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PRELIMINARY NOTE ON THE STERILIZATION OF SEEDS OF THE LEGUMINOSAE WITH HYDROGEN PEROXIDE

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In the scientific study of many bacteriological problems it is necessary to deal with pure cultures of the organism with which the worker is interested. This has been true in our studies on the nodule producing organisms of leguminous plants. Before any morphological or physiological studies are made on these organisms they should be tested on the host plant for their inoculating ability, and in order to do this all possible chances for contamination by other organisms must be eliminated. If, in testing out a certain culture for its inoculating ability on the host plant, the plants were grown under conditions that were not sterile, and certain results were obtained, we would have no right to say that these results were due to the culture used, because they may have been due to contamination from the air, from the soil used, or from using seeds that were not sterile. In our studies on these organisms we have attempted to overcome these difficulties and sources of error, and it is the purpose of this paper to present the results of some preliminary work on the sterilization of seeds of the *Leguminosae*.

A number of methods have been used for the sterilization of legume seeds. The most common, however, has been the use of mercuric chloride in a concentration of 1 to 500. In using this method certain difficulties have been encountered, the chief of which is the necessity of washing the seeds a number of times to free them of the disinfectant. This is undesirable because it increases the chances for contamination and it is a tedious and time-consuming process. It was decided, therefore, to attempt to devise another method of sterilizing legume seeds that would overcome these difficulties.

In choosing a disinfectant for use in our work the following characteristics of the reagent were kept in mind as being desirable. Most important of all, it should be absolutely effective in killing all organisms. Next in importance, it should not have an injurious effect on the germination of the seeds. Aside from these two very essential qualifications, it would be desirable for the disin-

fectant to be a liquid, convenient to use, and requiring a minimum time for sterilization. Also, a disinfectant was desired which would not require the washing of the seeds after treatment. This would necessitate either that it be volatile or quickly decomposable after use. Such a disinfectant should also be easily obtainable at a reasonable cost, and should be of such a nature that it could be kept on hand without deterioration.

With these points in mind a number of chemicals suggested themselves; among which were hydrogen peroxide, alcohol, chloroform, formaldehyde, and certain hypochlorites. Of these hydrogen peroxide seemed to present the greatest possibilities for measuring up to the desired qualifications. Previous experiments led to the belief that it would be efficient in sterilizing the seeds without injuring germination. It was also believed that the hydrogen peroxide would be decomposable to the extent that it would not be necessary to wash the seeds free from it after it had been allowed to drain from them. Therefore, experiments were planned for testing out the value of hydrogen peroxide as a sterilizing agent for legume seeds.

Soybean, field pea, red clover, and alfalfa seeds were treated with hydrogen peroxide of various concentrations for thirty minutes at room temperature and also at 60° C. As a convenient apparatus for carrying out these tests 100 c.c. graduated cylinders were cut off at the 50 c.c. mark Twenty-five of the larger and about 100 of the smaller seeds were placed in one of these cylinders. From 10 to 15 c.c. of hydrogen peroxide were poured over the seeds in the glass cylinders which were then stoppered with a one-hole rubber stopper, the hole being plugged with cotton. This was necessary in order to permit the escape of gas from the cylinder and at the same time to prevent any possible entrance of micro-organisms. The hydrogen peroxide was secured, freshly prepared, from the Central Scientific Co. under the trade name of "Superoxol." This consisted of approximately 30 percent hydrogen peroxide. In cases where concentrations less than the original "Superoxol" were desired it was diluted by the addition of distilled water.

The seeds were left in contact with the hydrogen peroxide for thirty minutes in all cases. At the end of that time the solution was poured off and the seeds immediately transferred to sterile petri dishes 150 m.m. in diameter. Sterile yeast-water mannitol agar medium was then poured over the seeds, after which they were incubated at 29° C. for about one week. At the end of that time observations were made to see if the seeds were sterile and

also to record the effect of the treatments on germination. The results of these tests are presented in table 1.

The results presented in this table show that hydrogen peroxide in concentrations of 10, 15, and 30 per cent was effective in sterilizing legume seeds when they were treated for 30 minutes either at room temperature or at 60° C., while 5 per cent hydrogen peroxide was not effective under these conditions. The results also show that the germination of the seeds was not hindered when the hyrogen peroxide was applied at room temperature, but the percentage of germination was decreased considerably when the treatment was made at 60° C. The results appeared very favorable from the beginning. The time required for germination of the seeds was really shortened by about one-half when the peroxide was used at room temperature, and the percentage of germination was increased over the checks. Furthermore, it was found unnecessary to heat the peroxide to 60° C. to sterilize the seeds, and in fact it was more desirable not to heat it, because the heating proved injurious to germination, and the chances for contamination were increased during the treatment. This is shown by the two instances of contamination when the seeds were treated at 60° C. Treating the seeds at room temperature was also found to be much more convenient and the chances for contamination were fewer.

As to the relative efficiencies of the various concentrations of hydrogen peroxide used, it is at once noticeable that the treatment with 10 per cent hydrogen peroxide was just as effective as the 30 percent in sterilizing the seeds. At 5 per cent, however, the treatment was not effective in all cases. After germination the seedlings grew very well in the agar medium, except in the cases where the seeds had previously been treated with 30 per cent hydrogen peroxide. The amount of hydrogen peroxide remaining on the seeds after treatment with this high concentration, apparently had a toxic effect on the development of the roots in the agar medium, with the result that the seedlings grew very poorly.

The results may be summarized as follows:

1. Legume seeds treated with hydrogen peroxide at concentrations of 10, 15 and 30 per cent for 30 minutes at room temperature or at 60° C. were found to be sterile.

2. Five per cent hydrogen peroxide was not effective as a sterilizing agent under these conditions.

3. Treatment at 60° C. was injurious to seed germination and was not as convenient as treatment at room temperature.

4. When 30 per cent hyrogen peroxide was used the seedlings

were injured by the amount of peroxide remaining on the seeds after treatment.

These results seem to justify the conclusion that hydrogen peroxide at concentrations of 10 or 15 per cent is an efficient and desirable solution for destroying all microorganisms which may be present on legume seeds when they are treated for 30 minutes at room temperature. However, other experiments are being carried on to investigate further the value of hydrogen peroxide as a sterilizing agent for legume seeds. Particular attention is being directed toward such phases of the problem, as the strength required to sterilize seeds of various legumes, its efficiency when seeds are inoculated with an infusion of microorganisms from various sources, and the keeping qualities and the variability of different lots of the commercial hydrogen peroxide.

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TABLE I

RESULTS OF TESTS ON THE STERILIZATION OF LEGUME SEEDS WITH HYDROGEN PEROXIDE

	30% H_2O_2		15% H_2O_2		10% H_2O_2		5% H_2O_2	
	ROOM TEMPERATURE	60° C.	ROOM TEMPERATURE	60° C.	ROOM TEMPERATURE	60° C.	ROOM TEMPERATURE	60° C.
Soybean	96 —	68 —	92 —	100 —	96 —	48 —	88 +	76 —
Soybean	96 —	68 —	100 —	100 +	100 —	44 —	88 +	76 —
Field Pea	96 —	72 —	88 —	60 —	92 —	68 —	96 —	60 —
Field Pea	100 —	60 +	96 —	60 —	92 —	40 —	92 +	56 —
Red Clover	52 —	0 —	43 —	50 —	Good —	0 —	Good +	....
Alfalfa	58 —	0 —	59 —	50 —	Good —	0 —	Good —	Poor —

Figures represent percentage of germination.
— Means the seeds were completely sterilized.
+ Means the seeds were not sterilized.