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The effect of kacip fatimah (*Marantodes pumilum*) plant density under semi-controlled environment for high production of biomass and bioactive content

[Kepadatan tanaman bagi kacip fatimah (*Marantodes pumilum*) untuk pengeluaran hasil dan kandungan biojisim dan bioaktif yang tinggi di bawah persekitaran separa-terkawal]

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Abstract

Marantodes pumilum or kacip fatimah is one of the most sought-after valuable herbs in Malaysia and is commonly used by Malay women after childbirth. *M. pumilum* is a jungle species and difficult to cultivate ex situ. Therefore, *M. pumilum* needs to be adapted to the agricultural environment ex situ before it can survive and grow optimally. Currently, a huge volume of the plant raw materials is obtained directly from the jungle or imported from neighbouring countries. There is an urgent need to develop good cultural practices for *M. pumilum* such as plant density or plant spacing. This criterion is important to ensure high yields both in terms of biomass and phytochemical contents. Plant spacing refers to the between-row spacing and interplant spacing. In this study, between-row spacing was fixed at 25 cm and interplant spacing was tested with three different distances of 12.5 cm (D1), 25 cm (D2) and 37.5 cm (D3), respectively. Population density was tested in the following ranges: 320,000 plant/ha, 160,000 plant/ha and 106,666 plant/ha, respective at D1, D2 and D3. The interplant spacing study was carried out under a field nursery system with an 80% shade level. The study was statistically designed in a randomised complete block design (RCBD) with five replications. Plant growth, biomass, total polypeptide content, total phenolic content and total flavonoid content data were recorded. The results revealed that an interplant spacing of 12.5 cm is the best for *M. pumilum* as it produced 54 – 65% higher fresh yield, 50 – 69% higher dry yield and 51 – 66% higher total polypeptide yield compared to the other two interplant spacings. This translates to a higher overall bioactive content in terms of its phytochemical and polypeptide production per hectare.

Key words: kacip fatimah, biomass, total polypeptide, total phenolic, total flavonoid

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Introduction

Marantodes pumilum, locally known as kacip fatimah is one of the most important herbs in Malaysia. It is a small woody plant with creeping stems and is a member of the Myrsinaceae family. The three main varieties of *M. pumilum* identified in Malaysia are *alata*, *pumila* and *lanceolata* (Jamia 2003). From these three varieties, only *pumila* and *alata* have been commonly cultivated and widely used in traditional medicine and research as they are more readily available than the *lanceolata*. The plant can be naturally found in the tropical forests of Malaysia (80 – 100 m above sea level), Thailand, Indonesia, Philippines, and New Guinea (Stone 1988). *M. pumilum* has been proven to have antimicrobial properties that could be attributed to bioactive compounds such as flavonoids, phenolics and saponin (Ehsan, et al. 2011). Jamia (2003) found that *M. pumilum* can be used to delay fertility, regain body strength and for the treatment of flatulence, dysentery, dysmenorrhoea and gonorrhoea. In Malay traditional medicine, water decoction of *M. pumilum* roots or whole plant is often given to pregnant women before delivery to induce and expedite labour (Muhamad and Mustafa Ali 1994).

Currently, the raw materials of *M. pumilum* are not easily obtained due to diminishing supply in the wild supplemented with limited efforts in replanting and cultivation. This in turn is limiting the supply of raw materials for the domestic market and thus encourages the importation of raw materials of which the quality is not always guaranteed. It is timely that aggressive efforts are made towards the research, development and generation of cultivation technologies to ensure production of good quality raw materials thus spurring the growth of the kacip fatimah industry in Malaysia.

M. pumilum is difficult to cultivate ex situ due to the plant's high sensitivity to its environment. It can be grown ex situ under an agricultural environment that is similar to its natural forest habitat. Standard environmental requirements for *M. pumilum* in its natural habitat are 80% shade, 70% humidity and a temperature of 25 – 35 °C (Mohd Noh 2006). *M. pumilum* cultivation under an open field has not been successful because the odd environment exposed the plant to environmental heat stress (Mohd Noh 2006). Therefore, the only available cultivation option for *M. pumilum* for commercial production is under a field nursery system (Musa et al. 2010). According to Mohd Hafiz (2012), higher bioactive contents (total phenolic, flavonoid and anthocyanin contents) were observed when *M. pumilum* was planted under shade with a light intensity of 225 μmol/m^2/s. The manipulation of environmental conditions such as light intensity, humidity, and temperature to produce similar attributes to the natural habitat of *M. pumilum* can be done under the field nursery system with a semi-controlled environment.

Planting distance or plant spacing is among the important factors of cultural practices that highly affect the biomass, primary and secondary metabolites yield of *M. pumilum*. Various studies have been reported on the plant spacing of other herbs but there has been no such documented report on *M. pumilum*. Plant spacing can determine the plant population density. Generally, yield/plant decreases as plant spacing decreases to a very short distance (Jhon and Poul 1995). Yield can be increased by increasing population density i.e. by reducing the plant spacing to an optimum distance. The current practice among farmers such as those in Kota Tinggi is planting *M. pumilum* at a space of 30 cm x 30 cm. However, Mohd Noh (2004) suggested a plant spacing of 20 cm x 20 cm. According to Konfranek (1980), optimum plant spacing depends on cultivars, seasons and other cultural management and this has not been comprehensively studied for kacip fatimah. This study was therefore conducted to determine the optimum interplant spacing for good growth, high biomass yield and quality of *M. pumilum* under a semi-controlled environment.

Materials and methods

The study was carried out under a rain shelter and netting structure in FRIM Kepong and MARDI Serdang, Selangor. *Marantodes pumilum* var. *alata* was used in this study with the mother plants obtained from a farm in Tasek Gelugor, Penang. Identification of the *M. pumilum* variety was carried out by Dr. Mohd. Nor Faizal Ghazalli, a taxonomist from the Malaysian Agricultural Research and Development Institute (MARDI) headquarters. Leaf cuttings were used as the planting material and propagation was carried out at FRIM. The leaf cuttings were propagated in 100% sand for 2 months and then transferred into polybags for another 4 months to ensure better root and bud development. The matured 6-month old seedlings were transplanted under a netting structure with a semi-controlled environment of 80% shade, maintained humidity of about 70% and temperature range of 25 – 35 °C. Planting troughs [1.2 m (width) x 2.5 m (length) x 0.3 m (height)] were filled with a medium of cocopeat:topsoil:organic fertiliser at a ratio of 3:2:1.

The experiment was statistically designed in a randomised complete block design (RCBD) with five replications. Each treatment consisted of three different planting distances where the distance between rows was fixed at 25 cm for each treatment. Interplant spacing was set at 12.5 cm (D1), 25 cm (D2) and 37.5 cm (D3) which translated to four rows of 72, 36 and 28 plants respectively for D1, D2 and D3. The plant was watered using a mist irrigation system at 30 minute intervals to maintain the temperature and humidity. Weeds were controlled manually and there was no application of pesticide as no major pest and disease were observed throughout the study. Plant growth parameters such as plant height, number of branches, number of leaves and plant canopy were recorded biweekly. The direct measurement method was used to measure the plant canopy according to its axis distance using a measuring tape as described by Ross (1981). The whole plants were harvested at 10 months after transplanting. The harvested plants were separated according to the plant components of leaves, stems and roots for bioactive content analysis. Data on biomass (fresh and dry yield) for the whole plant was determined. Fresh samples were dried immediately at 50 °C until the moisture content dropped to 10 – 12%. Immediate drying is important to preserve the bioactive compounds in the plant and prevent them from deteriorating. Dried leaf samples were ground to obtain the total polypeptide, total phenolic and total flavonoid contents in each plant part. Total polypeptide content was determined according to the approach developed by Masuko et al. (2005). Total flavonoid content was measured using a colorimetric assay developed by Zhishen et al. (1999). Statistically, all data were analysed using SAS statistical package 9.1 (SAS 2002) and treatment means were compared using the Duncan Multiple Range Test (DMRT).

Results

Plant growth measurement at harvest

The plant growth characteristics evaluated in this study were plant height, plant canopy and number of leaves. All the plant growth parameters of *Marantodes pumilum* were very significantly affected by interplant spacing (D) (*Table 1*).

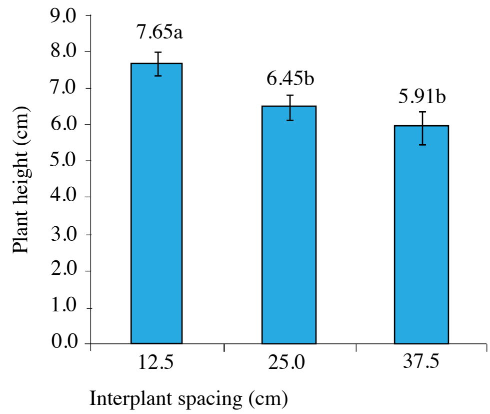
Plant height

Plant heights of *M. pumilum* at 10 months after transplanting at interplant spacings of D1, D2 and D3 were 7.65 cm, 6.45 cm, and 5.91 cm, respectively. The plant height was significantly different only between D1 and D2/D3 (*Figure 1*). The results of plant height in *M. pumilum* seem to indicate a trend where the plant becomes taller as the plant spacing becomes narrower.

Table 1. ANOVA for plant height, plant canopy and number of leaves of *M. pumilum* on different interplant spacings

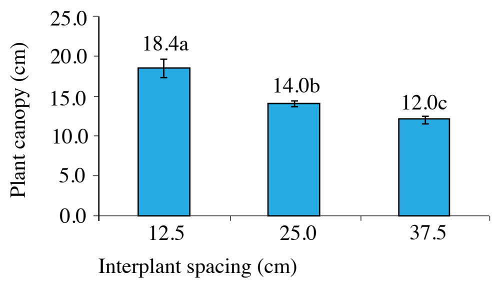
Source	DF	Plant height (cm)			Plant canopy (cm)			Number of leaves		
		Mean square	F value	PR > F	Mean square	F value	Pr > F	Mean square	F value	Pr > F
Interplant spacing (D)	2	3.17	29.23	0.0008**	42.86	78.08	<.0001**	7.2133	42.71	0.0003**
Replication	4	0.19	1.77	0.25ns	0.53	0.96	0.47ns	0.1222	0.72	0.5737ns

ns = not significant
**significant at $p < 0.01$



*Means with the same letter for each interplant spacing are not significantly different from one another by DMRT at $p < 0.01$. Error bars represent standard deviation.

Figure 1. Plant height of M. pumilum at different interplant spacings



*Means with the same letter for each interplant spacing are not significantly different from one another by DMRT at $p < 0.01$. Error bars represent standard deviation.

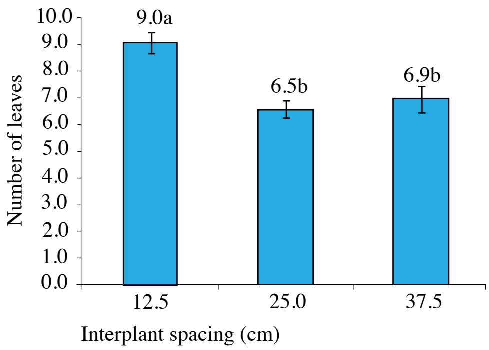
Figure 2. Plant canopy of M. pumilum at different interplant spacings

Plant canopy

The mean plant canopy of *M. pumilum* at different interplant spacings is presented in *Figure 2*. The plant canopy was significantly different between the treatments of interplant spacings. The D1 interplant spacing showed the widest canopy with 18.43 cm. It is followed by the control of D2 with 14 cm. The smallest canopy was found under the wider distance of D3 at 12.01 cm. These results indicate that the closer the interplanting distance, the larger the canopy will expand.

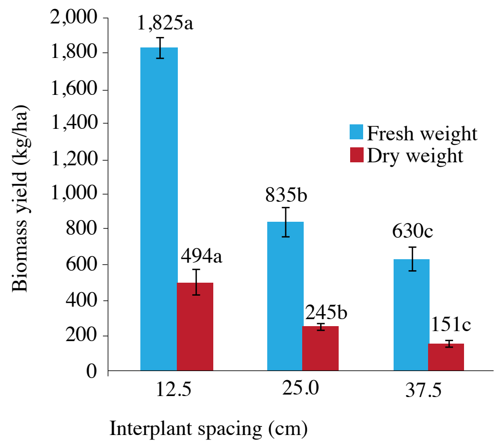
Number of leaves

The mean of leaf number/plant for *M. pumilum* at harvest is shown in *Figure 3*. The number of leaves was significantly different between the treatments of interplant spacings. The highest leaf number was found at D1 with 9 leaves. It was followed by the spacing of D3 with a leaf number of 6.9. The control of D2 had the lowest number of leaves at 6.5 but it was not significantly different to D3.



*Means with the same letter for each interplant spacing are not significantly different from one another by DMRT at p <0.01. Error bars represent standard deviation.

Figure 3. Number of leaves of M. pumilum at different interplant spacings



*Means with the same letter for each interplant spacing are not significantly different from one another by DMRT at p <0.01. Error bars represent standard deviation.

Figure 4. Plant canopy of M. pumilum at different interplant spacings

Biomass

Biomass refers to the fresh and dry yields of the whole plant (leaf, stem and root) of *M. pumilum*. The yield/plot and yield/plant were recorded and converted to kg/ha. *Table 2* shows that the fresh and dry yields of *M. pumilum* were very significantly different at different interplant spacings. The data on the biomass at different interplant spacings in kg/ha are presented in *Figure 4*.

Fresh yield

The fresh yield of *M. pumilum*, harvested at 10 months old, was significantly different for different interplant spacing dimensions (*Figure 4*). The highest fresh yield was 1,825 kg/ha when planted at the closest distance (D1). The lowest yield was found at the widest distance (D3) with a 630 kg/ha of fresh yield. Meanwhile, the fresh yield at control (D2) was 835 kg/ha. This means that an interplant spacing of 12.5 cm produced 54% higher yield than 25 cm and 65% higher yield than 37.5 cm.

Dry yield

The dry yield shows a similar trend to the fresh yield (*Figure 4*). Significant differences were shown between the treatments. The highest dry yield was 494 kg/ha for D1, followed by interplant spacings of D2 and D3 which gave 245 kg/ha and 151 kg/ha, respectively. The interplant spacing of D1 produced 69% higher dry yield compared to D2 and 50% higher dry yield compared to

Table 2. ANOVA for plant height, plant canopy and number of leaves of *M. pumilum* on different interplant spacings

Source	DF	Total yield (kg/ha)					
		Fresh			Dry		
		Mean square	F value	Pr > F	Mean square	F value	Pr >F
Interplant spacing (D)	2	1632705.08	286.68	<.0001**	125605.75	66.55	<.0001**
Replication	4	1971.64	0.35	0.79ns	1996.56	1.06	0.43ns

ns = not significant
**significant at p <0.01

D3. From these results, the best interplant spacing for fresh and dry yields is at 12.5 cm.

Bioactive content

The effect of planting distance on phytochemical content (total polypeptides, total phenolics and total flavonoids) in the leaf, stem and root of *M. pumilum* was determined.

Total polypeptide content and total polypeptide yield

The results of total polypeptide content at different plant spacings and plant parts are presented in *Table 3*. There was no significant difference of total polypeptide content in all plant parts or their combinations at the different interplant spacings. However, the leaf content was 34% higher than the stem content and 8% higher than in the root (*Table 3*).

Total polypeptide yield per hectare was determined by calculating the total dry yield per ha multiplied with the total polypeptide content in the whole plant. The results revealed that the total polypeptide yield per hectare was significantly higher (111.71 kg/ ha) at the interplant spacing of D1 followed by D2 (54.27 kg/ha) and D3 (37.84 kg/ha) (*Table 3*).

Total phenolic content and total phenolic yield

The total phenolic content at different interplant spacings shows that there was no significant difference for all plant parts (*Table 4*). However, the total phenolic content was 18% higher in the root compared to the leaf and 20% higher in the stem. The results also revealed that the total phenolic yield per hectare was significantly higher at the interplant spacing of D1 followed by D2 and D3 (*Table 4*).

Total flavonoid content and total flavonoid yield

The total flavonoid content was not significantly different between the spacing treatments (*Table 5*). However, the highest total flavonoid content was in the leaf followed by the root and stem. The results also revealed that the total flavonoid yield per hectare was significantly higher at the interplant spacing of D1 followed by D2 and D3 (*Table 5*).

Discussion

The cultural practices of *M. pumilum* such as plant spacing are important criteria to be considered for high production of the herbs. This translates to higher biomass and phytochemical yields per unit area.

Table 3. Total polypeptide content and total polypeptide yield of different plant parts of *M. pumilum* at different interplant spacings

Interplant spacing	Total polypeptide content (mg/100g)				Total polypeptide yield (kg/ha)
	Leaf	Stem	Root	Whole plant	
12.5 cm (D1)	10,499 ± 1,301a	2.684 ± 1.007a	9,431 ± 3,152a	2,2614 ± 2,031a	112.43 ± 24.68a
25 cm (D2)	10,991 ± 1,307a	2.692 ± 1.344a	8,466 ± 3,224a	2,2149 ± 3,353a	54.39 ± 10.56b
37.5 cm (D3)	11,492 ± 2,337a	4.220 ± 1,773a	9,345 ± 3,148a	2,5057 ± 6,323a	38.41 ± 12.65b
Mean	10,994	3.199	9,081	23,273	68.41
Significance	ns	ns	ns	ns	**
CV (%)	16.37	26.22	10.13	13.11	19.05

ns = not significant

**significant at p <0.01

Means with the same letter for each interplant spacing are not significantly different from one another by DMRT at p <0.01

The results revealed that a shorter interplant distance within a row resulted in better plant growth and biomass of *M. pumilum*. This is different from most crops where close plant spacing would lead to limited leaf expansion and decreased plant performance and also plant biomass. However, the phenomena in *M. pumilum* can be explained by the fact that *M. pumilum* requires high humidity. A higher plant density increased the humidity of the plant microclimate and this in turn may have caused *M. pumilum* to grow better. The microclimate soil temperature at all depths decreased with increasing plant density (Mwangi 2000). Population density at the closest spacing (12.5 cm) was 320,000 plant/ha compared to 160,000 plant/ha at the medium spacing (25 cm) and 106,666 at the wider spacing (37.5 cm). Increasing the plant density from 106,666 to 320,000 plant/ha resulted in a biomass increase of 69% in dry weight/hectare. According to Jones (1985), the productivity of plants is ultimately dependent upon the influence of the microclimate on plant processes such as photosynthesis, respiration, transpiration and translocation.

Although an 80% shade was provided to *M. pumilum* in order to mimic its natural habitat, the plant still competed for the remaining 20% of light for optimal growth. Generally, in a crowded population, a higher interplant competition exists as plants try

Table 4. Total phenolic content and total phenolic yield of different plant parts of *M. pumilum* at different interplant spacings

Interplant spacing	Total phenolic content (mg/100g)				Total phenolic yield (kg/ha)
	Leaf	Stem	Root	Whole plant	
12.5 cm (D1)	2,029 ± 55a	2,086 ± 697a	3,817 ± 381a	7,932 ± 943a	38.73 ± 2.36a
25 cm (D2)	2,044 ± 145a	1,814 ± 320a	3,401 ± 386a	7,260 ± 565a	17.83 ± 2.34b
37.5 cm (D3)	2,308 ± 574a	2,195 ± 538a	3,550 ± 488a	8,052 ± 1484a	12.26 ± 3.21c
Mean	2,127	2,032	3,589	7,748	22.94
Significance	ns	ns	ns	ns	**
CV (%)	14.52	30.9	11.2	14.57	11.41

ns = not significant
**significant at $p < 0.01$
Means with the same letter for each interplant spacing are not significantly different from one another by DMRT at $p < 0.01$

Table 5. Total flavonoid content and total flavonoid yield of different plant parts of *M. pumilum* at different interplant spacings

Interplant spacing	Total flavonoid content (mg/100g)				Total flavonoid yield (kg/ha)
	Leaf	Stem	Root	Whole plant	
12.5 cm (D1)	1,414 ± 253a	736 ± 270a	1,213 ± 172a	3,363 ± 651a	16.38 ± 2.15a
25 cm (D2)	1,353 ± 240a	680 ± 142a	1,118 ± 344a	3,151 ± 701a	7.72 ± 1.85b
37.5 cm (D3)	1,634 ± 277a	885 ± 2,88a	981 ± 83a	3,500 ± 516a	5.28 ± 0.99b
Mean	1,467	767	1,104	3,338	9.79
Significance	ns	ns	ns	ns	**
CV (%)	17.92	30.89	22.37	20.45	19.41

ns = not significant
**significant at $p < 0.01$
Means with the same letter for each interplant spacing are not significantly different from one another by DMRT at $p < 0.01$

to outgrow each other while trying to attain sufficient sunlight, leading to the tendency of becoming taller and thus having a greater amount of biomass. In line with this finding, Wan Zaki and Musa (2007) also found that the *dukung anak* (*Phyllanthus niruri*) plant grows taller and with a higher level of biomass as the number of rows per bed increased. However, a reverse trend was reported in the dragonhead (*Dracocephalum moldavica* L.) where the widest distance within a row (40 cm) had the maximum mean values of plant height, number of branches/plant and biomass (Hussein 2006). This may be explained by the fact that plant characteristics have a great influence on their growth performance. A widely branched plant like the dragonhead needs a bigger distance between plants for the expansion of its branches. *M. pumilum* on the other hand does not have such a characteristic as it is a small woody plant with a smaller canopy and fewer branches and therefore needs relatively much less space for its growth.

The findings of this research seem to indicate that the phytochemical and polypeptide contents in *M. pumilum* were not influenced by plant spacing. However, since the biomass was drastically increased as the plant density increased, this then translated to higher total flavonoid, phenolic and polypeptide yields per hectare. A number of studies have revealed that the phytochemical and polypeptide contents in plants are more readily influenced by environmental conditions such as light intensity, temperature, carbon dioxide level, fertilisation, biotic and abiotic factors rather than plant density (Wei 2016). These environmental factors are known to directly affect the enzyme activity regulating the metabolic pathways of the metabolites thus affecting their synthesis.

The leaves and roots of *M. pumilum* were found to have higher primary (polypeptides) and secondary metabolites (flavonoids and phenolics) contents as compared to the stem. This finding is an important indication to identify economically important plant parts that should be harvested for medicinal use. This finding is in line with that of Mohd Hafiz (2011) who worked on the same plant species.

Conclusion

The best planting distance of *M. pumilum* planted under a semi-controlled environment (with 80% shade, 70% humidity and temperature of 25 – 35 °C) is 25 cm between rows and 12.5 cm within rows (interplant spacing).The recommendation is based on the highest plant growth performance (plant height, plant canopy and number of leaves), biomass (fresh and dry yield), and total polypeptide, phenolic and flavonoid yields. It is also concluded that the leaves and roots are the most economically valuable parts of *M. pumilum* with the highest total contents of polypeptides, phenolics and flavonoids.

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Abstrak

Marantodes pumilum atau kacip fatimah merupakan salah satu herba bernilai tinggi di Malaysia dan biasanya digunakan oleh wanita Melayu selepas bersalin. *M. pumilum* adalah spesies hutan dan dengan itu, ia perlu disesuaikan dengan persekitaran pertanian sebelum dapat hidup dan tumbuh dengan optimum. Oleh itu, ia sukar untuk ditanam secara ex situ. Pada masa ini, sejumlah besar bahan mentah diperoleh terus dari hutan atau diimport dari negara jiran. Terdapat keperluan mendesak untuk membangunkan amalan kultur yang baik untuk *M. pumilum* seperti kepadatan tumbuhan atau jarak tanaman. Kriteria ini adalah penting untuk memastikan hasil yang tinggi dari segi biojisim dan fitokimia. Jarak tanaman merujuk kepada jarak antara baris dan jarak dalam satu baris. Dalam kajian ini, jarak antara baris ditetapkan pada jarak 25 cm dan jarak dalam satu baris telah diuji dengan tiga jarak berbeza terdiri daripada 12.5 cm (D1), 25 cm (D2) dan 37.5 cm (D3). Kepadatan populasi tanaman yang diuji adalah dalam julat berikut: 320,000 pokok/ha, 160,000 pokok/ha and 106,666 pokok/ha pada D1, D2 dan D3 masing-masing. Kajian jarak tanaman telah dijalankan di bawah struktur jaring dengan 80% teduhan. Kajian ini telah disusun secara statistik dalam susunan blok lengkap secara rawak (RCBD) dengan lima replikasi. Data pertumbuhan pokok, biomass, jumlah kandungan polipeptida, jumlah kandungan fenolik dan jumlah kandungan flavonoid telah direkodkan. Keputusan kajian menunjukkan jarak tanaman dalam satu baris pada 12.5 cm adalah terbaik untuk *M. pumilum* yang telah menghasilkan sebanyak 54 – 65% lebih tinggi hasil basah, 50 – 69% lebih tinggi hasil kering dan 51 – 66% lebih tinggi hasil polipeptida berbanding dengan dua lagi jarak tanaman. Ini menunjukkan kandungan bioaktif secara keseluruhan adalah lebih tinggi dari segi pengeluaran fitokimia dan polipeptida sehektar.