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Evaluation and Standardization of Growing Media for Adventitious Root Formation in Lemon (*Citrus Limon* L.) Through Air Layering Technique

Muhammad Adeel Zaman Khan
Department of Horticulture, Gomal University, D.I. Khan, Pakistan
adeelzaman346@gmail.com
Aziz Ullah*
Agriculture Research Sub-Station, District Bajaur, KP, Pakistan
azizullahkhan60@yahoo.com
Sheheryar Hafeez
Horticulture Section, Agriculture Research Institute, D.I. Khan, KP, Pakistan
sherekhan4444@gmail.com
Farkhanda Khan
Horticulture Section, Agriculture Research Institute, D.I. Khan, KP, Pakistan
khanfarhanda60@gmail.com
Gohar Hayat
Agriculture Research Station, Kohat, KP, Pakistan
goharktk539@gmail.com
Zubair Shah
Department of Botany, Hazara University Mansehra, KP, Pakistan
zubairshah_bjr@yahoo.com

Abstract: *The experiment was carried out at the Horticulture Orchard of the Faculty of Agriculture, Gomal University, Dera Ismail Khan. The study aimed to evaluate different types of growing media prepared from locally available materials. Since Lemon plants are widely traded in the study area, therefore it was necessary to explore an extended propagation period using air layering and to identify the most suitable material that could provide maximum success in lemon plant production. The growing media evaluated in this study included Cinnamon, Aloe vera, Leaf mold, Farmyard manure and Silt. Parameters studied were root diameter (mm), number of roots, number of root bud, maximum length of root (cm), branches plant^{-1}, fresh root weight plant^{-1} (gm), and dry root weight plant^{-1} (gm). Leaf mold and mixture of FYM + silt was found to be the best material for air layering in lemon. Correlation studies revealed that growing media that had high moisture retaining capacity resulted in best root development in the layers.*

Keywords: *Lemon, Growing media, Silt, Aloe vera, FYM and root diameter.*

INTRODUCTION

Lemon (*Citrus limon* L.) belongs to the family Rutaceae and is an important fruit of citrus group. It is cultivated throughout tropical and subtropical regions of Pakistan. Lemon fruit is round and slightly elongated. Lemon juice is good for asthma, headache, pneumonia, and arthritis. It is a good general purifier for blood and body. Lemon is rich in vitamin, fiber, and several other beneficial plant compounds. It is a popular fruit in the world due to its taste, aroma and healing properties (Gaurav and Richa, 2012). Among people who consume a large number of certain nutrients, the risk of asthma seems to have low. Lemon is also a rich source of potassium, calcium, phosphorus, and magnesium. Its fruit also has a unique and pleasant taste and smell which makes it an excellent complement to food and drink (Helen West, RD, 2019). Lemon trees can be planted in a variety of soils, including deep sandy loam, loam and sticky soil with good drainage. However, they grow poorly in very heavy soils, clay, sandy and alkaline soils. pH should be between 6.5 and 7.5. If the acidity is high, lime must be applied to achieve the optimum level (Khan et al., 2017). Citrus are cultivated in four provinces of Pakistan. Districts like Sargodha, Sahiwal, Lahore, Sialkot, Jhang, Minwali, Multan and Gujranwala of Punjab, Mardan, Peshawar, Swat, Swabi, Nowshera, Hazara of KPK, Sukkur, Khairpur, and Nawabshah of Sindh, Mekran, Sibi and Kech of Balochistan are the major areas of citrus cultivation (Khan et al., 2017).

Lemon (*Citrus limon*) can be propagated through seeds, budding, grafting, and layering; however, air layering has emerged as one of the most reliable vegetative techniques for rapid and true-to-type multiplication. The method ensures continuous supply of carbohydrates and endogenous growth substances from the mother plant, thereby promoting enhanced adventitious root formation and the development of a balanced root system. Compared with seed propagation and cuttings, air layering produces vigorous and uniform plants within a shorter period. Rooting success in woody perennials is strongly influenced by environmental factors such as temperature and humidity, and previous studies have highlighted the importance of favorable seasonal conditions and growth regulator application for improved rhizogenesis (Bose et al., 1986; Tyagi and Patel, 2004).

Despite the commercial importance of lemon cultivation in D.I. Khan, scientific standardization of propagation practices particularly growing media remains inadequate under local agro-climatic conditions. Growers traditionally rely on a narrow seasonal window for air layering without experimental validation of substrate suitability. Locally available organic and inorganic materials such as cinnamon, aloe vera, leaf mold, farmyard manure (FYM), sawdust, and silt may offer cost-effective alternatives for improving root initiation and overall propagation success. Therefore, the present study was undertaken to standardize suitable growing media for air layering of lemon, with the aim of enhancing rooting efficiency and providing evidence-based recommendations to support sustainable nursery production in the region.

MATERIALS AND METHODS

An experimental study with the title "Evaluation and standardization of growing media for adventitious root formation in lemon (*Citrus Limon* L.) through air layering technique" was conducted at the well-established orchard at the Faculty of Agriculture Gomal University, D.I Khan. This research was carried out on the lemon trees of same age and size. Trial was conducted in Randomized Complete Block Design with three replications. Branches of about 2-3 cm in diameter were selected to perform the air layering. The detail of treatments are: Cinnamon + Silt; Aloe vera + Silt; Saw dust + Silt; Silt; FYM + Silt and leaf mold.

Data were recorded on these parameters for the experiment. Root diameter (mm), number of root, number of root bud, maximum length of root (cm), dry weight (g), fresh weight (g), number of branches and rooting percentage.

Statistical analysis

Analysis of Variance technique was applied to analysis the data collected during the studies. Statistix 8.1 was used to analyze the data. Means were compared using LSD technique at 5% level of significance.

RESULTS

Root Diameter (mm)

Root diameter as affected by various growing media is presented in Table-1. Diameter of roots was significantly affected by using several types of soil media used for air layering in lemon. Results showed that a combination of FYM and silt significantly improved the root diameter and resulted in 1.20 mm of root diameter. Root diameter measured for the media cinnamon + silt was at par with above mentioned treatment and produced roots having diameter of 1.13 mm. Similarly, combination of silt+ Aloe vera resulted in 0.94 mm of root diameter. Leaf mould and silt alone produced roots with statistically similar diameter with 0.83 mm and 0.75 mm of diameter respectively. Saw dust+ silt produced minimum diameter of 0.73 mm.

Number of roots plant^{-1}

Data collected for number of root plant^{-1} is presented in Table-1. It was found out that diverse growing media significantly influenced the roots produced. Maximum number of roots 59.11 was noted when FYM and silt were used as medium in ratio of 50:50 followed by leaf mould and sawdust+silt for number of root were found statistically similar. Growing medium prepared by mixing the cinnamon and silt produced 35.00 roots plant^{-1}. Using silt alone (T3) and mixing with Aloe Vera (T6) resulted in least number of root with 28.78 and 24.89 roots plant^{-1} respectively.

Table 1: **Effect of different growing media on the root diameter, number of roots plant^{-1}, number of root buds and length of root for air layering in lemon.**

Media	Root diameter (mm)	Number of root per plant	Number of root buds	Length of root (cm)
Leaf mould	0.83 cd	51.33 ab	3.00Ns	4.25 a
Saw dust+silt	0.73 d	50.00 ab	3.00	3.32 ab
Silt	0.75 cd	28.78 c	0.67	3.04 ab
Cinnamon+silt	1.13 ab	35.00 bc	3.22	3.39 ab
FYM+silt	1.20 a	59.11 a	3.33	4.13 a
Aloe Vera+silt	0.94 bc	24.89 c	2.77	2.77 b
LSD at 0.05	0.19	20.86	1.37	1.29

Mean having same letter (s) do not differ significantly at 5% level of significance

Number of root bud

Although some growing media showed an increase in the number of buds formed on roots,

the data presented in Table 1 indicated that the differences among treatments were statistically non-significant. The highest number of buds (3.33 buds plant^{-1}) was recorded in the treatment consisting of FYM mixed with silt. Cinnamon + silt resulted in 3.22 buds plant^{-1}, while the treatments consisting of leaf mold and sawdust mixed with silt produced 3.00 buds plant^{-1}.

Length of root plant^{-1}

Using various growing media significantly affected the root length in air layers. Data presented in table1 showed that leaf mould T1 and FYM+ Silt T5 resulted in longest roots producing 4.25cm and 4.13cm root length, respectively other treatment like sawdust + silt T2, Silt T3 Cinnamon +Silt T4 produced statistically similar root length having 3.32cm, 3.04cm and 3.39cm of root length, statistically. The other medium used in T6 Aloe Vera + silt resulted in root length of 2.77cm.

All roots produced in each air layer with different growing media were removed and cleaned. Weight of roots was obtained and presented in Table 1. Combination of FYM and silt resulted in the highest fresh weight of 1.99g which was statistically at par with fresh weight of 1.74g produced by leaf mould. Saw dust + silt also performed better and helped improve fresh weight by having 1.39g of weight. Silt alone, Cinnamon + silt and Aloe Vera + silt showed poor results in term of fresh weight of roots producing 0.65g, 0.75g and 0.59g of fresh root weight, respectively.

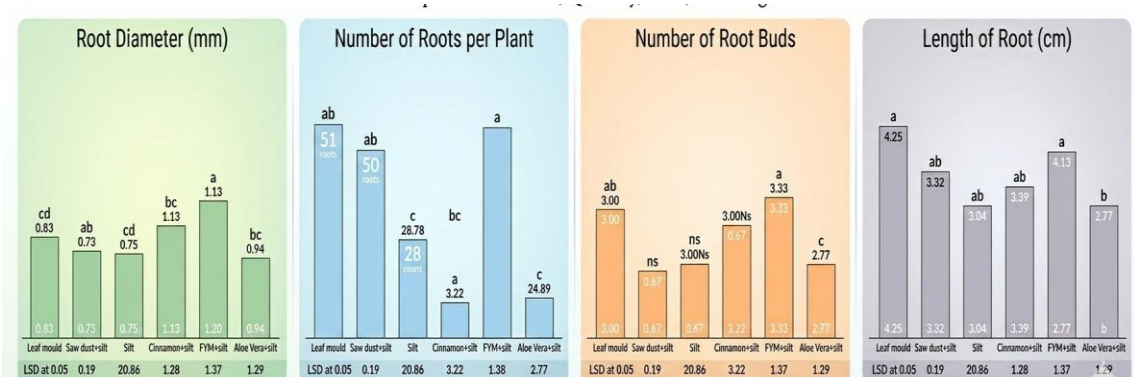


Figure-1: Effect of growing media on the root diameter, number of roots plant^{-1}, number of root buds and length of root for air layering in lemon.

Root dry weight plant^{-1}

Fresh roots obtained were dried out to observed the dry mass/matter present in the roots. Data

collected are presented in Table-2. Similar trend was observed as found for fresh weight of roots. Maximum dry weight of 0.65g and 0.63g was recorded for the leaf mould and F.Y.M + Silt, respectively. Both of the treatments were similar statistically. Minimum dry weight was recorded for the layers grow with cinnamon + silt and Aloe vera + silt having 0.25g and 0.22g grow media of saw dust + silt resulted in 0.53g of root dry weight while silt alone resulted in 0.32g of root dry weight.

Table 2: Shows the effect of different media on the root fresh weight (g), root dry weight (g) and number of branches of air layering in lemon.

Media	Root fresh weight (g)	Root dry weight (g)	Number of branches
Leaf mould	1.74 a	0.65 a	5.22 a
Saw dust + silt	1.39 ab	0.53 ab	4.89 a
Silt	0.65 b	0.32 bc	4.67 a
Cinnamon + silt	0.75 b	0.25 c	4.44 a
FYM + silt	1.99 a	0.63 a	5.67 a
Aloe Vera + silt	0.59 b	0.22 c	5.11 a
LSD at 0.05	0.87	0.27	1.43

Mean followed by similar letter (s) do not differ significantly at 5% level of significance

Number of branches plant^{-1}

Effect of growing media on a number of branches above the air layer was also observed. The data are presented in Table-2. However different growing media could not have any effect on branches above the layers, Maximum number of branches (5.22) counted in layers grown with leaf mould and with FYM + silt (5.67 branches per layer). Minimum branches (4.44) were counted when cinnamon + silt was used as a growing medium.

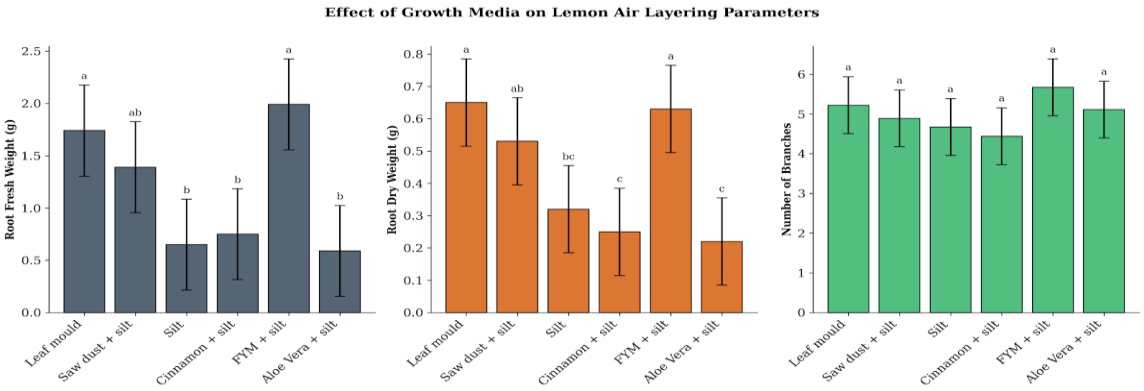


Figure-2: Shows the effect of different media on the root fresh weight (g), root dry weight (g) and number of branches of air layering in lemon.

Correlation between Moisture Content and Root Characteristics

Linear correlation coefficient between moisture retaining capacity and other parameters was calculated and is summarized in Table-3. Moisture content retained by the growing media was found positively correlated with number of roots layer^{-1}, root dry weight, root fresh weight and root length. That means that increasing rate of moisture content in the growing media or high capacity of growing media for retaining moisture results in the improved root growth in the layers.

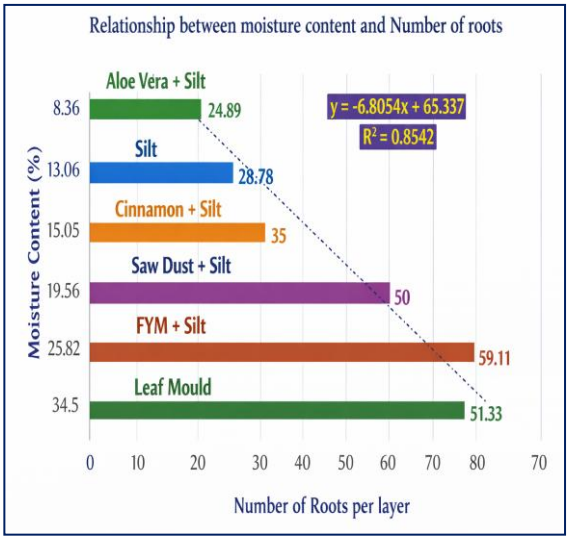
Table 3: Correlation coefficients between moisture content in growing media and root characteristics.

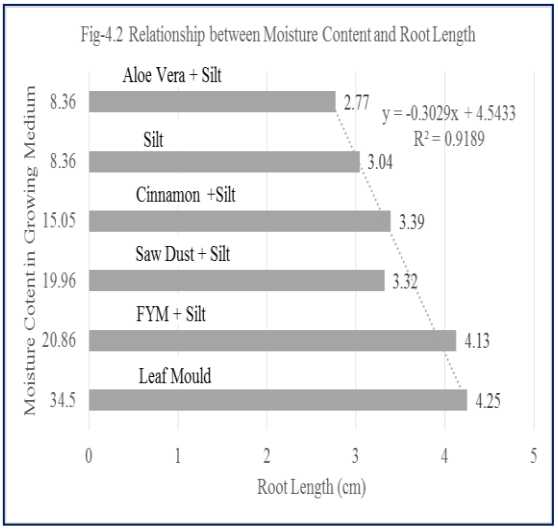
	Number of Roots	Root Dry weight	Root Fresh Weight	Root Length
Moisture	0.784*	0.8475**	0.8127*	0.8855**
Number of roots		0.9405**	0.9768**	0.8706**
Root Dry Weight			0.9679**	0.8698**
Root Fresh Weight				0.9038**

Relationship between moisture content and number of roots

As shown in Fig-3 it was observed that a positive and statistically significant correlation (Table-3) was found between the moisture content and number of roots per layer which means that increasing amount of moisture content for longer period of time increase the number of roots in the layer. Maximum number of roots was counted for layers performed using FYM + Silt and Leaf Mould that resulted in 59.11 and 51.33 roots per layer, respectively. Similarly, the medium having low moisture retaining capacity i.e. 8.36 (Silt and Aloe Vera +Silt) resulted in 28.78 and 24.89 roots per layer.

Figure-3 **Figure-4**





Relationship between moisture content and root length (cm)

Relationship between moisture content and root length is presented in Fig-4 and its correlation coefficient is summarized in Table-3. A highly significant positive correlation was recorded between the moisture retaining capacity of the growing media and root length inside the layers. Root length increased with gradual increase in the moisture retaining capacity of growing media. The longest roots of 4.25 cm in length

were measured for layers performed using Leaf Mould that had moisture retaining capacity of 34.5% which was closely followed by 4.13 cm of root length measured for the roots of layers performed with a mixture of FYM + Silt. Minimum root length of 2.77 cm 3.04 cm was measured for the media having Aloe Vera + Silt and Silt only that retained the least moisture content of 8.36%.

Relationship calculated between moisture content and root dry weight

Relationship calculated between moisture content and root dry weight is presented in Fig-5. positive and significant correlation was noted between moisture content and root dry weight (Table-3). It means that growing medium that had higher water retaining

capacity resulted in higher root dry weight. The figure shows that Leaf Mould and FYM + Silt having with maximum moisture retaining capacity (34.50% and 20.86%%) produced maximum root dry weight of 0.65 g and 0.63 g, respectively. Growing media of Aloe Vera + Silt and Silt only with minimum moisture retaining capacity of 8.36% resulted in 0.22 g and 0.32 g of root dry weight.

Relationship between moisture content and root fresh weight

Relationship between moisture content retained by the growing media and root fresh weight is presented in Fig-6. As mentioned in Table-3. It was observed statistically significant positive correlation was recorded between moisture content and root fresh weight. Higher retaining capacity of Leaf Mould (34.50%) and FYM + Silt (20.86%) resulted in higher root fresh weight i.e. 1.74 g and 1.99 g, respectively. Least weight of fresh roots (0.59 g) per layer was recorded for the growing media having mixture of Aloe Vera + Silt that had least moisture retaining capacity of 8.36%.

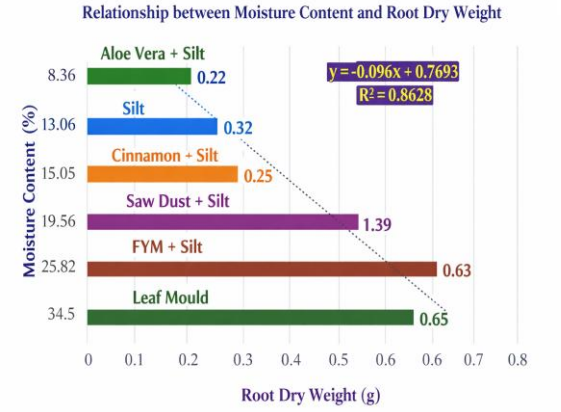


Figure-5

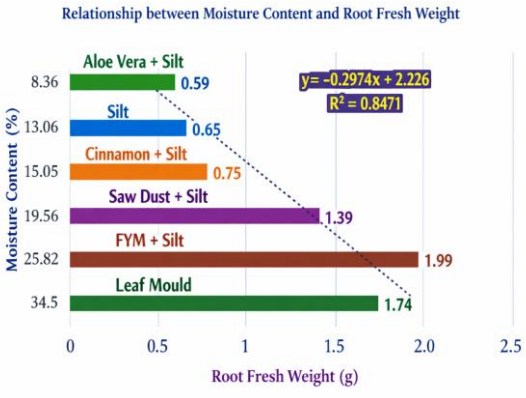


Figure-6

Relationship between number of roots and root fresh weight

Highly significant relationship between number of roots and root fresh weight was found (Table-3). Fig-7 shows the relationship between the two factors. It was noted that rising number of roots resulted in increased root fresh weight. Minimum fresh root weight of 0.59 g was recorded for growing medium having Aloe Vera + Silt, that had minimum number of roots. Growing media that had higher number of roots resulted in higher root fresh weight.

Growing media with higher number of roots like FYM + Silt and Leaf Mould resulted in higher root fresh weight i.e. 1.99 g and 1.74 g, respectively.

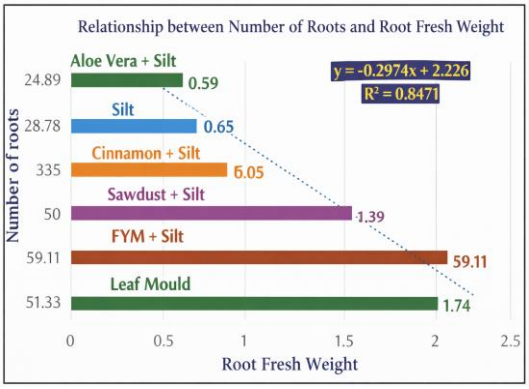


Figure-7

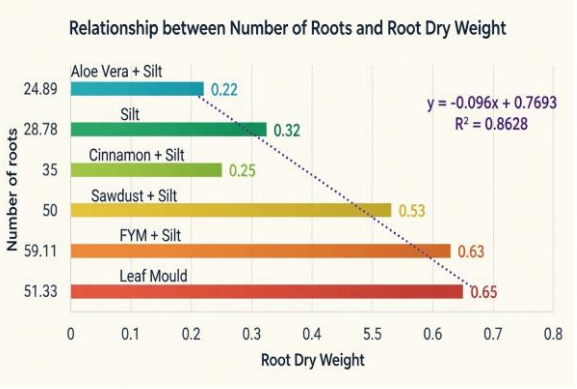


Figure-8

Relationship between Number of roots and root dry weight

Number of roots per layer and root dry weight were had highly significant positive correlation (Table-3). Fig-8 shows the relationship between number of roots and root dry weight. Both the growing media i.e. FYM + Silt and Leaf Mould, having maximum number of roots resulted in 0.63 g and 0.65 g of root dry weight, respectively. On the other hand, Alow Vera + Silt and Silt only having least number of roots per layer (24.89 and 28.78) resulted in least root dry weight of 0.22 g and 0.32 g, respectively.

Relationship between root length and root fresh weight

Highly significant correlation coefficient was observed between root length and root fresh weight (Table-3). Relationship between root length and root fresh weight is presented in Fig-9 Trend line shown in the Fig clearly reveals the trend of increasing fresh root weight as a result of increased root length by various growing media. Highest root fresh weight of 1.74 g and 1.99 g was recorded for growing media having highest root length of 4.13 cm and 4.25 cm by using FYM + Silt and Leaf Mould, respectively. Similarly, the lowest root fresh weight of 0.59 g was recorded against the least root length (2.77 cm) by growing medium of Aloe Vera + Silt.

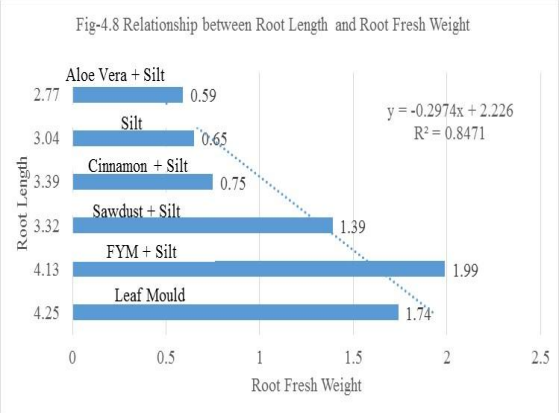


Figure-9

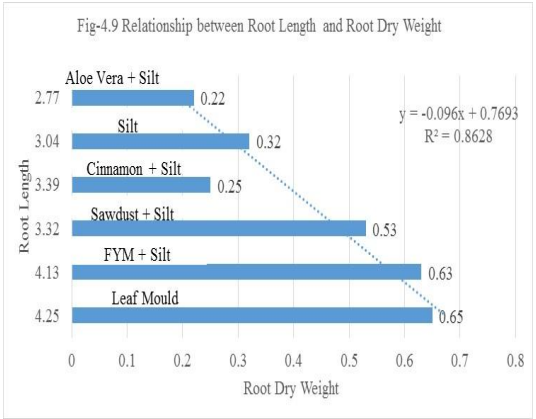


Figure-10

Relationship between root length and root dry weight

As found for relationship between root length and root fresh weight correlation coefficient for root length and root dry weight was found positive and highly significant as well (Fig-10). The highest root dry weight of 0.65 g and 0.63 g was recorded for the highest root length of 4.25 cm and 4.13 cm resulted by using Leaf Mould and FYM + Silt, respectively. Alow Vera + Silt and Silt alone produced roots with minimum length and hence resulted in the least root dry weight. Using Aloe Vera + Silt resulted in 0.22 g of root dry weight while Silt alone produced roots having 0.32 cm length.

Relationship between root fresh weight and root dry weight

Correlation coefficient analyzed for root length and root dry weight showed highly significant statistically having $R^2 = 0.8628$ (Fig-11). Relationship shown in the following figure reveals that the highest root dry weight of 0.65 g and 0.63 g was obtained by using Leaf Mould and FYM + Silt that had high moisture content and resulted in maximum fresh root weight of 1.74 g and 1.99 g, respectively. Aloe Vera + Silt and Silt only that showed minimum moisture retaining capacity produced least root fresh weight and hence resulted least root dry weight of 0.22 g and 0.32 g, respectively.

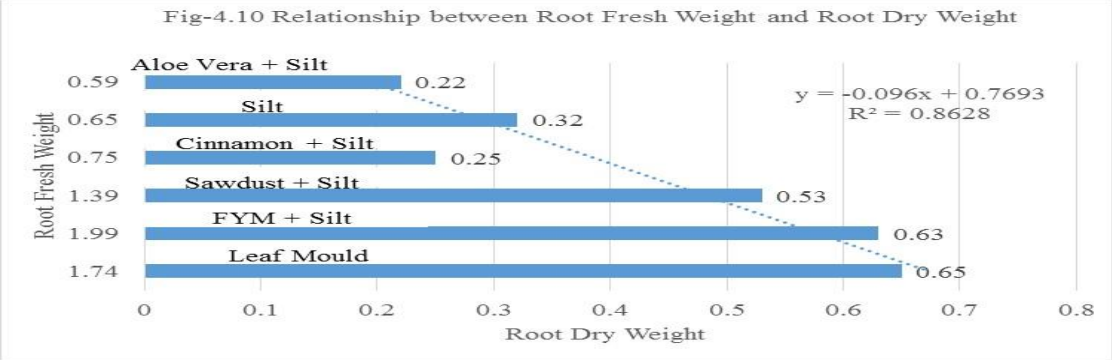


Figure-11

DISCUSSION

The results of the present investigation revealed that different growing media significantly influenced rooting characteristics and survival of lemon air-layers. Among the tested media, the combination of FYM + silt exhibited the best performance by producing the maximum number of roots, highest root diameter and maximum number of buds. The superior performance of FYM-based media may be attributed to improved aeration, water-holding capacity and nutrient availability, which collectively promote root initiation and development during air layering. Organic substrates such as FYM are known to enhance the physical and biological properties of rooting media, thereby improving root growth and establishment of propagules (Singh et al., 2016; Kumar et al., 2024).

Leaf mold was found to be the second most effective medium in producing a higher number of roots per layer. Furthermore, both FYM + silt and leaf mold showed statistically similar results with respect to root length and fresh and dry root weight**,** indicating that organic matter-rich substrates create favorable conditions for root initiation and development. Similar observations have been reported in citrus propagation studies where organic growing media improved rooting percentage, root length, and root biomass due to improved moisture retention and nutrient supply (Rymbai et al., 2012; Singh and Pathak, 2012; Ali et al., 2016).

The combinations of sawdust + silt and cinnamon + silt formed the next group showing moderate performance for the parameters studied. Sawdust can improve soil structure and aeration, which may facilitate root formation; however, its low nutrient content may limit root growth compared to organic manure-based media. Similar findings were reported in

studies on air layering where media containing organic amendments produced better rooting characteristics than inert substrates alone (Kumar et al., 2024; Dubey and Mishra, 2024).

In contrast, the treatment consisting of aloe vera paste combined with silt showed poor performance and produced results almost similar to silt alone**.** Although aloe vera contains certain growth-promoting compounds and natural hormones, the results of the present study indicate that its effect was insufficient to significantly enhance rooting parameters under the prevailing experimental conditions. Similar observations were noted in studies evaluating plant-based extracts for vegetative propagation where natural extracts showed limited effectiveness compared to nutrient-rich rooting substrates or synthetic growth regulators.

Several researchers have emphasized the importance of selecting suitable rooting media and growth-promoting substances to improve root development during air layering of citrus species. Studies conducted on lemon air-layers reported that application of growth regulators and appropriate rooting media significantly improved rooting percentage, root length and survival rate of air-layers (Rathour et al., 2021). Similarly, recent research on citrus propagation reported that combinations of soil with organic substrates such as cocopeat or vermicompost significantly enhanced root formation, root thickness and survival percentage of air-layers (Kumar et al., 2024).

Furthermore, recent studies on citrus and other fruit crops have shown that improving root growth through appropriate substrates or beneficial microorganisms can significantly enhance plant vigor, photosynthetic activity and overall plant development (Abd-El-Moneim et al., 2024). These findings support the results of the present investigation, where organic substrates such as FYM and leaf mold provided better rooting responses compared with mineral substrates alone.

Overall, the findings of this study are in agreement with previous research indicating that organic media improve the physical, chemical, and biological properties of the rooting environment, thereby enhancing root initiation and development in air-layers. Consequently, the combination of FYM + silt can be recommended as the most suitable growing medium for successful propagation of lemon through air layering.

CONCLUSION

The findings of the present study indicate that FYM + silt proved to be the most effective rooting medium for air layering of lemon under the agro-climatic conditions of D.I. Khan. This medium produced the highest number of roots, greatest root diameter, and maximum number of buds compared with the other treatments. Among the remaining media, leaf mould ranked second**,** showing comparatively good performance for the same parameters, although it was slightly inferior to the FYM + silt combination.

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