Effect of Cannabis (Hashish) on Mitosis of *Allium cepa* L. Root Tips

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Abstract. Treatment with cannabis induces a partial inactivation of the spindle apparatus, and an unequal separation of the chromosomes. There is more or less a tendency to c-mitosis at higher concentrations and to multipolar anaphases at lower concentrations like so many other narcotic substances. The M value decreases linearly and sharply with the increase of concentrations used, i.e. cannabis had a severe mitodepressive effect. Long treatment for 24 h caused the death of all cells examined.

Cannabis causes a complete dissolution of the matrix substance of the chromosomes and breaks down the chromatin material of the nucleus to minute granules. This phenomenon was observed in all root tips treated with the cannabis. The same picture was observed after letting the roots recover in water.

The application of cannabis may disturb the extrachromosomal mechanism. This disturbance is restricted to the abnormal diffusion of the spindle organizers in many directions instead of two poles.

An attempt was made in this investigation to examine the cytological effect of cannabis upon the process of mitosis and structure of chromosomes in the root tips of *Allium cepa* (onion bulb).

Material and Methods

The crude hashish (cannabis) was obtained from the Anti-narcotics administration office in Cairo.

Onion bulbs (*Allium cepa* cv. ‘Giza’) of the same size and age were used for the present cytological studies, the bulbs were germinated in sandy soil irrigated with tap water. When the roots had reached a length of about 1.5—2.5 cm the bulbs were removed from the pots and the roots were washed thoroughly with tap water. Throughout the different experiments in the present study roots of the same length were taken. The roots, while still intact with the bulbs, were immersed in small vials containing various concentrations of cannabis. Each series of experiments included a simultaneous control treated in an identical way, where tap water was used instead

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of the alkaloid. All treatments were carried out at room temperature ranging between 20—25 °C. Roots were treated with various concentrations of cannabis ranging from 15 mg ml⁻¹ to 0.75 mg ml⁻¹.

The roots were kept in the chemical for 4 h and fixed after one or after 24 h.

The Feulgen squash technique was applied for the cytological work. The root tips (1 cm) were cut after each treatment and fixed in Carnoy’s solution (acetic acid: alcohol 1:3) for 24 h. The roots were then transferred to 70 % ethyl alcohol and kept in the refrigerator until required. They were stained with Leucobasic fuchsine after hydrolysis in 1 N HCl for 8 min and permanent slides were then prepared. For each treatment scoring of the mitotic indices (MI — percentage of dividing cells) was carried out from 10 roots of at least 3 bulbs. Each determination was based on 10 fields taken at random from each root.

Results

A. Effect of Cannabis on Root Tips of Allium cepa after 4 h Treatment

The cytological effect of cannabis manifested itself in a range of concentrations between 15—0.75 mg ml⁻¹. Eleven concentrations were used: 15, 13.5, 12, 10.5, 9, 7.5, 6, 4.5, 3, 1.5 and 0.75 mg ml⁻¹.

The prophase frequencies decrease while those of metaphase increase in all of the applied concentrations, only the concentrations 4.5 and 9 mg ml⁻¹ had a slight effect on prophase stages where its frequencies were raised to 62.40 % and 63.71 % respectively, compared with that of the untreated roots 56.13 % (Fig. 1). Similarly P/M ratio was raised to 3.21 : 1 and 4.89 : 1 at the two previous concentrations while its value in all other concentrations applied shows a decrease compared with the control value.

The ana+ telophase frequencies slightly increase with the increase of cannabis concentrations till the concentration 7.5 mg ml⁻¹, then decrease to a minimum at the sub-lethal concentration compared with that of the control 20.85 %.

Data on the frequency of aberrations observed at metaphase and ana+ telophase in the different treatments indicate that the chromosomal aberrations tend to increase as the concentration of the crude substance increases (Fig. 1).

In concentration 10.5 mg ml⁻¹ only three normal metaphases were recorded of 58 metaphase plates examined. Consequently, the percentage of abnormal metaphase reached 94.83 % in 10.5 mg ml⁻¹ and 100 % in concentrations 9, 12, 13.5 and 15 mg ml⁻¹, then the frequencies of abnormal metaphases decrease with the decrease of concentrations used, reaching a minimum of 5.06 % at the lowest concentration 0.75 mg ml⁻¹ which still has some influence on the spindle.

Some of the anaphases resulting from treatment with cannabis show a characteristic feature; the chromosomes being able to reach the pole in the mean time are unable to build normal poles and appear as disturbed anaphases. Also slight disturbances in orientation of the spindle fibre apparatus were observed after treating Allium cepa roots tips with cannabis. The asters which are polarised regions in the cytoplasm (Darlington 1937) are normally found at the centre of the cytoplasm at the two poles; where two asters meet a spindle is formed. The previous abnormality in orientation of