UNIVERSITI PUTRA MALAYSIA

IN VITRO CATHARANTHUS ROSEUS (L.) G. DON CALLUS RESPONSES TO CHITOSAN TREATMENTS

NUR ATIKAH ISMAIL

FP 2015 89

IN VITRO CATHARANTHUS ROSEUS (L.) G. DON CALLUS RESPONSES TO CHITOSAN TREATMENTS

By:

NUR ATIKAH BINTI ISMAIL

A project report submitted to the Faculty of Agriculture,

Universiti Putra Malaysia

in fulfillment of the requirement of PRT 4999 (Project)

for the award of degree of Bachelor of Horticultural Science

2014/2015

IN VITRO CATHARANTHUS ROSEUS (L.) G. DON CALLUS RESPONSES TO CHITOSAN TREATMENTS

NUR ATIKAH BINTI ISMAIL

FACULTY OF AGRICULTURE

UNIVERSITI PUTRA MALAYSIA

SERDANG, SELANGOR

2014/2015

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious and Merciful.Alhamdulillah, to Almighty Allah SWT who has showered his blessings that enable me to complete my research project successfully.

I owe my most sincere gratitude to my supervisor, Assoc. Prof. Dr. Saleh Bin Kadzimin, Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, for his valuable advice, guidance, and comments in carrying out the project and his patience to review my report.

Sincere thanks also to Encik Aziz Bin Ismail, Science Officer at Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia for his kindness in assisting and guiding me in use of laboratory facilities and his endless support throughout the project.

I also wish to thank all my beloved friends for their help and support in completing my project and always being there whenever I needed them. Lastly, special thanks to my family who always give me the spirit and encouragement to accomplish my project, also who incessantly prayed for my success. May all of you always be in His care.

CERTIFICATION

This study report entitled "*IN VITRO CATHARANTHUS ROSEUS* (L.) G. DON CALLUS RESPONSES TO CHITOSAN TREATMENTS" is prepared by NurAtikahBinti Ismail and submitted to the Faculty of Agriculture in fulfillment of requirement of PRT 4999 (Project) for the award of degree of Bachelor of Horticultural Science.

Student's name: Student's signature

NurAtikahBinti Ismail

Certified by:

(ASSOC. PROF. DR. SALEH KADZIMIN)

Project Supervisor,

Department of Crop Science,

Faculty of Agriculture,

Universiti Putra Malaysia

Date:

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
Df	Degree of freedom
et al	And others
g	Gram
mg/L	Milligram per litre
m	Milli
mm	Millimetre
µm	Micrometer
mL/min	Millilitre per minute
MS	Murashige and Skoog
LSD	Least Significant Difference
SAS	Statistical Analysis System
GCMS	Gas chromatography-mass spectrometry

ABSTRACT

The present study investigates the effects of chitosan on callus growth and development of*Catharanthusroseus* under light treatment. In the study, chitosan was supplemented in culture medium. Callus of *C. roseus*, previously initiated on MS medium before the onset of the experiment was cultured. Various levels of chitosan (0, 1, 2, 3, 4, 5 mg/L) were used. The experiment was conducted in a completely randomized design with 3 replications. Study parameters include dry weight of callus andboron level.

Data recorded that the control, 0 mg/L chitosan concentration gave the highest dry weight of callus 0.210 g. Treatment with 1 mg/L chitosan concentration increased boron level in callus of *C. roseus*to 0.097 mg/L. The study suggests that the application of chitosan in culture medium did not give significant increase in dry weight of callus compared to the control. Chitosan treatment did not give significant increase or decrease in boron level of callus.

The study concludes that chitosan had no significant effect on dry weight and boron level in callus of *C. roseus* under light treatment.

ABSTRAK

Kajian ini menyiasat tentang kesan kitosan pada pertumbuhan kalus dan pembangunan Catharanthus roseus dibawah rawatan cahaya. Dalam kajian itu, kitosan telah ditambah dalam medium kultur. Kalus C.roseus, yang sebelum ini dimulakan pada medium MS sebelumbermulanya eksperiment ini dikulturkan. Pelbagai tahap kepekatan chitosan (0, 1, 2, 3, 4, 4, mg/L) telah digunakan. Eksperimen ini dijalankan dalam rekabentuk yang benar-benar rawak dengan ulangan. Parameter kajian termasuk berat keringk kalus dan tahap boron.

Data direkodkan bahawa kawalan 0 mg/L kepekatan kitosan memberikan berat kering kalus yang tertinggi iaitu 0.210 g. rawatan dengan kepekatan kitosan 1 mg/L meningkatkan boron dalam kalus C.roseus iaitu 0.097 mg/L. kajian ini mencadangkan bahawa penggunaan kitosan dalam medium kultur memberi peninkatan yang tidak ketara dalam berat kering kalus berbanding dengan kawalan. Rawatan kitosan tidak memberikan peningkatan atau penurunan yang ketara dalam tahap boron di dalam kalus.

Kesimpulannya, kitosan tidak memberikan kesan yang ketara kepada berat kering dan tahap boron di dalam kalus C.roseus di bawah rawatan cahaya.

CHAPTER 1

INTRODUCTION

Catharanthusroseus is an ornamental plant belonging to the family Apocynaceae and has been widely planted in several countries for the ornamental purposes. Besides its use as an ornamental plant, it isan important source of one hundred and thirty valuable alkaloids. These include alkaloids which are routinely used in the treatment of cancer, leukemia, and diabetes. However, the alkaloids are produced in minute amounts making them high in demand and cost. As a consequence numerous efforts to develop alternative strategies for their production have been documented.

The present study examines responses in growth and development of callus of *C. roseus* on chitosan treatments. Callus was previously initiated from culture of leaf explants on Murashige and Skoog medium. Resulting callus was cultured on same medium and supplemented with various levels of chitosan.

REFERENCES

Aslam, J., Khan, S. H., Siddiqui, Z. H., Fatima, Z., Maqsood, M., Bhat, M. A. (2010).Catharanthusroseus (L.) G. Don. an important drug. Its application and production.*PharmacieGlobale,* 4: 104-110.

Banos, S.B., Lauzardoa, A.N.H., Vallea, M.G.V.D., Lopeza, M.H., Barkab, E.A., Molinac, E.B., Wilsond, C.L. (2006). Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities.*Crop Protection*, 25: 108–118.

Benhamou, N., Kloepper, J.W., Tuzun, S. (1998). Induction of resistance against Fusarium wilt of tomato by combination of chitosan with an endophytic bacterial strain: ultrastructure and cytochemistry of the host response. *Planta*, 204 : 153–168.

Blevins, D. G., and Lukaszewski, K. M. (1998).Boron in plant structure and function.*Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 49: 481-500.

Borax, (2012). Retrieved 18 October 2014, from http://www.borax.com/docs/agronomy-notes/functionsofboroninplantnutrition-final-feb2012.pdf?sfvrsn=2

Cheng, X.Y., Zhou, H.Y., Cui, X., Ni, W., Liu, C.H. (2006). Improvement of phenylethanoid glycosides biosynthesis in *Cistanchedeserticola* cell suspension cultures by chitosan elicitor. *Journal of Biotechnology,* 121: 253–260.

Dutta, A., Batra, J., Pandey-Rai, S., Singh, D., Kumar, S., Sen, J. (2004). Expression of terpenoidindole alkaloid biosynthetic pathway genes corresponds to accumulation of related alkaloids in *Catharanthusroseus* (L.) G. Don. *Planta*220: 376-383.

ETI products, (2014). Retrieved 18 October 2014, from http://www.etiproducts.com/boron-in-agriculture-5s.htm

Etimine USA, Inc., (2014). Retrieved 12 October 2014, from http://www.etimineusa.com/en/boric-acid

Fraser, R.S., Loening, U. E., and Yeoman, M. M. (1967).Effect of light on cell division in planttissue cultures.*Nature,* 215:873.

Freepons, D., 1991. Chitosan does it have a place in agriculture. *Proc. Plant Growth Regulators Soc. Am*., pp.11–19.

George, E.F. and Sherrington, P. D. (1984).Plant propagation by tissue culture.*Journal of Basic Microbiology*, 25: 475.

Gilman, E.F., and Howe, T. (1999).*Catharanthusroseus* Periwinkle, Madagascar Periwinkle.University Florida IFAS Extension. Retrieved 13 July 2014, from http://edis.ifas.ufl.edu/fp112

Goldbach, H. E., and Wimmer, M. (2007). Boron in plants and animals: Is there a role beyond cell-wall structure. J. *Plant Nutr. Soil Sci.*, 170: 39-48.

Gupta, R. (1977). Periwinkle-produces anticancer drug.*Indian Farming* 7: 11-13.

Hadrami, A. E., Adam, L. R., Hadrami, I. E., and Daayf, F. (2010).Chitosan in plant protection.*Marine Drug*, 8(4): 968-987).

Hirano, S. (1988). The activation of plant cells andtheir self-defence function against pathogens inconnection with chitosan (in Japanese with English summary). *Nippon Nogeikagaku*kaishi, 62:293–295.

Hisiger, S., and Jolicoeur, M. (2007).Analysis of *Catharanthusroseus* alkaloid by HPLC.*Phytochemistry Reviews*, 6: 207-234.

Jaramillo, J., and Summers, W. L., (1991). Dark–Light Treatments Influence Induction of Tomato Anther Callus.*Hort Science,* 26(7): 915-916.

Lata, B. (2007). Cultivation, mineral nutrition and seed production of *Catharanthusroseus*(L.) G. Don in the temperate climate zone. *Phytochemistry Reviews*, 6: 403-411.

Kew, (2014). Retrieved 25 August 2014, from http://www.kew.org/science-conservation/plants-fungi/catharanthus-roseus-madagascar-periwinkle

Mathias, R. J., Fukui, K., and Law, C. N. (1986).Cytoplasmic effects on the tissue culture response of wheat *(Triticumaestivum)* callus. *TheorAppl Genet,* 72: 70-75.

Murashige, T., and Skoog, F. (1962).A revised medium for rapid growth and bio-assays with tobacco tissue cultures.*Physiol Plant,* 15(3): 473-497.

National Tropical Botanical Garden, (2003). Retrieved 9 November 2014 from http://www.ntbg.org/plants/plant_details.php

Nejat, N.,Valdiani, A., Cahill, D.,Tan, Y.H., Maziah, M., and Abiri, R. (2014).Ornamental exterior versus therapeutic interior of madagascar periwinkle (*Catharanthusroseus*): The two faces of a versatile herb**.***The Scientific World Journal*. Retrieved from http://www.hindawi.com/journals/tswj/aa/982412/

Nge, K. L., Nwe, N., Chandrkrachang, S., Stevens, W. F. (2006).Chitosan as a growth stimulator in orchid tissue culture.*Plant Science,* 170: 1185–1190.

Noth, M.H. and Abel, W. O. (1971).Zurentwicklunghaploiderpflanzenausunreifenmikrosporenverschiedener*Nocotiana*arten.*Z. Pflanzenszucht.,* 65: 277-284.

Ohta, K., Taniguchi, A., Konishi, N., and Hosoki, T. (1999). Chitosan treatment affects plant growth and flower quality in*Eustomagrandiflorum*. *Hort science*, 34(2):233–234.

Orlita, A., Gorycka, M.S., Paszkiewicz, M., Malinski, E., Kumirska, J., Ewa M. Siedlecka, E.M., Lojkowska, E., and Stepnowski, P. (2010).Application of chitin

and chitosan as elicitors of coumarins and furoquinolone alkaloids in *Rutagraveolens* L. (common rue).*Biotechnol. Appl. Biochem.,* 51: 91–96.

Parrek, S.K., Singh, S., Srivastava, V.K., Mandal, S., Maheswari, M.L., and Gupta, R. (1981). Advances in periwinkle cultivation.*Indian Farming,* 31(6): 18-21.

Pinho, P.G.D., Goncalves, R.F., Valentao, P., Pereira, D.M., Seabra, R.M., Andrade, P.B., and Sottomayor, M. (2009).Volatile composition of Catharanthusroseus (L.) G. Don using solid-phase microextraction and gas chromatography/mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, 49: 674-685.

Pretto, F. R., and Santarem, E. R. (2000).Callus formation and plant regeneration from *Hypericumperforatum*leaves. *Plant Cell, Tissue and Organ Culture,*62: 107–113.

Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. *Prog.Polym.Sci.,* 31, 603–632.

Rodriguez, M. B. H., Fontes, A. G., Rexach, J., Cristobal, J. J. C., Maldonado, J. M., and Gochicoa, M. T. N. (2010). Role of boron in vascular plants and response mechanisms to boron stress.*Plant Stress,* 4 (2): 115-122.

Saleem, M., Khanif, Y.M., Fauziah, I., Samsuri, A.W. and Hafeez, B. (2011). Importance of boron for agriculture productivity: A review. International Research *Journal of Agricultural Science and Soil Science,* Vol. 1(8) pp.293-300.

Shehata, S. A., Fawzy, Z. F., and El-Ramady, H. R. (2012).Response of cucumber plants to foliar application of chitosan and yeast under greenhouse conditions. *Australian Journal of Basic and Applied Sciences,* 6(4): 63-71.

Tariq, M.,and C. J. B. Mott, C. J. B. (2006).Effect of boron supply on the uptake of Micronutrients by radish (*Raphanussativus L.*).*Journal of Agricultural and Biological Science,* 1(2): 1-8.

Tsugita, T., Takahashi, K., Muraoka, T., and Fukui, H.(1993).The application of chitin/chitosanfor agriculture (in Japanese).*7th Symposium on chitin and chitosan*, p. 21–22.

Vasconcelos, M. W. (2014). Chitosan and chitooligosaccharide utilization in phytoremediation and biofortification programs: current knowledge and future perspectives. Front. *Plant Sci*., 5: 616.

Verma, A. K., Singh, R. R., and Singh, Seema.(2012). Improved alkaloid content in callus culture of *Catharanthusroseus.BotanicaSerbica*, 36(2): 123-130.

Wikipedia (2014). Retrieved 12 July 2014 from http://en.wikipedia.org/wiki/Chitosan

Zibbu, G., and Batra, A. (2011). GC-MS analysis of the desert plants of Apocynaceae family: *Nerium oleander* L. and *Thevetiaperuviana* (pers.) schum. *International Journal of Pharmaceutical Research and Development*, 3(10): 49 – 62.