The Horticulture Journal 88 (3): 420–426. 2019.
doi: 10.2503/hortj.UTD-087

The Japanese Society for
Horticultural Science
http://www.jshs.jp/

Pulsing with Magnesium Oxide Nanoparticles Maintains Postharvest Quality of Cut Lotus Flowers (*Nelumbo nucifera* Gaertn) 'Sattabongkot' and 'Saddhabutra'

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Two main problems of cut lotus flower buds are petal blackening and petal discoloration, resulting in short vase life and loss of postharvest quality. In this study, we investigated the effect of magnesium oxide nanoparticles (MgO NP) in terms of maintaining postharvest quality of the lotus cut flower buds (*Nelumbo nucifera* Gaertn) 'Sattabongkot' and 'Saddhabutra'. Cut flower buds of each cultivar were pulsed with different concentrations of an MgO NP suspension (0, 0.05, 0.1, and 0.3%), for 6 h followed by transfer to distilled water as the vase solution at 27 ± 1°C and 80–85% RH. The longest vase life of 90.0 h was found in 'Sattabongkot' when cut flowers were pulsed with 0.1% MgO NP compared with control was 64.8 h. 'Saddhabutra' showed the second longest vase life of 87.0 h when pulsed with 0.3% MgO NP and control was 62.2 h. Pulsing with an appropriate concentration of MgO NP (0.1% for 'Sattabongkot' and 0.3% for 'Saddhabutra') increased water uptake, delayed fresh weight decrease, suppressed ethylene production, and reduced petal blackening in the cut flower buds of both cultivars. Moreover, MgO NP inhibited microbial growth in vase water as indicated by a reduction in microbial population on agar plates as compared with the control. Based on our results, we suggest that 0.1 and 0.3% MgO NP suspensions may be applied as an alternative way to preserve the postharvest quality of cut lotus flower buds.

Key Words: ethylene, flower bud, vase life, microbial growth, stress treatment.

Introduction

The lotus (*Nelumbo nucifera* Gaertn) flower is a symbol of Buddhism and is commonly used for decoration as cut flower buds. In Thailand, there are two major commercial cultivars: 'Saddhabutra' with green outer petals, and 'Sattabongkot' with pink outer petals (Salaemae et al., 2018a). Lotus flowers are climacteric in pattern having a high respiration rate and ethylene production that induce rapid senescence (Imsabai et al., 2010). Currently, cut lotus flower buds have two main problems: petal blackening and petal discoloration. These symptoms lead to a short vase life and loss of postharvest quality. A rapid blackening of the petals occurs within 24 h of harvest without flower opening when lotus flowers are placed in water at ambient temperature (Imsabai et al., 2010). Green petal discoloration was reportedly due to chlorophyll degradation (Salaemae, 2017). Impaired water uptake is another physiological stress that may be associated with blackening. Lack of water uptake or excessive water loss

Received; March 11, 2019. Accepted; April 19, 2019.
First Published Online in J-STAGE on June 8, 2019.
This work was supported by a research fund from the Faculty of Technology and Community Development, Thaksin University, Prince of Songkla University (grant no. NAT6202117-0, and the Center of Excellence in Agricultural and Natural Resources Biotechnology (CoE-ANRB) phase 2. Ube Material Industries, Japan provided MgO NP.
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from the flowers are causes of short vase life and petal blackening (Imsabai and van Doorn, 2013). These quality problems in lotus are exacerbated due to stresses induced by harvesting. Wounding by cutting flowers can induce xylem occlusions and increase bacterial populations in vase water (Vaslier and van Doorn, 2003; Loubaud and van Doorn, 2004).

In order to maintain postharvest quality several methods have been applied to reduce senescence and prolong vase life. For instance, 1-methylcyclopropene (1-MCP) was applied to delay petal blackening and inhibit the expression of genes involved in ethylene biosynthesis, *Nn-ACS* (*Nn*-1-aminoacyclopropane-1-carboxylate synthase), and *Nn-ACO* (*Nelumbo nucifera*-1-aminocyclopropane carboxylate oxidase) in the lotus 'Saddhabutra' (Imsabai et al., 2010). Also, 1-MCP, commercially obtained as an EthylBloc sachet, combined with 2,4-pyridinedicarboxylic acid (2,4-PDCA) could extend the vase life of lotus flowers by reducing ethylene production and the respiration rate (Salaemae et al., 2018a). Further, application of gibberellic acid (GA_3) was reported to delay petal blackening and to extend the vase life of the lotus 'Saddhabutra' (Imsabai and van Doorn, 2013).

Nanotechnology has the potential to be used in agricultural fields for plant protection (Ghormade et al., 2011). Recent investigations have shown that several inorganic metal oxide nanoparticles exhibit strong germicidal activity against bacteria (Paret et al., 2013; Liu et al., 2014), fungi (Liu et al., 2017), and a few viruses (Mishra et al., 2011). Among different inorganic metal oxides, the magnesium oxide nanoparticle (MgO NP) is a strong antimicrobial agent that has been recognized as a safe material by United States Food and Drug Administration. Furthermore, MgO NP has been reported to induce systemic resistance to bacterial wilt disease in tomatoes by activating salicylic acid and jasmonic acid (Imada et al., 2016). However, there is no report on the application of MgO NP to maintain the postharvest quality of cut lotus flower buds. We hypothesized that MgO NP would maintain the postharvest quality of cut lotus flower buds at high level by inhibiting bacterial growth in the xylem and reducing physiological stress and ethylene biosynthesis. Therefore, this research aimed to evaluate the effects of MgO NP on the postharvest quality of 'Sattabongkot' and 'Saddhabutra' lotus cut flower buds.

Materials and Methods

Plant materials

Lotus (*Nelumbo nucifera* Gaertn) flower buds of 'Sattabongkot' and 'Saddhabutra' at commercial stage 5 of flowering, i.e., buds of 6.0–6.5 cm in diameter (Netlak and Imsabai, 2013) were sampled from a private field in Phatthalung province, located in southern Thailand. Cut lotus flower buds were brought to the laboratory of Plant Science, Faculty of Technology and Community Development, Thaksin University, Phatthalung campus where experiments were conducted. Stems of lotus flower buds were re-cut to 25-cm in length under tap water for further use. The flowers were stored in water (control) or pulsing nanoparticle solutions in glass bottles (one flower per bottle), and incubated at 27 ± 1°C and 85–90% RH under natural day light (from about 6 am to 6 pm).

Pulsing with magnesium oxide nanoparticles

MgO NP was obtained from Ube Material Industries, Japan. MgO NP was suspended in distilled water and sonicated for 5 min before use in all experiments. Morphological observation of MgO NP was conducted by scanning electron microscopy (JEOL, JSM-6100, Tokyo, Japan). To evaluate the effects of MgO NP on postharvest quality, pulsing with different concentrations (0.05, 0.1, and 0.3% (w/v) was performed on cut flowers for 6 h. Pulsing with distilled water served as the control. The flowers were then transferred into 200 mL glass bottles (one flower per bottle) with their stem ends in distilled water as the vase solution. Experiments were conducted with 15 replicated flowers per treatment. The vase life and postharvest quality parameters were determined every 6 h.

Determination of water uptake, weight change and ethylene production

The rate of water uptake was measured by placing stems in graduated cylinders sealed with parafilm and measuring the water level every 6 h. Water uptake was determined by monitoring the reduction in water volume per cut flower every 6 h. Water was replenished every 6 h for measurements. Flower fresh weight was determined during the experiment. Weight change was determined as the percentage of initial fresh weight. Ethylene production from cut lotus flowers was determined according to Imsabai et al. (2010). Stems of lotus flowers of both cultivars were cut to 7 cm in length, enclosed in 2.0 L plastic bottles (one flower per bottle), and left for 10 min. The experiment was conducted in 15 replicates. Head-space gas samples (1 mL) were taken from the gas sampling port on the lid of the bottles. Concentrations of ethylene in the gas samples were analyzed using a gas chromatograph (Shimadzu GC 8A, Kyoto, Japan) equipped with an active alumina column and flame ionization detector. Data were expressed per gram of flower fresh weight.

Evaluation of petal blackening and vase life

During vase life, petal blackening was determined every 6 h. To assess the degree of blackening, the internal scale of the blackened-area in petals was scored as a percentage: 10% = onset of petal blackening, 25% = black patches on the edges of petals, 50% = whole edges covered by black tissues, 75% = blackening region expanding, and 100% = whole petals blackened

(Imsabai et al., 2010). The length of vase life was defined as the period until half of the visible petals showed black patches or reached 50% petal blackening.

Measurement of microbial populations in vase solutions

To examine the microbial populations in the vase water of cut flowers of both cultivars, 10-fold dilutions were performed on nutrient agar (NA). After pulsing with different concentrations of MgO NP, the vase water for each treatment was collected and subjected to 10-fold dilution and spread on NA by a simple spread plate technique. The agar plates were incubated at 30°C for 24 h and colony forming units ($CFU \cdot mL^{-1}$) were measured.

Statistical analysis

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 calculated by the F-test one-way ANOVA. Statistical significance of vase life was evaluated using Tukey's Range Test, while water uptake, fresh weight, ethylene production, petal blackening, and microbial population were analyzed by Student's *t*-test (Gomez and Gomez, 1984). The difference between means was calculated by least significant difference at $P < 0.05$.

Results

Optimization of MgO NP in pulsing treatment

As shown in Figure 1, the MgO NP particles were spherical or irregularly shaped, and had a diameter in the range of 100–200 nm. To test the effect of MgO NP on vase life, cut lotus flowers buds were pulsed with different MgO NP suspensions (0, 0.05, 0.1, and 0.3%) for 6 h. The longest vase life of 90 h was observed for 'Sattabongkot' when cut flowers buds were pulsed with 0.1% MgO NP as compared with controls which achieved 64.8 h. 'Saddhabutra' had the longest vase life of 87.0 h when pulsed with 0.3% MgO NP and the control achieved 62.2 h (Table 1). The control flowers of both cultivars developed complete petal blackening (100%) at 84 h, whereas MgO NP-treated flowers showed no petal blackening up to 90 h in 'Sattabongkot' treated with 0.1% MgO NP and 'Saddhabutra' treated with 0.3% MgO NP (Fig. 2). Therefore, 0.1 and 0.3% MgO NP treatment of 'Sattabongkot' and 'Saddhabutra', respectively, were selected as the optimal treatments and used for further investigation.

Effect of MgO NP on water uptake, fresh weight and ethylene production

The water uptake of both 'Sattabongkot' and 'Saddhabutra' treated with MgO NP at 0.1 and 0.3%, respectively, occurred at high levels from 12–18 h of vase life, then rapidly declined by the last day. Treatment with MgO NP maintained water uptake at a significantly higher level than that of the control ($P \leq 0.01$) during incubation in both cultivars (Fig. 3A, B). In both cultivars, the fresh weight of the control flowers declined rapidly during vase life, whereas MgO NP-treated flowers showed a delayed reduction in the fresh weight ($P < 0.01$) (Fig. 4A, B). The cut flowers of both cultivars showed similar climacteric patterns of ethylene production up to 48 h (Fig. 5A, B). The flowers pulsed with suitable concentrations of MgO NP produced less ethylene than the controls in both cultivars.

MgO NP delays petal blackening in cut lotus flower buds

To test the effect of MgO NP on petal blackening, an interval symptom percentage scale was used as described in the Materials and Methods section 2.4. In the control flowers of both cultivars, petal blackening rapidly developed from 0 to 80% during vase life. Pulse treatment with 0.1 and 0.3% MgO NP effectively

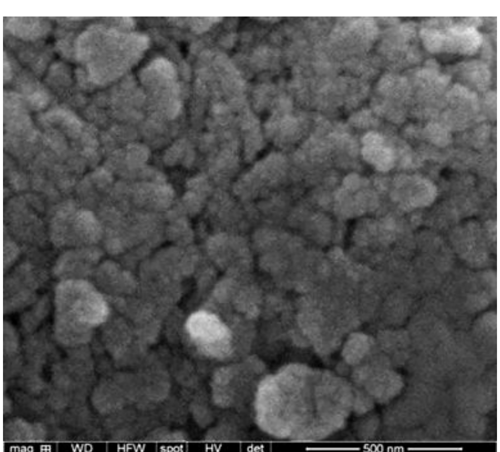

Fig. 1. Morphology of MgO NP observed by scanning electron microscopy.

Table 1. Vase life of cut lotus flowers 'Sattabongkot' and 'Saddhabutra' pulsed with different concentrations of MgO NP suspensions.

Treatment	Vase life (h) 'Sattabongkot'	Vase life (h) 'Saddhabutra'
Control	64.8 ± 0.4d	62.2 ± 0.4d
MgO NP 0.05%	75.6 ± 0.5b	75.6 ± 0.5c
MgO NP 0.1%	90.0 ± 0.4a	78.1 ± 0.7b
MgO NP 0.3%	73.2 ± 0.8c	87.0 ± 0.5a
F-test	**	**
C.V. (%)	0.8	0.7

The average values were calculated by the F-test one-way ANOVA with SE (n = 15). Different superscript letters (a–d) within the same column indicate significant differences between treatments. The asterisk (**) indicates that the value is significantly different from the corresponding control ($P < 0.01$).

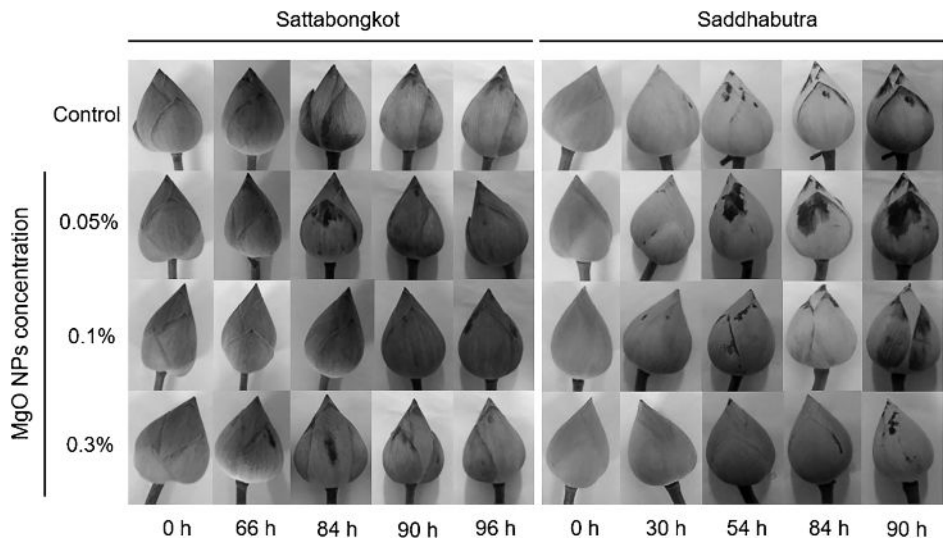

Fig. 2. Development of petal blackening in 'Sattabongkot' and 'Saddhabutra' lotus flower buds pulsed with MgO NP suspensions.

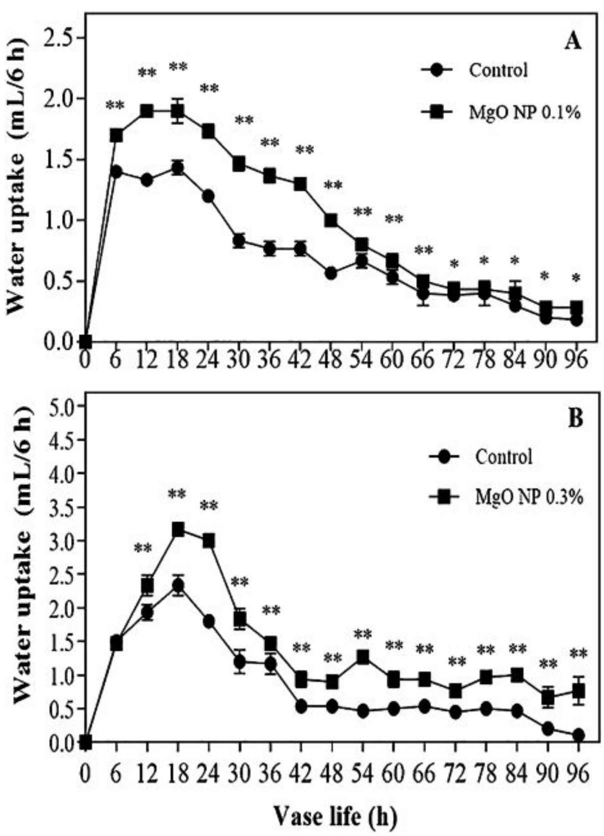

Fig. 3. Change in water uptake in 'Sattabongkot' (A) and 'Saddhabutra' (B) lotus flower buds pulsed with MgO NP suspensions. *: significant differences at $P \leq 0.05$, **: significant differences at $P \leq 0.01$ (n = 15).

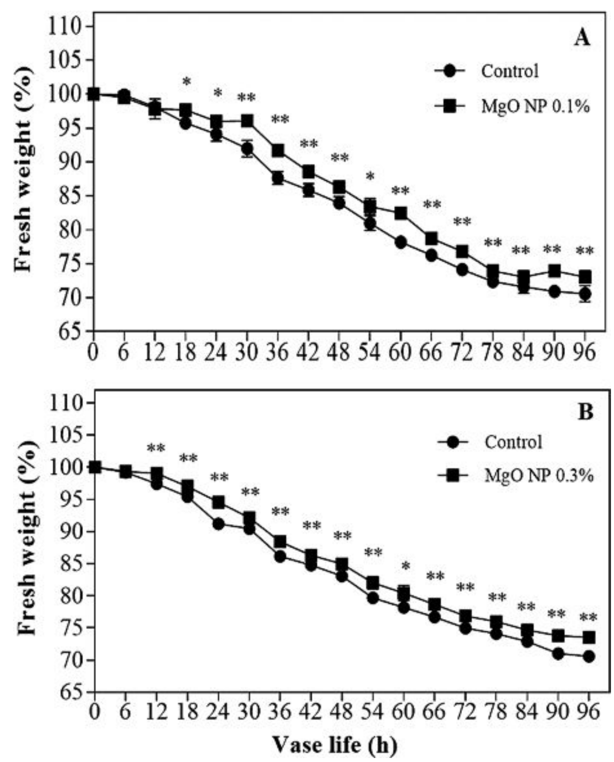

Fig. 4. Fresh weight of 'Sattabongkot' (A) and 'Saddhabutra' (B) lotus flower buds pulsed with MgO NP suspensions. *: significant differences at $P \leq 0.05$, **: significant differences at $P \leq 0.01$ (n=15).

delayed the petal blackening of 'Sattabongkot' and 'Saddhabutra', respectively (Fig. 6A, B).

MgO NP reduction of microbial populations in vase water

The effect of MgO NP on microbial populations in vase water of cut flower buds of both cultivars during vase life was assessed using a 10-fold dilution and simple spread plate technique. After incubation for 24 h, microbial populations in vase water obtained from both concentrations of MgO NP suspensions (0.1% and 0.3%) were lower than in the controls in both 'Sattabongkot' and 'Saddhabutra' in NA test plates (Fig. 7).

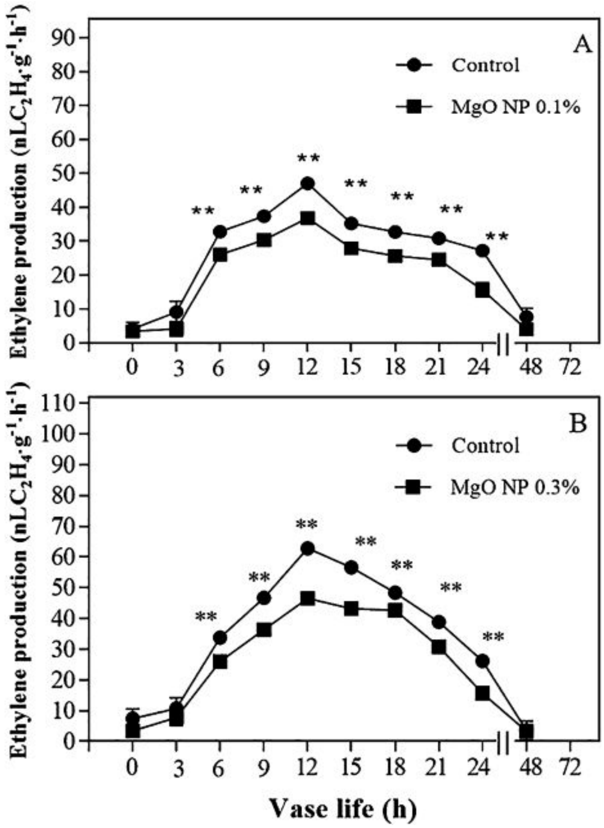

Fig. 5. Ethylene production of ‘Sattabongkot’ (A) and ‘Saddhabutra’ (B) lotus flower buds pulsed with MgO NP suspensions. *: significant differences at $P \leq 0.05$, **: significant differences at $P \leq 0.01$ (n = 15).

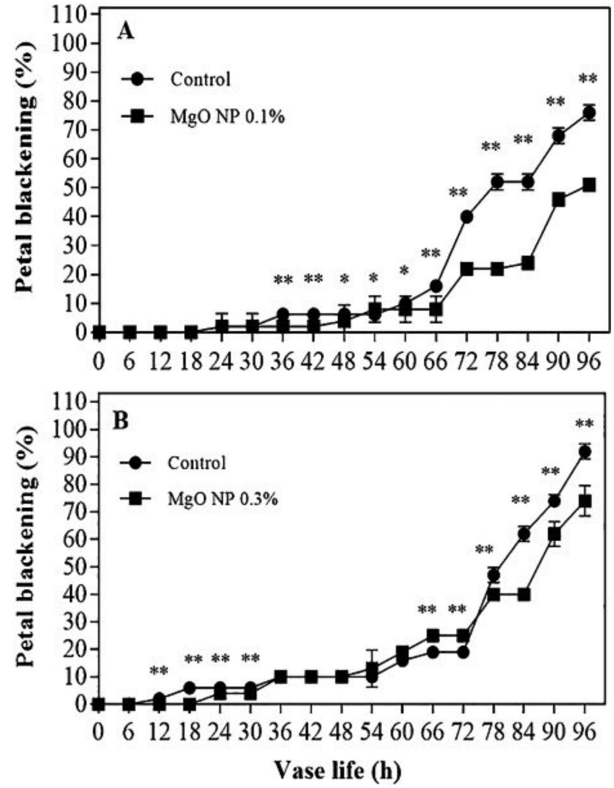

Fig. 6. Changes in petal blackening in ‘Sattabongkot’ (A) and ‘Saddhabutra’ (B) lotus flower buds pulsed with MgO NP suspensions. *: significant differences at $P \leq 0.05$, **: significant differences at $P \leq 0.01$ (n = 15).

Discussion

In this study we investigated the effect of MgO NP on postharvest quality of the cut flower buds of lotus ‘Sattabongkot’ and ‘Saddhabutra’. Our results showed that application of MgO NP by pulsing extended the vase life of flowers (Table 1), increased water uptake (Fig. 3), delayed fresh weight loss (Fig. 4), reduced ethylene production (Fig. 5), delayed petal blackening in both cultivars (Fig. 6), and reduced microbial populations in the vase water of cut flower buds (Fig. 7).

The lotus is a climacteric flower, which rapidly senescences after cutting, showing petal blackening, a symptom that causes a major loss of flower quality. Discoloration of lotus petals has been reported to be associated with chlorophyll degradation (Salaemae, 2017). Nowadays, several methods have been developed to maintain postharvest quality, including use of 1-MCP in cut flower buds of the lotus ‘Saddhabutra’ as previously described by Imsabai et al. (2010). The use of MgO NP in this study caused a reduction in petal blackening and an extension of the vase life of cut flower buds of ‘Sattabongkot’ and ‘Saddhabutra’ (Fig. 2). These findings suggest that application of MgO NP at appropriate concentrations could extend the vase life and maintain the postharvest quality of cut lotus flower buds.

Water deficiency after harvest is another problem causing petal blackening and short vase life (Imsabai and van Doorn, 2013). In some cut flower species, wounding due to cutting can induce xylem occlusions in the stem ends, which can cause further problems such as bacterial proliferation in the vase water (Vaslier and van Doorn, 2003; Loubaud and van Doorn, 2004). Lack of water uptake or excessive water loss from the flowers can result in a short vase life and petal blackening (Imsabai and van Doorn, 2013). In certain cut flowers, water uptake increased in the first 6 h after cutting, but thereafter declined markedly, and the fresh weight also declined during the same period (Rogers, 1973; van Doorn, 1997). Our results with ‘Sattabongkot’ and ‘Saddhabutra’ showed that treatment with MgO NP caused the highest water uptake and reduced fresh weight loss of cut flowers of both cultivars (Figs. 3 and 4). Therefore, application of MgO NP by pulsing could promote water uptake and reduce fresh weight loss in cut flower buds of both lotus cultivars.

It is known that physiological stress can be caused by a lack of water and an unsuitable environment, which in turn causes an increased transpiration rate and ethylene production (Imsabai and van Doorn, 2013). Ethylene is

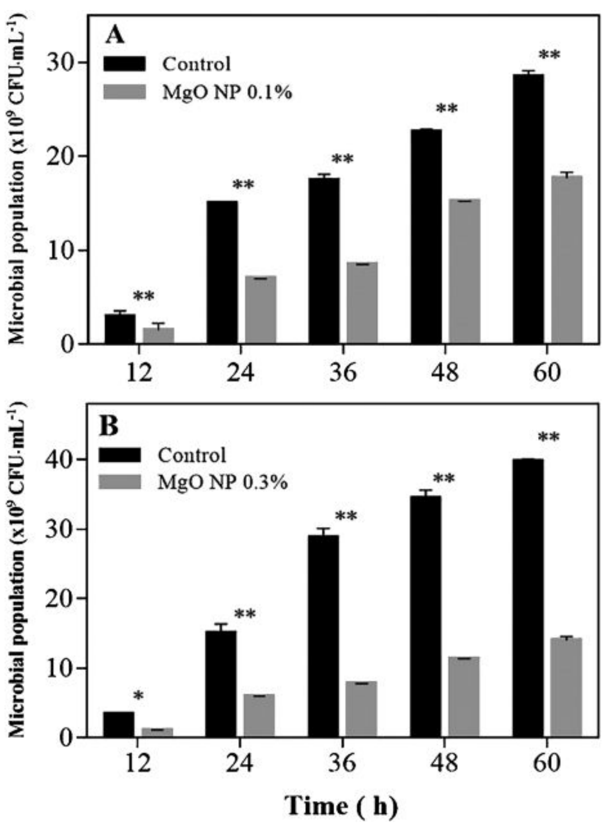

Fig. 7. Microbial population in vase water of cut lotus flower buds 'Sattabongkot' (A) and 'Saddhabutra' (B) pulsed with MgO NP suspensions.

a plant hormone that plays an important role in inducing senescence in several plants (Burg, 1968; Reid, 1995), as well as in lotus flower buds (Imsabai et al., 2010). Because the lotus is a climacteric plant, various types of physiological stresses are often accompanied by an increase in ethylene production that directly affects flower senescence (Abeles et al., 1992). Imsabai et al. (2010) reported that 1-MCP delayed the occurrence of petal blackening in flowers of the lotus 'Saddhabutra' by 2 d and inhibited the expression of two genes involved in ethylene biosynthesis, *Nn-ACS* and *Nn-ACO*. This study showed that pulsing with MgO NP resulted in significantly lower ethylene production than the non-pulsed controls in both cultivars (Fig. 5). The MgO NP-treated cut flower buds of both lotus cultivars developed petal blackening at a slower rate than the control flowers (Fig. 6). This finding suggests that pulsing with MgO NP reduced ethylene production and petal blackening development in cut lotus flower buds. The use of ethylene inhibitors such as 1-MCP or a combination of 1-MCP (EthylBloc Sachet) and 2,4-PDCA reduced petal blackening and prolonged the vase life of cut flower buds of the lotus cultivars, 'Sattabongkot' and 'Saddhabutra' (Salaemae et al., 2018b). It is known that ethylene production is related to ACS and ACO activities in plants (Peter et al., 1991; Kim, 2006). MgO NP was involved in ethylene biosynthesis in the present study. MgO NP may act as an ethylene inhibitor by reducing ACS and ACO activities, as well as ACS and ACO gene expression, resulting in reduced ethylene production in cut lotus flower buds. Future studies will focus on the effect of MgO NP on ethylene biosynthesis enzymes activities and the related gene expression of cut lotus flowers.

Petal blackening of cut lotus flowers shortens their vase life and reduces postharvest quality. It was found that a cultivar with a short vase life and rapid blackening had a large number of stomata in the petal epidermis (Salaemae, 2017), suggesting that inhibition of a change in fresh weight could prolong vase life and prevent petal blackening. In this study, pulsing of MgO NP effectively delayed petal blackening of both 'Sattabongkot' and 'Saddhabutra' compared with the non-pulsed controls (Fig. 6). These results showed that treatment with a suitable concentration of MgO NP could delay petal blackening development in cut lotus flowers.

Accumulation of microbes in lotus stems causes reduced water uptake and results in rapid petal blackening. Leethiti et al. (2014) found that holding cut lotus flower buds in 8-hydroxyquinoline sulfate (HQS) solution did not affect water uptake. Our results showed that application of MgO NP effectively reduced the microbial population in vase water, as well as reducing colony forming units in NA test plates (Fig. 7). The MgO nanoparticles can release reactive oxygen species (ROS) that can produce hydrogen peroxide that is toxic to bacterial pathogens. MgO NP is regarded as a strong antimicrobial agent, and it inhibits the growth of several bacterial strains (Liu et al., 2014; Paret et al., 2013; Sierra-Fernandez et al., 2017) by damaging cellular components such as proteins, lipids, and even nucleic acids (Hemeg, 2017); it can reduce bacterial populations to a similar level to typical bactericide activity (Cai et al., 2018; Jin and He, 2011). MgO NP has also been shown to destroy *Ralstonia solanacearum* bacterial cells (Imada et al., 2016). Therefore, application of MgO NP in this study may have inhibited bacterial growth and reduced bacterial populations in vase water. Accumulation of smaller microbial populations in the stem end of cut lotus flower buds (Fig. 7) may lead to higher water uptake and lower fresh weight during vase life.

Several recent studies have focused on maintaining the postharvest quality of cut lotus flower buds by using ethylene inhibitors and/or plant hormones (Imsabai and van Doorn, 2013; Imsabai et al., 2010). In this study, we used MgO NP to extend the vase life of cut lotus flower buds by one day, this is meaningful in the lotus industry as it could reduce postharvest loss by about 50%, maintain good quality and achieve a good price. In addition, MgO NP promoted a useful reduction in physiological stress by inhibiting the microbial populations in the stem, as well as reducing ethylene production. Application of MgO NP at 0.1 and 0.3% maintained postharvest quality by delaying petal blackening and prolonging the vase life of commercial cut flower buds of the 'Sattabongkot' and 'Saddhabutra' lotuses. To the best of our knowledge, this is the first report on using MgO NP in postharvest flowers. However, we have not yet clarified the role of MgO NP at the molecular level, and further studies on the effects of MgO NP on several enzymes involved in ethylene biosynthesis and free radical scavenging are necessary in the future.

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