CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Experiment I Effects of Leaf Maturity on Asiaticoside and Nutritional Content in Bua Bok

4.1.1 Growth of Bua Bok

The tree accessions of Bua Bok were grown under full sunlight, then their leaves were harvested on days 7, 14, 21, 28 and 35, after emerging to determine the leaf area. It was found that, in the same age, each accession of Bua Bok had different sizes of leaf areas. Nakhon Si Thammarat accession had the highest average leaf area and was statistically different from Ubon Ratchathani and Rayong accessions (Fig 4.1). However, leaf area of Ubon Ratchathani and Rayong accessions were not statistically different. Considering the leaf age of Bua Bok, the results indicated that increasing leaf area and leaf age to 28 and 35 days after emerging were not statically different. Therefore, it could be said that Bua Bok leaves were mature at approximately 28 days after emerging. The interaction between accessions of leaf age by leaf area was also found. The leaf of Nakhon Si Thammarat accession at age 35, 28 and 21 days after emerging had the largest average leaf areas which were 11.71, 11.70 and 11.01 cm^2, respectively (Fig. 4.3 and Fig.4.4). It could be noticed that the Bua Bok leaves at age 35 days after emerging started to turn yellow in some parts (Fig 4.2).

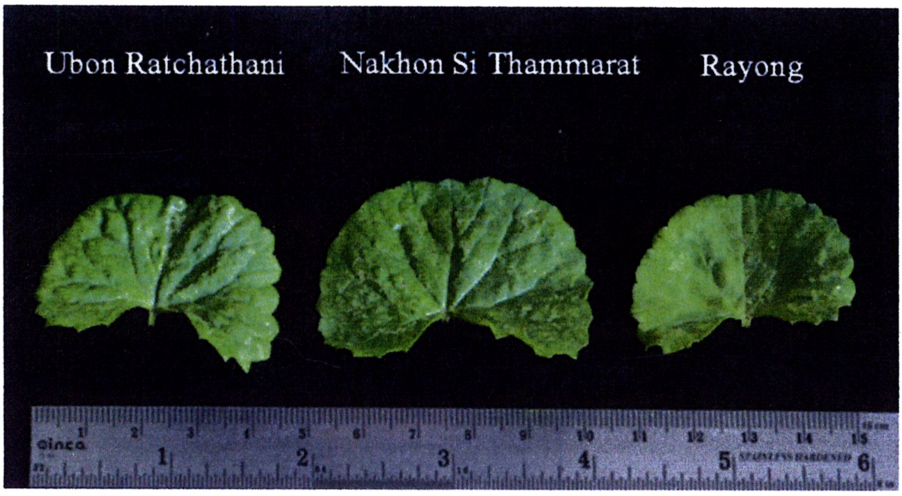
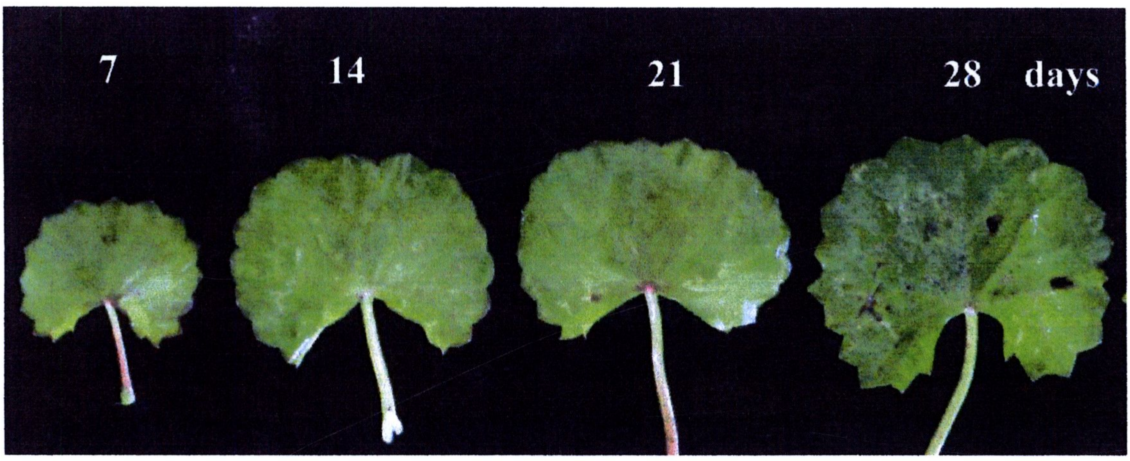


Fig 4.1 The leaves of three accessions of Bua Bok at 28 days after emerging

Fig. 4.2 Leaves of Bua Bok from the first day to yellow blade

The results of this experiment were in accordance with the increased leave areas of Spinach at higher age (Ratana, 2004). The plant developed further when it grew up as its cells divided and grew resulting in cell enlargement (Lilly, 2001). This growth would develop different parts of plants such as increasing leaf size and amount, and leaf area. Each age range during the leaf development was not at the same rate. The leaves at the point of maximum development would be the largest expansion and the leaf area would be much increased. After this period, leaves would turn into yellow color and fall (Hopkins, 1999).

Nakhon Si Thammarat accession



Rayong accession

Ubon Ratchathani accession

Fig. 4.3 The leaf of three accessions at different leaf age 7, 14, 21 and 28 days after emerging.

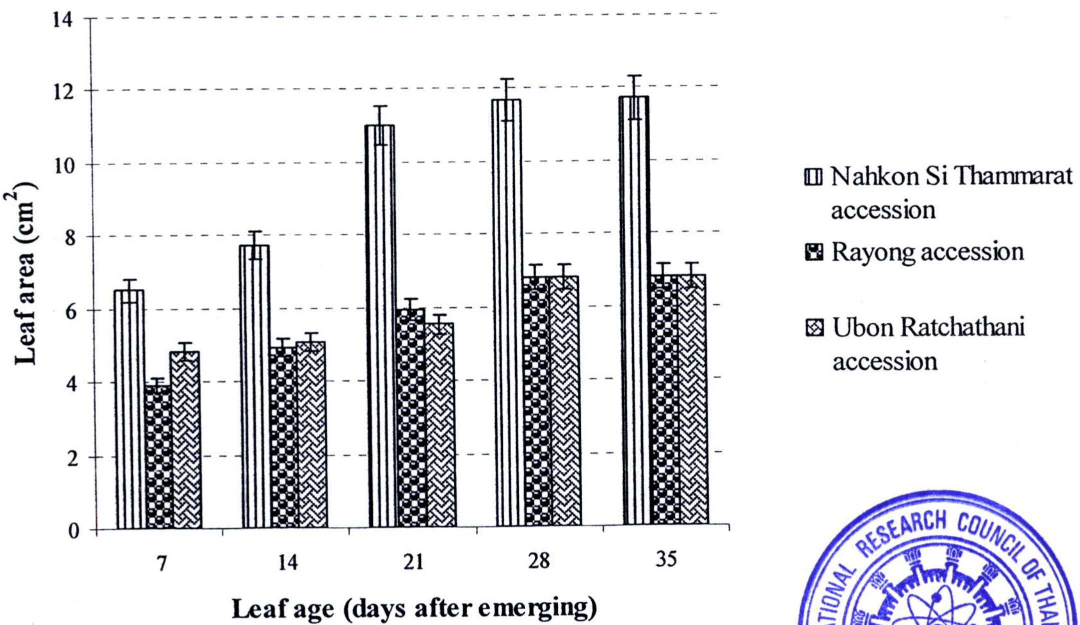


Fig 4.4 Leaf area of various accessions of Bua Bok and leaf age

4.1.2 Nutritional value with age leaf of Bua Bok

The 3 accessions of Bua Bok were planted at the full sunlight. The leaves were collected at different ages to determine the fiber, protein, calcium and beta-carotene contents. The results illustrated that all accessions and leaf age affected the fiber, protein, calcium and beta-carotene contents with statistical difference. Nakhon Si Thammarat accession had the highest average fiber content at 24.53 g/100 g dry weight while Ubon Ratchathani and Rayong accession contained fiber at 24.44 and 22.92 g/100 g dry weight, respectively. The results presented that the average age of the Bua Bok leaf with most fiber volume was 28 days after emerging. (Fig. 4.5).

This experiment was consistent with the comparative amount of fiber in Chinese Spinach (*Amaranthus tricolor*) which was analyzed to determine the fiber content from Chinese Spinach that harvested at different ages. It was found that the fiber content was statistically different. The volume of fiber was much greater when harvested at higher ages (Ratana, 2004).

The amount of fiber in plant was increasing during its lifetime, because the development of sections within plant would result in an accumulation of expanded fibers. The accumulation of fibers would be around branch of petioles which enlarged in each age of plant. So when resources were accumulated to develop more and more expansion, the fiber content of the cumulative rate would be multiplied as well (Lilly, 2001). For this experiment, the results revealed that the fiber content would be added by harvested age in Ubon Ratchathani accession while that of Rayong and Nakhon Si Thamarat accessions tended to be uncertain as it could be due to the accessions or sampling. However, when considered in conjunction with the accession at the same age, it also represented that each accession provided the different fiber volume as well.

The average fiber content of Bua Bok leaves at maturity period was 24.62 g/100 g dry weight which was remarkably higher than that Bua Bok grown in South Africa and India in that the fiber content was previously reported at 1.92 g/100 g fresh weight and 5.92 g/100 g fresh weight, respectively (Odhav *et al.*, 2007; Gupta *et al.*, 2005). This might be due to the differences of geography affecting the accumulation of fiber in Bua Bok.

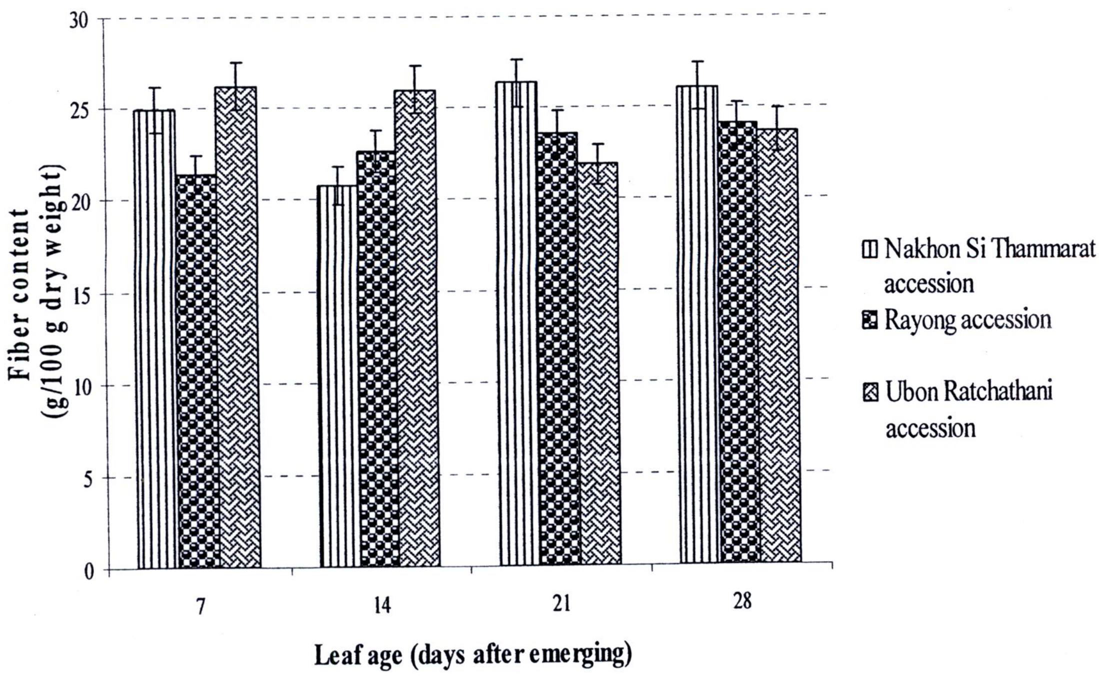


Fig 4.5 Fiber content of various accessions of Bua Bok and leaf age

Accessions and leaf age were significantly different in the protein content. Ubon Ratchathani accession had the most average amount of protein (14.83 g/100g dry weights) followed by Nakhon Si Thammarat and Rayong accessions which contained protein contents of 14.25 and 13.85 g/100g dry weight, respectively (Table A.3). It was found that there was a collaborative interaction between accessions and leaf age. The highest amount of protein (15.21 g/100g dry weight) was found in Ubon Ratchathani accession of 14 days after emerging (Fig 4.6). The protein content in Nakhon Si Thammarat and Ubon Ratchathani accessions tended to decrease with ages. The protein content in Rayong accession had variance with no direction. Hegart and Peterson (1973) found that the protein content in the leaves varied with plant species, varieties, age, fertilizing and density of sunlight, length of light, and the water content. Therefore, the protein contents in 3 Bua Bok accessions were various.

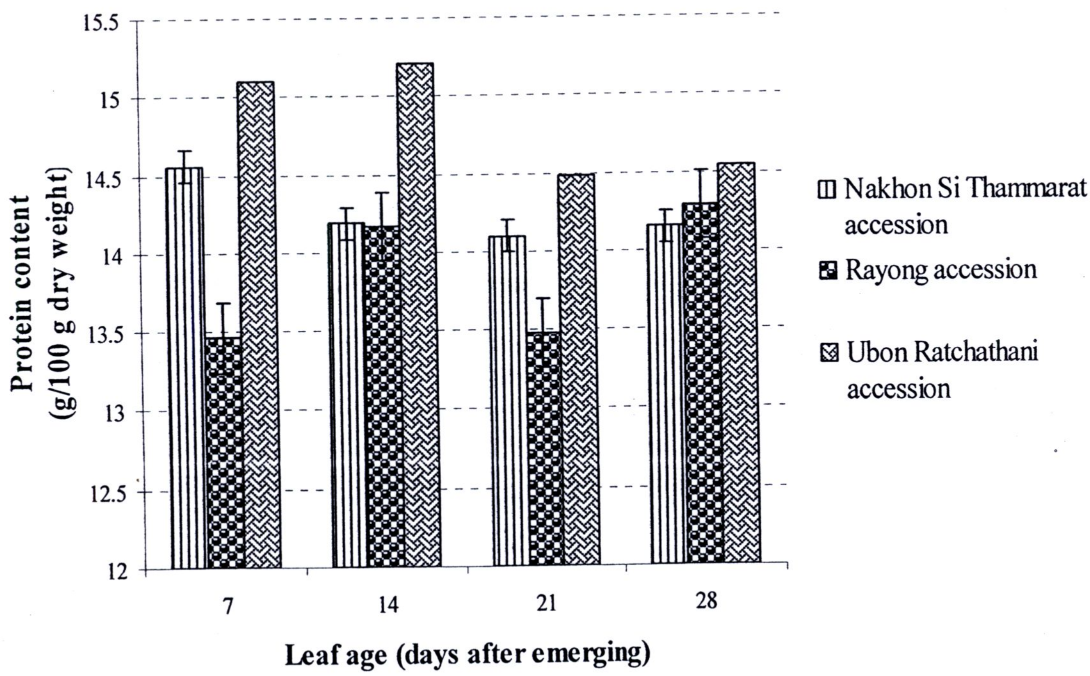


Fig 4.6 Protein content of various accessions of Bua Bok and leaf age

The highest average calcium content (1.41 g/100g dry weight) was found in Ubon Ratchathani accession and was statistically different from that of Nakhon Si Thammarat and Rayong accessions which had the calcium contents at 1.29 and 1.12 g/100g dry weight, respectively. Regarding to this result, it could be stated that the leaf age affected calcium content. It was found that the older leaves accumulated greater amount of calcium content. The leaves at average age of 28 days after emerging had more calcium content than 7, 14, and 21 days after emerging. It showed that there was a collaborative interaction between accession and leaf age on the calcium content. After emerging of Nakhon Si Thammarat accession, the calcium content in leaves was the highest at 28 days meanwhile the calcium contents in the leaves of Ubon Ratchathani accession at 28 and 14 days after emerging were at 1.54, 1.44 and 1.42 g/100 g (dry weight) respectively (Fig 4.7). Since calcium was non-movable element within the plant, therefore, the older leaves contained more calcium content than younger leaves.

However, the previous report indicated that calcium contents in Bua Bok leaves from South Africa and India, were at 24.25 g/100 g (dry weight) (Odhav *et al.*, 2007) and 174 mg/100 g fresh weight (Gupta *et al.*, 2005), respectively. On the contrary, the result of this experiment presented that the calcium content of Bua Bok leaves collected from full sunlight was 1.30-1.54 g/100 g (dry weight) was affected by geographical differences.

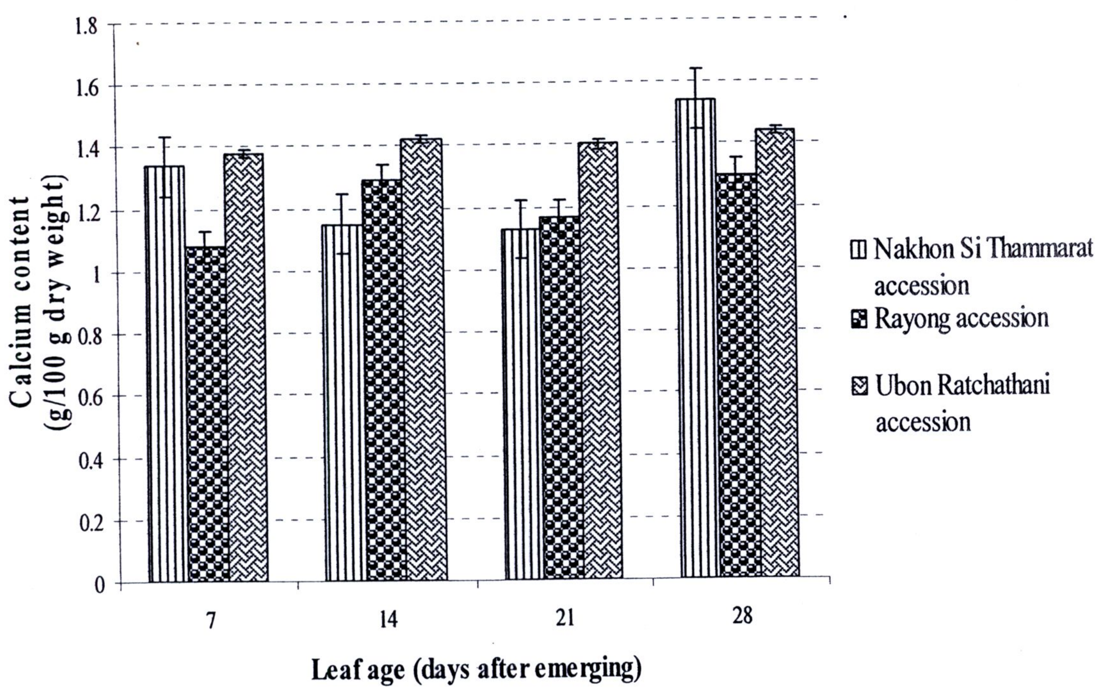


Fig 4.7 Calcium content of various accessions of Bua Bok and leaf age

The contents of beta-carotene in the 3 accessions of Bua Bok were statistically different. Rayong accession had the highest average beta-carotene content of 236.36 µg/100g dry weight, followed by those of Nakhon Si Thammarat and Ubon Ratchathani accessions at 231.88 and 225.88 µg/100g dry weight, respectively. This represented that the leaf age of Bua Bok affected the beta-carotene content. The collaborative interaction between accession and leaf age on beta-carotene content was found in the leaves of

Nakhon Si Thammarat accession at 28 days after emerging, whereas the leaves of Rayong accession at 14, 21 and 28 days after emerging were at 241.23, 241.57, 236.41, 238.97 μg/100g (dry weight), respectively (Fig 4.8).

This experiment was supported by Lefsrud *et al.* (2007), which noticed that the accumulation of beta-carotene content in the leaves of *Brassica oleracea* L. var. a*cephala* increased with higher leaf age. The beta-carotene content from mature leaves (28 days after emerging) was in a range of 229.15-241.23 μg/100 g dry weight, lower than that of Bua Bok grown in India (3.90 mg/100 g fresh weight).

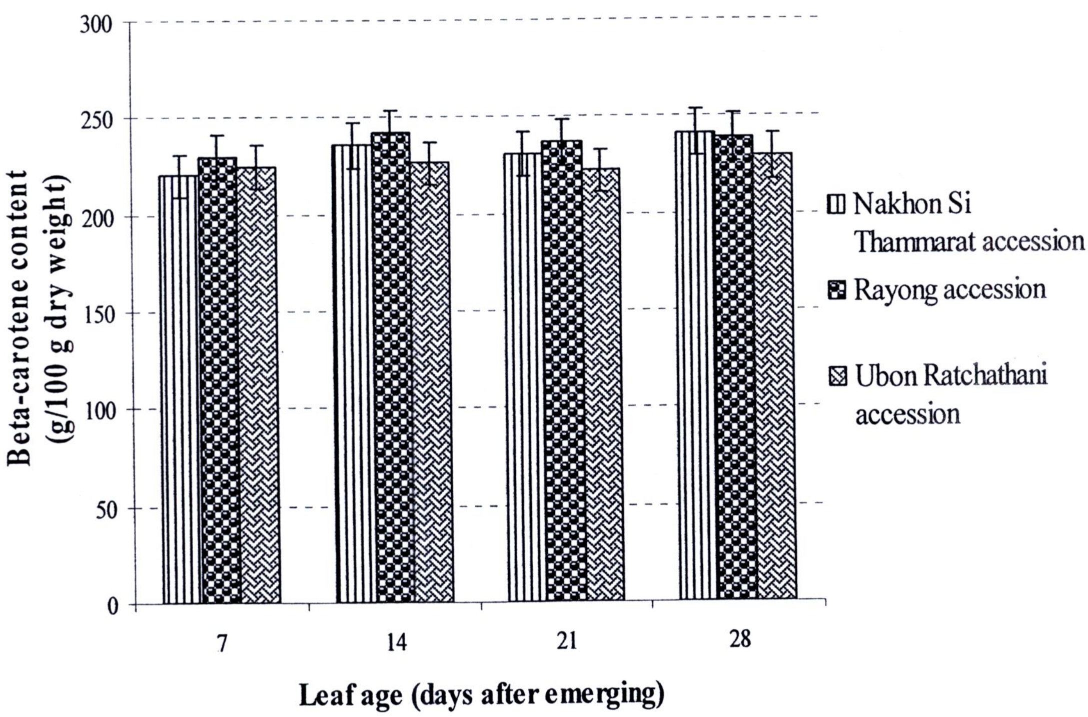


Fig 4.8 Beta-carotene content of various accessions of Bua Bok and leaf age

4.1.3 Asiaticoside content

The asiaticoside contents from the different leaf ages of 3 accessions of Bua Bok were statistically different. The accessions and leaf ages affected the asiaticoside content of Ubon Ratchathani accession which had the greatest amount of asiaticoside (3.94% dry weight), and was statistically different to those of Nakhon Si Thammarat and Rayong accessions. However, the asiaticoside contents in Bua Bok of Nakhon Si Thammarat and Ryong were not statically different. When considering the overall leaf age, it was found that the leaves of 21 and 28 days after emerging had the highest contents of asiaticoside at 3.89% and 3.77% dry weight, respectively. To consider the interaction between accessions with leaf age, the greatest asiaticoside content was found in leaves at age of 21 days after emerging. Ubon Ratchathani accession had asiaticoside content of 4.46% dry weight followed by the leaves of Ubon Ratchathani accession at age of 28 days after emerging (Fig. 4.9).

In general, the harvest of asiaticoside would gather fresh leaves and old leaves by the age of 90 days after cutting. Piriyapattharakit (2008) cultivated Bua Bok in organic agricultural systems, and then collected leaves at 90 days after cutting. The results indicated that asiaticoside content was in the range of 0.94 -3.58% (dry weight). Regarding to the experiment, it demonstrated that the asiaticoside content analyzed from the mature leaves (28 days after the start bud) at average of 3.03 – 4.32% (dry weight) had greater asiaticoside content than the leaves collected at 90 days after cutting. Notwithstanding, to collect only the mature leaves required more time and cost than collected all at 90 days after cutting.

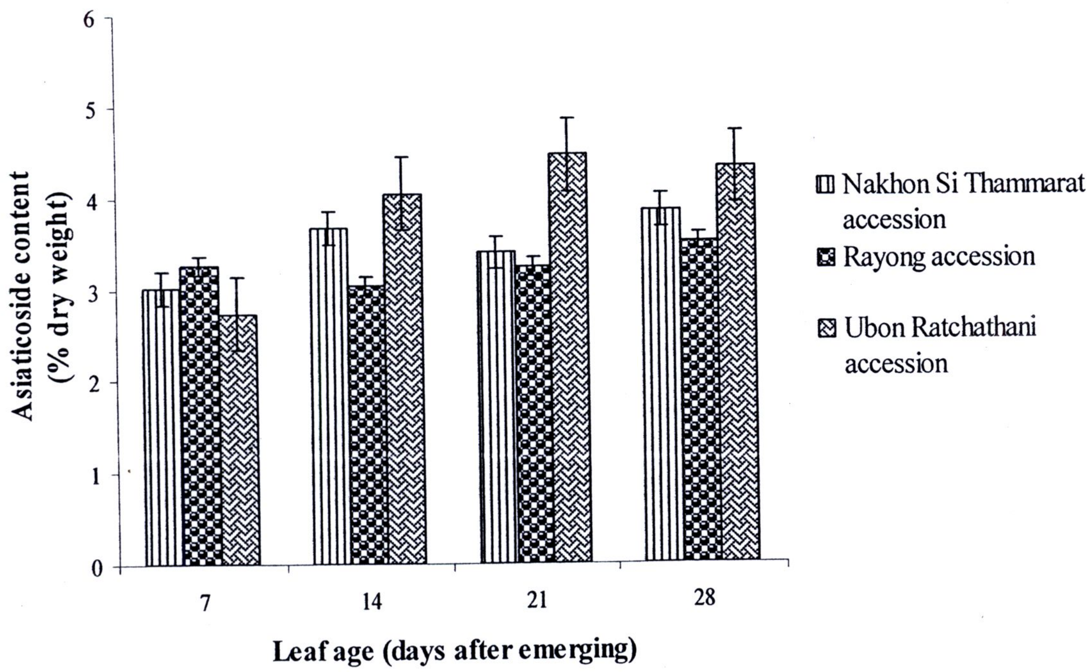


Fig 4.9 Asiaticoside content of various accessions of Bua Bok and leaf age

4.2 Experiment II Effect of Light Condition During Growing on Asiaticoside and Nutritional Content in Bua Bok

4.2.1 Growth of Bua Bok

Three accessions of Bua Bok were planted under 3 levels of light intensity, then mature leaves were harvested. Chlorophyll content, leaf area, petiole length, fresh and dry weight, fiber, protein, calcium beta-carotene and asiaticoside content were determined.

The Bua Bok grown under low light intensity possessed the highest chlorophyll content in all accessions (Fig 4.10). Their leaves were much greener when compared with those of Bua Bok grown under higher light intensity. At the same time, reduced light intensity resulted in the larger leaves, longer petioles (Fig 4.11 and 4.12). The relationship between accessions on leaf area showed Nakhon Si Thammarat accession had the largest leaf area, followed by Rayong and Ubon Ratchathani accessions, respectively. The leaf areas of Rayong and Ubon Ratchathani were not significantly different while the light intensity also affected the leaf area. All accessions had the largest leaf area at the light intensity of 93.30 μmol/m^2/s. Besides, the interaction between accession and light intensity showed that the largest leaf areas of 14.72, 15.87 and 12.66 cm^2 were found in Nakhon Si Thammarat and Ubon Ratchathani accessions that were grown under light intensity of 93.30 μmol/m^2/s and Nakhon Si Thammarat accession under 362.55 μmol/m^2/s. The longest petiole found in Rayong accession that were grown under light intensity of 93.30 μmol/m^2/s, was 14.7 cm (Fig 4.11). Also found Bua Bok grown under low light intensity resulted to decrease fresh weight.

From Fig. 4.13, the relationship between light intensity and accession on fresh weight per area is shown. It indicated that Ubon Ratchathani accession grown under light intensity of 933.07 μmol/m^2/s had the highest average fresh weight of 3.50 kg/m^2.

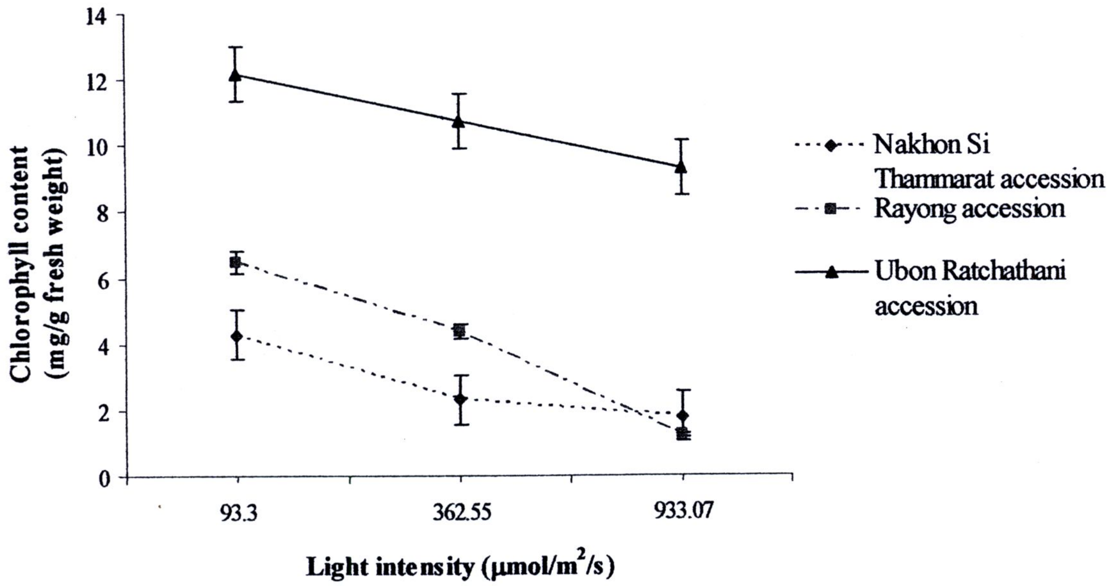


Fig 4.10 Chlorophyll content of various accessions of Bua Bok and light intensity

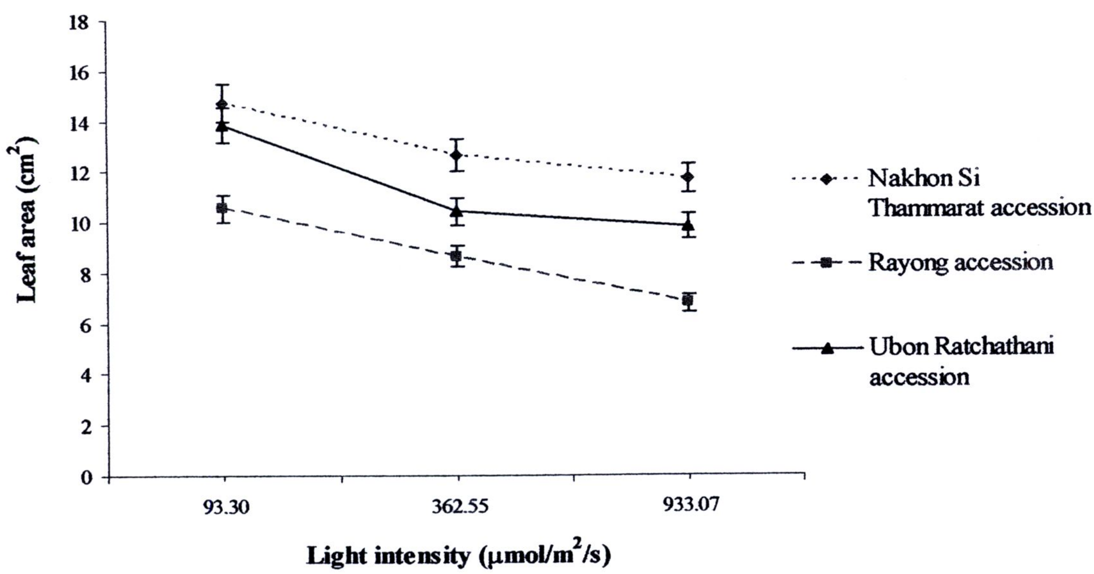


Fig 4.11 Leaf area of various accessions of Bua Bok and light intensity

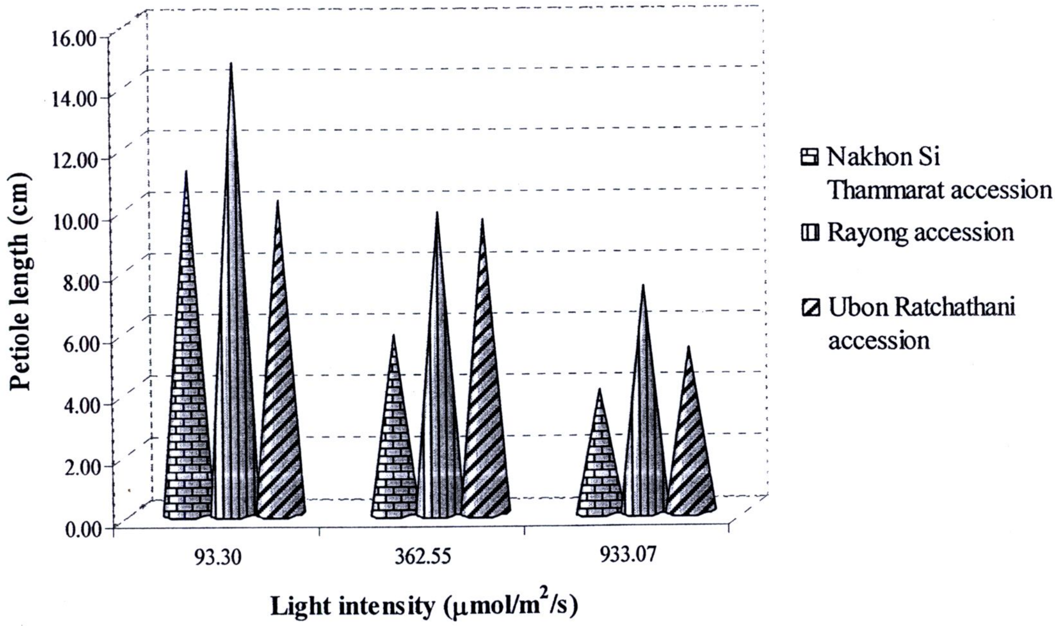


Fig 4.12 Petiole length of various accessions of Bua Bok and light intensity

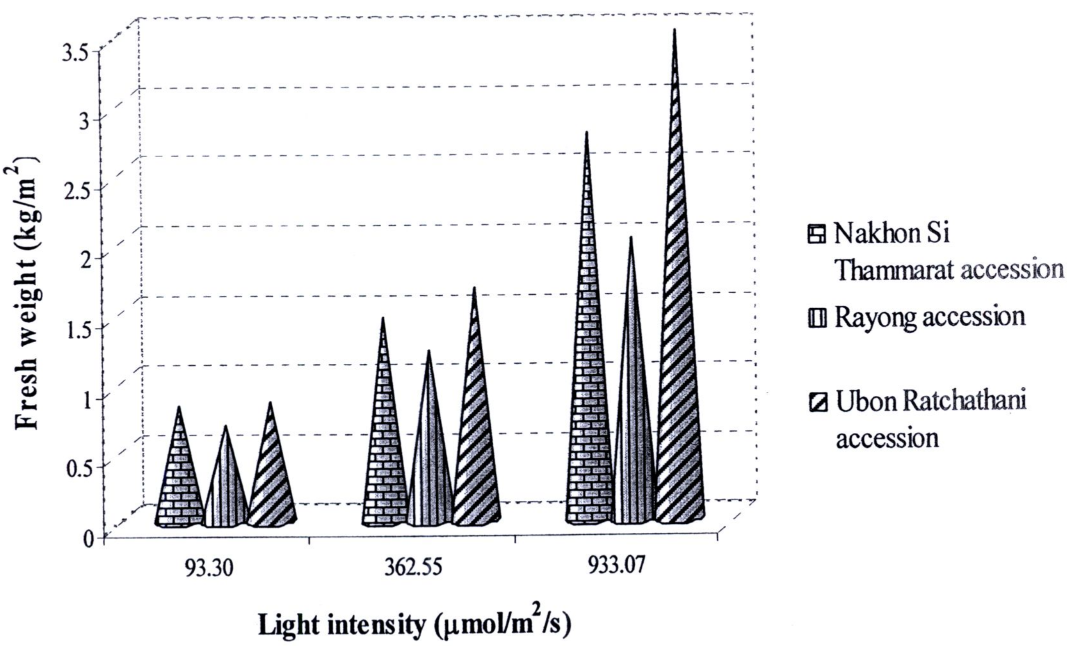


Fig 4.13 Fresh weight various accessions of Bua Bok and light intensity

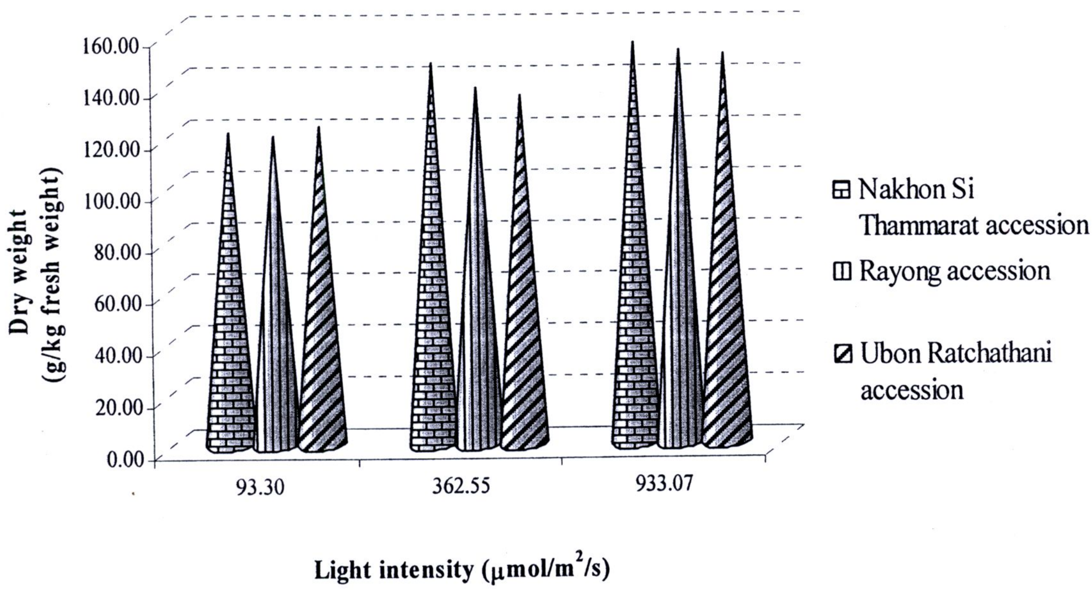


Fig 4.14 Dry weight of various accessions of Bua Bok and light intensity

The light is the limited factor for plant. Plants need light intensity at the appropriate levels. In too low intensity environment, the plants would adjust themselves to sufficient photosynthesis to live. The plants that received lower light intensity might adjust the photosynthesis rate that in lower condition (Barden, 1974; Boardman, 1977; Kappel and Flore, 1983; Noggle and Fritz, 1983; Phuwiwat, 1993). Therefore, plants must have mechanisms to adapt in the low light intensity. It was found in the low light area that the plants adapt to increase leaf area to receive more light and increase the petiole length to approach lighting. Wankhar and Tripathi (1990) studied the growth of Bua Bok by cutting in India and found the relationship between light intensity with leaf area and petiole length. In the low light intensity, Bua Bok would have larger leaves and longer petiole than that grown under high light intensity. In the same time, it rapidly accumulated high chlorophyll content (Collard, *et at.*, 1977; Conover and Poole, 1977; Rylski, 1986; Reglos, 1989; Phuwiwat, 1993). The adaptation mentioned above would help the plants to increase more light conditions. This would result in the overall photosynthesis in plants (Hale and Orcutt, 1987; Phuwiwat, 1993) and affect the growth of plants. In this study, Bua Bok was planted in an environment with low light intensity (93.30 μmol/m^2/s). The results showed the amounts of chlorophyll, leaf area and petiole length increased when compared with Bua Bok grown under high light or full sunlight (933.07 μmol/m^2/s).

Light intensity resulted in continued growth of Bua Bok grown under the various light intensity levels as above. This results show the fresh weight per area. The Bua Bok grown under light intensity of 933.07 μmol/m^2/s produced the best yield, followed by Bua Bok plants that were grown under light intensity 362.55 μmol/m^2/s and 93.30 μmol/m^2/s, respectively. It noticed that the best level of light intensity that Bua Bok grew well and gave very good yield was at 933.07-362.55 μmol/m^2/s.

Concealing the light too much would affect the growth of Bua Bok and cause the decrease of productivity, especially when Bua Bok received light intensity less than 362.55 μmol/m^2/s. These results were supported by Earley *et al.* (1966) who found that 30% light intensity reduction resulted in decreased corn yields. In addition, Ndamba *et al.* (1996) found that *Phytolacca dodecandra* grown under full sunlight had the greatest growth and higher yield than that under the shade.

For dry weight yields of three Bua Bok accessions grown under different light intensity, each accession had no effect on dry weight but the different light intensities could affect the different dry weights of Bua Bok. (Fig 4.13). In addition, the experimental results revealed that Bua Bok in each accession grown under the light intensity of 933.07 μmol/m^2/s had the most average of dry weight productivity, followed by Bua Bok grown under light intensities of 362.55 and 93.30 μmol/m^2/s, respectively. Nakhon Si Thammarat, Rayong, and Ubon Ratchathani accessions were grown under light intensity of 933.07μmol/m^2/s showed dry weights of 154.93, 151.45 and 150.56 g/kg fresh weight, respectively (Fig 4.14).

4.2.2 Nutritional values

From the study, the contents of fiber, protein, calcium and beta-carotene in 3 Bua Bok accessions under different light intensity were determined. The results showed that light intensity affected on the contents of protein, calcium and beta-carotene in two Bua Bok accessions. The highest fiber content was found in Rayong accession, followed by Ubon Ratchathani and Nakhon Si Thammarat accession, respectively. The light intensity did not affect the fiber content. All light intensities studied gave the fiber content without any significant difference but the highest fiber content was found in Bua Bok under light intensity of 933.07 μmol/m^2/s and that decreased at low light intensity. It was also found that there was the interaction between accessions and light intensity. The highest fiber contents were found in Rayong accession that was grown under light intensities of 933.07 and 93.30 μmol/m^2/s, and Ubon Ratchathani accession grown under light intensity of 93.30 μmol/m^2/s, were 12.19, 12.11 and 11.91 g/100g dry weigh, respectively (Fig. 4.15).

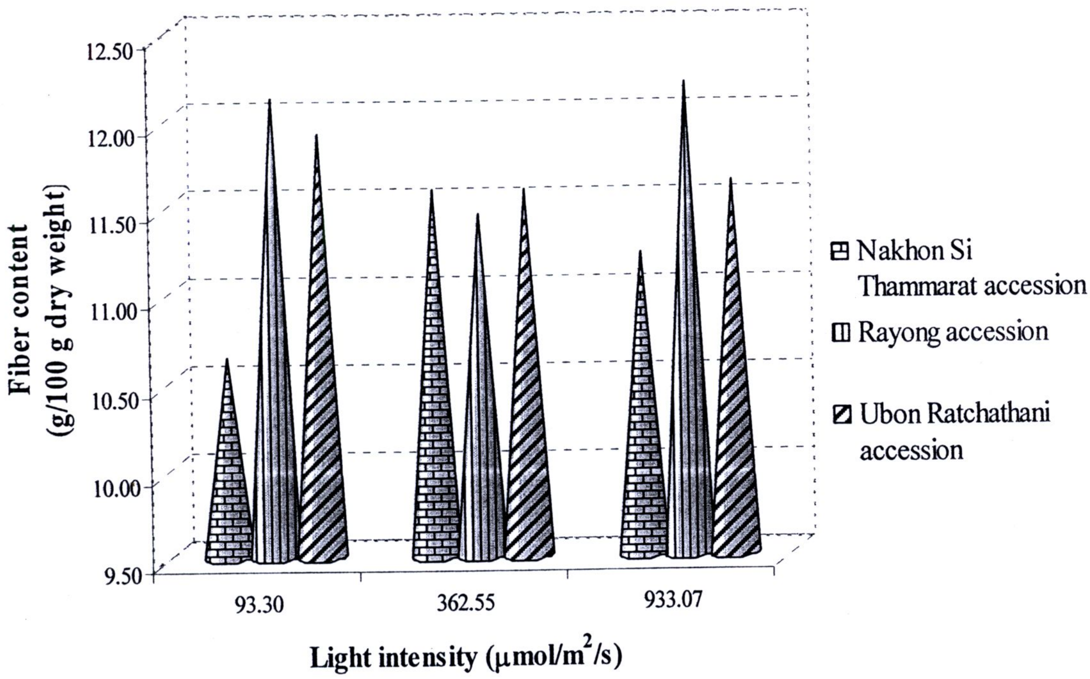


Fig 4.15 Fiber content of various accessions of Bua Bok and light intensity

The average amount of protein was significantly different in each accession. Nakhon Si Thammarat accession had the highest protein content, 18.63 g/100 g dry weight, followed by Ubon Ratchathani and Rayong accessions, respectively. The light intensity that Bua Bok produced the highest protein content was 933.07 μmol/m^2/s. It was significantly different with the amounts of protein at 362.55 and 93.30 μmol/m^2/ s light intensity, respectively. The interaction between the accessions and light intensity was found in Rayong accession grown under light intensity of 362.55 μmol/m^2/s which had the highest protein content, and significant difference with other treatments. The protein content in Rayong accession was 20.09 g/100 g dry weight followed by Nakhon Si Thammarat accession grown under light intensity of 93.30 μmol/m^2/s which had 19.84 g/100 g dry weight of protein content, respectively (Fig. 4.16).

At the low light intensity, Bua Bok accumulated much more protein which was in contrast to the reduced accumulation of protein in corn at 30% decrease of light intensity (Earley *et al.*, 1996). This could be due to other different kinds of plants.

The reports on analysis of proteins content in Bua Bok found that the protein content from Bua Bok grown in KwaZulu-Natal, South Africa in the sandy soil conditions and full sunlight had protein content of 3.0 g /100 g fresh weight (Odhav *et al.*, 2007). In India, Gupta *et al.* (2005) analyzed the content of protein in Bua Bok collected from local markets and local fields and found that the average amount of protein was 2.4 g/100 g fresh weight.

It was found that protein contents in Bua Bok from the 2 trials were lower in this experiment and were in the range of 17.78 – 20.09 g/100 g dry weight. This might be explained by the differences in growing environments. Hegart and Peterson (1973) reported that the content of protein in plant leaves was dependant on species, age, type of fertilizer, light intensity and amount of water.

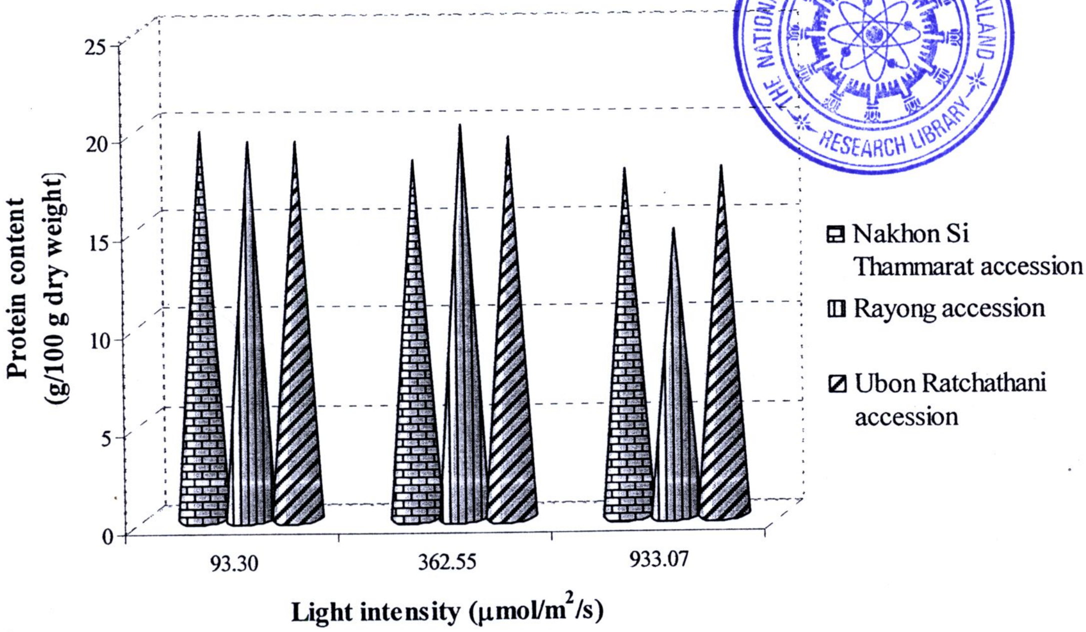


Fig 4.16 Protein content of various accessions of Bua Bok and light intensity

The amounts of calcium found in three Bua Bok accessions were significantly different. The average volume of calcium in Ubon Ratchathani accession was the highest, followed by Rayong and Nakhon Si Thammarat accessions, respectively (Fig. 4.17). Growing Bua Bok under different light intensity affected the volume of calcium. The average volume of calcium became the highest when Bua Bok was grown under light intensity of 93.30 $\mu mol/m^2/s$, followed by 362.55 and 933.07 $\mu mol/m^2/s$, respectively. The study of the interaction between accession and light intensity revealed that the most calcium content in Ubon Ratchathani accession grown under light intensity of 93.30 $\mu mol/m^2$ /s had 2.17 g/100 g dry weight with significant difference with other treatments, followed by Rayong accession grown under light intensity of 362.55 $\mu mol/m^2/s$, and Ubon Ratchathani grown under light intensity of 933.07 $\mu mol/m^2/s$ which were 1.76 and 1.75 g/100 g dry weight of calcium content, respectively.

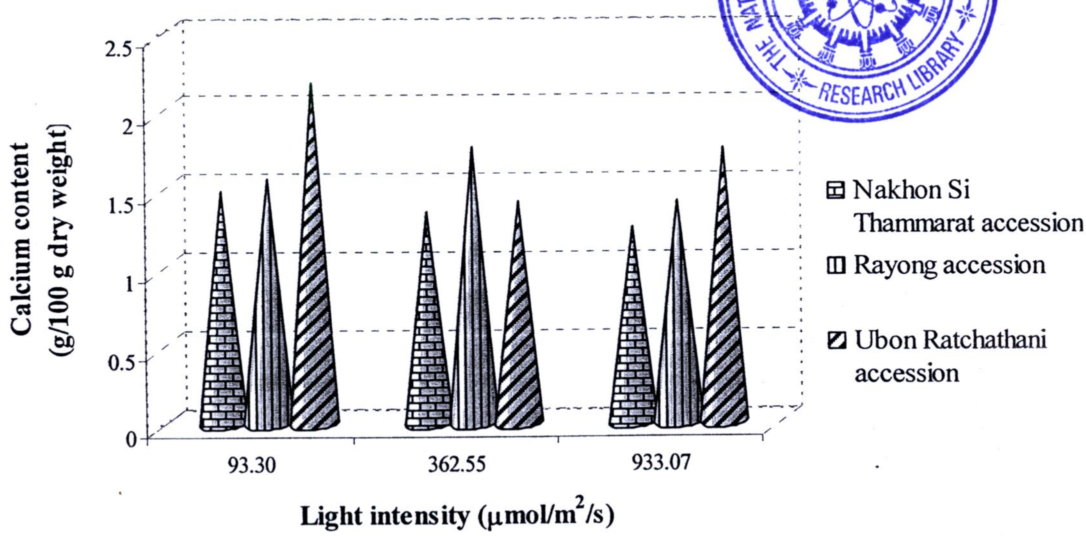


Fig 4.17 Calcium content of various accessions of Bua Bok and light intensity

According to the report by Gupta *et al.* (2005), found that the amount of calcium accumulated in Bua Bok collected from local markets and planted by local fields had the volume of 174 g/100 dry weight which was higher than that of this experiment. This might be due to the differences in geography. In addition, the result of light intensity in rate to the quantity of calcium in Bua Bok was opposite to the result of light intensity in rate to collecting calcium in kiwifruit. It was analyzed that concealing of light affected the quantity of calcium in kiwifruit (Montanaro *et al.*, 2006).

The beta-carotene content in each accession was significantly different. The Rayong accession had the highest average beta-carotene content, followed by Ubon Ratchatani and Nakhom Si Thammarat, respectively (Fig. 4.18). Similarly, the light intensity had the effect on beta-carotene content. At lower light intensity (93.30 $\mu mol/m^2/s$), Bua Bok accumulated higher beta-carotene content than at higher light intensity. The interaction between accession and light intensity showed the great beta-carotene content in Rayong accession grown under light intensity of 362.55$\mu mol/m^2/s$, followed by Ubon

Ratchathani accession grown under light intensity of 93.30 μmol/m^2/s which were 20.12 and 19.80 μg/100 g dry weight, respectively.

Gupta *et al*., (2005) reported that the contents of beta-carotene in Bua Bok collected from local market and local field of India were 3.90 mg/100g fresh weight. Moreover, another report found that Bua Bok from local market of Khon Kaen, Thailand had the volume of beta-carotene of 1.0 μg/g fresh weigh (Speek *et al*., 1988) These two reports had the higher content of beta-carotene than that in this experiment (15-18 μg/100g dry weight). This might be the result of using dried Bua Bok in the analysis.

Oyama *et al*. (1999) studied the effects of air temperature and light intensity on beta-carotene in spinach and lettuce and found that spinach and lettuce exposed to sunlight had highest beta-carotene content. The results of this study showed that the amount of beta-carotene in Bua Bok, however, was differed from the studies of spinach and lettuce. This might be due to the variation in plant material, experimental location, climate or some other undetermined factors.

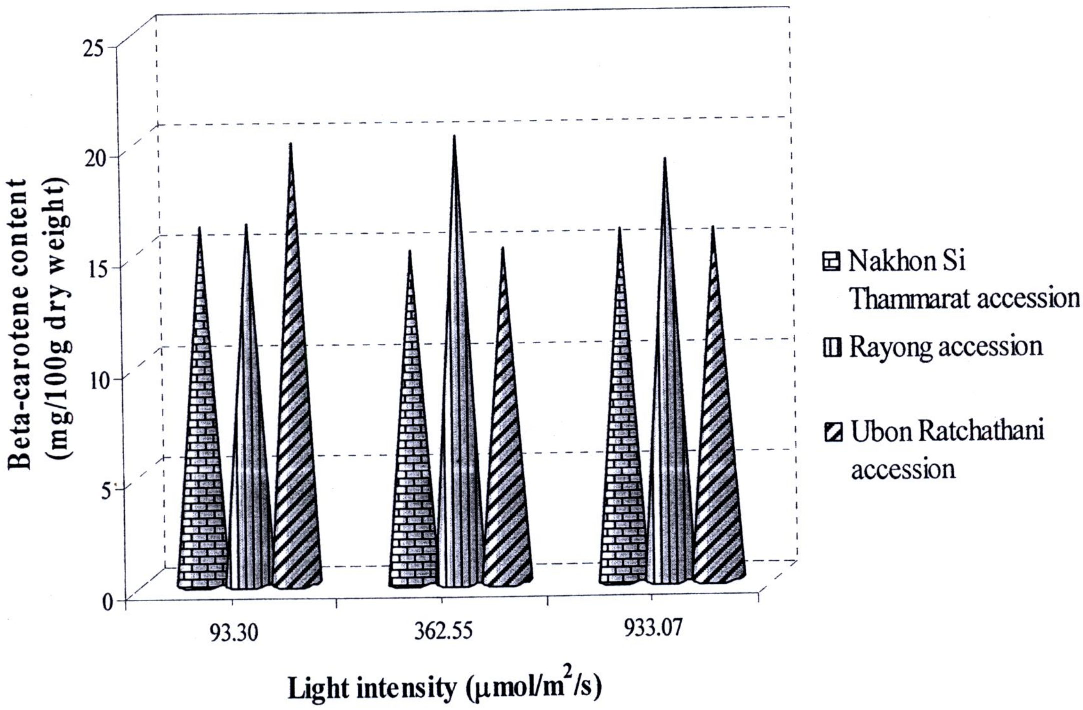


Fig 4.18 Beta-carotene content of various accessions of Bua Bok and light intensity

4.2.3 Asiaticoside content

The asiaticoside content was analyzed from 3 Bua Bok accessions grown under different light intensity. The results showed that the variation of asiaticoside content depended on the accession and light intensity. The highest average content of asiaticoside found in Ubon Ratchathani accession, following by Rayong and Nakhon Si Thammarat accessions, respectively. In other side, it was found that Bua Bok grown under light intensity of 933.07 $\mu mol/m^2/s$ had the highest asiaticoside content. There was interaction between accession and light intensity. Rayong accession grown under light intensity at 933.07 $\mu mol/m^2/s$ had the highest asiaticoside content which was 4.38% dry weight (Fig 4.19).

The finding of asiaticoside content of Bua Bok analyzed in this study was in accordance to the previously published literature as reported by Mathur *et al.* (2000). They collected

Bua Bok from different regions of India and screened for the asiaticoside yield under different levels of shading in sub-tropical field. They found that shading affected the asiaticoside content. Plants grown under normal light (100% light intensity) produced high asiaticoside content. In other report, *Phylacca dodecandra* cultivated in full sunlight gave higher saponin content, compared to that under shading (Ndamba *et al*, 1996). Secondary metabolite biosynthesis is regulated genetically and by environmental factors. Light is very important as a consequence of its significance and complex mechanism of action on the metabolism, and influence on the accumulation and quality of the main ingredients (De Moura *et al*., 2002; Bernath and Tete′nyi, 1987) since all substances produced by the plant were involved directly or indirectly with photosynthesis (de Casto *et al*., 2006). This experiment showed that the optimum light intensity to produce and accumulate asiaticoside content in Bua Bok was not lower than 362.55 $\mu mol/m^2/s$. Randriamampionoma *et al*. (2007) found the highest asiaticoside content measured in sample collected from Mangoro where the climate was characterized by the highest degree of humidity. The accessions showed the variation in asiaticoside content. In addition, the asiaticoside content differed among the ecotype reflecting their genome variation and season (Das and Mallick, 1991; Mathur *et al*., 2000; Somwong, 2006; Aziz *et al*., 2007; Randriamampionoma *et al*., 2007; Jame *et al*., 2008; Piriyapattharakit, 2008).

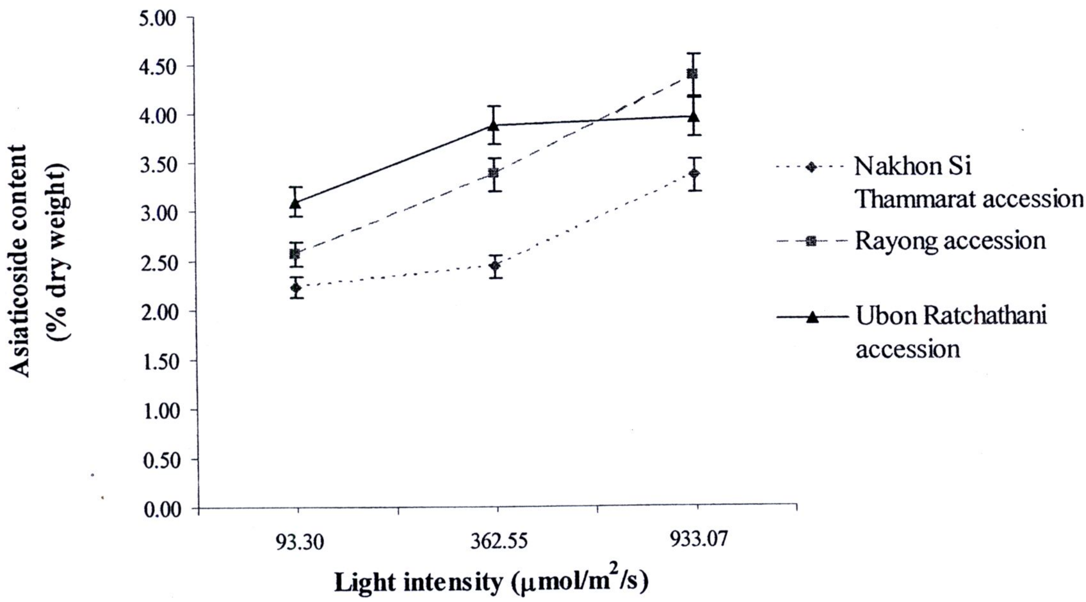


Fig 4.19 Asiaticoside content of various accessions of Bua Bok and light intensity

4.3 Experiment III The Influence of Time and Temperature on The Storage of The Asiaticoside Content of Bua Bok.

The storage of Bua Bok accession dried at 50°C for 24 hours in the plastic bag and kept at 4°C for 1 and 4 months indicated that the quantity of asiaticoside content of Nakhon Si Thammarat accession stored for 1 and 4 months decreased. However, the declined asiaticoside content had no significant difference at 95% confidence level (Table 4.1) while the asiaticoside content of Ubon Ratchathani accession stored for 1 and 4 months obviously decreased and had significant difference at 95% confidence level (Table 4.1). Moreover, the dried Bua Bok stored at 4°C and room temperature for 4 months had the higher asiaticoside content than that of Nakhon Si thammarat accession stored at 4°C and room temperature. However, there was no significant difference at 95% confidence level (Table 4.1). Meanwhile, the asiaticoside content of Ubon Rathchathani accession stored at 4°C was significantly higher than that stored at room temperature (Table 4.2). According to the experiment, it represented that the different accession, storage duration and temperature influenced the asiaticoside content. The longer duration of storage during the test affected the declined asiaticoside content of Ubon Ratchathani accession which was concordant with the trial of Muangnoi (2007) who found that the asiaticoside content decreased because the asiaticoside content changed into asiatic acid by enzyme in the plant when stored for a longer period.

Table 4.1 The asiaticoside content of two accessions of Bua Bok that stored at 4°C for 1 and 4 months

Months	Asiaticoside content (% dry weight)	
	Nakhon Si Thammarat accession	Ubon Ratchathani accession
1	3.84	4.32
4	3.56	3.96
T-test	ns	*
C.V. (%)	2.92	1.57

* = Significantly different to each other at P= 0.05

ns = Not significantly different

In addition, the storage temperature also influenced the asiaticoside content of Ubon Ratchathani accession. The asiaticoside content stored at the average room temperature 25-30 °C was less than that at 4°C. It might be because the higher temperature in such duration which affected the degradation of the asiaticoside content. This result was relevant to the storage saponin content which was a considerable matter of yam (*Dioscorea pseudojaponica* Yamamoto). Moreover, the storage at 25°C affected the higher degradation of the saponin content than at 17 and 4°C, respectively (Deng-Jye and Jau-Tien, 2008). On the other hand, the low temperature also resulted in the degradation of the matter by decelerating the enzyme function and microbe destruction (Schwarzbach *et al.*, 2006). Furthermore, the dried Bua Bok accession stored at the room temperature affected fade color of both 2 accessions. Perhaps, it might be because of the storage at the room temperature which resulted in physical change of the dried Bua Bok accession.

Regarding to this experiment, it demonstrated that Nakhon Si Thammarat accession could be maintained as dried Bua Bok, both at room temperature and 4°C while there was no difference of the asiaticoside content. However, the dried Bua Bok storage of Ubon Ratchathani accession stored at room temperature would have less effect on the asiaticoside content than that stored at 4°C about 16.41% which there was little decrease of the asiaticoside content during 4 months. In addition, the dried Bua Bok should be kept in light-resistant container or black container to prevent the discoloration of Bua Bok. Changes in physical and chemistry properties of powder of Bua Bok during storage were affected by storage temperature and time. The powder colors became paler (Sapkoet, 2007). The dry herb was stored in dark color packing material to maintain their color (Hettiarachchi *et al.*, 2005)..

Table 4.2 The asiaticoside content of two accessions of Bua Bok that stored for 4 months at different temperatures.

Temperature (°C)	Asiaticoside content (% dry weight)	
	Nakhon Si Thammarat accsession	Ubon Ratchathani accession
4.0	3.56	3.96
ambient temperature	3.36	3.31
T-test	ns	*
C.V. (%)	2.42	2.24

* = Significantly different to each other at P= 0.05

ns = Not significantly different