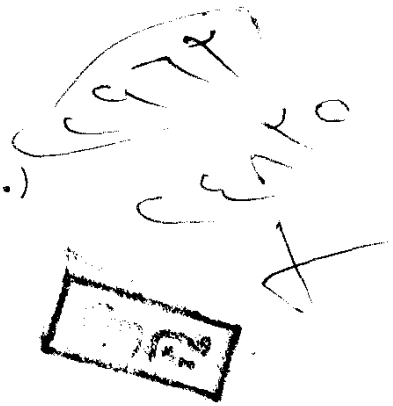
METABOLISM IN HYOSCYAMUS MUTICUS (L.)



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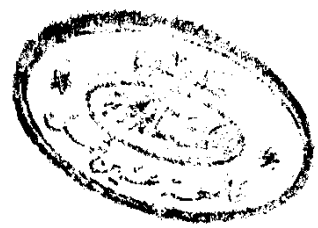

A THESIS

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the requirements for the degree

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in Agricultural Biochemistry

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PREFACE

The work embodied in this dissertation was done in the Experimental Farm of the Medicinal and Aromatic Plant Department, Ministry of Agriculture, and the Laboratory of the Agricultural Biochemistry Department of the Faculty of Agriculture, Ain Shams University, starting from 1964.

The present investigation was suggested and directly supervised by Professor Dr. Mohamed Abdel Moneim Kamal, Chairman Professor of Agricultural Biochemistry, Faculty of Agriculture, Ain Shams University, to whom the author is greatly indebted for his inspiration, guidance and encouragement throughout the whole course of the research.

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ABSTRACT

M.NABIL AHMED EID (Name) For the Ph.D (Degree) in Agri. Biochemistry (Major)

Date of thesis is presented:

Title: METABOLISM IN HYOSCYAMUS MUTICUS (L.)

In view of the stimulant effect of nitrogen fertilizer or colchicine and its modification of various metabolic products,it was decided whether any of this fertilizer or colchicine would induce a favourable effect on the metabolism in Hyoscyamus muticus (L.) leading to high alkaloid production.

Two complete randomized block experiments of fertilization with nitrogen fertilizer in five levels and colchicine application to the seed in six concentrations, were carried out. The metabolic changes were followed in the various organs of the plant including flowers, leaves, stems, and roots at ten days intervals after 74 days of sowing the seeds. Alkaloid, nitrogen and carbohydrate fractions together with the dry weight were investigated under the influence of the different treatments.

There was an increase in the dry matter of the various organs of the plant during growth. The gain was high at the

full-blooming stage before maturity. The increase of dry matter was more stimulated by colchicine-pretreatment than by nitrogen fertilizer application. The colchicine concentrations of 0.2 % and 0.4% yielded the highest dry matter. Nitrogen fertilizer slightly increased the growth and dry matter at a certain level of nitrogen after which higher levels were found to reduce the yield of dry matter.

Hyoscyamine was the main alkaloid constituent in the various organs of the plant, while hyoscine was present only in traces. Hyoscyamine seemed to be biosynthesized in the leaves, then translocated to the roots. Hyoscine was probably biosynthesized in the roots, then translocated to above organs, or it might be formed in aerial organs by transformation of hyoscyamine.

Nitrogen fertilizer or colchicine application did not considerably affect the percentage of total-alkaloids and the specific percentages of alkaloids in so far as concerns the quality of alkaloids. However, the content of total-alkaloids per plant was highly increased due to application of high colchicine concentration of 0.4% than the control. The leaves of the colchicine pretreated plants contained the highest quantities of alkaloids followed by stems, then flowers and finally the roots. On the other hand, nitrogen

fertilizer did not consinerably affect the content of alkaloids, but nigh nitrogen levels decreased it.

There were remarkable increases in the total-nitrogen content of the plant by nitrogen fertilizer and colchicine pretreatment especially when applied in high doses. On the other hand, the content of total-carbohydrates was decreased due to nitrogen fertilization, while colchicine did not significantly affect it.

The role of some amino acids and sugars in the biosynthesis of alkaloids was discussed.

INTRODUCTION

The investigation of alkaloidal plants is still very active, in spite of intensive work over the last 160 years, and it is likely to continue as an important branch of chemistry. The processes by which alkaloids are biosynthesized in plants have long been the subject of study by physiologists and biochemists. Little is known about their formation together with other metabolites occurring alongside them in the plants. However, a study of the actual biosynthetic pathway of alkaloids in the plant might help to deduce precisely what can be done to a drug-producing plant to direct the biochemical reactions into desired direction. Insight into the biochemical pathways and the exact steps in the formation of tissue constituents, is important to increase the yield and lower the cost of production the drugs.

The expansion of biochemical research showed that all living systems are strikingly alike in their basic metabolism. This situation should not obscure the fact that certain chemical differences do exist, not only in the quantity of constituents but also in the nature of these constituents. Thus, some species produce abnormal or secondary substances such as alkaloids which seem to have no apparent function.

These deviating compounds are quite numerous, although the basic metabolites from which all of them are formed are few and present in nearly all cells. In plants, some secondary products are formed as a result of biochemical imperfection of cells, or failure of an enzymatic link in a metabolic chain leading to unusual accumulation of specific metabolites. These abnormal substances cannot follow any of the normal metabolic pathways of elimination, so they suffer spontaneous transformation into compounds that are non-toxic to the plant.

The pharmacological importance of medicinal plant is of no doubt depending on their contents of specific compounds of these secondary substances. However, the form in which a plant appears at a given moment depends not only on its genetic constituents and stage of development, but also on the external condition under which it grows. It is also known that the external conditions can have a modifying effect on the external and internal characteristics of a plant.

Hyoscyamus muticus (L.) is one of the most important alkaloidal plants of the Solanaceae, indiginous to the U.A.R. The cultivation of medicinal plants in general or the industry of their products in the U.A.R. does not hold its desired position it deserves among other industries ,

although the environmental conditions prevailing are most suitable for its cultivation and propagation.

The present work is concerned with a phytochemical investigation of the metabolism of Hyoscyamus muticus (L.) plant during the whole growth season. It will be of great interest to study the metabolic changes of the alkaloid fractions, the nitrogen fractions, and the carbohydrate fractions of the different organs of Hyoscyamus plant during the growth season to find a relation between the accumulation of the alkaloids from other constituents.

It will be also of interest to study the effect of fertilizer treatments upon growth and other constituents of Hyoscyamus plant during the growth season. Moreover, an attempt was made in this work to treat Hyoscyamus seeds before cultivation with different concentrations of colchicine, hoping to induce some favourable changes in the metabolism of the resulting plants leading to the production of higher alkaloidal content.

REVIEW OF LITERATURE

The literature concerning the alkaloidal plants is so enormous since the last 180 years when Sertuerner (1806) isolated morphin, the first alkaloid to be isolated and characterized from opium. The literature reviewed in the present work covers the following aspects:

1. Botanical origin.
2. Agricultural requirements
3. Metabolism
4. Inducing polyploidy by the aid of colchicine.

1. Botanical Origin:

Bailey (1943) reported that Hyoscyamus belongs to the family Solanaceae, the plants are annual, biennial herbs and the genus contains about 15 species of the Old World. The English name is "Henbane" and it is known locally in the U.A.R. as "Sakaran".

Trease (1946), Gathercoal and Wirth (1947), and Wallis (1960) stated that much of the European drug of Hyoscyamus was said to consist of Hyoscyamus niger (L.) and Hyoscyamus albus (L.), while Hyoscyamus muticus (L.) was indiginous to Egypt.

[illegible] Stevens [illegible] indicated that some investigators obtained a favorable effect from certain fertilizer in a certain alkaloid containing crop, while others obtained a negative effect. However, the same author (1957) concluded that a dressing of nitrogen generally stimulated growth especially in what form this dressing was given. Manuring with the elements phosphorus, potassium and calcium often had a much less marked effect on growth. This may be attributed to deficiency of those elements in soils. The problem is still less pronounced though it is difficult to arrive at a uniform conclusion.

Hiltner and Boshart (1923), Klan (1930), Guillaume (1931), Dafert et al (1931 - 1932), Boshart (1938 - 1939), Salgues (1938), Esdorn (1940), Dijkstra (1943), Prasad (1946, 1947, 1948), James (1947), Brewer and Hiner (1950), Ustirner (1950), Rowson (1950), Zderkiewicz (1958), Shpilenya (1959), De Maggio (1961), and Malik et al (1963) found that fertilizers rich in nitrogen had been to increase the dry weight of Datura, Hyoscyamus, Solanum, Atropa, Lobelia, and Nicotiana. On the other hand, Boshart (1938 - 1939), and [illegible] and Naunez-Melendez (1942) determined that the [illegible] due to fertilizers treatments fluctuated between limits that were pharmacologically, hardly important. In the same trend, Balbaa et al (1963) found that the dry weight and alkaloid content of Hyoscyamus muticus (L.) were not

affected by nitrogen fertilization.

Clan (1930) found that the alkaloid content of Hyoscyamus niger (L.) was increased by nitrogen and phosphorus dressing; especially in combination with calcium and magnesium, while it was decreased by potassium addition. On the other hand, Dafert et al (1931 - 1932) found that manuring with nitrogen, potassium and phosphorus did not affect the alkaloid content in Datura stramonium (L.) and Hyoscyamus niger (L.); but the dry matter was increased . Dafert et al (1936) also found that only a heavy dressing with nitrogen might cause an increase in the alkaloid content, while potassium, phosphorus, and manganese seem to have an adverse efiect in Hyoscyamus niger (L.), Atropa belladonna (L.), and Datura stramonium (L.)

Asdorn (1940) reported that plants of Lobelia inflata (L.) manured with nitrogen, phosphorus, potassium and calcium, grew better than those grown in untreated soil, although their alkaloid content was lower.

Oxford Medicinal Plant Report (1942, 1943, 1945) declared that at high levels of ammonium sulphate, the Atropa belladonna (L.) plants were being stunted and the young shoots showed signs of scorching; nevertheless the percentage of alkaloids; even the total amount of alkaloid

per plant was increasing.

Prasad (1946) found that nitrogen and phosphorus were important for attaining higher shoot and dry matter of Hyoscyamus niger (L.) . He (1946) also added that nitrogen produced significant effects on height of the stem and dry matter of the leaf and the entire plant at all stages. There was a significant influence on the dry matter of the root and stems in later stages of growth. On the other hand, potassium and phosphorus showed comparatively a little effect on these character. The ratios of top/root, leaf/root, and stem/root were higher in early stages but became lower with advance in age; the reverse was noted in leaf/stem ratio.

The same author (1946) found that nitrogen and potassium significantly increased the alkaloid content. This was also noticed due to the interaction of nitrogen, potassium and phosphorus. Later on, Prasad (1947) reported that Hyoscyamus niger (L.) needed nitrogen fertilizers at the beginning of the life cycle to give significant effect on alkaloid formation. The growth of the leaves and branches became greater and sturdier than those received nitrogen at last. It was also noticed that fractionation of the complete normal quantity of fertilizers into three doses, did not response to as compared when divided into two