## Effect of Gibberellic Acid on Chenopodium ambrosioides var. anthelminticum

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The application of gibberellic acid, also known as GA<sub>3</sub>, to Chenopodium ambrosioides L. var. anthelminticum (L.) A. Gray, induced increases of approximately 25 and 33% in the volatile oil content and yield of fruits. Vegetative growth was also increased. The per cent of ascaridole  $(C_{10}H_{16}O_2)$  in the treated and control plants was not altered appreciably.

URING the course of investigations on the effect of gibberellic acid (GA) with various important aromatic plants, the authors studied the effect of this substance on the growth and production of volatile oil in the chenopodium plant. The per cent of ascaridole in chenopodium oil was also determined. This paper describes the results of our preliminary study with this plant.

### EXPERIMENTAL

Young chenopodium seedlings, about 20-30 cm. tall and bearing 15 to 20 leaves, were transplanted during the second week of May 1961 into wellmanured beds of six square meters. The first treatment with GA at an average rate of 10 ml. per plant was given on May 28 at the preflowering stage. The same treatment was repeated 1 week later. GA was sprayed in concentrations of 25, 50, 100, and 200 p.p.m. Chenopodium seedlings which were planted in individual clay pots (10  $\times$ 12 inches) were simultaneously given GA-spray treatments at fortnightly intervals for 8 weeks in order to determine growth differences.

Observations on Growth.-The plants responded favorably to the various concentrations of GA. Increased internodal elongation was noted in the main shoots of the treated plants. Two weeks after the treatment, the axillary and side shoots appeared in all the treated plants, but in the untreated ones they were found after 4 weeks. After 8 weeks the maximum height (70 cm.) was observed in the plants given a dose of 100 p.p.m. of GA, as compared with 0, 25, 50, and 200 p.p.m. The corresponding figures for these groups were 26, 42, 48, and 68 cm., respectively. Maximum growth responses were observed in plants treated with 100 p.p.m. of GA until the sixth week, but thereafter the differences in length resulting from treatments with the higher concentrations of GA became less pronounced. The leaves were narrower and chlorotic in the treated plants. By the end of June, almost half of the flowers had opened. However, fruit formation was not complete until the end of August. The increased number of nodes and increased elongation of internodes in the treated plants resulted in a large number of axillary shoots bearing more fruit. It was also observed that if the seedlings were sprayed with high concentrations of GA in winter (December to January) the treatment did not initiate a characteristic growth response. However, when the length of day and the temperature were increased, a favorable growth response from GA is controlled by specific temperature and photoperiodic condi-

Received January 15, 1962, from the Regional Research

Accepted January 15, 1962, from the Regional Research Laboratory, Jammu and Kashmir, India. Accepted for publication March 5, 1962. The authors thankfully acknowledge the keen interest of Dr. I. C. Chopra in the work and I. C. I., Ltd., London, for supply of samples of gibberellic acid.

tions. The optimum period of growth for chenopodium is from April to the end of August.

Fruit and Oil Yield.-The plants were harvested in the first week of September and dried in the shade for a period of 2 weeks. The fruits were then gathered from the dry plant material. The most favorable response on fruit development was induced by treatment with 50 p.p.m. of GA. An increase of about 33% in the dry weights of this group was noted (Table I). The increased amount of fruit was due mainly to the development of a greater number of fruits. This was caused by the formation of additional flowering shoots by the GA treat-The per cent of volatile oil, determined by ment. steam distillation of the fruits, was found to be up to 25% greater in the treated plants (Fig. 1).

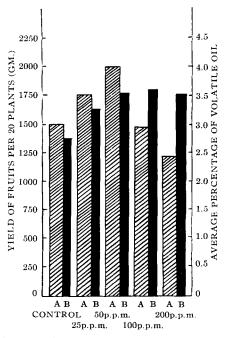


Fig. 1.-Histogram showing the per cent of volatile oil and yield of chenopodium fruits; A = fruit yield, B = % volatile oil.

The oil fractions from the variously treated groups were analyzed for ascaridole  $(C_{10}H_{16}O_2)$  according to the *Pharmacopoeia* of *India* (2). The per cent of ascaridole in the oil of treated plants was about the same as in the controls (Table I). It is interesting to note that the ascaridole content for all plants is above the 65% recommended in the British Pharmacopoeia.

TABLE I.—VOLATILE OIL, ASCARIDOLE, AND FRUIT YIELD IN CHENOPODIUM FOLLOWING TREATMENT WITH GIBBERELLIC ACID

	Treatment with GA, p.p.m				
Determination	0	25	50	100	200
Volatile oil in					
fruits	a	0.05	0 45	0.50	9 90
(v/w), <i>ª %</i>	2.80	3.25	3.45	3.50	3.20
Ascaridole					
content of					
oil, % <sup>b</sup>	79.6	79.4	79.0	78.5	78.0
Dry fruit yield,					
Kg. <sup>c</sup>	1.50	1.75	2.00	1.45	1.25
-					

<sup>a</sup> Mean from 10 determinations. <sup>b</sup> Average from three terminations. <sup>c</sup> Average yield from 20 plants occupying determinations. an area of 6 sq. meters.

#### DISCUSSION

From the foregoing observations it appears that, like Anethum sp. (4), the volatile oil of the chenopodium fruits also registers an increase with the application of GA. The fruit yield was also increased, which may be due to longer and more numerous flowering shoots. The increase in percentage of oil in the fruits suggests the possibility that GA may enhance the biosynthesis of essential oils in certain specific organs by either increasing the size and number of the cells which store the essential oil or prolonging the process of synthesis of The ascaridole content in the oil fractions was oil. not, however, altered appreciably by GA treatment but the increase in oil percentage in the chenopodium and anethum is in contrast with the observations on Mentha spicata (5) and M. arvensis (6) where the percentage of oil was decreased following GA treatment. The difference in behavior by the two categories of aromatic plants is of interest and needs further study.

The observations of Stevens, et al. (3), and Kaul and Kapoor (4) suggest that the increase may be due to prolonged biosynthesis of volatile oil and also the increased cell dimensions in the fruits containing the oil.

#### SUMMARY

1. The plants of Chenopodium ambrosioides Linne var. anthelminticum responded favorably to the treatment of GA.

2. It induced 25 and 33% increase in the volatile oil and the yield of the fruits, respectively.

3. GA did not appreciably alter the ascaridole content of the oil fractions from the various treatments.

4. The increased yield of fruits was due to increased number of axillary shoots which consequently bore more fruits.

5. The increased oil content in the treated plants may be due to increased cell size of fruits or prolonged biosynthesis of the essential oil.

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*Communications* 

# A Modified Method for Preparing Litmus Solution

### Sir:

The procedure prescribed by N.F. XI for the preparation of litmus T.S. (1) is tedious and results in a product which is subject to deterioration due to microbial growth. These difficulties may be removed by (a) preparing the solution by means of a continuous extractor and (b) dehydrating the product by means of freeze-drying. The resulting powder is not subject to microbial growth and may be used to prepare the test solution as needed. The following extraction procedure was used: Place 25 Gm. of powdered litmus in a paper extraction thimble and insert

the thimble into a Soxhlet extractor. Add sufficient alcohol to allow for free siphoning into the receiving flask, immerse the flask up to its neck in a steam bath, adjusting the flow of steam to maintain the extracting alcohol at a gentle boil, and continue the extraction for 3 hours. Raise the Soxhlet apparatus so the steam heats enough of the receiving flask to boil only the alcohol in the flask and permit the extraction to continue with the condensor-cooled alcohol for 30 minutes. Draw off all the alcohol in the system and discard. Reassemble the apparatus as it was originally, add sufficient water, arrange for heating with a Bunsen burner, and wash the litmus powder with reflux-cooled water for 3 hours. Discard the water. Remove the extraction thimble with the litmus powder from the extractor, place it in the emptied receiving flask,