The effects of plant spacing on yield and quality of butterfly pea (*Clitoria ternatea* L.) cultivated in organic-oriented farming system on grey soil

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ABSTRACT

Clitoria ternatea L. is a plant species that can be used in food and pharmaceutical industry. This study was conducted to evaluate the effects of different plant spacing on the productivity and quality of butterfly pea grown on grey soil in Thu Duc, Ho Chi Minh City. Six treatments correspond to plant spacing of 80 x 15 cm, 80 x 20 cm, 80 x 25 cm, 80 x 30 cm, 80 x 35 cm and 80 x 40 cm. The results showed that the highest flower amount (296.8 flowers/plant) was obtained with butterfly pea planted at the spacing of 80 x 15 cm, commercial flower weight (7.86 g/100 flowers), theoretical yield of fresh flower (1,779.0 kg/1,000 m^2), actual yield of fresh flower (841.9 kg/1,000 m^2), theoretical yield of commercial flower (194.6 kg/1,000 m^2) and actual yield of commercial flower (89.0 kg/1,000 m^2). Nevertheless, plant spacings did not affect the dry matter ratio, anthocyanin and tannin content in the commercial butterfly flowers.

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1. Introduction

Clitoria tenatea L. also known as butterfly pea, is a species belonging to Fabaceae family. Currently, the flowers from butterfly pea are being used in food, medicine as well as in cosmetics (Morris, 2009). Especially, the dried butterfly pea flowers can also be used as tea. Butterfly pea tea is characterized by a rich source of natural antioxidants (Kamkaen & Wilkinson, 2009), which is also highly safe (Luu, 2005) and satisfied most requirements of the consumers. Consequently, the flowers are consumed increasingly as healthy food. However, the research on farming techniques which are necessary for butterfly pea reaching high yield and quality, are still limited.

Plant spacing is an important determinant of plant growth, development and productivity (McMurray, 2004; McRae et al., 2008; Khaliq et al., 2009). The impact of crop density is mainly due to differences in solar radiation distribution. An optimization of solar radiation uptake is the most important for photosynthetic efficiency. An appropriate plant density or spacing helps plants take advantage of sunlight energy, reducing pests and diseases, paving the way for high productivity. In addition, appropriate spacing can also save the seedlings, labor and other costs, those turn out to improve the economic efficiency. Contreras et al. (2012) concluded that when planting butterfly pea plant with a distance of 25 x 25 cm gave highest total grain yield, grain yield per plant, number of pods per plant, number of pods per m^2, fruit length, number of seeds in pod as well as the seed weight. However, there is no recommendation for suitable plant spacing for butterfly pea flower used as tea. Therefore, the aim of this research was to identify suitable plant spacing for butterfly pea growing on grey soil in organic-oriented farming system.

2. Materials and Methods

2.1. Experimental design

The experiment was conducted at the Experimental field of Faculty of Agronomy, Nong Lam University, Ho Chi Minh City (September 2019 to January 2020).

The seeds of double-winged butterfly pea variety (collected in Pham Van Coi Commune, Cu Chi District, Ho Chi Minh City) was sown.

Nutrient used for the whole experiment was well composed cow dung, that supplied by the Experimental field of Animal Science and Veterinary Medicine Faculty, Nong Lam University in Ho Chi Minh city. The manure was applied at a rate of 5.0 tons/ha at 15 days before planting.

Insects and butterflies occurrences on the experimental field were cached manually. No other chemical fertilizers or pesticides was used before and during cultivation period.

One-factor experiment was arranged in a Randomized Completed Block Design (RCBD) included 6 treatments with 3 replicates. The plant spacing in the experiment included: 80 x 15 cm (8,333 plants/1,000 m^2), 80 x 20 cm (6,125 plants/1,000 m^2), 80 x 25 cm (The control) (5,000 plants/1,000 m^2), 80 x 30 cm (4,167 plants/1,000 m^2), 80 x 35 cm (3,571 plants/1,000 m^2) and 80 x 40 cm (3,125 plants/1,000 m^2). Total number of experimental plots was 18 plots; a single plot area was 16.8 m^2; The spacing between two neighbouring plots was 0.5 m; The whole experimental area was 302.4 m^2.

2.2. Land preparation and field management

Beds were established with a size of 6.0 x 2.8 m, each bed consisted of 3 single rows, each bed was 80 cm apart and 20 cm from the aisle; Composted cow dung was applied at the rate of 5 tons/ha 15 days before planting.

Staking setup: U-shaped staking was made of bamboo, with a height of 1.5 m, each row consisted of 7 bamboo poles separated by 1.0 m; a black film was used to cover along the rows for weed preventing.

Seeds were sown on a nursery. After 15 days, the seedlings reached 3 pairs of leaves which then were transplanted onto the experimental field. At 60 days after planting, the plant tips were cut off for branch boosting. Experimental field was watered once/day. Weed control was conducted manually once in every 15 days.

When butterfly pea at flowering stage, new opening flowers were harvested every 2 days. The fresh flowers were left on open air in 48 hours for naturally dryness, then finally, dried at 95°C for 40 minutes (Luong, 2004).

2.3. Data collection and statistics

Data from following parameters were collected, including:

Total numbers of flowers per plant (flowers/plant): Count the average number of flowers on the target plants at all harvests until the end of the experiment; Fresh flowers weight (g/100 flowers): Weigh 100 fresh flowers at harvest time 60, 75 and 90 days after planting, then average; Commercial flower weight (g/100 flowers): Weigh 100 dried flowers at harvest time 60, 75 and 90 days after planting, after drying at 95°C for 40 minutes and then average; Theoretical fresh flower yield (kg/1,000 m^2): [Total number of flowers/plant (flower/plant) * fresh flower weight (g/100 flowers) * number of plants/1,000 m^2]/105; Actual fresh flower yield (kg/1,000 m^2): [Total fresh flowers weight/plot (kg) x 1,000]/16,8; Theoretical commercial flower yield (kg/1,000 m^2): [Total number of flowers/plant (flower/plant) * commercial flower weight (g/100 flowers) * number of plants/1,000 m^2]/105; Actual commercial flower yield (kg/1,000 m^2): [Total commercial flowers weight/plot (kg) x 1,000]/16,8.

Anthocyanin content in commercial flowers was determined using the method TCVN 11028:2015; Tannin content was determined by Leventhal method.

Data analysis was conducted with ANOVA test and Duncan rank at significance level $\alpha = 0.01$ using SAS 9.1 software.

Table 1. Soil characters of the experimental plots[1]

Indices	Unit	Result	Method
$pH_{KCl_{(1:5)}}$		5.501	pH meter
$EC_{(1:5)}$	mS/cm	0.367	EC meter
Total Organic Carbon	%	0.718	Tiurin
Total N	%	0.062	Kjeldahl
$N\text{-}NH_4^+$	mg/100 g	0.597	Devardar – Alloy
C/N		11.581	
Total P_2O_5	%	0.053	Colorimeter
Availability P_2O_5	mg/100 g	4.390	Bray #1
Total K_2O	%	0.154	Flame photometer
Exchangeability K_2O	mg/100 g	5.085	Flame photometer
CEC	meq/100 g	7.801	Acetate Amonium
Soil texture	%	Sand: 82.20 Loam: 13.05 Clay: 4.75	Densitometrer

[1]Analyzed by Department of Soil Science, Faculty of Agronomy, Nong Lam University, 2019.

Table 2. Amount and mass of butterfly pea flower under the influence of plant spacings

Plant spacing (cm)	Flower amount (flowers/plant)	Fresh flower weight (g/100 flowers)	Commercial flower weight (g/100 flowers)
80 x 15	296.80[a]	73.23[ab]	7.86[a]
80 x 20	290.70[a]	73.98[a]	7.88[a]
80 x 25 (Control)	277.23[a]	72.95[ab]	7.92[a]
80 x 30	249.20[b]	70.20[b]	7.22[b]
80 x 35	204.50[c]	70.18[b]	7.33[b]
80 x 40	180.00[c]	69.95[b]	7.46[b]
CV (%)	4.27	1.67	1.48
F value	60.93**	6.95**	22.77**

[a-c]In the same column, numbers with the same character are statistically insignificant difference.
**: the difference is statistically significant at $\alpha = 0.01$.

3. Results and Discussions

3.1. Evaluation of soil quality at the experimental field

Physical and chemical analysis results of the experimental soil (Table 1) suggested that the experiment plot soil has texture containing 82.20% of sand, 13.05% of loam and 4.75% of clay. According to García-Gaines & Frankenstein (2015) the soil at the experimental field is belonging to loamy sand texture. The soil was highly acidic ($pH_{KCl_{(1:5)}} = 5.501$) and not saline ($EC_{(1:5)} =$ 0.367 mS/cm) (Slavich & Petterson, 1993). It was recommended that the soil pH ranged from 5.5 to 8.9, which was acceptable for butterfly pea (Singh et al., 2017).

The soil organic C content was low (0.718%) and the C/N ratio was 11.581. The soil had low levels of macronutrients (Rayment & Lyons, 2011). Furthermore, cation exchange capability was also low. However, butterfly pea is a native plant, it is highly adaptable to various soil types therefore this location was acceptable for butterfly pea cultivation. Even those, organic fertilizer supplement is necessary to provide nutrients for plants during cultivation.

3.2. Influence of plant spacing to amount and mass of butterfly pea flower

The number of flowers and flower weight are most important factor correlating to butterfly pea flower yield. At the same plant spacing, the

Table 3. Theoretical and actual yields of butterfly pea under the influence of plant spacing

Plant spacing (cm)	Theoretical fresh flower yield (kg/1000 m^2)	Actual fresh flower yield (kg/1000 m^2)
80 x 15	1,812.6^a	841.9^a
80 x 20	1,345.5^b	721.9^b
80 x 25 (Control)	1,011.0^c	562.2^c
80 x 30	728.5^d	511.0^c
80 x 35	511.8^e	442.3^{cd}
80 x 40	395.0^e	371.6^d
CV (%)	6.3	7.7
F value	233.9**	47.3**

[a-e]In the same column, numbers with the same character are statistically insignificant difference.
**: the difference is statistically significant at $\alpha = 0.01$.

greater the number of flowers and the heavier weight, the higher the yield will be. Results presented in Table 2 showed that the total number of flowers per plant was significantly different between plants grown at different spacing in the experiment. Planting at the spacing of 80 x 15 cm gave the most flowers (296.8 flowers/plant), but not statistically different from the plant spacing of 80 x 20 cm (290,70 flowers/plant) and 80 x 15 cm (277.23 flowers/plant). Planting at the spacing of 80 x 40 cm obtained lowest number of flowers (only 180 flowers/plant), the difference was 116.80 flowers/plant lower as compared to planting at the spacing of 80 x 15 cm.

Fresh flower weight and commercial flower weight of butterfly pea plants were significantly different under the influence of different plant spacing. Butterfly pea plants grown at a spacing of 80 x 20 cm gained the highest fresh flower weight (73.98 g/100 flowers), event it was not statistically different from planting at the spacing of 80 x 15 cm or 80 x 25 cm. The lowest fresh flowers weight gained when planting at the spacing of 80 x 40 cm (69.95 g/100 flowers). It was 4.03 grams lower than when planting at the spacing of 80 x 15 cm.

Similarly, the highest commercial flower weight of butterfly pea was obtained when planting at the spacing of 80 x 20 cm (7.88 g/100 flowers) even it was not statistically different from planting at the spacing of 80 x 15 cm or 80 x 25 cm. Planting at the spacing of 80 x 40 cm gained lowest commercial flower weight (7.46 g/100 flowers) but which was not statistically different from planting at the spacing of 80 x 30 or 80 x 35 cm; It was only 0.42 g lower if compared to planting at spacing of 80 x 15 cm.

Because the experiment conducted at the end of rainy season, most of growth season was during dry and hot weather, plant population at higher density perhaps made microclimate not as hot as in lower density population. The result shown that at higher plant spacing (80 x 15 cm, 80 x 20 cm, 80 x 25 cm), butterfly pea plants grown better, giving more flowers and higher flower mass. This result were in accordance with a report by Tran & Pham (2018) on *Limnophila rugosa* (Roth) Merr. when they found that growing in a long spacing, especially when the growing substrate covered so it is less affected. Because of high temperature at the experimental area, the plants grew well and formed more leaves.

3.3. Influence of plant spacing to theoretical and actual yields of butterfly pea

Results presented in Table 3 shown that butterfly pea grown at the spacing of 80 x 15 cm reached highest theoretical yield as well as actual flower yield (1,812.6 and 841.9 kg/1,000 m2, respectively). The actual yield of fresh flowers accounts for 46.4% of the theoretical fresh flower yield. Besides, butterfly pea grown at the spacing of 80 x 40 cm, yielding the lowest theoretical and actual fresh flower yields (395.0 and 371.6 kg/1,000 m^2, respectively). The actual yield of fresh flowers accounts for 94.1% of the theoretical fresh flower productivity. The difference in the ratio of actual yield and theoretical yield was due to the level of coverage of the pea plants. When the pea plants were higher density, it might lead to the plants being overlapped. In fact, plants at high density tended to be overlapped each other, which affected to actual numbers of harvestable flowers on the plot. As a consequence, there was a big difference between the theoretical fresh yield

Table 4. Commercial flower yields of butterfly pea under the influence of plant spacings

Plant spacing (cm)	Theoretical commercial flower yield (kg/1000 m^2)	Actual commercial flower yield (kg/1000 m^2)
80 x 15	194.6^a	89.0^a
80 x 20	143.4^b	75.9^b
80 x 25 (Control)	109.8^c	60.1^c
80 x 30	75.0^d	52.5^{cd}
80 x 35	53.6^e	45.7^{de}
80 x 40	42.0^e	39.0^e
CV (%)	5.7	7.3
F value	296.6**	55.2**

[a-e]In the same column, numbers with the same character are statistically insignificant difference.
**: the difference is statistically significant at $\alpha = 0.01$.

Table 5. Dry matter, anthocyanin and tannin contents of butterfly pea flowers under influence of plant spacings

Plant spacing (cm)	Contents (%)		
	Dry matter	Anthocyanin	Tannin
80 x 15	10.753	0.538	1.817
80 x 20	10.661	0.546	1.824
80 x 25 (control)	10.856	0.538	1.820
80 x 30	10.295	0.540	1.824
80 x 35	10.490	0.540	1.827
80 x 40	10.635	0.536	1.829
CV (%)	1.930	1.745	0.483
F value	2.831^{ns}	0.380^{ns}	0.760^{ns}

ns: non-significant.

and the actual yield. Less shading between plants reduced the difference.

3.4. Influence of plant spacing to commercial flower yields of butterfly pea

Commercial flower ratio is a determinant of economic efficiency for the farmers. In this research, butterfly pea flowers were naturally dried for 48 hours at room temperature then transferred to temperature of 95°C for 40 min in order to reach commercial quality level with moisture content was about 12%. The results presented in Table 4 shown that the difference of theoretical and actual commercial flower yield was statistical significance between flower collected from plants grown in different spacing. Butterfly pea plants grown at the spacing of 80 x 15 cm obtained the highest theoretical commercial yield (194.6 kg/1000 m^2); the plants grown at the spacing of 80 x 40 cm (42.0 kg/1000 m^2), was 152.6 kg/1000 m^2 lower than that.

The actual harvest of commercial flowers was also highest with the butterfly pea planted at the spacing of 80 x 15 (reaching 89.0 kg/1,000 m^2). It was statistically significant higher as compared to plants in all other treatments. Plant grown at the spacing of 80 x 40 cm, showed the lowest commercial flower (39.0 kg/1,000 m^2); it was 40 kg/1,000 m^2 lower as compared to plants grown at the spacing 80 x 15 cm.

In general, it was obvious that the plant density greatly affected to both theoretical and commercial flower yields. The butterfly pea when grown at a higher density obtained a higher yield. At the same time, the difference between theoretical yield and actual yield was high.

3.5. Influence of plant spacing to dry matter, anthocyanin and tannin contents of butterfly pea flowers

Results in Table 5 indicated that different plant spacing did not affect the quality of butterfly pea flower indices including dry matter ratio, anthocyanin and tannin content in dried flowers. The dry matter ratio of the butterfly pea flower ranged from 10.3 to 10.86%. In the commercial flowers, anthocyanin content ranged from 0.54 to 0.55%. This result suggested that anthocyanin in butterfly pea flowers is higher than that in some fruits such as blueberries (0.08 to 0.53%), cherry (0.35 to 0.45%), black raspberry (0.08 to 0.18%) (Horovitz et al., 2008). Anthocyanin related to the intensity of plant colour, the darker the colour, the higher the anthocyanin content. Nevertheless, tannin content in commercial butterfly peas ranged from 1.82 to 1.83%, is much lower than that in black tea (13.36%), green tea (2.65%) and Oolong tea (8.66%) (Khasnabis et al., 2015). Tannin is polyphenol compounds in plants that help to against bacteria and induce acrid taste, it plays an important role in the quality of tea products.

4. Conclusions

Pea flower of plants grown at the spacing of 80 x 15 cm gained highest number of flowers on plant (296.8 flowers/tree), dry flower weight (7.86 g/100 flowers), the theoretical fresh flower yield (1,779.0 kg/1,000 m^2), the actual fresh flower yield (841.9 kg/1,000 m^2), the theoretical commercial flower yield (194.6 kg/1,000 m^2) as well as the actual commercial flower yield (89.0 kg/1,000 m^2). The different plant spacing did not affect quality criteria such as dry matter, anthocyanin and tannin content in commercial butterfly pea flower.

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