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Effect of *Aloe vera* Gel for Inducing Rooting of Stem Cuttings and Air layering of Plants

Mirihagalla, M. K. P. N. and Fernando, K. M. C.
Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka

Abstract: Plant growth regulators (PGRs) are important to facilitate root formation and firm plant establishment hence used in vegetative propagation for many years. Farmers use natural alternative compounds to induce rooting of cuttings. The present study was conducted to identify the effect of *Aloe vera* gel on root formation of stem cuttings and air layering of plants. Three experiments were conducted to compare *A. vera* gel with commercially available PGR for root induction of semi hard wood cuttings(*Citrus aurantifolia)*, softwood cuttings (*Coleus* spp.) and air layering plants (*Syzygium jambos*). Average root length, number of roots and root vigour were recorded as root traits. Effect of PGR and *Aloe vera* gel on root traits was not significantly different for *C.aurantifolia* and *S.jambos*. However, effect of PGR was significantly different on root traits compared to cuttings did not receive either *A. vera* gel or PGR. Therefore, synthetic PGRs can be replaced by *A. vera* gel, a natural alternative root inducing substance, for semi hardwood cuttings of *C. aurantifolia* and air layering of *S. jambos* but not for softwood cuttings of *Coleus spp*. These finding could be used in developing natural root inducing substance and resolving and reducing the risk of chemical toxicity in plants due to PGRs.

Keywords**:** *Aloe vera* gel, Air layering, Plant growth regulators, Rooting, Stem cutting

Introduction

Plant growth regulators (PGRs) are often referred as synthetic plant hormones and used in agriculture for many years. PGR s categorized into five main groups such as auxins, gibberellins, cytokinins, abscisic acid and ethylene. These PGRs alter plant physiological and functional processes. Most of PGRs are used in commercial agriculture including horticulture and other fields of agriculture. Success of rooting of cuttings depends on the physiological stage of the mother plant (Day and Loveys, 1998), time which cutting was taken (Darwesh, 2000) and the type of PGRs used (Rowzack, 2001).

Corresponding Author:K. M. C. Fernando, menaka@crop.ruh.ac.lk

Replacement of synthetic PGRs with natural alternatives are becoming popular because of high cost of synthetic PGRs, risk of toxicity in plants, human and animals due to the application of overdoses (Cutler *et al*., 1990). Alternative natural plant extracts rich in plant hormones and natural antioxidants can be used to improve, induce and stimulate growth of another plant species. Coconut water is one of the natural sources which could be used to stimulate rooting of cuttings (Karunarathna and Kumuthini, 2016). Coconut water contains plant growth hormones such as auxin, gibberellins, cytokinis and some natural inhibitors and regulators which consist of ethylene, abscisic acid, phenols and flavonols (Juanita *etal*., 1988).

Among many of the natural alternatives, the extract from the leaves of *Aloe vera* are also being used. *Aloe vera* is an important medicinal plant which belongs to the family Asphodelaceae originated in Africa. It is a shrubby succulent herb grows in many countries around the world (Pandey and Singh, 2016). Its large leaves consist of three layers with inner clear gel, middle layer of latex and outer thick layer. Inner clear gel consists of 99% of water and some glucomannans, amino acids, sterols and vitamins. Middle parenchymatic cells contain liquid of yellow latex of a bitter sap, which is rich in essential amino acids, mono-and polysaccharides, lignin, macronutrients, micronutrients, vitamins, gibberellins and salicylic acid (Surjushe *et al*., 2008). The outer thick layer of 15-20 cells stored synthesized carbohydrates and proteins (Ramachandra and Rao, 2008).

Furthermore, Aloe leaf extract has been used to improve vegetative growth of some crop species. *Aloe vera* leaf powder, as a bio stimulant, was used to enhance growth and yield of *Abelmoschus es culentus* (Padmajaya *et al*., 2007). Also, many basil varieties were treated with *Aloe vera* as bio-fertilizer and some plant extracts on growth and yield (Ahmed *et al.*, 2014).

Since *Aloe vera* gel extract contains plant hormones such as auxins and gibberellin, and plant root growth promoters such as salicylic acid (Surjushe *et al*., 2008), it could be used as a source of natural hormone instead of synthetic growth regulators or purified natural hormones to induce rooting of cuttings. At present, many farmers and villagers use fresh *Aloe vera* gel for inducing rooting of stem cuttings and air layering of plants. Rooting of cuttings may be facilitated due to its antibacterial properties and/or it composition which includes root inducing substances like growth regulators or hormones. However, written scientific evidences are limited on the effect of *Aloe vera* gel as a root inducing substances hence systematically designed experiments are needed to prove the use of *Aloe vera* as a root inducing substances.

Therefore, the present study was conducted with aims of studying the effect of *Aloe vera* gel on root initiation of stem cuttings with softwood cutting of *Coleus* spp., semi- hardwood cuttings of lime (*Citrus aurantifolia*) and air layering of rose apple (*Syzygium jambos*) plants and compare the effect of *Aloe vera* gel with a commercial growth regulator for root initiation.

Materials and Methods

Experiment 1

Effect of Aloe vera gel on root in duction of semi-hardwood cuttings of *Citrus aurantifolia*

Completely randomized design was used to arrange the experiment with four treatments (T1-cuttingenddippedin *Aloe vera* gel for two minutes,T2-cutting end dipped in *Aloe vera* gel for five minutes, T3-apply growth regulator in cutting end (control) (Rapid root®, 0.3% Indole 3-butyric acid; IBA), T4- without applying *Aloe vera* gel or growth regulator to cutting) to determine the effect of *Aloe vera* gel on root induction of semi-hardwood cuttings of *Citrus aurantifolia.* Single propagators were used in the experiment where 6- 8 inches long cuttings were established. General media of coir dust: top soil: sand: compost (1:1:1:1/4) was used as a rooting media of the single propagator. All propagators were kept in shade house to provide cool and shade environment. Average root length (cm), number of roots and root vigour scale (no callus formation =0, callus formation = 1, callus and root initiation= 2, callus and few adventitious roots= 3,less than ten adventitious roots =4, more than ten adventitious roots =5)were recorded using destructive sampling at two and three months after establishment of the single propagators.

Survival rates of cuttings were calculated according to equation 1.

$$\text{Survival rate } \% = \frac{\text{Number of cuttings having roots and callus}}{\text{Total number of cuttings established}} \times 100 \quad \text{Equation 1}$$

n = 15 for each treatment

Experiment 2

Effect of *Aloe vera* gel on root inducti on of soft-wood cuttings of *Coleus* spp.

Same treatments as in experiment1were evaluated in this experiment to determine the effect of *Aloe vera* gel on root induction of soft-wood cuttings. Soft- wood cuttings of *Coleus* spp. (6 - 8 inches long) were established in trays. General media of coir dust: top soil: sand: compost (1:1:1:1/4) was used in the experiment. Completely randomized design was used for above same four treatments with five replicates (trays) for each treatment. Each tray consists of twelve cuttings and kept inside a protected house.

Data were collected destructively in every week after establishment in a random manner and Survival rate, average number of roots, average length of roots (cm) and root vigour scale was recorded (same vigour scale was used as in Experiment 1).

Experiment 3

Effect of *Aloe vera* gel on root induction of air layering of *Syzygium jambos*

Three treatments (T1-apply *Aloe vera* gel on the layering surface, T2-apply growth regulator on the layering surface (control) (Rapid root®, 0.3% Indole 3-butyric acid; IBA), T3- without applying *Aloe vera* gel or growth regulator on layering surface) were compared on rooting of air layering plants. Experiment was set up according to randomized completely block design. Four *Syzygium jambos* plants were selected to apply three treatments with five replicates in randomly selected branches. A single tree was considered as a block. Moist coir dust was used as media. All recommended practices for air-layering technique were followed. Data were collected after three months of layering of branches. Survival rate**,** average number of roots, average length of roots and root vigour were recorded (same vigour scale was used as in Experiment 1). Survival rate was calculated using equation 2.

$$\text{Survival rate } \% = \frac{\text{Number of Successful air layers}}{\text{Total number of air layered branches}} \times 100 \quad \text{Equation 2}$$

Statistical Analysis

Data were subjected to Analysis of General Linear Model (GLM) using SAS Package. The Duncan Multiple Range Test (DMRT) at probability of 5% was used to compare treatment means and Dunnett'st test was used to compare the mean values of the treatments by considering PGR treatment as the control.

Results and Discussion

Experiment 1

Average root length was not significantly different among treatments at two months after establishment. However, root vigor was significantly different ($P<0.01$) among treatments at two and three months after establishment. Average length of roots in third month was significantly different ($P<0.05$) among treatments where the highest mean root length was observed in PGR applied cuttings (6.48 cm). However, it was not significantly different from the cuttings which were dipped in *Aloe vera* gel in five minutes (5.02 cm) and two minutes (3.58 cm) (Table 1). Therefore, it is possible to use *Aloe vera* gel to induce rooting as same as commercially available PGR hence could be replaced easily.

PGR applied cuttings showed the highest survival rates of 53% and 93% at two and three months after establishment, respectively (Figure 1). Most of the cuttings had well developed callus while weakly developed few roots in PGR applied treatment at two months than in three months after establishment. Survival rate of the cuttings which were dipped in *Aloe vera* gel for two minutes showed 33% and 53% in second and third months, respectively. Cuttings which were treated and dipped in *Aloe vera* gel for five minutes showed 40 % and 60% of survival rates in second and third months, respectively. Survival rate of the cuttings treated with *Aloe vera* gel was greater than the cutting did not

Table 1: Average root length, number of roots and root vigor of semi hard wood cuttings of *Citrus aurantifolia* in single propagator after two and three months of establishment where T1-cutting end dipped in *Aloe vera* gel for two minutes, T2-cutting end dipped in *Aloe vera* gel for five minutes, T3- apply growth regulator in cutting end (control) (Rapid root®, 0.3% Indole 3-butyric acid; IBA), T4- without applying *Aloe vera* gel or growth regulator to cutting

Treatment	Two months			Three months		
	Average root length (cm)	Number Of roots	Root vigor	Average root length (cm)	Number Of roots	Root vigour
T_1	1.79^{a}	1.59^{a}	2.06^{a}	3.58^{ab}	1.71^{a}	2.43^{ab}
T_2	1.71^{a}	1.58^{a}	1.16^{b}	5.02^{a}	1.72^{a}	3.00^{ab}
T_3	1.62^{a}	1.58^{a}	0.52^{c}	6.48^{a}	2.44^{a}	2.95^{a}
T_4	1.54^{a}	1.58^{a}	0.13^{d}	2.26^{b}	1.61^{a}	1.95^{b}
Pvalue	0.859	1.000	0.001	0.010	0.248	0.027
CV%	14.02	14.17	15.38	31.79	27.32	15.02

(Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to Duncan's Multiple Range Test)

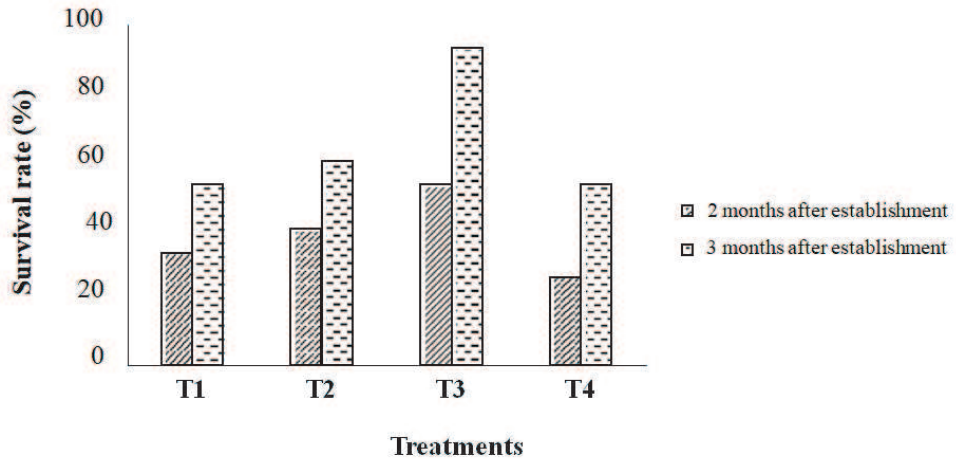

Figure 1: Effect of different root inducing treatments on survival rate of semi-hard wood cuttings of *Citrus aurantifolia* (Two and three months after establishment in single propagators) T1-cutting end dipped in *Aloe vera* gel for two minutes, T2-cutting end dipped in *Aloe vera* gel for five minutes, T3- apply growth regulator in cutting end (control) (Rapid root®, 0.3% Indole 3-butyric acid; IBA), T4- without applying *Aloe vera* gel or growth regulator to cutting

receive either PGR or *Aloe vera* gel. In general, the greater survival rate was observed after three months of establish ment of cuttings in single propagators than two months.

The mean values of root traits of the cuttings dipped in *Aloe vera* gel to gether with the cuttings did not receive *Aloe vera* gel or PGR were compared with the mean value of the cuttings received PGR (as a control). None of the root traits were significantly different between control and other treatments after two months of establishment. However, after three months, average root length and root vigour were significantly different in above mentioned treatments when compared to the control (Table 2). However, after three months of establishment, treatment 4 showed significant difference from treatment 3, however treatment 1 and 2 did not show significant difference from treatment 3.This indicates that treatment 1 and 2 where cuttings dipped in *Aloe vera* gel for 2 and 5 minutes was not signi ficantly different from PGR applied cuttings (Table 2). Hence, there is a possibility to replace PGR with *Aloe vera* gel as an alternative root induction substance for semi-hard wood cuttings of *Citrus aurantifolia* after 3 months of establishment (Plate 1).

Similar experiments have been conducted by different researchers related to *Citrus* species on various parameters affiliate with root formation. Most of them have found that auxin application enhances the histological features of the cuttings like formation of callus and tissue and

Table 2: Summary of Dunnett'st Test applied in GLM procedure after three month sof establishment in single propagators of semi hard wood cuttings *Citrus aurantifolia*. Mean values of root traits of the cuttings received PGR (T_3- control) was compared with other treatments

Parameter	Treatment comparison	Difference between means	Simultaneous 95% Confidence Limits	Significant indication
Average root Length (cm)	2-3	-0.3053	-1.0251 0.4145	*NS*
	1-3	-0.6536	-1.4335 0.1263	*NS*
	4-3	-1.0414	-1.7881 0.2947	***
Number of roots	2-3	-0.2494	-0.6545 0.1556	*NS*
	1-3	-0.2536	-0.6925 0.1853	*NS*
	4-3	-0.2925	-0.7127 0.1277	*NS*
Root vigour scale	2-3	-0.11556	-0.36923 0.13812	*NS*
	1-3	-0.16000	-0.43485 0.11485	*NS*
	4-3	-0.33875	-0.60190 -0.07560	***

Comparisons significant at the 0.05 level are indicated by***, *NS*–not significant

Plate 1: Effect of different root inducing treatments on root traits of semi hard wood cuttings of *Citrus aurantifolia* after three months of establishment in single propagators. T1-cutting end dipped in *Aloe vera* gel for two minutes, T2- cutting end dipped in *Aloe vera* gel for five minutes, T3- apply growth regulator in cutting end (control) (Rapid root®, 0.3% Indole 3-butyric acid; IBA), T4- without applying *Aloe vera* gel or growth regulator to cutting

differentiation of vascular tissue (Mitra and Bose 1954; Satpal *et al*., 2014). Hence this research showed application of PGR as the treatment can be replaced by *Aloe vera* gel. Hence, *Aloe vera* gel can be used to induce callus formation, tissue formation and root initiation of semi-hard wood cuttings of *Citrus aurantifolia.*

Experiment 2

Soft wood cuttings of *Coleus* spp didn't record significant difference among treatments for average root length and number of roots per cutting but significantly different for root vigour in 1st week after planting. However, in 2nd week average root length was significantly different among treatments where the highest value was recorded in control treatment and lowest value was recorded in T2 treatment. There was no significant difference between treatments for number of roots per cutting and root vigor in 2nd week. Treatment effect was significant for average root length and number of roots per cutting in 3rd week but not for root vigour. None of the root traits were significant among treatment at 4 weeks after planting. However, average root length was significantly different among treatment in 5th week (Table3). This result indicates that treatment effect on some root traits of softwood cutting of *Coleus* spp vary with time.

Highest survival rate 100% was observed in 2nd week after planting of *Coleus* cuttings. There was no considerable difference among survival rates of treatments used.

Table 3: Average root length (cm; ARL), number of roots (NR) and root vigor scale (RVS) of softwood cuttings of *Coleus* spp at five destructive sampling (T1-cutting end dipped in *Aloe vera* gel for two minutes, T2- cutting end dipped in *Aloe vera* gel for five minutes, T3- apply growth regulator in cutting end (control) (Rapid root®, 0.3% Indole 3-butyric acid; IBA), T4- without applying *Aloe vera* gel or growth regulator to cutting)

Treatment	1st week			2nd week			3rd week			4th week			5th week		
	ARL	NR	RVS	ARL	NR	RVS	ARL	NR	RVS	ARL	NR	RVS	ARL	NR	RVS
T_1	1.36^{a}	8.08^{a}	1.76^{a}	5.34b^{a}	17.55^{a}	4.08^{a}	10.95^{a}	26.31^{a}	4.70^{a}	8.47	16.48^{a}	3.68^{a}	10.62^{a}	20.25^{ab}	4.20^{a}
T_2	1.08^{a}	7.29^{a}	1.90^{b}	3.72^{b}	20.79^{a}	3.50^{a}	11.63^{a}	21.72^{a}	4.66^{a}	9.00	14.21^{a}	3.72^{a}	11.22^{a}	18.92^{b}	4.41^{a}
T_3	1.15^{a}	7.10^{a}	2.55^{ab}	6.35^{a}	25.70^{a}	3.81^{a}	9.24^{a}	20.07^{a}	4.75^{a}	9.73	16.69^{a}	4.28^{a}	13.47^{a}	25.70^{a}	4.67^{a}
T_4	1.93^{a}	6.66^{a}	1.51^{b}	4.58^{ab}	18.06^{a}	3.84^{a}	6.38^{b}	12.32^{b}	4.62^{a}	8.94	16.64^{a}	4.49^{a}	10.21^{a}	19.18^{b}	4.24^{a}
P value	0.47	0.93	0.04	0.05	0.18	0.66	0.002	0.001	0.93	0.95	0.86	0.25	0.2	0.01	0.55
CV %	31.85	26.26	22.28	16.12	23.68	15.47	18.22	19.74	5.3	29.97	26.81	13.86	15.99	15.75	9.43

(Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to Duncan's Multiple Range Test)

Therefore, application of PGR or *Aloe vera* gel has not definite effect when considering about the survival rates observed during this five weeks period (Figure 2). Root formation of soft wood cuttings of *Coleus spp*. are shown in Plate 2

Comparison among four treatments was not significant at any sampling point. Root characteristics of the cuttings treated with PGR were not significantly different with *Aloe vera* gel applied treatments as well as with the cuttings did not receive either PGR or *Aloe vere* gel. So, application of PGR or *Aloe vera* as root inducing material doesn't have any significant effect on rooting of *Coleus* soft wood. This might be due to *Coleus* spp fast rooting ability and it presence of apical leaves. It has found that stem cuttings of *Coleus* rooting did not vary according to IBA concentrations (PGR concentrations). Also, have found that root length and number of roots were higher in stem cuttings which have apical leaves in comparison to leafless ones, regardless to IBA concentrations of the treatments used. Therefore, the presence of apical leaves is even fundamental for coleus stem cuttings rooting and the use of IBA or PGR or any other alternative root inducer is not required for propagation (Belniaki *et al.*, 2018). The new leaves emerged during the rooting period is an essential feature for roots initiation because this new leaves and shoots produce plant hormones such as indoleacetic acid, rooting cofactors which essential for

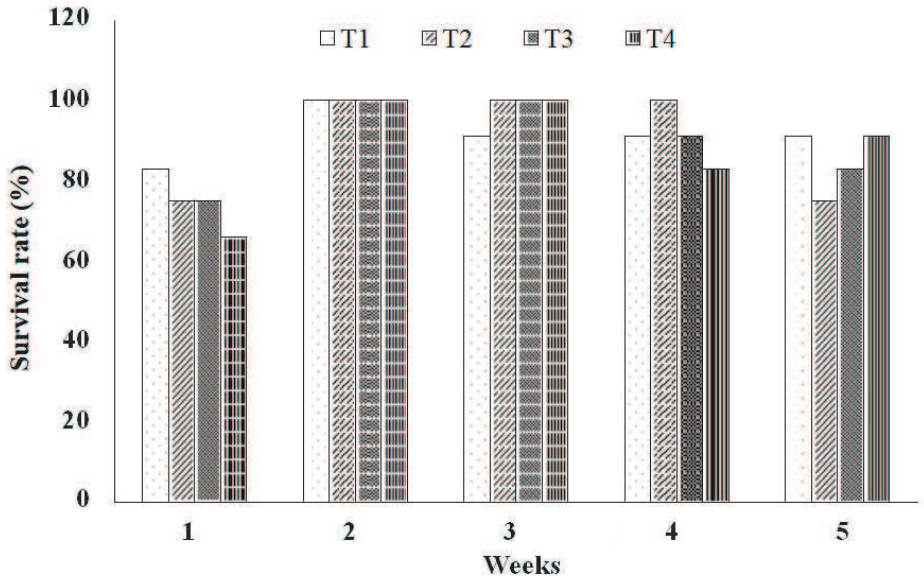

Figure 2: Effect of different root inducing treatments on survival rate of *Coleus* spp for five week period. T1-cutting end dipped in *Aloe vera* gel for two minutes, T2- cutting end dipped in *Aloe vera* gel for five minutes, T3- apply growth regulator in cutting end (control) (Rapid root®, 0.3% Indole 3-butyric acid; IBA), T4- without applying *Aloe vera* gel or growth regulator to cutting

Plate 2: Effect of different root inducing treatments on root traits after (A) two weeks and (B) five weeks after establishment of soft wood cuttings of *Coleus*spp. T1-cutting end dipped in *Aloe vera* gel for two minutes, T2- cutting end dipped in *Aloe vera* gel for five minutes, T3- apply growth regulator in cutting end (control) (Rapid root®, 0.3% Indole 3-butyric acid; IBA), T4- without applying *Aloe vera* gel or growth regulator to cutting

adventitious root formation in vegetative propagation (Fachinello *et al*., 2005). Since the potential for *Coleus* spp. vegetative propagation is highly associated with the presence of leaves in softwood stem cuttings of it promotes better results for rooting, roots development and sprouting, PGR or *Aloe vera* like alternative root inducer treatments might not have significant effect.

Experiment 3

Figure 5 shows root formation of air layering branches of *Syzygium jambos*. There was no significant effect of treatments on average root length and root vigour. But number of roots was shown a significant difference among treatments ($P< 0.05$) two months after layering (Table 4). Therefore, there is a possibility to use *Aloe vera* gel to induce rooting of air layered *Syzygium jambos* as same as commercially available PGR. However, the highest survival rate of air layers of *Syzygium jambos* was observed in PGR applied branches (83%). *Aloe vera* gel applied branches showed 55 % of survival rate and layered branches did not receive either *Aloe vera* gel or PGR only gave 35 % of success rate (Plate 3

Table 4: Average root length, number of roots and root vigor in air layering of *Syzygium jambos* after 2 months of establishment (T_1- apply *Aloe vera* gel on the layering surface, T_2- apply growth regulator on the layering surface (control) (Rapid root®, 0.3% Indole 3-butyric acid; IBA), T_3- without applying *Aloe vera* gel or growth regulator on layering surface)

Treatment	Root length (cm)	Number of roots	Root vigour scale
T_1	1.50^b	1.10^b	1.60^b
T_2	3.76^a	3.02^a	3.67^a
T_3	1.11^b	0.96^b	1.56^b
P value	0.1924	0.0418	0.3428
CV %	36.26	32.47	26.57

(Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to Duncan's Multiple Range Test)

and Figure 3). In general, it can be assumed that the *Aloe vera* treatment also a successful alternative root inducing method in air layering of *Syzygium jambos* when considering the survival rates. Also, the comparison between treatment 2 and 1, where PGR was applied as control treatment was not significantly different with treatment 1, where branches were treated with *Aloe vera* gel. However, treatment 2 showed a significant difference between T_3 where air layered branches did not receive either PGR or *Aloe vera* gel (Table5). Accordingly, those air

Plate 3: Effect of different root inducing treatments on root traits after second month of air layering of *Syzygium jambos* (T_1- apply *Aloe vera* gel on the layering surface, T_2- apply growth regulator on the layering surface (control) (Rapid root®, 0.3% Indole 3-butyric acid; IBA), T_3- without applying *Aloe vera* gel or growth regulator on layering surface)

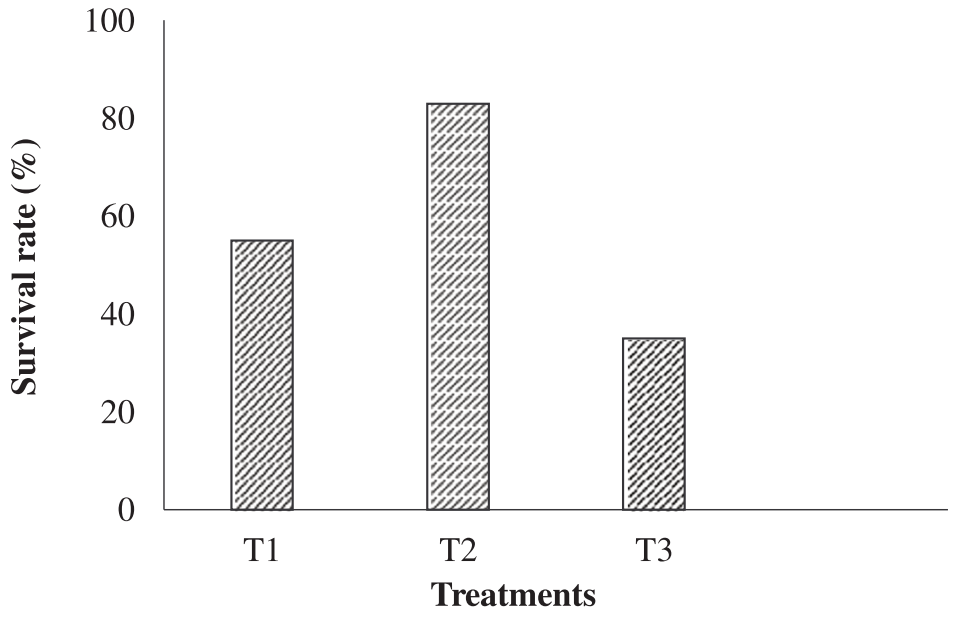

Figure 3: Effect of treatments on survival rate on air layering of *Syzygium jambos* two months after establishment; where T1-apply *Aloe vera* gel on the layering surface, T2-apply growth regulator on the layering surface (control) (Rapid root ®, 0.3% Indole 3-butyric acid; IBA), T3- without applying *Aloe vera* gel or growth regulator on layering surface

layered branches don't show any significant difference between T_1 and T_2, which again justify that there is a possibility to replace PGR with *Aloe vera* gel as an alternative root inducer for air layering of *Syzygium jambos* (Table 5).

As an objective to protect retention of desirable characteristics of the rootstock and the ability of mass production of identical plants quickly and efficiently air layering has been identified as suitable asexual propagation (Adriance and Brison, 1955) method for *Syzygium jambos*. PGRs as well as the rooting media, play a vital role in facilitating and improving the rooting of *Syzygium jambos* air layers. Hence, according to the results of experiment 3, it could be mentioned that we can successfully replace PGR with *Aloe vera* gel as an alternative material to induce rooting of *Syzygium jambos* air layering.

Conclusions

According to the results of the study, *Aloe vera* leaf gel can be recommended as an alternative root inducing substances to induce rooting of semi hard wood cuttings of *Citrus aurantifolia* and air layering plants of *Syzygium jambos*. It is not essential to use root inducing substances to induce rooting of soft wood cuttings of *Coleus* spp. In the present

Table 5: Summary of Dunnett's t Test applied in GLM procedure after two months of establishing air layering of *Syzygium jambos* mean values of root traits of the cuttings received PGR (T2- control) was compared with other treatments

Parameter	Treatment comparison	Difference between means	Simultaneous 95% confidence Limits	Significant indication
Average root	1-2	0.0721	-0.2661 0.4104	*NS*
Length (cm)	3-2	0.7600	0.4289 1.0911	***
Number of roots	1-2	0.1729	-0.2948 0.6405	*NS*
	3-2	0.8851	0.4273 1.3429	***
Root vigour	1-2	0.01500	-0.27857 0.30857	*NS*
Scale	3-2	0.66625	0.37887 0.95363	***

Comparisons significant at the 0.05 level are indicated by *, *NS*- not significant**

study freshly harvested *Aloe vera* leaves were used to collect gel substances. However, best results could be obtained by using leaves of *Aloe vera* harvested 5 - 7 days before being used them as root inducing material. Since, *Aloe vera* leaves produce more rooting hormones after separated from the plant as an anti-inflammatory actions.

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