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AZADIRACHTA INDICA A. JUSS. (NEEM) : SEED PHARMACEUTICALS RELATED TO GROWTH REGULATOR TREATMENTS

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Endogenous titres of "neemrich' was stimulated with exogenously applied hormones. In our efforts to study and control the commercial seed yield harvest, results distinctly indicated the regulatory influence of hormone combinations: kinetin-GA_3 and kinetin-spermidine in maintaining higher limonin levels during maturation relative to control samples. Variations in the amount of limonoid was observed during the five classes of growth regulator applications, i.e., putrescine, spermidine, spermine, GA_3 and kinetin. Limonoid content was generally high in samples treated with kinetin 0.2 mM and spermidine 0.2 mM. The levels decreased in 0.5 mM concentration treatments in spermine, putrescine and spermidine. Therefore, high concentrations of polyamines proved inhibitory to limonin levels. A steady rise in limonin was noted with kinetin while increasing the exogenous supply from 0.2 mM to 0.5 mM levels. Thus, the results are of potential pharmaceutical significance in terms of understanding and enhancing reproductive potential and seed yield.

Key Words: *Azadirachta indica*, GA_3, kinetin, neem, putrescine, spermidine, spermine.

Neem, a prodigious multipurpose tree of the tropics, has immense potential to benefit mankind and to protect the environment (Kraus 2002, Kaaya *et al.* 2003). Often called 'Gift of the Gods' or 'Nature's Pharmacy', the tree is exploited as a commercial medicine and antibiotic. Extracts from its extremely bitter seeds may, in fact, be the source of a new generation of chemicals for use in Integrated Pest Management (Walia *et al*. 2002, Koul and Wahab 2007).

Previous studies indicate the involvement of certain growth regulators during regenerative growth flush (Tamas 1987, Dewittee *et al.* 1999, Angeles Botella *et al.* 2000). Foliar application of plant growth regulators on cash crops has enhanced plant growth, development and yield (Abd El-Waheb and Gamal El-Din 2004, Vila *et al.* 2004). Kinetin and GA_3 have now been recognized as plant growth regulators which can mediate reproductive differentiation in various plants (Dhir *et al.* 1986, Metzger 1995, Koul and Wahab 2007). These reports are based on their ubiquity, abundance in growing tissues and their effects on growth and development (Martineau *et al.* 1995, Kaminek *et al.* 1997, Durdan *et al.* 2000). Spermidine (a low molecular weight, aliphatic triamine) has also been recognized as a plant growth regulator which in addition to various responses produced at molecular level; influences protein, nucleic acid and mitotic activity (Martin-Tanguy 2001, Aziz 2003) and also plays a role in cell division, growth and induction of invertase and phosphatase syntheses (Pandey *et al.* 2000, Vila *et al.* 2004). Considering the role of plant growth regulators in regeneration and development, an experiment was designed as an extension to our previous work (Sabherwal and Dhir 2009,10). The principle aims of this study were:

to determine the regulatory role of exogenously applied growth regulators on the active principles in seed,

to assess the role of the plant growth substance in the secondary metabolic pathway of biologically active compounds and

to exploit the potential of growth regulator in increasing the pharmaceutical yield.

This was taken as a significant research problem since the product of the reproductive phase, that is, the Neem seed; is commercially important in Integrated Pest Management and pharmaceuticals. Moreover, Neem is native to the Indian sub-continent; and inferences from

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Table I: Effective endogenous seed ingredients with growth regulator treatments

Profiled limonin µg/ml/g seed / Treatment	Aza F M.W. 664.0 m.p.(°C) 165 R.T. 5.47	Aza A M.W. 720.0 R.T. 7.65	Aza I M.W. 618.0 m.p.(°C) 200 R.T. 6.03	Aza H M.W. 662.0 m.p.(°C) 248 R.T. 6.26-6.46	Aza D M.W. 662.0 R.T. 7.0-8.73	Aza B M.W. 662.0 m.p.(°C) 204-6 R.T. 8.0-9.8	Azadirachtin R.T. 12.5	Azadiradione R.T. 25.32	Deacetylnimbin R.T. 18.02	Nimbin R.T. 27.18	Salannin R.T. 31.76	Eproxy-azadiradione R.T. 58.53
Cyt/GA	0.328_{Pk7}	Nd	5.848_{Pk8}	3.218_{Pk9}	Nd	6.031_{Pk10}	$0.622_{Pk11,12}$	0.416_{Pk16}	0.114_{Pk15}	Nd	0.191_{Pk17}	Nd
Cyt/Spd 1	0.496_{Pk12}	Nd	Nd	Nd	Nd	1.599_{Pk13}	0.600_{Pk14}	4.965_{Pk20}	0.600_{Pk17}	0.232_{Pk21}	$0.265_{Pk23,24}$	2.327_{Pk32}
Cyt/Spd 2	Nd	1.352_{Pk8}	Nd	Nd	0.283_{Pk7}	2.886_{Pk9}	0.379_{Pk11}	2.715_{Pk18}	0.926_{Pk15}	0.409_{Pk19}	0.265_{Pk21}	0.155_{Pk26}
Put 0.2mM	0.352_{Pk4}	0.148_{Pk7}	Nd	1.625_{Pk5}	0.526_{Pk6}	Nd	1.798_{Pk8}	Nd	0.392_{Pk9}	5.076_{Pk10}	0.203_{Pk11}	Nd
Put 0.5mM	0.084_{Pk9}	0.225_{Pk11}	Nd	0.240_{Pk10}	Nd	Nd	0.994_{Pk12}	Nd	1.202_{Pk13}	Nd	0.216_{Pk14}	Nd
Spd 0.2mM	0.798_{Pk7}	1.921_{Pk11}	1.123_{Pk8}	1.498_{Pk9}	1.533_{Pk10}	1.809_{Pk12}	0.471_{Pk17}	Nd	5.060_{Pk18}	Nd	0.669_{Pk19}	0.127_{Pk20}
Spd 0.5mM	1.254_{Pk3}	0.075_{Pk6}	1.074_{Pk4}	0.399_{Pk5}	Nd	0.712_{Pk7}	0.738_{Pk9}	0.190_{Pk12}	0.486_{Pk10}	Nd	0.219_{Pk13}	Nd
Spm 0.2mM	0.216_{Pk4}	0.057_{Pk6}	Nd	Nd	0.116_{Pk5}	Nd	5.882_{Pk7}	Nd	4.063_{Pk8}	1.056_{Pk9}	1.677_{Pk10}	Nd
Spm 0.5mM	0.200_{Pk5}	Nd	0.771_{Pk6}	0.479_{Pk7}	Nd	Nd	0.580_{Pk9}	0.096_{Pk11}	0.207_{Pk10}	Nd	0.482_{Pk12}	$Negligible_{pk14}$
Cyt 0.2mM	0.229_{Pk5}	0.219_{Pk8}	0.338_{Pk6}	0.352_{Pk7}	Nd	0.275_{Pk9}	1.186_{Pk12}	0.210_{Pk14}	2.087_{Pk13}	1.164_{Pk15}	0.315_{Pk16}	0.588_{pk19}
Cyt 0.5mM	0.204_{Pk21}	0.253_{Pk26}	0.773_{Pk22}	1.613_{Pk23}	0.193_{Pk24}	2.489_{Pk27}	1.978_{Pk38}	0.255_{Pk60}	0.353_{Pk43}	1.421_{Pk63}	0.597_{Pk73}	Nd
GA 0.2mM	1.040_{Pk22}	1.828_{Pk26}	4.068_{Pk23}	2.086_{Pk24}	0.834_{Pk25}	4.102_{Pk27}	0.616_{Pk37}	Nd	3.113_{Pk58}	Nd	Nd	Nd
GA 0.5mM	1.390_{Pk6}	0.062_{Pk10}	0.510_{Pk7}	0.332_{Pk8}	0.102_{Pk9}	3.584_{Pk11}	1.841_{Pk15}	1.652_{Pk17}	0.919_{Pk16}	Nd	0.806_{Pk18}	Nd
Control	Nd	Nd	2.310_{Pk10}	4.002_{Pk11}	Nd	Nd	Nd	Nd	0.013_{Pk17}	Nd	Nd	Nd

previous work on some other plants indicate that there may be a definite possibility to enhance the yield.

MATERIALS AND METHODS

Young reproductively mature trees of 5-6 years age were developed in the pharmaceutical garden nursery in Panjab University, Chandigarh (Lat. 30.5 N Long. 77.0 E). Thirty healthy plants of a single genotype strain were available in different years ((in consorted efforts with with Department of Biotechnology, Panjab University). Replicates of five plants were taken up in our experiments in one year. Just prior to onset of the regeneration phase in March, apices of the test branches were sprayed *in vivo* with standardized kinetin, polyamine and GA_3 solutions with a few drops of Tween 20.

Preliminary experimentation : In accordance to previous test analyses and studies (Sokal and Rohlf 1973, Devakumar and Sukhdev 1993), the growth regulators were applied. The number of seed/fruit per panicle, their size, weight and quality indicating Seed Yield and Economics were recorded (Sabherwal and Dhir 2009, 10). Accordingly, in continuation of our previous work, the best treatments were correlated and standardized for the present study on seed pharmaceuticals.

Main experimentation : The individual treatments selected were putrescine, spermidine, spermine, kinetin and GA_3 applied in concentration 0.2 mM and 0.5 mM, respectively. Also, 0.5 mM kinetin was applied in combinations with spermidine 0.2 mM, 0.5 mM and GA_3 0.5 mM to separate test branches (in replicates of five) in three consecutive years. Free limonoids tetranortriterpenoids were determined by the method of Devakumar and Sukhdev (1993). In all the treatments, qualitative and quantitative profiles were recorded for the effective endogenous seed limonoids, such as azadirachtins, aza isomers, nimbin, salannin and the modified active principles in 'Neemrich' after prior soxhlet extraction of the seed post-anthesis. The active principle in the samples were detected by Shimadzu HPLC using ODS chromosorb column (250.0 mm x 4.6 mm) with a mobile phase of methanol:water (60:40, v/v). Each run was set to 60 minutes. The calibration was as per the peak-area data of HPLC elutions with standards (Sigma chemicals and IARI, New Delhi).

The statistical analysis of data was done by ANOVA tests available in software packages as outlined by Sokal and Rohlf (1973).

RESULTS AND DISCUSSION

Azadirachta indica A. Juss. (Family Meliaceae) has a single annual growth flush with budding and flower blooms in March developing to post-anthesis fruit set-seed. The ripe fruit set in July coincides with the heavy monsoons and is subject to insect attack, feeding birds and human harvest.

The total bioactive compounds comprised the limonoid evaluations, that is, azadirachtins, nimbin and salannin together. The independent evaluations of these compounds were also measured. Although no inter adjustment was found among the compounds, they correlated with the exogenous growth regulator treatments (Table 1). Our precise objective was 'to test effects of PGRs in increasing pharmaceutical yield of neemrich'.

Limonoid evaluations : Neem seeds are normally sold by weight while their commercial value depends upon their mean limonoid content per unit weight (Govindachari *et al.* 1990, Devakumar and Sukhdev 1993). Therefore, our results were expressed as compound mg per unit g seed (Table 1). Azadirachtins, nimbin and salannin comprising the 'Neemrich' elution was influenced with growth regulator treatments indicating their possible role in limonoid metabolism.

Limonoid content varied, ranging from 0.003-0.006 units in spermidine and putrescine test analyses as compared to control (0.010 units). Hence, the polyamine samples recorded poor 'Neemrich' levels. The content gradually increased with kinetin (0.011-0.012 units) and GA_3 (0.015-0.054 units). Significant increase

was observed with combination of hormones; kinetin-GA_3 (0.091 units) and kinetin-spermidine (0.016-0.020 units). Therefore, GA_3 treatment individually or in combination with spermidine considerably influenced the increase in limonoids. This suggested that GA_3 had profound effect upon endogenous titres of 'Neemrich' in commercial seed product. While the influence of polyamines was profound only in certain cases as spermidine (0.017-0.021 units) and in the combination kinetin-spermidine (0.016-0.020 units) test samples. Application of kinetin and spermidine induced processes integral to fruit development (Palni *et al.* 1990, Jacqmard *et al.* 1994). Exogenously applied hormones promoted fruit set, increased fruit size and growth of developing fruit. An increased growth regulator supply during early fruit growth (the period of cell division) was expressed as large increase in size as well as quality with enhanced dry matter. However, in certain cases (GA_3 0.5 mM and kinetin 0.5 mM) no increase in fruit size was observed despite the treatments. This appears to be due to extra fruit set that otherwise get abscised later on (Angrish and Dhir 1996, Tucker 1996).

In our effort to commercially as well as pharmaceutically define the harvest seed yield, worthwhile ratios of seed production were obtained.

Mainly owing to its various effects on insects, azadirachtins are considered the most important active principle in neem seed kernels (Friend 1998). However, the quantity of this compound present in neem seed kernels vary considerably with exogenous growth regulator treatments; the hormone and its concentration. Higher yield of azadirachtins were obtained in tissues treated with putrescine, kinetin as well as GA_3. Azadirachtin is formed by a group of closely related isomers, designated aza A to aza G (Kraus *et al*. 1985, Broughton *et al.* 1986). While aza A is the most important compound in terms of its quantity in neem seed kernel extracts, all others are also effective insect growth regulators (Rembold 1990).

Azadirachtins

The peaks of azas A and I were resolved within 7.65 minutes and 6.03 minutes as Retention Time (R.T.), respectively. Aza F appeared at 5.47 R.T.

Earlier, Yamasaki *et al.* (1986) isolated and purified azadirachtins by normal-phase as well as reverse-phase HPLC, while Govindachari *et al.* (1990) purified azadirachtins on preparative HPLC and resolved as analytical HPLC with aqueous methanol. However, we found that the solvent on preparative HPLC improved separation of aza isomers. Relative proportions of azas A, B, D, F, H and I in seeds from different treatments are presented in Table1.

During most test elutions, aza F was the major metabolite in the seeds as compared to azas I and A. GA_3 samples recorded 0.062 units aza A and 1.39 units aza F. Relative proportions were recorded with spermine 0.2 mM (0..057/0.216 units), spermidine 0.5 mM (0.075/1.254 units) and putrescine 0.2 mM (0.148/0.352 units) test samples. However, aza F and aza A were sometimes matched close as in kinetin 0.02 mM (0.219/0.229 units) and kinetin 0.05 mM (0.204/0.253 units) quantifications. While putrescine 0.5 mM and spermidine 0.2 mM treated samples recorded enhanced aza A (0.225/1.921 units, respectively) as compared to aza F (0.084/0.798 units). Total azadirachtins were high in samples treated with kinetin (1.186-1.978 units), GA_3 (0.616-1.841 units) and putrescine (0.994-1.798 units) (Table 1).

Aza A (peak R.T. 7.65) and aza B (peak R.T. 8.0-9.8) were the major metabolites in 'Neemrich' analyses as compared to aza I (peak R.T. 6.03) and aza C (failed to profile) according to previous studies (Rembold 1989, Govindachari *et al*. 1990). In fact, aza A was found to occur many times more than some other aza isomers as H (peak R.T. 6.26-6.46), I (peak R.T. 6.03) and D (peak R.T. 7.0-8.73), which were barely recovered or recovered with great difficulty. However, we found a significant increase in the syntheses of azas B

and F with spermidine (0.712/1.254 and 0.798/1.809 units) and kinetin (0.204/2.489 and 0.229/0.275 units) relative to spermine 0.5 mM (0.200 units), putrescine 0.2 mM (0.352 units) and putrescine 0.5 mM (0.084 units) test samples.

Previously reported results on increased hormonal levels during regeneration (Bernier *et al.* 1990, Lejeune *et al.* 1994, Koul and Wahab 2007), point to their putative role in fruit evocation-transition phase. Therefore, reproductive phase is characterized by enhanced growth regulator stimulated activity, in contrast to untreated shoot apices which showed comparatively slow organogenesis and associated metabolism. Furthermore, the differences in distribution of metabolites at reproductive levels may be indicative of their specific physiological role in re-mobilization during regenerative processes.

A considerable number of other active compounds were isolated from neem seed kernels, such as salannin, nimbin and deacetylnimbin. These compounds show anti-feedant activity in bio-test (Schwinger *et al*. 1984, Jones *et al*. 1990). Some other minor ingredients profiled are azadiradione and epoxyazadiradione. Similar pesticidal properties are assigned to such elutions (Kubo *et al*. 1986, Kraus *et al*. 1987).

Nimbin and its conjugate deacetylnimbin

Nimbin (peak R.T. 27.18) was distinctly eluted in putrescine 0.2 mM (5.066 units), kinetins (1.421-1.164 units) and spermine 0.2 mM (1.056 units). Smaller proportions were profiled in kinetin-spermidine samples (0.232-0.409 units). The nimbin profiles were undetected in case of spermidine, GA_3 and untreated control.

Salannin

Salannin (peak R.T. 31.76) was adequately detected in most test samples, however, we could not trace them in untreated controls as well as GA_3 treatments. Hormone combinations evaluated 0.191 units (kinetin 0.5 mM-GA_3 0.5 mM), 0.265 units (kinetin-spermidine) elutions; while higher levels were recorded with kinetin (0.579,0.315 units) and spermine (1.677, 0.482 units). GA_3 0.5 mM profile registered 0.806 units salannin. Favorable counts were reported in putrescine (0.203, 0.216) as well as spermidine (0.216, 0.669 units) liquid chromatograms.

Results suggested a positive coordination of salannin metabolites with exogenous growth regulators as cytokinins, spermine and spermidine. Good results were also reported with combinations of hormones like kinetin, GA_3 and spermidine.

Minor compounds: azadiradione, epoxyazadiradione

These minor compounds (peaks R.T. 25.32, 58.53) were only partially detected. Azadiradione was adequately profiled with hormone combinations. 0.416, 4.965 and 2.715 units were the respective elutions for kinetin 0.5 mM-GA_3 0.5 mM, kinetin 0.5 mM-spermidine-0.2 mM and kinetin 0.5 mM-spermidine 0.5 mM. Other important profiles included GA_3 0.5 mM (1.652 units) and spermidine 0.5 mM (0.190 units). In fact, azadiradione was successfully detected in kinetin samples as small quantities, 0.210 units (kinetin 0.5 mM) and 0.255 units (kinetin 0.2 mM). Minute profiles appeared in spermine 0.2 mM with 0.096 units azadiradione. While epoxyazadiradione was favored with kinetin, either alone (0.5 mM: 0.588 units), or in combination with spermidine 0.2 mM (2.327 units) and spermidine 0.5 mM (0.155 units). Spermidine 0.2 mM profiled the conjugate compound as 0.127 units. Negligible quantities were detected in spermine elutions.

CONCLUSIONS

Azadiradione and epoxyazadiradione chromatographic elutions were incomplete and only partially successful. We could get positive effects with combination of hormones and, to some extent, with individual applications; kinetin and spermidine.

According to Robins and Sweeney (1979), growth regulators form a metabolic link with limonoids (alkaloids). Potential polyamine precursors are efficiently incorporated into the

alkaloids. This pathway is, in fact, well characterized in several higher plant systems (Smith 1985). Synthesis of putrescine via arginine-agmatine pathway is in accordance with alkaloid metabolism. Putrescine is a dynamic branching point, being a substrate for both reversible spermidine formation and alkaloid synthesis (Hartmann *et al.* 1988). In contrast to the highly dynamic character of putrescine/spermidine, alkaloids accumulate as a virtually inert metabolic product. Therefore, the regulation of substrate flow from the dynamic polyamine pools was enhanced. Limonoid analyses indicate that spermidine might be an important control element, since the polyamine by itself or in combination was quite effective. The flow of metabolic pathway from arginine into the alkaloid-specific pathway seem to be sensitively controlled in the presence of putrescine.

According to the eco-physiological function of alkaloids, that is, as an insect repellent, there is only the need to maintain a suitable concentration of alkaloids within tissues to fulfill this function (Hartmann *et al.* 1988). A metabolic coupling of alkaloid synthesis to polyamine metabolism would offer a simple mechanism to adjust the required alkaloid level to growth rate. Active growth and cell division have been found to be correlated with increased rates of growth regulators (Sabherwal and Dhir 2009,10).

REFERENCES

Abd El-Waheb M S A & Gamal El-Din K M 2004 Stimulation of growth, flowering, biochemical constituents and essential oil of chamomile plant with spermidine and stigmasterol application. *Bulg J Plant Physiol* **30** 89-102.

Angeles Botella M, Amor A, Serrano M, Martinez V & Cerda A 2000 Polyamines, ethylene and other physio-chemical parameters in tomato (*Lycopersicon esculentum*) fruits as affected by salinity. *Physiol Plant* **109** 428-434.

Angrish R & Dhir K K 1996 Flowering process in generative buds of woody perennials: an experimental approach. *Curr Sci* **70** 81-84.

Aziz A 2003 Spermidine and related-metabolite inhibitor modulate sugar and amino acid levels in *Vitis vinifera* L.:Possible relationships with initial fruitlet abscission. *J Exp Botany* **54** 355-363.

Bernier G, Lejeune P, Jacqmard A & Kinet J M 1990 Cytokinins in flower initiation: In *Plant Growth Substances*, (eds RP Pharis and S Rood), Springer-Verlag, Berlin, p 486-491.

Broughton H B, Ley S V, Slawin A M Z, Williams D J & Morgan E D 1986 X-ray crystallographic structure determination of detigloyldihydroazadirachtin and reassignment of the structure of the limonoid insect antifeedant, azadirachtin. *J Chem Soc Comm* 46-47.

Devkumar C & Sukhdev 1993 Chemistry: In *Neem Research and Development Society of Pesticide Science*, (eds NS Randhawa and PS Parmar), IARI, New Delhi, p 63-96.

Dewittee W, Chiappetta A, Azmi A, Witters E, Strand M, Rembur J, Noin M, Chriqui D & Onckelen HV 1999 Dynamics of cytokinins in apical shoot meristems of a day-neutral tobacco during floral transition and flower formation. *Plant Physiol* **119** 112-121.

Dhir KK, Angrish R & Pubby S 1986 Cytokinin effect on inflorescence development from generative buds of *Salix tetrasperma* cultured *in vitro*. *J Plant Sci Res* **2** 109-111.

Durdan SF, Herbert RJ, Rogers HJ & Francis D 2000 The determination time of the carpel whorl is differentially sensitive to carbohydrate supply in *Pharbitis nil*. *Plant Physiol* **123** 189- 200.

Friend JA 1998 Twenty years of horticultural research with neem against diamond-back moth, *Plutella xylostella* L. (family: Ponemeutidae). Syn: *Plutella maculipennis* (after Streets 1975). *Pestology* **22** 33-40.

Friend, J.A. (1998). Positive impacts and influence of neem and azadirachtin in soil environments. *Pestology* **22** 41-44.

Govindachari TR, Sandhya G & Ganeshraj SP 1990 Simple method for the isolation of azadirachtin by preparative high performance liquid chromatography. *J Chromatogr* **513** 389-391.

Hartmann T, Sander H, Adolph R & Toppel G 1988 Metabolic links between the biosynthesis of pyrrolizidine alkaloids and polyamines in root cultures of *Senecio vulgaris*. *Planta* **175** 82-90.

Jacqmard A, Houssa C & Bernier G 1994 Regulation of the cell cycle by cytokinins: In *Chemistry, activity and function*, (eds DWS Mok and MC Mok), CRC Press, Boca Raton, USA, p 197-221.

Jones PS, Ley SV, Morgan ED & Santafianos D 1990 The chemistry of the neem tree: In *The Neem Tree Focus on Phytochemical Pesticides*, (ed M Jacobson), CRC Press, Boca Raton, USA, p 19-45.

Kaaya G P, Saxena RC & Solomon G 2003 Potential of neem products for control of three species of ticks affecting livestock in Africa. *J of Economic Entomol* **96** 78-86.

Kaminek M, Motyka V & Vankova 1997 Regulation of cytokinin content in plant cells. *Physiol Plant* **101** 689-700.

Koul O & Wahab S 2007 Neem: In *Neem: Today and in the New Millennium*, (eds O Koul and S Wahab), Kluwer, Netherlands, p 122-147.
Kraus W 2002 Biologically active ingredients: In *The Neem Tree*, (ed H Schmutterer), The Neem Foundation, Mumbai, p 39-111.
Kraus W, Bokel M, Klenk A & Pohnl H 1985 The structure of azadirachtin and 22,23-dihydro-23-B-methoxyazadirachtin. *Tetrahedron Letters* 6438.

Kraus W, Bokel M, Bruhn A, Cramer R, Klaiber I, Klenk A, Nagi G, Pohnl H, Sadio H & Vogler B 1987 Structure determination by NMR of azadirachtin and related compounds from *Azadirachta indica* A. Juss. (Meliaceae). *Tetrahedron* **43** 2817-2830.
Kubo I, Matsumoto A & Matsumoto T 1986 New insect ecdysis inhibitory limonoid, deacetyl azadirachtinol isolated from *Azadirachta indica* (Meliaceae) oil. *Tetrahedron* **42** 489-496.
Lejeune P, Bernier G, Requier MC & Kinet JM 1994 Cytokinins in phloem and xylem saps of *Sinapis alba* during floral induction. *Physiol Plant* **90** 522-528.
Martineau B, Summerfelt KR, Adams DF & de Verna JW 1995 Production of high solids tomato through molecular modification of levels of the plant growth regulator cytokinin. *Bio Tech* **13** 250-254.
Martin-Tanguy J 2001 Metabolism and function of polyamines in plants. *Plant Growth Regul* **34** 135-148.
Metzger J D 1995 Hormones and reproductive development: In *Plant Hormone Physiology, Biochemistry and Molecular Biology*, (ed PJ Davies), Kluwer, Boston, p 617-648. .
Palni LMS, Nandi SK & Singh S 1990 The physiology and biochemistry of cytokinins: In *Hormonal Regulation of Plant Growth and Development*, (ed SS Purohit), Agro-botanical, Bikaner, p 1-60.
Pandey S, Ranade SA, Nagar PK & Kumar N 2000 Role of polyamines and ethylene as modulators of plant senescence. *J Bioscience* **25** 291-299.
Rembold H 1989 Medicinal Research: In *Economic and Medicinal Plant Research Vol. 3*, (eds H Wagner and R Norman), Academic Press, New York, p 57.
Rembold H 1990 Isomeric azadirachtins and their mode of action: In *Focus on Phytochemical Pesticides of the Neem Tree*, (ed M Jacobson), CRC Press, Boca Raton, USA, p 47-67.

Robins DJ & Sweeney JR 1979 Pyrrolizidine aldaloids: evidence for the involvement of spermidine and spermine in the biosynthesis of retronecine. *J Chem Soc Chem Commun* 120-121.
Sabherwal S & Dhir KK 2009 *Azadirachta indica* A. Juss. (Neem): Reproductive studies in relation to kinetin application. *The Ind For* **135** 505-516.
Sabherwal S & Dhir KK 2010 *Azadirachta indica* A. Juss. (Neem): Reproductive studies in relation to spermidine application. *The Ind For* **136** 245-256.
Schwinger M, Ehhammer B & Kraus W 1984 *Proc, 2nd International Neem Conference*, Rauischholzhausen. GTZ, Eschborn, p 181.
Smith TA 1985 Polyamines. *Annu Rev Plant Physiol* **36** 117-143.
Sokal R R & Rohlf FJ 1973 Estimation and hypothesis testing: In *Introduction to Biostatistics*, (ed W H Freeman), Freeman, San Francisco, USA, p 94-112.
Tamas I A 1987 Hormonal regulation of apical dominance: In *Plant Hormones and Their Role in Plant Growth and Development*, (ed PJ Davies), Martinus Nifhoff, Dordrecht, p 393-410.
Tucker S C 1996 Floral evolution and development: testing hypothesis. *Amer J Bot* **83** 200-205.
Vila S, Gonzales A, Rey H & Mroginski L 2004 *In vitro* plant regeneration of *Melia azedarach* L. :Shoot organogenesis from leaf explants. *Biologia Plantarum* **47** 13-19.
Walia S, Kumar J & Parmar BS 2002 Ecologically sound botanical pesticides: In *Development and Use in Biopesticides and Pest Management*, (eds O Koul, SS Marwaha and JK Arora), Campus Book International, New Delhi, p 56-63.
Yamasaki RB, Klocke JA, Lee SM, Stone GA & Darlington 1986 Isolation and purification of azadirachtin from neem (*Azadirachta indica*) seeds using flash chromatography. *J chromatogr* **356** 220-226.