Effects of various carbohydrates on the *in vitro* pollen germination of *Vinca rosea* and *Cucumis melo* var. *utilissimus*

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Abstract

Pollen germination is crucial for the process of plant development which strongly depends on the presence of carbohydrates as a primary source of energy. In this study, we analyzed the differential effects of four sugars with varying concentrations on the pollen germination of *Vinca rosea* Linn. and *Cucumis melo* var. *utilissimus* Duth. & Fuller. using Brewbaker and Kwack's medium as germination medium and hanging drop method after an incubation period of 1h. Sucrose and glucose supported the pollen germination but galactose and fructose did not support and even considerably inhibited the pollen germination of *Vinca rosea*. Whereas in pollen germination of *Cucumis melo* var. *utilissimus*, all the four sugars supported the pollen germination. The study suggests that 15% sucrose, for *Vinca rosea*, and 12% galactose, for *Cucumis melo* var. *utilissimus*, supports in achieving the highest pollen germination percentage when added to the pollen germination medium.

Keywords: Pollen grains, carbohydrates, *in vitro* pollen germination, *Vinca rosea*, *Cucumis melo* var. *utilissimus*, Brewbaker and Kwack's medium, Hanging drop method.

Introduction

The development of pollen grains on stigma or in the *in vitro* conditions is a time-bound and high energy requiring process (Selinski and Scheibe, 2014). The recognition of compatible pollen and the growth of pollen tube largely depends upon the receptivity of stigma and other recognition factors. The further growth of pollen tube, till it reaches embryo sac, is facilitated by the environment of stylar canal (Boavida et al., 2005). Carbohydrates, act as an osmotic regulator and also as a substrate for primary energy source, supporting pollen germination and the pollen tube growth in stylar canal (Reinders, 2016).

Most of the studies conducted on *Vinca rosea* are based on its therapeutic properties. In Ayurveda extracts of both roots and shoots are used to treat several diseases. Many vinca alkaloids including vinblastine and vincristine are used for treating leukaemia and Hodgkin's lymphoma (Moudi *et al*., 2013). *Cucumis melo* var. *utilissimus* is commonly called as American cucumber or "kakri" in India. The fruits are used for its juice or made into powder for cooking (Khare, 2007). Extract from the fruits are medicinally used to promote skin hydration, treat light burns and act as a stomach tonic. Seeds are used for suppressing cough, reducing fever and act as digestive aid (Gill *et al*., 2010).

The present study investigates the effects of various sugars with varying concentrations on the *in vitro* pollen germination of *Vinca rosea* and *Cucumis melo* var. *utilissimus* by using a pollen germination medium and calculating pollen germination percentage. Different effects of sugars and their respective concentrations were observed on the pollen germination of *Vinca rosea* and *Cucumis melo* var. *utilissimus.* 15% sucrose and 12% galactose prove to be the best sugar concentrations along with the pollen germination medium to achieve the highest pollen germination

percentage after an incubation period of 1h for *Vinca rosea* and *Cucumis melo* var. *utilissimus* respectively.

Materials and Methods

Pollen collection: Flowers of *Vinca rosea* and *Cucumis melo* var. *utilissimus*, at anthesis, were collected from the botanical garden of Miranda House, University of Delhi during daytime. Pollen grains were carefully collected on separate clean petri dishes by tapping and brushing the anthers of each flower.

Preparation of Pollen Germination Medium: Standard solution (100ml) of Brewbaker and Kwack's medium (Brewbaker and Kwack, 1963) was prepared by using 0.01g of boric acid, 0.01g of potassium nitrate, 0.02g of magnesium sulphate heptahydrate and 0.03g of calcium nitrate. The medium was prepared by using four different sugars: sucrose, glucose, fructose and galactose. For each sugar, four different concentrations were made: 5%, 10%, 12% and 15%. The germination medium was autoclaved to maintain the sterility.

Experimental Set-up: Hanging drop method (Shivanna and Rangaswamy, 1992) with slight modifications was used for performing the *in vitro* pollen germination. A small drop of pollen germination medium was taken on a glass slide and the pollen grains were transferred onto it with the aid of a nylon brush. The glass slide was inverted and placed on a glass cavity block. The edges were sealed with the help of petroleum jelly. The set-up was incubated in light with temperature set at 30°C.

Observation of pollen germination: Observations for pollen germination was taken by microscopy four times, each with 15 mins interval. Three microscopic field views per replicate, that contained a minimum of 30 pollen grains, was observed. Pollen grains were considered germinated when the length of the pollen tube was greater

than the diameter of the pollen grain. The total number of pollen grains that germinated was calculated and the percentage germination was calculated by dividing the number of pollen grains germinated by the total number of pollen grains. The experiment was replicated thrice.

Statistical analysis: Descriptive statistics was used to determine mean and standard deviation for pollen germination percentage. One-Way Analysis of Variance (ANOVA) was used to determine the significant differences in pollen germination percentage among different sugar concentrations.

Results

Pollen germination in *Vinca rosea*: The pollen germination percentage for *Vinca rosea* under the effect of various sugars with varying concentrations was obtained as shown in Table 1. As presented, pollen grains germinated in every media except when fructose was used (Fig. 1a.-1d.). 5% sucrose concentration had an inhibitory effect on pollen germination

Using 15% sucrose in germination medium resulted in highest pollen germination percentage (67.99%) while the lowest pollen germination (3.06%) was obtained by using 10% galactose in germination medium after an incubation period of an hour. Lower concentrations of both glucose and galactose had an inhibitory effect on the pollen germination of *Vinca rosea*. Fructose, in any concentration, inhibited the pollen germination.

Pollen germination in *Cucumis melo* var. *utilissimus*: The pollen germination percentage for *Cucumis melo* var. *utilissimus* under the effect of various sugars with varying concentrations was obtained as shown in Table 2.

As depicted, every germination medium irrespective of the sugar and its concentration used, was successful in pollen germination (Fig. 2a.-2d.). Pollen grains showed the highest germination percentage (65.33%) when 12% galactose was used along with the germination medium and the lowest germination percentage (17.71%) was obtained when 5% galactose was used along with the germination medium after an incubation period of an hour.

Table 1. Effect of various sugars with varying concentration on the pollen germination of *Vinca rosea*

Time (min)	Sucrose Concentration*				
	5%	10%	12%	15%	Control
15	0 ± 0	0 ± 0	0 ± 0	25.7936 ± 0.8361	1.6269 ± 1.4102
30	0 ± 0	2.0634 ± 1.8028	3.4555 ± 1.2370	31.7272 ± 0.5662	1.7105 ± 1.4827
45	0 ± 0	9.1547 ± 0.7364	15.1532 ± 0.7780	42.1530 ± 0.6243	3.5776 ± 1.2426
60	0 ± 0	12.5216 ± 1.1040	23.2478 ± 0.4986	67.996 ± 1.1547	4.4014 ± 1.2714

Time (min)	Glucose Concentration*				
	5%	10%	12%	15%	Control
15	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.6269 ± 1.4102
30	0 ± 0	0 ± 0	0 ± 0	7.4853 ± 0.7090	1.7105 ± 1.4827
45	0 ± 0	0 ± 0	0 ± 0	10.4323 ± 0.9278	3.5776 ± 1.2426
60	0 ± 0	0 ± 0	0 ± 0	23.4508 ± 0.8336	4.4014 ± 1.2714

Time (min)	Galactose Concentration*				
	5%	10%	12%	15%	Control
15	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.6269 ± 1.4102
30	0 ± 0	0 ± 0	3.0786 ± 0.2806	0 ± 0	1.7105 ± 1.4827
45	0 ± 0	0 ± 0	5.6903 ± 0.4949	0 ± 0	3.5776 ± 1.2426
60	0 ± 0	3.0693 ± 0.1905	12.8835 ± 1.2903	0 ± 0	4.4014 ± 1.2714

Time (min)	Fructose Concentration*				
	5%	10%	12%	15%	Control
15	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.6269 ± 1.4102
30	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.7105 ± 1.4827
45	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3.5776 ± 1.2426
60	0 ± 0	0 ± 0	0 ± 0	0 ± 0	4.4014 ± 1.2714

Values (in %) are represented as Mean ± S.D, P-value ≤ 0.05 is represented by *.

136 Table 2. Effect of various sugars with varying concentration on the pollen germination of Cucumis melo

Time (min)	Sucrose Concentration*				
	5%	10%	12%	15%	Control
15	10.932 ± 0.6062	7.9194 ± 0.5337	16.4781 ± 0.7762	12.6515 ± 0.6200	2.7329 ± 0.1515
30	25.0221 ± 1.1519	12.4689 ± 0.9166	25.4757 ± 1.1330	20.3921 ± 0.3396	5.6198 ± 0.3202
45	33.9432 ± 0.5295	23.6438 ± 0.7673	36.8434 ± 0.5884	29.9636 ± 0.8164	10.0637 ± 1.1559
60	36.6987 ± 0.5306	28.0373 ± 0.8773	53.4214 ± 0.9768	34.2811 ± 0.9045	12.9308 ± 0.7233
Time (min)	Glucose Concentration*				
	5%	10%	12%	15%	Control
15	15.6545 ± 0.2337	18.9507 ± 0.5462	9.0535 ± 0.8325	2.7834 ± 0.1548	2.7329 ± 0.1515
30	26.6025 ± 1.0612	30.2455 ± 0.7299	14.2483 ± 0.7924	9.5750 ± 1.0785	5.6198 ± 0.3202
45	32.4059 ± 0.0459	46.1841 ± 1.0849	21.1655 ± 1.0173	18.6612 ± 0.695	10.0637 ± 1.1559
60	41.7055 ± 0.7001	56.3911 ± 1.1185	31.9536 ± 0.6112	25.7282 ± 0.7353	12.9308 ± 0.7233
Time (min)	Galactose Concentration*				
	5%	10%	12%	15%	Control
15	5.5814 ± 0.4667	13.7774 ± 0.5721	28.828 ± 0.6515	18.4244 ± 0.9229	2.7329 ± 0.1515
30	8.1438 ± 0.6624	21.5488 ± 0.5831	41.6864 ± 1.0166	24.5261 ± 1.1647	5.6198 ± 0.3202
45	12.6119 ± 0.4348	40.2146 ± 0.7107	53.5372 ± 0.8779	37.7841 ± 0.8211	10.0637 ± 1.1559
60	17.7137 ± 0.6764	54.8933 ± 0.6366	65.3399 ± 0.7144	48.9583 ± 1.8042	12.9308 ± 0.7233
Time (min)	Fructose Concentration*				
	5%	10%	12%	15%	Control
15	11.6799 ± 0.7277	19.2885 ± 0.8009	5.8207 ± 0.6638	9.6757 ± 0.8514	2.7329 ± 0.1515
30	18.0022 ± 1.1831	33.3505 ± 0.9267	10.6516 ± 0.7224	12.7688 ± 0.2327	5.6198 ± 0.3202
45	22.87 ± 0.5724	46.9229 ± 0.5268	16.0279 ± 1.0168	18.1152 ± 0.5700	10.0637 ± 1.1559
60	35.7376 ± 0.6145	52.2535 ± 0.7847	25.9454 ± 0.3239	25.9726 ± 0.4390	12.9308 ± 0.7233

137 Values (in %) are represented as Mean ± S.D, P-value ≤ 0.05 is represented by *.

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Discussion

The present study demonstrates that the germination of *Vinca rosea* and *Cucumis melo* var. *utilissimus* pollen grains is largely influenced by the exogenous supply of various sugars with varying concentrations.

Numerous studies have shown that sucrose exhibits a strong stimulatory action on the growth of pollen grains (Patel, 2017). Both glucose and fructose have reported to show varied effects on pollen germination of various plant species (Okusaka and Hiratsuka, 2009). A higher concentration of sucrose as well as glucose promotes the in vitro pollen germination of *Vinca rosea* (Bandyopadhyay and Mukherjee, 1977). Although, in case of *Vinca rosea*, the lower concentrations of glucose inhibit the pollen germination which supports the involvement of hexokinase mediated pathway of inhibiting sucrose mediated pollen germination (Godt *et al*., 1995; Sinha *et al*., 2002). Sucrose promotes the pollen germination both in case of *Vinca rosea* and *Cucumis melo* var. *utilissimus* and this can be supported by the fact that sucrose is the most common form of sugar that is translocated to non-photosynthetic tissue, such as flower, and thus is also excreted by the stigma as well as the stylar canal for hydrating the pollen and supporting pollen tube growth (Hopkins and Hunner, 2009). Glucose supports the pollen germination minimally in *Vinca rosea* as well as in *Cucumis melo* var. *utilissimus*. This could be due to the weak expression of AtSTP9, a glucose-specific monosaccharide transporter, in the mature pollen grain (Schneidereit *et al*., 2003, 2005).

Fructose inhibits the pollen germination of *Vinca rosea* and not *Cucumis melo* var. *utilissimus*. Presence of fructose causes the pollen grains to completely utilise their stored sugars as respiration substrates and thus fail to develop (Okusaka and

Hiratsuka, 2009). On the other hand, in case of *Cucumis melo* var. *utilissimus*, fructose promotes the pollen germination which can be supported by the studies which states the presence of fructose in the stigmatic fluid of plants promote pollen germination (Portnoi and Horovitz, 1977). Like that of fructose, the same effect can be clearly seen with galactose which inhibits the pollen germination in *Vinca rosea* but largely promotes the pollen germination in *Cucumis melo* var. *utilissimus*. The inhibitory effect of galactose can be explained by the hexokinase mediated pathway of sucrose mediated pollen germination inhibition (Godt *et al*., 1995; Sinha *et al*., 2002).

This study establishes the fact that the influence of various sugars on the germination of pollen grains also depends on the physiology of the pollen grains. This study adds to the information of developmental biology of pollen grains of *Vinca rosea*, which is a known ornamental and medicinal plant, and *Cucumis melo* var. *utilissimus*, which is a popular vegetable crop and medicinal plant of India. This study can further be used for extensive cultivation of these plants around the world.

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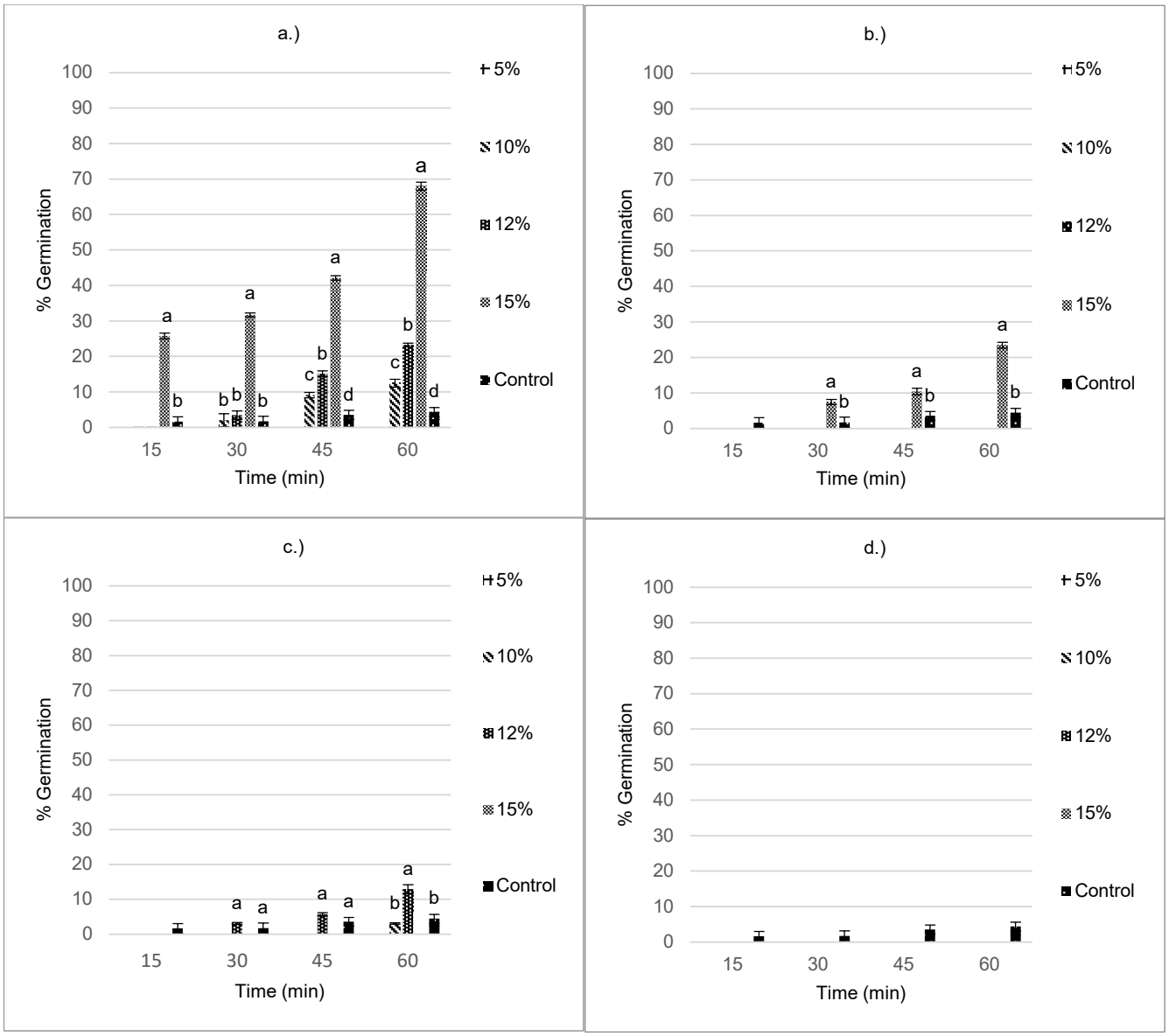

Fig. 1. Effect of varying concentrations of a.) Sucrose, b.) Glucose, c.) Galactose and d.) Fructose on the *in vitro* pollen germination of *Vinca rosea* . Data are shown by Mean ± S.D. Mean values with the same letter are not significantly different at P=0.05 by Tukey's multiple-range test.

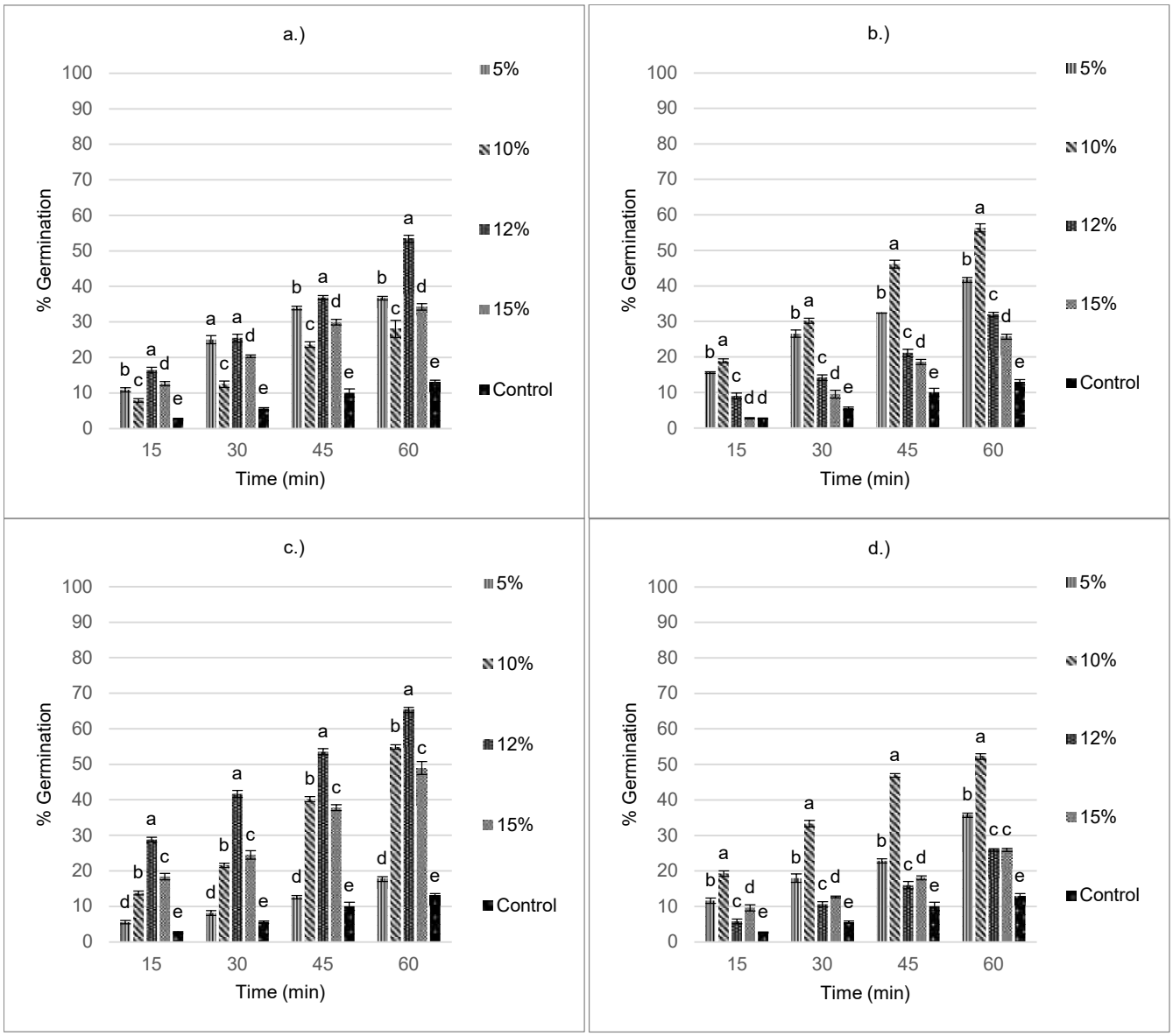

Fig. 2. Effect of varying concentrations of a.) Sucrose, b.) Glucose, c.) Galactose and d.) Fructose on the *in vitro* pollen germination of *Cucumis melo* var. *utilissimus*. Data are shown by Mean ± S.D. Mean values with the same letter are not significantly different at P=0.05 by Tukey's multiple-range test.