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Thesis Advisory Committee : Assoc.Prof.Dr. Sanha Panichajakul, and
Assoc.Prof. Boonyuen Kijwijan

Abstract

The method for quantification and identification of azadirachtin from neems (*Azadirachta siamensis*) seed was developed using HPLC reverse phase column and isocratic condition. Partial purification of azadirachtin A, 1-tigloyl-3-acetylazadirachtol and azadirachtin B from methanol neem'seed extract carried out by fractionation through silica gel column eluted with ethyl acetate obtained the average of 1 fold improvement with 97.8% yield. The purified product exhibited slightly lower in insecticidal activity (LD_{50}) using young *Plutella xylostella* larvae bioassay in comparison to toxicity of azadirachtin A. The pattern of azadirachtin A production during the maturation period of neem's fruits has been investigated with Khon Kaen local neem trees. The accumulation of azadirchtin A was early detected at the young flower stage and gradually increased to the maximum within 7^{th} week at the average level of 3-3.5 mg/g dry weight while the fruit color turn from green in to deep-yellow. The level of azadirachtin A was rapidly disappeared after 10 weeks which was equivalent to the deep-brown color of surface.

The procedures for callus induction and plant regeneration from callus and tissues of *A. siamensis* were studied and developed. *A. siamensis* callus when cultured in Murashige and Skoog (MS) medium plus 1 mg/l 2,4-D and 2 mg/l BA under 2,000 luxes light illumination at 25±2 ^{o}C produced the average of 69 µg azadirachtin A/g dry weight. Cultivation of cell suspension cultured in the medium containing glucose (30 g/l) under the standard condition yielded slighly higher content of azadirachtins. Regeneration of 10 months old *A. siamensis* stem piece calluses were established by cultured on MS medium plus 0.5 mg/l IAA and 1 mg/l BA for the period of 9 weeks. Successfully regeneration of 24 months old cotyledon calluses was also demonstrated by culture on Linsmaier and Skoog (LS) medium plus 2 and 3 mg/l BA. About 10 % shoot was obtained within 3 weeks and increased to 50-80 % yielded after cultivation continuously for 9 months. Multiple shoots of *A. siamensis* can be easily obtained by axillary buds cultured in MS medium supplemented with various concentration of BA. The level of 3 mg/l BA yielded highest number of multiple shoots at 15 shoots/explant. Root induction in MS medium was greatly enhanced by IAA (0.5-1.5 mg/l) within 45 days. Azadirachtins content in regenerate plantlets and calluses were monitored. The highest level of azadirachtin A content was detected in regenerated stems at the level of 193.4 µg/g dry weight. The LD_{50} of 0.784 µg/g body weight was established for regenerated plantlet extract while the methanol extracted from callus exhibited LD_{50} 68.95 µg/g body weight.