ผลของนาโนซิงค์ร่วมกับอะลูมิเนียมซัลเฟตและซูโครสต่อการยับยั้งเชื้อจุลินทรีย์ ในน้ำยาปักแจกันและปรับปรุงคุณภาพของกล้วยไม้สกุลแวนด้าพันธุ์เพียวแว็กซ์พิงค์

Effect of ZnO NPs Combined with $Al_2(SO_4)_3$ and Sucrose on Inhibiting Microbial Growth in the Vase Solution and Improving the Quality of Cut *Vanda* Hybrid 'Pure Wax Pink'

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บทคัดย่อ

การศึกษาผลของการพัลซิ่งด้วยนาโนซิงค์ (ZnO NPs) ร่วมกับการปักแช่ในสารละลายอะลูมิเนียมซัลเฟต ($Al_2(SO_4)_3$) และซูโครส (Suc) ต่อการยับยั้งเชื้อจุลินทรีย์ในน้ำยาปักแจกันและปรับปรุงคุณภาพของกล้วยไม้สกุลแวนด้าพันธุ์เพียวแว็กซ์พิงค์ โดยทำการพัลซิ่งช่อดอกกล้วยไม้ด้วยสารละลาย ZnO NPs ความเข้มข้น 0.25% นาน 3 ชั่วโมง แล้วย้ายมาปักแช่ในสารละลาย ($Al_2(SO_4)_3$ ความเข้มข้น 150 ppm + Suc ความเข้มข้น 2 % ที่อุณหภูมิ 21 $\pm$ 2 องศาเซลเซียส ตลอดระยะเวลาการทดลอง ตรวจวัดการเปลี่ยนแปลงน้ำหนักสดของช่อดอก อัตราการดูดน้ำ การบาน ของดอกย่อย และการยับยั้งการเจริญของเชื้อจุลินทรีย์ในน้ำปักแจกัน เปรียบเทียบกับกล้วยไม้ที่ไม่ได้พัลซิ่งด้วย ZnO NPs แล้วปักแช่ในน้ำขจัดไอออน (deionized water: DI) และ $Al_2(SO_4)_3$ + Suc (ชุดควบคุม) จากการศึกษา พบว่า การใช้ ZnO NPs สามารถยับยั้งเชื้อจุลินทรีย์ในน้ำปักแจกันหลังจากการพัลซิ่งได้อย่างมีนัยสำคัญและในระหว่างการปักแจกันพบปริมาณเชื้อจุลินทรีย์ในน้ำปักแจกันน้อยกว่าชุดควบคุม ถึงแม้ว่ากล้วยไม้ในชุดควบคุมที่ปักแช่ในน้ำ DI และ $Al_2(SO_4)_3$ + Suc มีน้ำหนักสดและเปอร์เซ็นต์การบานของดอก รวมทั้งอัตราการดูดน้ำสูงกว่ากล้วยไม้ที่พัลซิ่งด้วย ZnO NPs แต่การใช้ ZnO NPs ร่วมกับ $Al_2(SO_4)_3$ + Suc ช่วยชะลอการเสื่อมสภาพของดอกและยืดอายุการปักแจกันได้นานถึง 14 วัน ในขณะที่กล้วยไม้ในชุดควบคุมที่ปักแช่ในน้ำ DI และ $Al_2(SO_4)_3$ + Suc มีอายุการปักแจกัน เท่ากับ 9.8 และ 10.3 วัน ตามลำดับ

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Abstract

This study aimed to obtain the positive influence of ZnO NPs pulsing combined with aluminium sulfate ($Al_2(SO_4)_3$) and sucrose (Suc) holding on inhibition of the microbial growth and improvement of the quality of cut *Vanda* hybrid 'Pure Wax Pink'. Flower stems were pulsed with 0.25% ZnO NPs for 3 h, then transferred to 150 ppm $Al_2(SO_4)_3$ + 2 % Suc at 21 $\pm$ 2 °C. Throughout the experimental period, the qualities of pulsed flowers were determined for changes in relative fresh weight, water uptake rate, flower bud opening and inhibiting the growth of microorganisms in vase solution compared with untreated flowers holding in deionized water (DI) and $Al_2(SO_4)_3$ + Suc as control. The result showed that ZnO NPs completely inhibited the microbial growth after pulsing and showed a minimum population of microbial in the vase on the other days as compared to the control. Although the control flowers holding in DI water and $Al_2(SO_4)_3$ + Suc had higher percentage of fresh weight and floret opening as well as higher water uptake than that of ZnO NPs treatment, the combination between ZnO NPs pulsing and $Al_2(SO_4)_3$ + Suc holding treatment significantly delayed floret senescence and extended the vase life to 14 days, whereas the control flowers holding in DI water and $Al_2(SO_4)_3$ + Suc had 9.8 and 10.3 days, respectively.

Keywords: Vanda, zinc oxide, preservatives, biocide.

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Introduction

Orchid cultivation is an international business that has great potential to take part in the economic growth of countries. The postharvest handling in the supply chain under dire care provides a suppressive impact on the physiological and physical mechanisms leading to microbial development in vase solution and quality loss (Kazemi *et al*., 2011). To prevent bacterial proliferation, preservatives, mainly comprised of antimicrobial compounds, are needed in the wet transport of cut flowers (Ichimura *et al*., 2009). When storing flowers, it is recommended to use aluminum sulphate $(Al_2SO_4)_3$ as a germicide which was reported to have a potential impact on floral color by reducing pH, reducing bacterial and fungal growth (Halevy and Mayak, 1981), and enhance longevity in cut roses (Ichimura *et al*., 2009). Use of ZnO nanoparticles provides an antagonistic relationship against bacterial growth and is commonly recognized as an eco-friendly metal in comparison to nano silver (NS) and silver thiosulphate (STS) (Prabawati *et al*., 2023). The present study aimed to investigate the impact of ZnO NPs on suppressing the microbial growth in cut *Vanda* orchid flowers cv. Pure Wax Pink and how it influences the abrupt alteration in their physiological cycle inducing postharvest longevity and the potential to supersede the commonly used floral preservatives.

Materials and Methods

Freshly cut *Vanda* Orchid *cv*. Pure Wax Pink were harvested from a commercial orchard (Kanchanaburi province). Orchid inflorescences bearing 5-8 open florets and 2-4 buds were selected for the experiment, and transported to King Mongkut’s University of Technology Thonburi (KMUTT), Bangkhuntien campus, Bangkok within 1.5 h. Upon arrival to the laboratory, flower stems were re-cut under water to a 20-cm length from the stem end to the lower first flower. The flowers were pulsed for 3h in 0.25% ZnO NPs, then placed in holding solution containing 150 ppm $Al_2(SO_4)_3$ + 2% Sucrose ($Al_2(SO_4)_3$ + Suc), compared with untreated flowers holding in deionized water and in $Al_2(SO_4)_3$ + Suc solution, as control. All flowers were maintained at 21±2°C; RH 60-75% throughout the experimental period. The experiment was arranged in a completely randomized design (CRD), with 8 replicate stems for each treatment for analyzing relative fresh weight, water uptake, microbial growth, floret senescence and vase life, and 3 replicates for anthocyanin content. Data were analyzed using ANOVA, and differences among means were compared using the Duncan Multiple Range Test (DMRT). For microbial plate count, all equipment used was sterilized prior to use and samples were collected at every 3 days interval from the vase solution diluting in 10-fold serial dilution before spreading on media (Jowkar, 2006).

Results and Discussion

ZnO NPs have proved to be bacteriostatic in nature (Deepshikha *et al*., 2018) which was found to be apprehensible after observing the effect of ZnO NPs on bacterial growth held in vase solution. Although the flowers treated with ZnO NPs did not have a significant influence when compared to $Al_2(SO_4)_3$ treated flowers (except for day 0), they showed a significant decrease in bacterial count throughout the vase period when compared to the control (Figure. 1). Zero incidence of bacterial colonies were observed after pulsing and holding at day 0 in the vase solution of ZnO treated flowers with a gradual increase at every three days interval. The decline in bacterial growth for treatment of ZnO NPs could be due to the synergistic effect between ZnO and HCl solvent that made it incompatible for bacteria to sustain and multiply (Dizaj *et al*., 2014). The initial pH level in ZnO solution without HCl was about 7.26 and decreased to 2.21 when mixed with HCl solvent. To avoid toxicity on the flower stem when pulsed with lower acidic preservatives, the duration for pulsing treatment was only 3 hours. Low values in pH have a direct influence on reducing the bacterial surge (Ichimura *et al*., 2003). Acidifying agents such as citric acid and ascorbic acid are used to lower the pH of vase solution to prolong vase life of cut flowers (Mashhadian *et al*., 2012; Sheikh *et al*., 2014).

However, when transferred to $Al_2(SO_4)_3$ + Suc holding solution, flowers showed some visible signs of bacterial growth which may be due to its higher pH value than ZnO NPs pulsing solution. Although, sucrose was added into holding solution for the maintenance of metabolic activities such as respiration, flower opening etc., sucrose in holding solution was also ideal for the growth of microorganisms. As a result, the relative fresh weight and water uptake were found to be less in comparison to control throughout the vase life (Figures 2A and 2B) which were observed in chrysanthemum flowers treated with NZn (Prabawati *et al*., 2023). It was also observed that ZnO NPs did not have a profound influence on their respiration rate but instead maintained an almost stable flow with that of control (Figure. 2C). Anthocyanin contents were found to have a better stability and better response (Figure. 2D) when exposed to treated solutions which were being found to alter a decline in pH making it favourable for anthocyanin pigment synthesis as observed in *Rosa hybrida cv*. Tereasa treated with chemical preservatives (Shanan, 2017). No significant differences were also observed for bud opening among treatments (Figure 2E) but the eminent reduction in floral senescence for ZnO NPs treated flowers and 30% senescence could be seen only after day 14 showing a profound significance against control which could not maintain 30% severity above 9.8 days (Figure. 3A and 3B). Through this can also be brought to an estimation that the vase life of ZnO NPs treated flowers had a sustaining vase period of 14 days which had a better performance as compared to 9.8 and 10.3 days for control. The influence on floral longevity maybe because of its known recognition as an activator of certain enzymes that can aid in regulating antioxidant activity. Also, the holding solutions treated with $Al_2(SO_4)_3$ must have aided in maintaining floral rigidity and shape (Halevy and Mayak, 1981) along with 2% sucrose providing the necessary food for cellular activity.

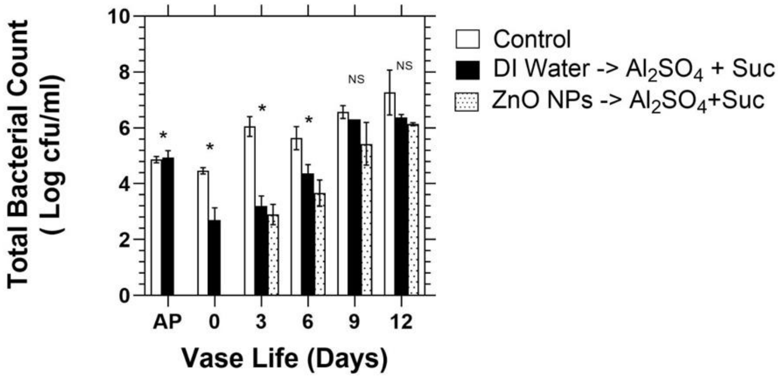

Figure 1. Effects of ZnO NPs pulsing prior to holding in $Al_2(SO_4)_3$ + Suc solution on bacterial growth of *Vanda* Orchid cv. Pure Wax Pink, compared to untreated flowers held in deionized water and in $Al_2(SO_4)_3$ + Suc solution. NS = not significantly different; * = significantly different at $P \leq 0.05$.

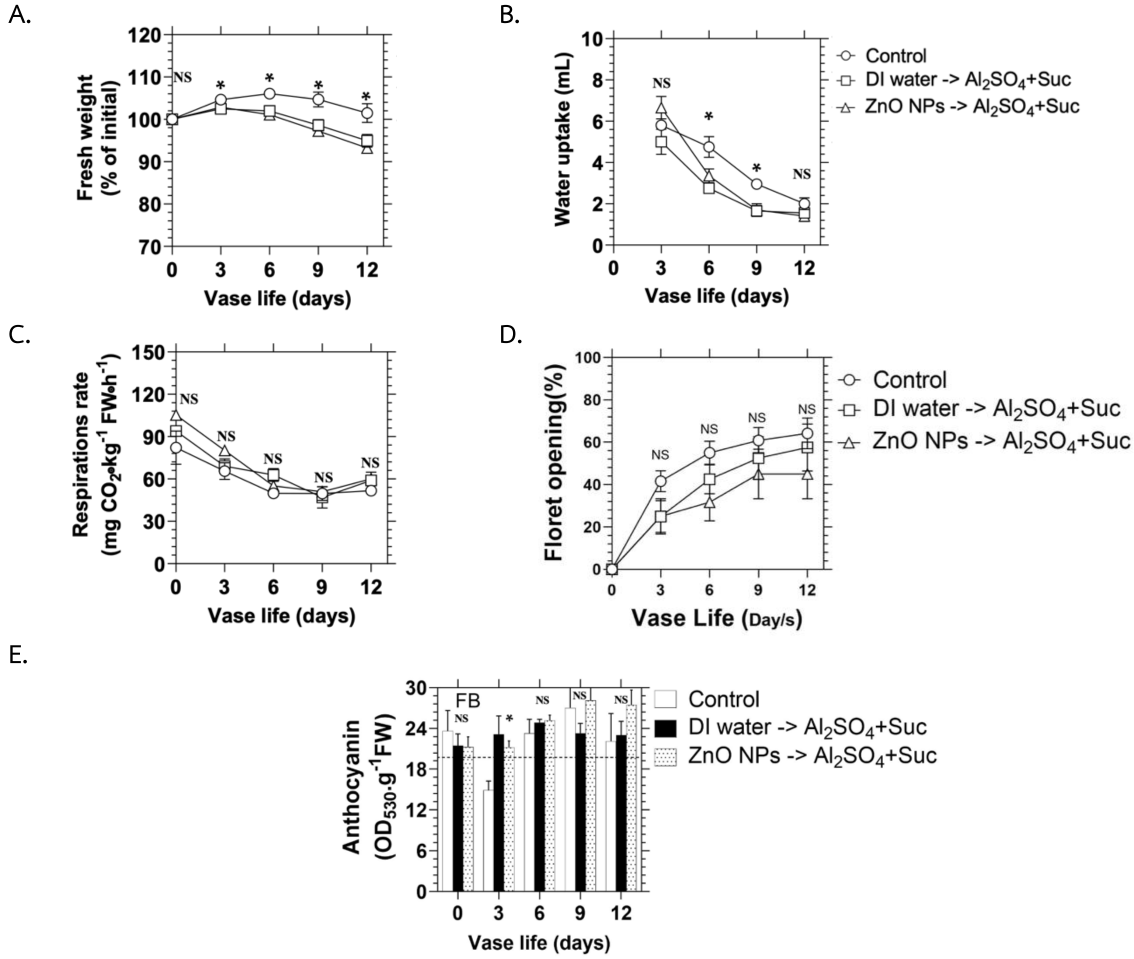

Figure 2. Effects of ZnO NPs pulsing prior to holding in $Al_2(SO_4)_3$ + Suc solution on relative fresh weight (A), water uptake (B),respiration (C), floret opening (D) and anthocyanin content (E) of *Vanda* Orchid cv. Pure Wax Pink, compared to untreated flowers held in deionized water and in $Al_2(SO_4)_3$ + Suc solution. The line in (E) represents anthocyanin content read at time zero. NS = not significantly different; * = significantly different at $P \leq 0.05$.

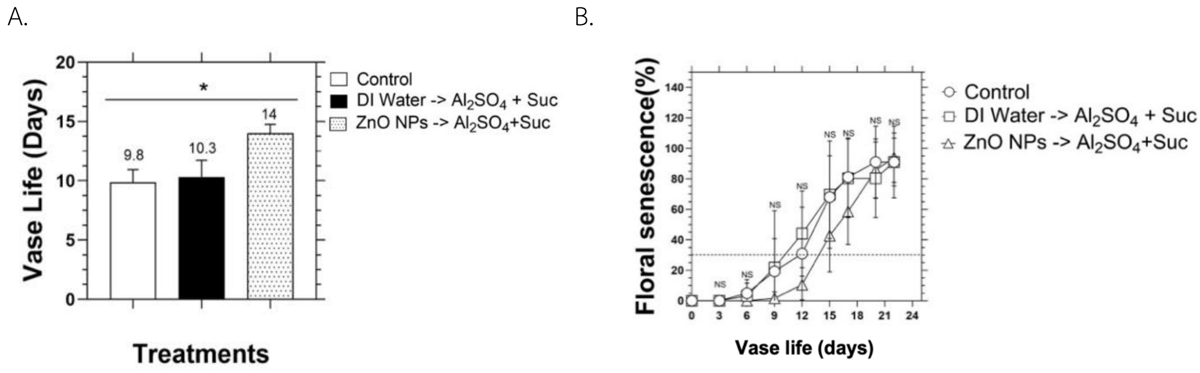

Figure 3. Effects of ZnO NPs pulsing prior to holding in $Al_2(SO_4)_3$ + Suc solution on vase life (A) and floral senescence (B) of *Vanda* Orchid cv. Pure Wax Pink, compared to untreated flowers held in deionized water and in $Al_2(SO_4)_3$ + Suc solution. NS = not significantly different; * = significantly different at $P \leq 0.05$. The line in (B) represents 30% severity beyond which the flowers are regarded as unfit for vase quality.

Conclusion

The application of ZnO NPs as a pulsing agent reduced the abrupt increase in bacterial colonies for cut *Vanda* Orchid cv. Pure Wax Pink, resulting in delayed floral senescence and prolonging vase life. Therefore, ZnO NPs at the concentration of 0.25% can be used as an effective biocide to mitigate against bacterial contamination for Pure Wax Pink *Vanda* Orchid in vase solution.

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