World Journal of Agricultural Research, 2019, Vol. 7, No. 2, 49-55
Available online at http://pubs.sciepub.com/wjar/7/2/2
Published by Science and Education Publishing
DOI:10.12691/wjar-7-2-2

Effect of Different Spacing and Fertilizer Levels on Physical and Chemical Yield of Different Parts of *Pogostemon heyneanus* Benth. (Lamiaceae)

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Received November 03, 2018; Revised January 06, 2019; Accepted March 05, 2019

Abstract *Pogostemon heyneanus* Benth. (Lamiaceae) is an aromatic, perfumery important, industrial crop widely cultivated in many Asian countries for its distinguished fragrance and other therapeutic purposes.Therefore, the present study was undertaken to determine the effect of different spacing and fertilizer levels on physical and chemical yield (total antioxidant capacity (TAC), total phenol content (TPC) and total flavonoid content (TFC) in different parts (Leaf, Stem & Root) of *Pogostemon heyneanus.* Nine treatment combinations consisting of three levels of space (S1-90cm×45cm, S2-90cm×60cm and S3-90cm×90cm) and three levels of fertilizer (F1-Organic, F2-Inorganic and F-3 Control) was used for the field experiment in a completely randomized block design with three replicates. The plant height, canopy spread, number of leaves, number of branches, length of branches, number of roots and length of roots were recorded at two weeks intervals. The fresh weight and the dry weight of leaves, stems and toots of uprooted plants were recorded in each month. TPC, TFC and TAC of leaf, stem & root were determined by colorimetric Folin-Ciocalteu method, Aluminium Nitrate method and Ferric Reducing Antioxidant Power (FRAP) assay respectively. The highest values for all TAC, TPC and TFC (55.5 ± 0.58^{AB}, 11.6 ± 0.25^{A} and 86.1 ± 2.83^{A}) were found in leaves of *Pogostemon heyneanus* planted in the treatment combination S3F1. The order of increase TPC, TFC and TAC of *P. heyneanus* was leaf > root > stem.The highest number of leaves, leaf area, leaf fresh weight and dry weight (928 ± 6.2^{A}, 9484 ± 4.9^{A}, 516.2 ± 4.9^{A} and 70.3 ± 0.7^{A}) recorded in the treatment combination S3F1. Therefore, it can suggest to use 90cm×90cm space and organic fertilizer (Compost) for cultivation of *P. heyneanus* in commercial scale. Presence of higher amount of dry matter content and chemical yield (TPC, TFC and TAC) in the leaves scientifically validate traditional claims of harvesting the leaves and value of leaf for the development of newer effective drugs instead of roots and stem.

Keywords: *Pogostemon heyneanus, Lamiaceae, total antioxidant capacity, total phenolic content, spacing levels*

Cite This Article: R.M.D.H. Rathnayake, R.M. Dharmadasa, and D.C. Abeysinghe, "Effect of Different Spacing and Fertilizer Levels on Physical and Chemical Yield of Different Parts of *Pogostemonheyneanus* Benth. (Lamiaceae)." *World Journal of Agricultural Research*, vol. 7, no. 2 (2019): 49-55. doi: 10.12691/wjar-7-2-2.

1. Introduction

Pogostemon heyneanus belonging to family Lamiaceae is a perennial herbaceous plant, distributed throughout Indo-Malaysian and Sino-Japanese regions and grown in various South East Aisin Countries [1,2]. Leaves of the plant contain sweet smelling oil which is accredited for a number of medicinal and cosmetic applications [3]. The commercial oil of patchouli is obtained by steam distillation of the shade-dried leaves and is one of the most important naturally occurring essential oil used in the perfumery industry because of its strong fixative properties [4,5]. Patchouli oil is a secondary metabolite produced by this plant contains a unique and complex composition of more than 24 different sesquiterpenes. The sesquiterpenepatchoulol (patchouli alcohol) is the key element and is the most important factor responsible for the typical patchouli aroma [6]. The patchouli oil possesses a powerful herbaceous aromatic, spicy fragrance, which improves with aging [6]. The oil is widely used in the manufacturing of soaps, scents, body lotions and detergents [7]. It also possesses antibacterial and antifungal properties [6,8]. Essential oil of patchouli already has demonstrated insecticidal activity [9] including repellency against mosquito species [10], whitefly [3] and termites [11].

Dry patchouli leaves have also been found to possess moth repellent properties and therefore, are used to scent wardrobes and protecting clothes especially woolens from insect damage [12]. As there is no synthetic substitute available to replace the oil of Patchouli, its value and position in the perfumery market are further enhanced [8].

Synthetic substitute for patchouli oil is difficult to produce because of its complex mixture of perfumery constituents like sesqueterpenes and hydrocarbons such as, patchouli alcohol, patchouline, bunessene, guaiene, caryophyllene, elemene and copaene and other minor constituents [13]. Among all the essential oil yielding plants, Patchouli (*Pogostemon cablin* Benth.) is considered to have tremendous business potential [6,14].

In aromatherapy, it is used to calm nerves, relieve depression and stress [15]. Fibrinolytic and anti-hrombotic [16,17] activity of this essential oil is also been reported. The patchouli essential oil also shows medicinal properties that include anti-inflammatory, aphrodisiac, anti-depressive, astringent, carminative, febrifuge, sedative, diuretic, tonic, antiemetic, trypanocidal, antibacterial, and antifungal activity [4]. It is estimated that Patchouli Alcohol has neuroprotective [18], anti-influenza [19] and anti-inflammatory activities [20]. Recently, it has reported that patchouli alcohol has anti-inflammatory activities in macrophage and colorectal cancer cells [21]. The plant *Pogostemon cablin* has been used as Chinese herbal medicine to remove dampness, relieve summer heat, exterior syndrome, stop vomiting and stimulate the appetite [22].

The crop is sensitive to extremely high and low temperatures, low relative humidity, unequal distribution of rainfall and poor soils that affect biomass production [23]. Earlier investigation has revealed that high herbage and oil yield were obtained in patchouli grown under partial shade [24,25]. However, not only essential oil, but patchouli extracts also used in many Ayurveda and traditional systems of medicine. Also scientific information on antioxidant capacity, phenol and flavonoid contents of different parts of Patchouli is scattered. Therefore, present investigation was carried out first time in Sri Lanka to study the effect of different spacing and fertilizer levels on physical and chemical yield of different parts of *Pogostemon heyneanus*.

2. Materials and Method

2.1. Location

Experiment was carried out in the experimental plots and the laboratory of the Department of Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP) from March to October 2014. The experimental site was situated in the Low Country Intermediate-Zone (IL_{1a}), at an elevation of 25 m above mean sea level [26]. The soil of the experimental field was with a pH range from 6.5-7.5.

2.2. Planting Materials

Single nodal semi hard wood stem cuttings were obtained from six months old, well maintained, healthy *P. heyneanus* mother plants, planted at university research plots. They were propagated in poly bags (10cm×15cm) using the potting medium consisted of top soil, sand and compost (1:1:1). Planted cuttings were managed providing water, shade and other aftercare operations. Six weeks old healthy, vigorously grown rooted cuttings were taken for field planting.

2.3. Land and Beds Preparation

The land was ploughed using a two wheel tractor at a depth of 30cm. Then a fine tilth was obtained after harrowing the soil. The experimental plots were prepared as raised beds (15cm height) according to the different spacing. Water drainage channels and safety fence were prepared around the experimental plots.

2.4. Treatment Combinations

Nine treatment combinations consisting of three levels of space and three levels of fertilizer as given bellow were used for the experiment.

Table 1. Different Factors and the Levels of Treatments Used.

Factors	Levels
Space	S1- 90cm×45cm
	S2- 90cm×60cm
	S3- 90cm×90cm
Fertilizer	F1- Organic (compost)
	F2- Inorganic
	F3- Control

2.5. Field Planting

Six weeks old rooted stem cuttings were planted in flat beds with relevant space in a completely randomized block design with three replicates. Each plot occupied 6.48m^2, 8.64 m^2 and 12.96 m^2 land area. Each plot consisted of 16 plants. 1500g of compost fertilizer per each plant was added at field planting and each month. The basal dressing of inorganic fertilizer was added for each plant at the field planting stage. Fertilizer was mixed with soil in the planting holes before planting the cuttings. The rest portions of inorganic fertilizer were added as given bellow.

Table 2. The Amount of Inorganic Fertilizer Applied for *Pogostemon heyneanus* at Different Maturity Stages

	Urea (kg/ha)	TSP (kg/ha)	MOP (kg/ha)
Basal dressing	75	325	85
Top dressing 1 (1 MAP)	75		
Top dressing 2 (2 MAP)	75		85
Top dressing 3 (3 MAP)	75		

TSP =Triple Super Phosphate, MOP = Muriate of Potash, MAP = Month after Planting.

2.6. After Care Operations

Immediate after planting watering was done and later twice a day. Then water application gradually reduced as once a day, once in two days, once in three days and weekly interval depending upon the climatic conditions. Weeds were removed by hand whenever necessary. Soil earthen up was done during the heavy rainy period.

2.7. Data Collection

The plant height, canopy spread, number of leaves, number of branches and length of branches were recorded at two weeks intervals form the selected plants in the plots. One plant was uprooted in each month and data were collected as, number of leaves, number of branches, length

of branches, number of roots and length of roots from the uprooted plant. The fresh weight and the dry weight of leaves, stems and toots of uprooted plants were recorded in each month.

2.8. Sample Collection and Preparation for Chemical Analysis

Samples of leaves, stems and roots were collected in each month from uprooted plants. All samples washed and dried first at room temperature (28°C±2) for 3-5 days and then using an oven for 2 hours at 40°C. The dried samples were ground into powder using motor and pestle. Ground samples were sieved (2 mm size) and stored in a refrigerator after packing in polythene until use.

2.9. Preparation of Extract

Pre-prepared samples were weighed (0.1g) into 15mL centrifuge tube and 5ml of 80% methanol was added. The sample was vortex for 15 min and placed in a water bath at 60°C for 40 min and vortex procedure was repeated in 10 min interval. Then the samples were centrifuged at 4000rpm for 5 min and supernatant was decanted into 15 mL centrifuge tube and the remaining was re-extracted with 5mL of 80% methanol. Both supernatants were collected and stored at -20°C.

2.10. Determination of Total Antioxidant Capacity

Total antioxidant capacity was determined using Ferric Reducing Antioxidant Power (FRAP) assay as described by Benzie and Strain [27]. Methanolic extract (100µL) of extract was mixed with 900µL of freshly prepared FRAP reagent of pH 3.6 containing 2.5mL of10 mmol/L, 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mmol/L, HCl plus 2.5 mL of 20 mmol/L FeCl3and 25 mL of 300 mol/L acetate buffer. Absorbance of the reaction was measured at 593 nm using the spectrophotometer (Shimadzu, UV Mini1240, Japan) after incubating for 4 min. The Trolox was used as the standard solution.

2.11. Determination of Total Phenolic Content

The total phenolic content was determined by modified Folin–Ciocalteu colorimetric method [28]. Four milliliters of distilled water and 0.5 mL of extract were added into a test tube. Then the same amount of (0.5 mL) of 0.5 N Folin–Ciocalteu reagent was added and allowed to react for 3 min. Then 1 mL of saturated sodium carbonate solution was mixed and samples were incubated in a water bath for 2 h at 30°C. The absorbance was measured at 760 nm using UV–visible spectrophotometer (ShimadzuUV-160). Gallic acid was used as the standard. The total phenolic content in 1 g of plant extract was calculated and expressed as Gallic acid equivalents (GAE).

2.12. Determination of Total Flavonoid Content

The flavonoid content of *Pogostemon heyneanus* samples were measured by a colorimetric method with modifications. A volume of 0.5 mL of methanolic extract was added to a test tube containing 3.5 mL of distilled water and mixed with 0.5 mL of 5% sodium nitrite ($NaNO_2$). After 6 minutes, 0.3 mL of 10% aluminium nitrate (AlNO3) solution was added and the mixture was allowed to stand for another 6 minutes. Then, 2 mL of 2M sodium hydroxide (NaOH) was added. The reaction mixture was diluted with 1.4 mL distilled water and the absorbance of the mixture at 510 nm was measured immediately using a spectrophotometer (Shimadzu, UV Mini -1240 Japan). Rutin was used as the standard solution.

2.13. Statistical Analysis

Statistical comparison of mean values was performed by General Linear Model (GLM) of ANOVA followed by Turkey Multiple Range Test using Minitab 16 version and presented as means ± SD with 95% confidence level.

3. Results and Discussion

The fresh weight and dry weight of leaves in different treatment combinations showed significant differences between the resulted values. The highest fresh weight and the dry weight resulted from the treatment combination S3F1 (516.2±4.9g and 70.3±0.7g). There is no any significant difference in fresh weight between the treatment combination S1F1, S1F2 and S1F3. Space level 90cm×90cm has given higher leaf weight when compared with rest spaces. When increase the space between plants the leaf weight has increased due to production of more leaves. Plants have the ability to intercept more sunlight and grow freely with in an enough space by producing more branches may be a reason for having higher fresh and dry weight in leaves.

Table 3. Physical and Chemical Yield of Leaves of *Pogostemon heyneanus* under Different Treatment Combinations

Treatment	Physical yield		Chemical yield		
	Fresh weight (g)	Dry weight (g)	TPC (mg GAE per g of dry matter)	TFC (mg RE per g of dry matter)	TAC (mg TE per g of dry matter)
S1F1L	323.1 ± 5.1^{F}	51.3 ± 3.0^{F}	10.9 ± 0.1^{AB}	78.1 ± 0.44^{BCD}	42.7 ± 0.39^{D}
S1F2L	381.2 ± 6.6^{E}	60.0 ± 1.1^{DE}	10.8 ± 0.11^{AB}	72.1 ± 1.43^{E}	41.5 ± 1.23^{D}
S1F3L	376.5 ± 5.0^{E}	57.5 ± 3.5^{EF}	11.1 ± 0.15^{A}	80.0 ± 0.66^{BC}	43.1 ± 0.38^{D}
S2F1L	401.0 ± 5.8^{DE}	62.0 ± 1.2^{CDE}	10.8 ± 0.37^{AB}	75.1 ± 0.37^{DE}	47.8 ± 1.18^{C}
S2F2L	419.6 ± 5.4^{CD}	$64.0\pm.0^{BCD}$	10.1 ± 0.29^{B}	72.3 ± 0.58^{E}	49.1 ± 1.31^{C}
S2F3L	433.2 ± 7.2^{BC}	65.7 ± 1.4^{ABCD}	10.9 ± 0.03^{AB}	82.0 ± 0.65^{AB}	48.6 ± 0.60^{C}
S3F1L	516.2 ± 4.9^{A}	70.3 ± 0.7^{A}	11.6 ± 0.25^{A}	86.1 ± 2.83^{A}	55.5 ± 0.58^{AB}
S3F2L	452.1 ± 6.0^{B}	68.4 ± 0.7^{AB}	11.0 ± 0.05^{A}	85.2 ± 0.73^{A}	56.7 ± 0.43^{A}
S3F3L	438.1 ± 4.0^{BC}	67.0 ± 0.9^{ABC}	11.4 ± 0.45^{A}	77.7 ± 0.91^{CD}	53.4 ± 0.72^{B}

The highest fresh weight and dry weight of leaves from three fertilizer levels (organic, inorganic & control) observed in organic fertilizer treatment irrespective to the spacing. Better moisture availability and edaphic environment under organic mulching appears to have enhanced plant growth and ultimately oil yield. Similar results were reported in *Mentha arvensis* [29].

Antioxidants, flavonoids and phenolics are secondary metabolites which are mainly responsible for the defense mechanisms of a plant [30]. The highest total antioxidant capacity resulted in treatment combination S3F2 (56.7±0.43). There is no significant difference in TAC between S1F1, S1F2, S1F3 and S2F1, S2F2, S2F3. There are no higher variations in TPC in leaves. S3F1, S3F2 and S3F3 treatments have given comparatively higher TPC in the leaves compared with other treatments. Significantly S3F1 and S3F2 treatments resulted the higher TFC in the leaves extract (86.1±2.83 and 85.2±0.73). As shown in Table 1, the TAC, TFC and TPC of leaf extracts of *Pogostemon heyneanus* have increased with the increase of spacing. The higher TAC in wider spacing levels might be due to plant exposure to the better light conditions. These findings are in agreement with [31], who reported the higher content of secondary metabolites and antioxidant capacity in plants grown in fully sunlight conditions compared to plants grown under shady conditions. According to the Table 1, treatment combinations with higher dry matter contents showed the higher chemical yield (TPC, TFC & TAC).

The stem weight pattern in different treatments showed marked variations. The higher stem fresh weight and dry weight recorded in S1F2 treatment combination (718.4±2.0g and 117.3±1.6g). Lower values in fresh and dry weight resulted in higher spaces than the lower spaces. This may be due to growth of main stem of plants vertically to absorb more sunlight and to prevent mutual shading.

The highest TAC was resulted in S3F1 treatment combination when compared with others. Similarly, higher TFC resulted in S3F1 and S3F2 treatment combinations. There were no significant differences in TPC in the stem compared with TAC and TFC. Chemical yield in stem has increased with the increase of spacing.

There were no major significant differences in fresh weight of roots under different treatment combinations. Comparatively higher space resulted higher fresh weight than lower space. There was no proper pattern in changing the dry weight of roots. Significantly, S3F1 treatment resulted the highest root dry weight (10±0.6g). S2F3, S3F1, S3F2 and S3F3 treatment combinations have given higher TPC than the other treatments. S1F1 treatment resulted the lowest TPC in roots. TAC in S3F1 and S3F2 treatments recorded the higher values compared with others. Comparatively taking all the treatment combinations together, S3F1 reflected the higher chemical yield of TPC, TFC and TAC.

Secondary metabolites are responsible for the therapeutic properties of a plant and it may vary from part to part in the same plant. Preliminary investigation of phytochemicals is important for the quantitative estimation and for the locating of pharmacologically active chemical compounds [32]. The phenolic contents were significantly varied in leaves, stems and roots of the plant (Table 1, Table 2, & Table 3). In general, the antioxidant capacity of plant extracts is associated with group of compounds, such as phenol, flavones, flavonols, carotenoids and pigments like pro-anthocyanidins etc. [33]. Out of above group of compounds, phenolics act as stabilizing agent of scavenging radicals [34]. The results demonstrated that phenolic content, flavonoid content and antioxidant capacity were increased as leaf > root > stem. This study revealed the significantly high antioxidant capacity, flavonoid content and total phenolic contents in leaves validating the use of leaves in pharmaceutical and medical purposes.In the present study it has also observed a positive relationship between TPC and TAC of different extracts of *P. heyneanus.*

Table 4. Physical and Chemical Yield of Stems of *Pogostemon heyneanus* under Different Treatment Combinations

Treatment	Physical yield		Chemical yield		
	Fresh weight (g)	Dry weight (g)	TPC (mg GAE per g of dry matter)	TFC (mg RE per g of dry matter)	TAC (mg TE per g of dry matter)
S1F1S	542.9±5.5^{C}	91.7±0.6^{C}	4.0±0.15^{ABC}	3.0±0.17^{C}	9.2±0.68^{CDE}
S1F2S	718.4±2.0^{A}	117.3±1.6^{A}	4.2±0.14^{ABC}	3.0±0.33^{C}	7.8±0.25^{EF}
S1F3S	640.9±5.0^{B}	103.9±1.9^{B}	3.8±0.21^{BC}	2.9±0.37^{C}	6.6±0.33^{F}
S2F1S	404.4±7.3^{F}	71.1±1.9^{DE}	3.8±0.09^{BC}	3.5±0.39^{BC}	8.8±0.52^{DE}
S2F2S	454.7±7.0^{DE}	72.4±1.4^{DE}	3.6±0.42^{C}	3.6±0.22^{BC}	9.8±0.32^{CD}
S2F3S	394.1±5.8^{F}	61.4±1.6^{F}	4.5±0.25^{AB}	3.8±0.86^{BC}	10.2±0.58^{BCD}
S3F1S	439.6±5.6^{E}	75.6±2.2^{D}	4.4±0.38^{ABC}	5.6±0.46^{A}	12.2±0.69^{A}
S3F2S	461.9±5.5^{D}	73.0±2.4^{DE}	4.5±0.04^{ABC}	5.6±0.19^{A}	11.7±0.41^{AB}
S3F3S	435.8±9.6^{E}	69.3±1.6^{E}	4.9±0.33^{A}	4.6±0.43^{B}	10.7±0.43^{ABC}

Table 5. Physical and Chemical Yield of Roots *Pogostemon heyneanus* under Different Treatment Combinations

Treatments	Physical yield		Chemical yield		
	Fresh weight (g)	Dry weight (g)	TPC (mg GAE per g of dry matter)	TFC (mg RE per g of dry matter)	TAC (mg TE per g of dry matter)
S1F1R	56.8±4.5^{AB}	9.9±0.2^{ABC}	8.4±0.27^{C}	58.8±0.82^{CD}	27.7±0.99^{D}
S1F2R	58.5±3.5^{AB}	10.1±0.3^{AB}	9.0±0.25^{BC}	60.0±1.45^{CD}	28.6±0.36^{D}
S1F3R	53.5±2.0^{AB}	8.7±0.4^{CD}	9.5±0.27^{AB}	57.1±0.4^{D}	29.5±1.10^{D}
S2F1R	49.6±4.6^{B}	8.3±0.3^{D}	9.7±0.37^{AB}	59.3±3.37^{CD}	39.7±1.17^{ABC}
S2F2R	59.6±2.3^{AB}	9.7±0.4^{ABCD}	9.9±0.57^{AB}	62.3±0.7B^{C}	38.7±1.61^{BC}
S2F3R	53.5±5.7^{AB}	8.9±0.6^{BCD}	10.1±0.13^{A}	65.1±0.74^{B}	37.9±0.43^{C}
S3F1R	63.9±3.3^{A}	10.4±0.6^{A}	10.1±0.04^{A}	78.4±0.16^{A}	42.2±0.54^{A}
S3F2R	60.7±2.8^{AB}	9.3±0.3^{ABCD}	10.1±0.04^{A}	75.3±0.27^{AB}	42.7±0.42^{A}
S3F3R	62.2±3.7^{A}	10.2±0.4^{AB}	10.3±0.05^{A}	71.0±0.49^{B}	41.8±0.62^{AB}

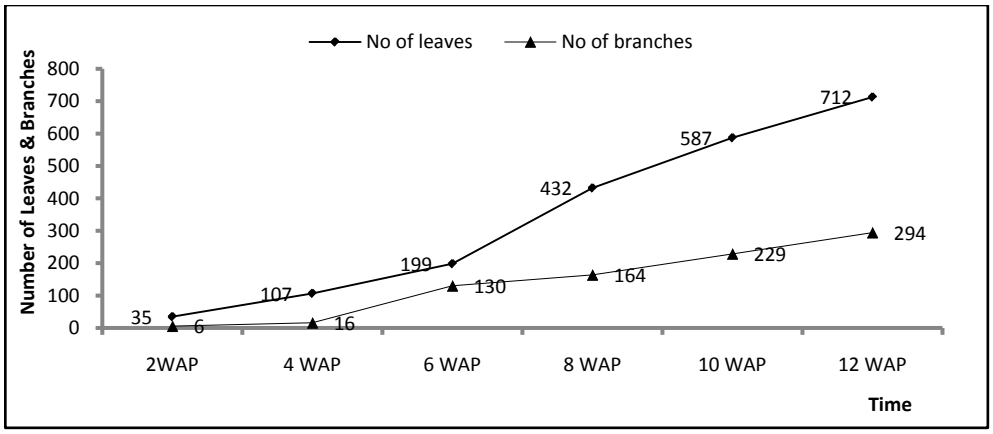

Figure 1. Variation of Number of Branches and Number of Leaves of *Pogostemonheyneanus* at Two Weeks Intervals

Number of branches and number of leaves of *P. heyneanus* have increased with the increase of time. There was a rapid increase of number of branches four weeks after planting. Number of branches has gradually increased from six weeks onwards. A rapid increase of number of leaves started from six weeks, after a gradual increment from second week. When increase the branches of a plant, the leaves also increased simultaneously due to the production of more growing points. Production of secondary metabolites increases when there are more leaves and more photosynthetic assimilates.

All the values of three parameters increased with the time. There was a rapid increase of length of branches after sixth week. Spread of the plant increased with the time due to increase of the number of branches and length of branches. Increasing the spread of canopy is important to absorb more sunlight by preventing mutual shading. Finally it affects to produce more carbohydrates and secondary metabolites with in the plant. A rapid increase of plant height can be observed after fourth week.

There are significant differences between the treatment combinations for the responses of number of leaves ($P<0.05$). The highest number of leaves recorded in the treatment combination S3F1 (928±6.2) while the lowest observed in S1F1 (550±8.2). Number of leaves increases with the increase of space of the plant. The reason may be having enough space to develop more branches and produce more leaves in the plants. Higher leaf areas observed in the plants which had higher number of leaves. Similarly wider spaces recorded the higher leaf areas. Organic fertilizer applied treatment gave the highest number of leaves and highest leaf area (928±6.2 and 9484±4.9cm^2) irrespective to the space. Application of compost (organic fertilizer) improved the physical status of soil with the reduction of bulk density and by increasing water holding capacity.

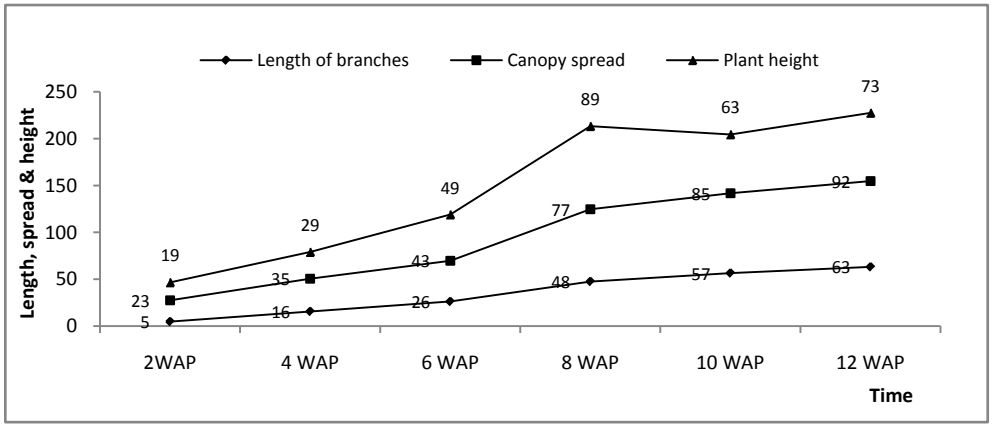

Figure 2. Variation of Length of Branches, Canopy Spread and Plant Height of *Pogostemon heyneanus* at Two Weeks Intervals

Table 6. Variation of Number of Leaves, Leaf Area, Length of Main Stem, Stem Diameter and Length of Main Root of *Pogostemon heyneanus* Three Months after Planting

Treatments	No of Leaves	Leaf area (cm^2)	Length of main stem (cm)	Stem diameter (mm)	Length of main root (cm)
S1F1	550±8.2^F	7468±12.3^H	82.7±4.1^{AB}	11.8±0.54^C	34.0±0.82^A
S1F2	611±6.2^D	8141±7.2^E	85.0±2.1^A	12.9±0.22^{ABC}	32.3±1.25^{AB}
S1F3	535±7.3^F	7254±6.3^I	84.0±2.1^{AB}	12.5±0.25^{ABC}	32.0±0.82^{ABC}
S2F1	579±9.0^E	7542±8.7^G	74.7±3.4^{BC}	12.3±0.09^{BC}	30.0±0.82^{BCD}
S2F2	590±8.2D^E	7653±7.9^F	72.3±2.0^C	12.9±0.55^{ABC}	32.0±0.82^{ABC}
S2F3	736±4.5^C	8356±9.2^D	70.3±2.0^C	12.7±0.53^{ABC}	29.0±0.82^{BCD}
S3F1	928±6.2^A	9484±4.9^A	68.7±4.0^C	13.6±0.17^A	28.7±1.25^{CD}
S3F1	788±6.2^B	8757±8.5^B	67.7±2.0^C	13.4±0.33^{AB}	29.0±0.82^{BCD}
S3F3	757±6.1^C	8531±6.9^C	66.7±2.6^C	13.3±0.38^{AB}	28.3±1.25^D

The lower spaces recorded the greater lengths of main stem in the treatment combinations. The highest length observed in S1F2 (85.0±2.1cm) treatment. There were no significant difference between the treatments S2F2, S2F3, S3F1, S3F2 and S3F3. Significantly different observations recorded for stem diameter in treatment combinations. The low spaces recorded the lower stem diameters and higher stem heights. The reason may be the elongation of stem more vertically to receive basic plant needs (sunlight, oxygen) by preventing the competition between neighboring plants.

The highest length of main root recorded in the treatment combination S1F1 (34.0±0.82cm) and the lowest in S3F3 (28.3±1.25). S2F1, S2F3 and S3F1 recorded significantly similar observations. Due to less space, root systems of the plants compete for nutrients and water in the soil. Therefore, plants try to absorb them from the deep layers of soil by making lengthier roots.

4. Conclusions

The present study demonstrated the effect of spacing and fertilizer levels for the physical and chemical yield of *P. heyneanus* for the first time in Sri Lanka. Spacing level 3 (90 cm×90 cm) and inorganic fertilizer (compost) is the most suitable treatment combination for cultivation of *P. heyneanus* in commercial scale as it produced the best results in all growth parameters and chemical yield tested. Presence of higher amount of dry matter content and chemical yield (TPC, TFC and TAC) in the leaves scientifically validate traditional claims of harvesting the leaves and value of leaf for the development of newer effective drugs instead of roots and stem.

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