Attempts were made to find out if it was necessary to repeat the operations once a month to control thrips effectively. Treatments once in two months with nicotine sulphate and Gammexane were tried and compared with treatments once a month. The object of the trial was to reduce the cost of operations without loss of efficacy. Such attempts proved unwise. The yield was reduced when the intervals between operations were prolonged beyond a month. The loss thus caused was more than the saving in the cost of treatments.

The experiments detailed so far go to show that Gammexane D·025 as a dust and nicotine sulphate 0·05% as a spray are quite effective in controlling thrips. Tata's Torch Brand DDT 0·25% is in the trial stage against cardamom thrips. This insecticide is promising, but final conclusions can be drawn only after completing the experiments now in progress.

The authors feel it a pleasure to acknowledge the valuable work done by their predecessors in the Cardamom Scheme in the campaign against this serious pest on cardamoms.

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Testing Seed Viability by Chemical Means

(A biochemical method of testing seeds for germinability)

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Introduction: The standard method of testing the germination capacity of seeds is by germinating 100 seeds in either moist sand or blotting paper and counting the number that actually germinates. There are however two drawbacks in this method. One is that a period of 4 to 10 days is often needed to get the full count of seeds that germinate out of every 100 that are kept; the actual number of days required being different for different crops. The other is that in the case of certain crops and varieties, the seeds seem to have a period of "after-ripening" or dormant stage, during which the germination is poor and often nil. In such cases, the ordinary germination tests become inconclusive and valueless.

In recent times various attempts have been made to assess seed viability more rapidly without having to use the ordinary germination tests. Organic dyes like methylene blue and indigo carmine have been tried, where dead seeds get stained and live ones remain uncoloured, and also various salts of selenium and tellurium and chemicals like dinitrobenzene and 2, 3, 5 triphenyl - tetrazolium chloride and bromide, where viable seeds get stained and non - viable ones remain uncoloured

The use of such chemicals for rapid assessment of seed viabillity has progressed considerably in recent years and the present paper is an account of tests made to see how far the chemical 2, 3, 5 triphenyltetrazolium chloride would prove useful in assessing the viability of some important grains and seeds.

Previous work and literature: The earliest record that is available of the use of a chemical for detecting seed viability is in 1876, when Dimitriewicz observed, on applying sulphuric acid to cut sections of grain seeds, that a deep rose colour developed in viable seeds within 2–5 minutes, while in poor seeds the colour appeared only after 15 minutes. Loew and Bokorny (1882) used a very dilute solution of alkaline silver nitrate for the same purpose and Molisch (1918) used silver sulphate solution. Lesage (1922) reported that viability could be tested within four hours by soaking seeds of *Lepidium sativum* in certain concentrations of potassium hydroxide. Attempts have also been made to utilise the relationship between germinative capacity and the respiratory activity of grains (Qvam, 1906) and by measuring the amount of heat given off by the seeds under conditions favourable for germination (Darsie et al, 1914) but these methods have the drawback that some special equipment is needed, for measuring respiration and temperature variations. Electrical methods were tried out by Waller (1901) and Fraser (1916) who found that "blaze current" response of seeds could be used as an indication of viability.

Fick and Hubbard (1926) reported that a correlation existed between seed viability and electrical conductivity. Besides these, a number of workers have attempted to make use of the activity of various enzymes and especially of catalase in seeds, as there was always a good deal of catalase activity when there was vitality in seeds. But these attempts were not in general very successful as tests of seed viability, due to the fact that dead seeds also contain catalase.

Another line of study was the effect of organic dyes on seeds, as for example, indigo carmine (Neljubow, 1925) which are capable of staining dead tissue, but do not penetrate living tissue. The chief difficulty in this method is that for each species the relation between the intensity of staining and germinative capacity has to be worked out separately (Mirov. 1936). Thus Sakata (1933) found that when the soaking period was too long in indigo carmine, even viable seeds got stained.

Other chemicals like para and ortho-dinitrobenzene have the property of getting reduced through the respiratory activity of living cells, into compounds that develop a characteristic colour reaction in the presence of ammonia (Gurewitsch 1935, Weise, 1937, and Dobrokhotov, 1937).

Even bacteria have been pressed into service for testing seed viability. Thus Scheurlen (Ref. Eidman 1936) is reported to have found that anthrax bacteria were able to reduce selenium and tellurium salts to form the free elements which could be readily distinguished by their colour reactions.

Sakata (1933) and Hasegawa (1935) reported satisfactory results with selenium and tellurium compounds on seeds of Japanese Cedar, cypress and pine, but Eidmann (1938) found that the selenium salts were more satisfactory. The selenium method was later modified and improved by Johnson (1947), as Hao (1939) pointed out that the selenium reaction was dependent on a number of factors such as respiration rate, temperature and sulphur content of seeds. Flemion (1936, 1938, 1941) found that a quicker assessment of viability was possible, by excising the embryos (peach, apple, pear, hawthorn etc.) and germinating them in moist peat moss or on moist filter paper in petri-dishes at room temperatures.

The chemical 2, 3, 5 triphenyl-tetrazolium chloride was synthesised (Pechmann and Runge) as long as 1894, but it was not until 1940, when Kuhn and Jorchel prepared a number of tetrazolium salts that its usefulness as an indicator of germinability was realised. Since then, a number of workers (Cottrell, 1947, Porter et al 1947, Dufrenoy and Pratt 1948, Goodsell, 1948 and Hyde 1949) have tested this compound and reported on its utility as an indicator for seed viability. The salt is colourless in aqueous solution, but in contact with soaked seeds, it gets reduced phytochemically to the insoluble, red (or carmine-coloured) triphenyl-formazan (Dufrenoy and Pratt). Microscopical examination of longitudinal freehand sections showed that the precipitate form (which indicates reducing activity) was localised at the sites of plasmodesmata and in lipidic parts of the cytoplasm. It was also observed that many other viable materials, in addition to seeds and yeast will reduce this chemical to give the staining reaction, at pH 6·9; such as the fleshy parts of apples, oranges, white and sweet potatoes; young leaves; the stigmas and ovaries of certain pollinated flowers; bull sperm and the blastoderm of hens' eggs (Mattson et al, 1947).

Material and Methods: A representative collection of seeds of important crops was first obtained, to serve as material for testing out the method, and 2, 3, 5-triphenyl-tetrazolium chloride was tried in various concentrations and soaking periods, following preliminary presoaking in

distilled water. The effect of light and darkness on the staining response of different seeds was also studied and the effect of dehusking the seed, particularly in paddy was also sought to be determined

The general method was as follows: A constant number of seeds was taken and soaked in distilled water for a specific period and then kept immersed in different strengths of the reagent 2, 3, 5 triphenyl tetrazolium chloride for a definite number of hours, six or eight hours as the case may be, according to the schedule of treatments drawn up beforehand. At the end of the period, the seeds were taken out and examined, where necessary under a hand lens or dissecting microscope, after longitudinal bisection, to note the depth of red colouration developed. The percentage of viability thus obtained was compared in each case, with actual germination tests that were started at the same time as the tetrazolium treatments.

The details of crop seeds and the treatments tried are given below:

(a) *Seeds*

(i) Paddy - Varieties G. E. B. 24, Co. 3, 4, 10, 13, 25, 26, AKP, 3, 4, 8; ADT 2, MTU. 7, 18, PTB. 10

(ii) Millets - Sorghum Co. 1, 2, 3, 5
Cumbu Co. 1, 3
Ragi Co. 1, 2
Tenai Co. 1.

(iii) Pulses - Redgram No. 37, Greengram No. 62, Blackgram No. 212, Cowpea No. C. 57

(iv) Oilseeds - Groundnut TMV. 1, 2, 3
Gingelly TMV. 1, 2, 3

(v) Cotton - MU. 1, No. 4453

(b) *Treatments:*

(i) Concentrations - 10, 20, 50, 100, 200, 500, 1000 parts per million and 0·1%, 0·2%, 0·5%, 1·0%

(ii) Husking - Paddy - intact; Cotton - intact
,, dehusked; ,, testa removed;

(iv) Soaked - (1) dry seed treated with chemical
(2) Soaked in distilled water for 1 hour before treatment in chemical
(3) Soaked in distilled water for 2 hours, 4 hours,
(4) Soaked in distilled water for 16 hours

(v) Light x Darkness.

Results: The results are presented in detail in tables in the Appendix and summarised below;

1. The chemical is capable of giving an index of viability which is closely similar to what can be determined by ordinary germination tests.

2. This result can be achieved more speedily than by ordinary germination, i. e., 24 hours' time as against four to ten days' time by ordinary methods.

3. The percentage of viability as indicated by the reagent is nearly always lower by about 3 to 4%, than the percentage obtained by straight germination tests. This can be considered as an advantage, as it gives a safer estimate in assessing the viability of seeds of improved strains that are intended for distribution to farmers.

4. Dehusking the seeds, especially in paddy, gives a higher percentage of stained seeds and lower concentrations of the reagent are sufficient, but dehusking does not seem to be essential to secure a reliable index of viability, except in the case of dormant varieties as in Co. 3 in paddy and in the case of cotton. This again may be considered as an advantage, since, if dehusking had really been necessary for a correct assessment of viability in all seeds by this chemical, the method would be too tedious for general adoption.

Further work is however, needed to settle the point whether dehusking is essential for certain crop seeds and especially dormant seeds i. e., seeds that need a certain period of "after-ripening" before they germinate.

5. The optimum soaking period in the chemical would be 8 hours, after a preliminary soaking in distilled water. Six hours in the reagent is also sufficient in some cases, but taken as a whole, an eight-hour period is preferable as a general rule.

6. Soaking in the reagent for 24 hours, as was suggested by some previous workers does not appear to be necessary, but a preliminary soaking in water is necessary.

7. Tests were also carried out to see if there is any difference in response between seeds kept in the dark and those kept in light, after treatment with the reagent. No difference was perceptible in the staining response and it may therefore be concluded that seed treatment with triphenyl tetrazolium chloride may be done in ordinary light without any extra precautions to ensure darkness.

8. A certain amount of varietal difference appears to exist, at least in paddy, in the staining response to tetrazolium chloride. This aspect needs further study on a more exhaustive basis, to assess the extent of these varietal differences in various crops and decide how far it is related to dormancy and how far such differences could be avoided or overcome.

9. In view of the high cost of the chemical, namely Rs. 21—8—0 for 5 grams, further work is also desirable to find out means of ensuring a reliable viability index with lower concentrations of the chemical than the 0·5% and 1% that are now found necessary. The use of some other chemicals, acids or oxidation-reduction reagents as adjuvants and the use of different temperatures are also possibilities that deserve exploration.

Discussion: The potentialities of tetrazolium salt as a reagent for testing viability in living tissues in general, are sufficiently promising to deserve a fuller investigation. The work of Mattson et al (1947) das shown that the enzyme systems responsible for the reduction of tetrazolium salts are present in a wide variety of living tissues. Thus Dufrenoy and Pratt (1948) utilised the reagent for testing the viability of sugarcane culms. Lakon soaked the seed for several hours in water to initiate the germination process and bisected the seed longitudinally and then treated with 1% tetrazolium for 2 to 10 hours in the dark at room temperature. Porter and his co-workers soaked rice grains in water for 16 hours, then bisected them longitudinally and treated them with 1% tetrazolium in the dark for 4 hours. A large variety of grains was tested viz., corn, wheat, barly, oats, rice, sorghum, cotton, legumes, soybean, pea, buckwheat and bahie grass; using different concentrations ½, 1 and 2% and different periods of soaking in the reagent. They concluded that the method was likely to prove useful in physiological experiments, although it was not found to be entirely dependable in the case of legumes and in sorghum. The sectioning method adopted both by Lakon and Cottrell involves considerable time and labour and in the light of subsequent work does not seem to be quite essential. Similarly it is not also necessary to keep the treated seeds in the dark, to get staining reactions comparable to actual germination tests.

Dehusking was recommended by L. Venkataratnam (1951) as necessary in rice and particularly in the case of rice varieties that have an appreciable dormant period; this aspect requires a fuller investigation before definite conclusions can be made regarding its necessity. In view of the time and labour involved in dehusking the seed without injuring the embryos it seems advisable to explore methods where dehusking can be omitted even in the case of dormant varieties where it is now considered to be necessary. Further work is also needed to settle the limits of reliability of the tetrazolium method. Thus Goodsell (1948) found the

method unreliable when applied to immature maize seed which had been exposed to frost and Porter et al (1947) found that this method needed considerable modification when used for *Paspalum notatum.*

Summary and Conclusions: The results of tests made on representative collection of crop seeds, namely rice, millets, pulses, oilseeds and cotton with the chemical 2, 3, 5-triphenyl-tetrazolium chloride are presented and discussed.

The use of this chemical is helpful is assessing seed viability more expeditiously than by the ordinary germination tests.

The lines along which further study is needed to make the fullest use of the potentialities of the chemical as a test reagent for viability in living tissues are briefly indicated.

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APPENDIX.

TABLE I.

Experiment to determine if presoaking in water is needed, before tetrazolium treatment.

	Dry seeds soaked in 1% Tetrazolium chloride for 16 hours	Treatments 16 hours in water + 8 hours in 1% Tetrazolium chloride	Control
Paddy :			
AKP 3	4%	92 (in 0·5%)	98%
AKP 4	Nil.	90 (in 0·5%)	100
AKP 5	12	86	100
AKP 8	4	96 (in 0·5%)	100
ADT 2	4	96	100
Co 3	4	44	100
Co 4	4	90 (in 0·5%)	100
Co 10	8	96	100
Co 13	4	96	100
Co 25	4	90	96
Co 26	8	96	98
GEB 24	8	90	96
Ragi :			
Co 1	46	96	100
Co 2	78	98	98
Gingelly :			
TMV 1	30	78	96
TMV 2	38	86	89
TMV 3	24	92	100
Cumbu :			
Co 1	82	94	98
Co 1	90	82	94
		92 (0.5%)	

Conclusion : Presoaking of the seeds in distilled water is necessary, to give reliable staining by subsequent soaking in tetrazolium chloride solution

Note : In this table as well as in those that follow, the figures given represent percentages of seed-viability as indicated either by actual germination in the case of "Control" or by the number of seeds that develop the carmine red strain after treatment in tetrazolium chloride.

TABLE II.

Experiment to determine the presoaking period in water that is necessary before tetrazolium treatment.

	Concentrations of triphenyl tetrazolium chloride										
	10 ppm	20 ppm	50 ppm	100 ppm	200 ppm	500 ppm	1000 ppm = (1·0%)	0·2%	0·5%	1·0%	Control
Paddy ; GEB 24 :											
(1) Dry seed in tetrazolium for 8 hours.	Nil	Nil	Nil	Nil	Nil	Nil	2	4	6	6	96%
(2) D. Water 16 hours + tetrazolium 8 hours.	24	28	40	76	92	92	92	96	98	90	96%
Co 4 :											
(1) D. Water 1 hour + tetrazolium 8 hours.	Nil	Nil	Nil	Nil	Nil	6	24	28	18	12	100%
(2) D. Water 2 hours + tetrazolium 8 hours.	Nil	Nil	Nil	Nil	Nil	4	10	10	20	2	100%
(3) D. Water 16 hours + tetrazolium 1 hour.	8	20	26	68	76	82	88	50	90	36	100%

Conclusion : A preliminary soaking in water for 16 hours followed by 8 hours in 0·5 or 1·0% solution of the reagent is best.

TABLE III (a) and (b)

Optimum concentration of Tetrazolium Chloride, for Paddy.

(The grains are soaked first in distilled water for 16 hours and then in the reagent for 8 hours and examined for red staining.)

(a) With Husk.

Concentration of reagent	10 ppm	20 ppm	60 ppm	100 ppm	200 ppm	600 ppm	1000 ppm	0·2 %	0·5 %	1·0 %	Control Germination percentage)
Strains											
1. GEB 24	24	28	40	76	92	92	96	98	96	90	96%
2. ADT 2	46	50	56	82	86	94	94	94	96	96	100%

TABLE III (a) and (b)
Optimum concentration of Tetrazolium Chloride, for Paddy.

(a) With Husk.

Concentration of reagent	10 ppm	20 ppm	50 ppm	100 ppm	200 ppm	500 ppm	1000 ppm (=1·0%)	0·2 %	0·5 %	1·0 %	Control (Germination percentage)
3. Co 3	44	40	44	44	46	44	58	48	58	44	100
4. Co 4	8	20	26	68	76	82	88	50	90	36	100
5. Co 10	84	100	100	98	94	98	100	100	96	96	100
6. Co 13	20	20	98	96	98	94	96	98	100	96	100
7. Co 25	18	20	40	50	74	84	82	92	84	90	96
8. Co 26	94	94	94	96	94	100	100	100	96	96	98
9. AKP 3	14	40	90	98	100	86	78	88	92	82	98
10. AKP 4	4	2	14	4	16	34	74	72	90	72	100
11. AKP 5	20	8	24	20	44	58	74	70	84	86	100
12. AKP 8	40	42	54	46	66	80	84	90	96	88	100
13. MTU 7	4	4	34	50	100	90	98	100	86	90	100
14. MTU 8	40	40	40	50	66	80	72	92	92	84	100
15. PTB 10	18	26	36	40	66	98	100	84	98	94	100

(b) Without Husk.

	10 ppm	20 ppm	50 ppm	100 ppm	200 ppm	500 ppm	1000 ppm	0·2 %	0·5 %	1·0 %	Control
1. GEB 24	68	70	70	80	90	94	92	96	96	96	100
2. ADT 2	54	54	60	82	92	96	96	94	100	96	100
3. Co 3	100	100	100	100	100	100	100	100	100	100	100
4. Co 4	80	62	92	72	96	96	96	94	88	90	100
5. Co 10	28	24	66	76	80	80	94	88	92	96	98
6. Co 13	14	14	72	90	94	94	88	94	96	96	100
7. Co 25	42	20	50	62	74	90	96	100	82	88	100
8. Co 26	86	30	50	64	70	86	90	90	98	90	100
9. AKP 3	94	28	60	80	82	94	88	94	94	100	100
10. AKP 4	8	...	20	6	20	40	92	78	88	72	100
11. AKP 5	24	6	28	20	56	70	80	84	84	90	100
12. AKP 8	52	48	72	68	78	78	98	92	96	88	100
13. MTU 7	10	16	58	86	88	88	88	90	94	98	100
14. MTU 8	6	8	40	60	72	74	76	76	88	84	100
15. PTB 10	20	30	34	40	70	100	100	100	100	98	100

Conclusions: (1) The optimum concentration i.e. the minimum strength of the reagent that gives the nearest approach to the ordinary germination test, lies between 0·2 and 0·5 per cent.

(2) Dehusking the grains before treating with tetrazolium chloride does not seem to be necessary; except probably in varieties that have a dormant period after harvest e.g. Co. 3. In Co 10, dehusking has not improved the percentage of staining.

(3) The germinative capacity indicated by the reagent is nearly always lower than in direct germination tests, by about 3–4 per cent.

TABLE IV (a) and (b).

Optimum Soaking Period — For Paddy.

(The seeds were soaked first in distilled water for 16 hours and then in 2, 3, 5 triphenyl-tetrazolium chloride, for various periods and examined for carmine-red staining. Three concentrations of the reagent were used in this trial.

(a) With Husk.

Concentration of reagent	Time 1 hour 0.2 %	0·5 %	1·0 %	2 hours 0·2 %	0·5 %	1·0 %	4 hours 0 2 %	0·5 %	1·0 %	6 hours 0·2 %	0·5 %	1·0 %	8 hours 0·2 %	0·5 %	1·0 %	Control (with husk) germination percentage
Strains	(Percentage of seeds — stained red)															
1. GEB 24	22	16	16	42	48	58	42	40	54	92	80	94	98	96	90	96
2. ADT 2	70	66	74	74	78	92	86	86	92	94	94	98	94	94	96	100
3. Co 3	8	12	18	8	12	18	28	22	22	34	44	44	48	58	44	100
4. Co 4	16	14	12	22	16	12	20	20	30	36	26	48	50	90	36	100
5. Co 10	40	40	38	44	40	36	42	42	40	58	62	64	100	96	96	100
6. Co 13	8	8	6	40	46	52	54	52	58	98	100	96	98	100	96	100
7. Co 25	...	...	...	...	8	10	4	12	12	4	12	22	92	84	90	96
8. Co 26	14	16	14	20	14	14	24	24	16	20	22	28	100	96	96	98
9. AKP 3	...	...	...	...	4	...	18	10	22	80	88	94	88	92	82	98
10. AKP 4	...	...	...	16	18	14	60	56	54	64	74	94	72	90	72	100
11. AKP 5	...	...	...	16	10	16	22	10	18	60	74	88	70	84	86	100
12. AKP 8	...	...	...	4	8	16	16	20	16	62	96	88	90	96	88	100
13. MTU 7	...	...	...	20	12	6	10	8	8	12	22	8	100	86	90	100
14. MTU 8	...	...	2	10	14	14	20	18	14	20	20	24	92	93	84	100
15. PTB 10	...	4	6	8	8	14	22	28	18	20	32	34	84	98	94	100

(b) Without Husk.

Concentration of reagent	Time 1 hour 0·2 %	0·5 %	1·0 %	2 hours 0·2 %	0·5 %	1·0 %	4 hours 0·2 %	0·5 %	1·0 %	6 hours 0·2 %	0·5 %	1·0 %	8 hours 0·2 %	0·5 %	1·0 %	Control (without husk)
Strains																
1. GEB 24	16	34	30	44	52	60	72	76	72	78	72	72	96	96	96	100
2. ADT 2	20	8	...	58	54	32	80	80	76	92	86	82	94	100	96	100
3. Co 3	58	82	58	88	96	88	92	98	90	94	98	96	100	100	100	100
4. Co 4	28	12	32	48	42	60	78	80	82	84	86	88	94	88	90	100
5. Co 10	2	8	4	12	34	34	62	50	62	80	88	94	88	92	96	100
6. Co 13	14	6	6	46	52	62	92	92	86	94	94	88	94	96	96	100
7. Co 25	...	2	...	40	40	56	54	56	60	90	80	86	100	82	88	100
8. Co 26	30	38	8	54	70	52	74	82	80	86	98	88	90	98	90	100
9. AKP 3	14	40	34	44	66	82	94	88	96	94	94	100	94	94	100	100
10. AKP 4	...	...	...	26	34	22	58	72	62	62	80	66	78	88	72	100
11. AKP 5	...	...	...	26	28	10	60	72	66	74	82	88	84	84	90	100
12. AKP 8	...	2	2	2	2	2	80	82	70	88	96	82	92	96	88	100
13. MTU 7	8	...	...	42	56	48	72	86	80	86	88	82	96	94	98	100
14. MTU 8	2	...	...	22	40	32	40	78	74	76	86	78	76	88	84	100
15. PTB 10	...	4	6	14	20	36	24	32	60	35	46	70	100	100	98	100

Conclusions: (1) A soaking period of 8 hours in the reagent at concentrations of 0·2 or 0·5% may be taken as optimum for paddy varieties.

(2) Dehusking the grains is not essential, except in certain varieties, as in Co 3.

TABLE V.

Optimum soaking period for different crops.

	Concentrations %	Hours: 1 hour 0·2	0·5	1·0	2 hours 0·2	0·5	1·0	4 hours 0·2	0·5	1·0	6 hours 0·2	0·5	1·0	8 hours 0·2	0·5	1·0	Control	Remarks
1.	Black gram 212	80	66	76	50	66	46	58	54	50	80	80	84	68	64	72	98	(Soaked in D.W. for 1 hour and then in reagent for varying periods)
2.	Cow pea Co 57	16	18	18	38	44	74	40	36	38	54	56	52	54	58	56	98	
3.	Red gram 37	10	22	26	10	20	26	28	52	80	36	46	68	70	60	62	94	
4.	Green gram 62	62	60	68	66	66	70	44	68	70	50	68	74	96	96	98	100	6 hours in reagent seem to be sufficient.
5.	Cholam Co 1	20	24	28	26	36	40	28	48	52	42	60	60	92	98	90	98	Soaked first in D. Water for 16 hours and then in reagent for varying periods.
6.	Cholam Co 2	60	64	74	64	78	86	86	96	86	94	92	94	98	100	100	100	
7.	Cholam Co 3	8	14	18	12	20	24	72	74	60	82	70	64	100	100	96	100	
8.	Cholam Co 6	14	18	24	16	24	24	62	74	80	66	70	80	82	90	96	98	
9.	Ragi Co 1	42	44	44	52	54	56	60	60	64	62	72	62	100	96	96	100	
10.	Ragi Co 2	38	42	42	70	78	62	88	92	84	88	96	94	88	98	98	98	
11.	Cumbu Co 1	34	38	36	60	82	72	88	86	72	88	94	90	88	92	94	98	
12.	Cumbu Co 3	8	18	22	28	30	34	46	38	38	46	40	38	88	86	90	92	
13.	Tenai Co 1	18	18	16	44	38	24	60	42	40	64	48	40	86	92	82	94	
14.	Gingelly TMV 1	2	6	10	8	14	16	42	54	64	60	82	84	52	78	78	96	
15.	Gingelly ,, 2	4	10	14	8	14	20	24	24	22	86	80	82	86	82	86	89	
16.	Gingelly ,, 3	0	4	8	4	8	8	16	18	14	90	84	92	86	86	92	100	
17.	Groundnut TMV 1	12	28	28	36	80	64	64	88	96	76	92	96	72	96	100	100	
18.	Groundnut ,, 2	60	60	68	100	96	96	100	100	100	100	100	100	100	100	100	100	4 hours soaking in reagent seems to be enough.
19.	Groundnut ,, 3	56	88	92	72	100	100	88	100	100	96	100	100	98	100	100	100	
20.	Cotton K 5	12	20	28	48	84	84	84	92	88	92	92	88	92	92	88	96	1950-51 seed } Presoaked first
21.	Cotton MU 1	8	8	24	8	8	28	8	24	32	44	44	60	48	48	64	64	1949-50 seed } in D. W. for 16
22.	Cotton ,,	4	4	12	12	12	28	36	32	36	44	44	66	92	92	92	96	1950-51 seed } hours and then
23.	Cotton 4463	12	16	16	20	24	28	44	44	56	76	84	72	76	88	92	100	1949-50 seed } in reagent for
24.	Cotton ,,	0	4	8	4	16	20	32	56	48	32	64	76	48	68	80	100	1950-51 seed } varying periods.

Conclusions: (1) In general, 8 hours of treatment with reagent solution is needed to get maximum staining.

(2) The exceptions are groundnut varieties TMV 2 and TMV 3 where 4 hours appear to be sufficient and Black-gram where 6 hours were found sufficient. More work is however needed to settle the optimum soaking periods for these crop plants.

TABLE VI

Optimum soaking period—For cotton

Varieties		Concentration 500 ppm		With testa 0·1%		0·2%		0·5%		1·0%		Control.	Testa removed (Dehusked) 500 ppm		1000 ppm = 0·1%		0·2%		0·5%		1·0%		Control
		Hours 6	8	6	8	6	8	6	8	6	8		6	8									
Cotton K.5	(1950 seed)	80	84	92	92	92	92	92	92	88	88	96	72	80	76	88	68	84	80	100	88	100	100
Cotton MU 1	(1949 seed)	16	24	20	28	44	48	44	48	60	64	88	68	96	76	100	80	100	80	100	84	100	100
Cotton MU 1	(1950 seed)	24	84	24	84	44	92	44	92	66	92	96	68	88	76	92	84	96	88	100	92	100	100
4562	(1949 seed)	56	68	68	72	76	76	84	88	72	82	100	60	80	72	84	72	92	100	100	92	100	100
4463	(1950 seed)	36	36	36	60	32	48	64	68	76	80	100	96	100	100	100	76	100	76	100	100	100	100

Conclusions:— (1) Removal of testa gives a higher percentage of staining with lower concentrations of reagent.

(2) Old seed has a lower percentage of germination than more recently harvested e.g. 1949 seed as compared to 1950 seed.

(3) 8 hours' soaking is preferable for cotton also.

TABLE VII

Effect of light and darkness on staining effect

Period	1 hour			2 hours			4 hours			6 hours			8 hours			Control
Concentration%	0·2	0·5	1·0	0·2	0·5	1·0	0·2	0·5	1·0	0·2	0·5	1·0	0·2	0·5	1·0	
Paddy (with husk)																
MTU 7, Light	0	0	0	20	12	6	10	8	8	12	22	8	100	86	90	100
,, Dark	14	16	16	18	18	18	18	20	18	18	20	18	24	24	24	100
AKP 3, Light	...	...	...	...	4	...	18	10	22	80	88	94	88	92	82	98
,, Dark	6	10	10	12	16	12	18	24	12	36	22	22	34	34	32	
Co. 3, Light	8	12	18	8	12	18	28	22	22	34	44	44	48	58	44	100
,, Dark	6	2	2	34	34	50	34	38	50	60	58	58	86	88	98	
GEB. 24, Light	...	...	...	...	...	...	42	40	54	92	80	94	98	96	90	96
,, Dark	...	...	...	...	...	...	40	42	28	46	46	48	74	72	72	
Paddy (without husk)																
MTU. 7, Light	8	...	...	42	56	48	72	86	80	86	88	82	96	94	98	100
,, Dark	8	10	20	26	30	40	44	44	80	62	62	88	76	68	96	
AKP. 3, Light	18	40	34	44	66	82	94	88	96	94	94	100	94	94	100	100
,, Dark	60	52	50	84	80	68	90	84	70	100	96	84	100	100	96	
CO. 3, Light	58	82	58	88	96	88	92	98	90	94	98	96	100	100	100	100
,, Dark	4	2	...	52	64	78	78	74	90	92	88	96	98	94	98	
GEB. 24, Light							72	76	72	78	72	72	96	96	96	105
,, Dark							58	64	66	76	72	68	82	72	76	

Mean values of 54 observations.

	Light	Dark
With husk ...	38·3%	32·4%
Without husk ...	76·7%	66·7%

Conclusions :— The seeds can well be treated in ordinary light with the reagent, and no advantage is gained by keeping them in the dark. On the other hand, staining is slightly better in light.

TABLE VIII

Optimum concentration of tetrazolium for different crops

	Concentrations	10 ppm		20 ppm		50 ppm		100 ppm		200 ppm		500 ppm		0·1%		0·2%		0·5%		1·0%		Control
	Time	6 hrs.	8 hrs.	6	8	6	8	6	8	6	8	6	8	6	8	6	8	6	8	6	8	
1	Blackgram	212	72	72	72	68	70	68	74	68	66	70	80	68	78	76	80	68	80	64	84	72	98
2	Cowpea	C57	32	16	22	32	18	26	58	52	26	44	40	50	36	46	54	54	56	68	52	56	98
3	Redgram	37	...	10	6	12	8	12	8	60	10	42	10	52	8	58	36	70	46	60	68	62	94
4	Greengram	62	26	92	48	92	56	94	54	92	46	96	54	96	68	88	50	96	68	96	74	98	100
5	Cholam	Co. 1	4	20	4	58	10	62	14	66	30	72	44	78	40	92	42	92	60	98	60	90	98
6	,,	Co. 2	50	96	58	96	62	98	64	96	60	96	88	98	92	98	94	98	92	100	94	100	100
7	,,	Co. 3	32	74	36	80	38	86	64	96	54	96	72	100	76	92	82	100	70	100	64	96	100
8	,,	Co. 6	26	42	32	48	36	54	40	60	24	62	54	74	54	80	66	82	70	90	80	96	98
9	Ragi	Co. 1	18	36	20	40	54	60	60	74	76	78	76	92	64	94	62	100	72	96	62	96	100
10	,,	Co. 2	26	38	34	48	46	52	60	64	74	76	84	88	76	92	88	88	96	98	94	98	98
11	Cumbu	Co. 3	22	24	34	38	58	60	66	68	86	88	88	88	88	92	88	88	94	92	90	94	98
12	,,	Co. 3	4	8	4	8	14	18	26	42	28	62	36	62	40	74	46	88	40	86	38	90	92
13	Tenai	Co. 1	12	32	14	38	20	54	40	60	54	92	60	84	60	86	64	86	48	92	40	82	94
14	Gingelly	TMV 1	18	22	20	28	26	30	32	38	34	42	40	84	44	52	60	52	82	78	84	78	96
15	,,	,, 2	14	20	18	26	26	34	30	46	54	64	84	86	78	80	86	86	80	82	82	86	89
16	,,	,, 3	34	36	44	46	46	52	60	66	70	72	74	78	82	84	90	86	84	86	92	92	100
17	Groundnut	,, 1	12	36	28	32	28	36	44	68	20	72	40	84	76	88	76	72	92	96	96	100	100
18	,,	,, 2	40	48	20	24	28	40	52	56	92	96	92	92	96	96	100	100	100	100	100	100	100
19	,,	,, 3	40	48	16	20	32	36	40	76	80	96	96	98	96	100	96	98	100	100	100	100	190
20	Cotton	K. 5											80	84	92	92	92	92	92	92	88	88	96
21	,,	MU. 1											24	84	24	84	44	92	44	92	56	92	96
22	,,	4463			Lower concentrations not tried								36	36	36	60	32	48	64	68	76	80	100
23	,,	4463											56	68	68	72	76	76	84	88	72	92	103

Conclusions :— (1) A concentration of 1·0% solution of 2, 3. 5 triphenyl-tetrazolium chloride would be the best for general adoption, although 0·5% seems to be sufficient in some cases.

(2) Soaking for 8 hours in reagent is preferable to 6 hours.