THE RELATION BETWEEN LOSS IN VIABILITY AND SEED-BORNE MICRO-FLORA IN RICE

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Introduction

When rice seeds are stored under wet and humid conditions, there is an increase in their moisture content and they rapidly lose their viability. It is generally held that such a loss in viability is brought about by the activity of moulds, but precise data to show the relationship between loss in viability and mould activity are lacking. Therefore, an investigation was carried out to determine the relationship, if any, between loss in viability of rice seeds under moist conditions of storage and mould activity.

Review of Literature

According to Johnson (1947) moulds spores found on surface of grains germinate and grow at critical moisture levels of the grains, particularly when air temperatures are warm; the fungi attack the carbohydrate, fats and proteins of grains by means of enzymes they secrete, resulting in raise of fat acidity, deterioration in quality and decrease in germinability. Loss in viability accompanied by increase in fat acidity was observed by Carter and Young (Johnson, *loc. cit.*) in wheat seeds but moulds were apparently not involved in this phenomenon. Crocker and Barton have reported (1953) that cotton seeds exposed to high moisture in field or to high moisture and temperature in storage showed marked correlation between the percentage of free fatty acid in the seed and viability. Sahadevan (1953) concluded that surface-borne moulds could not be the cause of deterioration in viability of rice seeds, since seeds treated with Agrosan GN also lost their viability in storage. On the contrary, Ramiah and Padmanabhan (1948) and Padmanabhan (1956) have shown that some fungicides like Cuprocide, Phygon and Spergon do help in preserving the viability of rice seeds stored under humid conditions.

Materials and Methods

Experimental.—Seeds of rice varieties were stored over different levels of relative humidity at room temperature. Over some of the levels the seeds lost their viability. The changes taking place in the moisture content, in germinability and the mould population carried in the seeds were recorded at periodical intervals. The investigation was carried out during the period, April–November, 1949.

The seeds of the following five varieties, T. 90, T. 812, T. 412, T. 1145 and T. 1242 were thoroughly sun-dried and their moisture percentage determined by standard methods. A sample of four oz. of seed of each variety was stored in May 1949 in large desiccators maintaining approximately 10, 25, 50, 75, 90 and 100% relative humidity levels over sulphuric acid dilutions. Fortnightly readings on the moisture content of the seeds were made till the end of July but afterwards the readings were taken only once a month. One hundred seeds of known moisture content were weighed and stored separately in the desiccators in small glass specimen tubes covered with muslin cloth. Variations in the moisture content of the seeds of each variety were calculated from the variations in the weight of the 100 weighed seeds determined at the stated intervals.

The germination percentage of the sun-dried seeds was nearly 100% in all cases before storage.

Four observations were taken on the germinability of the seeds during storage on:

6–6 to 12–6,
9–7 to 13–7,
4–8 to 7–8,
24–9 to 3–10.

The germination of the seeds were recorded on duplicate samples of 100 seeds drawn from the desiccators.

Isolation experiments to determine the nature and the type of microflora present internally were carried out on 5/5 to 16/5, (just prior to storage), 7/6 to 16/6, 19/7 to 27/7, 9/9 to 14/9 finally on 18/11 to 22/11. The determination of internally borne microflora were carried out with a sample of 100 seeds. The seeds were washed thoroughly in 10 ml. of sterile water, left to stand in the water for an hour, washed in a single change of sterile water, surface sterilised for a minute and a half in $HgCl_2$ of 1 : 1000 strength, washed once again in four changes of sterile water, and sown in thin Oatmeal agar plates. A week after sowing observations were recorded on the germination of the sterilized seeds and then fungus or fungi, if any, growing from the seeds were identified.

RESULTS

1. *Change in moisture content of seeds.*—The moisture contents of the sun-dried seeds of the five varieties were 13·5, 13·0, 12·5, 11·5 and 10% respectively, in T. 90, T. 812, T. 412, T. 1242 and T. 1145. Within a fort-

night of storage over the different R.H. levels when the first reading was taken the moisture content of the seeds attained equilibrium with the R.H. of storage. Further changes recorded were not large. When stored over 10, and 25% R.H. levels, the moisture content decreased in all varieties. Over 50% R.H. level also, there was a slight fall in the moisture content in the seeds of all varieties except T. 90, in which there was a slight increase. Over 75, 90 and 100% R.H. levels, the moisture content of the seeds of all varieties increased appreciably. The maximum increase recorded was in T. 90 (Table I).

2. *Relation between moisture content and germinability.*—As discussed more fully below, it may generally be said that increase in the moisture content of sun-dried seeds is sooner or later followed by deterioration in the germinability (Table I).

3. *Relation between loss in germinability and mould activity.*—There was no loss in viability of seeds stored over 10, 25, 50% R.H. levels. Over 75, 90 and 100% R.H. levels, loss in viability was seen to occur in all varieties sooner or later during the seven months of storage. Over 75% R.H. loss in viability was noticed in July test, two months after storage in T. 90, in August in T. 812, and in September only in other varieties. No moulds were recovered from any seed either in the second, third or even in the seventh month, when the seeds of some varieties had deteriorated appreciably (Table II).

Over 90% R.H. level, loss in viability was seen in June in T. 90 and in July in the other four varieties, but most prominently in T. 812. In the isolation test made in November there was no viable seeds left in any variety. Still no moulds were recovered from the seeds in any of the four isolation tests made in the second, third, fifth and seventh month of storage (Table III).

Over 100% R.H. level the seeds had deteriorated considerably even by the end of the first month of storage. In the third month there was practically no viable seed in any variety. But in the isolation tests made, no moulds were present in the seeds. In the isolation test made in the fifth month of storage, a couple of months after loss in viability had been recorded, *Penicillium* sp. could be isolated from 42, 34, 26, 20 and 4% of seeds of T. 812, T. 1242, T. 1145, T. 90 and T. 412, respectively. In the last isolation test made in the seventh month of storage, all the seeds tested were found to be colonised by *Penicillium* sp. (Table IV).

The isolation tests showed that there was an internal flora of a few species of fungi carried in almost all rice seeds (Padmanabhan, 1949). The population of internal flora remained unaltered during storage over 10, 25 and 50 per cent. R.H. levels (Table V, for results obtained over 10% R.H. of storage), *i.e.*, in the seeds which did not lose their viability. In the seeds

TABLE I

Showing the changes taking place in the moisture contents of sun-dried rice seeds when stored over different humidity levels during May–October 1949 and the germination per cent of the seeds during the same period recorded in duplicate sets of 100 seeds each

Month of testing	Germination per cent and moisture content (percentage) of seeds during storage over different relative humidity levels from May–October 1949					
	Relative humidity of storage					
	10 per cent	25 per cent	50 per cent	75 per cent	90 per cent	100 per cent
	T. 90 (*Original moisture content*—13·5 *per cent*)					
June ..	93·5	96·0	97·0	92·0	78·0	21·0
July ..	89·5	95·0	95·0	28·0	32·0	2·0
August ..	87·0	92·0	93·0	4·0	7·0	0·0
October ..	87·0	93·0	89·0	0·0	0·0	0·0
	(10·1–10·9)	(10·8–11·4)	(13·9–14·4)	(16·3–16·8)	(16·9–17·4)	(18·8–19·8)
	T. 812 (*Original moisture content*—13·0 *per cent*)					
June ..	99·0	97·0	96·0	97·0	94·0	12·0
July ..	99·0	97·0	98·0	88·0	39·0	0·0
August ..	98·0	95·0	94·0	48·0	5·0	0·0
October ..	97·0	94·0	96·0	19·0	0·0	0·0
	(9·7–10·8)	(10·1–11·1)	(12·0–12·4)	(14·0–14·7)	(14·6–15·3)	(16·8–17·9)

T. 412 (*Original moisture content*—12·5 *per cent*)

June	..	98·5	97·0	96·0	99·0	96·0	51·0
July	..	100·0	99·0	96·0	99·0	88·0	27·0
August	..	98·0	98·0	97·0	93·0	65·0	0·0
October	..	97·0	95·0	97·0	77·0	24·0	0·0
		(9·4–10·4)	(10·0–10·7)	(11·7–12·1)	(14·0–14·5)	(14·5–15·2)	(16·8–17·8)

T. 1242 (*Original moisture content*—11·5 *per cent*)

June	..	97·0	93·0	97·0	95·0	98·0	66·0
July	..	95·0	98·0	95·0	95·0	84·0	25·0
August	..	95·0	96·0	95·0	83·0	47·0	0·0
October	..	95·0	95·0	95·0	62·0	6·0	0·0
		(8·2–9·3)	(8·6– 9·5)	(10·4–10·9)	(12·7–13·3)	(13·6–14·2)	(15·9–17·0)

T. 1145 (*Original moisture content*—10·0 *per cent*)

June	..	99·0	97·0	96·0	99·0	99·0	44·0
July	..	99·0	99·0	100·0	100·0	91·0	10·0
August	..	99·0	97·0	94·5	97·0	58·0	0·0
October	..	97·0	100·0	99·0	85·0	6·0	0·0
		(6·8–7·7)	(7·2–8·1)	(8·1–8·5)	(11·3–11·9)	(11·8–12·5)	(14·3–15·4)

Note.—Figures in brackets indicate variation in moisture content during storage.

TABLE II

Showing the germination and the fungi isolated in 4 isolation tests made with seeds of 5 varieties of rice stored over 75% relative humidity level during 7 months of storage

Storage period in months	Germination per cent of seeds	Percentage of seeds observed with fungi	Total No. of fungi isolated from 100 seeds	*Trichoconis padwickii*	Other fungi†	Unidentified species*	Remarks
T. 90 (*Original moisture content*—13·5 *per cent*; *moisture content during storage*—16·3–16·8 *per cent*)							
2	92	56	66	32	14	20	Out of 80 seeds only
3	28	63	68	58	7	3	
5	4	..	..	..	..	..	No fungus growth; seedlings collapsed and died after germination
7	0	10	10	6	4	..	..
T. 812 (*Original moisture content*—13·0 *per cent*; *moisture content during storage*—14·0–14·7 *per cent*)							
2	98	100	100	78	4	18	
3	50	100	100	100	..	..	
5	16	48	48	24	..	24	
7	0	36	36	32	2	2	
T. 412 (*Original moisture content*—12·5 *per cent*; *moisture content during storage*—14·0–14·5 *per cent*)							
2	96	100	102	70	12	20	
3	94	100	118	80	31	7	
5	..	..	..	..	..	..	
7	60	72	74	15	12	47	

T. 1242 (*Original moisture content*—11·5 *per cent*; *Moisture content during storage*—12·7–13·3 *per cent*)

2	96	82	88	62	14	12	
3	92	100	100	45	..	55	Fungal growth from 55 seeds could not be indentified as plates became contaminated
5	44	36	36	36	..	..	
7	28	14	14	4	10	..	

T. 1145 (*Original moisture content*—10·0 *per cent*; *moisture content during storage*—11·3–11·9 *per cent.*)

2	100	86	87	52	26	9
3	100	100	112	82	14	16
5	64	84	88	60	12	16
7	38	54	54	34	6	14

* Unidentified fungi include growth from seeds which fall away from medium before the growth established themselves on the medium.

† Other fungi include *Helminthosporium oryzæ*, *Curvularia lunata*, *Pseudocercospora* sp., *Cephalosporium* species.

TABLE III

Showing the germination and fungi isolated in 4 isolation tests made with seeds of 5 varieties of rice stored over 90% R.H. level during 7 months of storage

Storage period in months	Germination per cent of seeds	Percentage of seeds observed with fungi	Total No. of fungi isolated from 100 seeds	*Trichoconis padwickii*	Other fungi†	Unidentified species*
T. 90 (*Original moisture content*—13·5 *per cent*; *moisture content during storage*—16·9–17·4 *per cent*)						
2	80	80	83	75	8	2
3	12	34	34	32	..	..
5	0	22	22	18	2	2
7	0	20	24	16	6	..
T. 812 (*Original moisture content*—13·0 *per cent*; *moisture content during storage*—14·6–15·3 *per cent*)						
2	92	94	94	66	6	22
3	10	84	84	84	..	..
5	0	70	70	68	..	2
7	0	90	90	90	..	..
T. 412 (*Original moisture content*—12·5 *per cent*; *moisture content during storage*—14·5–15·2 *per cent*)						
2	94	100	106	56	26	24
3	85	100	100	70	3	27
5	8	36	36	28	2	6
7	0	56	56	54	..	2

T. 1242 (*Original moisture content*—11·5 *per cent* ; *moisture content during storage*—13·6–14·2 *per cent*)

2	98	100	100	46	22	32
3	58	85	86	48	16	22
5	2	56	56	36	6	14
7	0	32	24	2	4	2

T. 1145 (*Original moisture content*—10·0 *per cent* ; *moisture content during storage*—11·8–12·5 *per cent*)

2	100	84	84	56	4	24
3	72	92	94	70	10	14
5	6	46	46	44	2	..
7	0	42	42	36	..	6

* Unidentified fungi include growth from seeds which fall away from medium before the growths established themselves on the m.

† Other fungi include *Helminthosporium oryzae*, *Curvularia lunata*, *Pseudocercospora* sp., *Cephalosporium* species.

TABLE IV

Showing the germination and the fungi isolated in 4 isolation tests made with seeds of 5 varieties of rice stored over 100% R.H. level during 7 months of storage

Storage period in months	Germination per cent of seeds	Percentage of seeds observed with fungi	Total No. of fungi isolated from 100 seeds	*Trichoncois padwickii*	Other fungi†	*Penicillium* species	Unidentified fungi*
T. 90 (*Original moisture content*—13·5 *per cent* ; *moisture content during storage*—18·8–19·8 *per cent*)							
2	21	70	70	39	3	..	28
3	2	20	20	20	..	..	..
5	0	20	20	..	..	20	..
7	0	100	100	..	..	100	..
T. 812 (*Original moisture content*—13·0 *per cent* ; *moisture content during storage*—16·8–17·9 *per cent*)							
2	12	96	96	76	12	..	8
3	0	69	69	58	..	..	11
5	0	42	42	..	..	42	..
7	0	100	100	..	..	100	..
T. 412 (*Original moisture content*—12·5 *per cent*; *moisture content during storage*—16·8–17·8 *per cent*)							
2	51	97	97	55	16	..	26
3	27	60	60	45	..	..	15
5	0	4	4	..	..	4	..
7	0	100	100	..	..	100	..

T. 1242 (*Original moisture content*—11·5 *per cent* ; *moisture content during storage*—15·9–17·0 *per cent*)

2	60	80	84	56	10	..	18
3	5	38	38	38	..	..	..
5	0	34	34	..	..	34	..
7	0	100	100	..	..	100	..

T. 1145 (*Original moisture content*—10·0 *per cent* ; *moisture content during storage*—14·3–15·4 *per cent*)

2	44	82	82	60	6	..	16
3	10	18	18	15	..	..	3
5	0	26	26	..	..	26	..
7	0	100	100	..	..	100	..

* Unidentified fungi include growth from seeds which fall away from medium before the growths established themselves in the medium.

† Other fungi include *Helminthosporium oryzæ*, *Curvularia lunata*, *Pseudocercospora* sp., *Cephalosporium* species.

TABLE V

Showing the germination and the fungi obtained in 4 isolation tests made with rice seeds of 5 varieties stored over 10% R.H. level during 7 months of storage (similar results were obtained with 25 and 50% R.H. levels)

Storage period in months	Germination per cent of seeds in isolation tests on sterile media	Percentage of seeds observed with fungi	Total No. of fungi isolated from 100 seeds	*Trichoconis padwickii*	Other fungi‡	Unidentified fungi*
T. 90 (*Original moisture content*—13·5 *per cent* ; *moisture content during storage*—10·1–10·9 *per cent*)						
2	87	53†	55	41	16	8
3	88	100	102	82	12	8
5	80	74	76	48	4	24
7	88	88	90	82	4	4
T. 812 (*Original moisture content*—13·0 *per cent* ; *moisture content during storage*—9·7–10·8 *per cent*)						
2	96	86	92	76	12	4
3	99	100	106	92	12	2
5	..	..	..	..	..	..
7	88	100	102	90	12	..
T. 412 (*Original moisture content*—12·5 *per cent* ; *moisture content during storage*—9·4–10·4 *per cent*)						
2	100	100	100	70	30	..
3	96	100	120	82	12	26
5	..	..	..	..	..	..
7	92	98	98	82	16	..

T. 1242 (*Original moisture content*—11·5 *per cent* ; *moisture content during storage*—8·2–9·3 *per cent*)

2	98	89	96	56	32	8
3	100	100	116	66	24	26
5	100	95	105	53	30	22
7	96	100	108	84	18	6

T. 1145 (*Original moisture content*—10·0 *per cent* ; *moisture content during storage*—6·8–7·7 *per cent*)

2	98	89	89	62	12	15
3	100	98	99	68	2	29
5	..	..	..	..	..	..
7	96	100	108	84	18	6

* Unidentified fungi include growth from seeds which fall away from medium before the growths established themselves on the medium.

† Out of 80 seeds only.

‡ Other fungi include *Helminthosporium oryzæ*, *Curvularia lunata*, *Pseudocercospora* sp., *Cephalosporium* species.

stored over 75, 90 and 100 % R.H. the flora gradually failed to appear in the seeds which had lost their viability (Tables II, III and IV).

DISCUSSION AND CONCLUSIONS

Loss in viability of rice seeds was found to follow absorption of moisture by the sun-dried seeds during storage. During the first three months of storage no moulds could be isolated from any of the seed samples, though deterioration in viability had set in from the first month in some cases. At the end of the seventh month when there was a considerable deterioration in the seeds of all varieties over 75 and 90% R.H., no moulds were found in the seeds stored over these levels. Over 100% R.H. there was practically no germination in the seeds after three months of storage, but in the isolation test made during this period, no moulds were present in the seeds. However, in the test made in the fifth month, 4% of seeds in T. 412, 20% in T. 90, 26% in T. 1145, 34% in T. 1242, and 42% in T. 812 yielded moulds on isolation. In the last test moulds could be isolated from all the seeds tested. Therefore, it may be concluded that the moulds began colonising the seeds between the third and fifth month of storage long after the germinability of the seeds were lost due to other causes. Loss in viability had preceded mould colonisation in the seeds stored over 100% R.H.

The normal internal flora also deteriorated along with the embryo when stored over high relative humidity levels.

Thus under high relative humidity of storage, loss of viability sets in gradually after absorption of moisture by rice seeds without the intervention of moulds. Increase in fat acidity is known to follow absorption of moisture by seeds (Carter and Young, *loc. cit.*, and Crocker and Barton, *loc. cit.*). Under normal germination in the presence of sufficient water, fatty digestion goes on *pari-passu* with increased metabolism of the growing embryo but when only lipolytic and other enzymatic activity are stimulated by increased moisture content without corresponding utilisation, the accumulation of fatty acid, etc., may prove toxic to the embryo. Further detailed investigations on the biochemical changes which take place inside the rice seeds following absorption of moisture under high humidity levels are necessary to throw more light on loss in viability of rice seeds over humid conditions of storage.

Only a few fungicides are effective in preserving the viability of rice seeds under humid storage condition. Whether the few effective fungicides interfere with the biochemical and enzymatic activity set up inside rice seeds when they absorb moisture has to be investigated to obtain an understanding of this aspect of the problem.

The experiment was carried out with small samples in the laboratory. Under bulk storage, the conditions and factors influencing deterioration may be somewhat different.

Summary

The changes taking place on the population of fungi internally borne in rice seeds during storage over relative humidity levels of approximately 10, 25, 50, 75, 90 and 100% were studied. Over 10, 25, and 50 % relative humidity levels there was a decrease in moisture content, but there was no deterioration in germinability within the period studied.

During storage over relative humidity levels of 75, 90 and 100% there was deterioration in germinability of seeds following increase in their moisture content.

No moulds could be isolated from the seeds stored over 75 and 90% relative humidity even after they had lost their vaibility during the course of the tests. Moulds were, however, isolated from seeds which had lost their viability during the storage over 100% R.H. level.

Deterioration in germinability was followed by decrease and disappearance of the normal internally borne fungi and this was followed by colonisation of the rice grain by principally *penicillia* in the case of seeds stored over water (*i.e.*, over 100% R.H.).

It is concluded that mould activity has no direct relation to the inactivation of embryo in rice seeds in storage under wet and humid conditions.

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