

## STUDIES ON PROPAGATION, OPTIMAL GROWTH CONDITIONS AND FRUIT FORMATION OF THE MEDICINAL PLANT *PIPER LONGUM* L.

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### ABSTRACT

This study was conducted to develop simple and rapid propagation techniques, and to understand the variation in pod formation and optimum conditions for growth of *Piper longum*. The performance of vegetative stem cuttings comprising the two uppermost nodes and the cuttings obtained from vertically growing reproductive parts of the plant showed that they were the appropriate propagule types for propagation. *Piper longum* plants grown under 50% shade (maximum instantaneous light intensity  $850 \mu\text{mol m}^{-2}\text{s}^{-1}$ ) performed well compared to plants grown under 25 and 75% shade respectively. Planting medium comprising sand, topsoil and farmyard manure mixed in the ratio of 1:1:1 was found to be the best substratum for the growth of *P. longum* plants. Plants raised from vertically grown branches produced fruit earlier compared to those from horizontally grown branches. However, nearly 50% of fruits were shed from the mother plant about 22 days after their emergence.

### Key words

*Piper longum*, Vegetative propagation, Light conditions, Potting mixtures

### INTRODUCTION

*Piper longum* L. belongs to the family Piperaceae. It is a dioecious, perennial herb with a thick, erect and branched rootstock and an ascending or prostrate stem (Huber, 1987). Stems are numerous and about 60-90 cm long. Flowers are unisexual, sessile in axils of fleshy bracts supported by two lateral bracts. Fruits are very small and sunk in the solid fleshy spike which is about 2.5-3.7 cm long (Jayaweera, 1982; Samuel *et al.*, 1983). Under natural growing conditions it propagates vegetatively by horizontally grown stems (vegetative branches) having rootlets at each node. Fruits are formed from vertically arising stems (reproductive branches), which have no rootlets.

*Piper longum* occurs in the warmer parts of India, Sri Lanka, Malay Peninsular, Timor and Philippine Islands. It is considered as a native species of northeast India (Jayaweera, 1982; Pandey, 2000). *Piper longum* grows naturally in a hot climate at 450-600 m altitude at a temperature range between 24-30<sup>o</sup> C and

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an annual rainfall over 250 cm per year. It grows in a sandy well-drained loam soil, rich in humus (Pandey, 2000).

Among species known from earliest times, *Piper longum* (long pepper) has been used for medicinal purposes. Ayurvedic publications including Sanskrit texts, have discussed in great detail the use of *P. longum* as flavoring agents in foods (Jayasinha, 1999).

Fruits, roots and stems of *P. longum* are used for the preparation of several drug in the Ayurvedic and Unani system of medicine. The alkaloid "Piparine" is the most active component in the plant, while many other constituents such as Sylvatin, Sesamin, Diaenolemin are present in different parts of the plant (Arambewela and Silva, 1999). Drugs prepared from spikes of *P. longum* are used for diseases of the respiratory tract, cough, bronchitis, asthma, muscular pains and inflammation (Glasby, 1982; Jayasinha, 1999).

Though this plant has been used in medicine for a very long time in Sri Lanka, interestingly no male plants have been found in the island (Samuel *et al.*, 1983). According to Samuel (1982) it is grown as a medicinal plant to a limited extent in village homesteads in Sri Lanka.

The statistics on the national demand for medicinal plants published by the International Union for the Conservation of Nature ranks *P. longum* as the tenth largest imported herbal medicine from India. Furthermore, it indicates that almost the entire demand of *P. longum* for the local market is supplied by imports from India or by the open market, but not by local cultivation. Each year more than 58,000 kg of immature dried spikes valued at Sri Lanka Rs. 270,000 are imported from India to meet the national demand of *P. longum* (Abeywardana and Hettiarachchi, 2001).

With the present demand for herbal remedies, the requirement for *P. longum*, a major constituent of many herbal medicines, is also increasing rapidly. However, cultivation of *P. longum* on a commercial scale in Sri Lanka has received little attention although the Ministry of Indigenous Medicine has categorized it as one of the twenty most used medicinal species in Sri Lanka.

The aims of the present study were to develop a simple vegetative propagation technique for *P. longum* using different sources of cuttings, develop an understanding of the light conditions required for optimum growth, evaluate the best potting media for the growth of the species under nursery conditions and to study the variation in timing and success of fruit formation using different types of cuttings (propagules).

## MATERIALS AND METHODS

The study was conducted in shade houses constructed at the University of Peradeniya, Sri Lanka during the period of February 2000 - August 2001.

### Performance of different types of cuttings

Vegetative propagation using different types of stem cuttings was examined. Five types of stem cuttings (propagules) were obtained from the stock plants raised in the nursery. They were cuttings of the young stem comprising the uppermost (i) first node, (ii) two nodes and (iii) three nodes, and (iv) the second and third nodes, and (v) the second, third and fourth nodes from the terminal ends of the stem. These cuttings represented different stages of maturity.

The stem cuttings were planted in regifoam boxes of size 1 m x 1 m containing sand and coir dust, mixed in equal proportion. Sixty cuttings from each type were randomly placed at an interplant distance of 10 cm. Plants were maintained for three months in a shade house under 50% shade where the maximum instantaneous light intensity was  $850 \mu\text{mol m}^{-2}\text{s}^{-1}$ . The cuttings were watered once every two days to maintain adequate moisture. The percentage survival and appearance of roots were recorded three months after commencement of the study. No artificial rooting hormones were used in this study.

The transformed data were used for analyses where means of percentages were used to plot the graphs.

### Optimal light conditions for growth

The optimal light condition for the growth of *P. longum* was examined by growing plants raised from cuttings taken from vertically growing branches under three shade levels. The shade levels tested, were improvised using UV resistant polythene and/or coir mesh and were as follows: (i) low shade - 25% shade (one layer of polythene only), (ii) medium shade - 50% shade (single layer each of polythene and coir mesh), (iii) deep shade - 75% shade (one layer of polythene and double layers of coir mesh). The respective maximum instantaneous light intensities in these shelters were 1850, 850 and  $450 \mu\text{mol m}^{-2}\text{s}^{-1}$  respectively. Each shade level was measured using a data logger (LI-1000, LiCor, Lincon, Nebraska) and quantum sensors (LI-190Z, LiCor, Lincon, Nebraska) over a period of two weeks, daily from 8.00 am to 5.00 pm.

All plants were grown in black polythene bags (30 cm x 15 cm) which contained sand, top soil and farm yard manure mixed in the ratio of 2:1:1 respectively. The experiment included three treatments (shade levels), three replicates per treatment and 20 cuttings per replicate. Growth performance of *P. longum* raised under these shade levels for 18 months was evaluated by recording leaf number/plant, number of branches/plant, plant height, leaf chlorophyll content (Arnon, 1949) and the number of fruits/plant in each

treatment. Root collar diameter of a plant was also measured at the ground level by using a vernier caliper.

### **Best potting mixture for optimum plant growth**

The best soil substratum for the species was examined by growing *P. longum* plants for a period of one year, in three different potting media where the sand, top soil and farm yard manure were mixed in 2:1:1, 3:1:0 and 1:1:1 ratios. These are referred to as substrata A, B, and C respectively in this paper. The plants were grown under coir mesh that provided 50% shade (maximum instantaneous light intensity was  $850 \mu\text{mol m}^{-2}\text{s}^{-1}$ ). Shade levels were measured by using a data logger (LI-1000, LiCor, Lincon, Nebraska) and quantum sensors (LI-190Z, LiCor, Lincon, Nebraska) over a period of two weeks, daily from 8.00 am to 5.00 pm.

The experiment included three treatments (soil medium), three replicates per treatment and 20 cuttings per replicate. The performance of *P. longum* plants in each of the treatments was evaluated by recording leaf number/plant, plant height and root collar diameter of a plant.

### **Fruit formation in plants grown from different types of cuttings**

In order to study the fruit production of *P. longum*, cuttings comprising the three uppermost nodes taken from both horizontally growing branches and vertically growing branches were planted separately in polythene bags (15 cm x 25 cm) containing sand, top soil and farm yard manure mixed in the ratio of 1:1:1 respectively. Plants were maintained under 50% shade (maximum instantaneous light intensity  $850 \mu\text{mol m}^{-2}\text{s}^{-1}$ ) by placing them beneath coir mesh shelters. The experimental design included two treatments (types of cuttings), three replicates per treatment and 15 cuttings per replicate.

The number of plants that produced fruiting spikes was recorded weekly over a period of 16 weeks. The changes associated with the progressive maturation of fruiting spikes were also observed in a sample of 40 tagged spikes, starting from the time they were 5 mm long until they were shed from the plants. These forty spikes were randomly selected from 40 plants raised from each type of cutting. The colour change and length of each spike during different stages of development were recorded over a period of 46 days, when they were all shed from the mother plant.

Data on mean plant height, leaf number/plant and leaf chlorophyll content were subject to the analyses of variance (ANOVA) procedure of the Statistical Analysis System (SAS) version 6.12 (SAS Institute Inc., Cary, N. C.).

## RESULTS

### Performance of different types of cuttings

The percentage of cuttings that survived and produced roots is shown in Fig. 1. The greatest survival (96%) was observed in cuttings comprising the two uppermost nodes and the lowest survival (86%) in cuttings with one node. In the remaining three types of cuttings, rooting success was 92%. As seen in Fig. 1, the results show that percentage rooting of all five types of cuttings was very high (more than 85%). Percentage survival was significantly higher ( $p < 0.01$ ) in cuttings comprising the two uppermost nodes.

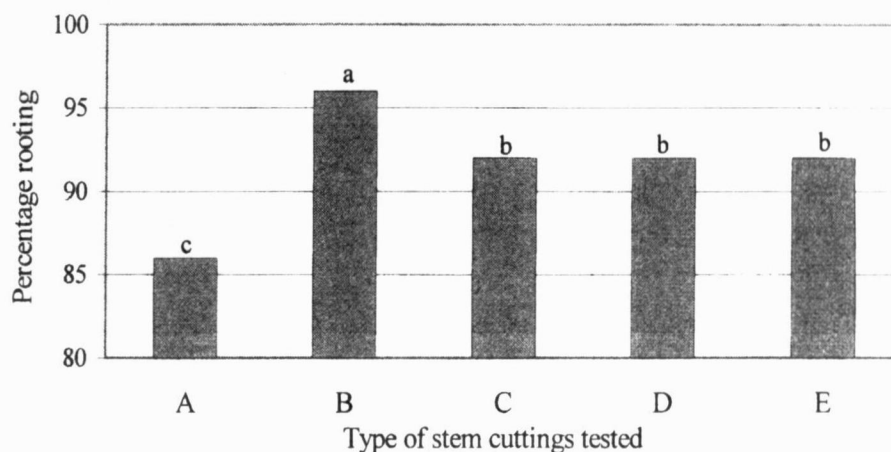
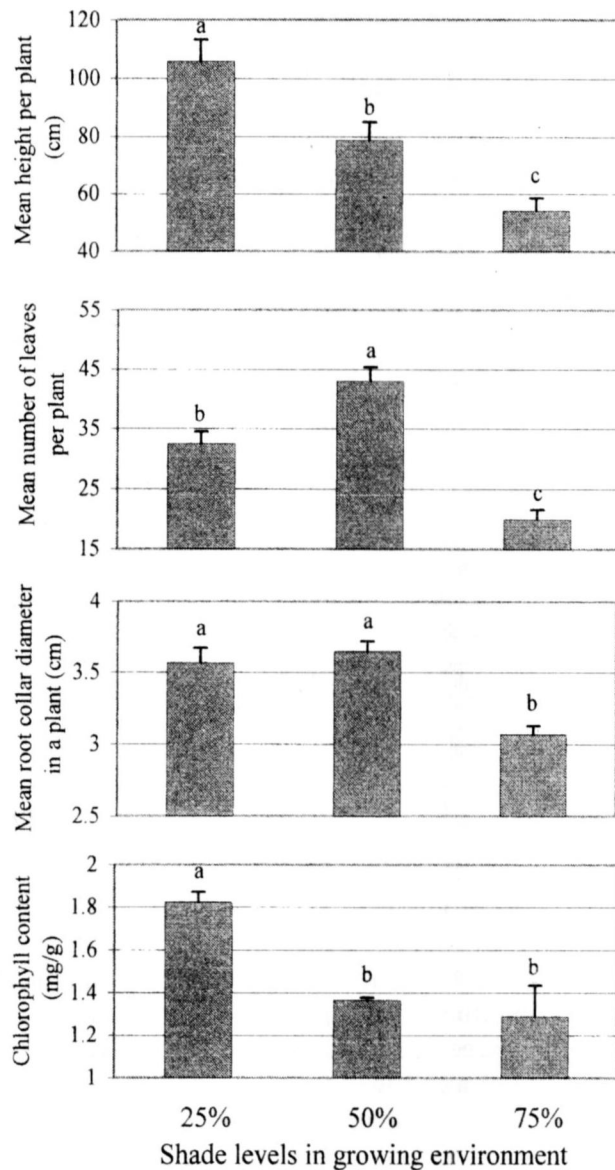


Figure 1. Rooting success of different types of stem cuttings in *P. longum* three months after planting. Cuttings tested comprised a part bearing one (A), two (B) or three (C) uppermost node/s only, the second and third nodes together (D) and the 2-4 nodes together (E). Means with similar letters shown above each bar were not significantly different at 0.05 probability level ( $n = 60$ ).

### Optimal light conditions required for growth

The shoots of *P. longum* grown under medium shade conditions were significantly taller ( $106 \pm 4$  cm) compared to those grown under low shade ( $79 \pm 6$  cm) and deep shade ( $54 \pm 4$  cm) (Fig. 2). Under medium shade, plants produced significantly higher number of leaves/plant ( $43 \pm 2$ ) compared to those grown under medium and deep shade ( $32 \pm 2$  and  $20 \pm 2$  respectively). The root collar diameter in plants grown under deep shade was significantly lower ( $3.1 \pm 0.1$  cm) than that in low and medium shade, where they were more or less similar ( $3.6 \pm 0.1$  and  $3.5 \pm 0.1$  respectively). The chlorophyll content was highest ( $1.8 \pm 0.1$  mg/g) in plants grown under medium shade and lower in those grown under low and deep shade ( $1.38 \pm 0.01$  and  $1.31 \pm 0.14$  mg/g respectively) (Fig. 2).



**Figure 2.** Variations in plant height, leaf number, root collar diameter and chlorophyll content of *P. longum* grown for 18 months, under low (25%), medium (50%) and deep (75%) shade, where the maximum instantaneous light intensities were 1850, 850 and 450  $\mu\text{mol m}^{-2}\text{s}^{-1}$  respectively. Means with similar letters shown above each standard error bar are not significantly different at 0.05% probability level.

### Best potting mixture for optimum plant growth

As shown in Fig. 3, substrata A and C [sand, top soil, farm yard manure (S/TS/FYM) mixed in the ratio of 2:1:1 and 1:1:1 respectively] produced significantly taller ( $p < 0.01$ ) plants compared to that in substratum (B), where S/TS/FYM were mixed in the ratio of 3:1:0. The mean heights of *P. longum* plants grown in substrata A and C ( $163 \pm 10$  and  $142 \pm 10$  cm respectively) were significantly taller compared to those grown in substrata B ( $20 \pm 2$  cm). The Mean

number of leaves per plant was significantly higher in plants grown in the mixture having equal parts of sand, top soil and farmyard manure ( $57 \pm 2$ ) whereas those grown in substratum B had the least ( $12 \pm 1$ ). The root collar diameters of plants grown in mixtures A and C were significantly higher ( $4.0 \pm 0.1$  and  $3.8 \pm 0.1$  cm respectively) than those grown in substratum B ( $2.9 \pm 0.1$  cm).

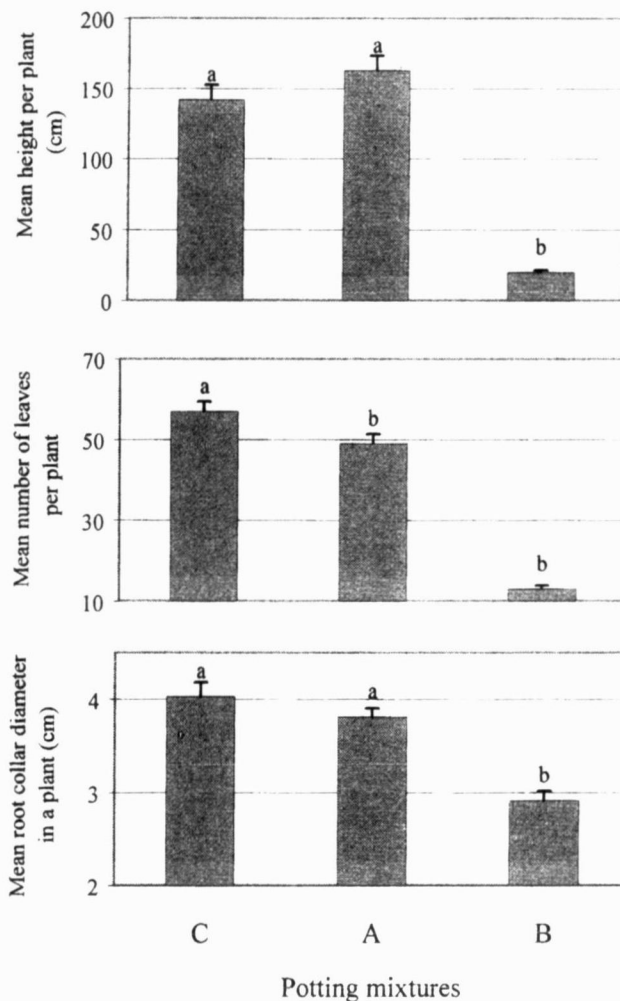
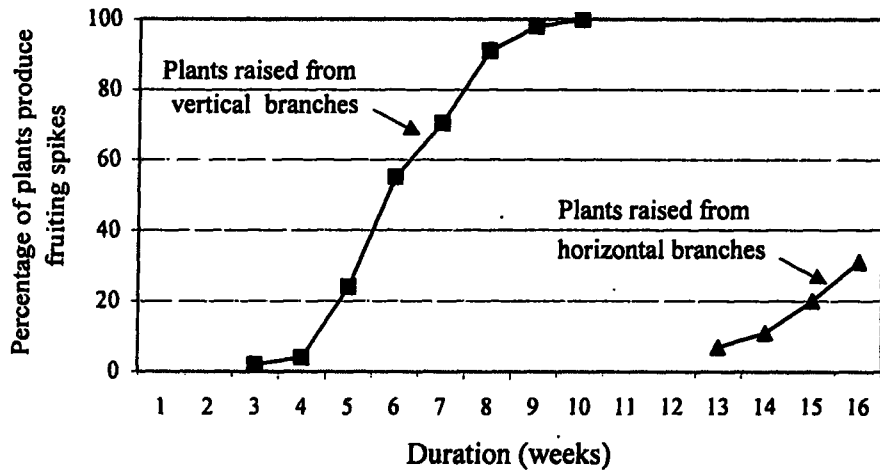


Figure 3. Means of plant height, leaf number and root collar diameter of *P. longum* grown in three different potting media comprising sand, top soil and farm yard manure in the ratio of 2:1:1 (A), 3:1:1(B) and 1:1:1(C), for a period of one year. Means shown by similar letters given above the standard error bars are not significantly different at 0.05 probability level.

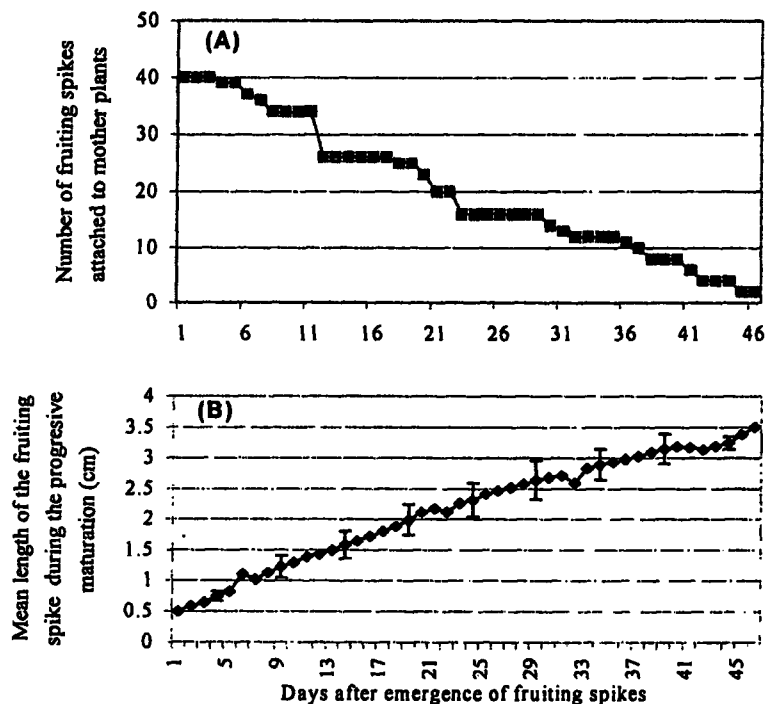
#### Fruit formation in plants raised from different types of stem cuttings

Plants obtained from cuttings of vertical branches started producing fruiting spikes from the third week after commencement of the experiment and all plants developed spikes within a period of 12 weeks (Fig. 4). In contrast, plants raised from the cuttings of horizontal branches started producing spikes only 12 weeks after commencement of the experiment. Even after 16 weeks, only 31 % of plants derived from horizontal branches produced fruiting spikes.



**Figure 4.** Variation in the time of fruit production in plants derived from cuttings taken from horizontal and vertical branches of *P. longum* and grown under 50% shade (n=45 plants).

As shown in Fig. 5A, 50% of the fruiting spikes produced by plants raised from cuttings of vertical branches were shed from the mother plants within 22 days after their emergence. At the time of shedding they were about 2.3 cm long and the colour was green. About 30% of the spikes developed (12 spikes out of 40) remained attached to the mother plant for about 35 days after emergence. When the spikes were about 35 days old they turned black, indicating that they were ripe and fully mature. All fruiting spikes were shed from the mother plants after 47 days, when their maximum average length was 3.5 cm (Fig. 5B).



**Figure 5.** The means and standard errors of the number of fruiting spikes of *P. longum* that remained attached to mother plants (n=40) (A), and the length of fruiting spikes (B) during their growth and development. Plants were grown under 50% shade.

## DISCUSSION

The results of the study provide information on the best type of cuttings (propagules) that can be used to obtain new plants rapidly, the optimum light and potting mixtures for growth and the pattern of pod formation of *P. longum*, which may be most useful for large-scale production for medicinal use.

According to information gathered by interviewing more than 20 Ayurvedic physicians and farmers, *P. longum* is usually propagated by stem cuttings comprising 3-4 nodes (Gunatilleke *et al.*, 2002). The Industrial Technology Institute of Sri Lanka has recommended the application of auxins (10 mg/l Indole-3-Butyric Acid or Naphthalene Acetic Acid) to increase root initiation of stem cuttings (Jayasinha, 1999). However, results of the present study showed that more than 85% of all stem cuttings tested survived and successfully produced roots. Furthermore, the survival rate of stem cuttings comprising the two uppermost nodes was 96% and it was significantly higher ( $p < 0.01$ ) compared to that of other stem cutting types examined. These results suggest that *P. longum* could be easily propagated from stem cuttings without any external hormonal application and this could be recommended as a most economical (cost effective) and simple method of propagating *P. longum*.

When light requirement of the species was considered it showed that it is a moderately shade-loving species. The amount of photon flux density received by plants grown under deep shade may not have been sufficient for plants to grow at optimum levels and this was substantiated by the chlorophyll levels. The higher light intensities at low shade may have destroyed the chlorophyll pigments in the plants due to photo oxidation. The yellowing of plants grown under low shade may be due to this effect. Low light intensities pose stresses on plants as irradiance limits photosynthesis. High light intensities may also be a stress for plants, particularly if other factors are not optimal (Lambers *et al.*, 1997). Furthermore high light environments particularly with other stresses such as drought or high temperature impose an entirely different set of constraints on resource acquisition and utilization by plants (Percy and Valladares, 1999; Taiz and Zeiger, 1998), thereby affect the growth of the plant. On the other hand, the low light levels received under deep shade usually retard leaf expansion thus causing a reduction of the amount of chlorophyll pigments as well. Hence, medium shade (maximum instantaneous light intensity  $850 \mu\text{mol m}^{-2}\text{s}^{-1}$ ) appears to provide the optimal light requirement for the growth of *P. longum*.

Among the three potting mixtures tested, substratum C and A (sand, top soil and farm yard manure (S/TS/FYM) mixed in the ratio of 1:1:1 and 2:1:1) gave significantly higher ( $p < 0.01$ ) growth levels compared to that in substratum B (S/TS/FYM in 3:1:0 ratio). This may be due to the presence of higher levels of top soil and farmyard manure, which provide adequate nutrients required by the plant. According to the Industrial Technology Institute *P. longum* plants can be grown successfully in a medium of top soil, compost, coir dust and coarse sand in 4:1:1:1 ratio (Jayasinha, 1999). In our study too the best results were obtained in the medium with less amount of sand and the poorest growth in the medium with high amount of sand. Substrates with sand may increase drainage but offer little in the

way of additional nutrients. The poorest growth was in the substratum with the highest amount of sand (sand, top soil and farm yard manure mixed in the ratio of 3:1:0). This confirms that *P. longum* requires farm yard manure and does not require a sandy soil for optimal growth.

Early fruit production was observed in vertically grown reproductive branches compared to that in horizontally grown vegetative branches of *P. longum*. Therefore, cuttings taken from vertical branches should be recommended for propagation in order to obtain fruits sooner, instead of the traditional method of using vegetative branches.

*Piper longum* fruits are harvested for Ayurvedic medicinal preparations while they are in the green unripe stage (Jayaweera, 1982; Arambewela and Silva, 1999). Our results show that fruits at this stage of development can be harvested when they are about 22 days old. Our results also showed that 50% of fruits were already shed by this stage. Hence, it would be very useful if a method is developed to minimize the premature fruit losses to enable growers to get the maximum fruit yields to meet the increasing demand whilst enhancing their own income levels.

## ACKNOWLEDGEMENTS

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