ISOLATION OF NIMBIN FROM AZADIRACHTA INDICA LEAVES AND ITS CALLUS CULTURES

Nimbin was isolated from *Azadirachta indica* Juss. Leaves and its callus cultures grown on Wood and Braun medium containing 3% sucrose and supplemented with Kn (2.0 µm/l) and NAA (6.0 µm/l) showed maximum synthesis/accumulation of nimbin.

Nimbin, a triterpene, white crystalline compound was isolated from stem bark\(^1\). The present investigation was undertaken to isolate nimbin if present from the leaves, its callus culture and to study the effect of phytochromones on the callus cultures for nimbin synthesis/accumulation.

Mature green leaves of a fifty year old plant were collected, washed in running tap water, air dried and powdered. The powder was extracted with methanol in soxhlet for 24 hours. The extract obtained was concentrated in rotary evaporator and subjected to column chromatography. The fractions eluted in chloroform were monitored on thin layer chromatography for the presence of nimbin (Benzene, Ethyl acetate solvent system 4:1). The spots were visualised by exposing the chromatogram plates to iodine vapours. Further quantitative estimations were carried out using colorimetric methods as described by Das & Banerjee\(^1\).

Leaf discs (15 mm) were inoculated on (30 ml) Wood and Braun medium\(^1\) containing 3% sucrose and supplemented with various doses of cytokinins — Kn (Kinetin), BAP (Benzyl α-guanine purine) and auxins — IAA (Indole 3-acetic acid), IBA (Indole butyric acid), α—NNA (Naphthlene acetic acid) and 2,4-D (2,4-Dichlorophenoxy acetic acid). Culture flasks were incubated at 25 ± 1°C in continuous white fluorescent light (1000 lux) for 16:8 hours photoperiod.

A white crystalline compound, nimbin was isolated from the leaves of *Azadirachta indica*. Presence of nimbin was confirmed by its IR spectrum comparing with the authentic sample. Stem bark of *Azadirachta indica* contained 0.04% of nimbin, while seeds contained 0.12%, on dry weight basis. In the present studies on leaves, the nimbin synthesised was comparatively less in quantity (0.03%). These results indicate that the nimbin synthesis might be occurring in leaves, while its accumulation in other bark or seeds.
Callus was initiated from leaf discs within four days in the presence of Kn (2.0 µ M/l) and IAA (4.0 µ M/l) in the medium. Callus was allowed to grow for some time and then subcultured on various test media containing cytokinin Kn; BAP (2.0 µ M/l) in combination with IAA/IBA/NAA; 2, 4-D (0-8.0 µ M/l).

Callus grown on Kn (2.0 µ M/l) combined with NAA containing media showed linear increase in its nimbint content corresponding to the increase in levels of NAA. But this increase was recorded up to 6.0 µ M/l NAA level, further increase to 8.0 µ M/l did not enhance nimbint synthesis/accumulation. This indicates that Kn 2.0 µ M/l with NAA 6.0 µ M/l might be the optimal dose of phytohormones required for nimbint synthesis/accumulation.

Callus grown on Kn (2.0 µ M/l) combined with IAA/IBA also showed linear increase in nimbint contents, corresponding to the increase in levels of them. But the amount of nimbint content was highest when NAA was present. Callus treated with Kn in combination with IAA;IBA/NAA which contained maximum nimbint content, was found to be compact, nodular with internal tracheary elements, differentiated. In few cases, roots were also initiated from such callus masses.

Callus subjected to BAP (2.0 µ M/l) and IAA/IBA/NAA interaction also showed linear increase in nimbint contents. However, the quantities of nimbint was comparatively less than that of corresponding Kn treatments. Moreover, the callus was fragile in nature. Callus subjected to Kn BAP with 2,4-D was ineffective in producing nimbint.

Results on the bark induced callus cultures have been corroborated with the results of leaf callus in the present investigation.

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