It is well established that cadmium has a high affinity for sulphydryl and hydroxyl groups and ligands containing nitrogen[7]. Thus binding to such groups in chemical system might affect various basic biochemical and physiological processes and thereby interfere with the central functions of the organism even at very low cadmium concentrations. The present results on tissue glycogen content in *L. rohita*, *O. punctatus* and *C. batrachus* indicate that cadmium in the water might produce dysfunctions of several physiological and biochemical processes in fish and such a mechanism may be responsible for the differential effect of cadmium on tissue glycogen content.

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PRODUCTION OF DIOSGENIN FROM *COSTUS SPECIOSUS* (KOEN) SM., AND *SOLANUM NIGRUM* L., SUSPENSION CULTURES

DIOSGENIN, an important sapogenin from economic point of view, has been reported from tissue cultures of several plant species[1-7]. Previous work on *Costus speciosus* and *Solanum nigrum* from *in vivo* and *in vitro* has been described[5-13] and in this communication we wish to report our findings on production and isolation of diosgenin from suspension cultures of these plant species.

Seedling calli of *C. speciosus*[7] and *S. nigrum* (hexaploid[5,6]) maintained for 18 months as static cultures were transferred in RT liquid medium supplemented with 0·1 ppm of 2,4-dichlorophenoxyacetic acid and grown as suspension cultures for 6–8 months by frequent subculturings of 4–6 weeks. The cultures of *C. speciosus* showed root formation, so callus was transferred to RT liquid medium supplemented with 1 ppm of 2,4–D, which resulted undifferentiated cells.

Suspension cultures of *C. speciosus* (differentiated) and *S. nigrum* were transferred to fresh liquid medium with 0·1 ppm of 2,4–D and allowed to grow for different time intervals of 2, 4 and 6 weeks. All the tissue samples were harvested separately, dried and growth indices calculated (GI = Final dry weight of tissue — Initial dry weight of tissue/Initial dry weight of tissue). Each of the dried tissue samples was powdered and extracted for its steroidal content[7]. Diosgenin was quantitatively estimated following a spectrophorometric method using thin layer chromatography[7], Absorbances were read on a Spectrophotometer (Carl Zeiss, JENA, DDR, VSU–2P) at 405 nm. Ten replicates were performed in each case and the mean values were taken.

Maximum growth index (6·8; 6·1) was observed in six and four weeks old suspenison cultures of *S. nigrum* and *C. speciosus* respectively.

Diosgenin was confirmed by its mp (204–206° C), mmp (203–204° C), Co-chromatography [TLC, Silica gel acctone benzene = 1 : 2 (R_f 0·71), chloroform–acetone = 8 : 2 (R 0·57); anisaldehyde and 50% H_2SO_4 as spraying reagent] and identical IR spectral studies.

Diosgenin content was low (0·15%) in differenciated cultures of *C. specicsus* (Table I) when compared with undifferentiated cells as suspension cultures (0·48%)[7] which supports the previous findings of Kaul and Staba[4] who have also found higher amount of diosgenin in undifferentiated suspension cultures of *D. deltoidea* as compared with differentiated liquid cultures. However in *S. nigrum* suspension cultures, maximum diosgenin content (0·20%) was noted in six weeks old cultures (Table I) which was low when compared with its static cultures (0·65%)[6].

TABLE I

Growth indices (GI) and diosgenin content in Costus speciosus and Solanum nigrum *suspension cultures*

Age of tissue (weeks)	*C. speciosus* (differentiated)		*S. nigrum*	
	GI	Diosgenin (%)	GI	Diosgenin (%)
2	2·8	0·036	3·5	0·143
4	5·3	0·10	5·5	0·174
6	3·4	0·15	6·8	0·200

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CHANGES IN CHOLESTEROL CONTENT IN 'KHESARI' (*LATHYRUS SATIVUS* L.) SEEDS INFESTED WITH *ASPERGILLUS FLAVUS* LINK

CHOLESTEROL is one of the steroids found in the vegetable kingdom usually in its isomeric form, phytosterol. The substance has been reported from many plants[1] and seeds[2]. However, no literature is available indicating its presence in the 'Khesari' seeds hence an attempt has been made to estimate cholesterol in healthy and *Aspergillus flavus* infested seeds of 'Khesari'. Healthy seeds of 'Khesari' were surface sterilized with 2% NaClO, treated with spore suspension of the *A. flavus* and incubated for 5, 10 and 15 days. Control was maintained to compare the result. The presence of cholesterol in healthy and infested seeds was confirmed by preliminary tests[3] and then it was estimated quantitatively by colorimetric method[4]. The results are presented in Table I.

TABLE I

Percentage of cholesterol in healthy and infested with Aspergillus flavus *seeds of 'Khesari'*

Days of incubation	Healthy seeds	Infested with *A. flavus*	Percentage of increase over control
0	0·1		..
5	0·1	0·12	20
10	0·1	0·18	80
15	0·1	0·44	340

It is evident from Table I that the healthy seeds had 0·1% cholesterol and no change was recorded within the 15 days of incubation whereas its amount increased in the infested seeds gradually with the increase of incubation periods. Fats (in ·95%) are known to be present in 'Khesari' seeds[5] which on account of fungal infestation may be hydrolysed to saturated fatty acids. These acids are reported to be the precursor for the synthesis of cholesterol[6,7]. This possibly may be the reason for the increase in the amount of cholesterol in infested 'Khesari' seeds.

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