PHYSIOLOGICAL STUDIES ON THE TEMPERATURE REQUIREMENTS OF *HYOSCYAMUS MUTICUS* L.

By

ZAKARIA JACOB KHALIL

B.Sc. (Agric.) Assiut Univ.

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INTRODUCTION

Attention has been paid, especially in the present decade to study the medicinal plants especially in Egypt where hundreds of medicinal plant species are grown.

Hyoscyamus muticus L. (Egyptian henbane), which is a member of Solanaceae naturally grown in Egypt and is one of the most important medicinal plants that contains tropane alkaloids. The plant is herbaceous perennial growing in the sandy districts of Egypt. It is indigenous to desert region in Arabia, Persia, Baluchistan, Sinai, western Punjab, and had been introduced into Algeria. It is cultivated in irrigated soils of southern California (Claus, 1961). The plant contains fifteen times at least of total alkaloidal content more than the foreign Hyoscyamus niger L. (0.7 to 1.5 % total alkaloids calculated as hyoscyamine). Hyoscyamine, the active principle of the drug, is sedative, anodyne, antispasmodic, stimulant and mydriatic and is used in mental excitement, insomnia, palpitation, hysteria and in irregular infections of the lungs, bowels and geinto-urinary organs. (Dutt, 1928). Beside hyoscyamine, the plant contains hyoscine (scopolamine) and poisonous oils in the seeds. However, very little is known about the physiology of growth and development in relation to the biosynthesis of the alkaloids of this plant. Although temperature is one of the major effective factors in determining the normal growth and development of plants, essentially little is known about its effect on alkaloid content of Hyoscyamus muticus. In addition, vernalization is a specific temperature treatment in which the activated seeds of winter annual and certain biennial plants are kept at a certain low temperature range for a limit period before sowing essentially to hasten flowering ~~in addition~~ and to altering some of the metabolic pathways. Similarly, the effect of this treatment on the active medicinal constituents of the plant is nearly lacking in the literature.

The present work aimed to study the temperature requirements in Egypt through the cultivation at different sowing dates to benefit from the natural variation of the climatological temperature at the successive developmental stages of the plant. The effect of temperature factor on growth, flowering and alkaloid content was evaluated to obtain the optimum yield of alkaloids. In addition, the effect of vernalization was studied on the growth and flowering and their relation to the alkaloidal accumulation and distribution in the different organs of

Hyoscyamus muticus L. plants. The most favourable temperature degree and duration of vernalization period was determined which allow for higher alkaloidal production.

Hyoscyamus muticus L. was chosen as the experimental plant for the following:

1. Its medical value as a source of mydriatic alkaloids.

2. The high content of alkaloids of this species which satisfactory compete, on a commercial scale, with the foreign *Hyoscyamus niger* L.

3. As far as the author is aware, the temperature requirements and the effect of vernalization were not studied for this plant in Egypt.

REVIEW OF LITERATURE

I. *Hyoscyamus muticus* L. and Alkaloid Biosynthesis:

Hyoscyamus muticus L., the experimental plant of this study was described in details by Ahmed and Fahmy (1949), Balbaa (1950), and Mahran (1967) who indicated that it is perennial, about 80 cm. heigh, occasionally attain up to 120 cm. in height. *H. muticus* L. is indigenous to desert regions in Egypt, Arabia, Persia, Baluchistan, Sind, Western Punjab and has been introduced into Algeria and is cultivated in Southern California (Trease and Evans, 1972). Ahmed and Fahmy (1949) reported that the highest concentration of alkaloid in *H. muticus* L. was in the floral parts, followed by the leaves and the stems, and the roots had the lowest content. They also added that the highest yield was obtained at the flowering stage.

According to Hegnauer (1951), the various *Solanaceae* plants contained only scopolamine (hyoscine) in their early stages of growth. Hyoscyamine predominated in the flowering season. However, the young shoots of *Atropa belladonna* L. contained primarily hyoscine. Balbaa *et al.* (1964) found that the highest total alkaloidal content of *H. muticus* L. was in the closed flowers. They added that hyoscyamine was the main alkaloid at all growth stages, but the hyoscine was only found in large amounts in the closed and unexpanded flowers of cultivated plants.

Using grafting technique, Wilson (1952), studied the biosynthesis of *Solanaceous* alkaloids in *H. niger* L., *H. muticus* L. and *Scopolia anomala* L., and found that the alkaloids were received by scions of *Physalis alkekengi*, potato or tomato when grafted on stocks of these species. Raymond and Saint Firmin (1967) reported that the flowers of *H. muticus* L. had the ratio 3 : 1 from hyoscyamine to hyoscine (scopolamine), while the amount of tetramethyl diaminobutane was half of hyoscine. Eid (1969) mentioned that hyoscyamine was the main alkaloid constituent in the various organs of *H. muticus* L. The best time for collection of leaves and flowers was one week after flower opening of *Hyoscyamus desertorum* (Sabri *et al.*, 1973).

Concerning the site of biosynthesis and distribution of alkaloids, Evans and Partridge (1953) concluded that the aerial parts of *Datura tatula* L.

and *Datura ferox* L. were able to synthesize hyoscyamine and metaloidine respectively and that in *Datura innoxia* L., hyoscine was mainly found in the roots and hyoscyamine in aerial parts. Thus, the author concluded that:

a) In *Datura ferox*, hyoscine was synthesized in both roots and aerial parts.

b) In *Datura tatula*, the aerial parts seemed to possess some ability for synthesizing hyoscine.

c) In *Datura ferox* and *Datura tatula*, the roots were incapable of synthesizing metaloidine and hyoscyamine respectively, or translocation of the alkaloids from the stalk to the scion did not occur.

Mothes *et al.* (1954), found that the bleeding sap obtained when the tops of *Datura*, *Hyoscyamus* and *Atropa* were removed, contained alkaloids irrespective of whether the plant was supplied with nitrogen through the roots or through the leaves. Initially, scopolamine (hyoscine) predominated, but subsequently the normal alkaloid mixture was present. They also found that *Atropa belladonna* L. grafted on tomato roots formed very little alkaloids, even if nitrogen was supplied directly to the leaf. They concluded that this limited alkaloid synthesis occurring in the shoot was located in the young leaves. Marion and Thomas (1955) found that when Datura stramonium L. was fed with C^{14}-methyl-labelled methionine, only the hyoscyamine, but not the hyoscine, was radio-active. There seemed to be little doubt, therefore, that as started by Trautner (1947), hyoscine was formed at a comparatively early stage in the development of the plant, after which hyoscyamine was formed exclusively. Reda (1963) found only hyoscine in early seedling stage (2nd. leaf) of Datura stramonium L. Underhill and Youngken (1962) found among several labelled compounds tested, phenylalanine-3-C^{14}, phenylacetic acid-1-C^{14} and sodium acetate-2-C^{14} were the most effective precursors. In a similar experiment, Turner (1964) explained the biosynthesis of scopolamine (hyoscine) from radioactive ornithine in young Datura stramonium plants. Tikhonov (1967) showed that c-labelled acetate, acetone and methylamine were used in the biosynthesis of tropane alkaloids in D. innoxia L. He added that the biosynthetic process had a number of maxima and the accumulation of hyoscyamine took place preferentially in the roots and scopolamine in the generative organs. Hamon and Eyolfson (1972) studied the biosynthesis of tropic acid in *D. innoxia* root and found that DL-phenylamine-1-C^{14}, Phenylacetic acid 1-C^{14}, DL-tryptophane-(2 indolyl)-C^{14}, DL-tryptophane-(benzene ring)-U-C^{14}, L-serine-3-C^{14} and formic acid-^{14}C were all utilized by *D. innoxia* root tissue as precursors of tropic acid, and each was incorporated in a specific manner.

II. Response of Plants to Temperature:

Temperature is a trigger environmental factor determining plant growth, development and metabolism, depending on the variation of genetical and physiological capacities of different plants. The present review considered most of the known effects of air-temperature in general and low temperature (vernalization) in particular on vegetative growth, flowering and metabolic processes, especially alkaloid biosynthesis.

1. Air-temperature:

Since the early pioneer reports of Sachs (1887), Voechting (1893) and Klebs (1918), many hypotheses had been postulated to explain the role of different environmental factors in controlling the growth and development of plants. However, none of these postulations yet was able to explain the developmental phenomenon especially in relation to temperature. Hanna (1924), dealing with the growth of corn and sunflower in relation to climatic conditions, reported that both the actual and relative growth rates showed a closer relationship with temperature than with any other climatic factor.

a. Effect of air temperature on seed germination:

The temperature at which a seed is placed may have significant effects, immediately and eventually, on the plant development. The first action of temperature is its direct effect on germination, and it is well known that seeds will germinate only if the prevailing temperature is suitable. Some seeds require an initial low temperature, or more rarely a high temperature as a preconditioning to facilitate germination subsequently at a more favourable temperature.

To determine the influence of different temperatures on the rate of seed germination, Beljdenkova (1946) designed an experiment with