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The combination of EthylBloc Sachet and 2,4-pyridinedicarboxylic acid reduces petal blackening and prolongs vase life of cut flowers of lotus (*Nelumbo nucifera* Gaerth) cvs. Sattabongkot and Saddhabutra

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ABSTRACT

Postharvest lotus (*Nelumbo nucifera* cvs. Sattabongkot and Saddhabutra) flowers show a rapid petal blackening which shortens the vase life of flowers. We studied the effect of EthylBloc Sachet and/or 2,4-pyridinedicarboxylic acid (PDCA) on the petal blackening and the vase life of two commercial cultivars. Cut flowers were treated with distilled water (control), EthylBloc 2 Sachet (active ingredient 0.014%, fumigation for 6 h at 256 ppb), vase solution of 2,4-PDCA (2.0 mM) or the combination of EthylBloc Sachet and 2,4-PDCA.

All treatments were kept at 28 ± 1 °C and 80–85% RH. The combination of 2,4-PDCA and EthylBloc Sachet caused the longest vase lives of 49.8 h with 'Sattabongkot' and 69.0 h with 'Saddhabutra', when the control values were 44.4 and 36.0 h, respectively. The combination of EthylBloc Sachet and 2,4-PDCA increased water uptake, delayed weight decrease and hue angle change, and reduced petal blackening. Moreover, the treatment caused less ethylene production and less respiration in the flowers than the other treatments. This method is useful for handling in commercial cut lotus flower.

1. Introduction

Thai lotus (*Nelumbo nucifera* Gaerth) flower is a symbol of Buddhism and used for decoration in hotel and spa business. It has two major commercial cultivars, and the cultivar with pink petals is called 'Sattabongkot' and that with white ones 'Saddhabutra' (Imsabai and van Doorn, 2013). The flower of both cultivars bears more than 50 petals. The commercial flowers are cut in the bud stage. 'Sattabongkot' has longer vase life than 'Saddhabutra'. Both 'Sattabongkot' and 'Saddhabutra' flowers start petal blackening in 6 h after cutting and have the vase life of 72 and 54 h, respectively (Salaemae, 2017). The petal blackening symptom occurs first with black patches at the margin of outer petals and spreads into the middle area. In addition, lotus petals show discoloration from green to yellow. These events result in short vase life. Ethylene is a plant hormone involved in senescence of cut lotus flowers, and the flowers are very sensitive to ethephon at 200 mg/L (Imsabai, 2010). The early petal blackening in lotus flowers is related to adverse water relation and suggests that the flowers may be deficient in sugar accumulation after harvesting (Imsabai et al., 2013; Netlak and Imsabai, 2016). Another possibility is the number of stomata which affects senescence in lotus flowers. Stomata number was larger in 'Saddhabutra' than 'Sattabongkot'. (Salaemae, 2017).

The maintenance of postharvest quality and prolonging the vase life of lotus flowers are very important commercially. Currently, cut lotus flowers (actually buds) are treated with ethylene inhibitors, such as 1-methylcyclopropene (1-MCP), which can delay the occurrence of petal blackening in 'Saddhabutra' lotus flowers and inhibits the expression of genes (*Nn-ACO* and *Nn-ACS*) involved in ethylene biosynthesis (Imsabai et al., 2010). 1-MCP is commercially available as EthylBloc Sachet, and it is used for potted carnations (*Dianthus caryophyllus* L.); it inhibits ethylene biosynthesis and action, keeps leaves green and improves their longevity (Burana et al., 2008). 2,4-Pyridinedicarboxylic acid (2,4-PDCA) is an inhibitor of ethylene biosynthesis, which inhibits ACC oxidase resulting in inhibition of ethylene production and eventually

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delays senescence in cut carnation flowers (Vlad et al., 2010; Fragkostefanakis et al., 2013). 2,4-PDCA has two distinct activities in cut spay carnation flower; it promotes flower opening and extends vase life (Satoh et al., 2014). The hypothesis of this research that EthylBloc Sachet was promoted by an inhibitor of ethylene action, while 2,4-PDCA probably inhibits ethylene biosynthesis. The combination of EthylBloc Sachet and 2,4-PDCA will be effectively reduced function of ethylene and lead to the long vase life of cut lotus flower.

Based on information described above, we aimed to see the effects of these agents on senescence of lotus flowers of 'Sattabongkot' and 'Saddhabutra' cultivars. The lotus flowers (buds) were treated singly or simultaneously with EthylBloc Sachet and 2,4-PDCA to see reduced petals blackening and prolonged vase life. The experimental procedure and obtained results would provide a useful method to be used and appropriate to commercial cut lotus flowers in Thailand.

2. Materials and methods

2.1. Plant materials

Lotus (*Nelumbo nucifera* Gaertn., cvs. Sattabongkot and Saddhabutra) plants were cultivated at a commercial field in Payakhan district, Phatthalung province, Southern of Thailand. Flowers (buds) were harvested in the early morning, and selected for the buds of normal commercial stage 5, 6.0–6.5 cm in diameters (Netlak and Imsabai, 2016). After harvest the flowers were transported in less than 1 h to the laboratory of Plant Science, Faculty of Technology and Community Development, Thaksin University, Phatthalung campus. The stems were cut in water to 25 cm in length. The flowers were held in water (control) or test solutions in glass bottles (one flower per bottle), and held at 28 ± 1 °C and 80–85% RH under natural day light (from about 6 a.m. to 6 p.m.).

2.2. Treatment with EthylBloc Sachet and/or 2,4-PDCA

Preliminary experiments were conducted with vase solutions containing 2,4-PDCA at 0, 2.0, 3.0 and 4.0 mM, and gave the best result with 2 mM. Also, EthylBloc Sachet [a commercial specimen purchased from Floralife, USA, active ingredient (a.i.) per Sachet is 0.014%] was tested to reduce petal blackening, and gave the best result with 2 sachets per treatment (a.i., 0.014% or 256 ppb). Then, experiments were conducted by treatment, singly or in combination, with two agents at these concentrations. The flowers were put in 200-ml glass bottles (one flower per bottle) with their stem ends in distilled water (control) or in the vase solution containing 2.0 mM 2,4-PDCA. The lotus were place in distilled water, they were treated with EthylBloc 2 Sachet (a.i., 0.014%) in a corrugated box (50 × 50 × 50 cm) for 6 h. Then, the flowers were removed from the box and left thereafter in 200 ml glass bottles by placing their stem in water or 2,4-PDCA. The vase life and postharvest quality parameter were determined every 6 h. The replications of the difference treatments of 15 flowers, individually placed in glass bottles; (n = 15).

2.3. Determination of water uptake, weight change and hue angle

The rate of water uptake was measured by placing stems in graduated cylinders and measuring water level every 6 h. The each treatment has data are means of 10 replicate flowers. The water uptake was calculated from the decrease of water level in graduated cylinders. Water was replenished every 6 h for measurement. Flower weight was determined initially and at given time of experiment. Weight change was expressed as the percentage of initial fresh weight. Hue angle was determined using Minolta Colorimeter: CR300. The Hue angle value indicates changes in color tone at various levels.

2.4. Measurement of ethylene production and respiration rate

Stems of lotus flowers were cut to 7 cm in length. The flowers were enclosed in 1.8 L plastic bottles (one flower per bottle), and left for 10 min. The experiment was conducted with three replicated flowers. Two 1- ml head gas samples were taken from the gas sampling port on the lid of the bottles. Concentrations of ethylene and carbon dioxide in the gas samples were analyzed using a gas chromatograph (Shimadzu GC 8 A, Kyoto, Japan) according to Imsabai et al. (2010), Imsabai (2010). Data were expressed per gram flower fresh weight.

2.5. Observation of petal blackening and vase life

During vase life, petal blackening was assessed every 6 h for flower 15 flowers individual treatment. The degree of blackening was defined as the percentages of blackened-area of total of petal area; 10% for onset of petal blackening, 25% for black patches on the edge of petals, 50% for whole edges covered by blackening, 75% for blackening region expanding, and 100% for whole petals blackening (Imsabai et al., 2010). The length of vase life was defined as the period until half of the visible petals showed black patches or attained 50% petal blackening.

2.6. Statistical analysis

The experiments were conducted in a completely randomized design. Data were analyzed by the General Linear Model program of the SAS system statistics data editor and means were compared by the F-test one-way ANOVA. The difference between means was compared by least significant difference at $P < 0.05$.

3. Results

3.1. Water uptake and weight change

The water uptake of both 'Sattabongkot' and 'Saddhabutra' cultivars treated with the combination of EthylBloc 2 Sachet (a.i., 0.014%) and 2,4-PDCA at 2.0 mM was high during 6–12 h of vase life, then decreased rapidly to low level. The combination treatment caused the highest water uptake, being significant at $P \leq 0.01$, during vase life in two cultivars (Fig. 1A and B)

The weight change of both cultivars showed the increase in the early stage of vase life (6–18 h), and a decline afterward with all treatments. The cut lotus flowers treated with the combination of EthylBloc Sachet and 2,4-PDCA exhibited the weight change larger than other treatments ($P \leq 0.01$) (Fig. 2A and B). While, the weight change in the control decreased rapidly in 'Saddhabutra' cultivar (Fig. 2B).

3.2. Change of hue angle

Fig. 3A and B show the change of hue angle (surface color) at outer most petals of flower of both cultivars treated with or without EthylBloc Sachet and 2,4-PDCA. The cut lotus flowers treated with the combination of EthylBloc Sachet and 2,4-PDCA showed the slowest delay in the reduction of hue angle as compared to the other treatments. The hue angle value in the control declined significantly during vase life with enhanced discoloration in both 'Sattabongkot' and 'Saddhabutra'.

3.3. Ethylene production and respiration

Ethylene production of cut flowers of 'Sattabongkot' and 'Saddhabutra' lotus showed similar climacteric patterns (Fig. 4A and B). The ethylene production rapidly increased in 6–12 of vase life, then gradually decreased until 48 h. The treatment with the combination of EthylBloc Sachet and 2,4-PDCA resulted in ethylene production less than the other treatments in both cultivars. While, the control flowers in both cultivars showed the highest ethylene production as compared

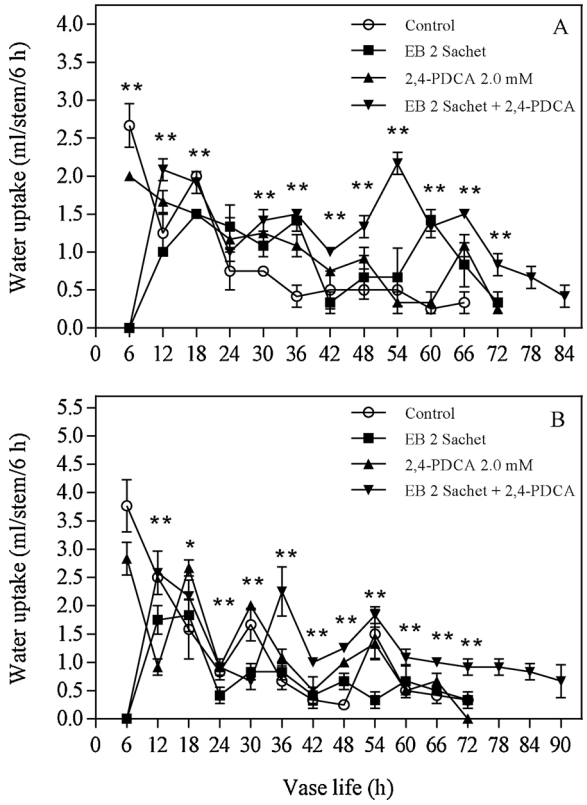

Fig. 1. Change of water uptake in'Sattabongkot' (A) and 'Saddhabutra' (B) lotus flowers treated with EthylBloc Sachet and/or 2,4-PDCA. *: significant differences at P ≤ 0.05, **: significant differences at P ≤ 0.01 (n = 15).

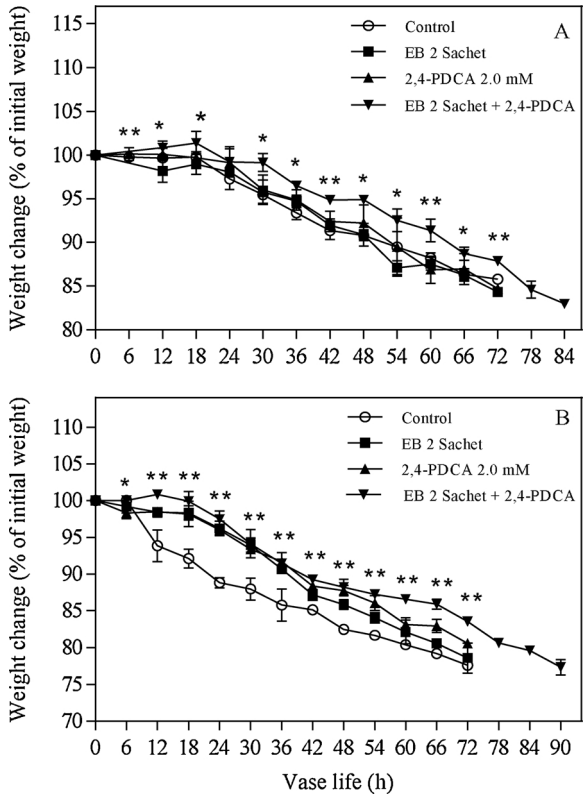

Fig. 2. Weight change in 'Sattabongkot' (A) and 'Saddhabutra' (B) lotus flowers treated with EthylBloc Sachet and/or 2,4-PDCA. *: significant differences at P ≤ 0.05, **: significant differences at P ≤ 0.01 (n = 15).

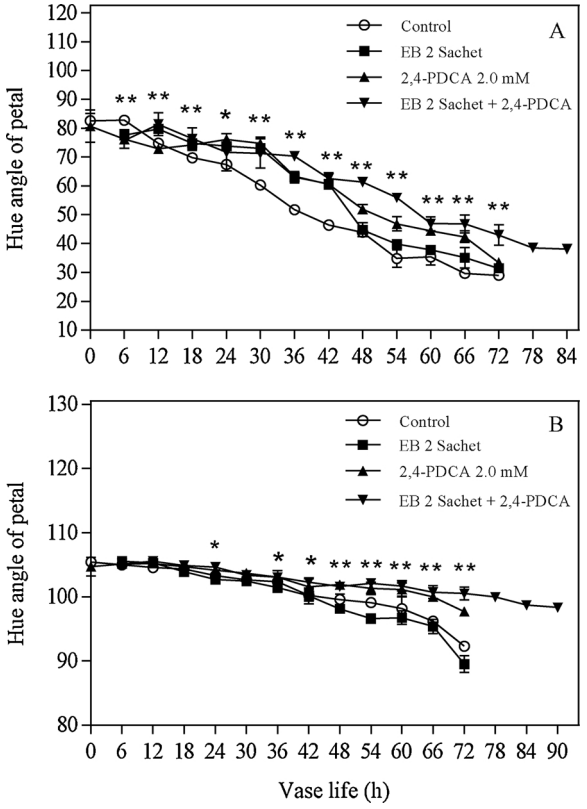

Fig. 3. Change of hue angle in 'Sattabongkot' (A) and 'Saddhabutra'(B) lotus flowers treated with EthylBloc Sachet and/or 2,4-PDCA. *: significant differences at P ≤ 0.05, **: significant differences at P ≤ 0.01 (n = 15).

with other treatments. EthylBloc Sachet and 2,4-PDCA reduced the climacteric ethylene production (Fig. 4A and B). The rate of respiration in both cultivars tended to increase during 0–9 h of vase life, then gradually decreased thereafter. The control flowers showed the highest respiration rate when compared with other treatments (Fig. 4C and D).

3.4. Development of petals blackening and vase life

Petal blackening in cut lotus flowers initially appeared at the margins of petals, then to the spread whole area of unopened buds in both cultivars. The combination of EthylBloc 2 Sachet (a.i., 0.014%) and 2.0 mM 2,4-PDCA effectively delayed petal blackening and prolonged the vase life of 'Sattabongkot' and 'Saddhabutra' cultivars (Fig. 5A and B). The control flowers started petal blackening in 6 h, while the treatment with the combination of EthylBloc Sachet and 2,4-PDCA delayed petal blackening to 18 h in 'Sattabongkot' and 36 h in 'Saddhabutra' (Fig. 6A and B). The vase life of both cultivars terminated when the black patches appeared on the half of surface of buds. The combination treatment gave the longest vase life of 49.8 h in 'Sattabongkot' and 69.0 h in 'Saddhabutra' (Tables 1 and 2).

4. Discussion

Two cultivars have the bootstrap value of 90% (Salaemae, 2017). Lotus cultivars are different not only genetically but also in the number of stomata, as shown in 'Sattabongkot' and 'Seika White' (Salaemae, 2017). 'Seika White' has less number of stomata and longer vase life than 'Saddhabutra', suggesting smaller water loss. However, lotus flowers show climacteric changes in respiration and ethylene production during postharvest period. Postharvest lotus flowers show a rapid petal blackening within 24 h and fail to open, resulting in a short vase life (Imsabai, 2010; Imsabai et al., 2010).

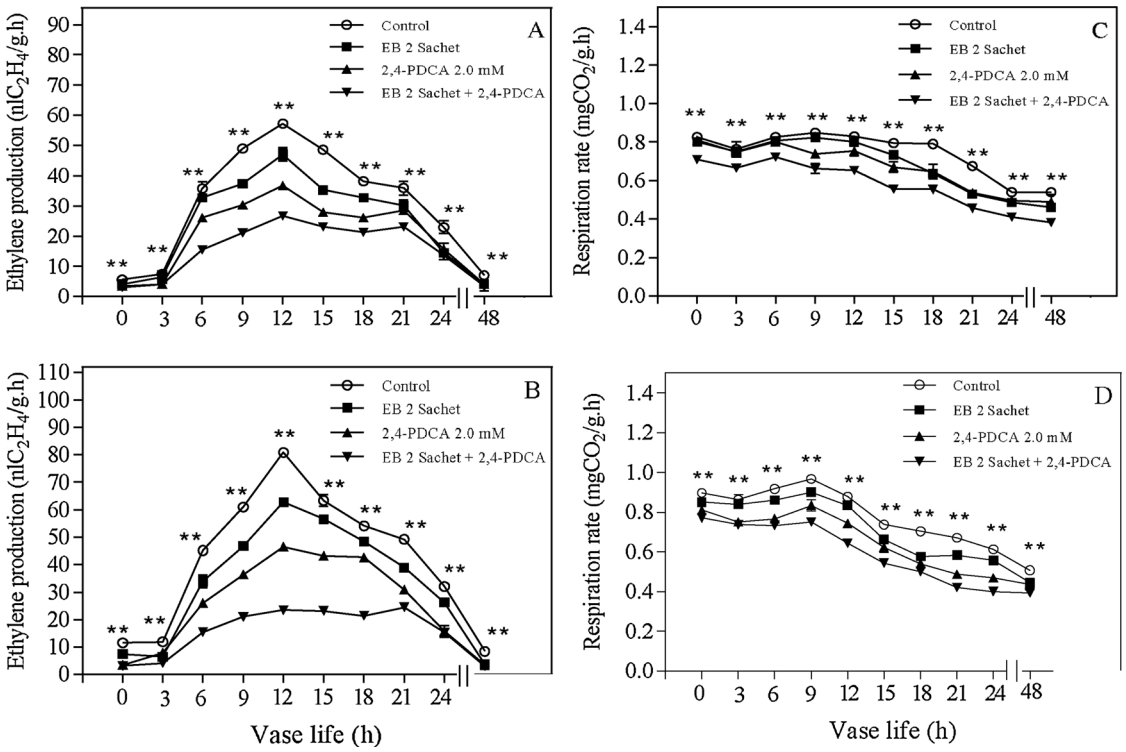

Fig. 4. Ethylene production and respiration rate in 'Sattabongkot' (A, C) and 'Saddhabutra'(B, D) lotus flowers treated with EthylBloc Sachet and/or 2,4-PDCA. *: significant differences at P ≤ 0.05, **: significant differences at *P* ≤ 0.01 (n = 15).

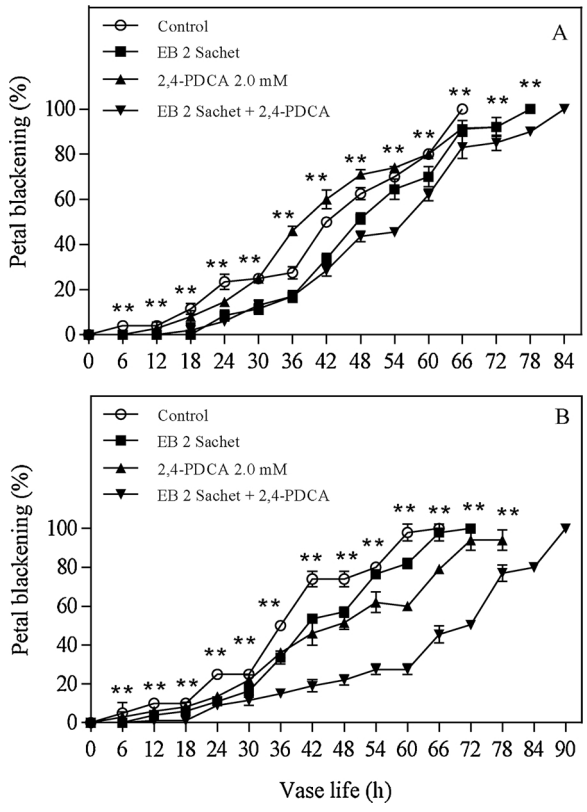

Fig. 5. Change of petal blackening in 'Sattabongkot' (A) and 'Saddhabutra' (B) lotus flowers treated with EthylBloc Sachet and/or 2,4-PDCA. *: significant differences at P ≤ 0.05, **: significant differences at P ≤ 0.01 (n = 15).

Currently, new chemicals are being used for postharvest quality maintenance and prolonging the vase life of cut flowers. 1-MCP delays the onset of petal blackening (Imsabai, 2010). 2,4-PDCA, which is a structural analog of 2-oxoglutarate (OxoGA), inhibits ethylene production of carnation flowers (Vlad et al., 2010; Fragkostefanakis et al., 2013; Satoh et al., 2014). In addition, PDCA has several structural analogs, including 2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-PDCA. All the analogs promote flower opening and delay senescence in carnation flowers. Moreover, 2,3-PDCA can promote root elongation in lettuce, carrot and rice seedlings (Satoh and Nomura, 2017).

The best treatment is the application of the combination of 1-MCP in a commercial agent EthylBloc Sachet and 2,4-PDCA. EthylBloc Sachet Technology is an EPA approved ethylene action inhibitor. The combination of EthylBloc and 2,4-PDCA at 2.0 mM caused the largest water uptake and weight change. Especially, it caused an increase in the initial 6 h of vase life, and a marked decline thereafter (Rogers, 1973; Van Doorn, 1997). The deficiency of water after harvest or water stress is another cause for petal blackening and short vase life (Imsabai et al., 2013). Also, lotus flowers may be deficient in sugar accumulation after harvest (Netlak and Imsabai, 2013). Petal blackening is the main cause, which affects postharvest quality and vase life. Imsabai, 2010 treated 'Saddhabutra' flowers with ethephon, and suggested that ethylene plays a role to accelerate senescence of lotus flowers. Moreover, Imsabai (2010) reported that ethylene production in 'Saddhabutra' flowers are climacteric, and the flowers showed the largest ethylene production in 9–12 h, and a marked decline thereafter, the respiration rate and ethylene production could not be measure after 48 h. In conclusion, the combination of EthylBloc Sachet and 2,4-PDCA effectively delayed ethylene production and respiration as compared to other treatments (Fig. 4). The increased ethylene production might be explained as the result of physiological stress, and so the rise in respiration rate. 2,4-PDCA might delay petal blackening by inhibiting ACC oxidase engaged in ethylene biosynthesis, as reported in cut carnation flowers (Fragkostefanakis et al., 2013). Satoh et al. (2014) reported that the treatment of 'Light Pink Barbara' carnation flowers with 2,4-PDCA at

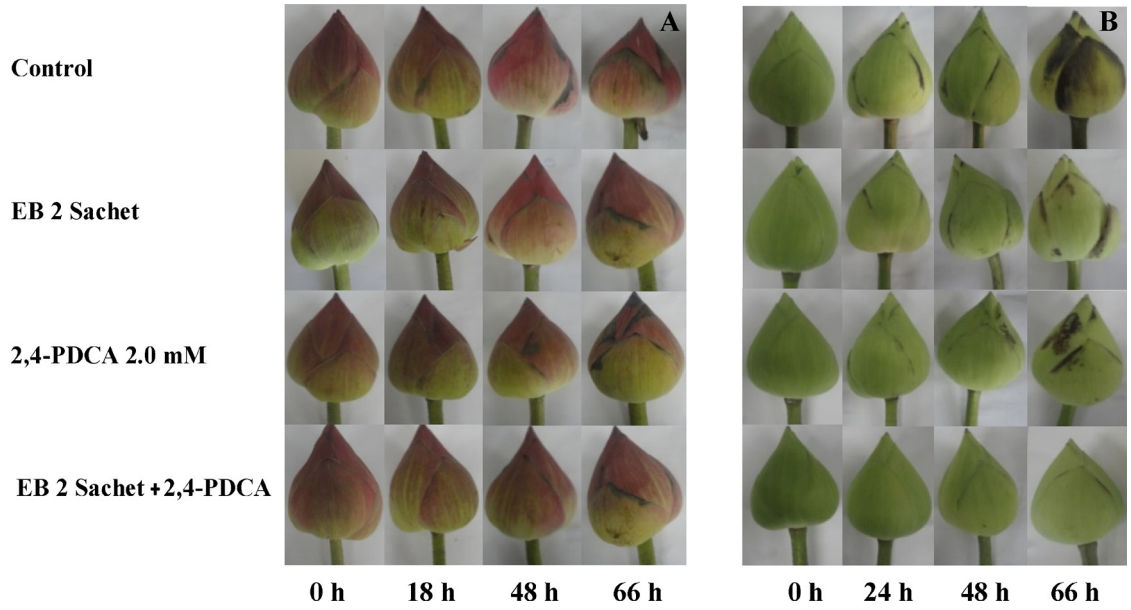

Fig. 6. Development of petal blackening in 'Sattabongkot' (A) and 'Saddhabutra' (B) lotus. flowers treated with EthylBloc Sachet and/or 2,4-PDCA. (n = 15).

Table 1
Vase life of bud-cut 'Saddhabutra' lotus flowers treated with EthylBloc Sachet and/or 2,4-PDCA.

Treatments	Vase life (h)
Control	36.0 ± 0.0c
EthylBloc 2 Sachet (a.i., 0.014%)	41.4 ± 7.5b
2,4-PDCA at 2.0 mM	43.8 ± 9.4b
EthylBloc 2 Sachet (a.i., 0.014%) + 2,4-PDCA at 2.0 mM	69.0 ± 4.2a
F-test	**
C.V. (%)	13.3

Table 2
Vase life of bud-cut 'Sattabongkot' lotus flowers as treated with EthylBloc Sachet and/or 2,4-PDCA.

Treatments	Vase life (h)
Control	44.4 ± 5.3bc
EthylBloc 2 Sachet (a.i., 0.014%)	48.6 ± 4.4ab
2,4-PDCA at 2.0 mM	39.6 ± 6.5c
EthylBloc 2 Sachet (a.i., 0.014%) + 2,4-PDCA at 2.0 mM	49.8 ± 7.5a
F-test	**
C.V. (%)	13.4

2.0 mM prolonged their vase life to 19.5 days, when that of non-treated control was 8.3 days. Also, 2,4-PDCA may act as an inhibitor of enzymes involved in gibberellin biosynthesis and metabolism. 2,4-PDCA may act as a competitive inhibitor of OxoGA-dependent dioxygenases with respect to OxoGA (Kivirikko and Myllyharju, 1998; Kivirikko and Pihlajaniemi, 1998; Vlad et al., 2010).

Previously EthylBloc Sachet treatment was studied with or without exogenous ethylene treatments on quality and longevity in potted carnations (*Dianthus caryophyllus* L.). EthylBloc Sachet delayed leaf yellowing and maintained plant longevity in potted carnations (Burana et al., 2008). This is due to the inhibition of chlorophyll degradation caused by ethylene. 1-MCP treatment could delay or inhibit the yellowing of many plant organs, such as broccoli florets and avocado fruits (Gong and Mattheis, 2003; Hershkovitz et al., 2005). The present findings revealed that the combination of EthylBloc Sachet and 2,4-PDCA effectively delayed petal blackening, which started at 18 h of vase life in 'Sattabongkot' and 36 h in 'Saddhabutra' (Fig. 6). Also the combination of two agents prolonged the vase life; from 44.4 h (the control) to 49.8 h in 'Sattabongkot' and from 36.0 h (the control) to 69.0 h in 'Saddhabutra'. From the result found that the combination of 1-MCP and 2,4-PDCA in Saddhabutra was effective more than Sattabongkot. Because of the lotus is climacteric flowers, it have high the respiration rate and ethylene production and induce to rapid senescence. Suggestion that, the combination of EthylBloc Sachet and 2,4-PDCA was promoted inhibit ethylene biosynthesis and ethylene action. Additionally, considering the ethylene production, suggest that the combination of EthylBloc Sachet and 2,4-PDCA reduced ethylene production in Saddhabutra from 80 down to 20 nL C_2H_4/g.h which were more than in Sattabongkot from 60 down to 20 nL C_2H_4/g.h. These findings suggested that 2,4-PDCA inhibited ethylene production in the early stage of vase life, and then EthylBloc Sachet inhibited ethylene action. 1-MCP tightly binds to ethylene receptor in plants, thereby blocks the action of ethylene. Thus, the present study suggest that EthylBloc Sachet treatment for 6 h is a useful for transportation of cut lotus flowers, and, in addition, 2,4-PDCA can be applied to reduce petal blackening extending their vase life.

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