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RESPONSES OF HOT PEPPER (*Capsicum chinense* Jacq.) CULTIVARS TO DIFFERENT CONTAINER SIZES FOR CAPSAICINOID PRODUCTION UNDER CONTROLLED HOUSE CONDITIONS

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SUMMARY

This study was to investigate the effects of container size on capsaicinoid production of hot pepper (*Capsicum chinense* Jacq.) cultivars under controlled house conditions. This experiment was conducted in the rainy season during the period from May to October 2015 and the dry season from October 2015 to March 2016. A factorial experiment in a randomize complete block design with three replications evaluated growth traits, the capsaicinoid production and its components. Factor A was two hot pepper cultivars, and factor B was three container sizes, i.e., 8 liter (L), 15 L and 21 L. Plant growth, dry fruit yield, capsaicinoid content, and capsaicinoid yield were recorded. The growing season affected all characteristics studied. Hot peppers grown in the rainy season had higher plant growth, dry fruit yield, and capsaicinoid yield than those plants grown in the dry season. Whereas, hot pepper fruits in the dry season contained more capsaicinoid content than those in the rainy season. Capsaicinoid yield of hot pepper depended on cultivars and the container size. Akanee Pirote grown in 21 L container produced the highest dry fruit yield (192 g/plant) and capsaicinoid yield (4,287 mg plant^{-1}) in both seasons. These results show that *C. chinense* F_1 hybrid with high pungency grown in a particular container size should be used to maximize the capsaicinoid production for food and pharmaceutical industries.

Key words: Chili, environment, genetic, heat sensation, pungency, season

Key findings: Hot pepper cultivars showed different responses to various container sizes for all traits. Akanee Pirote, an F_1-hybrid, produced the highest capsaicinoid yield grown in 21 L under controlled environment.

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INTRODUCTION

Pungency or the burning sensation of hot pepper is due to capsaicinoids, a unique alkaloid that are produced from the phenylpropanoid pathway and fatty acid synthesis pathway (Fujiwake *et al*., 1982; Blum *et al*., 2003). The two major capsaicinoids, capsaicin and dihydrocapsaicin, accounted for about 90% of the pungency in hot pepper fruits. Capsaicinoids have several health benefits including anti-oxidant, anticancer and antiarthritic benefits (Prasad *et al*., 2005), and are used in food, medicine and pharmaceutical industries, which increases its commercial value. The genus *Capsicum* contains five domesticated species: *C*. *annuum* L., *C*. *baccatum* L., *C*. *chinense* Jacq., *C*. *frutescens* L. and *C*. *pubescens* Ruiz & Pav with approximately 32 wild species (Bosland and Votava, 2012). *C*. *annuum* is the most commonly cultivated species for its pungent (chili) and non-pungent (sweet pepper) fruits valued as spices and vegetables. While, the world's hottest hot peppers are recognized in the *C. chinense* species such as 'Bhut Jolokia' (about 1,000,000 SHU) and Moruga Scorpion (Bosland and Baral, 2007; Bosland *et al*., 2012) and can be an excellent source of capsaicinoid for the extraction industry.

Due to various biotic (pest and disease), abiotic (rainfall, temperature, and light intensity) stresses, the crops grown in open-field conditions are often subjected to unfavorable environment that strongly affects crop production. Therefore, to obtain good quality and high crop yield, there is a need to cultivate crops under controlled house conditions. Unfortunately, investment and production costs are usually higher under the controlled house operation than in the field. Crop management under controlled house also requires more efficiency than those grown under open-field conditions. High value crops grown in efficient production systems need to ensure a high return in relation to investment costs.

Container size is one of the most important factors for growing plant in a controlled house to maximize fruit production per unit area of hot pepper. The physical restriction of the container for the root growth limits plant growth (Bouzo and Favaro, 2015). The decrease of the container size for the root growth decreases the photosynthesis of the plants because of physiological mechanisms similar to those of water stress (Peterson *et al*., 1991a; Ismail and Noor, 1996). A previous study reported that bell pepper (*C*. *annuum*) grown in container size 33 L produced higher fruit yield and larger fruit than those in 9 L and 8 L (Xu *et al*., 2001). However, Shaw *et al*. (2008) found that container size 11 L is appropriated for growing hot pepper

(*C*. *chinense*) under controlled house. Furthermore, the effect of container size may depend on hot pepper cultivars (Shaw *et al*., 2008). These results indicate that different hot pepper species and cultivars require different container sizes for maximizing capsaicinoid production.

Information on the effect of plant density on capsaicinoid production in hot pepper (*C*. *chinense*) is needed especially with hot pepper cultivars having varying pungency levels. Plant growth, fruit yield and capsaicinoid production of hot pepper is depended on genotype, environment, and their interaction (Jeeatid *et al*., 2018). The aim of this research was to study the response of two hot pepper cultivars (*C*. *chinense*) to three different container sizes in growth, dry fruit yield, accumulation of capsaicinoid and capsaicinoid yield characteristics under controlled house conditions.

MATERIALS AND METHODS

Hot pepper plants were grown under net house conditions. The experiments were conducted at Khon Kaen University, Thailand (latitude 16° 28′ N, longitude 102° 48′ E, 200 m above mean sea level) from May to October 2015 (rainy season) and from October 2015 to March 2016 (dry season). In order to synchronize the time of flowering for both cultivars, the sowing dates were adjusted based on the growth habits of each cultivar. The seedlings were transplanted to different sizes plastic containers filled with a mixture of rice husk, charcoal rice husk, filter cake (organic waste from the sugar industry), and composted cow manure in a ratio of 2:1:1:0.5 (v:v) and placed within the net house condition. The containers were placed in rows with 80 cm between the pots and 80 cm between rows. The plants were irrigated daily through a micro-drip irrigation system to field capacity. All plants received the same amount of fertilizer at three-day intervals by fertigation throughout the experiment (adapted from Patricia, 1999). The experimental used a factorial in randomize complete block design with three replications. The two hot pepper cultivars were assigned as Factor A, i.e. Bhut Jolokia and Akanee Pirote, and the three container sizes, i.e., 8 L, 15 L and 21 L, were Factor B.

In this study, two hot pepper cultivars were used. The F_1 hybrid, Akanee Pirote is one of the best known cultivars grown for capsaicin extraction for the pharmaceutical industry in Thailand. Akanee Pirote has a perennial growth habit, large canopy size, a relative large leaf size and an average heat level of 500,000 Scoville Heat Units (SHU). The Bhut Jolokia, one of the world’s hottest peppers, has a perennial growth habit, big canopy size, and a heat level of 800,000-1,200,000 SHU. Capsaicinoid extraction of Bhut Jolokia was used in food and pharmaceutical industries.

Throughout the experiment, air temperature and relative humidity (RH) were recorded every hour by data logger (LogTag HAXO-8, LogTag Recorders Limited, New Zealand). Plant height, canopy width, and stem diameter were recorded at the time of second fruit harvest, approximately 140 days after transplanting (DAT). Mature fruits were harvested at 45-50 days after flowering and total number of ripe mature fruits was recorded. For dry fruit yield, fruits were oven-dried at 70 °C until constant weight. For capsaicinoid quantification, five mature fruits per plant were harvested per replication, and ground to a uniform powder following the procedures of Collins *et al*. (1995). Capsaicinoids were extracted and quantified according to the short run method with high-performance liquid chromatography (HPLC), as described by Collins *et al*. (1995). The data were converted from parts per million to Scoville Heat Units (SHU) by multiplying the parts per million by 16. Only capsaicin and dihydrocapsaicin were measured and pooled as total capsaicinoids because they are the primary and majority of capsaicinoids found in hot pepper accounting for more than 90% of the capsaicinoid amount. Capsaicinoid yield was calculated based on dry fruit yield, and then multiplying the capsaicinoid content per plant.

Error variances were tested for homogeneity (Gomez and Gomez, 1984). A combined ANOVA was performed for all traits to estimate the effects of growing environment, cultivar, and growing environment-by-cultivar interactions. Least significant differences (LSD) compared the significant differences between cultivars and growing environment effect.

RESULTS

In the rainy season, the maximum air temperature was 41 °C and the minimum was 24 °C (Figure 1), with the monthly average RH averaging 80% throughout the experiment. During the experiment, the total rainfall was 738 mm in the rainy season. For the dry season, the maximum air temperature was 34 °C and the minimum was 20 °C, and the monthly average RH averaged 54% throughout the growing season. In the dry season, the total rainfall was 25 mm during the experiment.

Whether it was the rainy season or the dry season significantly affected all the traits studied ($P \le 0.05$) (Table 1). Container size also affected significantly all traits studied ($P \le 0.05$) except for the amount of dihydrocapsaicin and capsaicinoid content traits. The interactions between season and cultivar were significant for most traits except for stem diameter and capsaicin amount. Additionally, the interactions between cultivar and container size were significant for fruit number, dry fruit yield, capsaicin and capsaicinoid yield.

Hot peppers grown during the rainy season produced higher plant height, canopy width and stem diameter than the plants grown in the dry season (Table 2). In the rainy season, the higher plant height, canopy width, and stem diameter were found in 15 L and 21 L containers, however there were no significant differences between cultivars. For the dry season, plant height and canopy width of Akanee Pirote were higher than Bhut Jolokia, and both cultivars gave the highest plant height and canopy width in 15 L and 21 L containers. The largest stem diameter was obtained in the 21 L container.

In general, hot pepper plants during the rainy season produced more fruit number and dry fruit yield than those plants in dry season (Figure 2a-d), and Akanee Pirote showed higher fruit number and dry fruit yield than Bhut Jolokia in both seasons. The highest fruit number and dry fruit yield was obtained in Akanee Pirote with the 21 L container in both seasons. Fruit number of Akanee Pirote with the 15 L container was not significantly different from Akanee

Pirote grown in the 21 L container. In addition, the container size did not affect the fruit number and dry fruit yield of Bhut Jolokia.

The capsaicin, dihydrocapsaicin, and the capsaicinoid levels were increased during the dry season (Figure 3a-f). The two cultivars did respond differently under the different container sizes and seasons in terms of capsaicin, dihydrocapsaicin, and the capsaicinoid accumulation. The capsaicinoid accumulation under the dry season (1,160,135 SHU) was higher than when the plants were grown during the rainy season (857,629 SHU). In addition, capsaicinoid content of Bhut Jolokia (1,325,324 SHU) was higher than Akanee Pirote (692,440 SHU) in both seasons. Moreover, Bhut Jolokia gave the highest capsaicinoid content with the 15 L container, while the capsaicinoid content of Akanee Pirote with the 21 L container was higher than that with the 15 L container, but was not significantly different as compared to the 8 L container.

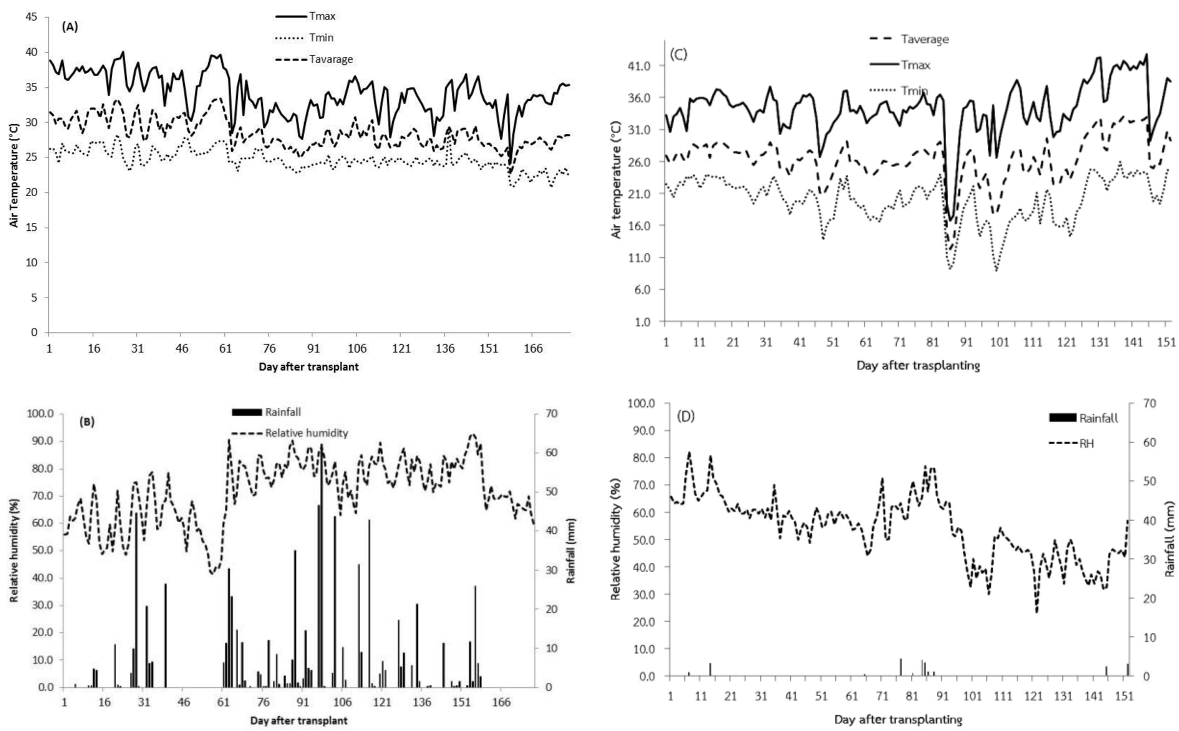

Figure 1. Maximum air temperature (T max), minimum air temperature (T min), average air temperature (T average), rainfall and relative humidity (RH) in rainy (A, B) and dry season (C, D) 2015 at research farm, Khon Kaen University.

In general, hot pepper grown in rainy season showed higher capsaicinoid yield than when grown in the dry season (5,350 and 1,725

Table 1. Combined analysis of variance for plant growth, fruit yield and pungency of two hot pepper cultivars grown under different container sizes in the rainy and dry seasons at Khon Kaen University, 2015 (d.f.: degree of freedom; **P* ≤ 0.05, ** *P* ≤ 0.01).

Source	d.f.	Plant height	Canopy width	Stem diameter	Fruit number	Dry yield	Capsaicin	Dihydrocapsaicin	Capsaicinoid	Capsaicinoid yield
Season (S)	1	3645.48**	5032.88**	0.8967**	139559**	62918.1**	2.72×10^{11}**	1.49×10^{11}**	8.24×10^{11}**	1.19×10^{8}**
Reps. within S	4	213.56	226.88	0.0234	505	240.0	5.31×10^{8}	4.02×10^{7}	4.66×10^{8}	1.03×10^{6}
Container (P)	2	1367.87**	3239.60**	0.3615**	7758**	4171.5**	7.04×10^{9}*	1.16×10^{9ns}	1.39×10^{9ns}	1.15×10^{7}**
S x P	2	27.51^{ns}	91.32^{ns}	0.0210^{ns}	1184**	524.4**	$5.92 \times 10^{8\ ns}$	3.56×10^{8ns}	1.22×10^{8ns}	7.80×10^{5ns}
Error (a)	8	20.70	34.63	0.0065	103	53.2	1.49×10^{9}	3.69×10^{8}	3.21×10^{9}	3.32×10^{5}
Cultivar (C)	1	447.46**	0.15^{ns}	0.0054^{ns}	108948**	37711.4**	1.04×10^{12}**	7.76×10^{11}**	3.60×10^{12}**	1.33×10^{7}**
S x C	1	1307.06**	943.96**	0.0013^{ns}	13702**	3392.4**	1.25×10^{9ns}	1.50×10^{10}**	2.50×10^{10}*	5.98×10^{6}**
P x C	2	54.74^{ns}	14.14^{ns}	0.0026^{ns}	3942**	2375.0**	7.83×10^{9}*	1.38×10^{9ns}	1.48×10^{10ns}	3.78×10^{6}**
S x P x C	2	14.49^{ns}	88.31^{ns}	0.0008^{ns}	567*	192.4*	4.91×10^{8ns}	4.89×10^{8ns}	9.53×10^{8ns}	3.87×10^{4ns}
Error (b)	12	31.51	59.60	0.0038	89	43.4	2.00×10^{9}	4.32×10^{8}	4.19×10^{9}	3.29×10^{5}
CV a (%)		3.54	4.31	4.95	9.27	10.13	5.61	5.99	5.62	16.27
CV b (%)		4.36	5.65	3.78	8.63	9.15	6.49	6.48	6.41	16.19

mg/plant, respectively) (Table 3). The highest capsaicinoid yield was obtained in Akanee Pirote with 21 L container (7,246 mg/plant) during the rainy season. While, Bhut Jolokia gave the highest capsaicinoid yield in 15 L and 21 L container (5,514 and 5,680 mg/plant, respectively). During the dry season, Akanee Pirote gave the highest capsaicinoid yield with the 21 L container (4,287 mg/plant). The capsaicinoid yield of Bhut Jolokia did not differ significantly within the various container sizes.

Table 2. Plant heights, canopy width and stem diameter of two hot pepper cultivars grown in different container sizes in rainy and dry seasons at Khon Kaen University, 2015.

Treatment		Plant height (cm)		Canopy width (cm)		Stem diameter (cm)	
		Rainy	Dry	Rainy	Dry	Rainy	Dry
Cultivar (C)							
Bhut Jolokia		141.2	109.0b	151.2	119.7b	1.78	1.49
Akanee Pirote		136.2	128.1a	143.2	129.8a	1.81	1.45
Container (P)							
8		124.7b[z]	107.9b	126.4b	108.9b	1.62b	1.25c
15		144.9a	124.0a	154.6a	132.9a	1.89a	1.52b
21		146.5a	123.8a	160.7a	132.5a	1.89a	1.65a
Interaction (C x P)							
Bhut Jolokia	8	126.9	100.6	128.6	106.9	1.60	1.23
	15	145.3	111.7	158.1	128.1	1.86	1.50
	21	151.4	114.9	166.9	124.2	1.89	1.64
Akanee Pirote	8	122.5	115.3	124.2	110.8	1.64	1.27
	15	144.4	136.4	151.1	137.8	1.91	1.55
	21	141.7	132.7	154.4	140.9	1.89	1.66
Means		138.7A[x]	118.6B	147.2A	124.8B	1.80A	1.48B

[z]Means within a column followed by the same common letter are not significantly different at ($P \leq 0.05$) by least significant difference.
[x]Different capital letter(s) indicate significant difference between seasons

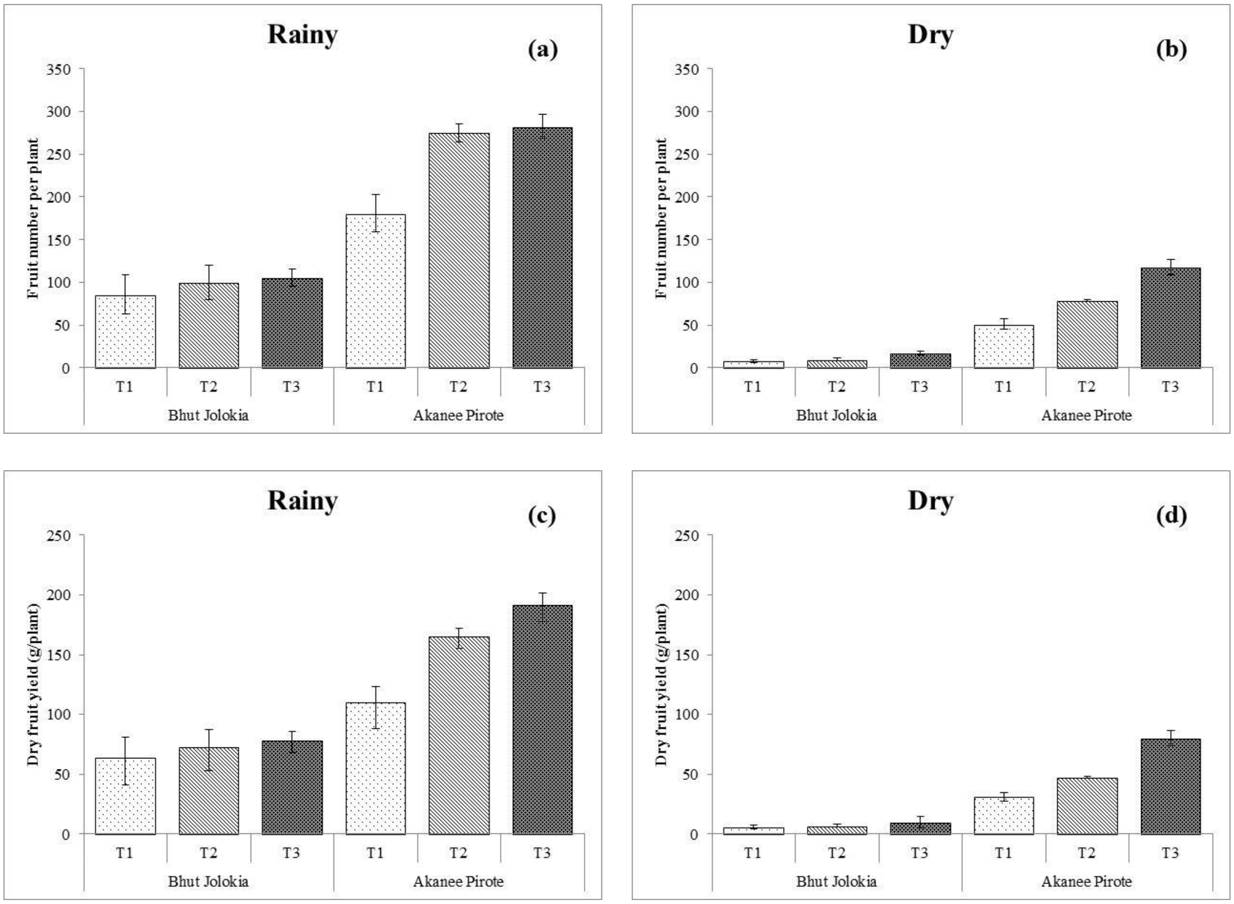

Figure 2. Fruit number (a, b) and dry fruit yield (c, d) of two hot pepper cultivars grown under different container sizes in rainy and dry season under controlled house at research farm, Khon Kaen University 2015.

Table 3. Capsaicinoid yield (mg/plant) of two hot pepper cultivars grown in different container sizes in rainy and dry season at Khon Kaen University, 2015.

Cultivar	Rainy			
	8 L	15 L	21 L	Means
Bhut Jolokia	4,283.8c[z]	5,514.2b	5,679.9b	5,159.3
Akanee Pirote	3,910.6c	5,528.2b	7,245.5a	5,561.4
Means	4,097.2C[x]	5,521.2B	6,462.7A	
	Dry			
Bhut Jolokia	544.4d	681.4d	902.1cd	709.3B
Akanee Pirote	1,549.3bc	2,388.0b	4,287.0a	2,741.4A
Means	1,046.9C	1,534.7B	2,594.6A	

[z]Means followed by the same common letter are not significantly different at ($P \leq 0.05$) by least significant difference.
[x]Different capital letter(s) indicate significant difference between cultivars and container sizes.

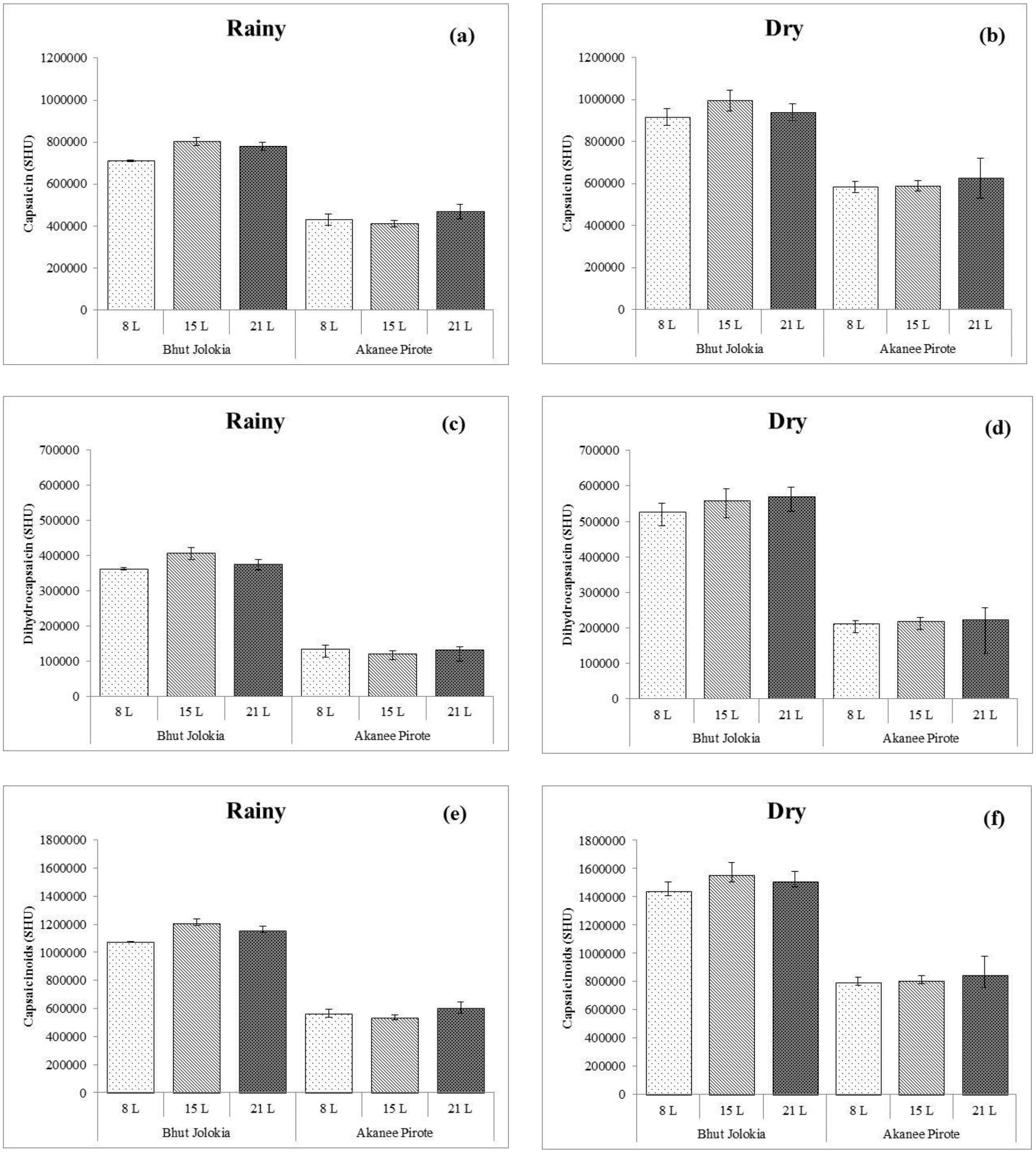

Figure 3. Capsaicin (a, b), dihydrocapsaicin (c, d), and capsaicinoids (e, f) of two hot pepper cultivars grown under different container sizes in rainy and dry season under controlled house at research farm, Khon Kaen University 2015.

DISCUSSION

With the two cultivars cultivated, the highest plant growth and fruit yield came during the rainy season. It has been shown that hot peppers grown under high humid conditions produces higher plant growth, fruit yield and capsaicinoid amount than plants grown under low humidity conditions (Tiwari *et al*., 2005; Jeeatid *et al*., 2017b). The cooler period during the dry season may have extended the period of early vegetative and reproductive growth because temperature has a strongly influence on growth and development (Wien, 1997). During the dry season experiment, there was also a large decrease in canopy growth and this could be because at the lower temperatures the net assimilation rate was low (De Swarte *et al*., 2006). Moreover, the reduction in yield during the dry season may be because the lower RH (60-70%) affected pollen development and pollen viability (Harel *et al*., 2014). The fruits that were produced during the dry season contained high capsaicinoid content which could be due to water stress suffered by the hot pepper plants (Phimchan *et al*., 2014; Jeeatid *et al*., 2018). Low humidity conditions induce high evapotranspiration rates in plants that cause water stress in hot peppers. Hot pepper fruits produced during the dry season in Thailand should be expected to have higher capsaicinoid content than those produced during the rainy season.

Small container size (8 L) strongly inhibited the canopy size of the two hot peppers cultivars tested in the experiment. Small containers limit root growth as well as nutrition supply and water availability to the hot pepper plants (Hameed *et al*., 1987). In addition, restriction of root growth imposed by the small container decreased root respiration, oxygen supply, photosynthesis and photoassimilates that affect plant growth and fruit yield of hot pepper (Robbins and Pharr, 1988; Peterson *et al*., 1991b). Our results demonstrated that Akanee Pirote gave higher dry fruit yield in 15 L and 21 L than in 8 L container, which may be because the Akanee Pirote in 15 L and 21 L had larger canopy size, increased number of nodes, which lead to increased fruit setting. Moreover, both cultivars have a perennial plant growth habit that made it possible to grow those cultivars in large containers for an extended harvesting period. The small container size affected the fruit number and dry fruit yield more in Bhut Jolokia than Akanee Pirote, because Akanee Pirote has a larger root system than Bhut Jolokia (Jeeatid *et al*., 2017a).

The two cultivars of hot peppers grown in the small container produced lower capsaicinoid content than those grown in the larger container sizes indicates that severe stress from small container probably reduced the accumulation of capsaicinoids (Jeeatid *et al*., 2018). An explanation of this outcome is that hot peppers grown under severe stress accumulate peroxidase enzyme (Phimchan *et al*., 2014), which is a key enzyme for oxidation of capsaicinoid synthesis (Contreras-Padilla and Yahia, 1998). The phenylalanine ammonia-lyase has been reported as key enzyme in capsaicinoid biosynthesis and is product of the shikimate pathway (Phimchan *et al*., 2014). When hot peppers are grown in the larger container size, those plants may accumulate more carbohydrates which is a substrate in the shikimate pathway resulting in high enzyme activities and capsaicinoid content. In addition, capsaicinoids of Akanee Pirote were more sensitive to the changes of container size and season than Bhut Jolokia. The sensitivity of this cultivar to different growing conditions may be explained by the fact that Akanee Pirote showed higher capsaicin/dihydrocapsaicin ratio (3.0) than Bhut Jolokia (1.8), and capsaicin is more sensitive to the change of environment than dihydrocapsaicin (Gurung *et al*., 2012).

The hybrid cultivar of *C. chinense*, Akanee Pirote, produced more capsaicinoid yield than the open-pollinated cultivar, Bhut Jolokia, may be because of heterosis, which leads to more fruit number and dry fruit yield than open-pollinated or pure line cultivars (Gelata and Labuschagne, 2004). Akanee Pirote had higher capsaicinoid yield than Bhut Jolokia. Although, Bhut Jolokia produced a 2-fold higher capsaicinoid contents than Akanee Pirote, but Akanee Pirote produced a 3-fold higher dry fruit yield than Bhut Jolokia. Thus, F_1 hybrid cultivars of hot peppers should be used for increasing capsaicinoid yield. On the other hand, different hot pepper cultivars show different responses to the growing environment with respect to capsaicinoids accumulation (Zewdie and Bosland, 2000). The highest capsaicinoid production for food and pharmaceutical industries comes from growing F_1 hybrid hot peppers under appropriate container size.

In this study, container size affected all characteristics studied. Moreover, hot pepper cultivars grown during the rainy season produced higher capsaicinoid yields than those in the dry season. It was also found that each hot pepper cultivar requires a different container size to maximize capsaicinoid yield. Akanee Pirote, an F_1 hybrid, grown in a 21 L container had the highest dry fruit yield and capsaicinoid yield in both seasons indicating that it can be used to produce hot peppers for the food and pharmaceutical industries.

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