CHAPTER 4

RESULTS AND DISCUSSION

Experiment 1: Identification of seed-borne fungi in barley seed

Before exposing barley seeds to RF, the detection of seed-borne fungi was carried out using the blotter method. The six species of fungi most frequently isolated and identified in our experiment were: *Alternaria* sp., *Aspergillus flavus*, *A. niger*, *Fusarium* sp., *Penicillium* sp. and *Rhizopus* sp., as shown in Figure 4.1. In accordance with ISTA (2006), both Potato Dextros Agar (PDA) and blotter methods were used to determine the pathogen infection status of the seed samples.

Experiment 2: Effect of radio frequency heat treatment to control seed-borne fungi in barley seed

2.1 PDA method

For the PDA method, the seeds were surface sterilized to eliminate external organisms, most of which were saprophytes (non-pathogenic), and the opportunity was taken to develop and identify internal pathogens. Figure 4.2 shows the percentage of fungi infection found from the barley seeds, which were screened using PDA media. Several fungal species became obvious from the growth media after incubation for 7 days. Before the seeds were treated with RF, the fungi species' identified included: *Alternaria* sp. (5.47%), *A. flavus* (9.94%), *A. niger* (2.71%), *Fusarium* sp. (10.3%), *Penicillium* sp. (13.9%) and *Rhizopus* sp. (11.33%). RF at a temperature of 65°C for 3 minutes resulted in complete eradication of *A. niger* from the barley seeds. Moreover, a temperature of 75°C for 3 minutes completely eradicated *Alternaria* sp. and *Fusarium* sp. This treatment was also the best at reducing the other seed-borne fungi infection in species of *A. flavus*, *Penicillium* sp. and *Rhizopus* sp. which were 0.51, 0.75 and 1.88% respectively (Table 4.1). The present study found that the level of reduction of the seed-borne fungi infections, depended on the temperature of the RF treatment and the application period used. High temperatures and long exposure times decreased the seed-borne fungi found in the seed sample.

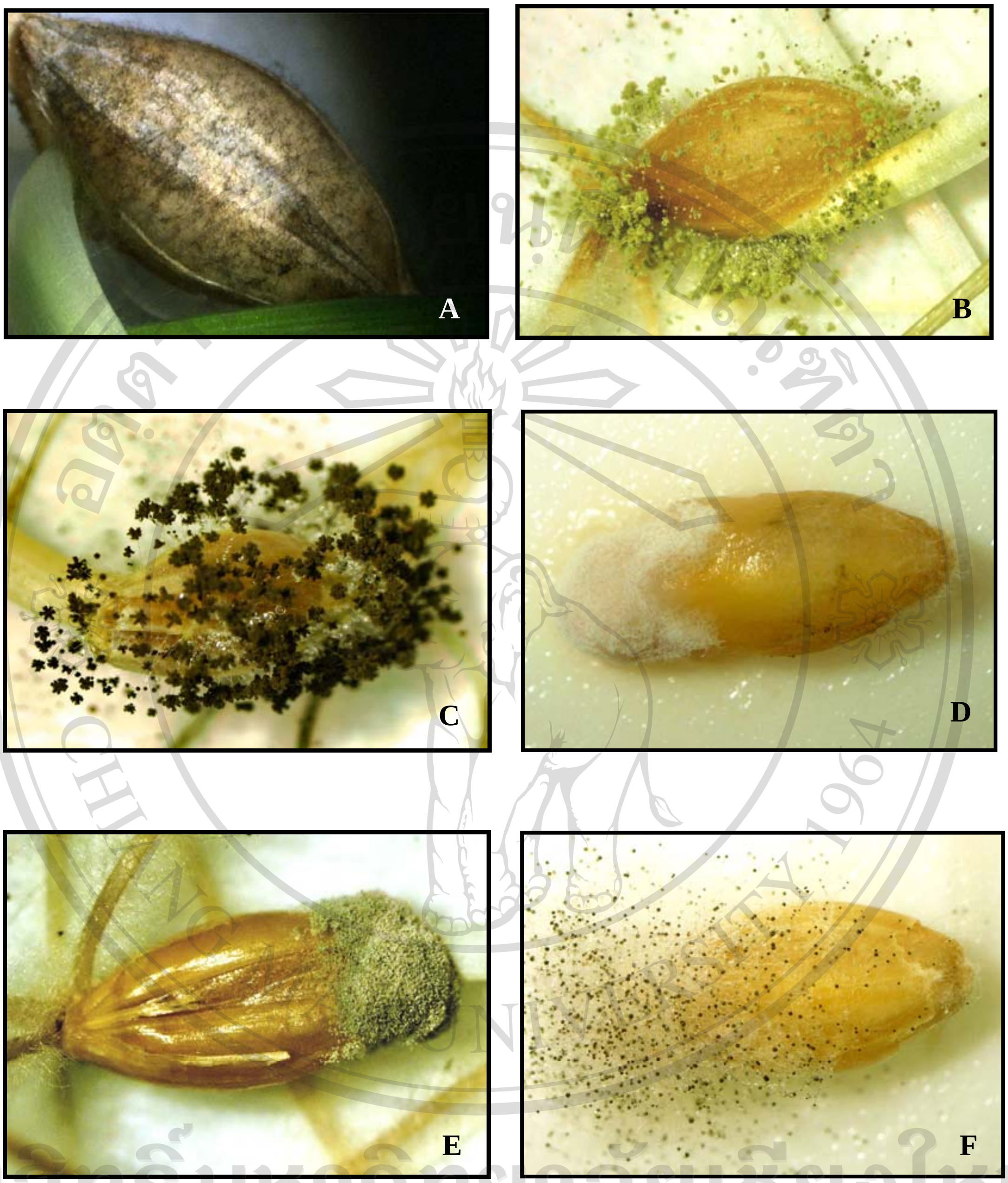

Figure 4.1 The infection of seed-borne fungi in barley seed: *Alternaria* sp. (A), *A. flavus* (B), *A. niger* (C), *Fusarium* sp. (D), *Penicillium* sp. (E) and *Rhizopus* sp. (F).

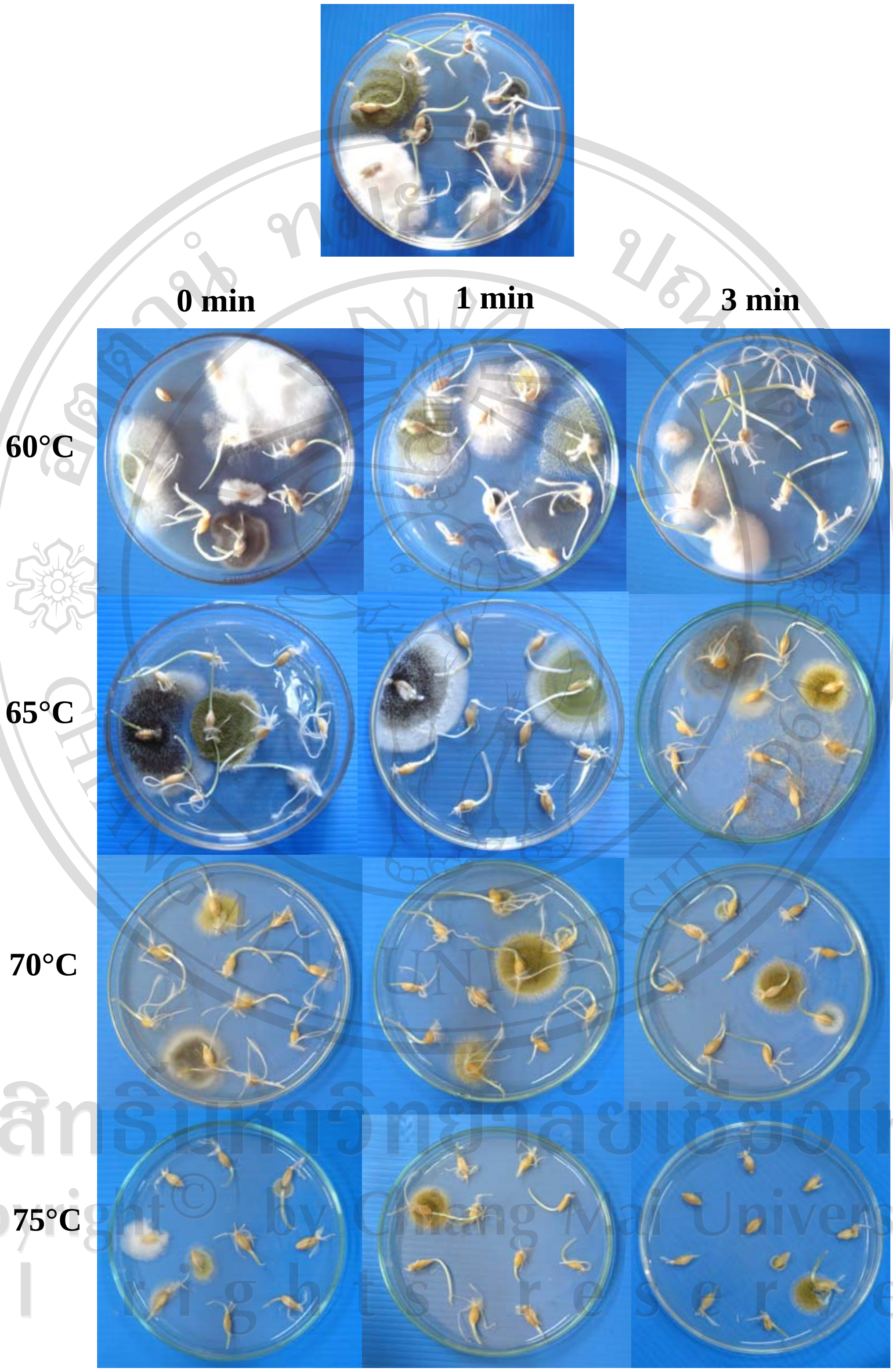

Figure 4.2 Growth inhibition of seed-borne fungi in barley seeds, detected by PDA method, after RF heat treatment at different temperature and exposure time

Table 4.1 Effect of RF heat treatment on fungal seed-borne pathogens in barley seed, evaluated by the PDA method.

Treatment		Seed-borne fungi infection (%) [1]					
Temperature	Time	*Alternaria* sp.	*A. flavus*	*A. niger*	*Fusarium* sp.	*Penicillium* sp.	*Rhizopus* sp.
Control		5.96 a	9.94 a	2.71 a	10.30 a	13.90 a	11.33 a
60°C	0 min	2.04 b	8.33 b	1.63 b	7.41 b	8.60 b	10.75 a
60°C	1 min	2.01 b	5.63 c	0.66 c	6.45 c	7.57 c	10.52 a
60°C	3 min	2.17 b	5.06 cd	0.13 d	3.84 d	7.27 c	9.16 b
65°C	0 min	1.54 bc	5.38 c	0.13 d	2.25 e	7.10 c	9.19 b
65°C	1 min	1.22 cd	3.66 de	0.04 d	1.91 ef	6.13 d	8.48 b
65°C	3 min	1.13 cde	2.50 ef	null	1.41 fg	4.13 e	6.50 c
70°C	0 min	0.66 def	2.47 ef	null	1.06 gh	3.44 e	5.37 cd
70°C	1 min	0.51 def	2.42 ef	null	1.25 g	1.90 f	4.89 d
70°C	3 min	0.66 efg	1.56 fgh	null	0.66 hi	1.57 fg	2.50 e
75°C	0 min	0.38 fg	2.03 fg	null	0.53 hij	1.38 fg	2.35 e
75°C	1 min	0.25 fg	0.94 gh	null	0.25 ij	0.88 g	2.50 e
75°C	3 min	null	0.51 h	null	null	0.75 g	1.88 e
CV%		28.71	26.11	44.29	14.16	11.63	10.84
F-test		**	**	**	**	**	**

[1] Seed-borne infection of pathogenic fungi in the barley seed sample

Means followed by the same letter(s) within a column did not differ significantly ($P<0.05$).

2.2 Blotter method

The blotter method results shown in Figure 4.3 reveal the different types of fungi found growing on barley seed samples after being treated with an RF regime. The results demonstrate that the reduction in seed-borne fungi infections depends on the temperature of the RF treatment and the heating time. High temperatures and long treatment times can decrease the levels of seed-borne fungi found in the seeds (Figure 4.3). Before the seeds were treated with RF, the fungi species found included: *Alternaria* sp. (5.41%), *A. flavus* (24.25%), *A. niger* (4.84%), *Fusarium* sp. (5.89%), *Penicillium* sp. (9.82%) and *Rhizopus* sp. (34.27%). Applying RF at a temperature of 70°C for 3 minutes resulted in a complete eradication of *Alternaria* sp. and *A. niger* from the barley seeds. Moreover, when using a temperature of 75°C for 3 minutes, this treatment also completely eradicated *Fusarium* sp. This treatment was also the best at reducing the other seed-borne fungi infection in species of *A. flavus*, *Penicillium* sp. and *Rhizopus* sp., which were reduced to 1.25, 0.25 and 6.32%, respectively (Table 4.2).

Table 4.2 Effects of RF heat treatment on fungal seed-borne pathogens in barley seed, evaluated by the blotter method.

Treatment		Seed-borne fungi infection (%) [1]					
Temperature	Time	*Alternaria* sp.	*A. flavus*	*A. niger*	*Fusarium* sp.	*Penicillium* sp.	*Rhizopus* sp.
Control		5.41 a	24.25 a	4.84 a	5.89 a	9.82 a	34.27 a
60°C	0 min	2.88 b	11.75 b	2.63 b	3.75 b	6.13 b	27.32 b
60°C	1 min	1.98 c	9.13 c	1.00 c	3.00 c	4.63 c	23.14 c
60°C	3 min	1.91 c	7.50 cd	0.88 c	2.05 d	3.38 d	23.69 c
65°C	0 min	1.63 c	5.75 de	0.63 cd	1.55 de	3.00 de	22.49 c
65°C	1 min	1.03 d	4.86 ef	0.37 de	1.13 ef	2.25 ef	19.99 d
65°C	3 min	1.00 d	4.38 efg	0.13 e	0.75 f	1.63 fg	19.49 d
70°C	0 min	0.38 e	4.75 ef	0.13 e	0.63 fg	1.38 gh	14.97 e
70°C	1 min	0.13 e	4.38 efg	0.13 e	0.75 f	1.00 ghi	15.66 e
70°C	3 min	Null	3.13 fgh	null	0.13 gh	0.63 hi	11.27 f
75°C	0 min	Null	2.25 gh	null	0.13 gh	0.51 i	7.27 g
75°C	1 min	Null	1.75 h	null	0.13 gh	0.38 i	7.77 g
75°C	3 min	Null	1.25 h	null	null	0.25 i	6.32 g
CV%		26.43	24.6	35.32	27.51	19.81	5.11
F-test		**	**	**	**	**	**

[1] Seed-borne infection of pathogenic fungi in the barley seed sample

Means followed by the same letter(s) within a column did not differ significantly (P<0.05).

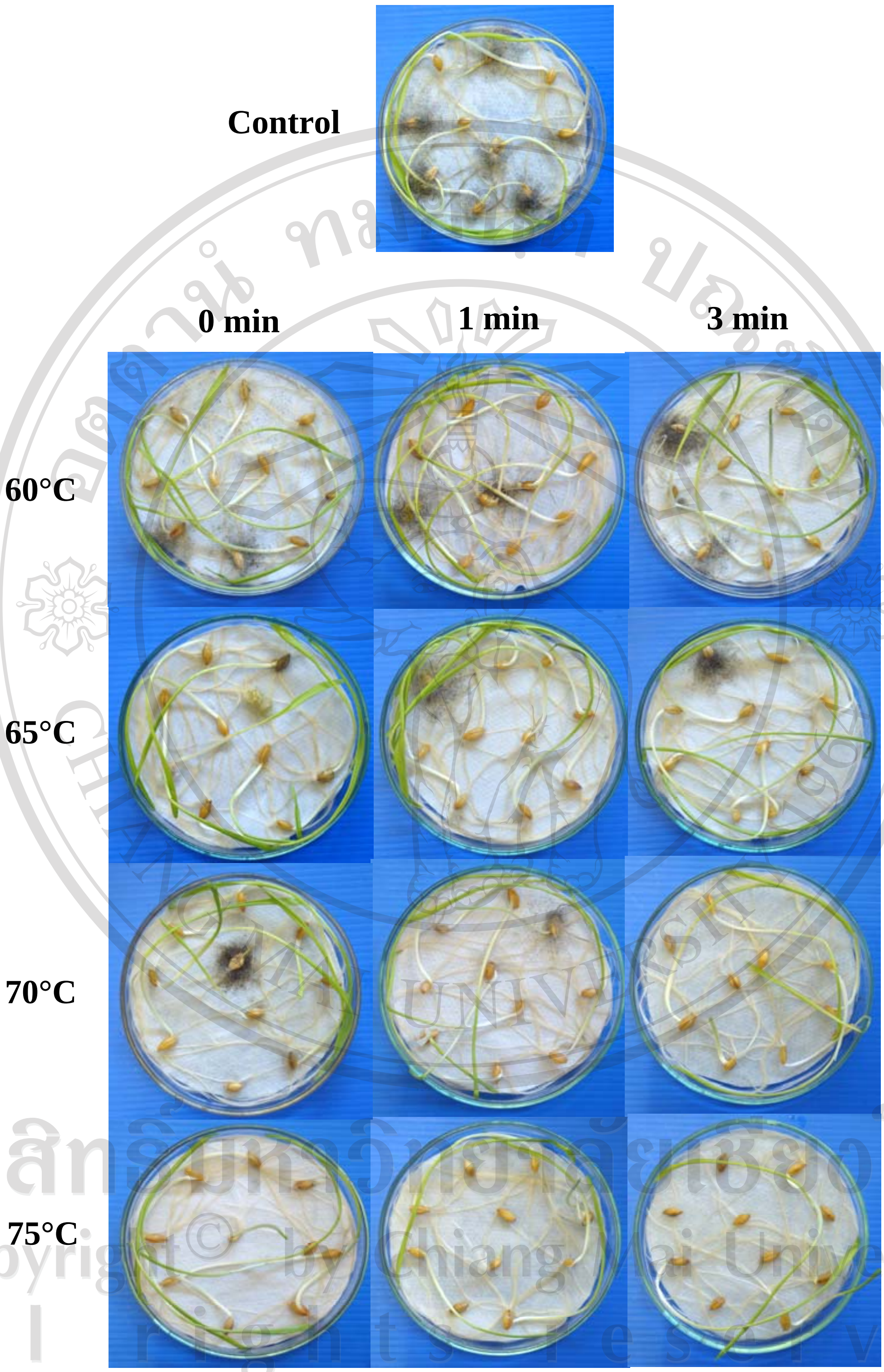

Figure 4.3 Growth inhibition of seed-borne fungi in barley seeds, detected by blotter method, after RF heat treatment at different temperature and exposure time

When a material containing water molecules is subjected to an electromagnetic field, the molecules rapidly change direction and rotate into alignment with the direction of the electrical field. The molecular friction that results produces heat within the material. For my experiment, this would suggest that the dielectric materials in the seed can store electric energy and convert electric energy into heat (Nelson, 1996; Wang and Tang, 2001 and 2005; Sacilik *et al.,* 2007). To control seed-borne fungi in seeds by dielectric heating, the dielectric properties of the seed and the fungi were important. Exposing seeds to an RF field can produce differential heating between the seeds and the fungi. Wang *et al.* (2003) stated that products with higher moisture content have a higher dielectric loss factor, which causes more of the absorbed RF energy to dissipate as heat, thereby causing the product to heat-up more quickly. Nelson and Walker (1961) reported that moisture content was very important factor in determining the maximum temperature of RF application. At high initial moisture contents, a larger proportion of energy was absorbed by free space inside the material. Because fungi contain more water more than the seeds, it is possible that the fungi heat up at a faster rate than the seeds they have infested. Cwiklinski and von Hörsten (1999; 2001) reported that the application of microwave and RF technology leads to a complete eradication of *Fusarium culmorum* (Smith) Saccardo in seeds, whilst maintaining germination levels. They reported a complete eradication of fungus on wheat seeds at temperatures of 70° to 75°C and treatment times of 150 to 180 seconds, when the initial seed moisture content was 15%. When using RF at 65°C, with an initial seed moisture content of 16%, this also completely eradicated *F. graminearum* whilst maintaining germination to 85%. Vassanacharoen (2005) treated sesame seeds with RF and found that the most effective level was 70ºC with a seed moisture content of 10%. By this point, all fungi invasions had decreased to 51%, whereas the percentage of seed germination stayed high, at 73%. Furthermore, using corn with an initial moisture content of 10.5 and 14%, and then treating the seed with an RF temperature of 65, 70, 75, 80 and 85°C for 10 minutes. showed that an increase in temperature led to a decreased percentage of *F. semitectum* infection, and at 85°C with a 14% moisture content, the percentage fungi infection found was only 2% (Vassanacharoen *et al.,* 2006). Janhang *et al.* (2005), used RF heat treatment at temperatures of 70, 75, 80 and 85°C for 180 seconds, to control seed-borne *Trichoconis padwickii* in rice seeds (*Oryza sativa* L.), and with initial seed moisture content 10.4%. These results suggested that the best temperature to used was 75 °C, for at this temperature *Trichoconis padwickii* infestation dropped to 17.8%, while the percentage seed viability was as high as 82% and the moisture content decreased to 9.5%.

The effects of heat on fungi are related to the chemical reactions within the fungal cells. For optimum growth, temperatures must be in a range that allows the most efficient progression of the chemical reactions necessary for growth. As temperatures progress above the optimum temperature, the chemical reactions occur less efficiently, and growth slows. Eventually, the temperature can reach a point where growth stops, and cell components actually begin to be damaged by the heat. Enzymes are proteins that change structurally when heated to their limit of tolerance. Similarly, membranes, which contain lipids, change in structure, and their function of protecting and regulating the internal environment of the cell becomes compromised.

Forsberg (2004) stated that the location of the pathogen inoculum that causes the fungi to develop in seeds, is not successfully removed during heat treatment. If the inoculum is situated deep within the seed, it will be partly protected from heat exposure. Another factor is the moisture content of the seed. Dry seeds resist higher temperatures, whereas the pathogen is rendered more sensitive by wetting obtained through the treatment. Moreover, Cavalcante *et al.* (1993) indicated that irradiation with microwaves (1420 W, 2450 MHZ) has the potential to eliminate fungal propagules in seeds. The irradiation of single-celled spores results in lower viabilities, but both multi-celled and dark spores were less affected than hyaline spores. Civello *et al.* (1997) also reported that the vegetative cells and conidia of most fungi were de-activated when exposed to 60°C for 5−10 minutes.

Experiment 3: Effect of radio frequency heat treatment on seed quality

3.1 Seed moisture content

Barley seeds were exposed to RF heat treatment at temperatures of 60, 65, 70 and 75°C, and for 0, 1 and 3 minutes. The seed moisture content was measured by the hot air-oven method (ISTA, 2006). The results showed that seed moisture content decreased with an increase in both exposure time and temperature.

The effects of various RF treatments on seed moisture content are shown in Figure 4.4. When seeds were not treated with RF, the moisture of the seed was 14.3%. After the seed sample was exposed, it was found that at a temperature of 60 and 65°C, for an exposure time of 0, 1 and 3 minutes, the level of seed moisture content did not differ significantly from the control, which was between 14.1 and 13.8%. However, RF applied at a temperature of 70 and 75°C resulted in a loss of barley seed moisture. In my study, the seed moisture content dropped by less than 1% during the experiment, but did not differ at all for the different exposure times in each treatment.

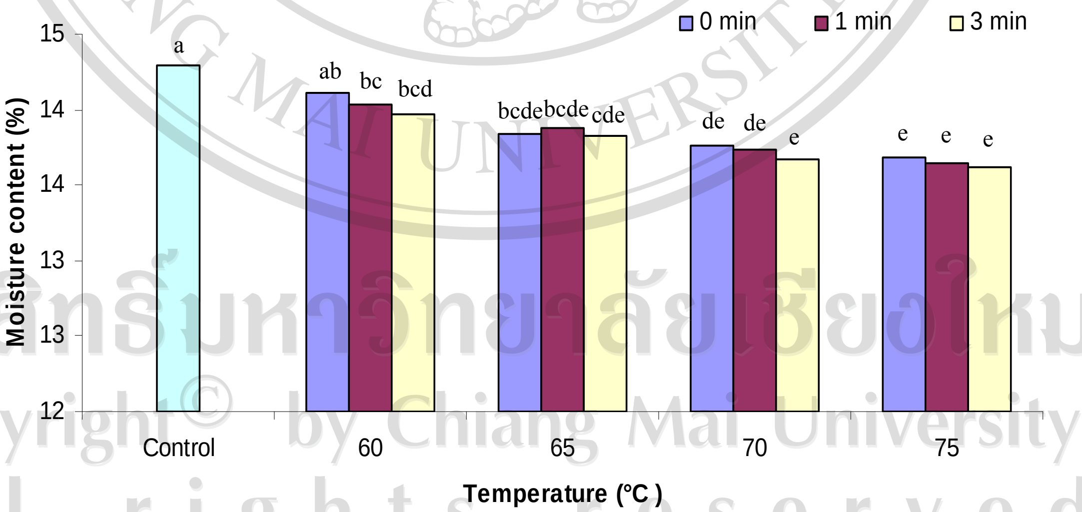

Figure 4.4 The moisture content of barley seeds treated with RF heat treatment at various temperatures and times

Lagunas-Solar *et al.* (2006) stated that during RF heating, the RF generator creates an alternating electric field between two electrodes. Polar molecules, such as water in the seed, try to align themselves with the polarity of the electric field. Since the polarity changes rapidly (at 27.12 MHz, the electric field alternates 27,120,000 cycles per second), the molecules try to continuously realign themselves with the electric field through a 'flip-flop' motion. The friction resulting from the rotational movement causes internal friction and generates heat within the seed (Yang *et al.,* 2003). The dielectric properties relevant during the RF heating are permeability, permittivity (capacitivity) and electrical conductivity of the heated material. The permittivity, which determines the dielectric constant, the dielectric loss factor and the loss angle, influences the RF heating process (Piyasena *et al.,* 2003).

As a result it can be assumed that when seeds were treated with RF energy, the moisture content was important because their dielectric loss factor increases with moisture content; therefore, their moisture content absorbed energy from the RF electric field. Also, the temperatures that seeds can tolerate without a loss of viability were dependent upon their moisture content. Orsat (1999), stated that treating seeds with a low moisture content of about 8%, resulted in a temperature of about 75-85°C and was close to the maximum exposure allowed to maintain germination quality and achieve appreciable levels of pest mortality. On the other hand, at a higher moisture content of around 14%, the final temperature should not exceed a maximum of 65-75°C. Beside that, Fanslow and Saul (1971) also reported that moisture removal was small in the first 60 seconds during the microwave drying of corn. They also noted that 31 to 56% of microwave power was consumed for moisture removal and the remaining power was utilized to raise the temperature of the grain and air. My results align with Hamid *et al.* (1968), who showed in experiments that the moisture content of wheat drops by less than 1%, for exposure times great enough to cause the total mortality of three wheat insects. Boulanger *et al.* (1969) achieved a moisture reduction of around 1–3% in their experiments. In addition Vadivambal *et al.* (2007) also demonstrated that a moisture loss of around 2%, when using microwave power of 500 watt, corresponds to 100% mortality of the insects present.

3.2 The percentage of germination

The germination percentage results of barley seeds treated with RF at the temperatures 60, 65, 70 and 75°C, for 0, 1 and 3 minutes, are shown in Figure 4.5. RF heat treatment had a significant effect on the barley germination. The germination percentage was reduced with increasing temperature and processing time.

The germination percentage for barley seeds was 92.9% prior to RF heat treatment. After the seed sample was exposed, it was found that temperatures of 60 and 65 °C for 0, 1 and 3 minutes had no significant effect on germination rates, which were between 92 and 90.5%. However, using a temperature of 70°C significantly reduced the germination rate of the seed sample, but did not differ with heating times, which were to 80.6, 77.8 and 75.9% respectively. Moreover, a temperature of 75°C over the same exposure time also affected a decline in the germination percentages, which were 62.9, 61.2 and 52.1%, respectively.

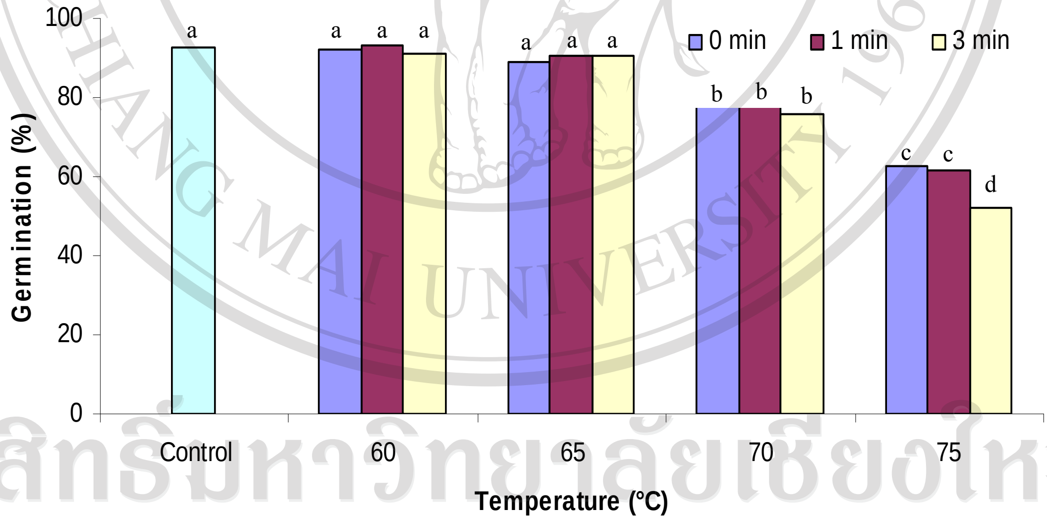

Figure 4.5 The germination of barley seeds treated with RF heat treatment at various temperatures and times

As a result, one can assume that the higher the temperatures and exposure times, the higher amount of energy was absorbed by the sample. High temperature and longer treatment times with RF may affect the embryo of the seed. Damage to the seed occurs more at the embryo than the endosperm level, because the embryo has a greater dielectric loss factor than the endosperm. This implies that the seed probably absorbs a greater amount of energy at the embryo level. Damage to the seed, especially in the part of embryo affected, causes a reduced germination capacity in the seed. The result was supported by Nelson and Stetson (1985), who reported that when seeds were exposed to RF energy of sufficiently high frequency and intensity, their temperature will rise due to dielectric heating. If samples of a given seed lot which contain an appreciable portion of hard seed were subjected to a sequence of exposures of increasing duration, germination will increase to a maximum as exposure and temperature increase; however, when increasing exposure continues, germination declines. Moreover, Campana *et al.* (1993) studied the physical, chemical and baking properties of wheat dried by microwave energy. They concluded that germination capacity was affected by exposure to microwave energy. The decrease in germination capacity was related to the final temperature and initial moisture content of the grain.

3.3 Tetrazolium test (TZ test)

Tetrazolium is a colourless chemical that reacts with living cells and stains them red. In this way, living tissue in seed embryos can be distinguished from non-living tissue. Seeds may suffer from heat-damage, which is lethal for the individual seed affected. Tetrazolium tests can detect the heat-damage within seeds, as it creates a unique staining pattern (Figure 4.6). The results of the seed viability of barley treated with RF treatments at temperatures of 60, 65, 70 and 75°C for 0, 1 and 3 minutes, are shown in Figure 4.7

When seeds were not treated with RF, the viability of the seed was 99.5%. After the seed sample was exposed, it was found that the application of RF at a temperature of 60 and 65°C for a time period of 0, 1 and 3 minutes, did not affect the viability of the seeds, which was between 99.3 and 98%. However, using a temperature of 70°C significantly reduced the viability of the seed sample, which declined to 97.8, 97.1 and 96.6%. Moreover, a temperature of 75°C for the same exposure time also affected a decline in the viability of the seeds, to 95.5, 85.6 and 84.1%.

From my study after the seed sample was exposed to RF at a temperature of 70°C, seed viability was impacted, because the seed was damaged by the temperature applied. This may indicate that damage to the seed occurs at the embryo superior level rather than in the endosperm, because the embryo has a greater dielectric loss factor than the endosperm; therefore, the seed probably absorbs a greater amount of energy than the embryo. Nelson (1976) reported that optimum germination response was related to elevation of seed temperature during dielectric-heating, and was about 75°C for alfalfa seeds of 6 to 7% moisture content. If the temperature exceeds this optimum level, the high temperatures damage seed viability. Janhang *et al.* (2005) also stated that rice seeds treated with RF temperatures of 75°C maintain high seed viability. However, increasing the temperature to 85°C results in a serious reduction in the viability of the seed.

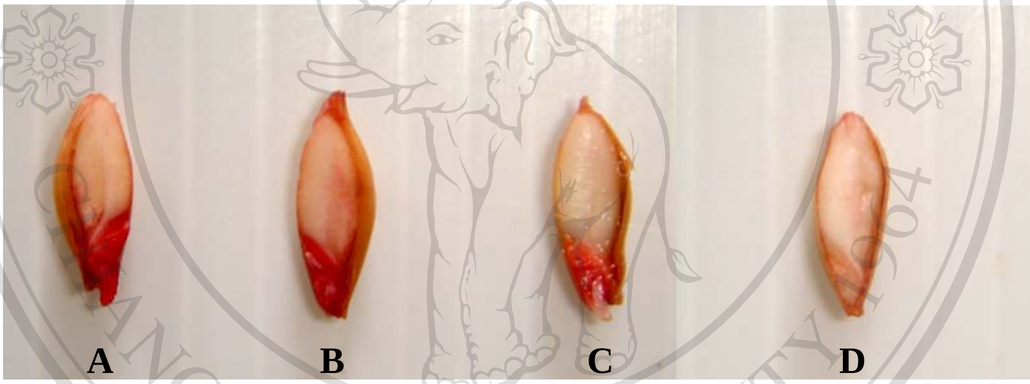

Figure 4.6 Different categories of barley seed viability characteristic determined by a tetrazolium test: **A**, **B** and **C** are viable seeds, while **D** is a non-viable or dead seed. This can be observed from the red color stained by formazan in the viable tissue.

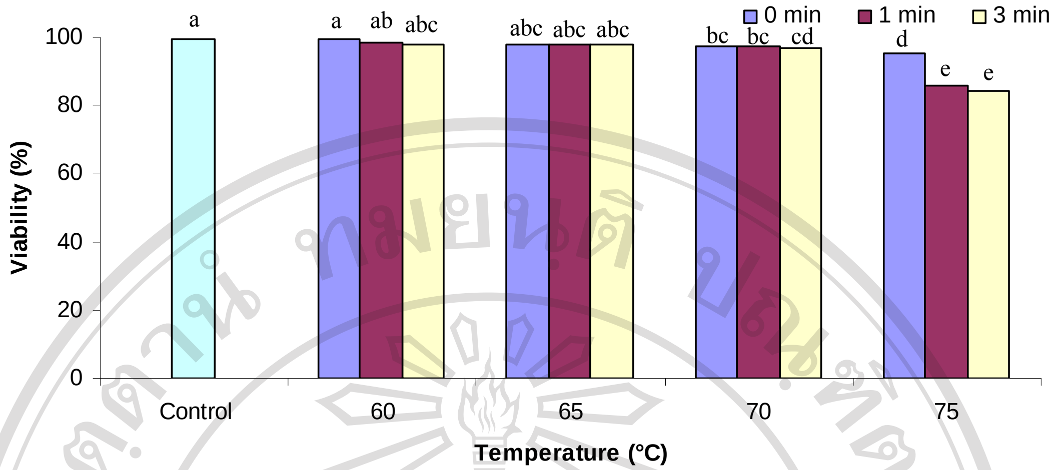

Figure 4.7 The viability of barley seeds treated with RF heat treatment at various temperatures and times

3.4 Accelerated aging test (AA test)

The degree of seed deterioration or vigour can be revealed through a stress test, such as the accelerated aging test that simulates long-term storage (AOSA, 2002). This method can be used to evaluate and predict the storability of the seed sample. Under favorable conditions, high-vigor seed lots will show only a slight decline in germination, while low-vigor seed lots will decline significantly after exposure to AA (Copeland and Mcdonald, 2001). The germination percentages resulting from the accelerated aging of barley seed treated with RF treatments at temperatures of 60, 65, 70 and 75°C for 0, 1 and 3 minutes, are shown in Figure 4.8. RF heat treatment has a significant effect on the barley seed viability, which reduces with increasing temperature and processing time.

When seeds were not treated with RF, the germination after accelerated aging was 42.3%, and after the seed sample was exposed to temperatures of 60 and 65 °C for 0, 1 and 3 minutes, there was no significant effect on the germination rates, which were between 38.1 and 34.4%. However, barley seed germination rates, after being treated at a temperature of 70°C and with the same procedure time, decreased significantly to 31, 28.9 and 26.5%. Moreover, when the temperature was increased to

75°C, this resulted in a significant decrease in the germination percentages under stress conditions, to 18.4, 18.1 and 16.6% respectively.

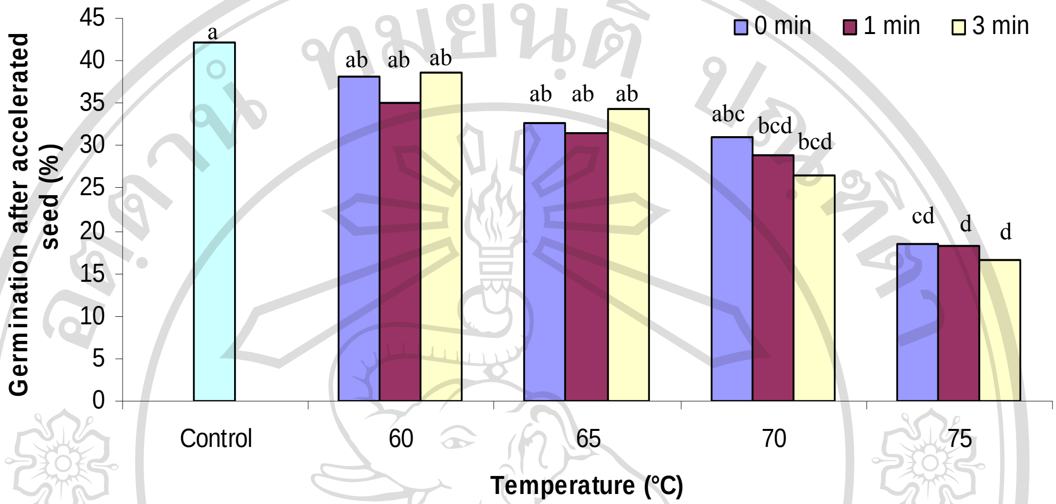

Figure 4.8 The germination after accelerated aging of barley seeds treated with RF heat treatment at various temperatures and times

In this experiment, once the seeds had germinated after accelerated aging, their vigor and storability was assessed. Exposing the barley seeds to RF over 70°C, the treatment heated up all the water molecules in the seeds, rapidly generating heat within them. A high temperature and a long exposure to RF caused overheating of the product, which suffered maximum damage during processing and demonstrated poor storage quality. This finding was in agreement with Vassanacharoen (2005) who found that treated sesame seeds with high RF temperatures (over 80°C) resulted in dramatically decreased vigor of the seeds. This suggestsed that high temperature RF treatment significantly reduced the vigor of seeds. Low seed vigor leads to increasingly large free fatty acid levels and free radicals productivity by lipid peroxidation. Priestley (1986) concluded that free fatty acids have deleterious effect on membranes, probably because they are detergents. Membrane disruption is one of the main reasons for seed deterioration. As a result, seed cells are not able to retain their normal physical condition and function. Crowe *et al*. (1989) showed that the addition of free fatty acids increases the fusion of plant vesicles, which leads to an increase in membrane leakage. McDonald (1995) reported that continual accumulation of free fatty acids culminates in a reduction of cellular pH and is detrimental to normal cellular metabolism. Furthermore, it denatures enzymes, resulting in loss of their activity. Individual seeds containing 1% or more free fatty acids will usually not germinate.

3.5 Germination index

The effect of RF treatment on the germination index was examined according to the exposures given by ISTA (2006). After treated barley seeds with temperatures of 60, 65, 70 and 75°C for 0, 1 and 3 minutes, the results showed that the germination index of the sample decreased in line with a corresponding increase in temperature and exposure time, as shown in Figure 4.9

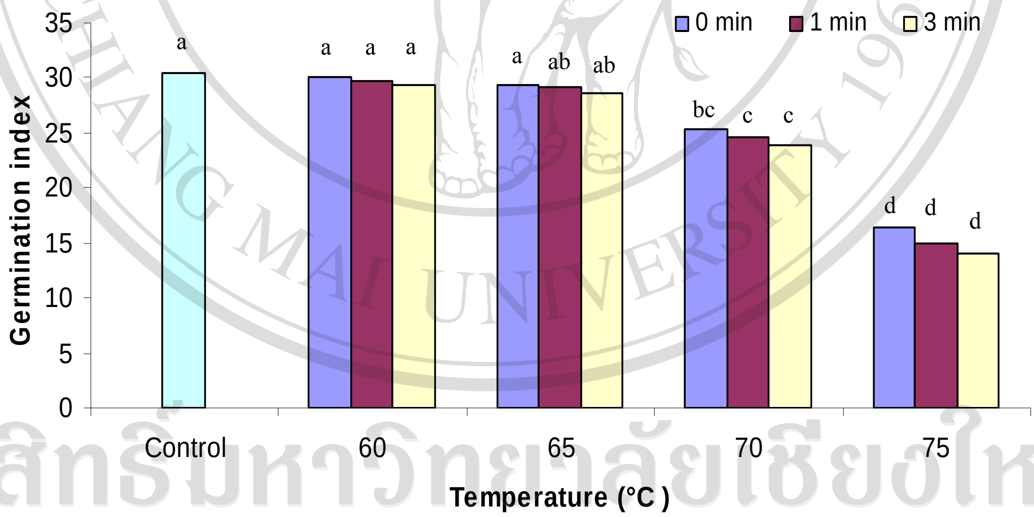

Figure 4.9 The germination index of barley seeds treated with RF heat treatment at various temperatures and times

The germination index of untreated barley seed was 30.5. After the seed sample had been exposed, it was found that an RF temperature of 60 and 65°C for exposure times of 0, 1 and 3 minutes kept the germination index level of the seeds as high as the control: between 30.1-28.7. However, the germination index declined to

25.4, 24.5 and 23.9 after applying a temperature of 70°C, but did not very in line with the heating times. Moreover, when the temperature was increased to 75°C, this treatment resulted in seriously reduced seedling establishment, which were 16.3, 15 and 14.1 respectively.

A seed that has a greater germination index is considered to be more vigorous. As a result, it could be assumed that high temperatures and long RF treatment times might damage the embryo of a seed sample, reducing seedling performance. McCormack (2004) stated that the microwave heating process speeds up a seeds metabolism, and may affect the permeability of the lipid-containing membranes, thereby causing cellular changes which enhance the germination of the seed. If the seed is heated too rapidly or for too long, certain biological molecules (proteins) such as enzymes are denatured, losing their enzymatic activity and as a result the seed is killed.

It is possible that the endosperm of the barley seed, which is made up principally of thin walled cells filled with protoplasm and starch granules, might have a lower resistance to moisture transfer than the non-porous pericarp and aleurone layer (Gunasekaran *et al.,* 1985). Therefore, the water vapour generated inside the seed due to heating, starts to accumulate within the endosperm. This is probably enhanced in dielectric heating, where volumetric heat generation may result in high temperatures. Furthermore, due to the different composition of various sections of the seed, it is likely that they possess different dielectric properties, leading to different levels of energy absorption. This may influence the location of damage within a seed (endosperm versus embryo), affecting the seed vigor and leading to lower seedling growth.

Experiment 4: The effect of radio frequency heat treatment on the chemical composition

4.1 Total protein content

The effect of RF treatment on the total protein content of barley seed treated with RF treatments at temperatures of 60, 65, 70 and 75°C, for 0, 1 and 3 minutes, are shown in Table 4.3. In barley seeds not treated with RF, the total protein content was 10.7%. After the seed sample was exposed, it was found that with the increasing time period and temperature of the RF treatment, there were no significant differences found in the total protein content in barley, seed which was between 10.4 and 10.7%.

This result was in accordance with Campana *et al.* (1993) who studied the physical, chemical and baking properties of wheat dried with microwave energy. They found that the protein content was not affected, but that the functionality of gluten was changed gradually with increasing exposure times. Walde *et al.* (2002) studied the microwave drying characteristics of wheat and reported no change in the total protein content of microwave treated wheat samples, but that the structural and functional characteristics of the wheat protein-gluten changed. The change in the functionality of gluten was observed through an absence of elasticity within the dough. They concluded that the microwave drying of wheat was not suitable where the final product made from flour which needs to be soft in its textural characteristics. Velu *et al.* (2006) studied the dry milling characteristics of microwave dried maize (*Zea mays* L.). The microwave drying did not alter the protein content, as measured by Kjeldahl's method. However, some structural changes in the starch and protein were noticed. The protein and starch content of microwave dried samples were 10.5% and 65.7%, respectively, whereas for the control sample, protein and starch were 10.06% and 60.6%, respectively. Based on my results, it can conclude that RF treatment did not affect the total nitrogen content in barley seeds.

Table 4.3 The total protein content of barley seeds treated with RF heat treatment at various temperatures and times

Treatment		Total protein content
Temperature	Time	(g 100g^{-1} DM)
control		10.7
60°C	0 min	10.4
60°C	1 min	10.5
60°C	3 min	10.5
65°C	0 min	10.6
65°C	1 min	10.7
65°C	3 min	10.6
70°C	0 min	10.7
70°C	1 min	10.6
70°C	3 min	10.6
75°C	0 min	10.5
75°C	1 min	10.6
75°C	3 min	10.6
F-test	=	ns
CV (%)	=	1.60
$LSD_{0.05}$	=	0.24

4.2 Dehydrogenase enzyme activity

The dehydrogenase enzyme activity results of barley seed treated with RF treatments at temperatures of 60, 65, 70 and 75°C, for 0, 1 and 3 minutes, are shown in Figure 4.10. RF heat treatment had a significant effect on the activity of the enzyme.

The result suggested that decreasing in dehydrogenase enzyme activity was related to the temperature of RF treatment more than the heating time. The RF at a temperature of 60°C for 0, 1 and 3 minute durations had no effect on the dehydrogenase enzyme activity of the seed sample, which remained as high as the untreated seed; between 104.1- 98.7 mMg^{-1}DM. However, the enzyme activity declined to 95.8, 95.9 and 93.8 mMg^{-1}DM after treating the seed sample with a temperature of 65°C for 0, 1 and 3 minutes, but did not differ between the heating times. When the seeds were exposed to RF heating over a temperature of 70°C, the activity of the enzymes dropped to 92.8, 89.8 and 86.1 $mMg^{-1}DM$. Moreover, when the RF temperature was increased to 75°C, it seriously reduced the activity of the enzyme, which was 16.3, 15 and 14.1 respectively.

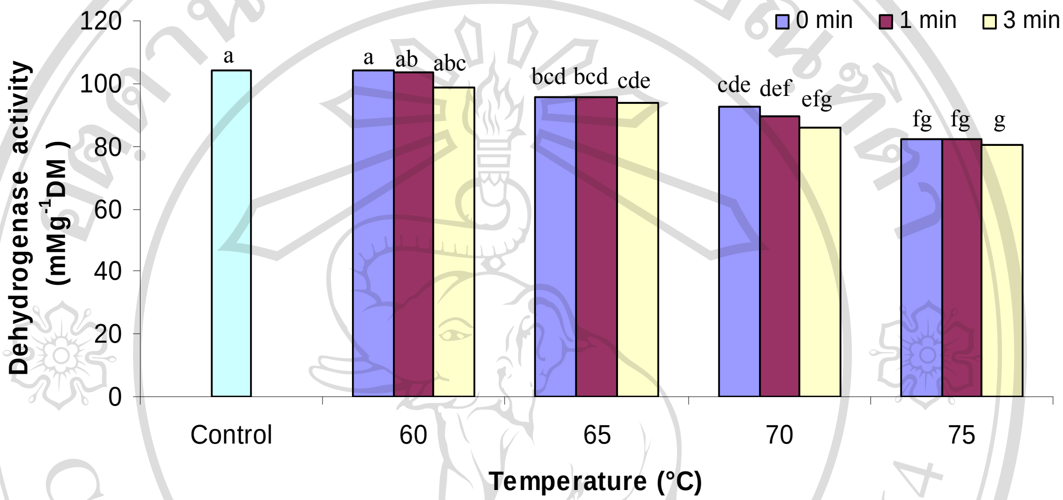

Figure 4.10 The dehydrogenase activity of barley seeds treated RF heat treatment at various temperatures and times

From my study, after the seed sample was exposed to RF at a temperature of 65°C, the seed was affected due to heat damage. This may indicate that damage to the seeds occurs at the embryo superior rather than endosperm level, because the embryo has a greater dielectric loss factor than the endosperm and so the seed probably absorbs a greater amount of energy at the embryo level (Orsat, 1999). The embryo is the major part of the seed and contains the highest amounts of protein and a large amount of enzyme. If heating the seed above a critical temperature, heat de-naturation leads to enzyme inactivation. Furthermore, it denatures the enzymes, resulting in a reduction in their activity levels. This reduction in enzyme activity results in un-germinated seed. Vassanacharoen (2005) reported that treating sesame seeds with RF at a temperature of 60 and 70°C did not affect the dehydrogenase enzyme activity of the seed. However, increasing the temperature to 80, 85 and 95°C result in decreased the activity of the enzyme. In addition enzyme activity decreased with an increase in the moisture of the seed.