

3-neck 5-liter Pyrex flask. The agitator is started, and 2000 Gm. zinc dust added. This precaution prevents any caking. The temperature is now raised to the boiling point of the diluted alcohol by a steam-bath, and 1600 Gm. of trimethylene dibromide (boiling at 162–164° C. uncorrected) which has been treated previously with 160 Gm. of zinc dust at room temperature is fed into the flask at a rate which will just maintain a smooth flow of gas. The gas so obtained was 99.5–100% absorbed by fuming sulphuric acid, and a 100-cc. sample left an acidified solution of 20 cc. *N*/5 KBrO_3 unaffected; indicating freedom from propylene.

We condensed the trimethylene from a typical experiment by a coil cooled by solid CO_2 and collected about 250 Gm. of liquid. This represents a yield of approximately 80%. No attempt was made to collect the largest possible yield.

Even though trimethylene is capable of producing anæsthesia when administered by inhalation, our results indicate that the margin of safety is too small for practical use.

SUMMARY.

A convenient method of preparing trimethylene with a high degree of purity and in substantial quantities has been developed, without recourse to burdensome methods of purification.

Preliminary tests indicate that this gas is not suitable for use as a general anæsthetic because of its low margin of safety.

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A THOUGHT ON THE PLACE OF VOLATILE OILS IN PLANT ECONOMY.*

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One of the most constant observations of the student of living things is the purposeful intent of the various devices of nature. While some seem quite obscure and some uncertain, the obviousness of others seems to guarantee a certain definite usefulness for all. Thus it is commonly accepted. The purpose of many devices is traditionally accepted, of many we find divergent and changing views, of some we will probably always be in doubt.

The question of the purpose of the class of products commonly termed volatile oils occurred to us several years ago. A class of secretions so widely distributed in the plant kingdom, consistently found in many species and produced with such definite intent, as the special structures which bear them would indicate, must certainly be developed for a very definite purpose to the plant. Certain general values have been ascribed to volatile oils and traditionally accepted, such as the attraction of insects to aid in pollination, the repulsion of animal life which might be detrimental to the species (6), etc. These go unchallenged. Nor is it the intent of this paper to challenge them, but we intend to show that volatile oils as a class serve an added and very important purpose.

In the literature of the past we find very few references made to the biological function of volatile oils. In Asa Gray's "Introduction to Structural and Systematic Botany,"

* Scientific Section, A. P. H. A., Rapid City meeting, 1929.

matic Botany and Vegetable Physiology" (2), published in the year 1858, we find the following statement regarding these:

These are some of the Proper Juices of plants, peculiar to certain species, and occurring under a great number of forms in different species. It is not known if they are of any account in vegetable growth or nutrition. . . . Not knowing of what use they are to the vegetable, we are inclined to regard them as of the nature of excretions.

In "Practical Plant Physiology" by Dr. W. Detmer (1), Professor of Botany of the University of Jena we read the following:

Etherial oils, in many cases, are certainly not to be regarded as excreta but as secretions, that is, as bodies with definite physiological function (to attract creatures necessary for the transference of pollen, to keep away injurious creatures, etc.) . . . It is further instructive to verify the fact that the fruits of many *Umbelliferae* are rich in etherial oil, occurring here in intercellular spaces and evidently functioning as a protection against injurious animals.

In most works on plant physiology we find that only casual reference is made to volatile oils and then only these traditional functions are ascribed.

In a search for further purpose we directed our inquiry to the effects produced by volatile oils, first in our own field, as used in medicine. Since they are so different in chemical composition we should expect to find a great variance in their medicinal action. This, of course, we know is true. But we find that practically all are said to have some antiseptic or germicidal action (7). This appeared to be a common property in a greater or lesser degree possessed by all members of the group. The thought then occurred that possibly many volatile oils actually serve a protective function for the plants which bear them. Since one of the most outstanding properties of living things is the power to resist chemical decomposition it did not seem unreasonable to suspect that volatile oils are devices of plants serving to protect against bacterial invasion.

Our problem, then, was to determine whether volatile oils were capable of affording such protection; if not all, whether those produced in certain organs of the plant possessed this power. It might be that those produced by certain types of structures were for protection.

The germicidal power of many volatile oils have been quite thoroughly studied. Most of these were studied by different individuals and the degree of toxicity arrived at in different ways. Thus it was found that available records did not furnish valid information for comparison. So it was decided to make new determinations from a selected list of oils using an identical technique in every case.

MATERIALS.

1. Volatile Oils.

Oil of Bergamot	Oil of Juniper	Oil of Peppermint
Oil of Cajuput	Oil of Lavender	Oil of Pimenta
Oil of Caraway	Oil of Mustard	Oil of Rosemary
Oil of Clove	Oil of Cubeb	Oil of Thuja
Oil of Dwarf Pine Needles	Oil of Myrcia	Oil of Thyme (White)
Oil of Eucalyptus	Oil of Sweet Orange Peel	Oil of Turpentine
		Oil of Orange Flower

The oils were supplied by a local wholesaler and were of official standard. Only one specimen of each oil was used.

2. *Bacteria.**Bacillus Anthracis**Bacillus Typhosus**Bacillus Coli Communis**Staphylococcus Pyogenes albus*

Organism A (a gram positive bacillus obtained from a decayed tomato)

Organism B (a gram negative bacillus obtained from a decayed pear)

3. *Medium.*

Nutrient Agar.

METHOD USED.

The method for obtaining Phenol Coefficient could not be used since the volatile oils could not be diluted with an inert diluent; they were either germicidal themselves or tended to prevent penetration. This made it imperative that time be used as the variable factor and that the pure oil be used in the tests.

Three drops of the oil were placed in a sterilized test-tube. A loopful of bacteria obtained from a 24-hour old culture was thoroughly mixed with the oil. From this inoculations were made upon an agar plate at intervals of one minute up to ten minutes, then every five minutes up to fifty and then every ten minutes up to ninety. The plate was incubated for 48 hours. The shortest period of time beyond which no growth was obtained was recorded. This was repeated for each organism with each oil. An old sporulated culture of *Anthrax* was also used. The figures obtained were used as the basis of comparison.

The results of the work are indicated in the following tables. The figures show the number of minutes beyond which no growth was obtained. Many of the figures do not indicate the even five- or ten-minute intervals. This is due to the fact that they represent the average of several determinations. The (*) indicates that growth took place after ninety minutes' exposure to the action of the oil.

TABLE I.—OILS DERIVED FROM LEAVES (8).

	Organism A.	Organism B.	<i>Bac. Anthracis</i> 24 hrs.	<i>Bac. Anthracis</i> spore.	<i>Bac. Typhosus.</i>	<i>Bac. coli.</i>	<i>Staph. Pyog. alb.</i>
Oil of Dwarf Pine Needles	23	15	60	90	37	23	30
Eucalyptus	*	53	90	*	53	53	*
Thuja	37	1	53	*	1	53	53
Cajuput	23	23	60	90	60	23	90
Myrcia	1	1	2½	60	10	10	1

TABLE II.—OILS DERIVED FROM LEAVES AND FLOWERING TOPS (8).

	Organism A.	Organism B.	<i>Bac. Anthracis</i> 24 hrs.	<i>Bac. Anthracis</i> spore.	<i>Bac. Typhosus.</i>	<i>Bac. coli.</i>	<i>Staph. Pyog. alb.</i>
Oil of Lavender	45	15	*	*	60	45	*
Peppermint	10	10	*	*	45	10	1
Rosemary	23	15	35	90	45	15	23
Thyme	10	10	37	*	10	10	2½

TABLE III.—OILS DERIVED FROM FRUITS (8).

	Organism A.	Organism B.	<i>Bac. Anthracis</i> 24 hrs.	<i>Bac. Anthracis</i> spore.	<i>Bac. Typhosus.</i>	<i>Bac. coli.</i>	<i>Staph. Pyog. alb.</i>
Oil of Bergamot	53	15	41	60	37	53	1
Cubeb	1	15	15	90	30	1	1
Juniper	37	1	45	*	45	37	90
Orange Peel	15	1	37	53	15	10	1
Caraway	22	22	60	90	60	45	22
Pimenta	1	1	37	53	23	23	1

TABLE IV.—OILS DERIVED FROM OTHER SOURCES (8).

	Organism A.	Organism B.	<i>Bac.</i> <i>Anthraxis</i> 24 hrs.	<i>Bac.</i> <i>Anthraxis</i> spore.	<i>Bac.</i> <i>Typhosus.</i>	<i>Bac.</i> <i>coli.</i>	<i>Staph.</i> <i>Pyog. alb.</i>
Oil of Orange Flower (petal)	30	15	45	*	45	15	45
Clove (receptacle)	15	1	22	37	1	30	1
Mustard (from glucoside in seed)	53	15	53	*	53	53	•

TABLE V.—OILS DERIVED FROM INTERNAL GLANDS.

	Organism A.	Organism B.	<i>Bac.</i> <i>Anthraxis</i> 24 hrs.	<i>Bac.</i> <i>Anthraxis</i> spore.	<i>Bac.</i> <i>Typhosus.</i>	<i>Bac.</i> <i>coli.</i>	<i>Staph.</i> <i>Pyog. alb.</i>
Oil of Caraway	22	22	60	90	60	45	22
Clove	15	1	22	37	1	30	1
Dwarf Pine Needles	23	15	60	90	37	23	30
Eucalyptus	*	53	90	•	53	53	*
Juniper	37	1	45	*	45	37	90
Orange Peel	15	1	37	53	15	10	1
Thuja	37	1	53	*	1	53	53
Turpentine	15	15	45	90	15	45	30
Bergamot	53	15	41	60	37	53	1
Pimenta	1	1	37	53	23	23	1

TABLE VI.—OILS DERIVED FROM GLANDULAR TRICHOMES.

	Organism A.	Organism B.	<i>Bac.</i> <i>Anthraxis</i> 24 hrs.	<i>Bac.</i> <i>Anthraxis</i> spore.	<i>Bac.</i> <i>Typhosus.</i>	<i>Bac.</i> <i>coli.</i>	<i>Staph.</i> <i>Pyog. alb.</i>
Oil of Lavender	45	15	*	•	60	45	•
Myrcia	1	1	2½	60	10	10	1
Peppermint	10	10	*	*	45	10	1
Rosemary	23	15	35	90	45	15	23
Thyme	10	10	37	*	10	10	2½

TABLE VII.—AVERAGES.

	Leaves.	Leaves and tops.	Fruits.	Int. glands.	Trichomes.
Ave. time in min. to kill saprophytes	19.7	17.2	15.3	17.5	14
Ave. time in min. to kill all vegetative forms	34.3	22.7	25.4	29.2	16.8
Percentage of vegetative forms killed within 90-min. period	93.3%	87.5%*	100%	96.6%	90%*

* The lowness of these figures may be due to the fact that spores had formed in the 24-hour old culture of *anthrax* as failure to kill was noted almost always in the 24-hour *anthrax* column.

According to Hiss & Zinsser (3), anthrax spores are killed by 1:2000 solution of mercuric chloride in 40 minutes but may remain alive as long as 40 days in 5% phenol; typhoid is killed in 5 minutes by either a 1:5000 solution of mercuric chloride or 5% phenol. The same authority gives ten minutes as the time required by a 1:1000 solution of mercuric chloride and 35 minutes as the time required by 1% phenol to kill the staphylococci. A comparison with our tables shows that many of our volatile oils are much more germicidal than these accepted agents, while some are relatively mild in their action.

CONCLUSIONS.

1. All the volatile oils investigated have some germicidal action.
2. Several are exceptionally powerful germicides.

3. There is a great variance in the power of a volatile oil to kill different organisms.

4. There is only a slight difference in the relative germicidal powers of oils from various plant organs. The fruit oils are most germicidal followed in order by those from leaves and flowering tops, leaves, and flower.

5. The volatile oils produced by glandular trichomes are relatively more germicidal than those produced by internal glands.

6. Volatile oils, as a class, are capable of affording protection against bacterial invasion irrespective of organ or structure from which they are derived. Some, however, seem to be much more highly specialized for this purpose than others.

REFERENCES.

- (1) W. Detmer, "Practical Plant Physiology," trans. S. A. Moor, The Macmillan Co., Philadelphia (1898), 338.
- (2) Asa Gray, "Introduction to Structural and Systematic Botany and Vegetable Physiology," Ivison & Phenny, New York (1858), 57.
- (3) P. H. Hiss and Zinnsser, "A Text Book of Bacteriology," D. Appleton & Co., New York and London (1918), 271.
- (4) Edwin Jordan, "General Bacteriology," W. B. Saunders, Philadelphia (1928), 338.
- (5) Henry Kraemer, "Scientific and Applied Pharmacognosy," John Wiley & Sons (1920), 283, 466.
- (6) Henry Kraemer, "Applied and Economic Botany," Kraemer (1916), 231.
- (7) T. Sollmann, "The Action of Drugs," W. B. Saunders Co., Philadelphia (1917), 121.
- (8) H. W. Youngken, "Pharmacognosy," P. Blakiston's Sons & Co., Philadelphia, Pa. (1926), 78, 152, 422.

ABSTRACT OF DISCUSSION.

George F. Reddish said the paper was rather surprising, especially from the standpoint of the germicidal action against anthrax spores. He asked the author whether, after transferring the mixture of essential oils and the culture to agar plates the plates were streaked so that the organisms would grow if not killed. He also asked the age of the anthrax spores, and on what medium they were grown, and remarked that the killing of anthrax spores is very difficult for any germicide—there have been specimens in which the spores survived several years. Tincture of iodine is one of our very strongest germicides and it does not kill within a half hour or more; so the condition of a spore, its resistance, and the medium on which it has been grown, is a very important point in this particular study.

The author replied that the anthrax was approximately a week old; he did not carry over from each week's work; fresh agar was made and the old culture used; they were transferred to agar plates and not spread over the plate, inoculations were short streaks.

George F. Reddish said that if the essential oils prove to be as highly germicidal as indicated in the reported work, the author has made a good start in the study of germicides; he expressed a desire to investigate.

The author stated that the work was prompted by an observation made by Professor Duffy, that anthrax spores were quite readily killed with oil of orange; he had carried the work further by adding the organism to egg albumen and then testing the germicidal value of oil of orange.

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