THE EFFECT OF STORAGE CONDITION ON VIABILITY OF PEANUT *(Arachis hypogaea L.)* SEEDS IN RELATION TO ITS GENETIC CONSERVATION

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Abstract

Storage experiments were carried out on peanut *(Arachis hypogaea L.)*. The seeds were stored under various storage conditions for a period from 2 hours to 4.5 months sealed in aluminium laminated packets. Temperature ranged from 4 to 55°C and moisture content 11 — 17 per cent. Seed stored at 35°C were mostly killed after 77 days of storage. Seeds stored at 4°C or 15°C and moisture content from 11 to 16 per cent did not deteriorate very rapidly over approximately 4.5 months. Storage at a temperature of 55°C and 17 per cent moisture content for 15 hours produced sigmoid curve, when germination percentage plotted against storage time.

Viability equations were derived for peanut and the constant Kv, C1 and C2 were calculated, was large. The significance of this is discussed.

Ringkasan

Telah dilakukan penelitian tentang pengaruh keadaan penyimpangan benih kacang tanah *(Arachis hypogaea* L.) terhadap viabilitasnya.

Tujuan dari penelitian ini ialah untuk mengetahui sejumlah mana keadaan lingkungan penyimpanan berpengaruh terhadap daya simpan (storage life) dan untuk memprediksikan daya simpan benih tersebut.

Untuk maksud tersebut dilakukan penyimpanan benih kacang tanah pada jangka penyimpanan berkisar antara 2 jam sampai dengan 4,5 bulan di dalam kantung-kantung aluminium yang ditutup rapat.

Temperatur penyimpanan berkisar antara 4 sampai dengan 55°C dengan kadar air benih berkisar antara 11 — 17%.

Rangkuman hasil penelitian tersebut ialah :

Benih yang disimpan pada keadaan penyimpanan dengan temperatur 35°C hampir semuanya mati setelah disimpan selama 77 hari.

Benih dengan kadar air benih berkisar antara 11 — 16% pada temperatur penyimpanan antara 4 — 15°C tidak memperlihatkan penurunan viabilitasnya secara cepat setelah disimpan 4,5 bulan.

Penyimpanan selama 15 jam pada temperatur 55°C dan KA benih 17% menghasilkan kurve ⇆ (sigmoid) bila persentase perkecambahan dihubungkan dengan temperatur penyimpanan.

Rumus viabilitasnya menunjukkan bahwa konstanta Kv, C1, C2 adalah besar.

1. Introduction

Legumes are regarded as an important sources of vegetable protein in Indonesia. Among the grain legumes soybean and peanuts are widely cultivated and they occupy a rather large area. The average yield of both crops in Indonesia are very low when compared to other countries. The important factors which affect yields are seed supply, pests and diseases and the cultural practices of farmers. Very rarely can farmers

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obtain good seeds. Low germinability of seeds naturally results in poor stand of the crop. Viability and germination capacity of the seed are important in attaining satisfactory stands (Wayne et al., 1973). The seed viability can drop very quickly during storage indicating the need for good storage facilities.

The major objective in the storage of seeds for genetic conservation are as follows :

— to maintain viabel seeds for long periods and
— to ensure that the genetic condition of these stocks remainds constant.

The duration of viability is one of the most important of plant life. Harrison (1966) stated that different rate of seeds deterioration are found between genera species and varieties, infering from this that genetic factors must be involved. He suggested that the speed of deterioration was controlled by storage temperature, the moisture content of the seeds and the gases surrounding the seeds.

Barton (1961) belived that the longevity of any particular seed was a function of the storage conditions under which it was kept rather than of its actual age.

In the vas majority of species which Roberts (1973a) designated as orthodox, there is predictable relationship between moisture content and viability period, where period of viability may be extend by lowering the temperature and moisture content during storage.

Loss of vaibility in orthodox seeds conforms closely to three basic viability equations applicable over a wide range of contitions (Roberts and Ellis, 1977).

In a population stored at constant conditions the frequency of individual deaths in time can be described by the normal distribution.

$$y = \frac{1}{\sigma} \left\{ (2\pi) \right\} \exp \left\{ - (p - \bar{p})^2 / 2\sigma^2 \right\}$$

where y is the relative frequency of deaths occuring at time p, $\bar{p}$ is the mean viability period, and σ is the standard deviation of the distribution of deaths in time. Thus seed survival curves are cumulative normal distribution of negative slope, which become straight lines if percentage values are transformed to probit. The spread of the distribution is directly proportional to the mean viability periode, that is :

$$\sigma = K_{\sigma}\bar{p}$$

where K is a constant. The relationship between moisture content, temperature and mean viability period is described by the equations :

$$\log \bar{p} = Kv - Cim - C2t$$

where m is moisture content, t is temperature (°C), and kv, C1 and C2 are constants. Once the four viability constants have been determined for a seed lot it is posible to define percentage viability after any period of storage (Roberts and Ellis, 1977). It is important in any circumstances to be able to predict the effect of environment on the longevity of seed ranging from the very rapid loss of viability to the very slow loss of viability in long term seed stores for genetic conservation.

Modification of these equation has been made which take into account variations in potential longevity due to genotype and pre-storage conditions and enables the equation to be applied over a far wider range of environmental conditions (Ellis and Roberts, 1980).

Seeds of peanut, *(Arachis hypogaea)*, are among the most unpredictable in their field performance and germination and emergence potential are easily impraired. The promtness of radical emergence can be used as a measure of seed viability and seedling vigour (Mixon, 1971).

In Indonesia peanut storage and to a lesser extent soybean from growing season to the next, is a problem for farmer because the seed viability declines very rapidly. It is of important both to farmers and seed producers, and those work with genetic resources to be able to either maintained their seed under better storage condition or to be able to predict the decline in viability they should expect under present storage conditions.

With this in mind, peanut seeds was stored under constant conditions of moisture and temperature. The level of moisture and storage temperature were selected in the hope that they would accelerate loss of seed viability so that it would be measured in the time available, that is to say several months. An attempt was also made to "age" peanut seeds using a very high temperature and moisture level in a period of hours, which has only previously been carried out with barley. The results were used to try to predict the storage life of their seeds.

This paper has two main objects: the first is to know how environmental factors effects the storage life of peanuts seeds; and the second object is to predict the storage life.

2. Material and Methods

Three cultivars of peanut (*Arachis hypogaea L.*) Macan, Kidang and Gadjah were tested. They were obtained from the Department of Agronomy Faculty of Agriculture University of Gadjah Mada Yogyakarta, Indonesia. The experiment were carried out at the University of Birmingham, UK, from June 20 up to Nov. 20, 1980.

Moisture Content Determination

The moisture of the seed was tested according to th2e International Seed Testing Association Rule (ISTA, 1976).

Germination Test

The germination of all the seeds of peanut were tested. Seeds were arranged in rows between moist germination papers which were then rolled up vertically and stored in plastic germination boxes containing water so that the seeds received moisture by capillary action. These were incubated at 25°C in the dark.

Germination was considered to have occured when the radicle length was more than 1 Cm long. Germination was observed daily and after 10 days germination percentage was scored.

Storage Experiment

Two storage experiments were carried out using peanut seeds. In the first experiment seed of high moisture content were stored at three temperature for approximately five months and in the second experiment seeds were stored for 15 hours at a very high temperature.

For the longterm storage experiment the seeds of all three varieties were divided into two parts. They were brought to moisture levels of 11% and 16% respectively.

Samples of 30 seeds at each of the two moisture levels were sealed hermatically in aluminium laminated foil packets. Three replicate packets of seed at each moisture level were stored at each of 3 temperature 4°C, 15°C and 35°C respectively, for 77 and 139 days.

Two cultivars, Kidang and Gadjah were used in the second experiment.

The moisture content of the seeds was brought to approximately 17%.

Samples of 10 seeds of each variety were sealed as previously. The packets were held in a water bath at temperature of 55°C and samples were withdrawn at 0 (a control), 2, 4, 6, 7, 8, 9, 11, 13, and 15 hours respectively. The germination and growth were examined as in the previous experiment.

Measurements were made of the root length, the hypocotyl length, the epicotyl length. The number of abnormal cotyledons was noted. The root length was measured from the tip of the root up to the base of the hypocotyl.

Tetrazolium Staining Test

Viability test were carried out at the same time as germination test, by using 1% tetrazolium salt solution over night. Evaluation of viability was according to the colouration of the embryo. The seeds are still viable when the embryos of the seeds stain red, but if the embryos are white the seeds are not viable (Copeland, 1976).

3. Results

Observation on the germination of peanut seeds after storage for months.

Germination are began on the third day after the germination test. The percentage germination of seeds from different storage regime after 10 days shows all seeds stored at 35°C were dead within the first sampling period of 77 days except for Kidang where 5% germinated after storage at 16% moisture content.

Seeds stored at low moisture content for only 77 days germinated rapidly but seeds stored at higher moisture content or higher temperature for a long period begin to show a delay in achieving their maximum germination. Gadjah is the variety which shows the greatest delay in germination due to storage rather light moisture and temperature conditions.

A two way analysis of variance was carried out on the raw data for germination after ten days for those treatments where germination occurred. There was a highly significant difference between storage times for all three varieties. Clearly the seeds were deteriorating with time. There was significant difference between treatments for

Gadjah only and the interaction between storage time and treatment was also significant. The treatment effects were clearly significant where the highest storage temperature conditions (35°C) are considered but in the time period for Gadjah response more rapidly than the other two varieties. The experiment were carried on the other conditions of moisture and temperature had indistinguishable effect on Macan and Kidang with respect to germination percentage. (Table 1).

The root length of seedling were measured after 5 days of germination. The analysis of the data shows there were highly significant difference between treatments for Macan and Kidang, but not for Gadjah, indicating the moisture content and the temperature of storage conditions affected the development of the root. Generally best root development resulted from seeds maintained at the lower temperature for the two varieties. Storage time also affected the variety Kidang. The interaction between storage time and treatment was also significant for Kidang. (Table 2).

Although the length of the primary root was generally greater at lower storage temperature. It was note that the growth of adventitious roots was good at the higher temperature and moisture contents.

To summarize the effect of storage temperature and moisture content, therefore on subsequent seedling development, it appears that while both high temperature and high moisture content produced several number of abnormal seedlings, high temperature effects were especially severe on shoot development and the total root development was also more adversely affected by high temperature than high moisture content. Figure 1. 2. and 3.

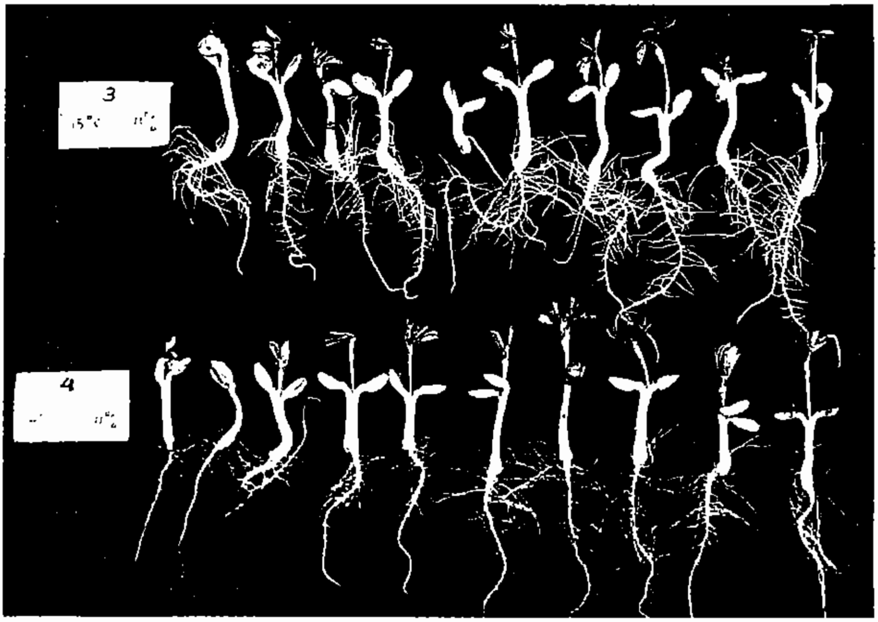

Figure 1. **8 day old seedlings of Macan after storage for 139 days at 11% moisture content at temperature of 4°C and 15°C.**
Top row left to right. Seedling 5 shows stunted development of both roots and shoots.

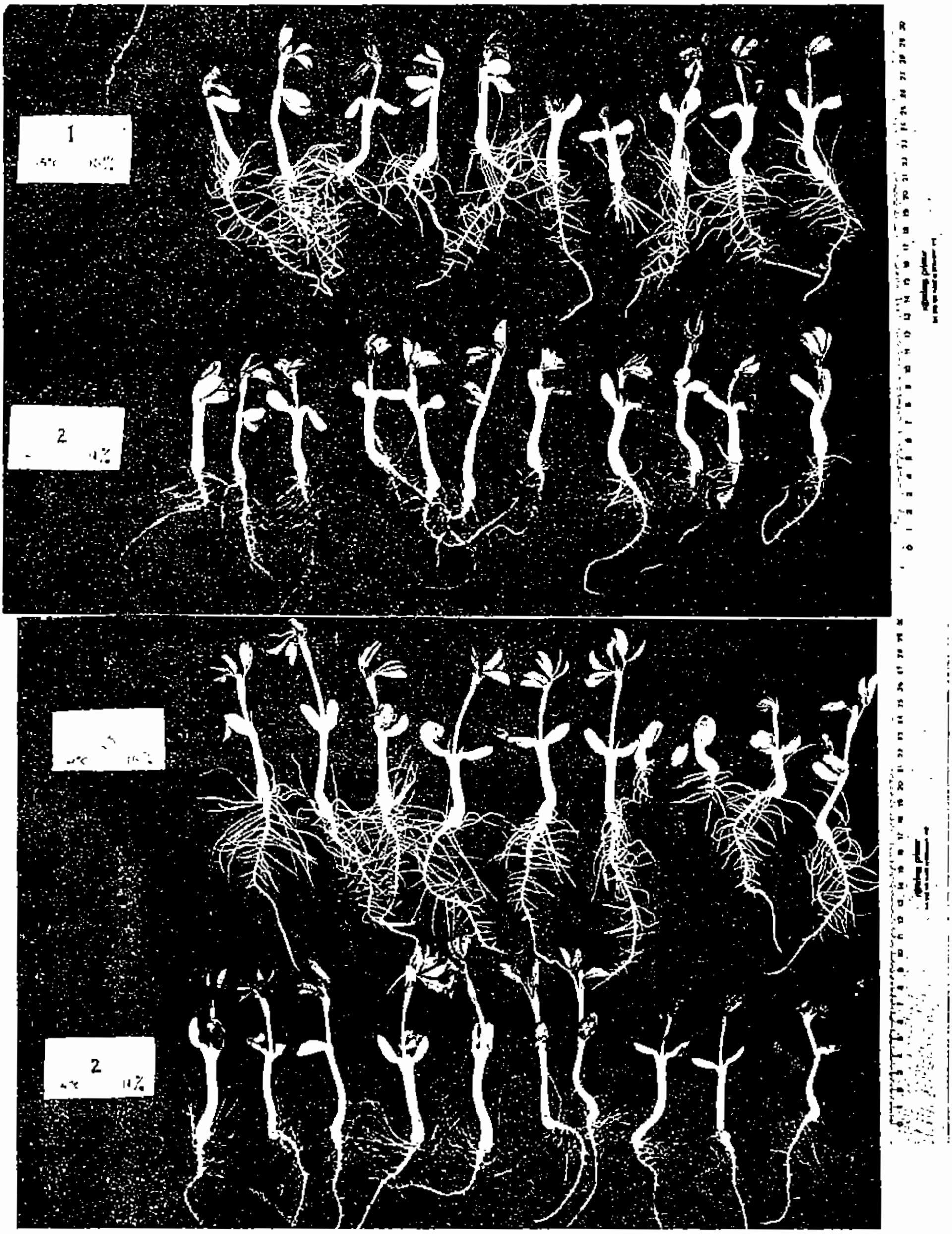

Figure 2. **8 day old seedlings of Gadjah after storage for 139 days under different conditions of temperature and moisture 4°C 11%, 4°C 16% and 11°C 16% respectively.**
Top row left to right seedlings 6 and 7 shows stunted development of shoots but three is some roots growth. Third row left to right seedling 7 and 8 shows stunted development of shoots but there is some roots growth.

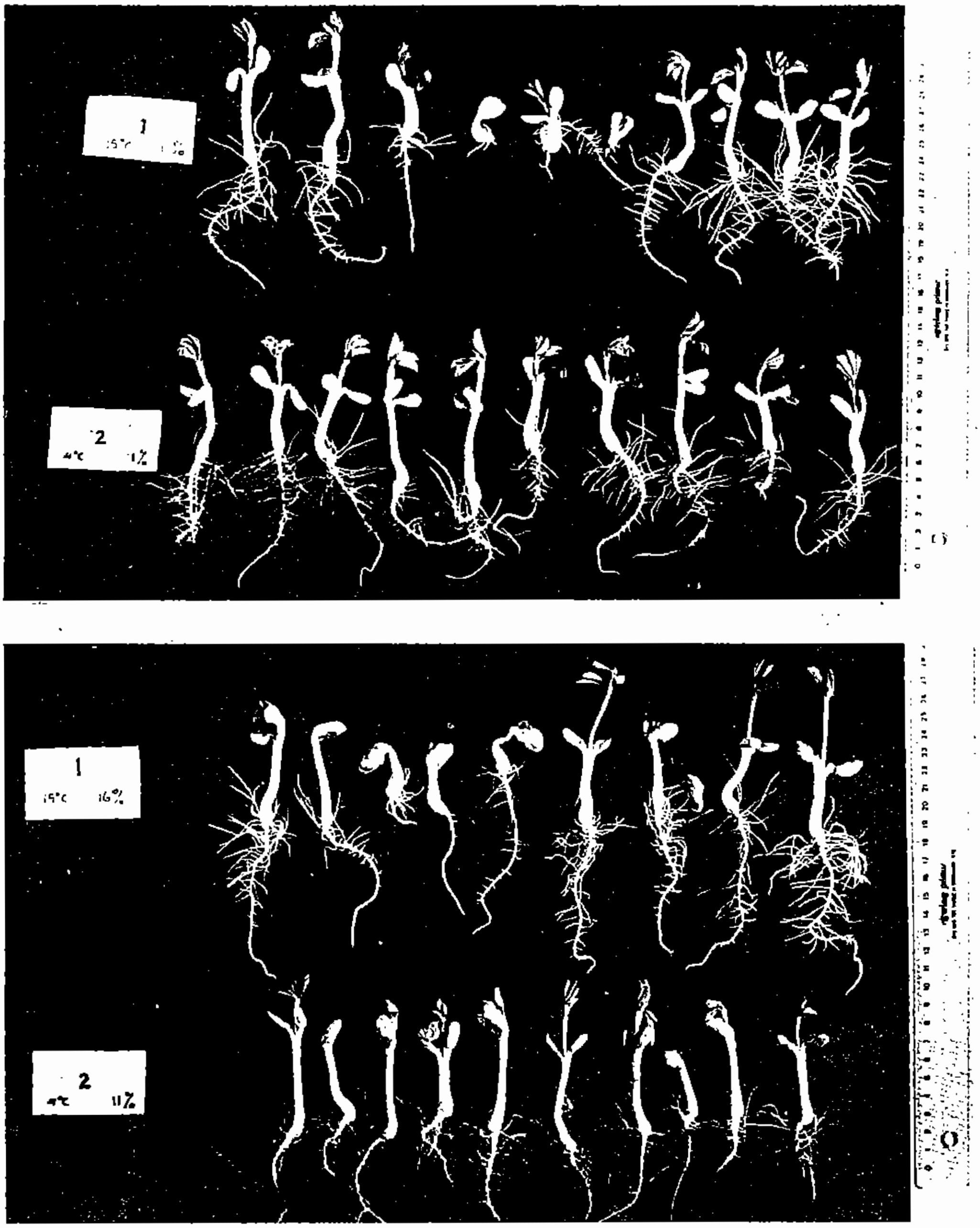

Figure 3. 8 day old seedlings of Kidang after storage for 139 days under different conditions of temperature and moisture: 4°C 11%, 15°C 11% and 15°C 16% moisture content respectively. Top row left to right seedlings 3, 4, 5 and 6 are abnormal. Shoot development ha been especially effected in 4 & 6 and the primary root is absent in 4,5 and 6. At third row left to right seedlings 3, 4, 5 and 8 are abnormal in both roots and shoots.

The hypocotyl length of seedlings was measured after 6 days of germination. The analysis of variance shows the effects of storage time and treatment for all of the varieties were not significant. It appears that the hypocotyl is less affected by the conditions of storage than either germination or root length.

Hypocótyl were least sensitive to storage and epicotyl lengths descriminated between treatment for only one variety. Germination percentage and root length gave a better indicator of deterioration during storage; but germination responded to storage time and root length to storage treatment in some varieties. Overall the respon to storage measured by germination percentage was the most consistent indicator of changes in the season for all varieties.

Observation on the germination of peanut seeds after storage for hours.

Germination began on the second days after the onset of the germination test. The percentage germination of seeds at 10 days after different storage periods at 55°C is shown in the "sigmoid" curve in figure 4. Germination declined with storage time.

An analysis variance was carried out on the data for germination after ten days. There was a significant difference between varieties, a highly significant difference between storage times.

The percentage germination values were coverted to a probit scale and plotted against time. The regression lines were fitted to the points. Figure 5 and 6. From the graphs the 50% viability period (p.50) the scales was obtained, Kidang p.50 = 13 hours, Gadjah p.50 = 12.3 hours. to enable prediction to be made about storage life of period for seeds under three different sets of conditions. The value of p.50 can be used in the three simultaneous equations.

$$\log p.50 = Kv - Cl - C2t$$

Where Kv, Cl and C2 are constant, m is the moisture content in percentage fresh weight and t is the storage temperature in degree centrigrade.

The conditions chosen for storage in the first storage experiment failed generally to show the sigmoid nature of the relationship between germination and storage time, Since the better conditions caused very little decline in seed viability and the worst condition, 35°C, killed all the seeds within the first samping period except for the variety Kidang. However using the results obtained for Kidang stored at 16% moisture, 35°C, the linear regression between germination on probit scale and time has been plotted and a p.50 value has been obtained, Figure 7.

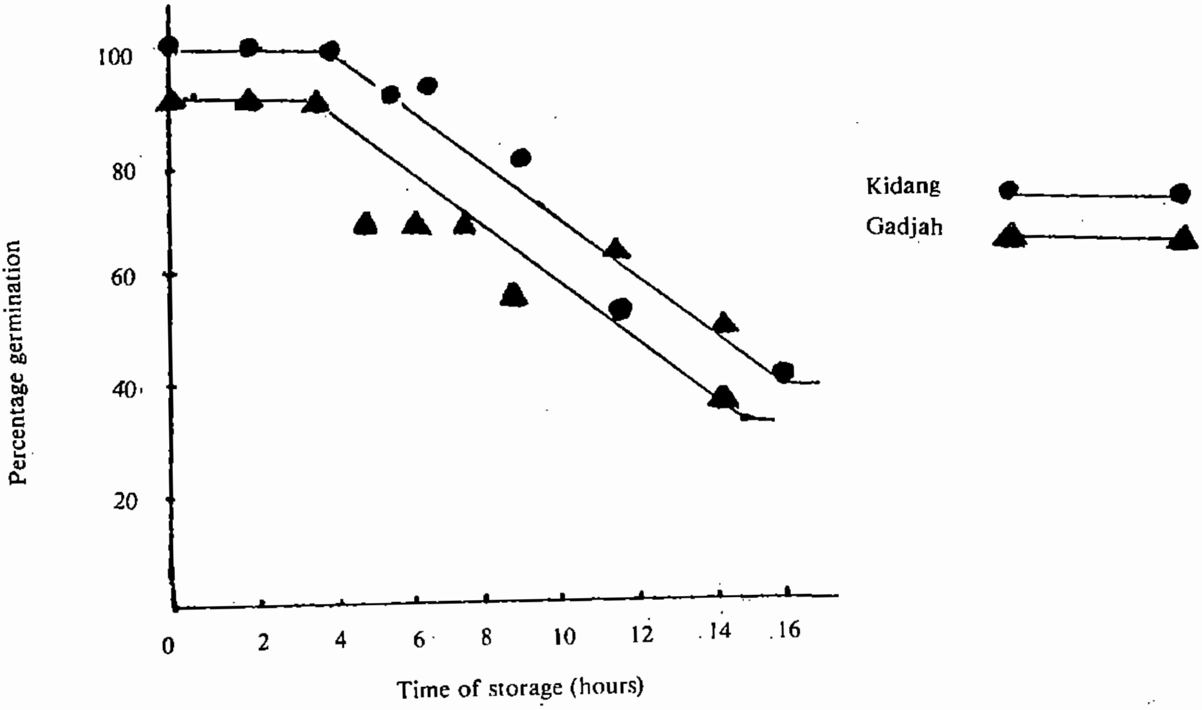

Figure 4. **Percentage germination of Kidang and Gadjah stored at 55°C, 17% moisture content.**

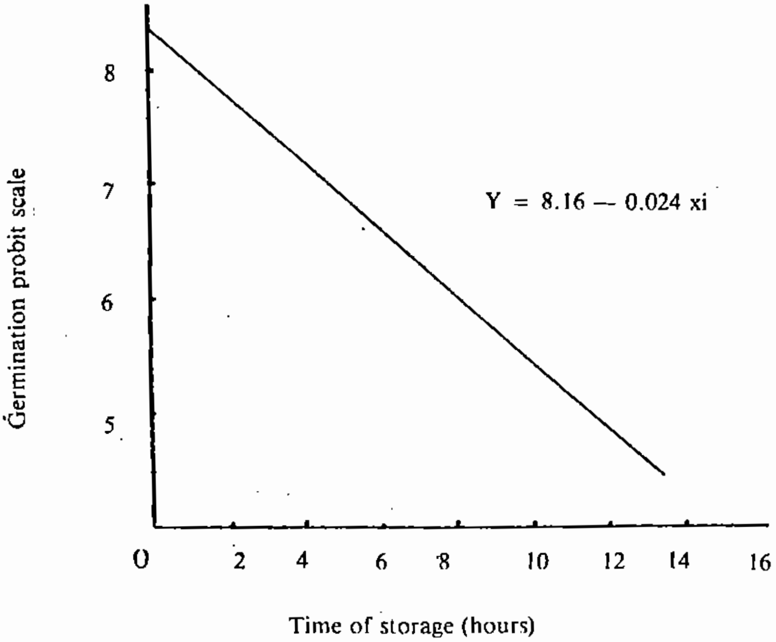

Figure 5. **Percentage germination of Kidang stored at 55°C, 17% moisture content.**

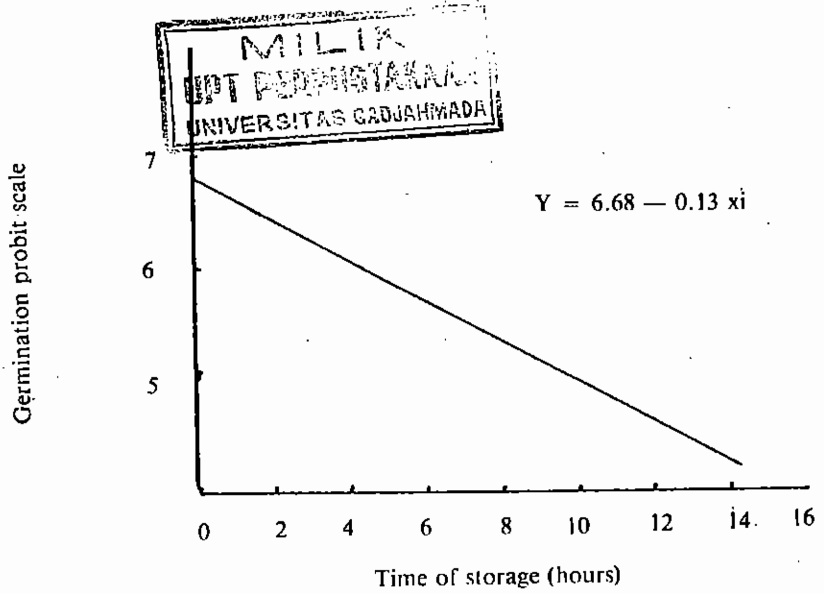

Figure 6. **Percentage germination of Gadjah stored at 55°C, 17% moisture content.**

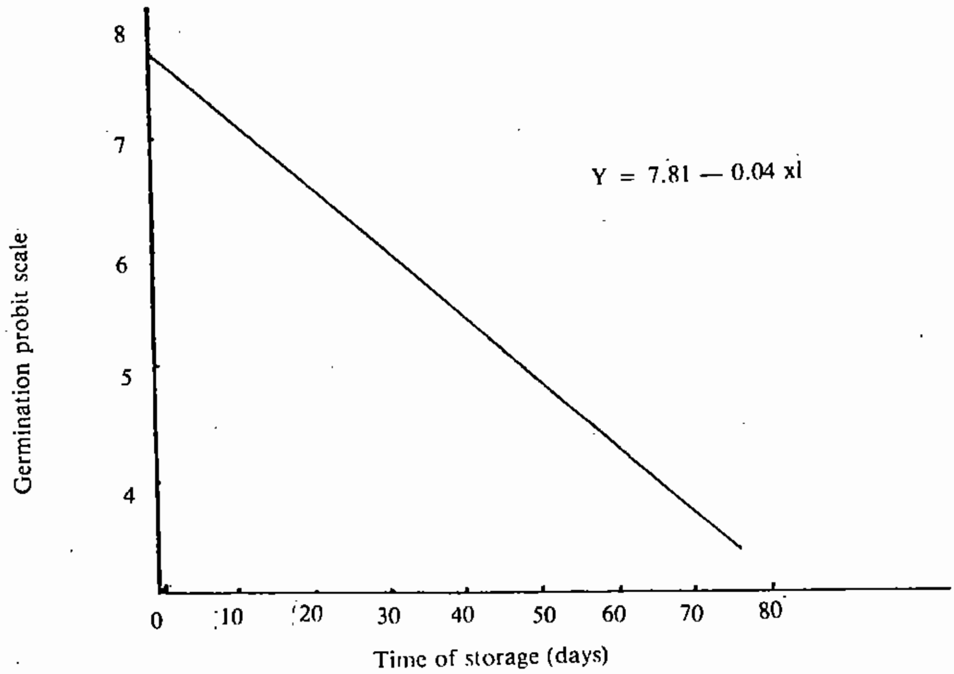

Figure 7. **Kidang stored at 35°C, 16% moisture content after 77 days.**

The p50 values available for use therefore :
Kidang stored at 16% moisture 35°C p50 = 47 days
Kidang stored at 17% moisture 55°C p50 = 0.541 days.

Gadjah stored at 17.8% moisture 55°C p50 = 0.512 days The Kv values for different varieties of the same species are generaly considered very similar (Roberts, 1972). Where, the three values of p50 obtained above has been used to solve the viability and calculate values for Kv, C1, C2 inspite of the fact that two were obtained with Kidang and one with Gadjah. It is appreciated that three values will not be exact but they gives a guide as to that might be expected with peanut generally. The values are : Kv = 5.4725 C1 = 0.021 C2 = 0.098

The value for C2 is greater than C1 indicating that each °C rise in temperature has a more damaging effect than each 1% increase in moisture, a trend observed earlier.

Using the viability constants the half viability period for peanut seed stored at different moisture centents and storage temperature can be calculated.

For example :

Moisture content %	Temperature of storage °C	Prediction time to reach 50% viability life (years)
5	4	271
5	26	2.819
15	26	1.11

Observations on the root, hypocotyle and epicotyl lengths of peanut stored for hours

The root lengths of seedlings were measured after 5 days of germination, hypocotyl and epicotyl lengths after six days, The analysis of data table 3 shows there was a highly significant difference between storage time for roots and epicotyl but no significant difference for hypocotyl. There was no significant difference between varieties.

4. Discussion and conclusions

In the first storage experiment attempted in this study two of six sets of storage conditions 11% moisture, 35°C and 16% moisture, 35°C both cause such rapid physiological deterioration of the seed that even at the first sampling these seeds had lost their ability to germinate. In spite of the known poor keeping quality of peanut seeds, this rate of the germination was not expecte, because although the temperature was higher than ambient the moisture level would well be consistent with seeds stored in places where relative humidity is not reduced. The remaining four sets of storage conditions, 11% moisture, 4°C and 15°C and 16% moisture, 4° C and 15°C did not reduce the germination enough within the 139 days sampling period for the sigmoid nature of the graph, when germination was plotted against storage time, to be confirmed. It was expected the higher the temperature, 15°C in combination with the higher moisture content, 16% would have had a more significant effect. The importance of the temperature component in survical of this seeds was shown by these results.

Although the results obtained were inappropriate for using in a viability equation for predictions about storage life of peanuts in particular storage conditions, there was clearly in reduction in germination with time. When germination results were compared with other parameters for assessing deterioration of the seeds it was found that viability as assessed by the tetrazolium staining method was a much better indicator of deteriorate changes occuring in the seed, even though all these changes might not prevent germination. Viability assessments distinguished between the storage treatment very effectively and more effectively than any of the measurement made on seedlings. Radical length was a useful parameter because it showed the difference in vigour that might be expected from seeds stored under different conditions. This was a significant effect of both treatments and time on primary root development.

The relationship between primary root development and the abundance of adventitious roots is one of interest. High temperature especially during storage adversely affected primary root growth in a manner comparable to its effect germinations. However a combination of high temperature and high moisture during storage increase the abundance of adventious roots. This might be explained as the protection of adventitious roots in a consequence in impaired primary roots growth just as when a primary root is mechanically damaged secondary roots are introduced.

The most reliable method of detecting the effects of storage therefore appears to be staining seeds and looking at the areas of dead cells. However, the scale used for interpreting the number of dead cells, on area one is difficult to define and relies on the eye of interpreter. Therefore, estimate of viability are likely to very greatly from person to person. For general therefore and consistency of viability scores, the germination test is perhaps preferable.

With this in mind a second experiment was conducted in this study in which viability was assessed again using germination percentage. The experiment was limited by quantities of seed available and time and therefore intended to be of a priliminary nature. Ellis and Roberts (1980) attempted to demonstrate that viability equation can be extended to a wide range of conditions and that of conditions of fairly high temperature and moisture are used, seed deterioration can be accomplished as a matter of hours rather than months or years. This experiment was carried out on barley only. The peanut seeds stored at 55°C, 17% moisture for fifteen hours confirmed their experience that a sigmoidal curve was obtained in a short time when germination percentage was plotted against storage time.

Although the data was sparse on attempt at planning a viability equation for these peanut seed lots was made. The viability constant for it within the range obtained for those of the other legumes seeds, peas and broad bean for which Roberts has published. The comparison of these viability constants is a follows :

	Kv	C1	C2
broad bean	5.766	0.139	0.056
pea	6.432	0.158	0.065
peanut	5.4725	0.021	0.098

It is apparent that the value for C1 and C2 however vary considerably from the other two legumes and their relationship to each other varieties. For the temperate legumes C1 is approximately 2 × C2 but for peanut C1 is very small and C2 about 4 × greater. This indicates that the temperature constant C2 is weighting the effect of temperature against moisture during the seed storage. This is consistent with the observations made that high temperature appeared to have more devastating effect than high moisture.

The only viability constants published to date are those for cereals and the legumes given above. Unpublished date for Psopocarpus seeds (Lubis and Mumford, 1979) indicates that these seeds also have relatively high C2 constans. Then we report in the literature covering the importance of moisture content level in peanut is equivalent to that of in other seeds. The difference in peanuts is the very high oil content which affect moisture relative. Viability constants have not been determined for any other seeds rich in oil, but soybean would be an ideal candidate. It is hope that future work will indicate whether there is a special need in oil seeds and peanut in particular to apply particular storage conditions to the seeds in order to maintain their viability on a trace scale comparable to other orthodox seeds.

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Table 1. **Analysis of variance of percentage germination of three peanut cultivars after storage in different environments.**

Variety	ITEM	df	SS	MS	F	P
Macan	Storage time	1	126.56	126.56	27.04	<0.01**
	Treatment	3	17.19	5.73	1.22	NS
	Treatment x storage time	3	17.19	5.73	1.22	NS
	Duplicate	8	37.5	4.68	—	
	TOTAL	15	198.44			
Kidang	Storage time	1	126.56	126.56	27.04	<0.01**
	Treatment	3	17.19	5.73	1.22	NS
	Treatment x storage time	3	17.19	5.73	1.22	NS
	Duplicate	8	37.5	4.68	—	
	TOTAL	15	198.44			
Gadjah	Storage time	1	400	400	84.2	<0.01**
	Treatment	3	181.25	60.41	12.71	<0.01**
	Treatment x storage time	3	87.5	29.16	6.31	<0.05*
	Duplicate	8	25	4.75	—	
	TOTAL	15				

Table 2. **Analysis of variance of root lengths of three peanut cultivars from different storage treatments.**

Variety	ITEM	df	SS	MS	F	P
Macan	Storage time	1	0.01	0.01	0.14	NS
	Treatment	3	3.38	1.126	15.85	<0.01**
	Treatment x storage time	3	0.21	0.07	1	NS
	Duplicate	8	0.57	0.071	—	
	TOTAL	15	4.17			
Kidang	Storage time	1	0.725	0.725	36.25	<0.01**
	Treatment	3	3.24	1.08	54	<0.01**
	Treatment x storage time	3	0.755	0.25	12.5	<0.01*
	Duplicate	8	0.16	0.02		
	TOTAL	15	4.88			
Gadjah	Storage time	1	0.725	0.725	1.6	NS
	Treatment	3	5.21	1.73	3.8	NS
	Treatment x storage time	3	2.705	1.10	2.4	NS
	Duplicate	8	3.62	0.45		
	TOTAL	15				

Table 3. **Analysis of variance of percentage germination, root length, hypocotyl length and epicotyl lenghth of two peanut cultivars Kidang and Gadjah after storage at 55°C for different periods.**

	ITEM		df	SS	MS	F	P
Germination	Variety		1	361.25	361.25	7.20	<0.05*
	Storage time		9	5251.25	583.47	11.63	<0.01**
	Remainder		9	451.25	50.13	—	
		TOTAL	19	6063.75			
Root lengths	Variety		1	0.57	0.57	0.522	NS
	Storage time		9	219.19	24.35	22.33	<0.01**
	Remainder		9	9.85	1.09		
		TOTAL	19	229.61			
Hypocotyl lengths	Variety		1	2.498	2.498	0.215	NS
	Storage time		9	103.59	11.51	1.	NS
	Remainder		9	104	11.61	—	
		TOTAL	19	219.088			
Epicotyl lengths	Variety		1	0.041	0.041	0.5	NS
	Storage time		9	93.31	2.591	32.32	<0.01**
	Remainder		9	0.799	0.08	—	
		TOTAL	19	24.15			