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The Effect of Chemicals on the Growth and Mitosis in Sesamum Orientale Linn (Til or Gingelly)

by

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Introduction: Drugs containing alkaloids are known for their outstanding physiological activity which is based partly on their antibiotic properties, and partly on the inhibition and poisoning of important physiological processes. Since the discovery of Colchicine by Blakeslee & Avery (1937) on the effectiveness of this drug as an agent for inducing chromosome duplication, much work on the effect of allied chemicals on the nuclear cycle has been reported. A review of the recent literature shows that a number of workers have been investigating the various aspects of the different chemicals like colchicine, Acenaphthene, Coumarine, Sulpha-drugs etc. and also the effect of growth promoting chemicals with regard to their activity on the mitosis, growth and cell development. This paper deals with the preliminary observations made during the investigations on the cytological effects of chemicals on the roots of *Sesamum orientale* L.

Material and Methods: Seeds of *Sesamum orientale* with black, brown, white and dull white coloured varieties were taken for study. The chemicals used are:— acenaphthene, α-naphthalene acetic acid. The concentrations used were 1000 p. p. m. (0·001%), 100 p. p. m. (0·0001%) and 10 p. p. m. (0·00001%) aqueous solution for 24 and 48 hrs. duration.

Seeds of Sesamum were grown on moist filter paper in petri dishes until the roots attained a sufficient length for handling. They were then transferred to solutions of different concentrations. Only the roots were actully immersed in the solution. After the required period of treatment, the roots were retransferred to distilled water and carefully washed with water so that the surface of the roots was free from the solution.

The root tips at the end of the treatment, either immediately or after keeping them in distilled water for 24 hrs. were fixed in alcoholic fixative (Acetic Alcohol 1:3). In all the cases only root tip squashes with Feulgen and acetic-orcein were employed.

Observations: (I) EFFECT OF CHEMICALS ON MITOSIS: (a) *Acenaphthene treatment:* When root tips of *Sesamum orientale*

were subjected to acenaphthene treatment at the different concentrations mentioned above for 24 and 48 hrs. duration, no significant effect was noticed in their growth and they were very similar to the control seedlings. But in a few cells there was slight disturbance in mitotic cycle in that there was ineffective clevage and abortive spindle formation. Nebel (1937), Kostoff (1938) have shown that a saturated solution of acenaphthene is effectively used as polyploidising agent in *Allium* and *Lilium*. So a saturated solution of this was applied to the roots and the treatments were given for 24 and 48 hrs. Even then no marked effect was produced by the chemical. The treated seeds were planted after carefully washing in water in small pots in order to observe whether any mutations would be produced. But none of the seedlings survived till the flowering stage. It has been inferred that *Sesamum* plants are not susceptible to the effect of acenayhthene.

(b) *∝−Naphthalene Acetic Acid* (NAA) & *β−Naphthoxy aceticacid treatment:* When the roots were treated with solutions at very low concentrations such as 0·00001% there is an appreciable increase in the mitotic rate, as compared to the control and there is stimulation of the growth of the roots. But at a higher concentration 0·0001% there is definite disturbance in the mitotic cycle. While many cells exhibited normal mitosis, in about 40% of the cases mitotic aberrations were seen.

The chromosomes of a cell undergoing mitosis remain scattered at metaphase instead of being arranged at the equatorial plate. The spindle if already formed at the time of treatment breaks down and the chromosomes do not show movement. Thus in the first stage of mitosis the chromosomes may be seen either scattered or in arrested metaphase or early anaphase positions without being separated. Some degree of contraction of chromosomes also takes place at this stage. The chromosomes then slowly pass into the interphasic condition and affected cell may contain either a single tetraploid nucleus or several nuclei with varying chromosome numbers. Arrested mitosis at anaphase may give rise to cells containing two normal diploid nuclei through failure to develope a complete dividing wall. The paired chromatids are frequently to be seen resulting in restitution polyploid condition. Chromosomes run together to form large, deeply staining pycnotic masses. The cells with high polyploid chromosome numbers were greatly enlarged in size and the walls were sometimes considerably thickened.

When the roots were treated with solution of 0·001% concentration there is a complete cessation of mitosis and an increase in the size of the nucleus and nucleolus. Further it is noticed that the time factor is not of so much consequence as the factor of the chemical used. But the experiments conducted by other workers show that at any concentration the number of 'metaphases' increases with lengthening treatment and with higher concentrations, reaches a maximum and then falls as nuclear disintegration takes place.

(c) *Phenyl acetic acid* (PAA) *treatment:* The effect of phenyl acetic acid on mitosis in the cells of root tips of sesamum was similar to that of other chemicals like α-naphthalene and β-naphthoxyacetic acid. Just like other growth promoting substances PAA also stimulates the cell enlargement and root growth at 0·00001% level as compared to that of control. At 0·0001% concentration there is considerable disturbance in mitosis and at 0·001% level, there is complete inhibition of cell division. Thus the roots of *Sesamum* are highly susceptible to the action of PAA in aqueous medium.

(II) EFFECT OF CHEMICALS ON GERMINATION AND GROWTH:— In order to study the effect of the three chemicals α-NAA and β-NAA and PAA, on the germination of seeds and their growth, they were soaked in three concentrations for 24 hrs, washed with water and kept in moist filter paper in petri dishes and observations made at required intervals. In general it has been observed that sprouting of seeds does not take place if the seeds are treated at 0·001% and a delayed sprouting of seeds at 0·0001%. At concentration of 0·00001% and less, sprouting is initiated even after 12 hrs. of soaking irrespective of the length of treatment and is found to be more significant than over the controls. This effect has been observed only in α-NAA and β-NAA and to a less extent in PAA. It has been found that germination and growth inhibitions are nearly always associated with one another. When seeds in different stages of germination are transferred from water to chemicals, their growth is inhibited. In a very advanced stage they continue to sprout but the radicles degenerate. Generally the roots are much more sensitive than the coleoptile, plumule or young sprouts to the effect of inhibition.

At higher concentrations elongations of the roots cease after a length of about 4–8 mm has been attained. In some cases the

root hair production is also stimulated. But it appears that although meristematic activity is inhibited by the above chemicals, differentiation as shown by root hair formation is unaffected and continues to the root apex. It is noticed that high concentrations cause some swelling of the roots so that the tip of a root which has stopped growing does not retain the dimensions of a normally growing tip, but increases to a thickness which equals or slightly exceeds that of fully differentiated roots of control plants. The hypocotyl swells considerably as seen in colchicine and may even reach thickness 3 to 4 times that of control seedlings. Similar effects have been observed when the seeds were sown in soil in petri dishes moistened with the chemicals.

The present study demonstrates that the effect of the various chemicals is not the same on the different varieties of the same species. The varieties which differ in the colour of the seed coats react differently towards the same chemicals. Kostoff (1938) found that *Vicia, Lathyrus* and *Medicago* species were less susceptible to the action of acenaphthene than were oats, barley and other graminaceous species. Levan & Ostergren (1943) also noted that leguminous plants were more resistant to naphthalene derivatives than some grasaes. In *Sesamum* it has been observed that the brown seeded variety is highly resistant to the action of chemicals, while the white seeded ones are highly susceptible to this effect. Hitchcock & Zimmerman (1940) has pointed out from the responses towards rooting of different plants, that the relative effects of chemicals vary considerably according to genera and species. The reason for this differential effect is of considerable value but the principles underlying this differential resistance are yet unknown.

Discussion: It is quite clear that mitotic irregularities are induced by the chemicals acenaphthene, α-napthalene acetic acid, β-naphteoxy acetic acid, and phenyl acetic acid. Such mitotic abberations appear to be closely similar to those reported in the literature due to colchicine and to a range of phenolic and amino compounds. Recent researches on mitotic poisons have demonstrated that two periods of the cellular cycle are outstandingly sensitive to chemical interference – the mataphase or more exactly the formation of a normal anisotropic spindle, and a certain period preceding prophase. The poisoning of the later stage corresponding probably to the increase in the thymonucleic acid content of the chromosomes, leads to nuclear destruction by 'pycnosis'. But there are deviations in effect and in most of the cases in the present

study no tetraploid was observed. The effects characteristic of colchicine which includes interference with centromere division, giving rise to typical paired chromosomes, and spindle suppression causing polyploidy were observed. The multipolar spindles, resulting as interphase nuclei of irregular shape and multinucleate cells seen are believed to be due to partial suppression of spindle action. Under the higher dosage of concentration complete suppression of cell division and in some cases over contraction of chromosomes were observed. Doxey (1949) in onion and rye and showed that morphological effects of the isopropyl phenyl carbamate treatment closely resemble those caused by colchicine and acenaphthene and other known mitotic poisons but were induced by a much lower concentration. Doxey & Rhodes (1949) showed the similarity of effects of acetic acid and of 4 – chloro – 2 methylphenoxy to those of x-radiation and mustard gas.

Koller (1947) has suggested that mitotic abnormalities prevailing in tumours are due to shortage of food and to toxic break down products of the neoplastic tissues. The present study however show that food supply to cells is not affected as the root structure is normal. The effects are more likely to be due to the toxic properties of the compound itself acting either on essential metabolic substrate or by chemical action on the enzymes essential for the processes. This mode of action would correspond with the direct chemical action as suggested by Lavan & Tjio (1948) as opposed to the theory of physical action put forward by Ostergren (1944). Dustin (1947) has considered the action of compounds affecting mitosis and suggests that carbamates interfere with purine metabolism and this would presumably affect the nucleic acid cycle of the cell. The enlarged nuclei and over contracted chromosomes seen in *Sesamum* root tips after treatment at 0.001% provides some evidence to the theory suggested by Dustin (1947). The evidence from the present study points out to the conclusion that interference with nucleic acid metabolism is primary or major cause of the mitotic irregularities.

All authors who have tried to elucidate the physiological action of different chemicals and extracts containing inhibitors have found that inhibition is accompanied by a stimulation of germination and growth. At sufficiently high dilutions, the inhibition of chemicals is replaced by stimulation. In the present study it has been noticed that at 0.001% concentration suppression of cell

division would be sufficient to prevent growth thereby promoting inhibition. At lower concentration 0.0001% rate of cell division is reduced and growth rate is also likely to be reduced. But the cell division may be prevented when polyploid tissues develop; cell enlargement continues though not in proportion to increase in chromosome number. Audus & Quastel (1948) observed that a concentration of 0.001%, sulphanilamide and sulphapyridine were more toxic to root growth than sulphaguanidine and sulphathiazole at the same concentration. Doxie (1249) has noticed with isopropyl phenyl carbamate similar effects on onion and rye with higher concentrations as well as lower concentrations. At concentration of 0.00001% there is marked tendency for the seeds as well as root tips for their germination and growth respectively. This indicates that stimulation takes place at very low dilutions. This relation between inhibition and stimulation can be explained in one of the three ways: (1) The same substance inhibits in high and stimulates in low concentrations. This is in agreement with well known fact that hormones and poisons inhibit at high and stimulate at low levels. The action of different dilutions of these chemicals seem to confirm this supposition. (2) Inhibition and stimulation are caused by two different substances whose action varies with respect to the concentration of the chemicals. The action of the inhibitor is sensitive to varying concentrations of the chemicals, while the action of the stimulator does not alter with the changes in the concentration of the chemicals. (2) The inhibitor is transformed into a stimulator by a chemical change during germination.

SUMMARY.

Growing roots of *Sesamum orientale* L, were treated with acenaphthene, α – naphthalene acetic acid, β – naphthoxy acetic acid, and phenyl acetic acid at concentrations of 1000, 100 and 10 p. p. m. for 24 and 28 hrs. Root tips were fixed and squash preparations made. Effects described include interference with spindle mechanism formation of multipolar spindles and enlargement of nucleoli. The effects are compared with those of colchicine. The effects of these chemicals on germination and growth are discussed.

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* Original note seen.