

## Original Research Article

# Effect of Putrescine and Different Media on Vegetative Growth, Floret and Some Biochemical Parameters of Gladiolus under Soilless Conditions

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## ABSTRACT

This experiment was conducted to evaluate the effect of putrescine (50 and 100 ppm) and coco peat: perlite medium with three ratios (v/v) (1:1, 3:1 and 1:3) on some vegetative growth, floret and some biochemical parameters of gladiolus cv. (Strong) under soilless condition in 2016. Data indicated that most criteria of vegetative growth expressed as plant height, leaf number and leaf area, floret parameters as floret number, floret fresh weight and floret dry weight and biochemical parameters as chlorophyll (a), chlorophyll (b) and total chlorophyll significantly increased by putrescine 100 ppm with (118.28 cm, 10.53, 72.11 cm<sup>2</sup>, 15.69, 10.64 g, 5.42 g, 3.12 mg/g, 1.60 mg/g and 4.72 mg/g) respectively. In terms of media the same traits (113.46 cm, 10.50, 47.18 cm<sup>2</sup>, 16.75, 10.57 g, 5.35 g, 3.15 mg/g, 1.71 mg/g and 4.87 mg/g) respectively, significantly increased by the coco peat: perlite medium with ratio (1:3). Interaction between putrescine and media in term of plant height, leaf area and floret number was significant (124.33 cm, 493.67 cm<sup>2</sup> and 18.32) respectively, by putrescine 100 ppm and coco peat: perlite medium with ratio (1:3).

## Keywords

Gladiolus,  
Putrescine,  
Soilless, Coco  
peat, Perlite

## Introduction

Gladiolus is very popular and important bulbous ornamental flowering plant of the world. It is known as queen of bulbous flowers. It belongs to the family Iridaceae and is a native of Mediterranean region. It is excellent for cut flowers as it lasts long in flower vase and has magnificent inflorescence with variety of colours (Mahadik and Neha, 2015). Gladiolus is the second most popular flower in the world, especially from the commercial point of view. Gladiolus has great economic value

and wide market in world (Khan *et al.*, 2012).

PAs are low molecular weight polycations, organic, biogenic amines that are found in all eukaryotic and most prokaryotic cells (Kumar *et al.*, 1997; Mahgoub *et al.*, 2006) and have profound effects on growth, development and senescence in eukaryotic cells (Casiro and Marton, 2007). In plants, di-amine putrescine (Put), triamine spermidine (Spd) and tetra-amine spermine

(Spm) are frequently present in amounts varying from micromolars to more than millimolars (Kakkar and Sawhney, 2002). Polyamines (Put, Spm and Spd) are recognized as a new class of plant growth bioregulators (Dantuluri *et al.*, 2008). They influence many biochemical and physiological processes such as cell division, cell elongation, flowering, fruit set and development, fruit ripening, senescence, storage life (Cohen, 1998).

In recent years, some problems in soil culture (such as salinity and unsuitable soil characteristics) and limitation of water resources in many countries, especially in Iran, causes the expansion of soilless culture. Soilless cultures an artificial means of providing plants with support and a reservoir for nutrients and water. The use of soil in protected agriculture is facing many limitations in this country. After years of cultivation, deterioration in soil fertility and increase in soil salinity, in addition to the increase of soil-borne diseases and limited productivity of crops, have often been observed. Therefore, utilizing substrate-based agriculture is a logical alternative to the current soil-based production approach in the country. Hydroponic scientist with a lot of examination had resulted that the growth of plant have not needed soil if grower supply nutrient elements for plants by fertilization and fertigation (Papadopolus, 1994). Dobrzanski (1981) reported that the yield of gladiolus flower was highest in peat and lowest yield was found in lignite soil. Leinfelder and Rober (1989) used peat + clay, rockwool, foam, perlite and clay for raising gladiolus. They found that flower quality was similar in peat + clay, rockwool and foam, but was very inferior in clay. Sorokina *et al.*, (1984) reported that bark and peat mixture was the best media for growing ornamental plants. Ahmed (1989) reported that sand + peat, sand + leaf mould

enhanced the flowering, number of flower and flower size significantly. Magnani *et al.*, (2003) reported that Lapillus was compared to a traditional substrate with perlite and alternative ones with coconut fibre, either single or in a mixture can give excellent productions for the bulbous species tested. Lapillus gave good results with gladiolus, similar to those with traditional perlite, with regards to the qualitative characteristics of the stem (fresh weight and height). Slight decrease in the qualitative characteristics of lily was observed when the lapillus was used singly, whereas it allowed us to obtain very satisfying results when used in a mixture with coconut fibre. Tribulato *et al.*, (2003) reported that among substrates, lavic basalt mixed with peat led to higher values of stem length and thickness and fresh weight of cut flowers. The highest plant density slightly decreased product quality, thus it seems possible to grow a high number of plants per square meter and increase the yield. Tehranifar *et al.*, (2011) reported that the effect of three soilless media on growth and development of two types of *Lilium* The media were 100% coco peat, 50% gravel + 50% sand and 40% peat + 60% perlite. In general, the media of 50% gravel + 50% sand was equal compared with two other media in most of the measured traits. The aim of this work was to study the responses Gladiolus to the interactive effects of Putrescine and different media.

### **Materials and Methods**

This experiment was conducted at the glasshouse of the Department of Horticultural Science and Landscape, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran, in 2016 to study the effects of putrescine (50 and 100 ppm) and two media (coco peat: perlite) with three ratios (v/v) (1:1, 3:1 and 1:3) on plant height, leaf number and leaf area, floret

parameters as floret number, floret fresh weight and floret dry weight and biochemical parameters as chlorophyll (a), chlorophyll (b) and total chlorophyll of gladiolus cv.(strong) under soilless conditions. The corms used in the experiment were purchased from a local commercial in (Mahallat). The mean size of these corms was 2.5 cm in circumference. Two factors was utilized in this study first factor putrescine (control, 50 and 100 ppm) and second factor two media (coco peat: perlite) with three ratios (v/v) (1:1, 3:1 and 1:3) were investigated. The pots were filled by the medium (10 kg/pot) with three ratios (v/v) (1:1, 3:1 and 1:3), and then three healthy corms were planted at the depth of 10 cm the size of pot was (25 cm X 40 cm) in May 2016 with an soilless open system. Plants were irrigated 2 times every day for 5 min (the amount of water was ½ liters per each pot per day).

Four weeks after planting plants were sprayed with different levels of putrescine in related treatments at the rates of 50 and 100 ppm and sprayed again before two weeks of flowering. To facilitate putrescine absorption, a few drops of twin 20 (Merk) were added to spray solutions. Bed leaching was done weekly to prevent the salt accumulation. Hoagland solution were set for pH=6 and EC=2 dS/ m<sup>-1</sup>. The glasshouse day and night temperatures were 24/20°C during the experiment. Relative humidity was adjusted at 50% and the light intensity averaged 90 mmol/m<sup>2</sup>/s<sup>-1</sup> during the day.

The standard cultural practices were followed during the entire growing period of the crop. The experiment was laid out in factorial based on completely randomized design with three replications. The observations related to the different physiological and biochemical parameters were recorded at the end of the experiment.

Plant height was calculated by measuring the length from the base of plant to the tip of the florets. Leaves number was measured by counting the leaves of 3 plants. Leaf area (cm<sup>2</sup>) was determined by leaf area meter device (AM 100, England). The total numbers of flowers per spike of 3 plants were counted and average was computed. The freshly harvested of 3 florets were weighed on an electric balance in grams. The harvested of 3 florets were oven dried well at 65 °C for 48 hour. The dried florets samples were weighed on an electric balance in grams. Chlorophyll contents were measured according to the method described by Rami and Porath (1980). Analysis of the data was performed by JMP8 software and mean values were compared by using Multiple Duncan's test.

## **Results and Discussion**

### **Vegetative Growth and Development**

#### **Plant height (cm)**

The results regarding height of plant showed the significant difference between the spraying putrescine, where higher plant height was obtained in putrescine 100 ppm with (118.28 cm) comparison with control with (91.56 cm). In terms of media the higher plant in coco peat: perlite medium with the ratio of 1:3 with (113.46 cm), while the lowest plant height found in coco peat: perlite medium with the ratio of 3:1 with (95.25 cm) Table. 1. As the results showed, there was a significant interaction between different treatments of putrescine and different media; the highest plant height was obtained by the putrescine 100 ppm and coco peat: perlite medium with the ratio of 1:3 with (124.33 cm) and the shortest plant height was obtained by control and coco peat: perlite medium with the ratio of 3:1 with (81.59 cm) Fig. 1.

### **Number of leaves per plant**

The results showed that, in terms of leaves number per plant, there was significant difference between the different treatments of putrescine, so that the putrescine 100 ppm showed higher number of leaves with (10.53 per plant) than control with (8.47 per plant). Furthermore, in media with different ratios, the highest number of leaves per plant was obtained in coco peat: perlite medium with the ratio of 1:3 with (10.50 per plant) when compared with coco peat: perlite medium with the ratio of 3:1 was the lowest with (8.41 per plant) Table. 1).

### **Leaf area**

According to the data exhibited in Table (1), the different treatments of putrescine, showed significant difference from each other in terms of leaf area, so that the putrescine 100 ppm had higher leaf area with (72.11 cm<sup>2</sup>) compared to the control with (63.63 cm<sup>2</sup>). Furthermore, among the media, plants produced in coco peat: perlite with ratio of 1:3 had the highest leaf area with (47.18 cm<sup>2</sup>), while plants in coco peat: perlite (3:1 ratio) showed the lowest leaf area with (42.22 cm<sup>2</sup>). Based on the results, there were significant interactions between the different treatments of putrescine and different media, the highest leaf area was obtained by the putrescine 100 ppm and coco peat: perlite medium with the ratio of 1:3 with (49.64 cm<sup>2</sup>) and the shortest leaf area was obtained by control and coco peat: perlite medium with the ratio of 3:1 with (40.55 cm<sup>2</sup>) Fig. 2.

### **Flowering parameters**

#### **Number of florets per spike**

As can be seen in Table (2), there was significant difference between the different treatments of putrescine regarding number

of florets per spike, so that the putrescine 100 ppm had higher number of florets per spike with (15.69 per spike) compared to the control with (11.64 per spike). Furthermore, among the media, plants produced in coco peat: perlite with ratio of 1:3 had the highest number of florets per spike with (16.75 per spike), while plants in coco peat: perlite (3:1 ratio) showed the lowest number of florets per spike with (11.64 per spike). The results also showed there were significant interactions between the different treatments of putrescine and different media, the highest number of florets per spike was obtained by the putrescine 100 ppm and coco peat: perlite medium with the ratio of 1:3 with (18.32 per spike) and the lowest number of florets per spike was obtained by control and coco peat: perlite medium with the ratio of 3:1 with (9.76 per spike) Fig.3.

#### **Florets fresh weight**

According to the data exhibited in Table (2), there was significant difference between the different treatments of putrescine regarding florets fresh weight, so that the putrescine 100 ppm had the largest florets fresh weight with (10.64g), when compared with control was the smallest with (8.29 g).

Furthermore, among the media, plants produced in coco peat: perlite with ratio of 1:3 had the florets fresh weight with (10.57 g), while plants in coco peat: perlite (3:1 ratio) showed the lowest number of florets per spike with (8.47 g).

#### **Florets dry weight**

As can be seen in Table (2), there was significant difference between the different treatments of putrescine regarding the florets dry weight, so that the putrescine 100 ppm had the largest florets dry weight with (5.42 g), when compared with control was the smallest with (3.23 g).

**Table.1** Main effects of putrescine and different media on height of plant, leaf number and leaf area (cm<sup>2</sup>) of gladiolus under soilless conditions

Putrescine	Plant height (cm)	Leaf number	Leaf area (cm <sup>2</sup> )
Control	91.56c	8.47c	63.63c
50 ppm	101.87b	9.40b	66.77b
100 ppm	118.28a	10.53a	72.11a
<b>Media</b>			
Coco peat: perlite (1:1)	103.00b	9.50b	44.09b
Coco peat: perlite (3:1)	95.25c	8.41c	42.22c
Coco peat: perlite (1:3)	113.46a	10.50a	47.18a

**Significance levels:**

Putrescine	*	*	*
Media	**	*	*
Putrescine x Media	*	ns	*

Columns and main effects followed by different letters are significantly different at P<0.05, Duncan's multiple range test. ns: not significant; \*, \*\* significant at P<0.05, P<0.01, respectively.

**Table.2** Main effects of putrescine and different media on floret number, florets fresh weight (g) and floret dry weight (g) of gladiolus under soilless conditions

Putrescine	Floret number	Floret Fresh weight (g)	Floret dry weight (g)
Control	11.64c	8.29c	3.23c
50 ppm	14.26b	9.60b	4.42b
100 ppm	15.69a	10.64a	5.42a
<b>Media</b>			
Coco peat: perlite (1:1)	13.22b	9.49b	4.34b
Coco peat: perlite (3:1)	11.64c	8.47c	3.37c
Coco peat: perlite (1:3)	16.75a	10.57a	5.35a

**Significance levels:**

Putrescine	*	*	*
Media	*	*	*
Putrescine x Media	*	ns	ns

Columns and main effects followed by different letters are significantly different at P<0.05, Duncan's multiple range test. ns: not significant; \*, \*\* significant at P<0.05, P<0.01, respectively.

**Table.3** Main effects of putrescine and different media on chlorophyll (A), chlorophyll (B) and total chlorophyll (mg/g) of gladiolus under soilless conditions

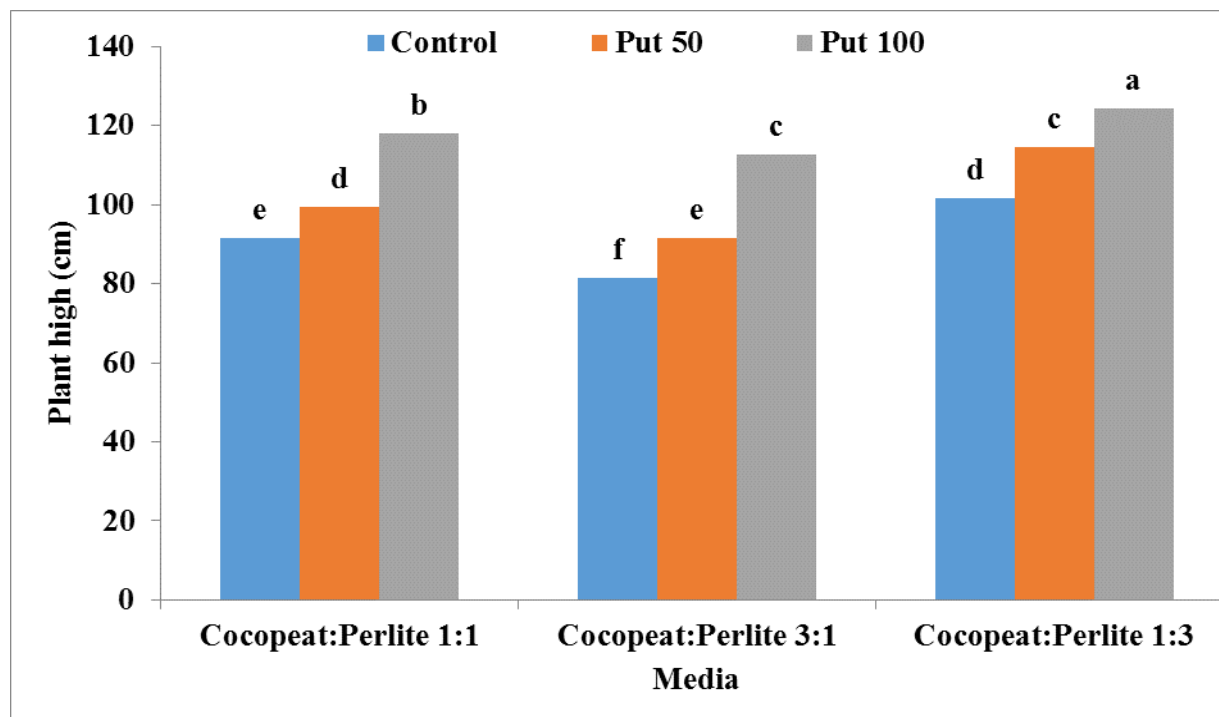
Putrescine	Chlorophyll (A) mg/g	Chlorophyll (B) mg/g	Total Chlorophyll mg/g
Control	2.76c	1.36c	4.12c
50 ppm	2.94b	1.48b	4.42b
100 ppm	3.12a	1.60a	4.72a
<b>Media</b>			
Coco peat: perlite (1:1)	2.93b	1.48b	4.41b
Coco peat: perlite (3:1)	2.73c	1.25c	3.98c
Coco peat: perlite (1:3)	3.15a	1.71a	4.87a

**Significance levels:**

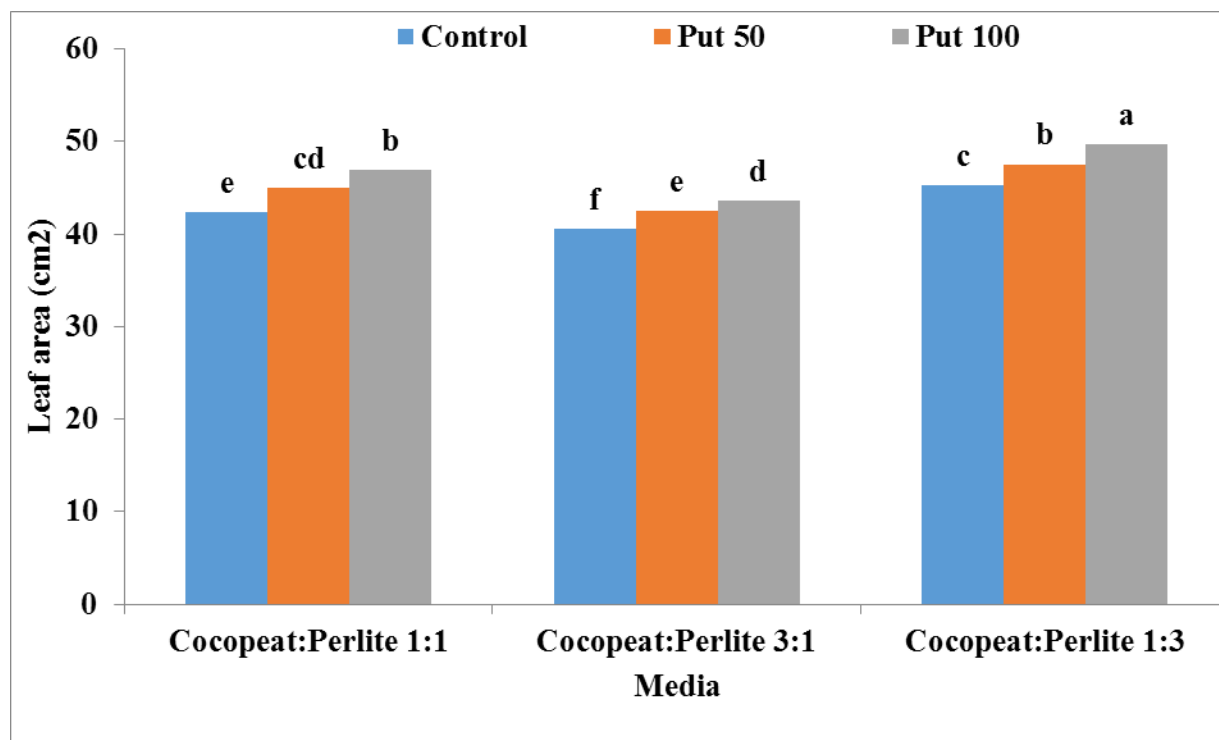
Putrescine	*	*	*
Media	*	*	*
Putrescine x Media	ns	ns	ns

Columns and main effects followed by different letters are significantly different at P<0.05, Duncan's multiple range test. ns: not significant; \*, \*\* significant at P<0.05, P<0.01, respectively.

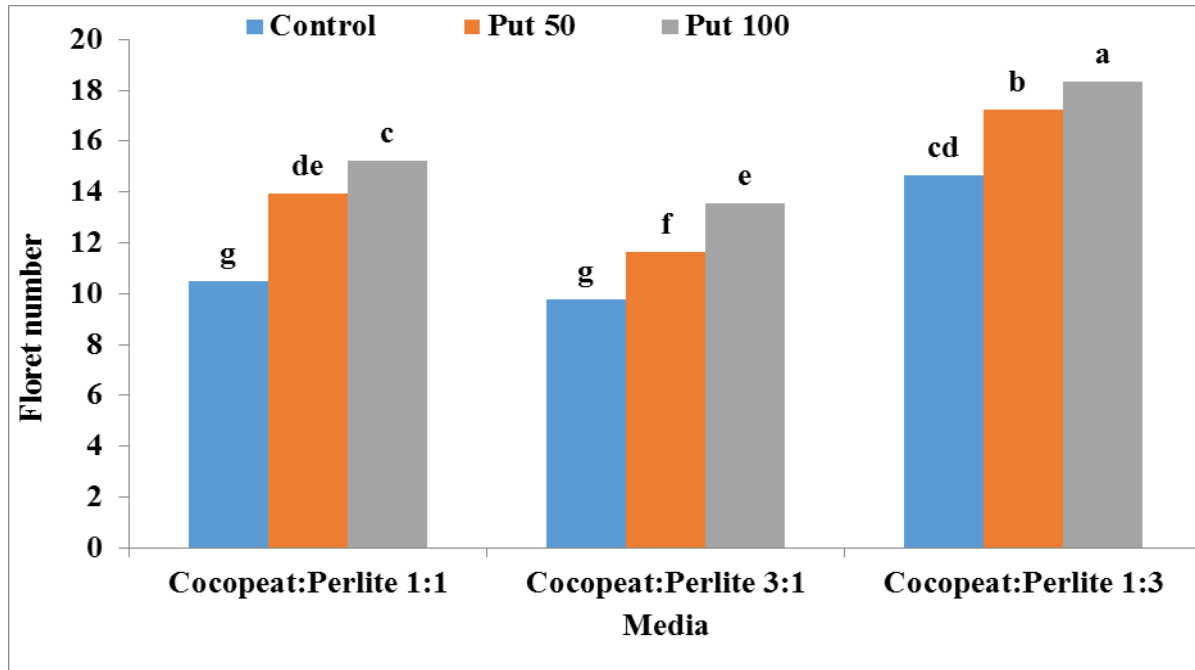
**Fig.1** Interaction effects of different treatments of putrescine and different media on plant high (cm) of gladiolus under soilless condition



**Fig.2** Interaction effects of different treatments of putrescine and different media on leaf area (cm<sup>2</sup>) of gladiolus under soilless condition



**Fig.3** Interaction effects of different treatments of putrescine and different media on floret number of gladiolus under soilless condition



Furthermore, among the media, plants produced in coco peat: perlite with ratio of 1:3 had the highest florets dry weight with (5.35 g), while plants in coco peat: perlite (3:1 ratio) showed the lowest florets dry weight with (3.37 g).

### Biochemical parameters

#### Chlorophyll A content

The results in Table (3) showed that the different treatments of putrescine significantly differed from each other in terms of chlorophyll (a) content, with putrescine 100 ppm showing higher chlorophyll a content (3.12 mg/g) in comparison with control with (2.76 mg/g). In addition, the highest chlorophyll a content (3.15 mg/g) was obtained in plants grown in coco peat: perlite medium (1:3 ratio), while the lowest content (2.73 mg/g) was observed in plants grown in coco peat: perlite medium with ratio (3:1).

#### Chlorophyll B content

Results presented in Table (3) suggested that chlorophyll (b) content in the different treatments of putrescine was significantly differed from each other, so that putrescine 100 ppm with (1.60 mg/g) had higher chlorophyll (b) content than control with (1.36 mg/g). Moreover, among the three medium ratios, plants grown in coco peat: perlite (1:3) had the highest content of chlorophyll (b) with (1.71 mg/g) compared to the plants grown in coco peat: perlite (3:1) with the lowest chlorophyll (b) content with (1.25 mg/g).

#### Total chlorophyll content

Regarding total chlorophyll content, there was significant difference between the different treatments of putrescine as shown in Table (3); the putrescine 100 ppm with (4.72 mg/g) had higher total chlorophyll content in comparison with control with

(4.12 mg/g). Furthermore, coco peat: perlite (1:3) showed to have the highest total chlorophyll content with (4.87 mg/g) among all the three medium ratios, while coco peat: perlite (3:1) showed the lowest total chlorophyll content with (3.98 mg/g).

As the results showed, there was a significant difference between the different media in all parameters were studied. From the result, it was evident that where perlite rate used more than cocopeat rate (3:1), it caused development vegetative growth, floret quality and biochemical parameters. Whereas cocopeat rate used more than perlite rate (1:3). The possible reason related physical properties of these two media. In perlite particles are loose and more porosity and absorbs water sufficiently, which can be utilized by plant. The cocopeat particles are closely linked with very little space for aeration and high water holding capacity; hence it hinders the vegetative growth and flower quality (Khan *et al.*, 2002). Coco peat is organic substrates and perlite is inorganic substrates and when mixed together become more effective in the composting process that can cause the mineralization of organic matter and change the organic forms of N and P to mineral forms (Michael and Heinrich, 2008). Coco peat has high water holding capacity which creates a poor relationship between air and water, leading to low aeration within the medium which affects oxygen diffusion to the roots (Abad *et al.*, 2002). Perlite substrate with very low cation exchange capacity (CEC), and good capacity of water absorption and coco peat substrate, with its high water holding capacity and nutrients can be considered as good growing media in soilless culture (Djedidi *et al.*, 1999). For these reasons also can obtain the maximum vegetative growth and flower quality. These results are in agreement with those obtained by (Mohamed, 1993) on *Nerium oleander*,

*Adhatoda vasica* and *Lantana camara* (Mahmood, 2005) on *Caesalpinia pulcherrima* and *Thevetia peruviana* and (Azza *et al.*, 2010) on *Jatropha curca* L. showed that mixture media significantly increased vegetative growth and flower quality. Using concentration of 100 ppm putrescine significantly increased vegetative growth, floret quality and biochemical parameters. These results may be to polyamine having been implicated in a wide range of biological process including growth development and biotic stress responses and cell division, differentiation (Kuechen *et al.*, 2005). Interaction between the mixture media and spraying putrescine at 100 ppm significantly increased vegetative growth, flower quality and biochemical parameters. These results are in accordance with those found by (El-Sallami, 2002) on *Chorisia speciosa* and *Leucaena leucocephala* seedlings, and (El-Khalifa, 2003) on *Dalbergia melanoxylon* plant. With regard to the effect of putrescine treatment, these results are in agreement with those obtained by (El-Quesni *et al.*, 2007) on *Bougainvillea* plants, (Abd El-Aziz *et al.*, 2009) on *Gladiolus* and (Singh *et al.*, 2017) on *Gerbera jamesonii*.

## References

- Abad M, Noguera P, Puchades R, Maquieira A, Noguera V. 2002. Physico-chemical and chemical properties of some coconut dusts for use as a peat substitute for containerized ornamental plants. *Biores. Technol.*, 82: 241-245.
- Abd El-Aziz, N. G.; Lobna, S. T. and Soad, M. M. L. 2009. Some Studies on the Effect of Putrescine, Ascorbic Acid and Thiamine of *Gladiolus* Plant. *Ozean J. App. Sci.*, 2 (2): 169 – 179.
- Ahmed K.K., 1989. Effect of different potting media on different rose cultivars under plastic tunnel. M.sc



- (Agric.) thesis, Dept. of Horticulture, NWFP Agricultural University Peshawar, Pakistan.
- Azza, A. M. M.; Nahed, G. A. and Habba, El- E. 2010. Impact of different soil media on growth and chemical constituents of *Jatropha curca* L. seedling grown under water regime. *Journal of American Science*, 6 (8): 549- 556.
- Casiro, R.A and Marton L.J. 2007. Targeting polyamines metabolism and function in cancer and other hyper-proliferative diseases. *Nat. Rev. Drug Disco.*, 6: 373-390.
- Cohen, S. 1998. A guide to the polyamines. Oxford University Press.
- Dantuluri, V.S.R., Misra, R.L and Singh V.P. 2008. Effect of polyamines on post-harvest life of gladiolus spikes. *Journal of Ornamental Horticulture*, 11(1): 66-68.
- Djedidi, M., Gerasopoulos, D. and Maloupa, E. 1999. The Effect of Different Substrates on the Quality of "Carmelo" Tomatoes (*Lycopersicon esculentum* Mill.) Grown under Protection in a Hydroponic System. *Cahier Option Mediterraneees*, 31, 379-383.
- Dobrzanski, J., 1981. Suitability of different substance for growing several tomato cultivars under glass. *Biuletyn Warzynizy*, 32: 393-404.
- El-Khalifa, K. F. 2003. Nursery establishment of abanus (*Dalbergia melnoxylon* Guill. and Perr). *Arab Gulf J. Sci. Res.*, 21 (3): 153 – 157.
- El-Quesni, F. E. M.; Magda, M. Kandil and Mona H. Mahgoup 2007. Some studies on the effect of putrescine and paclobutrazol on growth and chemical composition of *Bougainvillea glabra* L. at Nubaria. *American-Eurasian J. Agric. & Environ. Sci.*, 2 (5): 552 – 558.
- El-Sallami, L. H. 2002. Seedling responses of some ornamental trees to soil type and NPK fertilization. *Assiut J. Agric. Sci.*, 34 (1): 67 – 48.
- Kakkar, R.K and Sawhney, K.V. 2002. Polyamine research in plants-a changing perspective. *Physiologia Plantarum*, 116(3): 281-292.
- Khan, S., Abdul Humed. K., Aqib. L, and Main. J. R. 2002. Effect of different media on growth and quality of gladiolus (*gladiolus hortulanus*) cv. Jacksonvilla Gold). *Asian Journal of plant sciences*. 6: 670-675.
- Khan. F.N, Rahman M.M, Karim. A. J. M. S. K. M. and Hossain. 2012. Effects of nitrogen and potassium on growth and yield of gladiolus corms. *Bangladesh J. Agril. Res.* 37(4): 607-616.
- Kuechen, C. D. and Phillips, G. C. 2005. Role of polymines in apoptosis and other recent an advances in plant polyamines. *Critical Review Plant Science*, 24 (2): 123 – 130.
- Kumar, A., Altabella, T., Taylor, M.A and Tiburcio, A.F. 1997. Recent advances in polyamine research. *Trends Plant Sci.*, 2: 124-130.
- Leinfelder, J. and R. Rober, 1989. Environmental suitable gladiolus cultivation. Cut flower from a closed system. *Gartnerborse, und-Gartenwelt*, 89: 948\_953.
- Magnani, G., Grassotti, A. and Nesi, B. 2003. Lapillus growing medium for cut bulbous flowers in soilless culture. *Acta Hort.* 609:389-393.
- Mahadik, M.K and Neha Chopde. 2015. Influence of nitrogen and potassium on growth and Yield of gladiolus corms. *Plant Archives* Vol. 15 No. 1, pp. 193-196.
- Mahgoub, M. H.; El- Ghorab, A. H. and Bekheta, M. A. 2006. Effect of some bioregulators on the endogenous phytohormones chemical composition,

- essential and its antioxidant activity of carnation (*Dianthus caryophyllus* L.). *J. Agric. Sci. Mansoura Univ.*, 31 (7): 4229 – 4245.
- Mahmood, S. M. 2005. Effect of different soil media on seed germination, seedlings growth and NPK content *Caesalpinia pulcherrima* and *Thevetia peruviana*. *Univ. Aden J. Natur. and Appl. Sci.*, 9 (2) : 319 – 330.
- Michael Ravin, Heinrich Lieth J. 2008. *Soilless culture: Theory and Practice. Elsevier.* p. 571.
- Mohamed, M. F. 1993. Effect of salinity on growth and chemical composition of some ornamental shrub. M. Sc. Thesis, Fac. Agric. Cairo Univ., 301 p.
- Papadopolos, A.P. 1994. *Growing Greenhouse Seedless Cucumbers in Soil and in Soilless Media*, Agricultural Canada Publication 1902/E, Communications Branch, Agricultural and Agri-Food Canada, Ottawa, Canada. p. 108.
- Rami, M. and D. Porath, 1980. Chlorophyll determination in intact tissue using N, N. Dimethyl Formamid. *Plant Physiology*, 65: 478-479.
- Singh Vivek Kumar, Prasad V.M. Kumari Supriya, Rajoria Preeti and Misra Pragati 2017. Identification of the Suitable Hardening Protocol and Hardening Medium in Micropropagation of *Gerbera (Gerbera jamesonii Bolus)*. *International Journal Current Microbiology and Applied Sciences* 6(7): 2476-2484. doi: <https://doi.org/10.20546/ijcmas.2017.607.292>
- Sorokina, L. I., E. L. Kuclrnk and V. V. Torgasherer, 1984. Using bark in greenhouse. *Lesnaya promyshlennost*, 5: 92-99.
- Tehranifar, A., Selahvarzi, Y. and Alizadeh, B. 2011. Effect of Different Growing Media on Growth and Development of Two *Lilium* (Oriental and Asiatic Hybrids) Types in Soilless Conditions. *Proc. IInd IS on the Genus Lilium. Acta Hort*, 900-911.
- Tribulato, A., Noto, G. and Argento, S. 2003. Soilless culture on quality production in lily. *Acta Hort*.614.621.