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RESIDUAL EFFECTS OF SOME GERMICIDES USED IN STERILIZING LEGUME SEEDS

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In legume inoculation studies mercuric chloride is frequently used as a germicidal agent for sterilizing the legume seeds. While it is an effective disinfectant its use is often attended with objectionable results because of failure to remove all of the mercuric chloride from the seeds with the result that organisms surrounding the seeds may be killed thus preventing inoculation. In the course of some recent legume inoculation studies this difficulty became very apparent and as a result the extent of the toxic effect was investigated.

In the study in which the injurious influence of the chloride remaining on the seeds was first noted, field pea and soybean seeds had been sterilized with mercuric chloride, washed carefully with sterile distilled water and then allowed to germinate on yeast-mannitol agar plates which had previously been inoculated with legume nodule bacteria. After a short incubation period the plates were examined. On the plates in which field pea seeds had been placed, the development of bacterial colonies was normal over the entire plate but on the plates in which soybean seeds had been placed, no colonies developed in the vicinity of the seeds resulting in the formation of large, well defined sterile zones. This observation was in agreement with that of Haas and Fred (1) who noted the formation of sterile zones around soybean seeds which had been placed in petri plates seeded with Rhizobium. They attributed these zones to the sterilizing effect of mercuric chloride retained by the seed constitutents.

In the light of these observations experiments were conducted to determine the extent to which different legume seeds and legume nodule bacteria are affected by the mercuric chloride treatment. The toxic effects were measured by the extent of sterile zone formation around the seeds when placed in agar plate cultures of Rhizobium and also by the influence on seed germination. The effects of mercuric chloride and hydrogen peroxide were also compared, to determine whether or not the hydrogen peroxide is free from the objectionable features attending the use of mercuric chloride.

In order to verify the first observation, cowpea, soybean and Canada field pea seeds were used. These were sterilized for 3 minutes under reduced pressure with a 1 to 500 solution of mercuric chloride which is the solution strength generally recommended for this purpose. The seeds were thoroughly washed with sterile distilled water until the wash water was free of chlorides. The seeds were then placed in sterile petri dishes and allowed to dry. When dry, half of the plates were inoculated with a suspension of *Rhizobium leguminosarum* and the remainder with *Rhizobium japonicum*. Enough yeast-mannitol agar was then poured in the plates to practically cover the seeds.

After incubating for 3 days at 28° C. the plates inoculated with *Rhizobium leguminosarum* were examined. The plates containing the field pea seeds showed vigorous bacterial growth even in close proximity to the seeds and all of the seeds had germinated. On the plates containing cowpea and soybean seeds all of the seeds were surrounded by large, well-defined sterile zones. Some of these zones occupied more than half of the area of the plate. Bacterial growth surrounding the sterile zones was apparently normal and on some of the plates it was exceptionally heavy at the periphery of the zone, giving a ridgelike appearance to the growth. None of the cowpea or soybean seeds had germinated.

Due to the slow development of the soybean organisms, the plates inoculated with *Rhizobium japonicum* were incubated for six days before an examination was made. This series of plates gave the same results as the series previously examined. The field pea seeds showed no sterile zone formation and had all germinated normally, while the soybean and cowpea seeds were surrounded with characteristic zones and none of the seeds had germinated.

In order to determine whether or not the germicide had killed the cowpea and soybean seeds the plates were incubated for another six-day period and the plates examined a second time. The second examination showed that about half of the seeds were dead. The seeds which germinated developed rather abnormally and showed considerable necrosis of the cotyledons.

In the test only about 85 per cent of the seeds were sterile. All of the contaminated seeds were covered with a slimy bacterial growth which showed a tendency to spread out into the sterile zones.

Mercuric chloride and hydrogen peroxide were then compared as sterilizing agents. Hubam clover and field pea seeds were selected as representative of the horny coated legume seeds while the soybean and cowpea seeds were again used because of their

apparent ability to absorb the mercuric chloride. Half of the seeds were sterilized with mercuric chloride using the method previously described. Extreme care was exercised in washing the seeds in order to remove all traces of the chloride. The remainder of the seeds were sterilized with a 15 per cent solution of hydrogen peroxide under reduced pressure for 20 minutes then washed several times with sterile distilled water. No particular effort was made to remove all of the hydrogen peroxide from the seeds. After washing, the seeds were placed in sterile petri dishes and allowed to dry. With the larger seeds only two seeds were placed in each plate. This was necessary because of the large sterile zones which normally developed following the mercuric chloride treatment. With the hard coated seeds, approximately 15 seeds were placed in each plate. A total of 81 plates was prepared. Nine of the plates served as controls and the remainder were divided into three series of 36 plates each. One series of plates was inoculated with *Rhizobium meliloti*, the second with *Rhizobium leguminosarum*, and the third with *Rhizobium japonicum*. Yeast mannitol agar was then poured over the seeds and the plates incubated for 6 days at 28° C. The plates were examined, and seed germination, sterile zone formation and freedom from contamination noted. In order to simplify these data a recalculation was made on the percentage basis. The results appear in table I.

The results obtained with the mercuric chloride treatment were in entire agreement with those secured in previous tests. With the horny coated seeds, the mercuric chloride treatment did not reduce seed germination but a large part of the seeds retained enough of the germicide on the seed coats to bring about the formation of sterile zones around the seeds. With the hydrogen peroxide treatment, no sterile zones appeared and the germination was apparently stimulated.

The most striking results were secured with the larger seeds. When these seeds were treated with mercuric chloride a greater part were dead and all of them were surrounded by a large sterile zone indicating a rather high retention of the mercuric chloride by the seed constituents.

The hydrogen peroxide treatment gave entirely opposite results. Following this treatment the seeds showed vigorous germination and a total absence of sterile zone formation. For all practical purposes the two disinfectants showed the same germicidal efficiency. The results secured were the same with all species of Rhizobium.

The data which have been given indicate that the selection of the

Table I — Comparative Effects of Mercuric Chloride and Hydrogen Peroxide Treatments on Legume Seeds

Kind of Seed	Hubam Clover		Canada Field Peas		Soybeans		Cowpeas		Average for Large Seeds		Average	
	$HgCl_2$	H_2O_2	$HgCl_2$	H_2O_2	$HgCl_2$	H_2O_2	$HgCl_2$	H_2O_2	$HgCl_2$	H_2O_2	$HgCl_2$	H_2O_2
Percentage plates showing sterile zones	50	0	75	0	100	0	100	0	100	0	93	0
Percentage seeds germinating	51	50	92	92	25	100	0	100	38	97	38	67
Percentage seeds sterile	70	91	92	92	100	92	92	83	94	89	80	90

proper disinfectant for sterilizing the legume seeds in legume inoculation studies is of great importance. Hydrogen peroxide is apparently a safe and reliable disinfectant for sterilizing legume seeds but mercuric chloride should be used with caution particularly with the large seeds such as the cowpea and soybean seeds.

SUMMARY

A comparison was made of the effects of the mercuric chloride and hydrogen peroxide remaining on the seeds after they have been applied as seed sterilizing agents in legume inoculation studies. With the larger seeds, such as soybeans, the mercuric chloride treatment seriously reduced germination and resulted in the formation of large sterile zones around seeds placed in petri plate agar cultures which had been heavily seeded with Rhizobium. Following hydrogen peroxide treatment no sterile zones appeared and germination was apparently stimulated. With the smaller, horny-coated seeds no marked residual effects due to mercuric chloride retained on the seed coat, were noted. The disinfectants showed about equal sterilizing efficiency.

LITERATURE CITED

1. HAAS, A. R. C., AND E. B. FRED, 1919. The Effect of Soybean Germination Upon the Growth of Its Nodule-Forming Bacteria. Soil Sci. 7: 237-243.

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