Effect of root microbial inoculants and chitosan on growth of Aglaonema (*Aglaonema commutatum*) cuttings.

Abstract

A field investigation was carried at Floriculture Research Station, (Agricultural Research Institute) Rajendranagar, Hyderabad during October to April 2020-2021. The experiment was laid out in Factorial Completely Randomized Design with three replications. The experiment consisted of two factors. Factor I : Two levels in portion of cutting *i.e.* Top cutting (P_1), Middle cutting (P_2) and Factor II: Eight levels of bio-fertilizers, S_1 - VAM (Vesicular Arbuscular Mycorrhizae) - 5 ml / kg of media, S_2 - *Pseudomonas fluorescence* - 5 ml / kg of media, S_3 - Chitosan – 1000 ppm, S_4 – IBA (Indole-3-Butyric Acid) - 1000 ppm, S_5 – VAM - 5 ml / kg of media + IBA - 1000ppm, S_6 – *Pseudomonas fluorescence* - 5 ml / kg of media + IBA - 1000 ppm, S_7 – Chitosan – 1000 ppm + IBA - 1000 ppm and S_8- Control (without treatment). Among the different treatments P_1 (Top cutting) with S_5 (VAM - 5 g / kg media + IBA - 1000ppm) recorded best results in least number of days taken for sprouting (27.70 days), number of leaves per cutting (4.54), fresh weight of leaf (3.10 g), shoot length (28.96 cm) and shoot girth (13.00 mm) and days taken to finishing stage (125 Days). Whereas, P_1 - (Top cutting) with S_6 - (Pseudomonas fluorescence - 5 ml / kg media + IBA – 1000 ppm) recorded best results in growth rate (0.054 g).

Introduction

Aglaonema commutatum commonly called as Chinese evergreen belongs to the family Araceae. The genus name comes from the Greek words ‘aglaos’ meaning bright or clear and ‘nema’ meaning a thread in reference to the stamens. It is an evergreen perennial that generally resembles Dieffenbachia (dumb cane) in appearance (Chen *et al*., 2002) which commonly known as aroids. It typically grows to 20" tall and is characterized with thick, elliptic to lance shaped, dark green leaves (4-8" long and 2-3" wide) with attractive silver-grey blotches on erect, sometimes branched stems. It is a houseplant, which rarely flowers, each axillary flower (typical arum family) features a small creamy white spadix enclosed by a pale green spathe, usually in late summer to early fall, flowering is followed by clusters of red fruits.

In 1989, National Aeronautics and Space Administration (NASA) discovered that houseplants can absorb harmful toxins from the air, especially in enclosed spaces with little air flow. This study has been the basis for newer studies about indoor plants like Chinese Evergreen and their air cleaning abilities. While plants have less horsepower than air purifiers, they are more natural, cost effective, and therapeutic. NASA recommends two or three plants in 8 to 10-inch pots for every 100 square feet. Some plants are better at removing certain chemicals than others. Aglaonema grow best in fairly heavy shade of 73 to 90 percentage (approximately 1000 to 2400 foot-candles) with the highest shade level requirement where temperatures exceeds 95°F (Henny *et al*., 1991).

Production of planting material in bulk through vegetative propagation byusing cuttings is peremptory to meet the great demand of planting material of *Aglaonema*. Further, the root induction and growth is very low by conventional methods of propagation. There is need to find out different methods to encourage fast root induction and growth of the plant to produce more plants in finished stage. One among the different methods is use of various root inducing substances that can promote rooting and enhance fast growth of plants propagated through cuttings.

Chitosan is a biopolymer, a chitin derivative a compound which is completely safe for the environment. This compound is characterized by unique properties, such as bioactivity and biocompatibility (Dias *et al*., 2013). Chitosan can induce a multitude of biological processes in plant tissues, including the stimulation of chitinases, accumulation of phytoalexins, synthesis of proteinase inhibitors and increasing lignification.

Further, Pseudomonas belong to Plant Growth Promoting Rhizobacteria (PGPR), an important group of bacteria that play a major role in the plant growth promotion, induced systemic resistance, biological control of pathogens etc. Pseudomonas fluorescens BBc6 improve the rooting of de-rooted shoot hypocotyls of Norway spruce and enhance root elongation. (Karabaghli *et al*., 1998).

Another bioinoculant, Vesicular-Arbuscular Mycorrhizal fungi (VAMF) is one type of mycorrhizal fungi that is commonly associated with the roots of horticultural crops. Optimal uses for commercially available VAMF inoculum have not been well defined. The benefits from VAMF root colonization are thought to be highest when colonization occurs as early as possible during plant growth. In horticultural production systems, this means that to obtain maximum benefits from VAMF colonization, inoculum should be present during

radicle emergence in seed germination, during adventitious root formation in cutting propagation, or prior to the acclimation phase of tissue culture production (Chang, 1994). In horticulture, auxins, especially NAA and IBA, are commonly applied to stimulate root initiation for rooting in cuttings of plants. However, high concentrations of auxin inhibit root elongation and instead enhance adventitious root formation (Susaj and Irena, 2012).

Chitosan is having many biological activities in plant systems, *Pseudomonas* and VAMF has significant role in rooting of cuttings. *Aglaonema* is a very important ornamental house plant, which is propagated by stem cuttings but with slow growth. Hence, to study the influence of root microbial inoculant and chitosan effects solely and in combination with IBA in induction of rooting and growth of cuttings, the present investigation has been formulated.

Material and Methods

Experiment was carried out at Floriculture Research Station, (Agricultural Research Institute) Rajendranagar, Sri Konda Laxman Telangana State Horticulture University, Hyderabad during November 2020 to March 2021.

The experiment consisted of two factors. Factor I: Two levels in portion of cutting i.e.; Top cutting (P_1), Middle cutting (P_2) and Factor II: Eight levels of bio-fertilizers, S_1 - VAM (Vesicular Arbuscular Mycorrhizae) - 5 ml / kg of media, S_2 - Pseudomonas fluorescence - 5 ml / kg of media, S_3 - Chitosan – 1000 ppm, S_4 – IBA (Indole-3-Butyric Acid) - 1000 ppm, S_5 – VAM - 5 ml / kg of media + IBA - 1000ppm, S_6 – Pseudomonas fluorescence - 5 ml / kg of media + IBA - 1000 ppm, S_7 – Chitosan – 1000 ppm + IBA - 1000 ppm and S_8- Control (without treatment). Experiment was laid out in Factorial Completely Randomized Design with three replications. Top cuttings 15-20 cm long with 3-4 buds and Middle cuttings 10 cm long with 2-3 buds were selected for the treatment application. The cuttings were treated with one per cent Bavistin to prevent the occurrence of fungal disease. Later, they are treated with chitosan, microbial inoculants (*Psuedomonas fluorescence* and VAM) and IBA. The treated cuttings were placed in Red soil, sand, FYM (2:1:1) media filled in polybags. For application o treatments to cuttings liquid formulations of *Psuedomonas* and powder form of VAM were used. Treated cuttings were planted in polybags and the soil around cuttings were pressed firmly. Single cutting was planted in single polybags.

Five randomly selected rooted cuttings were taken out from polybags at 180 days after planting of cuttings with care and observations was recorded i.e., days taken for

sprouting, number of leaves per cutting, fresh weight of leaf (g), shoot length (cm), shoot length (cm), shoot girth (cm), days taken for finish stage and growth rate (gm^{-2} day^{-1}).

Result and discussion

Days taken for sprouting

The data recorded on number of days taken for sprouting as influenced by the portion of cuttings and root microbial inoculants, chitosan and IBA are presented in Table 1.

The interaction between portion of cuttings and root growth stimulants on number of days taken for sprouting of Aglaonema cuttings was significant. The treatment combination of P_1S_5 (Top cutting + VAM - 5 g / kg media + IBA - 1000ppm) recorded minimum number of days for sprouting (27.70 days), which was followed by P_1S_1 (Top cutting + VAM - 5 g / kg media) (29.21 days), P_1S_3 (30.00 days) and P_1S_6 (30.09 days). Whereas, the treatment combination P_2S_8 (Middle cutting + Control) recorded maximum number of days taken for sprouting (72.35 days). The other treatment combinations recorded intermediate values. The acceleration of sprouting with PGPR'S and auxins combination might be due to synergetic effect. Mycorrhizal infection alters the physiology of plants by increasing photosynthetic rates, changing the position of photosynthates in shoots and roots and changing the mineral uptake from soil, which consequently changes the nutritional composition of host tissues. This change in tissues in turn brings structural and biochemical changes in root cells and alters membrane permeability and auxins will help in cell division and differentiation this leads to increase the shoot growth, leaf area and plant dry weight (Gollan and Wright, 2006).

Number of leaves per cutting

The data recorded on number of leaves per cutting as influenced by the portion of cuttings and root microbial inoculants, chitosan and IBA are presented in Table 1.

The interaction between portion of cuttings and root growth stimulants on number of leaves per cutting of Aglaonema cuttings was significant. The treatment combination of P_1S_5 (Top cutting + VAM - 5 g / kg media + IBA - 1000ppm) recorded highest number of leaves per cutting (5.21), which was at par with P_1S_6 (Top cutting +(*P. fluorescence* 5 ml/kg + IBA – 1000 ppm) (4.94) and P_1S_2 (4.57). Whereas the treatment combination P_2S_8 (Middle cutting

+ control) recorded lowest number of leaves per cutting (1.00) and the other treatment combinations recorded intermediate values.

The result shows that the number of leaves were significantly increased with the application of VAM, P. fluorescence and IBA combinations (VAM + *P. fluorescence* + IBA 1000 ppm). More number of leaves were observed due to the application of PGPR'S, might be due to increase in efficiency of nutrient uptake by cuttings. These results are in accordance with the finding of Kumar *et al.* (2003) in China aster. Similar finding was also observed by Kumar *et al.* (2014). Increase in number of leaves may be due to vigorous growth and early initiation of root induced by the PGPR'S and IBA (Stancato *et al.* 2003). Similar finding was observed by Waseem *et al*. (2011) in chrysanthemum.

Fresh weight of leaf (g)

The data recorded on Fresh weight of leaf as influenced by the portion of cuttings and root microbial inoculants, chitosan and IBA are presented in Table 1.

The interaction between portion of cuttings and root growth stimulants on fresh weight of leaf of Aglaonema cuttings was significant. The treatment combination of P_1S_5 (Top cutting + VAM - 5 g / kg media + IBA - 1000ppm) recorded highest fresh weight of leaf (3.10 g), which was followed by P_1S_6 (Top cutting + *P. fluorescence* 5 ml/kg + IBA – 1000 ppm) (3.05 g). Whereas the treatment combination P_2S_8 (Middle cutting + control) recorded lowest fresh weight of leaf (0.67 g). The other treatment combinations recorded intermediate values.

Increase in number of leaves may be due to vigorous growth and early initiation of root induced by PGPR'S and IBA which absorbed more nutrients and thereby produced more leaves. Similar result was reported by Stancato *et al*. (2003) in Rhipsali grandiflora and Sohn *et al.* (2003) in chrysanthemum.

Shoot length (cm)

The data recorded on shoot length as influenced by the portion of cuttings and root microbial inoculants, chitosan and IBA are presented in Table 2.

The result shows that the shoot length was significantly increased with the application of VAM, *P. fluorescence* and IBA in combination. It is known that, PGPR'S increases both the plant uptake and accumulation of nutrients and therefore, acting as an important

biological factor that contribute to efficiency of both nutrient uptake and utilization. by Cruz *et al.* (2008) reported that, VAM inoculation to Senna spectabilis increased total shoot length.

Similarly, IBA increased the shoot length of terminal cuttings in the present study might be done the active root growth and more number of roots per cutting, which in turn increased the uptake of water and nutrients. Further, auxin enhances the cell division, cell elongation and production of protein synthesis which might have resulted in enhanced healthy vegetative growth (Kumaresan *et al*., 2019). Similar findings were observed by Ganjure *et al*. (2012) in chrysanthemum.

Shoot girth (mm)

The data recorded on shoot girth as influenced by the portion of cuttings and root microbial inoculants, chitosan and IBA are presented in Table 2.

The interaction between portion of cuttings and root growth stimulants on shoot girth of Aglaonema cuttings was significant. The treatment combination of P_1S_5 (Top cutting + VAM - 5 g / kg media + IBA – 1000 ppm) recorded maximum shoot girth (13.00 mm), which was followed by P_1S_6 (Top cutting + *P. fluorescence* 5 ml/kg + IBA - 1000ppm) (12.00 mm). Whereas the treatment combination P_2S_8 (Middle cutting + Control) recorded minimum shoot girth (3.68 mm). The other treatment combinations recorded intermediate values.

Application of PGPR'S develops special characteristic structures called as apostles and vesicles, these arbuscules helps in the transfer of nutrients from the soil into the root system and significantly effect on plant height, number of branches plant, leaf area and girth (Rabin and Chikkaswamy, 2014). The synergistic effect of PGPR'S and auxins (IBA) enhanced the rooting parameters and there by increased water and nutrient uptake. Further increase in cell division and differentiation enhanced vegetative growth.

Growth Rate

The data recorded on growth rate as influenced by the portion of cuttings and root microbial inoculants, chitosan and IBA are presented in Table 2.

The interaction between portion of cuttings and root growth stimulants on rooting percentage of Aglaonema cuttings was significant. The treatment combination of P_1S_6 (Top cutting + *P. fluorescence* 5ml/kg + IBA – 1000 ppm) recorded maximum growth rate (0.054).

Which was followed by P_1S_5 (Top cutting + VAM - 5 g / kg media + IBA – 1000 ppm) (0.046). Whereas, the treatment combination P_2S_8 (Middle cutting + Control) recorded lowest growth rate (0.009).

The result shows that the growth rate was significantly increased with the application of *P. fluorescence* and IBA combination (*P. fluorescence* + IBA-1000ppm). *P. fluorescence* improves the plant growth directly or indirectly by production of plant growth substances, improving the uptake of certain nutrients from the soil and additionally show antagonistic effects on some pathogenic microorganisms. Further, Pseudomonas strains with Arbuscular Mycorrhizal Fungi (AMF) induces the plant growth, probably due the hormone production and increased photosynthates which have a direct influence on growth of plant (Matheus *et al.,* 2018). Auxins stimulate cell elongation by stimulating wall- loosening factors, such as elastin's, to loosen cell walls and helps in growth of the plant (Karthik *et al*., 2017).

Days taken to finishing

The data recorded on days taken to finishing stage as influenced by the portion of cuttings and root microbial inoculants, chitosan and IBA are presented in Table 3.

The finishing stage (4 Leaf stage) ranged from 120 to 180 days with respect to top cuttings and with middle cuttings it reached to only 2 leaf stage. The interaction between portion of cuttings and root growth stimulants had influenced days taken to finishing stage. The treatment combination of P_1S_5 (Top cutting + VAM - 5 g / kg media + IBA – 1000 ppm) recorded minimum days taken to finishing (120 days), which was followed by P_1S_6 (Top cutting + *P. fluorescence* 5 ml / kg + IBA - 1000ppm) (130 days). Whereas, the treatment combination P_1S_8 (Top cutting + Control) has reached the marketable stage at 180 days.

Conclusion

Among the different combination of treatments, T_5 (P_1S_5) (Top cutting + VAM - 5 ml / kg of media + IBA - 1000ppm) proved to be the best treatment to improve growth parameters of Aglaonema cuttings.

Table 1: Effect of portion of cuttings and root growth stimulants (microbial inoculants, Chitosan and IBA) on days taken for sprouting, number of leaves per cutting and fresh weight of leaf (g).

Root growth stimulants (s)	Days taken for sprouting			Number of leaves per cutting			Fresh weight of leaf (g)		
	Portion of cuttings (p)			Portion of cuttings (p)			Portion of cuttings (p)		
	P_1 Top cutting	P_2 Middle cutting	Mean	P_1 Top cutting	P_2 Middle cutting	Mean	P_1 Top cutting	P_2 Middle cutting	Mean
S_1-VAM - 5 g / kg media	29.21	55.66	42.43	4.33	1.32	2.83	2.09	1.19	1.64
S_2-*P. fluorescence*- 5 ml / kg media	33.13	64.29	48.71	4.57	1.28	2.93	2.14	1.63	1.88
S_3-Chitosan – 1000 ppm	30.00	63.54	46.77	4.21	1.20	2.71	2.12	1.30	1.71
S_4-IBA - 1000 ppm	29.99	55.78	42.88	4.14	1.51	2.83	2.11	1.52	1.81
S_5-VAM-5 g /kg media + IBA-1000 ppm	27.70	52.14	39.92	5.21	2.10	3.65	3.10	1.81	2.45
S_6-*P. fluorescence* (5ml/kg) + IBA-1000 ppm	30.09	56.11	43.10	4.94	1.85	3.40	3.05	1.74	2.39
S_7-Chitosan–1000 ppm + IBA-1000 ppm	42.80	68.11	55.45	4.04	1.61	2.83	2.70	1.72	2.21
S_8-Control (untreated)	64.81	72.35	68.58	4.18	1.00	2.59	1.00	0.67	0.83
Mean	35.96	52.41		4.45	1.48		2.29	1.45	
	P	**S**	**P X S**	**P**	**S**	**P X S**	**P**	**S**	**P X S**
SEm ±	0.50	1.00	1.41	0.11	0.18	0.25	0.01	0.02	0.03
CD at 5%	1.44	2.88	4.08	0.31	0.48	0.70	0.04	0.06	0.08

Table 2: Effect of portion of cuttings and root growth stimulants (microbial inoculants, Chitosan and IBA) on shoot length (cm), shoot girth (mm) and growth rate

Root growth stimulants (s)	Shoot length (cm)			Shoot girth (mm)			Growth rate		
	Portion of cuttings (p)			Portion of cuttings (p)			Portion of cuttings (p)		
	P_1 Top cutting	**P_2 Middle cutting**	**Mean**	**P_1 Top cutting**	**P_2 Middle cutting**	**Mean**	**P_1 Top cutting**	**P_2 Middle cutting**	**Mean**
S_1-VAM - 5 g / kg media	16.90	5.10	11.00	10.00	4.21	7.10	0.037	0.019	0.028
S_2-*P. fluorescence*- 5 ml / kg media	19.89	6.31	13.10	10.11	4.64	7.37	0.043	0.018	0.031
S_3-Chitosan – 1000 ppm	16.78	5.90	11.34	9.21	3.79	6.5	0.041	0.021	0.031
S_4-IBA - 1000 ppm	20.12	7.24	13.68	11.17	5.01	8.09	0.041	0.017	0.029
S_5-VAM-5 g /kg media + IBA-1000 ppm	28.96	8.80	18.88	13.00	5.53	9.26	0.046	0.021	0.033
S_6-*P. fluorescence* (5ml/kg) + IBA-1000 ppm	28.06	7.94	18.00	12.00	5.31	8.65	0.054	0.022	0.038
S_7-Chitosan–1000 ppm + IBA-1000 ppm	25.62	7.64	16.63	11.79	5.24	8.65	0.045	0.017	0.031
S_8-Control (untreated)	6.01	4.80	5.40	4.51	3.68	4.09	0.024	0.009	0.017
Mean	20.29	6.71		10.22	4.67		0.041	0.018	
	P	**S**	**P X S**	**P**	**S**	**P X S**	**P**	**S**	**P X S**
SEm ±	0.09	0.19	0.26	0.05	0.07	0.15	0.0008	0.0010	0.0024
CD at 5%	0.27	0.54	0.77	0.15	0.21	0.44	0.0024	0.0029	0.0068

Table 3 : Effect of portion of cuttings and root growth stimulants (microbial inoculants, chitosan and IBA) on days taken to finishing stage of Aglaonema ((*Aglaonema commutatum* L.)

ROOT GROWTH STIMULANTS (S)	Number of days taken for marketable stage	
	P1 Top cutting	**P2 Middle cutting**
S1- VAM - 5 g / kg media	160 Days	2 Leaf stage
S2- *P. fluorescence*- 5 ml / kg media	150 Days	2 Leaf stage
S3- Chitosan - 1000 ppm	165 Days	2 Leaf stage
S4- IBA - 1000 ppm	165 Days	2 Leaf stage
S5- VAM-5 g / kg media + IBA-1000ppm	120 Days	2 Leaf stage
S6- *P. fluorescence* -5ml/kg + IBA-1000ppm	130 Days	2 Leaf stage
S7- Chitosan-1000 ppm + IBA-1000 ppm	135 Days	2 Leaf stage
S8- Control	180 Days	2 Leaf stage

References:

Chang DC. Management of Mycorrhizas in Agriculture, Horticulture and Forestry. Kluwer Academic Publishers. Netherlands. 1994; 65–81.

Chen J, Henny RJ and Mc Connell DB. Development of new foliage plant cultivars. Trends in new crops and new uses. 2002; 466-472.

Cruz RED and Husain TA. Effect of vesicular arbuscular mycorrhiza (VAM) fungi inoculation on coppicing ability and drought resistance of Senna spectabilis. Pak. J. Bot. 2008; 40(5): 2217-2224.

Dias AMA, Cortez AR, Barsan MM, Santos JB, Brett CMA and De Sousa HC. Development of greener multi-responsive Chitosan Biomaterials doped with Biocompatible ammonium ionic liquids. ACS Sustainable Chemistry and Engineering. 2013; 1(11): 1480-1492.

Ganjure SL, Gawande MB. and Golliwar VJ. Response of IBA and Rooting Media on Rooting of Cutting in Chrysanthemum. International Journal of Science and Research. 2012; 3(7): 1306-1310.

Gollan JR and Wright JT. Limited grazing pressure by native herbivores on the invasive seaweed Caulerpa taxifolia in a temperate Australian estuary. Marine and freshwater Research. 2006; 57: 685-694.

Henny RJ, Chase A R and Osborne LS. Aglaonema Production Guide. Apopka Foliage Plant Research. 1991; RH-91-2.

Karbaghil C, Frey PK, Sotta B and Tacon F L. Invitro effects of *Laccaria bocolor* S238N and *Pseudomonas fluorescens* strain BBc6 rooting of de-rooted shoot hypocotyl of Norway spruce. Tree physiology. 1998; 18: 103-111.

Karthik P and Mohapatra PP. Role of Auxin on growth, yield and quality of Tomato. International Journal of Current Microbiology and Applied Sciences. 2017; 6(11): 1624-1636.

Kumar KR, Singh KP and Raju DVS. Symbiotic Effect of Arbuscular Mycorrhizal Fungi on Growth and Flowering of Micro propagated Plants of Chrysanthemum (*Chrysanthemum dendranthemum*). International Journal of Bio-resource and Stress Management. 2014; 5(3): 369-374.

Kumar P, Raghava S PS and Misra R L. Effect of Biofertilizers on growth and yield of China aster. Journal of Ornamental Hort. 2003; 6(2): 85-88.

Kumaresan M, Kannan M, Sankari A, Chandrasekhar CN. and Vasanthi D. Phytochemical screening and antioxidant activity of *Jasminum multiflorum* (Pink Kakada) leaves and flowers. Journal of Pharmacognosy and Phytochemistry. 2019; 8(3): 1168-1173.

Matheus C A and Freitas SS. Effect of Pseudomonas putida on chrysanthemum growth under greenhouse and field conditions. African Journal of Agricultural Research. 2018; 13(6): 302-310.

Rabin CP and Chikkaswamy BK. Effects of VAM and Biofertilizers on some Medicinal Plants. International Journal of Current Microbiology Applied Science. 2014; 3(6): 1016-1027.

Sohn BK, Kim KY, Chung JS, Kim WS, Park MS, Kang JG, Rim YY, Kim TH. and Hyun LJ. 2003. Effect of the different timing of AMF inoculation on plant growth and flower quality of chrysanthemum, Scientia Horticulturae. 2003; 98(2): 173-183.

Stancato GC, Aguiar FFA, Kanashiro S and Tavares AR. Rhipsali grandiflora Haw. Propagation by stem cuttings. Scientia Agricola. 2003; 56: 185- 190.

Susaj EL and Irena K. Effect of different NAA and IBA concentrations on rooting of vegetative cuttings of Two Rose cultivars. Research Journal of Agricultural Science. 2012; 44 (3):121-127.

Waseem K, Jilani MS, Jaskani MJ, Khan, MS, Kiran M and Khan GU. Significance of Different Plant Growth Regulators on the Regeneration of Chrysanthemum Plantlets (*Dendranthema morifolium* L.) through Shoot Tip Culture. Pak. Journal of Botanical. 2011; 43(4): 1843-1848.