Maintaining storability of shelled rubber (*Hevea brasilliensis,* Muell - Arg) seed using potential osmotic solution and fungicide

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Abstract. Rubber seed will loose its storability in a short time. Seed germination and fungal attack were factors that barrier the storage period. The research aim was to test the ability of the potential osmotic solution and fungicides to reduce seed germination. Completely randomized design with two factors and three replications, i.e: PEG 6000 (w/v): 0%, 15%, 30%, 45%, 60% (potential osmotic solution) and fungicide (active ingredients were pyraclostrobin + metiram) (g / 1 kg seed): 0 g, 10 g, 20 g, 30 g and 40 g, were applied.The results showed that PEG 30% can inhibit seed germination up to 9.07% and 37.47% and fungicides 40 g/1kg can reduce fungal attack during storage of 12 and 16 days. Combination of PEG-6000 30% and fungicides 40g / 1 kg could maintain seed storability by pressing the seeds germination up to 10.67% and fungal attacks up to 18.00% during storage of 16 days with 96.80% germination.
Keywords: Osmotic potential solution, fungicide, rubber seed, storage period

Introduction

Rubber seeds have a low storage capacity (recalcitrant). They are susceptible to deterioration and not having dormancy time with high water content (36-90%) (King & Roberts, 1979) which triggers the process of respiration, and will lose its viability in a short time (Copeland & McDonald 1985, 1995; Pammenter & Berjak, 2000). Sembawa Research Institute (2009) reported that during storage, the seed germination will decrease to 0% after 14 days. During conventional storage seeds were mixed with moist sawdust, (1:1) in jute sacks and packed in wood containers. The procedure was expensive due to the addition of an exceptional delivery volume (Basuki *et al*. 1980; Rubber Research Center, 2003; Ladja, 2006; Sembawa Research Institute, 2009). The use of sawdust as a storage medium has been conducted by Zanzibar and Mokodompit (2007), with no satisfactory results, especially in soft-skinned mahogany seeds (recalcitrant) that cause negative effect on germination. The sawdust material used is relatively large (<600 µm), it will absorb and release water so that the treatment was similar to soaking of seeds for several days in unfavorable conditions; the atmosphere around the seed becomes more moist and also trigger the growth of fungi. The use of sawdust was more appropriately applied to impermeable seeds.

In order to increase the selectivity of the seed to water and air, the researchers tried to extract the shell of rubber seeds. As a replacement of the shells, seeds were coated with polyethylene glycol (PEG)6000 as a compounds which have osmotic potential that can limit the availability of water and oxygen in the storage medium (Krizek, 1985; Song, 2011). PEG ($HO\text{-}CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)x\text{-}CH_2\text{-}OH$) is a long-chain polymers compounds, inert, non ionic and non-toxic (Michele & Kaufman , 1973). The most commonly used in plant physiology study and seed treatment are PEG 4000 and PEG 6000 (Krizek, 1985; Sadjad, 1993). Sumayku (2002), successfully inhibit the germination of seeds of mango using PEG 6000. The purpose of the Research were to determine the ability of PEG 6000 to inhibit seed germination and the efficacy of fungicides to reduce fungal attack during storage, and maintaining seed viability.

Materials and Methods

The experiment was conducted at the Faculty of Agriculture, Seed Technology Laboratory USU Medan from May to August 2011. The materials used were PB 260 rubber seed (moisture content of 39.65%), polyethylene glycol 6000 (PEG-6000), a fungicide with active ingredient (a.i) of pyraclostrobin and metiram 55gr/1kg 550gr/1kg (Cabrio Top 60 WP). Insecticide used was Sevin 80S. Equipments used were: shell seed breaker , hand sprayer, perforated plastic bag and ventilated boxes, sterilized sand, germination box, thermohigrometer and other supporting materials. A complete randomized design in three replicates with two treatment were applied. The first factor was PEG 6000 (w/v): 0%, 15%,

30%, 45%, 60%. The second factor fungicide with a.i pyraclostrobin and metiram (g / 1 kg seeds): 0 g, 10 g, 20 g, 30 g, 40 g.

Implementation of research

PB 260 seeds were harvested from Institute garden crops Sungei Putih, Galang, North Sumatera, Indonesia. Seeds were selected by Rubber Research, seeds were mixed with moist sawdust then put into jute. On arrival at faculty of agriculture seed technology laboratory, the seeds were washed several times with clean water. Then the shells were peeled and shelled seed were directly selected. After that the seeds were dipped for 10 minutes in a mix of PEG 6000 and fungicide solution according to the level of treatments and then dried for 6 hours. After that the seeds were stored in a plastic bag (with holes), arranged in a cardboard box, closed with some ventilation. Then stored in a room with room temperature for 4, 8, 12, and 16 days. After storage, at each period the following was observed: seed germination and seed fungi at various concentrations of PEG (%), seed germination and seed fungi at various fungicide dose (%) and also the germination capacity (%). All data were then analyzed.

Results and Discussion

Effect of PEG 6000 and Fungicides in Storage.

Table 1 showed that the PEG 6000 and storage periods had no significant effected on fungus seeds, the PEG of 30% was the best option to protect the rubber seeds from fungal attack. It can be seen that at 12 and 16 days of storage the fungals was 18.53% and 42.27% respectively. Toruan (1982) and Sutjiati & Saenong (2002) found that the results of fungal infections in seed storage was not influenced by the metabolites that occur in the metabolism of seeds and suspected borne diseases in the tissue or with the seed.

Table 1. Average of fungus seed (%) in storage at various concentrations of PEG. Duncan Multiple Ranges Test of 5% in columns was used.

[PEG](w/v)	Storage period (days)			
	4	8	12	16
0%	2,94 a	10,67 a	20,93 a	42,80 a
15%	3,20 a	14,00 a	24,40 a	38,27 a
30%	2,80 a	10,80 a	18,53 a	42,27 a
45%	4,14 a	12,93 a	22,20 a	46,40 a
60%	3,87 a	12,13 a	20,00 a	58,93 a

Effect of Fungicides during Storage

Table 2 showed that the fungicide and storage periods had no significant effect. It was found that the fungicide of 40 g was able to protect seeds from fungal attack during storage at 12 days and 16 days (19.33 and 40.53% respectively). According to Copeland & McDonald (1995), the process of respiration is very high so that the seeds had a fast metabolism and the heat generated makes the seeds moist, so that they were easily contaminated with microbes and experiencing faster deterioration. The presence of fungus attacks was also found by Arief, *et al*.(2004) in cotton seeds (recalcitrant); Yuniarti, *et al*., (2008) in ebony seed storage and Melya & Indra, (2007) in resin storage test. The failure of germination and fungus attact were suspected due to high water content of seeds in store that trigger an increase in fat accidity. Hydrolysis of the seed or the lipase activity of the fungus in a store space, made the seeds having chronological deterioration.

In Table 3 the combined treatment that had the most moderate treatment was PEG concentration of 30% and fungicides of 40 g/1kg seed which able to protect seeds from fungal attack during storage up to 18.00% after 16 days of storage. Michel & Kaufmann, 1973; Emmerich & Hardegree, 1990, and Pristantho, *et al*., 2011, mentioned that the effectiveness of PEG 6000 in the study was able to inhibit imbibition and hydration of seeds, protect seed moisture content and support the exit and entry of oxygen and suppressed seed respiration and respiration heat, pressing the rate of deterioration and reduced moisture. Charloq (2011) reported that a systemic fungicide with active ingredient of pyraclostrobin +

metiram was the best for the storage of rubber seeds without shells. Fungicides can suppress the development of fungus by disturbing cell wall formation, cell membranes formation, protein synthesis and energy transformation reactions associated with the mitochondrial electron transport (Budiarti & Yulmiarti,1997).

Table 2. Average of fungus seed (%) in storage at various doses of fungicide. Duncan Multiple Ranges Test of 5% in columns was used.

Fungicide (g/1kgseed)	Storage period (days)			
	4	8	12	16
0 g	5,87 a	14,80 a	29,47 a	62,93 a
10 g	3,34 a	12,40 a	21,07 b	38,13 b
20 g	1,47 a	12,00 a	17,33 b	44,27 b
30 g	3,20 a	10,93 a	19,87 b	42,80 b
40 g	3,07 a	10,40 a	19,33 b	40,53 b

Interaction between PEG 6000 and fungicide during storage

Table 3. Average of fungus seeds on 16 days of the storage of (%). Duncan Multiple Ranges Test of 5% in the rows and columns was used.

PEG/Fungicide	0 g	10 g	20 g	30 g	40 g
0%	50,00 bcde	34,67 bcde	65,33 bcd	28,67 cde	35,33 bcde
15%	44,00 bcde	37,33 bcde	28,00 cde	42,67 bcde	39,33 bcde
30%	58,00 bcde	26,00 cde	65,33 bcd	44,00 bcde	18,00 e
45%	66,67 bc	37,33 bcde	43,33 bcde	53,33 bcde	31,33 cde
60%	96,00 a	55,33 bcde	19,33 de	45,33 bcde	78,67 ab
Average	62,93 a	38,13 b	44,27 b	42,80 b	40,53 b

Effect of PEG 6000 during storage

Table 4. Average of germinated seed (%) during storage at various concentrations of PEG 6000. Duncan Multiple Ranges Test of 5% in columns was used.

[PEG] (w/v)	Storage period (days)			
	4	8	12	16
0 %	3,20 a	9,73 b	13,60 b	41,33 a
15%	6,27 a	16,67 a	22,93 a	41,60 a
30%	0,94 b	5,47 c	9,07 c	37,47 a
45%	1,20 b	5,20 c	8,13 c	40,67 a
60%	0,40 b	3,07 c	5,33 d	55,07 a

Effect of fungicides during storage.

Table 5. Average of germinated seed (%) during storage at various doses of fungicide. Duncan Multiple Ranges Test of 5% in columns was used.

Fungicide (g/1kg seed)	Storage period (days)			
	4	8	12	16
0 g	0,40b	2,93c	5,47b	56,27a
10 g	3,20a	7,73b	14,67a	40,00a
20 g	3,07a	9,73ab	11,33a	39,47a
30 g	2,27a	11,20a	14,27a	40,27a
40 g	3,07a	8,53b	13,33a	40,13a

Table 4 showed that the PEG 30% treatment can reduced the percentage of seed germinated during storage. It can be seen that at 12 and 16 days the germination was 9.07% and 37.47% respectively. Table 5 showed that 40 g fungicide was able to maintain seeds germination. It can be seen that at 12 and 16 days of storage of the germination was 13.33% and 40.13% respectively. In table 6 interaction of 30% PEG and 40 g of fungicide at 16 days of storage can reduced seeds germination up to 10.67%. Emmerich & Hardegree (1990) reported that the role of PEG to maintain the osmotic potential of germ cells that can

limit the water content and oxygen on medium of seed storage, so that the PEG molecules outside the cell membrane formed a thin layer of seeds that protects and serves as a buffer to the entry and exit of water and oxygen, and preventing the germination of seeds without causing damage or decline in viability, through the osmotic pressure generated by the PEG (Copeland and Mc Donald, 1985), and the high osmotic pressure which is effective in inhibiting the germination of seeds (Bewley and Black, 1978). High osmotic pressure in the storage media resulted in water imbibition difficulty so that it will slow the germination (Copeland & Mc Donald,1985).

Interaction between PEG 6000 and fungicide during storage

Table 7 and Table 8 showed that the germination of the rubber seed was close to 90% after storage for up to 16 days on each unit of PEG-6000 treatment and fungicide and seed germination test was continued for 21 days in a sand box. This is in consistent with the report of Charloq (2004) that the 45% of PEG 6000 was able to maintain the viability of the rubber seeds (GT1 clone) up to 16 days of storage with 70% germination. Recalcitrant seeds storage was very complex and dilemma (Toruan, 1982). Pammenter and Berjak (2007) reported that the cacao seeds (recalcitrant) can not be dried below 30% moisture content, the seed will deteriorate and will not tolerant low temperature. The indoor storage of DR2 with relative humidity of 35%, 75% and 100% resulted in the decrease of fat content of the seeds, increase the fatty acid and sugar content, and membrane leakage rate and the highest alcohol content (35%).

Table 6. Average of germinated seed on 16 days of storage (%).Duncan Multiple Ranges Test of 5% in the rows and columns was used.

PEG/Fungicide	0 g	10 g	20 g	30 g	**40 g**
0%	36,00 bcdf	32,67 bcdf	65,33 abc	25,33 bcdf	47,33 bcdf
15%	38,67 bcdf	60,67 abcd	27,33 abc	45,33 bcdf	38,00 bcdf
30%	53,33 bcdf	18,00 cdf	65,33 abc	40,00 bcdf	10,67 df
45%	57,33 bc	34,67 bcdf	34,67 bcdf	50,67 bcdf	26,00 bcdf
60%	96,00 a	54,00 bcdf	4,67 f	42,00 bcdf	78,67 ab
Average	56,27	40,00	39,47	40,27	40,13

Sembawa Research Institute (2009) reported that a few days dryness will cause the seed not to grow. The decline of germination of stored seeds was related to the high water content which causes the irregular structure of mitochondria membrane so that membrane permeability increases. Increased permeability caused many metabolites (among others amino acids and sugars) leaked out of cell membrane. Thus the substrates for respiration were reduced so that energy produced for germination was reduced. The ability of PEG solution to retain water depends on its molecular weight and concentration (Sutjahjo *et al*., 2007). Ching *et al*., (1977) and Tatipata (2004) showed that seeds with high vigor contained ATP and total adenosine phosphate higher than those with low viability. ATP is necessary for the biosynthesis of new cells. The decrease in ATP is shown by lower germination. The result of this study was quite consistent with those mention by Harrington, et.al., (1972) and Kartasapoetra (2003) who said that seed deterioration can be reduced.

Table 9 shows that the germinated seeds was above 90% compared to 40,22% (Sulaiman, *et al*. 2010; Samjaya *et al*.(2010) and 0% (Sembawa Research Institute, 2009). It means that a substantial progress had been found. It can be recommended that PEG can be used as preservatives that can sustain the vigor and germination of rubber seeds at a high level. There are four fundamental difference of the above studies. Firstly seeds used in this study had been peeled, secondly, the seeds were treated with polyethylene glycol (PEG) as a preservative; thirdly, period of storage was not the same, fourthly the seeds were stored in a perforated plastic bag without using moist sawdust at room temperature. Provision of PEG at a concentration of 30% had a significant interaction with fungicide of 40 g. They can maintain the germination above 90%. There are four advantages, resulting from the extraction of seed shells i.e 1) smaller storage space, 2) reduce weight and delivery costs, 3) seed can absorb PEG easily and 4) easier seed quality evaluation. Table 9 showed the results of germination with several treatment combination.

Table 7. Average of germinated seed after storage at different concentration of PEG 6000 (%). Duncan Multiple Ranges Test of 5% in columns was used

[PEG] (w/v)	Germination period (days)			Average
	7	14	21	
0%	90,14 a	96,91 a	96,91 a	94,65 a
15%	78,44 a	81,01 a	81,01 a	80,15 a
30%	91,12 a	97,42 a	97,42 a	95,32 a
45%	95,29 a	97,43 a	97,43 a	96,72 a
60%	86,09 a	93,97 a	93,97 a	91,34 a

Table 8. Average of germinated seed after storage at different doses of fungicide(%). Duncan Multiple Ranges Test of 5% in columns was used.

Fungicide (g/1kg seed)	Germination period (days)			Average
	7	14	21	
0 g	80,40 a	94,78 a	94,78 a	89,99 a
10 g	87,65 a	88,49 a	88,49 a	88,21 a
20 g	93,11 a	96,88 a	96,88 a	95,62 a
30 g	92,76 a	98,21 a	98,21 a	96,39 a
40 g	87,17 a	88,38 a	88,38 a	87,98 a

Table 9. Average seed germinated after storage (%) at different PEG (%) and fungicide doses.

P/F	0 g	10 g	20 g	30 g	40 g	Average
0%	96,92	95,21	98,54	99,99	93,89	96,91
15%	84,96	61,34	90,46	93,89	74,96	81,01
30%	98,54	93,27	98,48	99,99	96,80	97,42
45%	96,27	97,67	99,98	99,97	93,26	97,43
60%	97,21	94,97	96,91	97,77	82,99	93,97
Average	94,78	88,49	96,88	88,38	88,38	93,35

Conclusions

The combination of PEG-6000 30% and fungicides 40g / 1 kg of seeds was very effective in reducing seed germination and fungal attack during the period of storage and was very effective in maintaining seed viability.

Acknowledgements

Thanks to the Rubber Research Institute staff and Agricultural Faculty colleagues USU, labor field and especially to the Promoters and Co-promoters.

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