ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

# Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 4 (2): 79-93, 2005
ISSN 1682-296X


# Useful Metabolites from Plant Tissue Cultures

Renu Sarin
Laboratory of Plant Physiology and Biochemistry,
Department of Botany, University of Rajasthan, Jaipur, India

**Abstract:** Phytochemical are now a days used as powerful medicine to cure many diseases. A number of bioactive substances have been reported from plant tissue cultures. The present study pertains to the important naturally occuring metabolites such as alkaloids, antimicrobials, flavonoids, insecticides, sterols and steroidal sapogenins reported from in vitro tissue cultures of a number of plant species.

**Key words:** Tissue culture, alkaloids, flavonoids, pyrethrins, rotenoids, steroids

## INTRODUCTION

In recent years, phytotherapy is rapidly evolving throughout the world. The beneficial effects of medicinal plants to cure many dreadful diseases were discovered long before scientific advancement. With the advancement of technology new commercial organizations switch on to study hitherto unknown medicinal plants. The study of modern herbalism has started selection of new seeds from cultures of plants in order to obtain qualitative and quantitative optimal yields and to elaborate new and scientifically tested drugs for the more effective treatment of specific illness. Phytochemical are naturally occuring biochemicals in plants that give plants their color, flavour, smell and texture. They may help to prevent diseases like cancer and heart diseases besides their role to inhibit the microorganisms causing many diseases in human beings. The active principles contained in the plants consist of a number of chemical compounds, which have a specific action on the organs of our body.

Now a days, there is a great decrease in plant resources due to human disturbances of the natural environment. Therefore, biotechnologists hope for a bypass to overcome this difficulty by introducing plant tissue culture technique and further multiplication of important plants by micropropagation technique. Now, the technique of tissue culture has been developed for large-scale cultivation of plant cell. The production of useful metabolites from plant tissue culture has created a new methodology for their commercialization. The useful metabolites from plant tissue cultures include alkaloids, antimicrobials, essential oils, flavonoids, pigments, proteins, phenols, pyrethrins, rotenoids, sterols and steroids. Several products are accumulated in cultured cells at a higher level than those in native plants e.g. shikonin by *Lithospermum erythrorhizon* and diosgenin by *Dioscorea*. For more than 30 years many researcher have been investigating plant cell cultures for the production of a variety of phytochemical. However, inspite of their many efforts only two products e.g. shikonin and ginseng cells are so far manufactured commercially. Mitsui Petrochemical Industries (Tokyo, Japan) has successfully produced shikonin, an antibacterial dye on commercial basis. A number of firms in US, Japan, Canada and Europe have been investigating intensively the production of a very promising anti-tumor compound, Taxol using the cell cultures. Thus, the chances of exploitation of tissue culture technique for large-scale production of useful metabolites are bright.

The beginning of production of useful metabolites from tissue culture in India dates back to 1964 when Mitra and Kaul at National Botanical Research Institute, Lucknow for the first time reported the production of reserpine from Rauwolfia serpentina tissue culture. Later on other laboratories like Central Drug Research Institute, Lucknow, Central Institute of Medicinal and Aromatic Plants, Lucknow, Bhabha Atomic Research Centre, Trombay, National Chemical Laboratory, Pune and Regional Research Laboratory, Jammu=Tawi and Universities like M.S. University, Baroda and Jawaharlal Nehru Technology University, Delhi carried out study on various metabolites from plant tissue cultures.

A number of reviews on plant constituents have been written[1-22]. The prospects and problems in large-scale production of metabolites from plant tissue cultures has been discussed[9,21].

**Natural plant metabolites:** The naturally occurring plant metabolites have been divided into two groups.

**Primary plant products:** Which are of prime importance and essentially required for growth of plants e.g. amino acids, ascorbic acid, carbohydrates, enzymes, lipids, nucleic acids and proteins, etc. They are of universal occurrence in plants.

**Secondary plant products:** Which are accessory, plant metabolites and seemingly not involved in the biosynthesis of primary metabolites. They are produced secondarily and derived biosynthetically from the metabolism of primary products such as carbohydrates, fats and amino acids. They are biosynthesized in selected few species of plants e.g. Alkaloids, coumarins, flavonoids, phenols, sterols and steroids. They are not of universal occurrence in plants.

**Primary products from plant tissue cultures:** Among primary products amino acids, ascorbic acid, carbohydrates, lipids and protein have been reported from tissue culture of a number of plant species.

Amino acids are the building blocks of proteins and many other secondary products. The concentration and amino acid composition of proteins from plant cells grown in suspension cultures has been reported[23]. The amino acids have been reported from tissue culture of a number of plant species such as *Datura metel*, *D. tatula, Momordica charantia* and *Trigonella foenum-graecum*[24], *Tephrosia purpurea, T. vogelii, Emblica officinalis* and *Sesamum indicum*[25], *Scopolia japonica*[26] *Ephedra foliata*[27], *Tagetes erecta*[28]. The effect of various amino acids on the production of tropane alkaloids in *Scopolia* species has also been investigated and it has been demonstrated that tryptophan is most effective amino acid in producing scopolamine and hyoscyamine[29].

Chemically ascorbic acid is more related to the monosaccharides as it is a hexose derivative. It controls the cholesterol metabolism and helps in the absorption and utilization of iron. It is widely distributed in plants mainly in citrus fruits. Production of endogenous ascorbic acid and effect of exogenous ascorbic acid on growth and metabolism has been reported in tissue cultures of *Emlica officinalis* and *Momordica charantia*[30], *Datura metel* and *D. tatula*[31] *Trigonella foenum graecum*[32], *Crotolaria burhia*[33], *Tagetes erecta*[34], *Ephedra foliata*, *Helianthus annuus*, *Tephrosia purpurea* and *T. vogelli*[35], *Papaver somniferum* [36] and *Solanum nigrum*[37].

Carbohydrates have been reported from tissue cultures[38,39]. All culture media require carbohydrates as a source of carbon. Utilization and metabolism of carbohydrates in cell and callus cultures has also been reported[40].

Fatty acid composition of lipids in various cell cultures have been reported[41-43]. Lipids in plant tissue cultures have been reported from a number of plant species[41-47] and the fatty acid composition of triglycerides in tissue cultures of rape and turnip rape[47], *Corchorous, Yucca, Dioscorea, Withania* and *Rivea*[48] have been investigated. Major lipid components in tissue cultures of *Euphoria* species has also been studied[49]. Biosynthesis of neutral lipids from malonate alongwith cardenolides in *Digitalis lanata* have been reported[50].

A hypoglycemic polypeptide-p, has been isolated from fruits, seeds and tissue culture of *Momordica charantia*[51,52]. Polypeptide-p, when administered subcutaneously (1.8 mg $mL^{-1}$) is very effective hypoglycemic agent[53].

The enzymes have been reported from tissue cultures of a number of plant species[54,55]. Physiology of enzyme production by plant cell cultures has also been investigated. Medora *et al.*[55] has reported proteolytic enzymes in papaya tissue cultures.

**Secondary products from plant tissue cultures:** The biosynthesis of secondary products from plant tissue cultures is of considerable interest and has become very important these days. A number of important secondary products such as alkaloids, flavonoids, pyrethrins, rotenoids, sterols and steroids have been isolated from different plant species grown *in vitro*.

**Alkaloids:** Alkaloids, a heterogenous group of basic nitrogen containing heterocyclic compounds are produced by plants as secondary metabolites. Some exceptions are colchicine and ricinine which are not basic and ephedrine and muscarine which are not nitrogen containing heterocycles. Their inclusion among the alkaloids is based mainly on their biosynthetic similarity with the true alkaloids. Alkaloids are very important in the medical world and are used as powerful drugs mainly due to their sedative properties and powerful effect on the nervous system. A variety of alkaloids have been used as pharmaceuticals. Alkaloids are produced in actively growing tissue and rarely occur in dead tissue. Research on production of useful alkaloids by plant tissue culture has also been carried out for more than 25 years. However, industrial production has not yet succeeded because of low producing ability of cultured cells. Vinblastine, an anti-tumor alkaloid is most likely to be produced commercially by a Japanese company using a combination process of plant cell culture and that of chemical synthesis, which was initially investigated by a Canadian company, Allelix Inc. At present time more than 3000 alkaloids are known which are distributed among almost 4000 plant species. A particular alkaloid is usually of limited distribution and confined to a specific genus or closely related group of plants. A number of alkaloids have been reported from tissue cultures of different plants.

Based on their chemical nature alkaloids can be classified into following 7 groups:

1. Pyrrolidine Alkaloids-This is a small group of alkaloids which can be further classified into 2 groups: (I) Simple Pyrrolidines e.g. Hygrine, hygroline, cuscohygrine, stachydrine, betanicine and turicine and (ii) Tropane alkaloids e.g. Atropine, hyocyamine, scopolamine, cocaine, etc.

These two types are related biogenetically and sometimes occur together in the same plant.

2. Pyrrolizidine alkaloids e.g. Senecio alkaloids
3. Pyridine and Piperidine alkaloids e.g. Nicotine and Anabasine
4. Polyacetyl Alkaloids e.g. Coniine
5. Isoquinoline Alkaloids e.g. Morphine and codeine
6. Indole Alkaloids e.g. reserpine, strychnine, vinblastine and quinine
7. Steroidal Alkaloids e.g. solasodine and solanine. They are usually classified with terpenoids as their C-skeleton is furnished by 5 C unit.

Alkaloids biosynthesis is a subject of very much greater inherent diversity due to their complex structure. They are derivatives of amino acids which supply nitrogen and C-skeleton. Amino acids-ornithine, lysine, phenylalanine, tyrosine and tryptophan are some of the primary metabolites from which various groups of alkaloids are formed.

Among pyrrolidine alkaloids tropane alkaloids are very important pharmaceutically particularly atropine and hyoscyamine which dilates the pupil of the eyes and therefore finds extensive use in ophthalmology. Tropane alkaloids act as sedatives and have been found in solanaceae, convolvulaceae and erythroxylaceae. The name comes from the tropane skeleton in their structure and each is the ester of an organic acid with a bicyclic base. Their biosynthesis starts from an amino acid, ornithine which is converted in pyrrolidine system. Biosynthesis of tropane alkaloids has been studied in root culture of *Atropa belladonna*[56-58], *Brugmansia candida*[59], *Datura innoxia*[60], *Datura stramonium*[61-64], *D. metel*[65-68], *D. tatula*[66], *Hyoscymus niger*[69,70], *Scopolia japonica*[71]. Tropane alkaloids from tissue culture of *Atropa belladonna* is shown by a number of researchers[72-74]. Jain and Khanna[75] have investigated the effect of amino acids on the production of atropine in suspension cultures of ***Atropa belladonna.*** Dmitruk[76] has also studied the effect of various amino acids on the production of tropane alkaloids in *Scopolia* sp.[76] and demonstrated that tryptophan was most effective in producing scopolamine and hyoscyamine. Cordan[77] has written a review on the production of tropane alkaloids in tissue cultures. Production of tropane alkaloids in genetically engineered root cultures was also investigated[78]. Kitamura *et al.*[79] reported that root differentiated from cultured cells accumulate scopolamine, hyoscyamine and nicotine but not accumulated in leaves of the regenerated plantlets[79]. The effect of various growth hormones on growth and production of tropane alkaloids in tissue cultures of *Datura metel* has been reported[80]. Sarker *et al.*[81] has studied the elicitation of tropane alkaloids in suspension culture of *Hyoscyamus*, *Datura* and *Atropa* by osmotic stress. Elicitation of tropane alkaloid biosynthesis in transformed root cultures of *Datura stramonium* has been studied[82]. Effect of jasmonic acid and aluminium on the production of tropane alkaloids in hairy root cultures of *Brugmansia candida* has also been investigated[83]. Inspite of many efforts to increase the yield using various approaches the concentration of tropane alkaloids in cultured cells are generally very low. Therefore, plant cell culture has not yet been employed to manufacture these alkaloids.

Pyrrolizidine alkaloids show a wide spectrum of biological activities against tumor and are hypotensive and muscle relaxant. They are known for their anti-tumor, carcinogenic, hepatotoxin and mutagenic properties. Pharmacodynamic importance of semisynthetic derivative of these alkaloids has been reported[84]. These alkaloids constitute a large family based on the pyrrolizidine nucleus and distributed in a number of plants belonging to asteraceae, boraginaceae and fabaceae families.

Pyridine (e.g. Nicotine and Nornicotine) and Piperidine (e.g. Anabasine) alkaloids occurs almost universally in tobacco plants. Nicotine and nornicotine consist of a pyridine ring to which a pyrrolidine ring is attached and they are the chief alkaloids of ***Nicotiana tabacum.*** In Anabasine, piperidine ring rather than a pyrrolidine ring is joined to the pyridine nucleus and it is the chief alkaloid of *Nicotiana glauca.* Nicotine is the principal constitute of tobacco leaves and occurs to the extent of 0.5-8%. The smoke of a cigarette can yield 6-8 mg of nicotine. It is a natural liquid alkaloid and is colorless, volatile and strongly alkaline. On exposure to air it turns brown and acquires the odour of tobacco. It has no therapeutic application and increases the heartbeat rate and raises blood pressure. The production of nicotine and anabasine in tobacco callus tissue has been investigated[85] and the regulation of nicotine biosynthesis by auxins has also been studied[86]. Regulation of nicotine biogenesis by urea in tobacco tissue cultures has also been investigated[87,88]. Pyridine alkaloids from cell cultures of *Nicotiana tabacum* has also been reported[89]. Trigonelline, another pyridine alkaloid has been reported from *Trigonella foenum-graecum* root callus culture[90] and the yield of this alkaloid was further increased by feeding the tissue with different concentrations of nicotinic acid[91].

Polyacetyl alkaloids includes coniine, Lycopodium and muscopyridine and carpaine alkaloids. Coniine, an alkaloid present in *Conium maculatum* (hemlock) is very poisonous and the extracts were used in ancient times for the execution of criminals. *Lycopodium* alkaloids are found in club mosses and muscopyridine is one of the few alkaloids from animals rather than plant source.

Isoquinoline alkaloids include most important benzylisoquinoline alkaloids. This group of alkaloids include major alkaloids of the genus Papaver e.g. morphine, codeine, thebaine, papaverine and narcotine which are pharmaceutically very important. Among these alkaloids morphine is purely narcotic and is normally reserved for severe pain when other analgesics fail to give relief. Codeine is widely used by general public as a mild analgesic because it is less toxic than morphine. The baine has almost the same analgesic effect as morphine but it is the most poisonous of the opium alkaloids and is scarcely used as such in therapy but is used in the form of its derivatives. Papaverine is used in the treatment of asthma and angina pectoric and narcotine is widely used in the preparation of cough linctus. Opium, the inspitated milky juice from unripe capsules of *Papaver somniferum* contains nearly 25 alkaloids. These alkaloids are absent in the seeds but are synthesized as soon as the plant is grown. Maximum amount is present in the capsules. Production of six major opium alkaloids has been reported from callus tissue cultures of *Papaver somniferum*[92-97], *P. bracteatum*[98], *P. rhoeas*[94,99,100] and four *Papaver* species[97]. Furuya *et al.*[101] have reported nine alkaloids from the callus tissue of *P. somniferum* and Ikuta *et al.*[102] have reported the presence of same alkaloids and the absence of morphine alkaloids in the callus tissues and redifferentiated plantlets of eleven species of papaveraceae. All these alkaloids are benzophenanthridine, protopine and aporphinetypes types of alkaloids. Formation of thebaine in the suspension culture of *Papaver bracteatrum* has also been reported[103] Effect of ascorbic acid, tyrosine and auxins on the production of these alkaloids is also studied in callus culture of *P. somniferum*[36,93]. Tetraploid tracheid containing callus of *P. somniferum* reportedly produced codeine, morphine and thebaine[104]. The high yielding tissues having large cells containing amorphous alkaloid contents were observed in *P. somniferum* and *P. rhoeas*[94]. Effect of tyrosine (a known precursor of opium alkaloids) on the production of alkaloids in high yielding cell strains of *P. somniferum* and *P. rhoeas* was also studied[95,105]. Effect of temperature stress on the production of alkaloids in cell suspensions from four *Papaver* sp. has also been investigated[97].

Berberine, a benzyl-isoquinoline (non-tryprophan indole alkaloid), used as a tonic and in stomachache. It is highly toxic to bacteria and is an intestinal antiseptic. Therefore, it is used for intestinal disorders. Berberine is obtained mainly from roots of *Coptis japonica* (Ranunculaceae) and it takes 5 to 6 years to produce *Coptis* roots as raw material. Furuya *et al.*[100] have investigated the production of berberine by *Coptis japonica* cell cultures. High berberine producing cultures of *Coptis japonica* were also reported[106-109]. Mitsui petrochemical has improved the productivity by addition of gibberellic acid into medium which stimulated berberine productivity upto 1.66 g $L^{-1}$ of medium. Khanna *et al.*[110] have reported berberine from *Argemone maxicana* tissue and cell cultures.

Indole alkaloids posses an indole nucleus often in reduced form and in case of some Cinchona alkaloids, altered almost beyond recognition. These alkaloids has received a great deal of attention from the pharmacologists, physiologists and physicians. The interest in indole alkaloids evolved from the discovery of the remarkable physiological properties of lysergic acid diethylamide on one hand and of reserpine, the sedative principle used for the treatment of hypertension, headache, asthama and dermatological disorders of Indian plant *Rauwolfia*, on the other hand. The extensive investigations of indole alkaloids as well as other constituents of the large family of flowering plants, Apocynaceae, were highlighted a few years ago by the introduction of two Vinca alkaloids, vinkaleukoblastine and leurocristine into the treatment of Hodgkin's disease and acute lukemia respectively. Indole alkaloids are found in plants of families apocynaceae, loganiaceae, rubiaceae and euphorbiacea. Production of indole alkaloids from callus cultures of *Catharanthus roseus* has been reported[111-115]. Influence of various chemical factors on the production of indole alkaloids in tissue cultures of *Ipomea*, *Rives* and *Arogrela* has been reported[116].

Another class of alkaloids is pseudoalkaloids resulting from the oxidation followed by the alkylation or acylation of certain amino acids. Such alkaloids are called protoalkaloids. Ephedrine, a phenylalkylamine alkaloid which dilates the bronchiolar muscle is very helpful in asthma. Pseudoephedrine, an isomer of ephedrine and an effective anti-aesthematic drug has been reported from tissue culture of *Ephedra foliata*[117]. An alkaloid, *momordicine* has also been reported in traces from tissue culture of *Momordica charantia*[118].

**Antimicrobials:** Important characteristics of chemical antimicrobial substances are their capability of inhibiting bacterial colonization, adhesion and their capacity to affect plaque growth metabolic activity. Plant products are very powerful antimicrobial agents[119]. Antimicrobial activity has also been shown in tissue cultures[120-129]. Khanna *et al.*[125] have screened ten plant species- *Atropa belladonna* Linn., *Brassica nigra* Koch., *Datura metal*, *D. tatula*, *Emblica officinalis*, *Hyoscymus niger*, *Momordica charantia*, *Sesamum indicum*, *Tagetes erecta* and *Trigonella foenum-graecum* grown *in vitro* as static cultures against a Gram positive and two Gram negative Bacteria and a fungus-*Candida albicans*. Later on, Khanna *et al.*[122] have screened tissues of ten other plant species-*Agave wightii, Aregemone mexicana, Calendula officinalis, Cheiranthus cheiri, Crotolaria burhia, Dahlia pinnata, Lycopersicon esculentum, Papaver rhoeas, Solanum luteum, S. tuberosum* and *Trigonella corniculata* for their antimicrobial activity against *Escherichia coli, Staphylococcus albus, Streptococcus faecalis* and a fungus *Candida albicans.* They have isolated and identified the possible antibacterial substances produced by them such as Apigenin, isorhamnetin, kaemferol, negretein, quercetine and scopoletin.

**Flavonoids:** Flavonoids are water soluble pigments which occur almost universally in higher plants and contribute to the flower and fruit colour. They impart mostly red, yellow, blue and violet colour to plant organs. Chemically they are phenolic compounds and most of them have flavone nucleus with two side aromatic rings. Flavones occur as glycosides in plants. Flavonoids are classified on the basis of the oxidation state of the central heterocycle of the flavone nucleus. Each subgroup is further grouped according to the pattern of substitution in side aromatic ring. Flavones such as luteolin and apigenin have the central heterocycle with two double bonds whereas flavonones such as naringenin and eriodictyol have one double bond. Flavonol result from the addition of a hydroxyl group at Carbon-3 of the central heterocycle. Kaempferol, quercetin and myericetin are common examples of flavonol. The precursors of flavonoid biosynthesis include shikimic acid, phenylalanine, cinnamic acid and p-coumaric acid.

The distribution of flavonoids in plant kingdom is more or less of taxonomic significance. Algae, fungi and bacteria lack any kind of flavonoid, whereas mosses have a few types of them. Ferns and gymnosperms have many types of simple flavonoids whereas angiosperms have a whole range of flavonoids. Highly complex forms of flavonoids e.g. quercetagetin occur in the highly evolved families like compositae. Production of flavonoids has been reported from tissue cultures of a number of plant species such as *Cicer arietinum*[130], *Citrus*[131,132], *Crotolaria juncea*[133], *Datura* sp.[134], *Emblica officinalis*[135], *Haplopappus gracilis* [136], *Trigonella foenum-grecum*[137], *Tephrosia purpurea*[138], *Tylophora indica*[139].

**Insecticides:** Naturally occuring insecticides fall under two major groups-Pyrethrins and rotenoids. Both are very toxic to insects and harmless to mammals. Thus, can be used safely as domestic insecticides.

**Pyrethrins:** Pyrethrin are economically most important of natural insecticides and are currently derived from Pyrethrum plant. Some biotechnology companies studied the possibility of industrial production of pyrethrins. Whether they are able to overcome the biological and technical constraints is still unclear.

Pyrethrins are the esters used in domestic insecticidal sprays as they are considered to be very toxic to flying insects and harmless to mammals and plants. They have an unusual paralytic effect, 'knock down', on flying insects and inhibit the mitochondrial electron transfer system of insects at characteristic site. Pyrethrins are present in floral heads of *Chrysanthemum cinerariaefolium* Vis. commonly called Pyrethrum[140] and *C. coccineum* Willd. Four closely related esters (Pyrethrin I and II and Cinerin I and II). Later on, Godin *et al.*[141-143] isolated and identified two additional pyrethrins (Jasmolin I and II) from the extracts of *Chrysanthemum cinerariaefolium.* Now, it is clear that there are at least six structurally related compounds collectively called 'Pyrethrins' responsible for the insecticidal activity of Pyrethrum flowers. All the six pyrethrins (Pyrethrin I and II, Cinerin I and II, Jasmolin I and II) have been reported from seeds, floral heads and tissue cultures of *Tagetes erecta*[144-146]. Khanna and Khanna[146] have also investigated the effect of ascorbic acid on the production of pyrethrins from *in vitro* tissue cultures of *Tagetes erecta* and reported that ascorbic acid plays an important role in growth and production of pyrethrins in *T. erecta* tissue culture. Later on, investigations into possible new sources of pyrethrins from a number of plant species such as *Calendula officinalis, Dimorphotheca sinuata, Zinnia elegans, Z. linearis*[147] and *Vernonia* species[148] belonging to family asteraceae were done *in vivo* and *in vitro*. Investigation on high pyrethrin producing tissues of *Chrysanthemum cinerariaefolium* has also been carried out (149=150). Studies of pyrethrum plant derived *in vitro* cultures revealed that unorganized tissue culture do not have secondary metabolism characteristics of the corresponding intact plant and only organized shoot cultures could be considered for pyrethrins production. Biological and technological obstacles have prevented the development of a large scale industrial process based on shoot cultures so far. Natural pyrethrins and biotechnological alternatives have been described[151]. Hitmi *et al*.[153] have written a critical review [152] on the production of pyrethrins by plant cell and tissue cultures of *Chrysanthemum cinerariaefolium* and *Tagetes* species. According to them although technology for plant cell culture exists, industrial applications have to date been limited due to both low economical viability and technological feasibility at large scale. Bioconservation of readily available precursors looks more attractive but more research is needed before this technique is used for industrial production of pyrethrins.

**Rotenoids:** Rotenoids, a group of ketonic compounds have become of agricultural and horticultural importance due to their insecticidal and pesticidal activity as well. The rotenoids are of special value for control of leaf chewing beetles, caterpillars and specially where toxic residues are not desired. With low toxicity and relatively long residual action to warm blooded animals rotenoids are also used as a fish poison. In South America rotenoids were used to control leaf-eating caterpillars one and a half century ago and three centuries prior to that to paralyse fish. Rotenoids are respiratory enzyme inhibitors acting between $NAD^+$ (a coenzyme involved in oxidation and reduction in metabolic pathways) and coenzyme Q (a respiratory enzyme responsible for carrying electrons in some electron transport chains) resulting in failure of respiratory functions. Chemically they contain cis-fused tetrahydrochromeno [3,4-b] chromenenucleus. Many rotenoids contain an additional ring e.g. rotenone.

Rotenoids are mainly produced in roots of two genras of fabaceae (Leguminosae) family-*Derris* and *Londrocarpus* grown in South America. Now, rotenoids have been reported *in vivo* and *in vitro* from a number of plant species such as *Crotolaria burhia*[153], *Cicer aeritinum*[154], *Derris* sp.[155], *Indigofera tinctoria*[156], *Abrus precatorius*[157], *Tephrosia purpurea*[158,159], *T. vogelii*[158,160], *T. falaformis*[161], *T. strigosa*[162] and *Trigonella foenum-graecum*[163].

**Steroids:** Steroids are the compounds known as terpenoids or isoprenoids. Terpenes are formed by the polymerization of isoprene units and steroids are triterpenes or triterpenoids. The term triterpene refers to a group of natural products containing 30 carbon atoms which are derived from six isoprene (5 C) units. Most terpenes posses carbon content in multiples of 5 C. Before the common biosynthesis of this class of products was recognized the terpenes was introduced for those compounds containing 10 C atoms and this base is still used for the modern classification of such natural products. The classification divides terpenes into six groups:

1. Hemiterpenes-(1x C5)
2. Monoterpenes-(C10 =2 x C5)
3. Sesquiterpenes-(C15 = 3 x C5)
4. Diterpenes-(C20 = 4 x C5)
5. Sesterpenes-(C25 = 5 x C5)
6. Triterpenes-(C30 = 6 x C5)

Hundreds of isoprenoids have been found and the actual number existing in the plant kingdom is probably in the thousands. Many of these are of interest because of their commercial uses and because they illustrate the ability of plants to synthesize a vast complex of compounds not formed by animals. Some steroids important to living world are:

1. Sterols
2. Bile acids
3. Steroid hormones (e.g., sex hormones and hormones of the adrenal cortex)
4. The Vitamins of D group
5. Steroid saponins (Saponin = Sapogenin + Sugar; Sapogenins are used in the commercial preparation of steroidal hormones)
6. Heart glycosides
7. Steroid alkaloid

All of these substances have skeletal structure of sterane or cyclopentoperhydrophenanthrene, which is then subjected to modifications which vary from group to group.

The sterols are most often discussed steroids in the plant literature. They are crystalline steroids which contain an alcoholic group and may be either saturated or unsaturated. Sterols have at least 2 functions.

As precursor in the formation of other steroids e.g., cholesterol and sitosterols are precursors in the formation of saponins As components of cell membranes e.g., cholesterol and phospholipids in animals and phytosterols, phospholipids, glycolipids and sulpholipids in plants.

Depending on their origin, they are called Zoosterol (from animals), phytosterols (from plants), mycosterols (from fungi) and marine sterols (from marine organisms e.g. sponges). Phytosterol have been isolated from a large number of plant species. They are also reported from tissue cultures of a number of plant species such as *Datura metel*[164], *Digitalis lanata*[165], *Dioscorea tokora*[166], *Helipterum roseum*[167], *Lindera strychnifolia*[168], *Momordica charantia*[169], *Sesamum indicum*[170], *Stephania cepharantha*[171], *Tephrosia purpurea*[172], Tobacco[173], *Yucca glauca*[174], *Uncaria tomentosa*[175].

Steroidal sapogenins are of economic importance as main precursors of many medicinally useful steroidal hormones such as sex hormones, corticosteroids and oral contraceptives. Economically steroidal sapogenins are isolated mainly from species of *Agave*, *Dioscorea* and *Yucca*. They have been reported from several other plant genra such as *Aspargus, Balanites, Costus, Lycopersicon, Solanum, Tribulus, Trigonella* and *Veleriana.* Tissue cultures of a number of plant species also produce them. Furuya *et al.*[176] have isolated saponins and sapogenins from callus tissue of *Panax ginseng.*

Among the steroidal sapogenins, diosgenin is most important and highly investigated. Diosgenin, is a major raw material in the commercial production of steroidal contraceptives and corticosteroids and is principally obtained from the underground portions of various *Dioscorea* species in 4-5% concentration on dry weight basis. Plant tissue cultures are the ideal system for studying the fundamental aspects of biosynthesis of diosgenin. Diosgenin has been reported from tissue culture of a number of plant species such as *Agave wightii*[177], *Costus speciosus*[178], *Dioscorea* sp.[179-186], *Daucus carota*[187], *Holarrhena antidysenferica*[188], *Solanum* sp.[189-198], *Trigonella foenum-graecum*[199], *Yucca aloefolia*[200], *Y. glauca*[201], *Y. shidigera*[202].

Increase in the yield of steroids, mainly steroidal sapogenins by incorporation of precursors had been a field of interest to tissue culturists. Effect of cholesterol, a precursor of sapogenins has been extensively investigated which showed an increase in diosgenin content in suspension cultures of *Costus speciosus, Dioscorea floribunda Solanum aviculare, S. xanthocarpus*[203], *S. elaeagnifolium*[204]. Effect of hormones on diosgenin biosynthesis in *Dioscorea deltoidea*[184] and *Trigonella foenum-grecum*[205-207] has also been found to increase the diosgenin and other steroid levels in tissue cultures.

In 1785, the English physician Withering used the red foxglove (*Digitalis purpurea*) as a remedy for heart diseases for the first time in Europe. Since then the genus Digitalis has become an indispensable accessory to the physicians. The efficacious substances of the plants are called glycosides after their field of application. They consist of a glycone with 23 carbon atoms which are linked with a varying number of sugars. The a glycone of the heart glycosides are known as cardinolides or cardiac glycosides as they have been employed in treatment of heart disease. A steroid with 21 carbon atoms, pregnenolone, derived from unknown intermediates is converted to digitoxigenin and other cardenolides. There are a number of papers describing production and biotransformation of cardiac glycosides in *Digitalis purpurea* and *D. lantana* tissue cultures[208-212]. Biosynthesis of cardenolide drom malonate in *Digitalis lanata* was studied by Groenveveld[213] and the effect of precursors and inhibitors on cardenolide metabolism in *D. lanata* was studied by Milek *et al.*[214]. Effects of digitoxigenin, digoxigenin and various cardiac glycoside[215] and effects of various pregnanes and two 23-nor-5-cholenic acids[216] on cardenolide accumulation in cell and organ cultures of *D. lanata* has also been reported. Moldenhaur *et al.*[217] have reported cardenolides in *Digitalis lanata* cells transformed with Ti plasmid. Although there are a number of papers describing production of cardiac glycosides in *Digitalis purpurea* and *D. lanata* tissue cultures, generally the yield was very low and moreover, during successive transfers of the cultured cells the amount of cardenolides often decreased and disappeared completely.

Steroidal alkaloids are the alkaloids whose carbon skeleton is furnished exclusively by 5 C unit. They have a fairly complex nitrogen containing nucleus and usually classified structurally with alkaloids but biosynthetically with terpenes. Two important classes of steroidal alkaloids are:

1. Solanum type-are found in the form of glycosides which are ethers that join a non-carbohydrate moiety the aglycone, by an ester bond to a carbohydrate. Solanidine is the nucleus (i.e. aglycone) for two important glycoalkaloids, solanine and chaconine occuring in plants of solanaceae family.
2. Veratrum type-There are more than 50 veratrum alkaloids including veratrmincyclopamine, cycloposine, jervine and muldamine occuring in plants of *Veratrum* sp.

Isolation and characterization of steroidal alkaloids from tissue cultures of some solanaceous plants such as *Solanum dulcamara, S. jasminoides*, *S. khasianum*, *S. nigrum, S. xanthocarpum*, has been reported[198,218-224].

Vagujfalvi *et al.*[225] have reported absence of solasodine but presence of diosgenin in tissue cultures of *Solanum laciniatum*[225].

## REFERENCES


1. Nickell, L.G., 1962. Submerged Growth of Plant Cells. In: Advances in Applied Microbiology. Vol. 4. Academic Press. New York, pp: 213-236.
2. Carew, D.P. and E.J. Staba, 1965. Plant tissue culture: Its fundamentals, application and relationship to medicinal plant species. Lloydia, 29: 1-26.
3. Puhan, Z. and S.M. Martin, 1971. The industrial potential of plant tissue cultures. Progr. Ind. Microbiol., 9: 13-29.
4. Stohs, S.J., H. Rosenberg and S. Billeta, 1975. Sterols of *Yucca glauca* tissue cultures. Planta Medica, 27: 257-261.
5. Butcher, D.N., 1977. Secondary Products in Tissue Cultures. In: Plant Cells and Tissue in Culture. Eds. Reinert, J. and Y.P.S. Bajaj, New York. Springer Verlag, pp: 668.
6. Chadha, M.S. and M.R. Heble, 1980. Applications of plant tissue and cell cultures. In: Biotechnological Applications of Plant Tissue and Cell Cultures: Problems and Prospects, Bombay, National Academy of Science, Bio-organic Division, Bhabha Atomic Research Centre, pp: 2.
7. Bohm, H., 1980. The formation of Secondary Metabolites. In: International Review of Cytology, Perspectives in Plant Cell and Tissue Culyure. Ed. X Vasil, I.K. New York, Academic Press Suppl., pp: 118.
8. Goldstein, W. *et al.*, 1980. Plant Tissue Culture as a Source of Biochemicals. Ed. Staba, E.J., 91-234 (1980) C.R.C. Press Boca Raton, Florida.
9. Hallsby, 1984. Prospects and problems in large scale production of metabolites from plant tissue culture. J. Am. Oil. Chem. Soc., 61: 1724-1728.
10. Khanna, P., 1982. Tissue Cultures and Useful Drugs. A Review of Thirty Eight Plant Species Grown ***in vitro.*** In: Cultivation and Utilization of Medicinal and Aromatic Plants (CUMAP) (Eds.) Atal, C.K. and B.M. Kapur, RRL, Jammu-Tawi, pp: 824.
11. Khanna, P., 1984. Useful metabolites from plant tissue cultures-fifty plant species. 16th Phillip R. White Memorial Lecture. In: Ind. Sci. Cong., Jan 2nd. 1984, Ranchi, Bihar.
12. Khanna, P., 1987. Antimicrobials and other useful metabolites from plant tissue cultures. Keynote address. Dae Symposium on plant-microbe interactions. Org. by Basic sciences committee-II. Board of Research in Nuclear Sci., Dept. of atomic energy, Govt. India, Nov. 11-13th, pp: 1-47.
13. Staba, E.J. and S.S. Lamba, 1963. Production of cardiac glycosides by plant tissue cultures. II. Growth of *Digitalis lanata* and *Digitalis purpurea* in suspension culture. Lloydia, 26: 29-35.
14. Staba, E.J., 1969. Plant tissue culture as technique for phytochemists. In: Phytochemical of North America, 1967, Sym. Appleton-Century-Crofts, New York.
15. Martin, S.M., 1980. In: Plant Tissue Culture as a Source of Biochemicals. (Ed. Staba, E.J.). C.R.C. Press Boca Raton, Florida, pp: 149-166.
16. Wetter, L.R. and F. Constabel, (Eds.), 1982. Plant tissue culture methods. Natl. Research council of Canada, Saskatoon.
17. Balandrin, M.F., J.A. Klocke, E.S. Wurtele and W.H. Bollinger, 1985. Natural plant chemicals: Sources of industrial and medicinal materials. Sciences, 228: 1154-1160.
18. Ulbrich, B., W. Weisner and H. Arens, 1985. In: Primary and Secondary Metablosm of Plant Cell Cultures. Eds. Neumann, K.H. and E. Reinhard, Springer-Verlag., Berlin, pp: 293-303.
19. Scragg, A.H., 1986. In: Secondary Metabolism in Plant Cell Cultures. Eds. Morris, A.H. ***et al.***, Cambridge Univ. Press, Cambridge, UK., pp: 202-207.
20. Shuler, M.L., 1981. Production of Secondary Metabolites from Plant tissue cultures-Problems and Prospects. In: Biochemical Engineering II Eds., Constantinides, W.R. Vieth and K. Venkatasbramanian. N.Y. Acad. Sci., 369: 65-79.
21. Shuler, M.L., J.W. Pyna and G.A. Hallsby, 1984. Prospects and Problems in large scale production of metabolites from plant tissue culture. J. Am. Oil Chem. Soc., 61: 724-1728.
22. Rhodes, M.J.C. *et al.*, 1989. In: Primary and Secondary Metabolism of Plant Cell Cultures. Ed. Kurz, W.G.W. Springer-Verlag, Berlin, Heidelberg, pp: 58-72.
23. Gamborg, O.L. and A.J. Finlayson, 1969. The concentrations and amino acid composition of proteins from plant cells grown in suspension cultures. Can. J. Bot., 47: 1857-1863.
24. Khanna, P. and T.N. Nag, 1973. Production of amino acids *in vitro* tissue cultures. Ind. J. Exp. Biol., 11: 310-311.
25. Khanna, P. and S.C. Jain, 1973. Presence of free and bound amino acids in plant tissue cultures. Ind. J. Pharm., 35: 63-65.
26. Misawa, M., K. Sakato, H. Tanka, M. Hayashi and H. Samejima, 1974. Production of Physiologically Active Substances by Plant Cell Suspension Cultures. In: Tissue Culture and Plant Science. (Ed. Street, H.E.), Academic Press, London and New York, pp: 405-432.

27. Aminuddin, 1977. Production of amino acids in *Ephedra foliata* suspension cultures. Curr. Sci., 46: 825-826.
28. Sarin, R. and P. Khanna, 1982. Free and protein-bound amino-acids from *Tagetes erecta* Linn. Tissue cultures. Bangladesh J. Bot., 11: 54-57.
29. Dmitruk, S.E., 1973. Role of amino acids in the formation of tropane alkaloids. Mter. Obl. Nauchin. Konf Becs. Khim. O. Va., Posuyashch. 1975-Letiya Khim-Technol. Fak. Toeusk. Politekh. Ins. 3rd 1972 (Pub. 1973), pp: 57-59.
30. Mohan, S., T.N. Nag, S.C. Jain and P. Khanna, 1974. Free ascorbic acid from plant tissue cultures. Vidya, 17: 9-13.
31. Nag, T.N., S.C. Jain, S. Mohan and P. Khanna, 1974. Ascorbic acid metabolism and Growth in *Datura* tissue culture. Ind. J. Pharm., 36: 49-50.
32. Jain, S.C., T.N. Nag, S. Mohan and P. Khanna, 1975. Effect of ascorbic acid and its estimation in plant tissue cultures. Sci. and Cult., 41: 439-440.
33. Aminuddin and Khanna, P., 1979. Ascorbic acid metabolism and growth of *in vitro* tissue culture of *Crotalaria burhia* Buch-Ham. Bangladesh J. Bot., 7: 17-19.
34. Khanna, P. and R. Khanna, 1976. Endogenous free Ascorbic acid and effect of exogenous ascorbic acid on growth and production of pyrethrins from *in vitro* tissue cultures of *Tagetes erecta* L. Ind. J. Exp. Biol., 14: 630-631.
35. Khanna, P., R. Kamal, R. Taparia and S.C. Jain, 1977. A quantitative estimation of free ascorbic acid and growth *in vitro* tissue cultures. Vidya B. Sci., pp: 1-6.
36. Khanna, P., R. Khanna and M. Sharma, 1978. Production of free ascorbic acid and effect of exogenous ascorbic acid and tyrosine on production of major opium alkaloids from *in vitro* tissue cultures of *Papaver somniferum*. Ind. J. Exp. Biol., 16: 110-112.
37. Rathore, A.K., G.L. Sharma and P. Khanna, 1979. Free ascorbic acid from *Solanum nigrum* L., tissue cultures. J. Bot., 2: 42-44.
38. Jaspars, E.M.J. and H. Veldstra, 1965. An α-amylase from tobacco crown-gall tissue cultures. II. Measurement of the activity in media and tissue. Physiol. Plantarum, 18: 626-634.
39. Lian, D.F. and W.G. Boll, 1972. Extracellular polysaccharides from cell suspension cultures of bush bean (*Phaseolus vulgaris* Cv. Conterdev). Can. J. Bot., 50: 2031-2037.
40. Chong, C. and C.D. Taper, 1972. Callus tissue cultures. I. Sorbitol (D-glucitol) as a carbon source for callus initiation and growth. Can. J. Bot., 50: 1399.
41. Staba, E.J., B.S. Shin and H.K. Mangold, 1971. Lipids in plant tissue cultures. I. The fatty acid composition of triglycerides in rape and turnip rape cultures. Chem. Phys. Lipids., (North Holland Publishing Co.), 6: 291-295.
42. Tattrie, N.B. and I.A. Veliky, 1973. Fatty acid composition of lipids in various plant cell cultures. Can. J. Bot., 51: 513-516.
43. Weete, J.D., 1971. Total fatty acids of habituated and teratoma tissue cultures of tobacco. Lipids, 6: 684-685.
44. Davies, W.H., E.I. Mercer and T.W. Goodwin, 1965. Presence of sulpholipids in callus cultures of Paul's Scarlet Rose. Phytochemistry, 4: 741.
45. Davyodova, I.M. and R.G. Butenko, 1970. Lipid metabolism in a tissue culture of flax endosperm Kul't. Izolirovannykh Organov, Tkanet Kletok Rast., T.r. Vses. Konf., 1st 1968: 265-269.
46. Shin, B.S., H.K. Mangold and E.J. Staba, 1972. Lipids of selected plant tissue cultures. Colleques Internationaux. C.N.R.S. No., 193: 52-69.
47. Stoller, E.W., S.J. Stohs, M.M. Elemy, Poly chen Yu and C.S. Tarang, 1974. Fatty acid composition of lipids of *Corchorous*, *Yucca*, *Dioscorea*, *Withani* and *Rivea* tissue cultures. Lloydia, 37: 309-312.
48. Thomas, D.R. and A.K. Stobart, 1970. Identification of various phospholipids and glycolipids in callus cultures of Kalanchoe crenata. J. Exp. Bot., 21: 274.
49. Lee, T.T. and Starratt, 1972. Growth substance, requirement and major lipid components totissue cultures of *Euphorbia esula* and *E. cyparissias*. Can. J. Bot., 50: 723.
50. Groenveveld, H.W. and T.L.J.P. Van *et al*., 1992. Cardenolide and neutral lipid biosynthesis from malonate in *Digitalis lanata*. Planta Medica, 58: 239-244.
51. Khanna, P., T.N. Nag, S.C. Jain and S. Mohan, 1974. Extraction of Insulin from a Plant Source. 3rd. IAPTC, Leicester, 21st-26th July, 1974.
52. Khanna, P., S.C. Jain, A. Pangariya and V.P. Dixit, 1981. Hypoglycaemic activity of polypeptide-p from a plant source. J. Natl. Prod., 45: 648-655.
53. Baldwa, V.S., C.M. Bhandari, A. Pangariya and R.K. Goyal, 1976. Clinical trials in patients with diabetes mellitus of an Insulin like compound obtained from plant source. Uppasala J. Med. Sci., 81: 39-41.
54. Jaspars, E.M.J. and H. Veldstra, 1965. An α-amylase from tobacco crown-gall tissue cultures. II. Measurement of the activity in media and tissue. Physiol. Plantarum, 18: 626-634.

55. Medora, R.S., J.M. Campbell and G.P. Mell, 1973. Proteolytic enzymes in papaya tissue cultures. Lloydia, 36: 214-216.
56. Hartmann, T. and L. Witte, 1986. Reinvestigation of alkaloid composition of *Atropa belladonna,* root cultures and suspension cultures. Planta Medica, 5: 390-395.
57. West, F.R., Jr. and E.S. Mika, 1957. Synthesis of atropine by isolated roots of root callus cultures of *belladonna.* Bot. Gaz., 119: 50-54.
58. Kamada, H. and Okamura, 1986. Alkaloid production by hair root cultures in *Atropa belladonna.* Plant Cell Reports, 5: 239-242.
59. Spoliansky, T.C., S.I. Pitta-Alvarez. and M. Gluliettl, 2000. Effect of jasmonic acid and Aluminium on production of tropane alkaloids in hairy root cultures of *Brugmansia candida*. EJB. Electronic J. Biotech., 3: 72-75.
60. Bereznegovskaya, L.N. and V.V. Smorodin, 1975. Effect of aeration on tissue growth and the formation of tropane alkaloids in a suspension culture of *Datura innoxia.* Rastit. Resur., 11: 528-531.
61. Gibson, M.R. and Abbott, 1963. The effect of proline on growth and alkaloid synthesis in *Datura stramonium* Var. *tatula.* Lloydia, 26: 125-132.
62. Gibson, M.R. and D.I. French, 1964. The effect of gibberallic acid on growth and alkaloid synthesisin *Datura stramonium* var. *tatula.* Lloydia, 27: 47-52.
63. Steinstra, T.M., 1954. Formation of mydriatic alkaloids in excised root cultures of *Datura stramonium* grown on a completely synthetic nutrient. Proc. Koninki Ned. Aka. Wetenschap, 57C: 584-593.
64. Zabetakis, I., R. Edwards and D. Hagan, 1999. Elicitation of tropane alkaloids biosynthesis in transformed root cultures of *Datura stramonium.* Phytochemistry, 50: 53-56.
65. Jain, S.C. and T.N. Nag, 1975. Scopoletin from *Datura metel* Linn., tissue cultures. Sci. and Cult., 41: 439-440.
66. Khanna, P. and T.N. Nag, 1972. Effect of Phenylalanine and tyrosine on growth and alkaloid production in *Datura metel* L., tissue cultures. Ind. J. Pharm., 34: 115-116.
67. Khanna, P. and T.N. Nag, 1974. Incorporation of dl-tyrosine-1-C14 in the biosynthesis of tropane alkaloids in the tissue cultures of *Datura metel* L. Ind. J. Exp. Biol., 12: 109.
68. Chokshi, S.J. and A.R. Mehta, 1976. Growth and alkaloid production in suspension cultures of *Datura metel* Anther. Ind. J. Pharm., 38: 13.
69. Dhoot, G.K. and G.G. Henshaw, 1977. Organisation and alkaloid production in tissue cultures of *Hyoscyamus niger*. Ann. Bot., 41: 943-949.
70. Telle, J. and R. Gautheret, 1947. Sur. La culture indefinie des tissue de la racine de jusquisma (*Hyoscymus niger* L.). Comp. Rend., 224: 1653-1654.
71. Mano, Y., S. Nabeshima, C. Matsui, and H. Ohkawa, 1986. Production of tropane alkaloids by hairy root cultures of *Scopodia japonica*. Agriculture and Biological Chemistry, 50: 2715-2722.
72. Heble, M.R., 1977. Atropine from Atropa belladonna tissue culturers. Second Cong. on Tissue culture Asso. Held at National Botanic Garden, Lucknow, 14-16th Feb., 1977.
73. Khanna, P., G.L. Sharma and Aminuddin, 1977. Atropine from *Atropa belladonna* tissue cultures. Ind. J. Exp. Biol., 15: 323.
74. Mitra, G.C., 1972. Atropine production and growth of excised belladonna root cultures. Ind. J. Exp. Biol., 10: 217-218.
75. Jain, M. and P. Khanna, 1982. Effect of amino acids on growth and production of atropine in suspension cultures of *Atropa belladonna* Linn. Ind. J. Bot., 5: 60-62.
76. Dmitruk, S.E., 1973. Role of amino acids in the formation of tropane alkaloids. Mter. Obl. Nauchin. Konf Becs. Khim. O. Va., Posuyashch. 1975- Letiya Khim-Technol. Fak. Toeusk. Politekh. Ins. 3rd 1972, (Pub. 1973), pp: 57-59.
77. Corduan, G., 1975. Production of tropane alkaloids in tissue cultures-A review. Planta Med. Supplement, pp: 163.
78. Hashimoto, T., Yun-D-J and Y. Yamada, 1993. Production of tropane alkaloids in genetically engineered root cultures. Phytochemistry, 32: 713-718.
79. Kitamura, Y., 1988. In: Biotechnology in Agriculture and Forestry 4th Edn. Bajaj,Y.P.S., pp: 419-436.
80. Khanna, P. and R. Khanna, 1976. Effect of various auxins on growth and production of tropane alkaloid from *in vitro* tissue cultures of *Datura metel* Linn. Ind. J. Pharm., 38: 120-123.
81. Saker, M.M. and M.R. Rady *et al.*, 1997. Elicitation of tropane alkaloids in suspension culture of *Hyoscyamus, Datura* and *Atropa* by osmotic stress. Fitoterapia, 68: 338-342.
82. Zabetakis, I., R. Edwards and D. Hagan, 1999. Elicitation of tropane alkaloids biosynthesis in transformed root cultures of *Datura stramonium.* Phytochemistry, 50: 53-56.
83. Spoliansky, T.C., S.I. Pitta-Alvarez and M. Gluliettl, 2000. Effect of jasmonic acid and Aluminium on production of tropane alkaloids in hairy root cultures of *Brugmansia candida*. EJB. Electronic J. Biotech., 3: 72-75.

84. Atal, C.K., 1978. Semisynthetic derivatives of pyrrolizidine alkaloids of Pharmaco dynamic importance: A review. Lloydia, 41: 291-384.
85. Furuya, T., H. Kojima and K. Syono, 1966. Nicotine and anabasine in tobacco callus tissue. Chem. Pharm. Bull., 14: 1189-1190.
86. Furuya, T., H. Kojima and K. Syono, 1971. Regulation of nicotine biosynthesis by auxins in tobacco callus tissues. Phytochemistry, 10: 1529-1532.
87. Ravishankar, G.A. and A.R. Mehta, 1981. Regulation of nicotine biogenesis 2: Increased production of nicotine by urea in tobacco tissue cultures. Experimentia, 37: 1143-1144.
88. Ravishankar, G.A. and A.R. Mehta, 1982. Regulation of nicotine biogenesis 3: Biochemical basis of increased nicotine biogenesis by ureain tissue culture of tobacco. Can. J. Bot., 60: 2371-74.
89. Lockwood, G.B. and A.K. Essa, 1984. Pyridine Alkaloids from cell cultures of *Nicotiana tabaccum.* Plant Cell Reports, 3: 109-111.
90. Antony, A., K.P. Gopinath and Vaidyanathan, 1975. Biosynthesis of trigonelline in root callus cultures of fenugreek (*Trigonella foenum-graecum* L). Ind. J. Exp. Biol., 13: 39-43.
91. Khanna, P. and S.C. Jain, 1972. Effect of nicotinic acid on growth and production of trigonelline by *Trigonella goenum-grecum* L. tissue cultures. Ind. J. Exp. Biol., 10: 248-249.
92. Khanna, P. and R. Khanna, 1976. Production of major alkaloids from *in vitro* tissue cultures of *Papaver somniferum* Linn. Ind. J. Exp. Biol., 14: 628-630.
93. Sarin, R., M. Sharma and P. Khanna, 1983. Effect of some growth regulators on the production of opium alkaloids in *Papaver somniferum* Linn. tissue cultures. J. Ind. Bot. Soc., 62: 73-76.
94. Sarin, R. and P. Khanna, 1989. High yielding tissue of *Papaver* sp. grown *in vitro* cultures. Ind. Bot. Soc., 68: 95-96.
95. Sarin, R., 1996. Effect of Tyrosine on the production of alkaloids in the high yielding cell lines of *Papaver somniferum* tissue culture. J. Plant Biochem. and Biotech., 5: 61-62.
96. Staba, E.J., S. Zito and M. Amin, 1982. Alkaloid production from *Papaver* tissue cultures. J. Natl. Prod., 45: 256-262.
97. Lockwood, G.B., 1984. Alkaloids of cell suspensions derived from four *Papaver* spp. and the effect of temperature stress. Zeitschriff Fur Pflanzenphysiologie, 114: 361-363.
98. Zito, S.W. and E.J. Staba, 1982. Thebaine from root cultures of *Papaver bracteatum.* Planta Medica, 45: 53-54.
99. Khanna, P. and G.L. Sharma, 1977. Production of opium alkaloids from *in vitro* tissue culture of *Papaver rhoeas* Linn. Ind. J. Exp. Biol., 16: 951-952.
100. Furuya, T., K. Shono and A. Ikuta, 1972. Isolation of berberine from callus tissue of *Coptis japonica.* Phytochemistry, 11: 175.
101. Furuya, T., A. Ikuta and K. Syono, 1972. Alkaloids from callus tissue of *Papaver somniferum.* Phytochemistry, 11: 3041-3044.
102. Ikuta, A., K. Syeno and T. Furuya, 1974. Alkaloids of callus tissues and redifferentiated plantlets in the papaveraceae. Phytochemistry, 13: 2175-2179.
103. Kamimura, S., M. Akasu and Nishkawa, 1976. Formation of thebaine in the suspension culture of *Papaver bracteatum.* Agr. Biol. Chem., 40: 913-914.
104. Sarin, R., 2001. Effect of tyrosine on the production of alkaloids in the high yielding cell strains of *Papaver somniferum* Tissue Culture. Flora and Fauna, 7: 71-72.
105. Sarin, R., 2003. Enhancement of opium alkaloids production in callus culture of *Papaver rhoeas* Linn. Ind. J. Biotech., 2: 271-272.
106. Sato, F. and Y. Yamada, 1984. High berberine producing cultures of *Coptis japonica.* Phytochemistry, 23: 281-285.
107. Sato, H., Y. Kobayashi, H. Fukui and M. Tabata, 1990. Specific differences in tolerance to exogenous berberine among plant cell cultures. Plant Cell Reports, 9: 133-136.
108. Sato, H., S. Tanaka and M. Tabata, 1993. Kinetics of alkaloid uptake by cultured cells of *Coptis japonica.* Phytochemistry, 34: 697-707.
109. Sato, F., N. Takeshita, H. Fujiwara, Y. Katagiri, L. Huan and Y. Yamada, 1994. Characterization of Coptis japonica cells with different alkaloid productivities. Plant Cell, Tissue and Organ Culture, 38: 249-256.
110. Khanna, P., O.P. Sharma and M. Saluja, 1982. Berberine from *Argemone mexicana* L. tissue cultures. Proc. 5th Intl. Assoc. Plant tissue and cell cultures, held in Japan from 11th to 16th July, 1982.
111. Doller, G., A.W. Alfermann and E. Reinhard, 1976. Production von indole-alkaloids in callus kulturen von *Cathranthus roseus.* Planta Med., 30: 14-20.
112. Doller, G., A.W. Alfermann and E. Reinhard, 1977. Serpentine in callus cultures of *Catharanthus roseus.* Planta Med., 31: 1-6.
113. Doller, G., 1978. Influence of the Medium on the Production of Serpentine by Suspension Cultures of *Cathranthus roseus* (L). G. DON. In: Proceedings of Intl. Sym. on Plant Cell Cultures (Eds. Alfermann, A.W. and E. Reinhard) held at Tubingen. 28-30th Sept., 1977, pp: 107-117.

114. Lee, S.L. and Scott, 1978. Biosynthesis of indole alkaloids in *Catharanthus* roseus tissue cultures. 4th IAPTC, Calgary, 20-25 Aug.,1978, Abst. 1418.
115. Toivonen, L., J. Balsevich and W.G.W. Kurz, 1989. Indole alkaloid production by hairy root cultures of *Catharanthus roseus.* Plant Cell Tissue and Organ Culture, 18: 79-93.
116. Dobberstein, R.F. and E.J. Staba, 1969. Ipomea, rives and aroyreia tissue cultures: Influence of various chemical factors on indole alkaloids production and growth. Lloydia, 32: 141-147.
117. Khanna, P., Aminuddin and M. Sogani, 1976. Pseudoephedrine from *in vivo* and *in vitro* tissue culture of *Ephedra foliata* Boiss. Ind. J. Pharm., 38: 140.
118. Khanna, P., Aminuddin and M. Sogani, 1976. Pseudoephedrine from *in vivo* and *in vitro* tissue culture of *Ephedra foliata* Boiss. Ind. J. Pharm., 38: 140.
119. Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., Oct., 1999, 12: 564-582.
120. Khanna, P. and E.J. Staba, 1968. Antimicrobials from plant tissue cultures. Lloydia, 31: 180-189.
121. Khanna, P., S. Mohan and T.N. Nag, 1971. Antimicrobials from plant tissue cultures. Lloydia, 34: 168-169.
122. Khanna, P., O.P. Sharma, C. Bhargava, M. Jain, A. Goswami, S. Singhvi and S.C. Jain, 1980. Antimicrobials active principles from in vitro tissue culture of some plant species. Ind. J. Pharm. Sci., 42: 112-117.
123. Khanna, P., 1980. Tissue Cultures and Useful Drugs: A Review on Thirty Eight Plant Species Grown *in vitro*. In: Cultivation and Utilization of Medicinal and Aromatic Plants (CUMAP) Eds. Atal, C.K. and B.M. Kapur, RRL, Jammu-Tawi, pp: 824.
124. Mathes, M.C., 1963. Antimicrobial substances from Aspan tissue grown *in vitro.* Science, 140: 1101-1102.
125. Mathes, M.C., 1967. The secretion of antimicrobial materials by various isolated plant tissues. Lloydia, 30: 177-181.
126. Mc.Cleary, J.A. and D.L. Walkington, 1964. Antimicrobial activity of the Cactaceae Bull. Torrey Botan. Club., 91: 17.
127. Misawa, M., K. Sakato, H. Tanka, M. Hayashi and H. Samejima, 1974. Production of Physiologically Active Substances by Plant Cell Suspension Cultures. In: Tissue Culture and Plant Science. (Ed. Street, H.E.), Academic Press, London, New York, pp: 405-432.
128. Nickell, L.G., 1958. Growth effects of antibiotics on plant cells and their reaction to a modified gram strain. Phyton, 11: 96-98.
129. Veliky, I.A. and R.K. Latta, 1974. Antimicrobial activity of cultured plant cells and tissues. Lloydia, 37: 611-620.
130. Sayagaver, B.M., N.B. Mulchandani and S. Narayanswami, 1969. Isolation of formonenetin from tissue cultures of *Cicer arietinum*. Lloydia, 38: 108.
131. Brunet, G. and R.K. Ibrahim, 1973. Tissue culture of *Citrus* peel and its potential for flavonoid synthesis. Z. Pflanzenphysiol., 69: 152-162.
132. Korden, H.A. and Morganstern, 1962. Flavonoid production by mature citrus fruit tissue proliferating *in vitro*. Nature, 195: 163.
133. Jain, S.C. and P. Khanna, 1974. Quercetine from *Crotolaria juncea* tissue cultures. Ind. J. Exp. Biol., 12: 466.
134. Jain, S.C., R. Khanna and P. Khanna, 1975. Quercetine from the seeds and *in vitro* cultures of *Datura* spp. Ind. J. Exp. Biol., 13: 83-84.
135. Khanna, P., R. Taparia and S.C. Jain, 1982. Flavonoids from *Emblica officinalis* gaertn tissue cultures. Ind. J. Bot., 5: 43-44.
136. Stickland, R.G. and N. Sunderland, 1972. Production of anthocyanins, flavols and chlorogenic acids by cultured callus tissues of *Haplopappus gracilis*. Ann. Bot., 36: 443-457.
137. Aminuddin, G.L. Sharma and P. Khanna, 1977. Flavonoids from *in vitro* seedling callus culture of *Trigonella foenum-graecum.* Ind. J. Pharm., 39: 142-143.
138. Khanna, P., R. Kamal and S.C. Jain, 1977. (β-sitosterol and quercetin from *in vivo* and *in vitro* tissue cultures of *Tephrosia purpurea* Pers. Sci. Cult., 43: 396.
139. Benjamin, B.D. and Mulchandani, 1973. Studies in biosynthesis of secondary constituents in tissue cultures of *Tylophora indica*. Planta Medica, 23: 394-397.
140. Chumsri, P. and E.J. Staba, 1975. Pyrethrin contents and larvicidal activity of *Chrysanthemum* Acad. Pharm. Sci., 50: 169.
141. Godin, P.J., R.J. Sleeman, Snarey and E.M. Thain, 1964. Jasmolin II. A new constituent of pyrethrum extract. Chem. Ind., pp: 371-372.
142. Godin, P.J., J.H. Stevenson and Sawicki, 1965. The insecticidal activity of Jasmolin II and its isolation from pyrethrum (*Chrysanthemum cinerariaefolium* Vis.), J. Econ. Entomol., 58: 548-551.
143. Godin, P.J., R.J. Sleeman, M. Snarey and E.M. Thain, 1966. The Jasmolins, new insecticidally active constituents of *Chrysanthemum cinerariaefolium.* J. Chem. Soc., pp: 332.

144. Khanna, P., R. Sharma and R. Khanna, 1975. Pyrethrins from *in vivo* and *in vitro* tissue culture of *Tagetes erecta* Linn. Ind. J. Exp. Biol., 13: 508-509.
145. Sarin, R., 2004. Insecticidal activity of callus culture of *Tagetes erecta*. Fitoterapia, 75: 62-64.
146. Khanna, P. and R. Khanna, 1976. Endogenous free Ascorbic acid and effect of exogenous ascorbic acid on growth and production of pyrethrins from *in vitro* tissue cultures of *Tagetes erecta* L. Ind. J. Exp. Biol., 14: 630-631.
147. Khanna, P., A. Goswami, A.K. Rathore, M. Sogani, S. Singhvi and U. Gupta, 1980. Investigations into possible new sources of pyrethrins. Pyr. Post., 15: 9-10.
148. Sarin, R., 1995. Pyrethrins from *Vernonia* species. Flora and Fauna, 1: 25-26.
149. Zeig, R.G., S.W. Zito and E.J. Staba, 1983. Selection of high pyrethrin producing tissue cultures. Planta Medica., 48: 80-91.
150. Staba, E.J. and S.W. Zito, 1985. The Production of Pyrethrins by *Chrysanthemum cinerariaefolium* (Trev.) Boccone. In: Neumann, Barz and Reinhard (Eds). Primary and Secondary Metabolism of Plant Cell Cultures. Berlin Heidelberg. Springer Verlag.
151. Jovetic, S., 1994. Natural Pyrethrins and Biotechnological Alternatives. Biotech. and Development Monitor No. 21, Dec.,1994.
152. Hitmi, A., A. Condret and C. Barthomeuf, 2000. The production of pyrethrins by plant cell and tissue cultures of *Chrysanthemum cinerariaefolium* and *Tagetes* species. Critical Reviews in Biochemistry and Molecular Biology, 35: 317-337.
153. Aminuddinn and P. Khanna, 1978. Rotenoids from *in vitro* tissue cultures of *Crotolaria burhia* Buch-Ham. Planta Med., 36: 181-183.
154. Khanna, P. and R.S. Joshi, 1986. New source of rotenoids-An insecticide. 2nd tissue cultures Seminar Held in Delhi Univ. on 2nd Jan., 1986.
155. Pinto, J.E.B.P., H.E.O. Conceicao, N.E.A. Castro, E.J.A. Santiago and O.A. Lameira, 2001. *In vitro* propagation and quantification of rotenoids in callus of *Derris* sp. *In vitro* Cell Dev. Biol. Animal., 37: 3 Part II March, 2001.
156. Kamal, R and M. Mangla, 1993. *In vivo* and *in vitro* investigations of rotenoids from *Indigofera tinctoria* and their bioefficacy against the larvae of *Anopheles stephensi* and adult of *Callosobrunchus chinensis*. J. Biol. Sci., 18: 93-101.
157. Kaushik, P. and P. Khanna, 1992. Insecticidal substances from *in vitro* and *in vivo* culture of *Abrus precatorius* L. Advances in Plant Sciences, 5: 464-469.
158. Sharma, R. and P. Khanna, 1975. Production of rotenoids from *Tephrosia* spp. *in vivo* and *in vitro* tissue cultures. Ind. J. Exp. Biol., 13: 84-85.
159. Zafar, R. and M. Mujeeb, 2002. Rotenoids and rutin in callus cultures of *Tephrosia purpurea* (L.) Ind. J. Pharmceutical Sci., 64: 217-221.
160. Lambert, N., M.F. Trouslot, C. Nef-Campac and H. Chrestin, 1993. Production of rotenoids heterotrophic and photomixotrophic cell cultures of *Tephrosia vogelii.* Hook Phytochem., 34: 1515-1520.
161. Kamal, R. and S.C. Jain, 1978. *Tephrosia falaformis*-a new source of rotenoids. Planta Medica, 33: 418-419.
162. Kamal, R. and S.C. Jain, 1980. Occurrence of rotenoids from *Tephrosia strigosa.* J. Agric. Biol., 44: 29-35.
163. Kamal, R. and R. Yadav, 1986. Insecticidal substances from *Trigonella foenum-graecum.* In: Intl. Cong. Plant Tissue Culture. Minneapolis (3-8th Aug.). Abst. 72.
164. Nag, T.N., 1976. *Datura* tissue culture: A source of sterol. Ind. J. Pharm., 38: 80-81.
165. Halmbold, H., U. Vollter and E. Reinhard, 1978. Sterols in cell cultures of *Digitalis lanata.* Planta Medica, 33: 185-187.
166. Tomita, Y., A. Uomori and H. Minato, 1970. Steroidal sapogenins and sterols in tissue cultures of *Dioscorea tokora*. Phytochemistry, 9: 111.
167. Singhal, V. and P. Khanna, 1994. Phytosterol from in vivo and *in vitro* tissue culture of *Helipterum roseum* benth. Ind. J. Pharmaceutical Sci., 56: 28-29.
168. Tomita, Y., A. Uomori and H. Minato, 1969. Sesquiterpenes and phytosterols in the tissue cultures of *Lindera strychnifolia*. Phytochemistry, 8: 2249.
169. Khanna, P. and S. Mohan, 1973. Production of steroids from *Momordica charantia* tissue cultures. Ind. J. Exp. Biol., 11: 58-60.
170. Jain, S.C. and P. Khanna, 1973. Production of sterols from *Sesamum indicum* Linn., tissue cultures. Ind. J. Pharm., 35: 163-164.
171. Itokawa, H., M. Akasu and M. Fujita, 1973. Several oxidised sterols isolated from callus tissue of *Stephania cepharantha.* Chem. Pharm. Bull., 21: 1386.
172. Khanna, P., R. Kamal and S.C. Jain, 1977. (β-sitosterol and quercetin from *in vivo* and *in vitro* tissue cultures of *Tephrosia purpurea* Pers. Sci. Cult., 43: 396.
173. Benveniste, P., 1968. Biosynthesis of sterols in tobacco tissue grown *in vitro.* Identification of Cycloeucalenol and Obtusifolial. Phytochemistry, 7: 451.

174. Stohs, S.J., H. Rosenberg and S. Billeta, 1975. Sterols of *Yucca glauca* tissue cultures. Planta Med., 27: 257-61.
175. Flores-Sanchez, I.J., J. Ortega-lopez, M.C. Montes-Horcasitas and A.C. Ramos-Valdivia, 2002. Biosynthesis of sterols and triterpenes in cell suspension cultures of *Uncaria tomentosa.* Plant and Cell Physiol., 43: 1502-1509.
176. Furuya, T., H. Kojima, K. Syono, T. Ishii, K. Uotani and M. Nishio, 1973. Isolation of Saponins and Sapogenins from callus tissue of *Panax ginseng*. Chem. Pharm. Bull., 21: 98-101.
177. Khanna, P. and O.P. Sharma, 1980. Studies on steroidal sapogenins from tissue cultures of *Agave wightii.* Natl. Prod., 43: 459- 462.
178. Rathore, A.K. and P. Khanna, 1978. Isolation and characterization of steroidal sapogenins from rhizome and stem callus of *Costus speciosus*. Lloydia, 41: 640-641.
179. Abroshnikova and V.V. Panina, 1972. Steroidal saponins and diosgenin in tissue culture of *Dioscorea deltoidea*. Khimiko-Farm. Zh., 6: 20-21.
180. Chaturvedi, H.C. and S.N. Srivastava, 1976. Diosgenin biosynthesis by tuber callus tissue cultures of *Dioscorea deltidea*. J. Natl. Prod., 39: 82-83.
181. Chowdhary, A.R. and H.C. Chaturvedi, 1980. Cholesterol and biosynthesis of diosgenin by tuber callus of *Dioscorea deltoidea*. Curr. Sci., 49: 237-238.
182. Kaul, B. and E.J. Staba, 1968. *Dioscorea* tissue cultures. I. Biosynthesis and isolation of diosgenin from *Dioscorea deltoidea* callus and suspension cultures. Lloydia, 31: 171.
183. Mehta, A.R. and E.J. Staba, 1970. Presence of diosgenin in tissue cultures of *Dioscorea composita* Hemsl. and related species. J. Pharm. Sci., 59: 864-865.
184. Marshall, Jo G. and E.J. Staba, 1976. Hormonal effects on diosgenin biosynthesis in *Dioscorea deltoidea* tissue cultures. Phytochemistry, 15: 53.
185. Khanna, P., A.K. Rathore and S.K. Manot, 1980. Production of steroids in static tissue cultures of *Dioscorea floribunda* Mart and Gal. Ind. J. Pharm. Sci., 42: 98-99.
186. Stohs, S.J., C.L. Wegner and H. Rosenberg, 1975. Steroids and sapogenins in tissue cultures of *Dioscorea deltoidea*. Planta Med., 28: 101-105.
187. Khanna, P., R. Khanna, M. Sogani, and S.K. Manot, 1977. *Daucus carota* L. Seedling callus, a new source of diosgenin. Ind. J. Exp. Biol., 15: 586-587.
188. Heble, M.R., S. Narayanswami and M.S. Chadha, 1976. Studies on growth and steroid formation in tissue cultures of *Holarrhena antidysenferica*. Phytochemistry, 15: 681-682.
189. Heble, M.R., S. Narayanswami and M.S. Chadha, 1968. Diosgenin and β-sitosterol: Isolation from *Solanum xanthocarpum* tissue cultures. Science, 161: 1145.
190. Vagujalvi, D., M. Maroti and Tetenyi, 1973. Presence of diosgenin and absence of Solasodine in tissue cultures of *Solanum laciniatum*. Phytochemistry, 10: 1389.
191. Khanna, P. and A.K. Rathore, 1977. Diosgenin and Solasodine from *Solanum nigrum* L. Complex. Ind. J. Exp. Biol., 15: 808-809.
192. Rathore, A.K. and P. Khanna, 1978. Production of diosgenin from *Costus speciosus* (Koen) Sm. and *Solanum nigrum* L. Suspension cultures. Curr. Sci., 47: 870-871.
193. Khanna, P., Aminuddin, G.I. Sharma, S.K. Manot and A.K. Rathore, 1976. Isolation and characterization of sapogenins and solasodine from *in vitro* tissue cultures of some solanaceous plants. Ind. J. Exp. Biol., 14: 694-695.
194. Khanna, P., S.K. Manot and A.K. Rathore, 1978. Isolation and characterization of sapogenins from *in vitro* static cultures of some *Solanaceous* spp. Ind. J. Exp. Biol., 16: 618-619.
195. Khanna, P., S.C. Jain and S. Sahu, 1981. *Solanum jasminoides* Paxt. tissue cultures. I Production of steroidal sapogenins and glycoalkaloids. J. Natl. Prod., 44: 125.
196. Jain, S.C. and S.L. Sahu, 1981. Growth and production of steroidal sapogenines and glycoalkaloids in *Solanum jasminoides* paxt. Suspension cultures. Agric. Biol. Chem., 45: 2909-2910.
197. Jain, S.C. and S.L. Sahu, 1981. Isolation and characterization of steroidal sapogenins and glycoalkaloids from tissue cultures of *Solanum verbascifolium* Linn. Chem. Pharm. Bull., 29: 1765-1767.
198. Jain, S.C. and S.L. Sahu, 1981. Suspension cultures part I Production of steroidal Alkaloids. Pharmazi, 36: 714-715.
199. Khanna, P. and S.C. Jain, 1973. Diosgenin, gitogenin and tigogenin from *Trigonella foenum-graecum* tissue cultures. Lloydia, 36: 96.
200. Khanna, P. and P.V. Purohit, 1981. Studies on Steroidal Sapogenins from *Yucca aloefolia* L. *in vivo* and *in vitro* Tissue Cultures. In: Plant Cell Culture in Crop Improvement. (Eds.) Sen, S.K. and L. Kenneth, Giles, New York, Plaenum, pp: 65-68.
201. Stohs, S.J., J.J. Sabatka, J.J. Obrist and H. Rosenberg, 1974. Sapogenins of *Yucca glauca* tissue culture. Lloydia, 37: 504-505.
202. Kaneda, N, H. Nakanishi and E. Staba, 1987. Steroidal constituents of *Yucca shidigera* plants and tissue cultures. Phytochemistry, 26: 1425-1429.

203. Khanna, P., G.L. Sharma, A.K. Rathore and S.K. Manot, 1977. Effect of cholesterol on *in vitro* suspension tissue cultures of *Costus speciosus* (Koen) Sm, *Dioscorea floribunda* Mart. and Gal., *Solanum aviculare* Forest. *S. xanthocarpum* Schrad and Wendl. Ind. J. Exp. Biol., 15: 1025.
204. Sharma, M., R. Sarin and P. Khanna, 1982. Effect of cholesterol on growth and production of diosgenin in tissue culture of *Solanum elaeagnifolium*. J. Ind. Bot. Soc., 61: 355-358.
205. Khanna, P., S.C. Jain and R. Bansal, 1975. Effect of cholesterol on growth and production of diosgenin, gitogenin, tigogenin and sterols in suspension cultures. Ind. J. Exp. Biol., 13: 211-213.
206. Khanna, P., R. Bansal and S.C. Jain, 1975. Effect of various hormones on production of sapogenins in *Trigonella foenum-graecum* L. suspension cultures. Ind. J. Exp. Biol., 13: 582-583.
207. Brain, K.R. and G.B. Lockwood, 1976. Hormonal control of steroid levels in tissue cultures from *Trigonella foenum-graecum*. Phytochemistry, 15: 1651-1654.
208. Buchner, S.A. and E.J. Staba, 1964. Preliminary examination of Digitalis tissue cultures for cardenolides. J. Pharm. Pharmacol., 16: 733-737.
209. Kaul, B., P. Well and E.J. Staba, 1967. Production of cardioactive substances by tissue cultures and their screening for cardiovascular activity. J. Pharm. Pharmacol., 19: 760.
210. Staba, E.J., 1962. Production of cardiac glycosides by plant tissue cultures. I. Nutritional requirements in tissue cultures of *Digitalis lanata* and *Digitalis purpurea*. J. Pharm. Sci., 51: 249-254.
211. Stohs, S.J. and E.J. Staba, 1965. Production of cardiac glycosides by plant tissue cultures. IV. Biotransformation of digitoxigenin and related substances. J. Pharm. Sci., 54: 56.
212. Furuya, T., M. Hirotani and Shinohara, 1978. Biotransformation of Digitoxin by suspension callus culture of *Digitalis purpurea*. Chem. Pharm. Bull., 18: 1080.
213. Groenveveld, H.W. and T.L.J.P. Van *et al*., 1992. Cardenolide and Neutral lipid biosynthesis from malonate in Digitalis lanata. Planta Med., 58: 239-244.
214. Milek, F. and E. Reinhard *et al*., 1997. Influence of precursors and inhibitors of the sterol pathway on sterol and cardenolide metabolism in *Digitalis lanata*. EHRH. Plant Physio. and Biochem. Paris, 35: 111-121.
215. Theurer, C. and W. Kreis *et al*., 1998. Effects of digitoxigenin, digoxigenin and arious cardiac glycosides on cardenolide accumulation in shoot cultures of *Digitalis lanata*. Planta Medica, 64: 705-710.
216. Hanssmann,W.W. and Kreis *et al*., 1997. Effects of various pregnanes and two 23-nor-5-cholenic acids on cardenolide accumulation in cell and organ cultures of *Digitalis lanata*. Planta Medica, 63: 446-453.
217. Moldenhaur, D. and B. Fuerst *et al*., 1990. Cardenolides in *Digitalis lanata* cells transformed with Ti Plasmids. Planta Medica, 56: 435-438.
218. Chaturvedi, H.C., A.R. Chowdury and Aminuddin, 1979. Solasodine biosynthesis in seedand seedling Callus of *Solanum khasianum* Clark. Grown *in vitro*. Ind. J. Exp. Biol., 17: 107-109.
219. Heble, M.R., S. Narayanswami, and M.S. Chadha, 1968. Solasodine in tissue cultures of *Solanum xanthocarpum*. Naturewisesenschaften, 55: 350.
220. Heble, M.R. and B. Bhatt, 1976. Solasodine from cultured spine mutent of *Solanum khasianum* Clarke. Ind. J. Exp. Biol., 14: 527-528.
221. Khanna, P., Aminuddin, G.I. Sharma, S.M. Manot and A.K. Rathore, 1976. Isolation and characterization of sapogenins and solasodine from *in vitro* tissue cultures of some solanaceous plants. Ind. J. Exp. Biol., 14: 694-695.
222. Khanna, P., S.C. Jain and S. Sahu, 1981. *Solanum jasminoides* Paxt. tissue cultures. I Production of steroidal sapogenins and glycoalkaloids. J. Natl. Prod., 44: 125.
223. Khanna, P. and A.K. Rathore, 1977. Diosgenin and Solasodine from *Solanum nigrum* L. Complex. Ind. J. Exp. Biol., 15: 808-809.
224. Kumar, P. and P. Khanna, 1986. Chemical investigations on plant parts and tissue cultures of *Solanum dulcamara* L. Acta Bot. Indica, 14: 136-138.
225. Vagujalvi, D., M. Maroti and Tetenyi, 1971. Presence of diosgenin and absence of Solasodine in tissue cultures of *Solanum laciniatum*. Phytochemistry, 10: 1389.