

# The Cultivation and Cucurbitacin Content of *Ecballium elaterium* (L.) A. Rich.

E. Attard and A. Scicluna-Spiteri

*Institute of Agriculture, University of Malta, Msida, MSD 06, Malta*

**Introduction:** Although *Ecballium elaterium* (L.) A. Rich., is considered as a minor crop in the Cucurbitaceae family. However, its medicinal virtues (3, 8) and its resistance to pests and diseases (6, 7), have made the plant a suitable candidate to improve crop quality in Cucurbitaceae species. Cultivation studies were aimed at assessing the growth of the plant and the variation of cucurbitacins in the plant tissue with a change in the seasons.

**Materials and Methods:** *Cultivation Studies.* Squirted *E. elaterium* seeds were obtained from mature fruit collected from the Southern region of Malta. The seeds ( $n=50$ ) were washed with distilled water, and seed coat cracked slightly (4) and placed overnight in a beaker with distilled water. The treated seeds were placed in seed trays and allowed for several weeks to germinate. The germinated seedlings were placed in Jiffy<sup>®</sup> pots (Sigma) and placed in a growth chamber at 24°C and a relative humidity of about  $95 \pm 5\%$ . When root tips emerged from the Jiffy<sup>®</sup> pots repotting was performed in normal pots. The plants were routinely watered twice daily. When an apical height of 90 – 100 mm was reached, the plants were planted in soil. They were allowed to grow for one year from September 1998 till August 1999.

*Elaterium and Cucurbitacin Contents.* At monthly intervals, two plants were sacrificed and the fruits, stems and leaves were gathered. The juice was extracted from each plant part by homogenization and filtration. In the case of stems and leaves, distilled water was added to aid the extraction of the juice. The juice obtained was dried in an oven at 40 °C, to obtain the dried elaterium. The cucurbitacin content was determined as described previously (1) using cucurbitacin E as the reference for the total cucurbitacin content.

*Statistical Analysis.* The results were analysed statistically by the one-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test for equality of means. Only  $p \leq 0.05$  were considered statistically significant.

**Results and Discussion:** *Cultivation Studies.* Although dormancy is the main problem with the seeds this was overcome by slightly cracking the seed coat (5) and then immersed in water overnight. It was noted that germination was two-staged (figure 1), with 40 % of the seeds germinating within the first 38 days. There was an average of 1 seed germinating per day for the first 45 days and 2 seeds per day for the following 10 days. The highest percentage of seed germination was 73.8 % achieved after 55 days, which persisted over the last 14 days of the experiment. Costich (5) determined that the maximum germination was 61 % by three months. The latter lacked stratification treatment. From figure 2, it was observed that there was constant growth for the plantlets with slight peaking at 21 and 63 days from seed germination. This peaking indicated a change in medium volume to accommodate better the growing roots. On transfer of the seedlings to the compost pots after 14 days, there was a rapid increase in growth, followed by a slowing down, probably related to root expansion. After 63 days, the roots reached maximum capacity in the medium, and so transfer to larger pots was necessary. This further enhanced plant growth. There was exponential growth between days 63 and 91, after which there was retarded growth. This indicated a change in the volume of medium. *Ecballium elaterium* is a plant that has a proliferative rooting system, therefore root expansion is essential for this plant.

*Elaterium and Cucurbitacin Contents.* High elaterium contents were mainly obtained in leaves compared to that in stems and fruit. Also accumulation of elaterium in leaves is temperature-dependent. In fact, the higher the temperature, the higher the accumulation of elaterium in the leaves, indicating that the metabolic and photosynthetic activities in the leaves are directly correlated with temperature. However, the extraction of elaterium from leaves is much more laborious and destructive. So the fruit still remain the plant part of choice for elaterium collection. In fruit, elaterium production was low in the flowering periods (March and

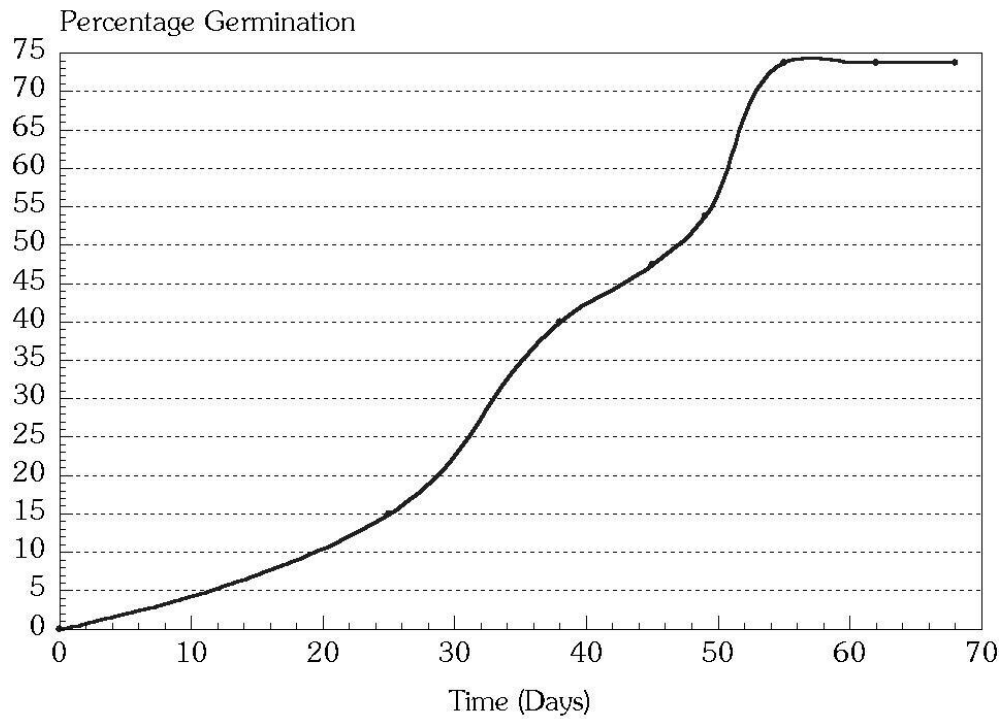


Figure 1. The percentage germination of *E. elaterium* seeds with time

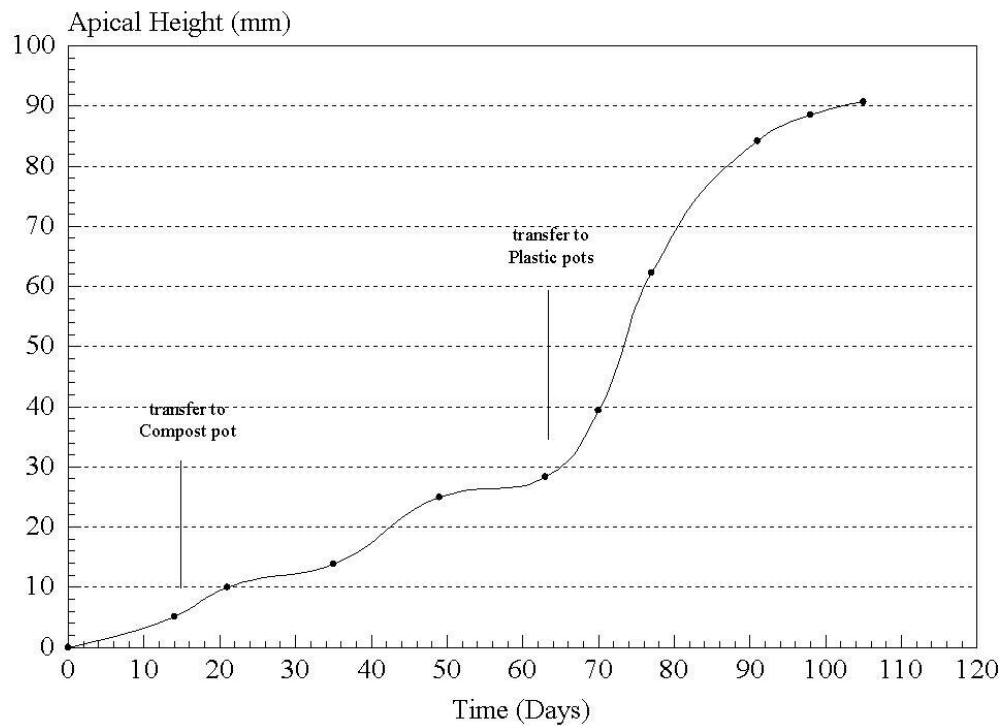


Figure 2. The apical height of *E. elaterium* seedlings with time

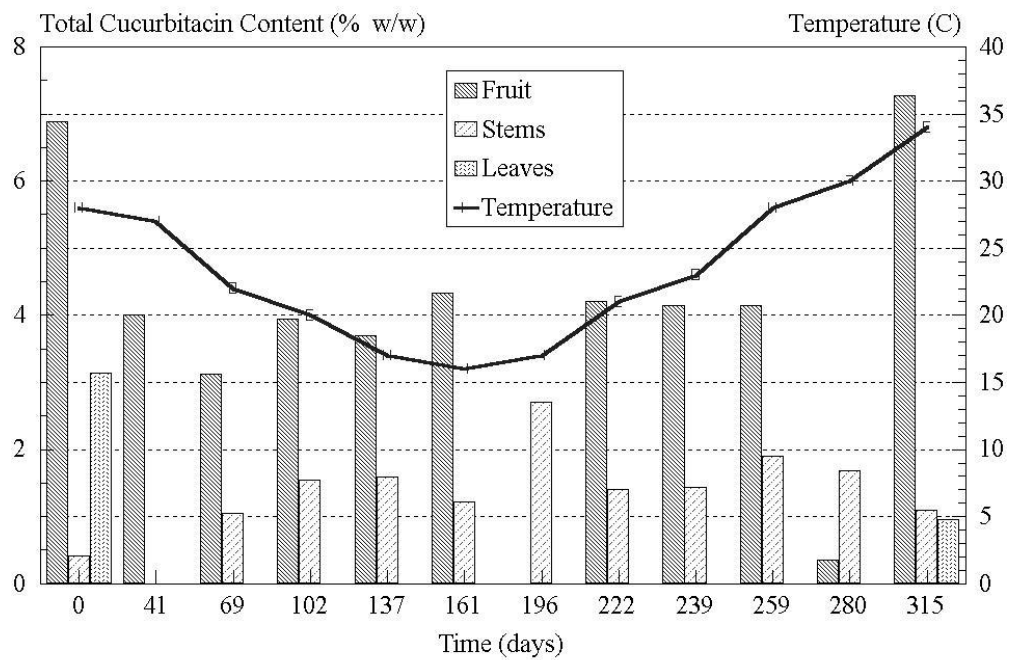


Figure 3. The total cucurbitacin content in elaterium produced from *E. elaterium* fruit, stems and leaves with time and temperature.

September, 0 and 0.297 % w/w respectively) but high in the fruiting periods (May-August and October-January, 1.4206 and 1.255 % w/w respectively). The constant but low yields of elaterium in the stem suggest that the elaterium is only transported through the stem to the different plant parts.

The total cucurbitacin content (figure 3) in elaterium produced from fruit, stems and leaves was also studied over the 315-day period. The fruit showed the highest mean total cucurbitacin content (3.84 % w/w), followed by the stems (1.34 % w/w) and then by the leaves (0.34 % w/w). The stems are involved in the transport of the cucurbitacins but only the fruit are associated with storage. Although the leaves contained a low concentration of cucurbitacins or none at all, the role of cucurbitacins is still important as antifeedants. For example, the bitterness of cucurbitacin E is experienced at a concentration of 10 ppb, (5), a concentration that is not detectable by the quantitative methods used. Balbaa and co-workers (2) determined a cucurbitacin content in the fruit 40 times greater than that in the leaves. In the present study, considering the fresh plant material, the cucurbitacin content in the fruit is about 22 times greater than that of the leaves. Therefore, the content of cucurbitacins in the leaves is adequate to promote the antifeedant and antimicrobial properties of the plant.

The elaterium and cucurbitacin contents of ripe and unripe fruit did not vary significantly ( $p > 0.1$ ,  $v = 11$ ). Lower elaterium contents in ripe fruit indicate a higher water content, a factor leading to the build-up of pressure inside the fruit and hence juice expression.

From this study, it can be concluded that the production of elaterium and particularly cucurbitacins reaches a peak during the active growth of the plant in the summer months, which coincides with the active growth and fruiting of several cucurbitaceous plants. As a result, *E. elaterium* can be cultivated

adjacent to edible cucurbitaceous plants in order to protect them from several pest and diseases.

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