

Effect of growing media and irrigation frequencies on early vegetative growth of *Piper longum* L

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Abstract

Systematic cultivation with well-developed agronomic practices for selected medicinal herbs involves conservation of wild populations from over harvesting, continuous supply and sustainable utilization. The present research was conducted to find-out the most suitable growing medium and irrigation frequency for commercial cultivation of *Piper longum* L. For this purpose, six different growing media along with three different irrigation frequencies were used. The experiment was arranged factorially with Completely Randomized Design in triplicates inside a protected plant house under controlled environmental conditions.

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Destructive measurements were taken at 12 weeks after planting. In addition, all growing media were analyzed for pH, electrical conductivity, available nitrogen, organic matter, phosphorous, available potassium using three replicates. Data analysis was carried out using analysis of variance with statistical analysis system while Duncan multiple range test was employed for mean separation. Based on the results, interaction effects between growing media and irrigation frequencies were not significant ($P \geq 0.05$) for plant height (cm), number of leaves, number of branches and shoot dry weight (g) of *P. longum* harvested at 12 weeks after establishment. Growing media and irrigation interval were significant on the growth parameters while the highest plant height of 43.3 cm was observed in topsoil: compost -1:1 closely followed by topsoil: sand: compost -1:1:1 (42.2 cm). *P. longum* grown in topsoil: sand: compost -1:1:1 (V/V) had the highest number of branches (7.6). Further, significantly ($P \leq 0.05$) higher number of leaves (43) and shoot dry weight (15.7g) were recorded in topsoil: sand: compost -1:1:1. Irrigation frequencies had no significant ($P > 0.05$) effect on growth parameters of *P. longum* (Figure 2). Shoot dry weights had no significant difference with the changing irrigation frequency. Based on the composition analysis and growth parameters, top soil: sand: compost (1:1:1) growing media and 9 days irrigation frequency appeared to be the most promising combination for early vegetative growth of *P. longum*.

Keywords: Composition analysis; Early vegetative growth; Growing media; Irrigation frequency; *Piper longum* L

Introduction

Medicinal plants are natural remedies for wide variety of diseases and disabilities having an exorbitant safety profile. There is a huge demand for medicinal plants in global and local market as they are natural, safe and effective remedies for various kinds of diseases and disorders (Khushbu *et al.*, 2011). In Sri Lanka, about 65% of the rural people use ayurvedic medicines as their primary health care (Perera, 2012). In order to cater the steadily increasing demand, most of the medicinal plants are collected from wild resources. Therefore, medicinal plant collectors have imposed great threat on wild resources of medicinal plants due to unsustainable harvesting (Mahindapla, 2004). In addition, Sri Lankan medicinal plant industry is in a risk due to irregular supply of medicinal plants (Perera, 2012), lack of appropriate

cultivation and processing guidelines as well as lack of continuous supply of quality materials (Kankanamalage *et al.*, 2013). Sri Lanka owned a variety of soil and climatic conditions and they are favorably influence on growth of many medicinal plants (Kuruppu *et al.* 2019).

Although, a rich genetic base exists for medicinal plants, their propagation and cultivation techniques of very few out of them have been examined (Russell-Smith *et al.*, 2006). Thus, extensive efforts are required to recognize their different means of propagation (Walisundara and Watawana, 2014). *P. longum* (family *Piperaceae*) is an imperative medicinal herb with extensive pharmacological characteristics and broad functions. It is one of the essential components in the Ayurvedic medicine (Khushbu *et al.*, 2011). It is also known as long pepper. Alkaloids, lignins, piperine, and volatile oils are the major chemical constituents of *P. longum* (Lu *et al.*, 2019). Whole plant has the ayurvedic importance (Department of Ayurveda, 2002). Roots contain piperine, pipartine or piperlongumine and mainly dihydro-stigmasterol like important active ingredients (Neelam and Krishnaswamy, 2000). It has been reported wide range of pharmacological properties which includes insecticidal and acaricidal activity, anti-fungal, hepatoprotective, anti-asthmatic, anti-microbial, diabetic, inflammatory, anti-cancer, antioxidant, analgesic and depressive properties (Zaveri *et al.*, 2010).

There are no large-scale organized cultivations of *P. longum* in Sri Lanka. In Sri Lanka *P. longum* is one among the 10 largely imported herbal materials due to its increasing demand for the ayurvedic preparations (Gunathilake *et al.*, 2002). Therefore, it is important to establish systematic commercial cultivations of *P. longum* to ensure continuous supply of raw materials to cater the steadily increasing demand. Current literature lacks information about the correct combinations of irrigation and proper potting media required for the *P. longum*. In addition, it is beneficial to develop medicinal plant cultivations with the use of organic growing substrates. Importantly, medicinal plants cultivated without synthetic chemical fertilizers possess high quality, efficacy and reliability of herbal materials and can extract authentic pure form of secondary metabolites (Kankanamalage *et al.*, 2013). Hence, present study was designed to find-out appropriate organic growing medium along with suitable irrigation frequency for better growth and development of *P. longum* at early vegetative growth.

Materials and methods

The present study was carried out inside a plant house under controlled environmental conditions located at Faculty of Agriculture, University of Ruhuna during December 2020 - March 2021. During the study period, average monthly temperature, RH and the light intensity of the protected plant house were 33°C, 70% and 25000 lux respectively. Six different growing media [T₁ - Topsoil: sand: compost (1:1:1); T₂ - Topsoil: sand: compost (1:1:2); T₃ - Topsoil: compost (1:1), T₄ - Topsoil: compost (1:2); T₅ - Topsoil only and T₆ - Topsoil: sand (1:1)] along with three irrigation frequencies (3, 6 and 9 days) were used as eighteen different treatments. They were factorially arranged in Completely Randomized Design with three replicates [MI₁, MI₂ and MI₃- growing media 1 with 3, 6 and 9 days irrigation frequency intervals; MI₄, MI₅, MI₆- growing media 2 with 3, 6 and 9 days irrigation intervals; MI₇, MI₈, MI₉ - growing media 3 with 3, 6 and 9 days irrigation intervals; MI₁₀, MI₁₁, MI₁₂ -growing media 4 with 3, 6 and 9 days irrigation frequency; MI₁₃, MI₁₄, MI₁₅- growing media 5 with 3, 6 and 9 days irrigation frequency; T₁₆, T₁₇, T₁₈- growing media 6 with 3, 6 and 9 days irrigation interval].

Six weeks old rooted plantlets of *P. longum* were planted in black polythene pots of 10"×10". These pots were filled with six different growing media as mentioned above. At each water application, about 250 mL water was applied to reach the field capacity determined by gravimetric method. Data on plant growth parameters such as plant height (cm), number of leaves, number of branches, shoot dry weight (g), root dry weight (g) and root volume (mL) were collected at 12 weeks after establishment. Physio-chemical parameters of six different growing media including pH, Electrical Conductivity (EC), organic matter content, available phosphorus content (mg/kg), potassium (mg/kg), ammonium nitrogen (mg/kg) and nitrate nitrogen (mg/kg) were collected. Data on pH and EC were determined using the pH and EC meter (HANNA HI 83099). Ammonium nitrogen, nitrate nitrogen and available phosphorous were analysed using UV visible spectrophotometer (Shimadzu UV160). Available potassium was measured using the flame photometer (Sherwood model 360).

Data were subjected to analysis of variance with the help of statistical analysis system (SAS statistical software 9.1 version). Duncan multiple range test was employed to compare treatment means. A Pearson correlation (SPSS software) was carried out to test the relationship/s among chemical composition and early vegetative growth of *P. longum* grown in different growing media.

Results and discussion

Interaction effects between growing media and irrigation frequencies were not significant ($P \geq 0.05$) for plant height (cm), leaves number, branches number and shoot dry weight (g) of *P. longum* harvested at 12 weeks after establishment. Therefore, results of main effects are presented for these parameters. However, significant ($P \leq 0.05$) interactions between growing media and irrigation frequencies were observed for dry weight of roots and volume of roots (mL) at the same stage.

Effect of different growing media on growth and yield of *Piper longum* L

Different growing media differently influenced on plant height (cm), number of leaves, number of branches and shoot dry weight (g) of *P. longum* harvested at 12 weeks after establishment. The highest plant height of 43.3 cm was observed in T₃ (topsoil: compost - 1:1) closely followed by T₁ (topsoil: sand: compost - 1:1:1) (Figure 1A). *P. longum* grown in T₁ (topsoil: sand: compost - 1:1:1) had the highest number of branches (7.6) (Figure 1C). Significantly ($P \leq 0.05$) higher number of leaves (43) and shoot dry weights (15.7g) were recorded in T₁ (topsoil: sand: compost - 1:1:1) (Figure 1B and D). Topsoil: compost (1:2) growing media showed the lowest values for plant height (31.7cm), leaves number (13), branches number (4) and shoot dry weight (4g) (Figure 1).

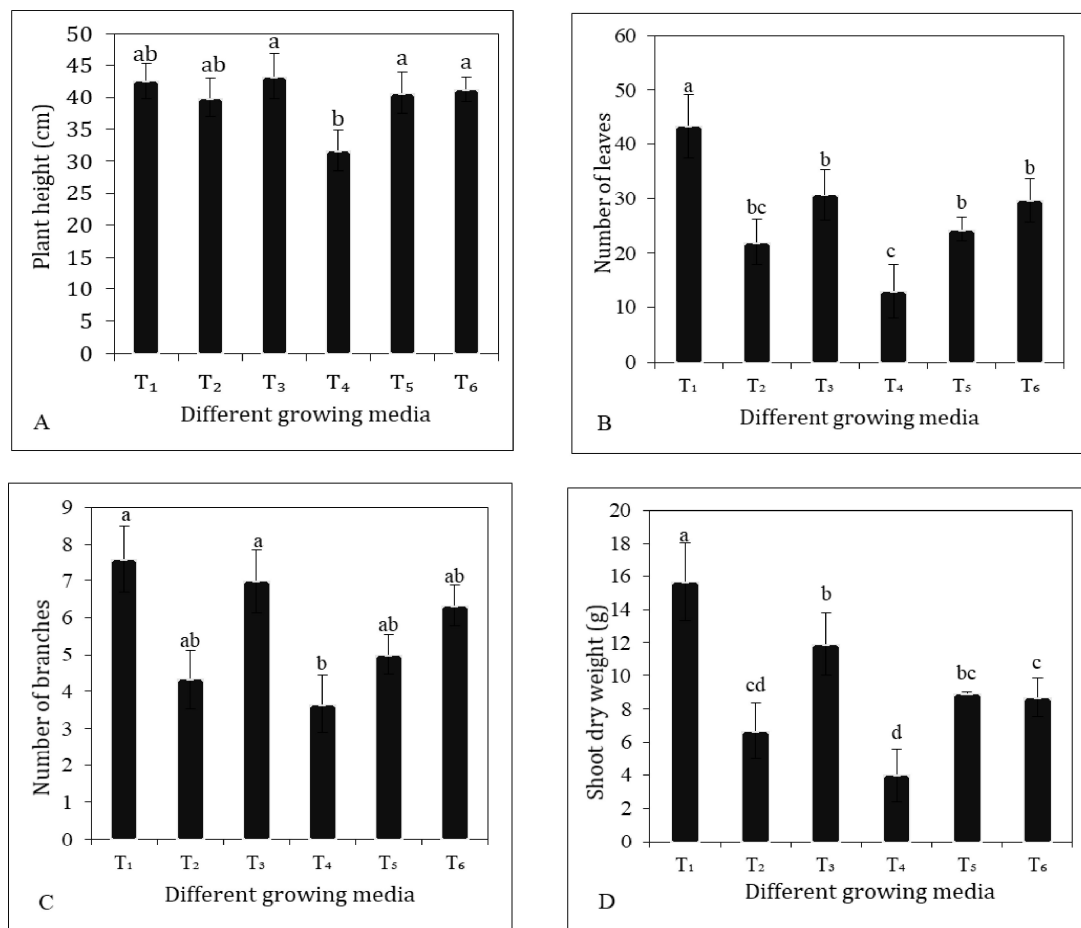


Figure 1: Influence of different growing media on plant height (cm)(A), number of leaves (B), number of branches (C), and shoot dry weight (g)(D) of *P. longum* grown under protected house conditions [T₁ - topsoil: sand: compost - 1:1:1, T₂ - topsoil: sand: compost - 1:1:2, T₃ - topsoil: compost - 1:1, T₄ - topsoil: compost - 1:2, T₅ - topsoil only and T₆ - topsoil: sand - 1:1].

Results of the present study report that topsoil: sand: compost - 1:1:1 and topsoil: compost - 1:1 as the most appropriate growing media for early vegetative growth performances of *P. longum*. Based on the literature, topsoil: sand: compost 1:1:1 was a recommended medium for different crop species including clove bean (Munasinghe and Fernando, 2021). Similarly, Weerasingha (2020) had used growing media consist of topsoil: sand: compost 1:1:1 as the standard growing media for *Capsicum annum*. Moreover, potting mixture containing topsoil: sand: compost: coir dust (1:1:1:1) and top soil: sand: compost: coir dust (1:1:1:1) showed the highest growth performances in betel belongs to same family of *P. longum*. The lowest values for all the above

parameters (Figure 01) were reported in T₄ (topsoil: compost 1:2) although, it consists of higher fraction of compost.

Observed growth retardation in the topsoil: compost 1:2 may be due to unfavorable effects from high amount of compost. The nutrient composition such as nitrate nitrogen, ammonium nitrogen, available phosphorus content, potassium and organic matter content of the compost used in the present study were respectively, 4.8 mg/kg, 0.36 mg/kg, 178.2 mg/kg, 908 mg/kg and 16%. Nitrogen mineralization rate was the main factor determining the growth and development of the plants and it appears that the use of compost at the correct proportion with other optimum nutrient levels can claim for a higher growth and yield (Raviv *et al.*, 2004). Hose *et al.*, (2012) also emphasized that growth characteristics and quality of crop yield were differed according to the components used to prepare compost and their proportions. Top soil: compost 1:2 (T₄) had significantly lower growth rate possibly due to poor drainage caused by the absence of sand and presence of high amount of compost. Growing media compaction was observed as fewer voids with lack of sand in the medium. Topsoil with compost help to retain water and provide nutrients and sand increase drainage and improve aeration. Plant may get optimum amount of moisture, aeration, nutrients and improved drainage as growing media 1 (T₁) contains equal amount of topsoil, sand and compost.

Effect of different irrigation frequencies on growth and development of *Piper longum* L.

Irrigation frequencies had no significant ($P>0.05$) effect on growth parameters of *P. longum* (Figure 2). There was no significant difference ($P>0.05$) in shoot dry weights with the changing irrigation frequency. Rao *et al.*, 2010 found that a significantly higher response in growth, yield and quality of *Piper longum* was with irrigation regime of 0.8 cumulative pan evaporation and emphasized that *P. longum* required comparatively high watering. Joshi *et al.*, 2013 emphasized that *P. longum* need to irrigate at least once a week if it grown as a sole crop. Irrigation and water content of the growing media has an influence on plant growth and development as well as runoff process. A potted plant must be able to equilibrate the atmospheric demand for water with the amount it absorbs from the growing media. Plants can withstand only for the range of readily available water in the growing media up to the refill point to the

container capacity (Wang 2013). Nowadays, irrigation frequency and growing media water content has an increasing concern as it transports chemicals and potentially can harm surface or groundwater (Smith, 2007).

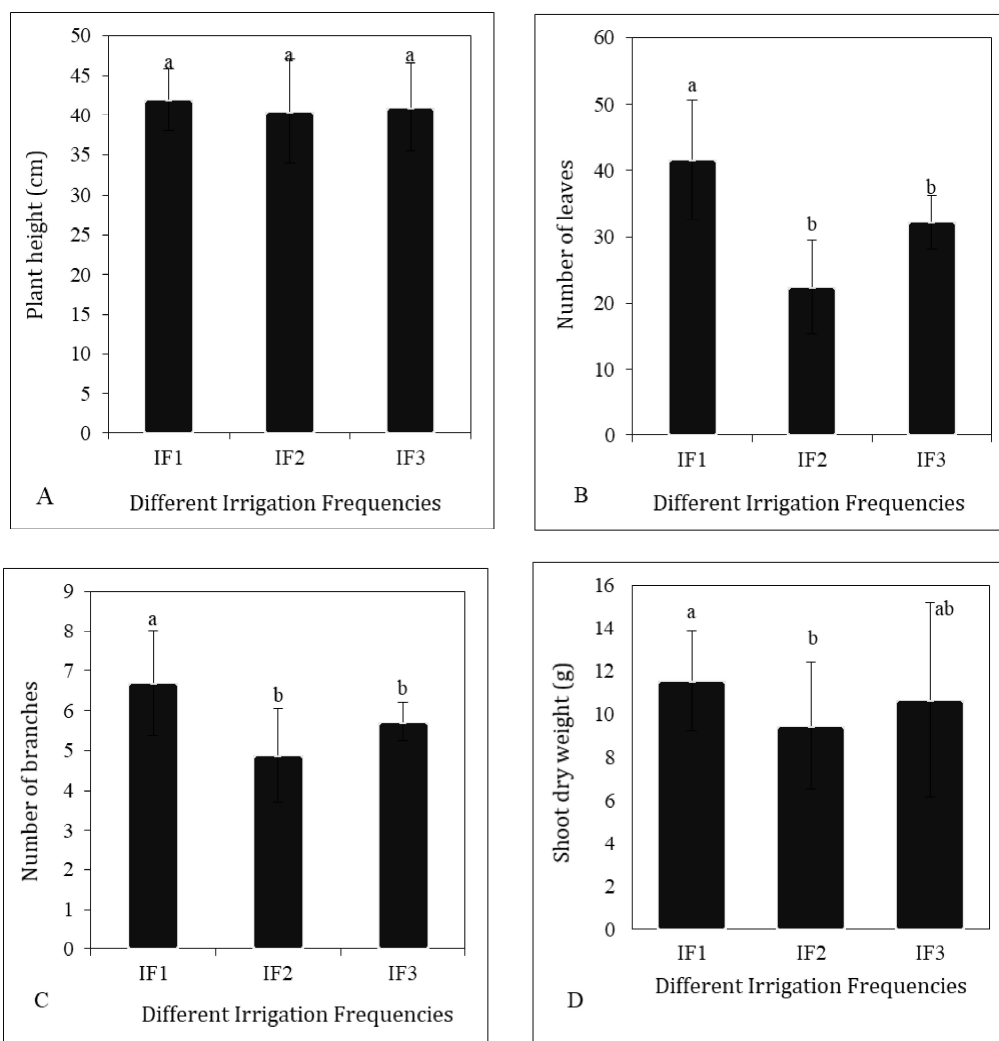


Figure 2: Influence of different irrigation frequencies on plant height (cm)(A), number of leaves (B), number of branches (C), and shoot dry weight (g) (D) of *P. longum* grown under protected house conditions [IF1, IF2 and IF3 - 3, 6 and 9 days irrigation frequencies respectively]

Effect of growing media and irrigation frequencies on root parameters

Root parameters had an interaction effect with growing media and irrigation frequency. Significantly ($p < 0.05$) highest root dry weight (4.6g) and the highest root volume (22.6 ml) were recorded in top soil: sand: compost 1:1:1 when irrigated at 9 days interval (MI₃) (Table 1).

Table 1: Interaction effect of growing media and irrigation frequency on root dry weight (g) and root volume (mL) of *P. longum* harvested at 12th weeks after establishment

Treatment	Root dry weight (g)	Root volume (mL)	Treatment	Root dry weight (g)	Root volume (mL)
T ₁	3.3 ^b	13.3 ^{bc}	T ₁₀	1.2 ^{def}	7 ^{cdef}
T ₂	2.8 ^{bc}	19.6 ^a	T ₁₁	0.5 ^f	2.6 ^f
T ₃	4.6 ^a	22.6 ^a	T ₁₂	0.8 ^{ef}	5.3 ^{ef}
T ₄	1.4 ^{def}	5.5 ^{ef}	T ₁₃	1.8 ^{cde}	12.3 ^{bcd}
T ₅	1.2 ^{def}	5 ^f	T ₁₄	2.2 ^{cd}	11.6 ^{bcde}
T ₆	1.9 ^{cd}	8.3 ^{bcdef}	T ₁₅	2 ^{cd}	13.6 ^b
T ₇	2.1 ^{cd}	7.6 ^{bcdef}	T ₁₆	1.3 ^{def}	6 ^{def}
T ₈	2.8 ^{bc}	12.6 ^{bc}	T ₁₇	1.6 ^{de}	8.3 ^{bcdef}
T ₉	1.2 ^{def}	5.6 ^{ef}	T ₁₈	1.8 ^{cde}	8 ^{bcdef}

Note: Means with similar letters in a column are not significantly different at $p < 0.05$

Composition analysis of different growing media

Composition analysis results indicated that (Table 2), different growth media showed significant differences ($P < 0.05$) for pH, electrical conductivity (dS/m), nitrate nitrogen (mg/kg), ammonium nitrogen (mg/kg), available phosphorus (mg/kg), available potassium (mg/kg) and organic matter content (%). Significantly higher pH was observed in T₁, T₂, T₃ and T₄. Potting media with topsoil: compost 1:2 (T₄) showed the highest nitrate nitrogen content. Ammonium nitrogen and phosphorus were greater in T₃ (Table 2). Various chemical and biological parameters such as pH, EC, total organic carbon content (TOC), nitrogen content, carbon nitrogen (C:N) ratio, humic like substances,

enzymatic activities, ATP content considered as indicator of compost stability (Mondini, 2004). Compost phytotoxicity is an important criterion as the application of immature compost will have higher amount of free ammonia, organic acids and other water-soluble compounds, which can cause plant growth retardation and root development (US Composting Council, 2002). These reasons may be associated with the growth retardation observed in media with high proportions of compost.

Table 2: Composition analysis of different growing media

Treatments	pH (w/v)	EC (dS/m)	Nitrate Nitrogen (mg/kg)	Ammonium Nitrogen (mg/kg)	Phosphorus (mg/kg)	Potassium (mg/kg)	Organic Matter (%)
T ₁	7.8 ^{ab}	1.21 ^d	1.6 ^e	0.76 ^c	126.5 ^d	193.0 ^e	4.6 ^f
T ₂	7.9 ^a	2.03 ^c	2.7 ^d	0.79 ^c	139.6 ^c	408.0 ^c	6.4 ^e
T ₃	7.7 ^{ab}	2.80 ^b	4.0 ^a	1.37 ^a	182.7 ^b	568.9 ^a	15.1 ^a
T ₄	7.7 ^{ab}	3.45 ^a	5.1 ^a	1.24 ^b	156.5 ^a	815.0 ^a	13.4 ^b
T ₅	6.6 ^c	0.11 ^e	3.3 ^c	0.76 ^c	13.4 ^e	3.6 ^d	7.9 ^c
T ₆	7.4 ^b	0.04 ^e	3.2 ^f	0.42 ^d	10.7 ^f	0.3 ^f	2.3 ^d
CV%	3.08	8.73	2.33	0.3	1.53	0.2	0.5
p	0.006	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

[T₁ - Topsoil: sand: compost 1:1:1, T₂ - Topsoil: sand: compost 1:1:2, T₃ - Top soil: compost 1:1, T₄ - Topsoil: compost 1:2, T₅ - topsoil, T₆ - Topsoil: sand 1:1]

Note: means with similar letters in a column are not significantly different at $p < 0.05$

Correlation coefficients were used to estimate the strength and direction of the relationship between the plant growth parameters and physio-chemical characters of different growing media (Table 3). Direction and the strength of association was nutrient dependent. For example, Nitrate N in growing media and K were negatively correlated with plant growth parameters. Further,

negative correlation of Nitrate N and K showed significant ($P \leq 0.01$) influence on shoot dry weight (g), number of branches and number of leaves (Table 3). Thus, it is important to select a growing medium with relatively low levels of Nitrate N and K in order to achieve the higher growth of *P. longum*. Increased application of P and organic matter had the positive relationship with improved growth. The availability of P in the potting media has a direct impact on nitrate metabolism (Linkohr *et al.*, 2002). One of the main reasons of nitrate accumulation in most plants is an uneven and greater supply of nitrogen and P (Perring *et al.*, 2018). Nitrate N is a significant form of nitrogen absorbed by plants, and Ammonium-N fertilizer can be converted to Nitrate-N relatively quickly when applied to aerobic soil, increasing the soil Nitrate-N content (Schroeder and Janos, 2005).

Table 3: Relationship between growth parameters of *P.longum* and physic-chemical characteristics of different growing media

	pH	EC	OM	Am N	Nitrate N	P	K
Root dry weight	0.24	0.09	0.16	-0.12	-0.12	0.16	-0.12
Root volume	0.27	0.11	0.14	-0.11	-0.02	0.14	-0.07
Shoot dry weight	0.4*	0.09	0.02	-0.03	-0.54**	0.02	-0.4*
Number of Branches	0.27	0.04	0.01	-0.07	-0.52**	0.01	- 0.48**
Number of Leaves	0.34	0.13	0.11	0.07	-0.57**	0.1	- 0.48**
Plant height	0.03	0.01	0.11	0.01	-0.24	0.11	-0.19

(Am N - Ammonium nitrogen, P - Available Phosphorus, K - Available Potassium)

** Significant level at $P \leq 0.01$ and * Significant level at $P \leq 0.05$

Conclusion

Topsoil: sand: compost (1:1:1) media and 9 days irrigation frequency appeared to be the most favorable growing media and irrigation frequency for *P. longum* early vegetative growth. Further studies are essential to evaluate influence of

growing media and irrigation frequencies on spike yield of *P. longum*. In addition, optimum compost level and toxic conditions with excess application of compost for *P. longum* warrant further investigations.

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