Research article

Effects of Chitosan and Lotus Extracts as Growth Promoter in *Dendrobium* Orchid

PIYAVADEE CHAROENWATTANA*
Rajamangala University of Technology Thanyaburi, Pathum Thani, Thailand
Email:piyavadeec@yahoo.com

UMNOUI PETPRAPAI
MahidolUniversity, Bangkok, Thailand

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Abstract The objective of this research was to investigate the effects of chitosan and lotus extracts to stimulate growth of orchid plantlets in greenhouse conditions. Chitosan was extracted from prawn shells (*Penaeus monodon*) with 90% deacetylation (DD) and 85-89% purity. The lotus extracts from the leaves of *Nelumbo nucifera* 'Roseum Plenum' were used as the sources of antimicrobial agents. The *Dendrobium* orchid 'Sureepeach' plantlets were treated with 10,30,50 and 100 mg L^{-1} chitosan and 1,3 and 5 g L^{-1} lotus extracts at 7 day intervals. The combination of 100 mg L^{-1}chitosan and 5 g L^{-1} lotus extracts gave the maximum number of leaves and shoots of the plantlets after 10 weeks of transplanting.

Keywords chitosan, lotus extracts, orchid, growth promoter

INTRODUCTION

Thailand is the world premier producer and exporter of orchids. The Thai orchid industry is well-known for both cut orchid flowers and the supplier of *in vitro* plantlets and pre-blooming plants. Most micro-propagated *Dendrobium* hybrids are the majority of Thai cut flowers. Key export markets are in Asia (China, Korea, and Japan), Europe (Italy and the Netherlands), the US and Australia. In 2011, the quantity of orchid exports was 23,392 tons or equivalent to Baht 2,111 million (Office of Economic Agriculture, 2012). The government's orchid strategy (2011-2016) envisages that Thailand will become the world's leading orchid exporter, with a target export value reaching 10,000 million Baht by 2016 (Than Sethakit. 2012). Increased demand for orchids could lead to an expansion of cultivated areas, especially for large scale production of orchid plantlets. To triple orchid production, the strategy aims to improve the quality of orchids and diversify their varieties. The orchid industry will benefit from the development of more efficient micro-propagation techniques, especially *Dendrobium* orchids.

Chitosan is wildly used in agriculture due to its positive effects on plants growth and development. Chitosan (poly [b-(1→4)-2-amino-2-deoxy-D-glucopyranose]), a biopolymer derivative of chitin, is mostly found in the exoskeleton of arthropods and crustaceans (Palpandi, *et al*., 2009). In Thailand, chitin can be extracted from the abundant prawn and crab shells that are sustainable and readily available as a natural waste product from the seafood industry. For the cut flower hybrid orchid, *Dendrobium* 'Earsakul', chitosan of 45 kDa molecular weight and a >90% degree of deacetylation (DD) applied at a concentration of 1–100 mg/L significantly increases both quantitatively and qualitatively inflorescence yields (Limpanavech et al., 2008). However, the impacts of chitosan on *in vitro* orchids and orchid propagation have been limited. Nge et al. (2006) found that the size and the origin of the chitosan as well as its concentration affected the number of *Dendrobium* and *Phalaenopsis* plantlets regenerated from a protocorm-like body (PLB). Additionally, the research also found that spraying with chitosan significantly reduced the severity of leaf spot diseases in orchids, promoted plant growth of some orchids, and increased the size and length of the *Dendrobium* florets and inflorescences, respectively (Uthairatanakij et al., 2007).

Moideen et al. (2011) found that the medicinal plant of *Nelumbo nucifera* (lotus) has a wide range of medicinal properties. Various techniques using different chemical solutions such as ethanol, methanol and water were applied to the leaves of *Nelumbo nucifera* to obtain some extracts for testing using preliminary phytochemical analysis. The antimicrobial susceptibility studies were conducted against gram (-) bacteria such as *E. coli, P. aeruginosa, K. pneumonia* and gram (+) bacteria such as *Staphylococcus aureus*. The results show that ethanol extract is anti-microbial.

Li and Xu (2008) tested the anti-microbial of the lotus leaf extracts against five microorganisms. The most active antimicrobial extract was subjected to spectroscopic analysis. The result shows that the minimum inhibitory concentrations of the most active extract were 0.625, 1.25, 1.25, 0.625 and 2.5 mg/mL for *Actinobacillus actinomycetemcomitans* Y4, *Actinomyces viscosus* 19246, *Porphyromonas gingivalis* 33277, *Fusobacterium nucleatum* 25586, and *Actinomyces naeslundii* wvl45. Quercetin from lotus leaves was found to have the greatest antimicrobial activity; this suggests it could be a potential antibacterial agent for periodontitis.

The use of chitosan and lotus leave extracts to stimulate growth and increase disease resistance is expected to increase orchid production and thus further develop the orchid export industry. They will replace chemical fertilizer and pesticide and are environmental friendly.

OBJECTIVE

This research aims to demonstrate that chitosan and lotus extracts can stimulate the growth of *Dendrobium* orchid plantlets.

METHODOLOGY

Extraction of chitin and chitosan

Chitin and chitosan were produced from prawn shells through demineralization, deproteinization and deacetylation (Burrows *et al*., 2007). Shells of prawns (*Penaeus monodon*) were washed, dried and then ground into small pieces. Approximately 25 g of prawn shells were demineralized with 100 ml of 1% (v/v) hydrochloric acid (HCl) for 24 h to remove the minerals (mainly calcium carbonate). To obtain the chitin, the washed and cleaned shells were transferred to 50 ml of 2% (w/v) sodium hydroxide (NaOH) and soaked for 1 hour in order to dissolve proteins and sugars. Subsequently, the chitin was cleaned and oven dried, resulting in a chitin product that was subsequently de-acetylated in 100 ml 50% (w/v) sodium hydroxide (NaOH) and boiled at 100°C for 2 h on a hot plate to obtain chitosan. The 1% (w/v) chitosan solution was obtained from dissolving 5 g of chitosan in 500 ml of 0.1M acetic acid and stirred continuously at 55°C for 12 h. The filtrated chitosan solution had the pH of 3.99, 90% deacetylation (DD), and 85-89% purity. Concentrated chitosan of 10, 30, 50 and 100 mg $L^{-1}$was obtained from diluted chitosan in 1% acetic acid.

Lotus extracts

The leaves of *Nelumbo nucifera* 'Roseum Plenum' were air dried for 2-3 days before cutting into small pieces (2-3 cm.) and ground up. The leaves were coldly extracted using ethanol at room temperature. 200-gram leaves were filled in a cloth bag and soaked in 3 L of ethanol three times during one week until no extracts came out. Subsequently, the ethanol was discarded using a rotary evaporator to get the brown and concentrated crude extracts that were kept in a cold dry place. Crude extracts were fractioned using the Column Chromatography technique to derive hexane crude extracts, which were then diluted into 1, 3 and 5 g L^{-1} concentrations.

Plantlets preparation

The study also used *Dendrobium* 'Sureepeach' plantlets from Mana orchid farm, Nakornpathom province. 2 to 4 leave plantlets with well-developed roots were removed from medium culture and washed thoroughly under running tap water to remove traces of agar. Plantlets were acclimatized in pots for 4 weeks, then transferred to a greenhouse and watered daily with 400 g L^{-1} of 21-21- 21 fertilizer every week for 10 weeks. The *Dendrobium* orchid 'Sureepeach' plantlets were treated with 10, 30, 50 and 100 mg L^{-1} chitosan and 1, 3 and 5 g L^{-1} lotus extracts at 7 day intervals.

Data analysis

All experiments were carried out in a completely randomized design with three replications under daily visual observations to record the number of leaves and shoots over the course of 10 weeks. Subsequently, an analysis of variance (ANOVA) and means were calculated and compared using Duncan's new multiple rang test (DMRT).

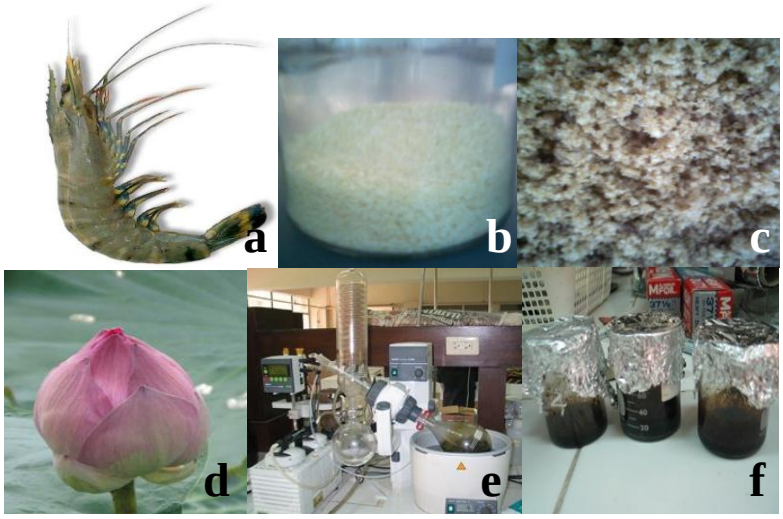

Fig. 1 Chitin (b) and chitosan (c) are produced from prawn shells (a) through demineralization, deproteinization and deacetylation, ethanol extracts come from lotus leaves (d), the crude extracts were fractioned by rotary evaporator (e) to obtain brown crude extracts (f)

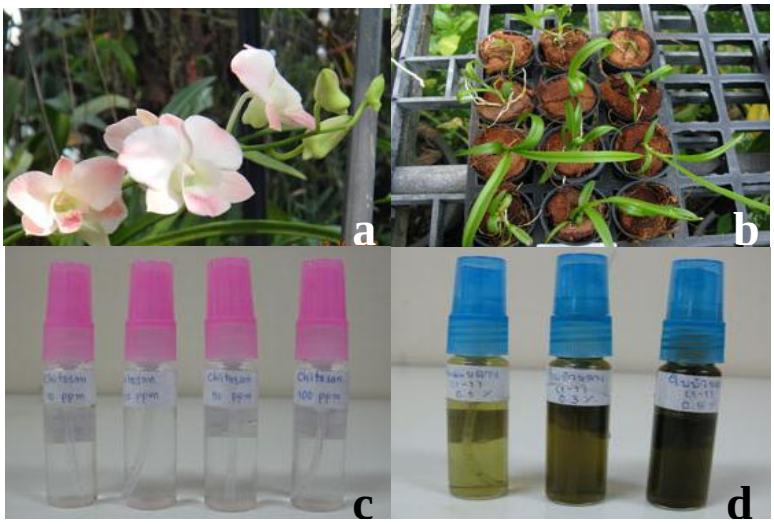

Fig. 2 Flowers of *Dendrobium* 'Sureepeach' (a), plantlets were transplanted to pots filled with small pieces of coconut husk (b), chitosan and lotus leave extracts (c, d)

RESULTS AND DISCUSSION

The study found that the number of leaves after 2 weeks of transplanting was not statistically different. However, the number of leaves after 4, 8 and 10 weeks of transplanting was significantly different. The orchid plantlets treated with 100 mg L^{-1} chitosan in combination with 5g L^{-1} lotus extracts gave the maximum number of 8.5 leaves. The control plantlets without the treatment of chitosan and lotus extracts gave an average number of 5.1 leaves. The new shoots and leaves that emerged after 8 weeks were strong compared to those without the chitosan and lotus extract treatment after 10 weeks. The application of 100 mg L^{-1} chitosan combined with 5g L^{-1}lotus extracts resulted in the maximum number of leaves on average. The concentrated chitosan used in this study was higher than in *Dendrobium* orchid 'Earsakul' that was applied to 1-100 mg L^{-1} chitosan.

Table 1 The effects of chitosan and lotus extracts on the number of *Dendrobium* 'Sureepeach' leaves within 10 weeks after transplanting in the greenhouse

Treatment		Number of leaves per pot				
Chitosan (mg L^{-1})	Lotus extracts (g L^{-1})	Week 2	4	6	8	10
0	0	3.0	3.1 a-e	3.2 a	3.9 a-d	5.1 b-f
	1	3.6	3.3 a-d	2.2 b-d	2.8 de	3.7 ef
	3	3.3	4.0 a	2.8 ab	4.9 ab	6.4 a-d
	5	3.8	2.7 c-e	2.7 ab	2.8 de	2.8 f
10	0	3.4	3.6 a-c	2.8 ab	3.4 c-e	4.9 b-f
	1	3.4	3.7 ab	2.8 ab	4.4 a-c	6.3 a-d
	3	3.0	3.0 b-e	2.7 ab	2.5 e	3.7 ef
	5	3.2	2.8 b-e	2.2 b-d	4.1 a-d	5.6 b-e
30	0	2.8	2.8 b-e	2.2 b-d	4.1 a-d	4.2 c-f
	1	3.2	2.7 c-e	2.1 b-d	3.3 c-e	4.5 c-f
	3	3.4	3.4 a-d	2.3 a-c	4.4 a-c	6.6 a-c
	5	3.3	4.0 a	2.9 ab	3.5 b-e	4.2 c-f
50	0	3.1	2.9 b-e	2.8 ab	4.5 a-c	5.7 b-e
	1	2.7	2.2 e	2.3 a-c	3.6 b-e	4.9 b-f
	3	3.4	3.2 a-d	2.6 a-c	3.6 b-e	4.7 c-f
	5	2.7	2.6 de	1.4 d	3.7 a-e	5.6 b-e
100	0	3.2	2.8 b-e	1.7 cd	3.4 c-e	4.0 d-f
	1	2.7	2.8 b-e	2.6 a-c	4.4 a-c	6.1 a-e
	3	3.4	2.7 c-e	2.6 a-c	3.8 a-e	7.4 ab
	5	3.3	3.1 a-e	2.5 a-c	5.0 a	8.5 a
F-test		Ns	**	*	**	**
CV (%)		15.7	15.9	18.9	18.6	4.2

Means followed by the same letter in the same column had no significant difference by DMRT
**, ** indicatessignificance at 5% and 1% levels of probability, respectively.ns = not significant*

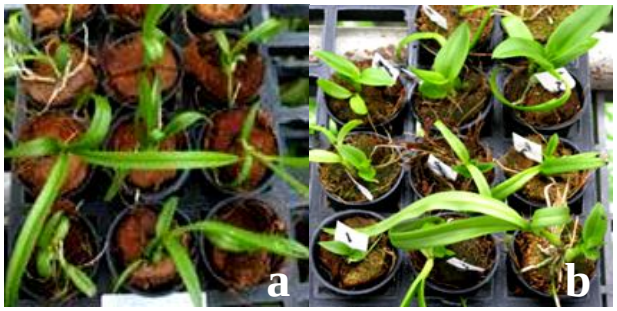

Fig. 3 Comparison of the effects of chitosan and lotus extracts on plantlet growth
controlled plantlets (a) and plantlets treated with chitosan and lotus extracts after10 weeks (b), Photograph is representative of three such independent replications.

It increased both the yield and quality of the inflorescence (Limpanavech *et al*., 2008). The plant responded differently to various type of chitosan molecule. The lower molecular weight of chitosan was more effective in inducing and disease resisting than the higher molecular weight of chitosan (Lin *et al*., 2005).

CONCLUSION

The appropriate concentrations of chitosan and lotus extracts were investigated for their effects on the growth of *Dendrobium* orchid plantlets in greenhouse conditions. According to the result obtained, prawn chitosan has the ability to enhance the survival rate and the growth of the plantlets. Chitosan of 100 mg L^{-1} are the most effective. The results indicates that plantlets treated with 100 mg L^{-1} chitosan and 5 g L^{-1} lotus extracts yielded the maximum number of leaves and shoots after 10 weeks of transplanting. However, further work is required to evaluate the effects of chitosan and lotus extracts interaction or alone for various stages of plant growth.

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REFERENCES

Burrows, F., Louime, C., Abazinge, M. and Onokpise, O. 2007. Extraction and evaluation of chitosan from crab exoskeleton as a seed fungicide and plant growth enhancer. American-Eurasian J. Agric. and Environ. Sci., 2(2), 103-111.

Li, M. and Xu, Z. 2008. Quercetin in lotus leaves extract may be responsible forantibacterial activity. Arch. Pharm. Res., 31(5), 640-644.

Limpanavech, P., Chaiyasuta, S., Vongpromek, R., Pichyangkura, R., Khunwasi, C. Chadchawan S., Lotrakul, P., Bunjongrat, R., chaidee, A., and Bangyeekhun, T. 2008. Chitosan effects on floral production, gene expression, and anatomical changes in the *Dendrobium*orchid. J. Scientia Horticulturae, 116, 65-72.

Lin, W., Hu, X., Zhang, W., John Rogers, W. and Cai, W. 2005. Hydrogen peroxide mediates defence responses induced by chitosans of different molecular weights in rice. Journal of Plant Physiology, 162, 937-944.

Moideen, M.M.J., Raffick.M., Kumar, S. and Kishore, N. 2011. Antimicrobial activity and phytochemical analysis of Nelumbonucifera leaves. Journal of Global Trends inPharmaceutical Sciences, 2, 404-410.

Nge, K.L., New, N., Chandrkrachang, S. and Stevens, W.F. 2006. Chitosan as a growth stimulator in orchid tissue culture. Plant Science, 170, 1185-1190.

Office of Economic Agriculture, 2012. The quantity of orchid exports.
(Available: http://www.oae.go.th/download/bapp/Q3-2555.pdf, accessed: November 15, 2012.)

Palpandi, C., Shanmugam, V. and Shanmugam, A. 2009. Extraction of chitin and chitosan from shell and operculum of mangrove gastropod *Nerita (Dostia)crepidularia* Lamarck. International Journal of Medicine and Medical Sciences, 1(5), 198-205.

Than Sethakit. 2012. Orchid production. (Available: http://www.thannews.th.com, accessed: November 3, 2012.)

Uthairatanakij, A., Teixeira da Silva, J.A. and Obsuwan, K. 2007. Chitosan for improving orchidproduction and quality. Orchid Science and Biotechnology, 1, 1-5.