The Journal of the Indian Botanical Society.

(Formerly "The Journal of Indian Botany".)

VOL. V. JANUARY, 1926. No. 1.

THE CAUSE OF COTTON WILT IN INDIA.

BY

S. L. AJREKAR, B.A., I. E. S.

Professor of Botany, Royal Institute of Science, Bombay.

In a preliminary account of the investigation of cotton wilt in the Central Provinces presented by J. F. Dastur (1)* read before the Agricultural Section of the Indian Science Congress at Bangalore in January 1924, the author sought to make two points which may be stated briefly as follows:—

1. That the workers on cotton wilt in India previous to him considered the fungus *Fusarium sp.* as the cause of the disease merely on the analogy of the similar American disease and not on indisputable local evidence.
2. That the accumulation of compounds of iron and aluminium in the tissues of wilting plants, of which evidence was obtained in micro-chemical tests, is probably the cause of cotton wilt in India.

The author of the above paper deserves congratulations on his efforts to get free from the traditional views on his subject, but one is sorry to find that he has secured his liberty only to lose it again. For we find him immediately afterwards almost blindly following the lead of the American workers, Hoffer and Carr (2) whose work on the root rot of corn has evidently inspired him to put forward his aluminium and iron salts accumulation theory of cotton wilt, although, strangely enough, we find no reference in his paper to this and other American work on the subject of iron and aluminium salts in soils and plants.

While the present writer is quite prepared to give up the fungus theory of the causation of cotton wilt in favour of any other when sufficient evidence for the same is forthcoming, he intends in this paper to give some additional evidence in support of the fungus theory which he has obtained since the publication of his last joint paper (3)

* The numbers refer to literature cited.

on the subject of cotton wilt in 1921. He will also try to clear certain points in that paper which have been misunderstood or mis-stated by Dastur. Dastur's reasons to doubt the parasitism of the cotton Fusarium and to believe in the iron and aluminium theory will also be examined.

Additional evidence of the parasitism of cotton Fusarium.

Since the publication of the results of the four months' work on cotton wilt in the Central Provinces which the writer was able to do under the special circumstances described in the previous joint paper[3], the writer has not had any opportunity to continue the work on the original plan. The additional evidence in support of the parasitism of the cotton *Fusarium* that he now intends to present is, therefore, such as was obtained incidentally during the course of other work and not during a systematic investigation of cotton wilt. It seems nevertheless very convincing and must be taken seriously into account by any one wishing to discard the fungus theory of cotton wilt and this is his justification for presenting it.

The evidence in question consists of the results of a series of inoculation experiments undertaken at the request of Messrs. Kottur and Patel, Cotton Breeders, working at Dharwar and Surat respectively, in connection with their variety trials for wilt resistance. The general plan cf these experiments was to sow cotton seeds which had been disinfected with mercuric perchloride in pots which had been cleaned thoroughly and washed with mercuric perchloride solution. The soil in the pots had been sterilised by heating in the autoclave at 120 C.° for half an hour. The inoculation was done by mixing the contents of a culture tube containing a pure culture of cotton *Fusarium* with the soil at the time of sowing the seeds. The plants were then allowed to grow naturally in the plant house.

The results are given below :—

Experiment No. 1

Pot No.	Date of sowing and inoculation.	No. of plants come up.	No. of wilted plants with date of examination.
1	7–6–1921	6	1. 6–8–1921 5. 8–9–1921
2	,,	6	5. 8–9–1921. All dead before 8–9–1921
3	Control 7–6–1921	6	*Nil.*

REMARKS :—The fungus used in this experiment had been isolated originally from a wilted plant from Dharwar. The fungus was found in all the wilted plants.

Experiment No. 2

Pot No.	Date of sowing and inoculation.	No. of plants come up.	No. of wilted plants with date of examination.
A	12-9-1921	12	1. 24- 9-1921 3. 21-10-1921 2. 25-10-1921 1. 26-10-1921 1. 31-10-1921 1. 30-11-1921 2. 3- 1-1922
	Control 12-9-1921	11	None up to 7-2-1922

Experiment No. 3

B	12-9-1921	11	1. 22- 9-1921 1. 23- 9-1921 1. 27- 9-1921 1. 17-10-1921 1. 19-10-1921 1. 13-11-1921 1. 3- 1-1922
	Control 12-9-1921	11	None.

Experiment No. 4

C	12-9-1921	13	1. 10-10-1921 1. 14-10-1921 1. 17-10-1921 2. 25-10-1921 1. 2-11-1921 2. 13-11-1921 1. 16-11-1921
	Control 12-9-1921	10	None.

Experiment No. 5

D	12-9-1921	10	1. 27- 9-1921 2. 19-10-1921 1. 29-10-1921 1. 13-11-1921 1. 22-11-1921 1. 7- 2-1922
	Control 12-9-1921	9	None.

REMARKS on Experiments 2, 3, 4 and 5.

The Fungus used in all these was a Nagpur strain of *Fusarium*. The controls to these in every case showed no case of wilt up to 7-2-1922 when the experiments were concluded. The wilted plants in every case showed the fungus in them. The varieties of cotton tried were as follows :—

Experiment No. 2	...	...	Suratee Broach Gi.
,, ,, 3	...	...	Selection 1A.
,, ,, 4	...	...	Ghoghari E5.
,, ,, 5	...	...	Ghoghari B21.

Experiment No. 6

Pot No.	Date of sowing and inoculation.	No. of plants come up.	No. of wilted plants with date of examination.
1	18–8–1922	8	1. 9– 9–1922 1. 12– 9–1922 1. 26– 9–1922 2. 28– 9–1922 1. 11–10–1922 1. 13–10–1922 1. 17–10–1692
1a	(Control)	9	*Nil* up to 8–12–1922 when the experiment was concluded.

REMARKS :—The variety of cotton seed used was "Broach Deshi plant" 4. The fungus used was a Dharwar strain and was found in all the wilted plants.

Experiment No. 7

2	18–8–1922	9	1. 29– 9–1922 1. 2–10–1922 1. 5–10–1922 1. 6–10–1922 1. 30–10–1922
2a	Control 18–8–1922	9	*Nil* up to 8–12–1922 (date of conclusion of experiment).

REMARKS :—Variety of cotton ... Broach Deshi plant 5.
Fungus used ... Dharwar strain.

Fungus found in all the wilted plants. The last plant examined shed all its leaves and this showed the fungus only in the petioles.

Experiment No. 8

3	18–8–1922	10	*Nil.*
3a	18–8–1922 (Control)	7	,,

REMARKS :—Variety of cotton ... Broach Deshi plant 6.
Fungus ... ... Dharwar strain.

General note on the experiments 6, 7 and 8 :—The results in in these confirmed in a striking manner the susceptibility of the Broach Deshi Plants 4 and 5 and the resistance to wilt of Broach Deshi Plant 6 observed in field conditions.

The results of these experiments point clearly to the parasitic nature of the Fusarium strains isolated from wilted plants from both Dharwar and Nagpur. It was fortunate that the fungus used in the the experiments numbers 2, 3, 4, and 5 happened to be a subculture of a strain of Fusarium originally isolated from a wilted plant from Nagpur. Otherwise it would have been easy to weaken the force of the above evidence by suggesting that the Dharwar strains might be parasitic but not the Nagpur ones.

Points in the previous joint paper misunderstood or mis-stated by Dastur.

Before proceeding to consider Dastur's views on this subject it is necessary to note that in his enthusiasm for the new iron and aluminium theory of cotton wilt and in his anxiety to prove that the fungus theory is wrong, it has not been always possible for him to understand clearly or state fairly the results of previous workers. For example, because it happens that the details of Butler's work on cotton wilt at Pusa have not been yet published, Dastur is quite ready to ignore the positive results of inoculation experiments reported seriously by that authority in the annual report of the Agricultural Research Institute, Pusa, for 1913-14. Little wonder then that he should pay no heed to the few but none the less positive results in the writer's experiments previously reported. Shutting his eyes coolly against these positive results Dastur feels no hesitation to state that his results were "very much similar to those of Ajrekar and Bal *viz.* that the fungus failed to inoculate the plants"! Similarly, Dastur can hardly be said to have done justice to the joint authors he criticises, when he ignores their cautious and suggestive remarks on the results of their inoculation experiments. He indeed quotes their remark "negative results in inoculation experiments do not prove anything" but omits the closely following clause "and these experiments must be repeated in any future work on this disease" and thus makes an essentially cautious statement appear a "bold assertion" Again, Dastur's statement that the joint authors "failed to verify their suspicion (that the fungus secretes a fatal toxin) in their experiments," is misleading. The joint authors state clearly that they made only one experiment (not experiments) and that "experiments with larger quantities of the supposed toxin and with younger plants seem . . . necessary to settle this point." It is hardly fair to rule the toxin theory out of court from the negative result of a solitary and imperfect experiment, as Dastur seems to have done.

The Parasitism of the cotton Fusarium.

Dastur's reasons for doubting the parasitism of the cotton *Fusarium* and its causal connection with the wilt disease may now be examined. He says he made "several inoculation experiments under varying conditions" with the result that "the fungus failed to inoculate the plants." But how is one to reconcile this statement with the very next sentence which informs us that "the more the inoculated plants were under normal conditions the more complete was the failure to inoculate. . . ." This means, if anything, that Dastur got varying degrees of failure or, to put it conversely, varying degrees of success in his inoculation experiments according to the conditions of experiment!

Even granting that Dastur got only negative results in his inoculation experiments, it is not possible for every one to follow him immediately to the conclusion that the fungus is non-parasitic on the strength of these negative results alone. If, instead of characterising as "bold" the writer's cautious statement "that negative results in inoculation experiments do not prove anything," Dastur had taken the trouble to grasp its full meaning, namely, that negative results, by themselves, do not give a final answer to the question whether the organism concerned is or is not a parasite, he would have hesitated before rushing to his conclusion. He would then perhaps have seen some of the difficulties in the way. He would have, for example, asked himself if the number of inoculation experiments made by him which he indicates by the adjective "several" was convincingly large; or whether the conditions of his experiments precluded other explanations of his negative results than the non-parasitic character of the fungus. Even from the scanty details given of his experiments we learn that his plants were grown in moist sand under abnormal conditions of "starvation and water-logging" in two of the three series of experiments described, and in the third, although the plants were grown in tank soil, "the upper lateral roots were cut." Might not the abnormal conditions of starvation and reduction in root surface (which means reduction in the number of vulnerable points of the host) help to account for the failure of inoculation in these cases? Again, Dastur seem to have omitted to ask the meaning of the constant occurrence of the same or at least similar strains of *Fusarium* in wilted plants collected from widely different localities. He himself has "isolated species of *Fusarium* from innumerable wilted plants from various localities." If he had kept in mind this constant association of the fungus with the wilt he would not have so lightheartedly ignored the positive results of inoculation experiments reported by others, and he would have been extremely cautious in

accepting his own negative results as conclusive. In his eagerness to disprove the causal connection of the fungus with the disease he seems to demand nothing less than cent per cent deaths among the inoculated plants as proof of parasitism. But it is the experience even with many undoubted bacterial and fungoid parasites that cent per cent deaths in inoculation experiments are not always obtained. In some cases, especially with newly discovered parasites, this is due to the imperfect knowledge of the conditions of successful infection. The total failure to produce infection of the Jowar plant in inoculation experiments with the spores of the long smut (*Tolyposporium filiferum*) so far is a case in point. Yet few would hazard the conclusion from this failure that the Long Smut organism is not a parasite. In other cases the obscure but none the less definite factor of individual resistance perhaps comes into play. It is quite a common experience with infectious or contagious diseases that some individuals will escape the disease although exposed to infection.

The results of inoculation experiments with the cotton *Fusarium* isolated from widely separated tracts presented in this paper leave little doubt as to the parasitic nature of the fungus. The high percentage of deaths in inoculated plants, the production of the characteristic symtoms of the disease in the infected plants and their absence from the controls, and the recovery of the introduced parasite from the plants showing the symtoms of wilt in these experiments will have to be kept in mind by any one who proposes to make a final statement of the cause of cotton wilt.

The Iron and Aluminium salts accumulation Theory of cotton wilt.

Now a few words about the Iron and Aluminium salts accumulation theory of cotton wilt advanced by Dastur. As the writer has not had any opportunity recently to continue his cotton wilt studies, his remarks will necessarily be based on general considerations only. As far as he has been able to ascertain from such literature on the subject as was available to him it seems that active aluminium salts occur in acid soils, and as far as he is aware the cotton soils are *not* acid either in the Central Provinces or in the Bombay Presidency. The probability, therefore, that aluminium salts are concerned in the causation of cotton wilt does not seem very great. Dastur bases his theory, as do Hoffer and Carr, on the evidence obtained in micro-chemical tests made on the tissues of diseased plants, but unlike these authors he does not seem to have verified by chemical examination the acid nature of the cotton soils or the differences in iron and aluminium contents of healthy and diseased plants. As regards the

colour reactions in the microchemical tests, it may be doubted how far they can be relied on as specific for iron and aluminium compounds. In any case the advocates of the iron and aluminium theory will find it very hard to explain the results of the inoculation experiments presented in this paper on that theory, for the soil in the inoculated pots and other conditions were exactly the same as in the controls excepting the fungus factor.

Dastur's experiments with injections of iron and aluminium salts as reported were obviously tentative and it would be hardly fair to criticise them at this stage. It must be noted, however, that in the series of experiments in which he injected one per cent solutions of aluminium and iron salts, the plants after showing "wilting the day following the injection . . . . . soon recovered." In other words, the results in these experiments were negative and cannot lend any support to the theory. Again, in the series in which the plants were transferred from pots to solutions containing different amounts of aluminium chloride, the only positive results obtained were with the plants kept in 0. 01% of the normal solution. One should like to have some evidence that such toxic concentrations of aluminium salts are actually absorbed by cotton plants growing in the ordinary field conditions, before the aluminium and iron theory can be accepted.

Summary.

1. Successful inoculations of cotton plants with *Fusarium sp.* isolated from wilted plants from Nagpur and Dharwar are reported.

2. J. F. Dastur's reasons for doubting the parasitism of cotton Fusarium as set forth in his preliminary account of the investigation of cotton wilt in the Central Provinces and Berar are examined and found unconvincing.

3. Some difficulties in the way of accepting the iron and aluminium salts accumulation theory of cotton wilt advanced in the same paper by Dastur are indicated.

Literature Cited.

1. Dastur. J. F. A perliminary account of the investigation of cotton wilt in the Central Provinces and Berar. Agr. Jour. Ind. XIX. pp. 251-260.

2. Hoffer. G. N. & Carr. R. H. Accumulation of Aluminium and Iron Compounds in corn plants and its probable relation to root rots. Jour. Agr. Res. Washington. S. C. Vol. XXIII. No. 10. 1923.

3. Ajrekar. S. L. & Bal. D. V. Observations on the Wilt disease of cotton in the Central Provinces. Agr. Jour. Ind. XVI. pp. 598-617.

cotton plants, for instance, up to about three and half weeks, test tubes 9" long and 1½" in diameter have been found to be quite suitable. These are carefully washed and dried. About 20 cc. of the necessary water culture solution are poured into the test tube. The concentration of the salts in a normal Knop or Detmer's solution is too high to permit the growth of young seedlings. The writer has used one of Shive's (1) three salt solutions for this purpose and so as to permit the growth of the parasite 1 per cent dextrose or cane sugar has also been added. The composition of this solution is as follows :

Acid potassium Phosphate :	...	...	18 cc. of M/1 solution
Calcium Nitrate :	...	...	5·6 cc. of M/1 solution
Magnesium Sulphate :	...	...	15 cc. of M/1 solution
Iron	...	...	a trace
Cane sugar	...	...	10 grams

Distilled water to make one litre of the solution.

After the above solution is poured into the test tube small pieces of broken glass are placed inside. The object which these glass pieces serve is to support the cotton wad which is now placed inside the test tube. The cotton wad just touches the solution and is not immersed in it which is due to the broken glass. A few cc. of the nutrient are again poured inside so as to moisten the cotton wad. The wad must be of absorbent cotton and not the usual non-absorbent cotton used in pathological laboratories. The test tubes are then plugged and sterilised for 15 minutes at 15 lbs. pressure.

The seed which is to be introduced into these test tubes has also to be sterilised. In the case of cotton seed, the writer has diluted it with strong sulphuric acid, washed with sterile water, and finally he has placed it in dilute Mercuric Perchloride solution (1 : 1000). It is transferred directly from this solution to the test tubes, proper care to see that there has not been any contamination having been taken. It has not been found necessary to wash the seed free of the perchloride solution. Instead of this disinfectant, Formalin, Copper sulphate solution, &c., may also be used.

The radicle appears in about three to four days and the cotyledons are released soon after. The former pierces through the cotton wad into the solution below. In due time the test tubes are inoculated with the pathogene. Figure one will serve to make the above clear.

References to literature :

(1) Shive, John W., Physiological Balance in Nutrient Media. Physiological Researches Vol. 1, No. 7, 1915.

(2) Tisdale, W. H., Two Sclerotial diseases of Rice. Journal of Agricultural Research, Vol. 21 No. 9, 1921.

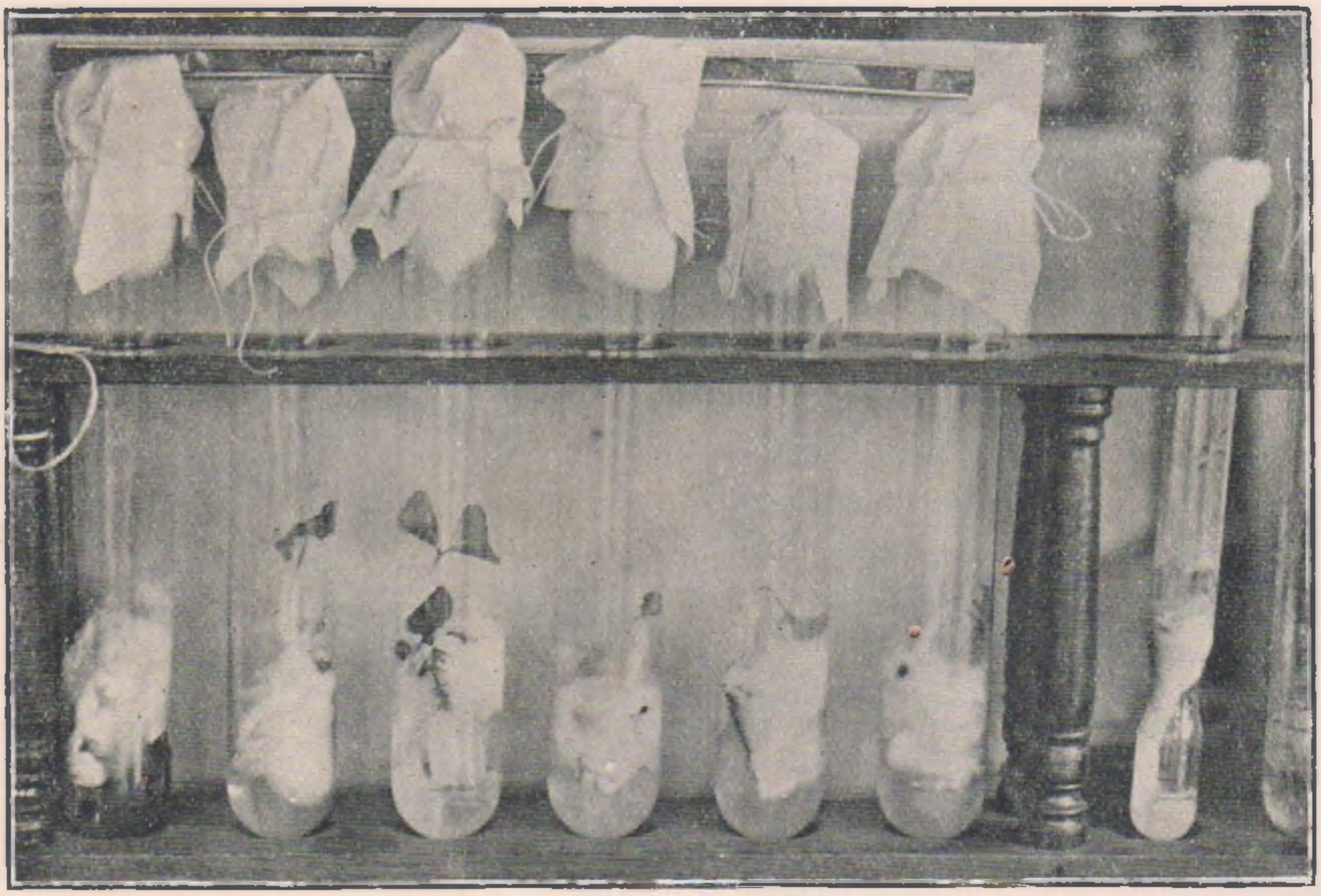

Fig. 1.—Cotton plants growing in sterile test tubes. The plants are wilting in consequence of their having been inoculated.