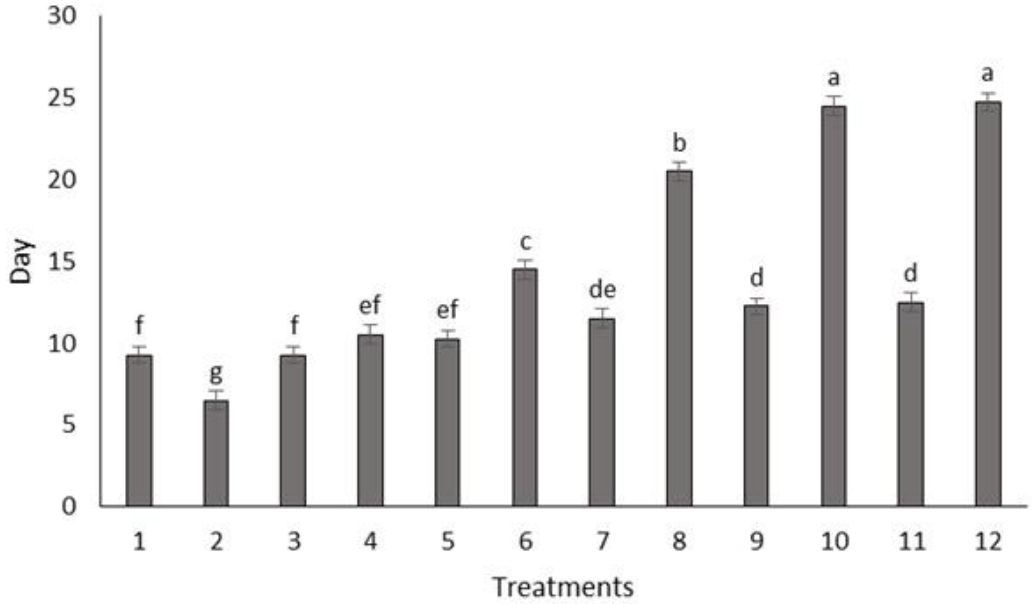

Fig.2: Cut flower vase- life. 1) 0%B+0%sucrose, 2) 0% B+0%sucrose, 3) 0.4% B+0%sucrose, 4) 0.4 % B+2%sucrose, 5) 0.8% B+0%sucrose, 6) 0.8% B+2%sucrose, 7) 1.2% B+0%sucrose, 8) 1.2% B+2%sucrose, 9) 1.6% B+0%sucrose, 10) 1.6% B+2%sucrose, 11) 2% B+0%sucrose, 12) 2% B+2%sucrose.

3-2 The length of rotten end stem:

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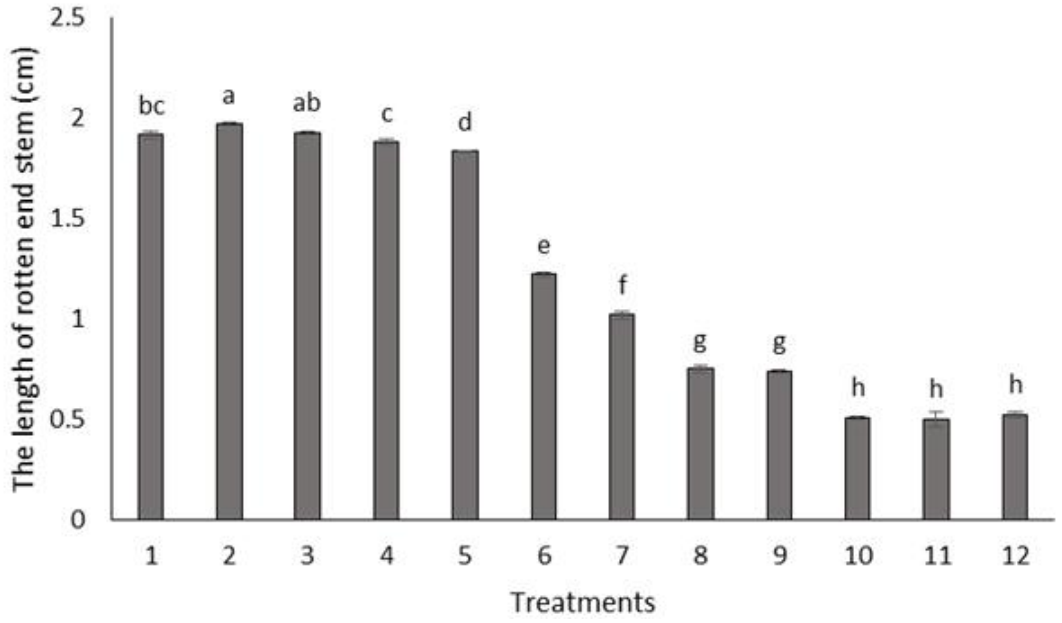

Fig.3: The length of rotten end stem in different treatments. 1) 0%B+0%sucrose, 2) 0% B+2%sucrose, 3) 0.4% B+0%sucrose, 4) 0.4 % B+2%sucrose, 5) 0.8% B+0%sucrose, 6) 0.8% B+2%sucrose, 7) 1.2% B+0%sucrose, 8) 1.2% B+2%sucrose, 9) 1.6% B+0%sucrose, 10) 1.6% B+2%sucrose, 11) 2% B+0%sucrose, 12) 2% B+2%sucrose.

3-3 Water absorption:

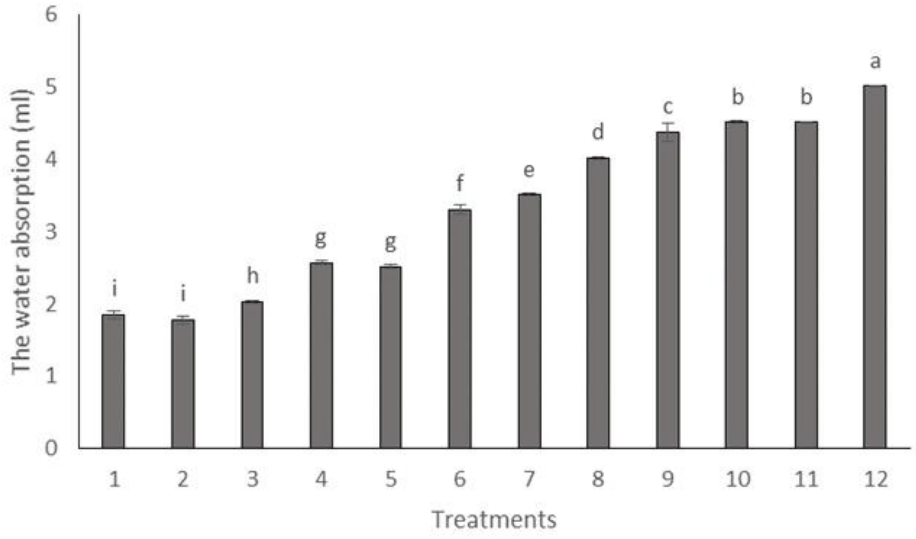

Fig.4: Water absorption by cut flower in the seventh day. 1) 0%B+0%sucrose, 2) 0% B+2%sucrose, 3) 0.4% B+0%sucrose, 4) 0.4 % B+2%sucrose, 5) 0.8% B+0%sucrose, 6) 0.8% B+2%sucrose, 7) 1.2% B+0%sucrose, 8) 1.2% B+2%sucrose, 9) 1.6% B+0%sucrose, 10) 1.6% B+2%sucrose, 11) 2% B+0%sucrose, 12) 2% B+2%sucrose.

3-4 Bacterial colony:

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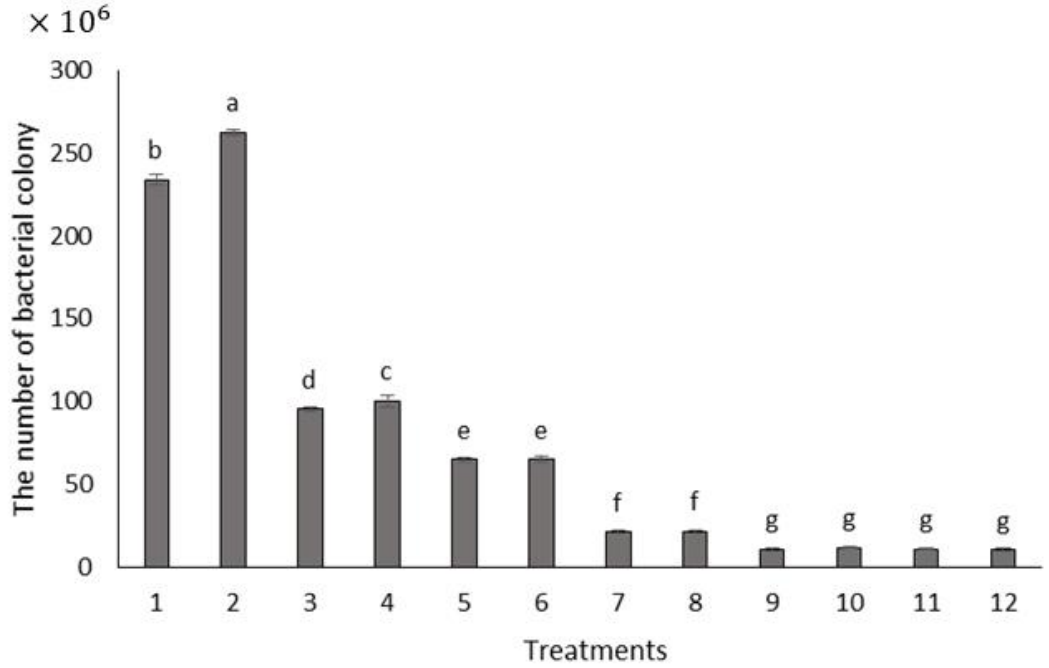

Fig.3: Number of Bacterial colony per ml of each treatment. 1) 0%B+0%sucrose, 2) 0% B+2%sucrose, 3) 0.4% B+0%sucrose, 4) 0.4 % B+2%sucrose, 5) 0.8% B+0%sucrose, 6) 0.8% B+2%sucrose, 7) 1.2% B+0%sucrose, 8) 1.2% B+2%sucrose, 9) 1.6% B+0%sucrose, 10) 1.6% B+2%sucrose, 11) 2% B+0%sucrose, 12) 2% B+2%sucrose.

3-5 Fungal spores:

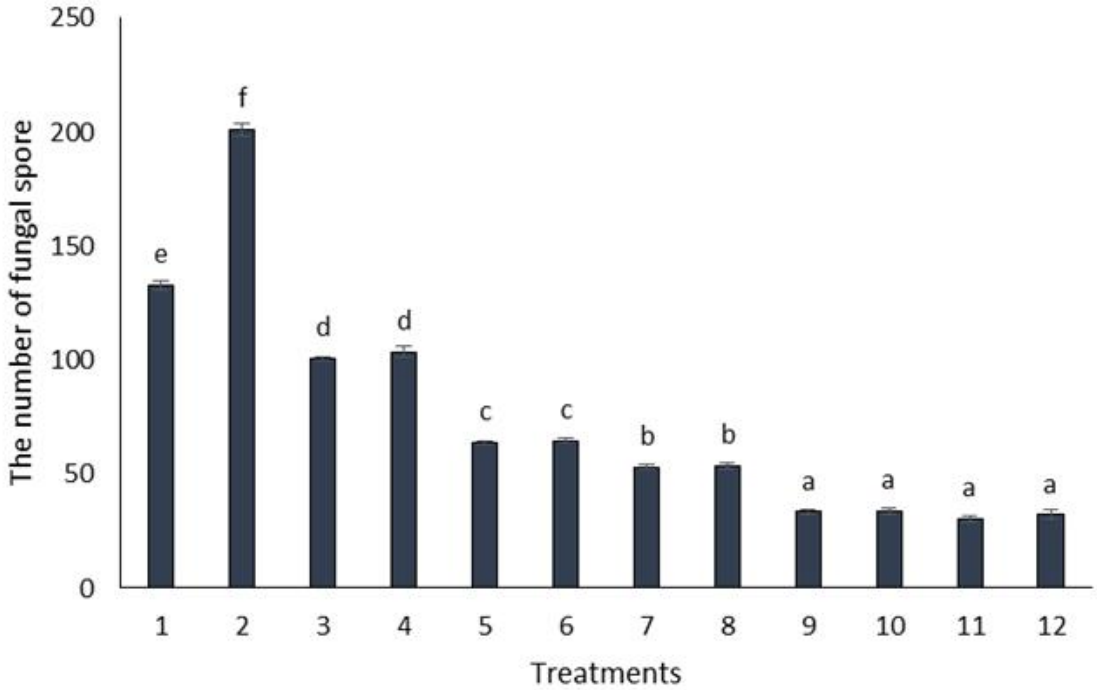

Fig.4: Number of fungal spore per ml of each treatment. 1) 0%B+0%sucrose, 2) 0% B+2%sucrose, 3) 0.4% B+0%sucrose, 4) 0.4 % B+2%sucrose, 5) 0.8% B+0%sucrose, 6) 0.8% B+2%sucrose, 7) 1.2% B+0%sucrose, 8) 1.2% B+2%sucrose, 9) 1.6% B+0%sucrose, 10) 1.6% B+2%sucrose, 11) 2% B+0%sucrose, 12) 2% B+2%sucrose.

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3-6 Effect of treatments on isolated fungi:

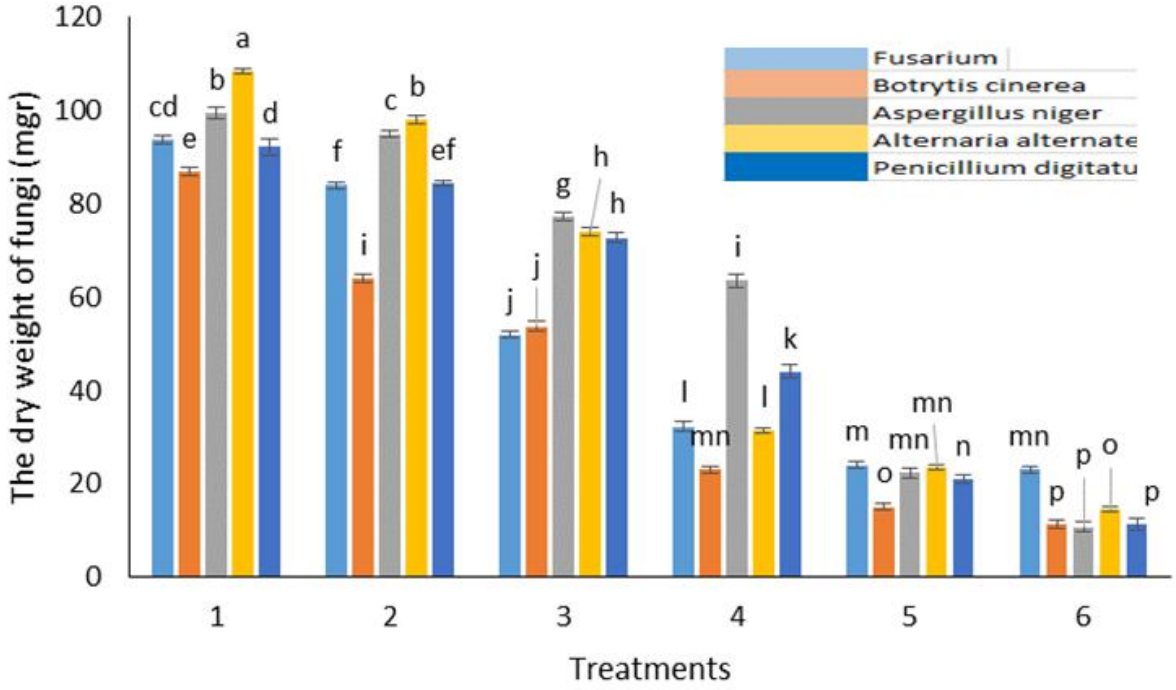

Fig.5: Effect of treatments on isolated fungi. 1) 0%B+0%sucrose, 2) 0% B+2%sucrose, 3) 0.4% B+0%sucrose, 4) 0.4 % B+2%sucrose, 5) 0.8% B+0%sucrose, 6) 0.8% B+2%sucrose, 7) 1.2% B+0%sucrose, 8) 1.2% B+2%sucrose, 9) 1.6% B+0%sucrose, 10) 1.6% B+2%sucrose, 11) 2% B+0%sucrose, 12) 2% B+2%sucrose.

The Fungi which we identified were *Penicilliumdigitatum*, *Alternariaalternate*, *Aspergillusniger* *Botrytis cinerea* and *Fusariumsolani.*

3-7 Correlation:

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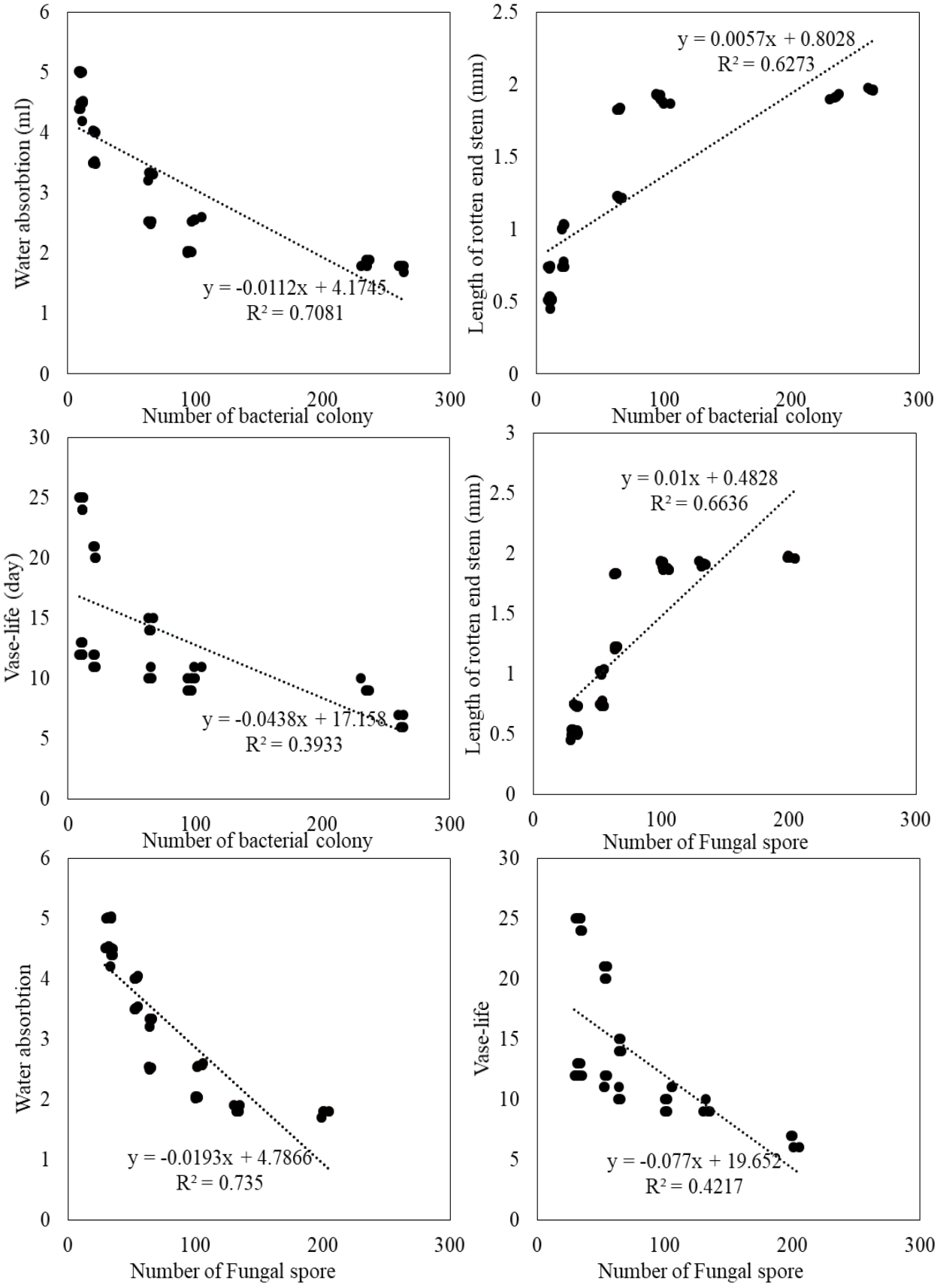

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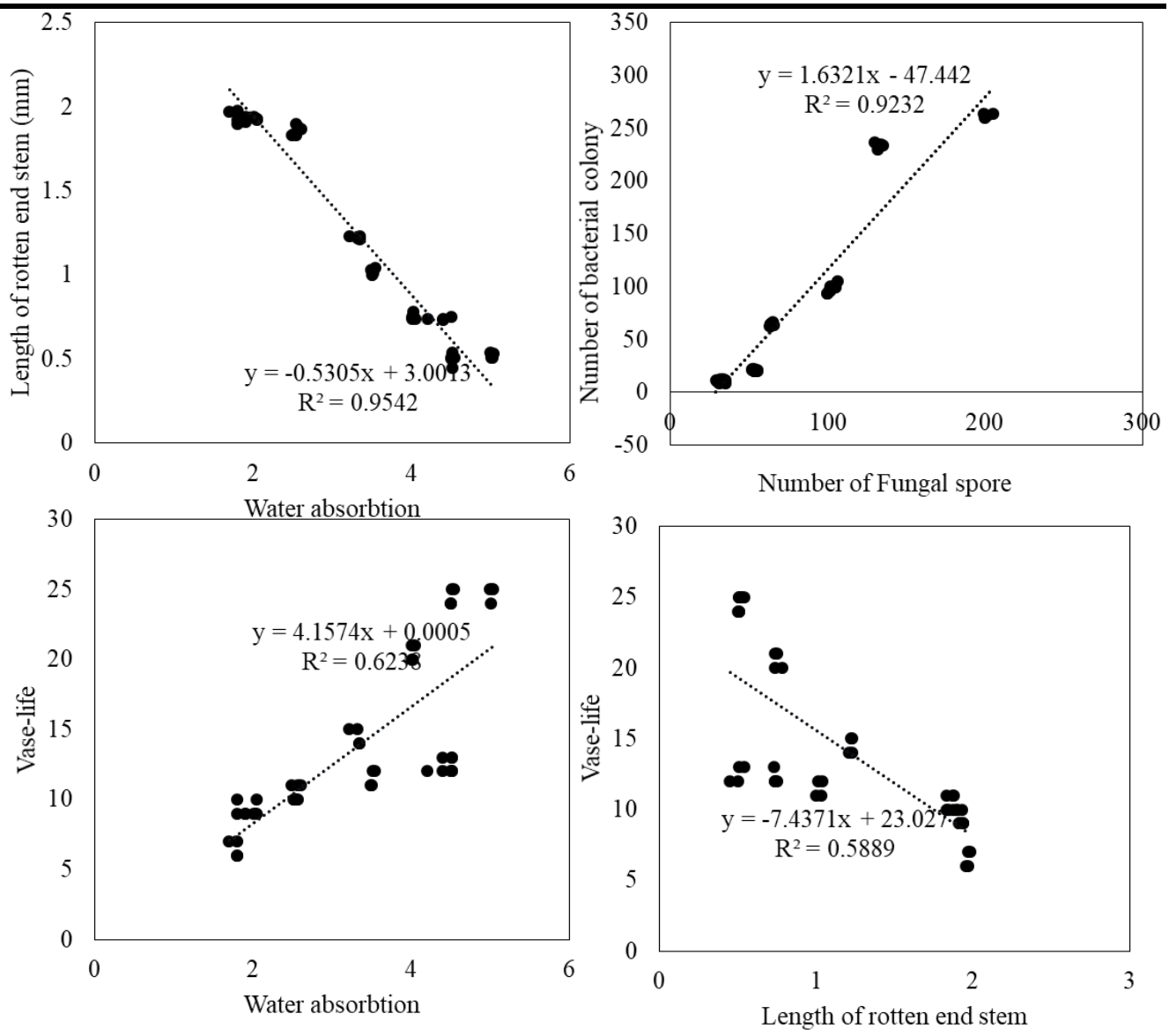

Fig.6: Correlation between factors.

IV. DISCUSSION

4-1 Cut flower vase-life

The highest life vase-life was related to the treatment of 1.6% B+2%sucrose and 2% B+2%sucrose and the lowest vase-life belongs to 0% B+2% sucrosetreatment. When Bconcentration increased from 1.6%, excessive increase in vase-life was not observed. And for this reason, the effective concentration must be 1.6%. According to the results, at a similar value of B, the treatment in that B exist with Sucrose in form of B-Sucrose complex, had a longer vase-life. This indicates the important effect of the presence of sucrose, near B, in form of B-Sucrose complex in an increase in the sustainability of the cut flower. The lowest vase-life was 2% sucrose treatment. The highest vase-life expectancy was treatments number 10 and 12 respectively.

In the graph (Figure 2), you can see that, in the high concentration of B, the vase-life of the cut flower decreased but in the same concentration of B, treatments of B-Sucrose complex, the vase life was increased. Yokota and Konishi(Yokota & Konishi, 1990) also concluded that, despite its high levels of toxicity for the plant, the formation of a complex with sugar, which results in the production of a sucrose-B complex, could potentially affect the sensitivity of the plant to a high level of B toxicity and enhance plant tolerant. Therefore, it can be justified to increase the cut flower vase-life in a uniform concentration of B, in treatments with B-sucrose complex.

Also, the formation of the complex between B and sucrose, according to Isabell et al. (1948), Zittle(2006)and Bowen(1969), could enhance B absorption by a plant. And because of the fact that B is one of the important elements involved in the cell wall of the plants, it can increase the

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tolerance of the plant to an unsuitable environmental condition.

4-2 The length of rotten end stem

As you can see in figure 3, in the high B concentrations (1.6% and 2%), the length of rotten end stem has decreased. A remarkable point to be noted is the significant decrease in the amount of rotten stem length in treatments that are containing B-sucrose complex. This emphasizes the better effect of this complex compared to the use of B lonely on the reduction of this rotten stem.

4-3 Water absorption

The highest water absorption was achieved at high B concentrations (1.6% and 2%) by the cut flower. And the lowest water absorption was observed in control and 2% sucrose treatment. It can be seen that among treatments, the treatments that had B-sucrose complex, water absorption by cut flower was the highest compared to its same B concentration treatments. Earlier studies also confirm that B can increase water absorption in the plant in various ways, including improving root and shoot growth and increase the yield of water channel activity. In the case of Anthurium cut flowers, this can be attributed to the role of improving the function of water channel activity (Warington, 1923; Huang, Ye, Bell, & Dell, 2005;Wimmer & Eichert, 2013).

4-4 Bacterial colony:

The highest reduction in the number of the bacterial colony in the vase solution occurred at higher B concentrations of 1.2%, 1.6%, and 2%. Also, the highest number of the bacterial colony was observed in control and 2% Sucrose treatment. The presence of sucrose in form of B-sucrose complex, at 0.4% concentration only, caused a significant increase in the number of bacterial colonies in the vase solution compare to non-sucrose treatment (control) indicated using of sucrose by bacteria.

Nelsona et al. (2007), also showed that increases in B levels of soil, caused a change in the soil microorganism's population. This effect was on bacteria in both plant and plant pathogenic conditions. This effect can be due to the change in the type and amount of plant secretions, and also, according to them, B, lonely, is toxic to these microorganisms. It also affects osmotic pressure. In our experiment, it has a toxic effect on bacteria and reduces the number of them in the solution.

4-5 Fungal spores:

The highest decrease in the number of fungal spores in the vase solution occurred at higher B concentrations of 1.2%, 1.6%, and 2%. Also, the highest number of fungal spores in control and 2% sucrose treatment was observed. The presence of sucrose beside, as a B-sucrose complex, did not significantly affect the number of fungal spores present in the solution without sucrose treatment.

4-6 Effect of treatments on growth of isolated fungi:

The results showed that growth of all isolated fungi decreased with increasing B concentration in the liquid medium. This decrease in growth at higher concentrations was higher than its lower concentrations.

The impact of B on microorganisms has not been widely studied, however, according to previous studies, it can prevent the growth of bacteria, fungi, and algae, and this effect is higher at higher B concentrations. B isn't an essential element in the growth of these microorganisms, and only some of the cyanobacteria are used it in their structure (Bowen, 1969; Butterwick, de Oude, & Raymond, 1989).

Terzi et al. (2017) also proved that B existence can have a negative effect on the growth of molds and reduce it.

Similar studies have been done on the fungi tested in this study. As in the present study, Bowen et al.also tested the effects of B on *Penicillium* and *Aspergillus* and achieved similar results. Also, in 2016, Dong et al. investigated the effect of B on *Fusarium* wilt and reduced the germination of spores and decreased the growth of this fungus as this experiment. In addition to these fungi, other studies on effects on other fungi have been carried out. As an example, in a study by Rolshausen and Gubler in 2005 on Eutypa dieback of grapevines, it has been shown that application of B can affect the growth of the fungi as well as the germination of fungal spores and reduced them.Also, application of B on wood discoloration fungi and *Monilinialaxa*, could also have a significant inhibitory effect (Keane & Sackston, 1970; Thomidis & Exadaktylou, 2010).

Correlation:

There was a significant correlation between all treatments (between 6.27 and 9.77) at the 0.01 level. By decreasing the number of fungal spores and the number of bacteria in the solution, the activity of water absorption by the plant increased and the longevity of the cut flower vase- life was increased. Subsequently, the length of the rotten end stem was reduced. Hu and Brown in 1997 said that the rate of absorption of water was directly correlated with the rate of B absorption (Hu and Brown, 1997). The lack of blockage of the veins by fungi and bacteria, as well as the arrival of sugars (sucrose here), and finally the existence of Bwhich plays an important role in the strength of the cell wall of the plants, increase the cut flower stem endurance to decay significantly. In the end,these factors could together increase the longevity of Anthurium cut flower vase-life when it is separated from its mother plant.

Extending the shelf life of cut flowers:

One way to prolong the vase life of our Anthurium cut flowers is by preparing a good holding solution, a holding solution which contains energy supplier components, such as sucrose in this experiment and also components with fungicidal and bactericidal properties such as B. Sucrose

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as an energy source, in many cases, can also be used by the other microorganisms and increase their growth. Therefore, it leads to more pollution of the holding solution.

Despite the benefits of sucrose for the plant, it could promote the growth of microorganisms that are pathogen to the plant or inhibit its growth. These pathogens can cause stem rot. Such cut flowers, especially Anthurium, are very susceptible to disease especially diseases causing vessel closure. Therefore, it is better to complex it with antifungal and antibacterial agents to prevent the use of sucrose by such microorganisms and of course their growth. The results showed that the bore containing treatments improved stem water uptake by protecting the cut stem ends by inhibiting the growth of Fungi and bacteria.

Sucrose pollution caused by these fungi and bacteria might be an environmental pollution. Nevertheless, if we use both sterilized water and condition, this pollution can be reduced.

Despite the role of B in inhibiting the sucrose pollution, this element has other beneficial properties in relation to plant.

Roles of B in the plant:

B moves through the Phloem in plants. It affects metabolic pathways through the cell walls and membranes and can counteract the toxic effects of some elements in the plant (Blevins & Lukaszewski, 1998).B inhibits the starch phosphorylase reaction and it may be the only one of the many reactions it can do. Any reactions involving a substrate capable of complexing with B may be influenced by the element (Dugger, Humphreys, & Calhoun, 1957).

The complex of B and Sugar:

The high B level can be toxic to plants, but this toxicity can be reduced by the formation of a sugar-borate complex (Yokota & Konishi, 1990). Transfer of sugars as a sugar-borate complex is another role of B in the plant. This property of B can improve the absorption of sucrose from water that has added sugar (Keane and Sackston, 1970). According to Gauch and Dugger, (1953) B affects the translocation of sugar in two ways: (1) in the form of the borate-sugar complex; (2) increasing the speed of sugar movement through cellular membranes.

V. CONCLUSION

According to the data of this research and available information about these two elements, the sucrose-B complex can increase the vase life of cut flowers such as Anthurium that have economic importance. Also, use of this compound has economic profits in two ways: 1) Increasing the vase life and being inexpensive. 2) Sucrose is accessible everywhere. So, this holding solution can be used instead of many other holding solutions in markets. B alone can increase the vase life in terms of reducing pollution and reducing vessel blocking caused by bacteria and fungi. However, in complexes with sucrose, it can reduce sucrose absorption by plant too.

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