CHAPTER 4

RESULTS AND DISCUSSION

The control of seed-borne pathogen with ozone treatment in rice seed.

To examine the effect of ozone application on the infection of fungi in rice, ozone doses of 1.25 mg/g rice seed/hr was used. All ozonation experiments were conducted on dry and wet rice seed sample for 2, 4, 6 and 8 hrs. The result showed that ozone treatment significantly controlled seed-borne fungi in the wet seed (18% MC) higher than the dry seed. The ozone treatment for 8 hr was most effective in reduction of seed-borne fungi from 83.88% to 22.06% (Table 4.1 and Figure 4.1)

After seed-borne fungi observation, it was found that *Alternaria padwickii* and *Fusarium moniliform* were mostly appeared on the rice seed cv. Khao Dawk Mali 105. The ozone treatment was efficiently controlled *A. padwickii* and *F. moniliform* especially in the wet seed, as the percentage of *A. padwickii* decreased from 66% to 14.38% (Table 4.2 and Figure 4.2) and *F. moniliform* decreased from 5.38% to 1.00% (Table 4.3 and Figure 4.3). So, ozone treatments were special to control the seed-borne fungi. But their effectiveness depended on the seed moisture contents. The high efficiency was found in wet seed. The increases of seed moisture contents also promoted the seed-borne fungi growth rate. According to Raila *et al*. (2006), it has been established that ozone, in reaction with the more moist grain surface, the mycological pollution of which is usually higher than that of dry one, splits more rapidly. As ozone is a strong oxidizer, at the same time it destroys microorganism of the grain surface, ozone penetrates its layer slower, with a longer reaction with the grain surface and microflora on this surface, the destruction of which is more effective because of longer exposure. Zhao and Cranston (1995) found that when ozone was used to inactivate microorganism on black pepper, the effectiveness of ozone on the reduction of fungal spores was strongly influenced by the moisture content of the ground black pepper. Higher moisture content led to more reduction in fungal spore. It is known that ozone could owe its biocidal effectiveness

to the direct lysing of cellular walls. Ozone readily oxidizes organic materials in microbial membrane, which weakens the cell wall and leads to cell rupture (Pryor and Rice, 1998). Hoigne and Bader (1983) have proved that the presence of water often accelerates ozone reaction with organic substance, because ozone decomposes more readily in water than in the atmosphere to form free radicals, which are more powerful oxidants than the molecular ozone (Mason *et al*., 1997). Allen *et a*l. (2003) and Kim *et al*. (2003) confirms assertion that ozone in the media with a greater surface moisture content is a more active oxidizer, while the ozone with 280 ppb., ozone concentration in dry agent, the change of grain mycological pollution, compared with ventilation without ozone in air, was not statistically significant (Raila *et al*., 2006)

Table 4.1 Percentage of rice seed infected by fungi in dry and wet seed treated with ozone 1.25 mg/g rice seed/hr at various times

Ozonation time (hr)	Seed infected by fungi (%)		Average
	Dry seed (11% MC)	Wet seed (18% MC)	
0 (Control)	87.50	80.25	83.88a
2	75.50	64.88	70.19b
4	58.00	47.13	52.56c
6	34.50	26.39	30.44d
8	26.25	17.88	22.06d
Average	56.35a	47.30b	
LSD .05 (Ozonation time)	9.82		
LSD .05 (Seed condition)	5.19		
CV (Ozonation time)	17.39%		
CV (Seed condition)	14.85%		

* The different letters in the same row and column indicate the statistically significant by LSD at 5% level

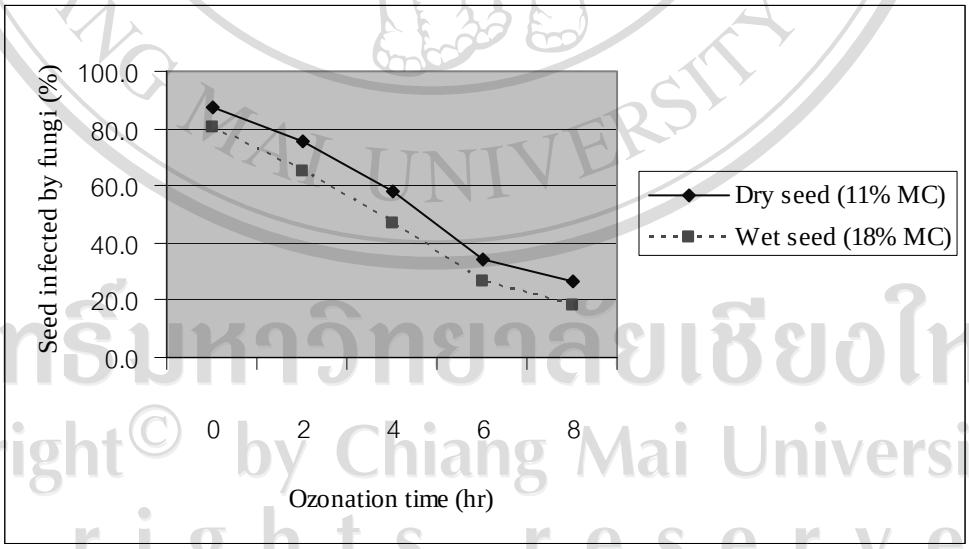

Figure 4.1 Percentage of rice seed infected by fungi in dry and wet seed treated with ozone 1.25 mg/g rice seed/hr at various times

Table 4.2 Percentage of rice seed infected by *Alternaria padwickii* in dry and wet seed treated with ozone 1.25 mg/g rice seed/ hr at various times

Ozonation time (hr)	Seed infected by *A. padwickii* (%)		Average
	Dry seed (11% MC)	Wet seed (18% MC)	
0	69.63	66.00	67.81a
2	56.75	52.75	54.75b
4	43.63	38.25	40.94c
6	27.38	21.57	24.47d
8	20.38	14.38	17.38d
Average	43.55a	38.59b	
LSD .05 (Ozonation time) 8.56			
LSD .05 (Seed condition) 4.35			
CV (Ozonation time) 19.13%			
CV (Seed condition) 15.72%			

* The different letters in the same row and column indicate the statistically significant by LSD at 5% level

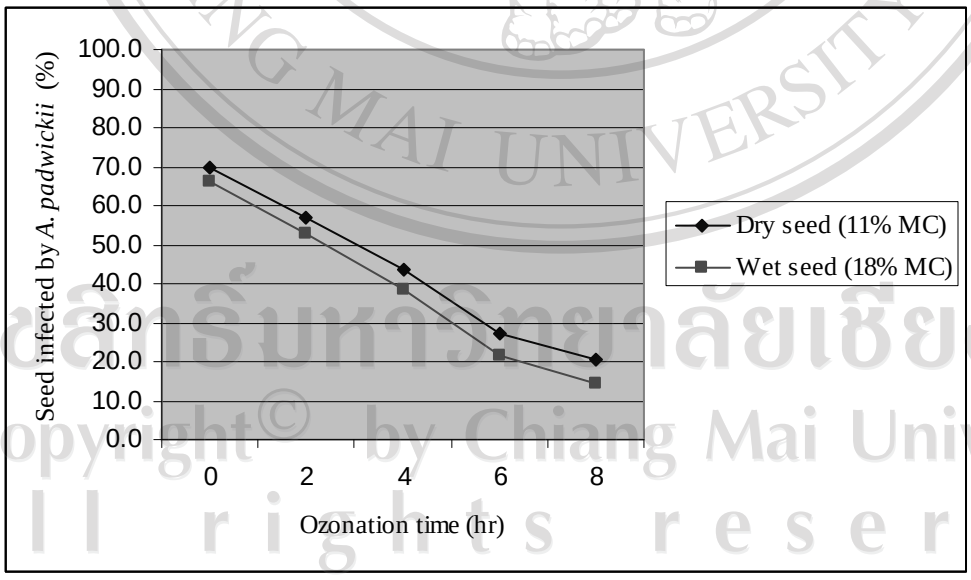

Figure 4.2 Percentage of rice seed infected by *Alternaria padwickii* in dry and wet seed treated with ozone 1.25 mg/g rice seed/hr at various times

Table 4.3 Percentage of rice seed infected by *Fusarium moniliforme* in dry and wet seed treated with ozone 1.25 mg/g rice seed/hr at various times

Ozonation time (hr)	Seed infected by *F. moniliforme* (%)		Average
	Dry seed (11% MC)	Wet seed (18% MC)	
0 (Control)	3.63	5.38	4.5a
2	2.75	3.75	3.25a
4	1.50	2.25	1.88b
6	0.63	1.01	0.82b
8	0.63	1.00	0.81b
Average	1.83b	2.68a	
LSD .05 (Ozonation time) 1.26			
LSD .05 (Seed condition) 0.45			
CV (Ozonation time) 51.42%			
CV (Seed condition) 29.54%			

* The different letters in the same row and column indicate the statistically significant by LSD at 5% level

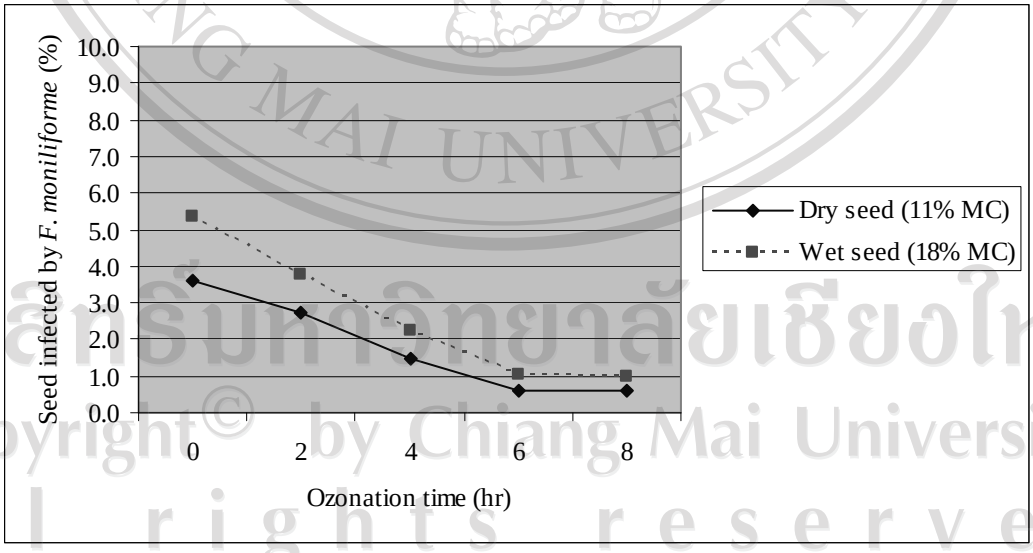

Figure 4.3 Percentage of rice seed infected by *Fusarium moniliforme* in dry and wet seed treated with ozone 1.25 mg/g rice seed/hr at various times

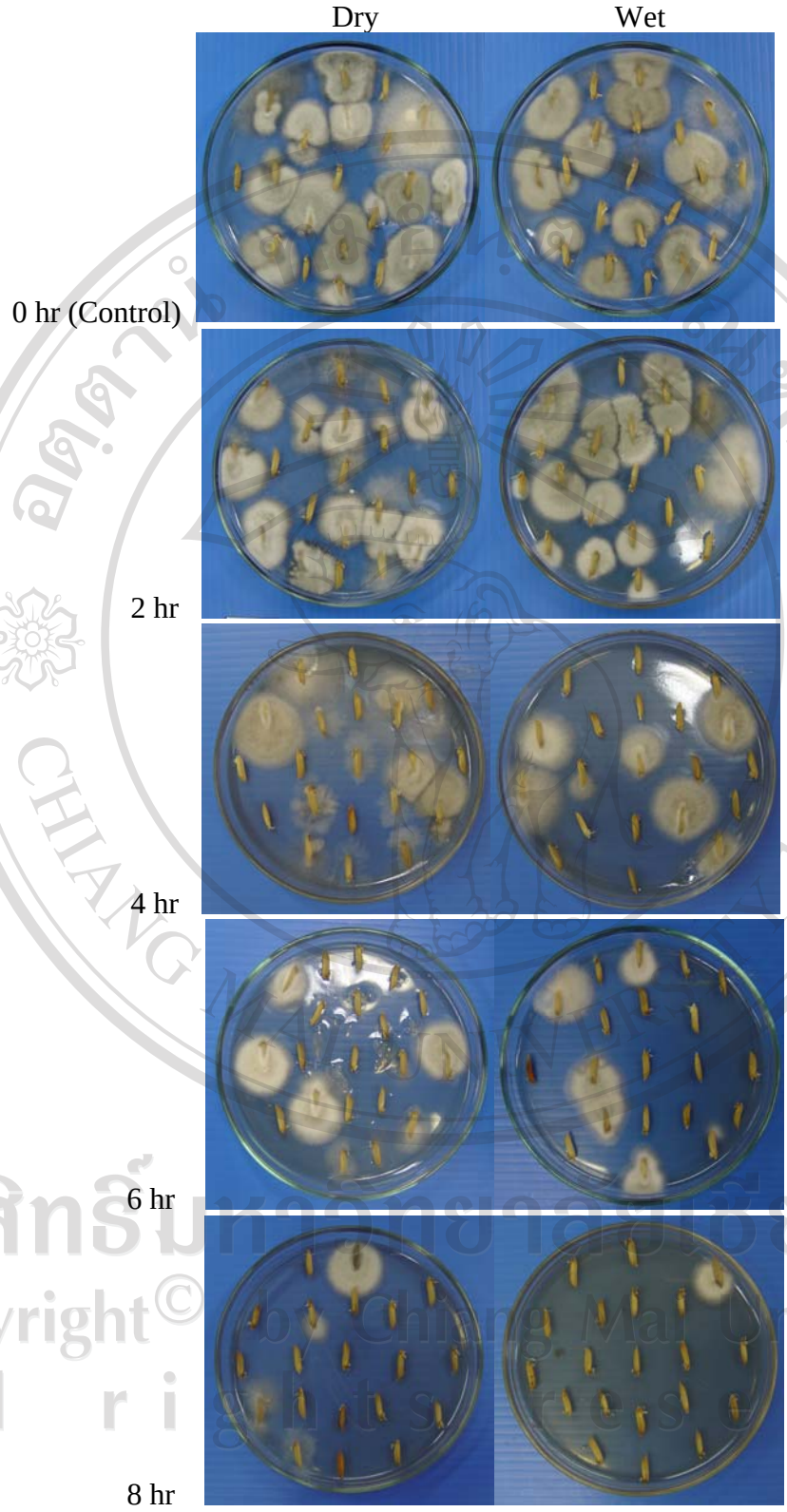

Figure 4.4 Colonies growth of fungi culture on PDA in dry and wet seed treated with ozone 1.25 mg/g rice seed/hr for 0, 2, 4, 6 and 8 hr

Effect of ozone treatment on the changes of seed moisture content

The influence of ozone on the changes of seed moisture content was investigated. Using 1.25 mg/g rice seed/hr ozone in dry (11% MC) and wet seed (18% MC) for various durations: 0, 2, 4, 6 and 8 hr. The result showed that the decreases of seed moisture content related to the increases of ozonation times. The ozone treatment was effective to decrease seed moisture content from 18.46% to 17.51, 16.83, 16.77 and 16.13% in the wet seed, and from 11.30 to 11.12, 11.08, 10.87 and 10.80% in the dry seed after ozonation for 2, 4, 6 and 8 hrs respectively (Table 4.4, Figure 4.5). The result was supported by Raila *et al*. (2006) who reported that the more intense reduction of grain moisture with surface exposed to ozone was possible because of the interaction of ozone with the water or moisture content of material exposed, caused changes in the physical, chemical and thermal properties of water (Glushchenko and Glushchenko, 2003).

Table 4.4 Percentage of rice seed moisture content in dry and wet seed treated with ozone 1.25 mg/g rice seed/hr at various times

Ozonation time (hr)	Seed moisture content (%)		Average
	Dry seed (11% MC)	Wet seed (18% MC)	
0 (Control)	11.30e	18.46a	14.88a
2	11.12ef	17.51b	14.31b
4	11.08ef	16.83c	13.95c
6	10.87ef	16.77c	13.82c
8	10.80f	16.13d	13.46d
Average	11.03b	17.14a	
LSD .05 (Ozonation time) 0.29			
LSD .05 (Seed condition) 0.23			
LSD .05 (Ozonation time*Seed condition) 0.51			
CV (Ozonation time) 1.89%			
CV (Seed condition) 2.39%			

* The different letters in the same row and column indicate the statistically significant by LSD at 5% level

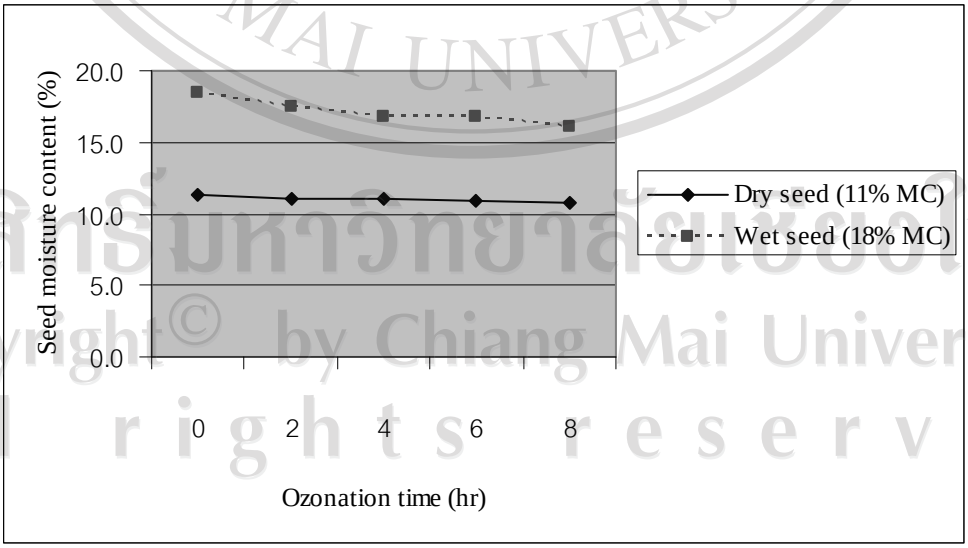

Figure 4.5 Percentage of rice seed moisture content in dry and wet seed treated with ozone 1.25 mg/g rice seed/ hr at various times

The effect of ozone treatment on germination and viability of rice seed

The influence of ozone treatment on seed germination and seed viability were examined according to the method proposed by the International Seed Testing Association. The dry (11% MC) and wet (18% MC) rice seed samples were applied with ozone in the different times. The results showed that the germination and viability of rice seed were almost unaffected by 2 hr ozone treatment. The increase of ozonation times led to slowly decrease the percentages germination and viability. The ozone treatment for 8 hr resulted in the lowest of germination and viability percentages which were 81% in dry seed (11% MC) and 80% in wet seed (18% MC) (Table 4.5, Figure 4.6). The trend of viability was similar to that of the germination. The viability were 86% and 83% in the dry and wet seed respectively (Table 4.6, Figure 4.7)

The result reported by Bender *et al.* (2006) who studied on the responses of biomass production and reproductive development to ozone exposure, indicated the differences between European wild plant species when applied with a range of ozone concentrations from 20 to 55 ppb (seasonal 8 hrs daily mean). The result showed that germinability of seed was affected by ozone application as germination rate of ozone treated plants was 30% or more lower than that of the control plants. Moreover, Jiangning *et al*. (2006) investigated the application of gaseous ozone as a fungicide to preserve stored wheat. The ozonation dose of 0.98 mg/g wheat/ min applied for 0, 5, 10, 15, 20, 30 and 45 min. They found that the germination capacity of the wheat was almost unaffected by 15 min ozonation, but, the ozonation for 20 and 30 min reduced the germination percentages to 85.4% and 80.0%, respectively. The wheat germination was reduced to 61.3% by 45 min of ozonation.

Table 4.5 Percentage of rice seed germination in dry and wet seed treated with ozone 1.25 mg/g rice seed/hr at various times

Ozonation time (hr)	Germination (%)		Average
	Dry seed (11% MC)	Wet seed (18% MC)	
0 (Control)	89	88	89a
2	87	88	88a
4	81	88	85b
6	83	86	85b
8	81	80	81c
Average	84	86	
LSD .05 (Ozonation time) 2.54			
CV (Ozonation time) 2.74%			
CV (Seed condition) 4.07%			

* The different letters in the same column indicate the statistically significant by LSD at 5% level

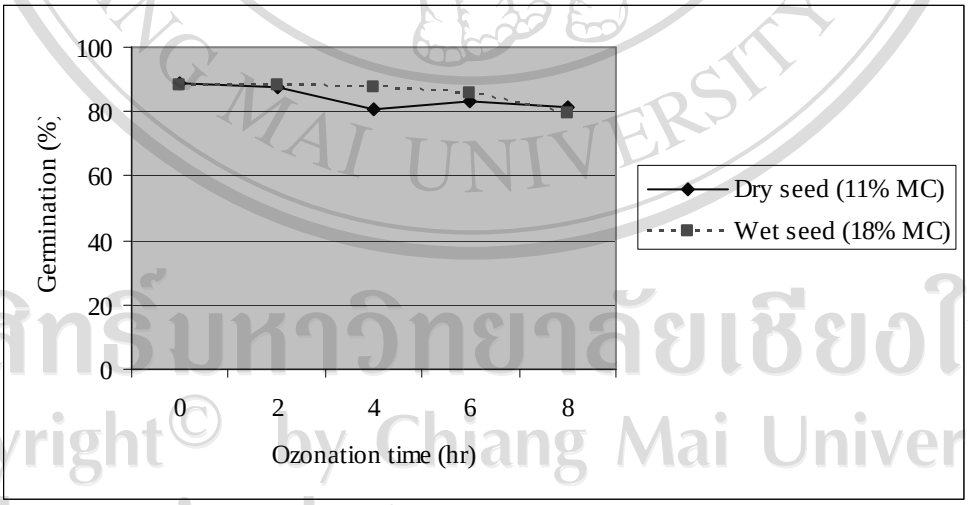

Figure 4.6 Percentage of rice seed germination in dry and wet seed treated with ozone 1.25 mg/g rice seed/hr at various times

Table 4.6 Percentage of rice seed viability in dry and wet seed treated with ozone 1.25 mg/g rice seed/hr at various times

Ozonation time (hr)	Seed viability (%)		Average
	Dry seed (11% MC)	Wet seed (18% MC)	
0 (Control)	97	97	97a
2	96	96	96a
4	92	93	93b
6	90	89	89c
8	86	83	85d
Average	92	92	
LSD .05 (Ozonation time) 1.87			
CV (Ozonation time) 1.86%			
CV (Seed condition) 1.77%			

* The different letters in the same column indicate the statistically significant by LSD at 5% level

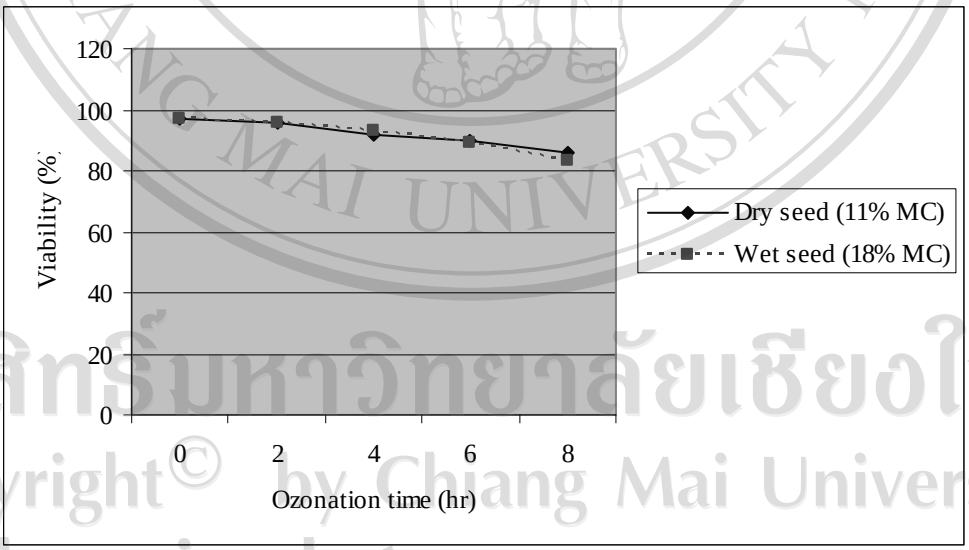

Figure 4.7 Percentage of rice seed viability in dry and wet seed treated with ozone 1.25 mg/g rice seed/hr at various times

Effect of ozone treatment on seed vigor

The general vigor testing strategy has been to measure some aspect of seed performance or condition that reflects the stage of seed deterioration and to provide information about the planting value for a wide range of environmental and/or the storage potential of seed lot. The observation of seed vigor after ozonation with 1.25 mg/g rice seed/hr during ozonation time for 2, 4, 6 and 8 hr, were determined with the speed of germination, the seedling growth rate and the cold test. The result showed as follows.

1. The consequence of ozone treatment on the speed of germination.

The speed of germination after ozone treatment at various time are shown in table 4.7 It can be observed that the ozonation time for 2 hr had the higher speed of germination, it was 15.49 seedling/day. While ozonation time for 8 hr had the lowest speed of germination, it was 13.03 seedling/day. In the difference of seed condition, it was found that ozone treatment in the wet seed (18% MC) had the higher speed of germination more than the dry seed (11% MC) in all of ozonation time treatment. The speed of germination were 15.00 seedling/day in wet seed and 14.06 seedling/day in dry seed.

2. The consequence of ozonation on the seedling growth rate.

The results of the seedling growth rate after ozone treatment at various time are shown in table 4.8. It can be observed that the seedling growth of rice seed was almost unaffected by ozonation times for 2, 4 and 6 hr, and there was no difference between the treatments and control group which were 6.22, 5.99 and 5.91 mg/seedling/7 days, respectively. Whereas ozone treatment with 8 hr had the most effect on decreasing the seedling growth rate to 5.39 mg/seedling/7 days. In addition, ozonation in the wet seed (18% MC) had higher seedling growth rate than that of the dry seed (11% MC). The seedling growth rates were 6.10 mg/seedling/7 days in wet seed and 5.68 mg/seedling/7 days in dry seed.

3. The consequence of ozonation on the germination percentage by cold test.

The cold test determines the ability of seed to germinate and produce normal seedling after exposure to the stress environment in cold and moist conditions, and the report of the normal seedling after germination. The results of germination by cold test

after ozone treatment at various time are shown in table 4.9. It can observed that the increases of ozonation time significantly affected to reduce the percentage of seed germination. The ozonation for 2 hr was 79%, which had the least effect to decrease seed germination. Whereas, under ozonation for 8 hr had the most effect to decrease the germination from control group (94%) to 65%. The ozone treatment had the effect to decrease germination in dry seed (11% MC) more than wet seed (18% MC), the germination were 74% and 79%, respectively.

The vigor of rice seed was effected by ozonation time increased. The ozonation time for 8 hr. was the critical point which significantly decreased seed vigor. The present results agreed with Bender *et al.* (2006) who studied on the responses of biomass production and reproductive development to ozone exposure which differed between european wild plant species. As such experiment, the ozone treatments covered a range of concentration from 20 to 55 ppb ozone (seasonal 8 hr daily mean), the results revealed significant differences between species with respect to the sensitivity of different end points toward ozone exposure. Ozone caused a significant reduction in leaf biomass of more than 20% in six species. These result supported by Black *et al.* (2007), who studied on the ozone effects on gas exchange, growth and reproductive development in *Brassica campestris*. The results demonstrated that exposure to ozone during the vegetative phase might affect physiological processes, vegetative growth and reproductive development in *B. campestris*, even though the reproductive organs were not directly exposed. The nature and extent of the short- and long-term effects induced were apparently influenced by ozone dose and uptake, effectiveness of physiological repair and acclimation mechanisms, and compensation mechanisms operating at the reproductive level. Exposure to 70 ppb ozone for 7 hr/day for 2 or 10 days reduced stomatal conductance and net assimilation and induced visible injury, consistent with reports for other species (Reiling and Davison, 1992; Sanders *et al*., 1992; Hassan *et al*., 1994; Reiling and Davison, 1995; Donnelly *et al*., 2001; Zheng *et al*., 2002; Crous *et al*., 2006; Iriti *et al*., 2006; Rämö *et al*., 2006; Souza *et al*., 2006). The reductions in stomatal conductance and net assimilation were greater and extensive visible injury was observed when pre-exposure stomatal conductance was relatively high (Stewart, 1998).

Table 4.7 Speed of germination (Seedling/day) of rice seed in dry and wet seed treated with ozone 1.25 mg/g rice seed/hr at various times

Ozonation time (hr)	Speed of germination (seedling/day)		Average
	Dry seed (11% MC)	Wet seed (18% MC)	
0 (Control)	15.03a	15.00a	15.02ab
2	15.07a	15.90a	15.49a
4	13.60b	15.67a	14.63b
6	13.66b	15.34a	14.50b
8	12.95b	13.11b	13.03c
Average	14.06b	15.00a	
LSD .05 (Ozonation time) 0.71			
LSD .05 (Seed condition) 0.44			
LSD .05 (Ozonation time*Seed condition) 0.98			
CV (Ozonation time) 4.47%			
CV (Seed condition) 4.46%			

* The different letters in the same row and column indicate the statistically significant by LSD at 5% level

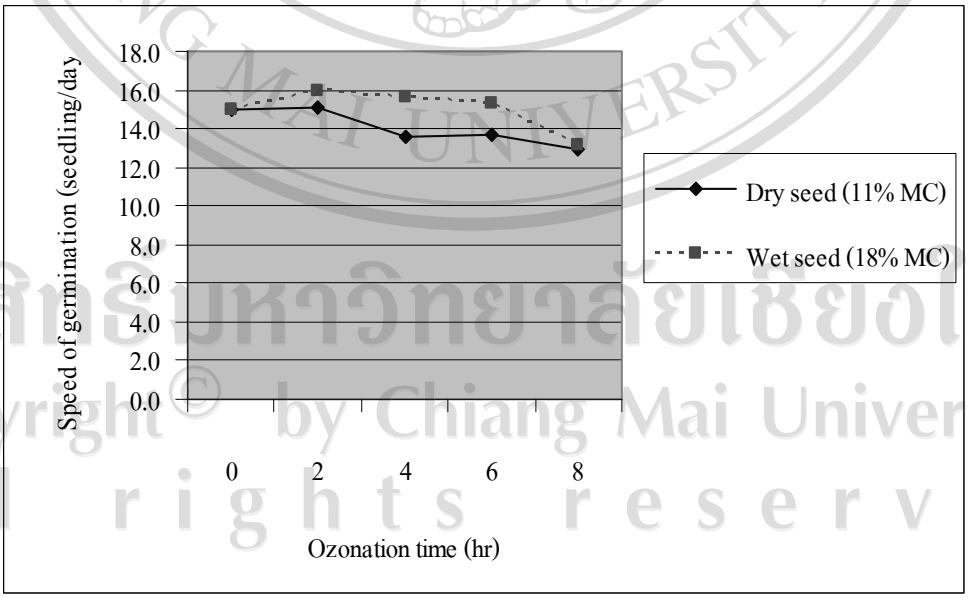

Figure 4.8 Speed of germination (Seedling/day) of rice seed in dry and wet seed treated with ozone 1.25 mg/g rice seed/hr at various times

Table 4.8 Seedling growth rate (mg/seedling/7 days) of rice seed in dry and wet seed treated with ozone 1.25 mg/g rice seed/hr at various times

Ozonation time (hr)	Seedling growth rate (mg/seedling/7 days)		Average
	Dry seed (11% MC)	Wet seed (18% MC)	
0	6.03	5.84	5.94a
2	6.00	6.45	6.22a
4	5.71	6.28	5.99a
6	5.62	6.20	5.91a
8	5.06	5.73	5.39b
Average	5.68b	6.10a	
LSD .05 (Ozonation time) 0.39			
LSD .05 (Seed condition) 0.23			
CV (Ozonation time) 6.01%			
CV (Seed condition) 5.84%			

* The different letters in the same row and column indicate the statistically significant by LSD at 5% level

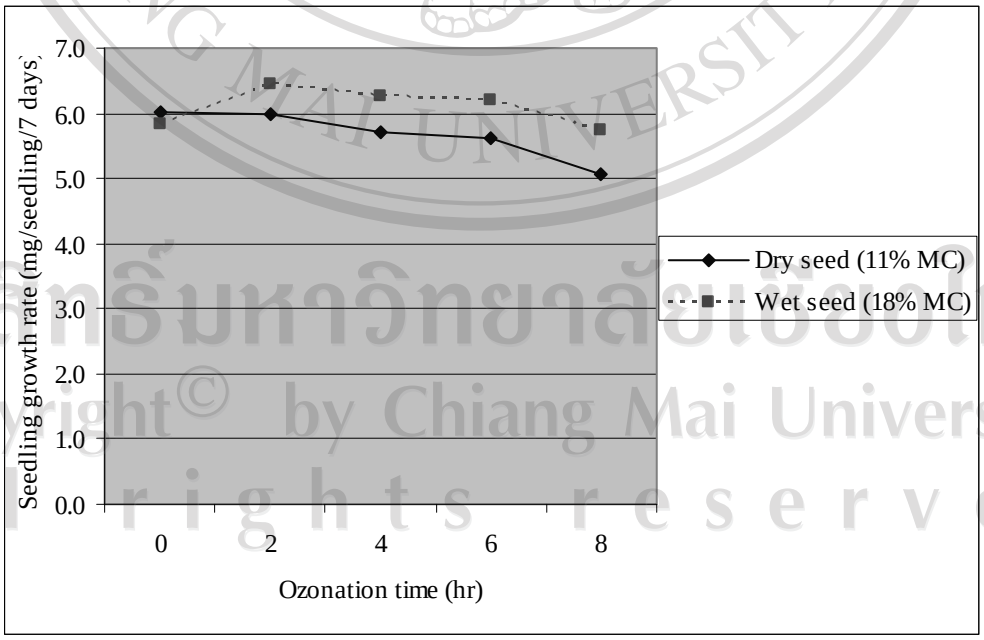

Figure 4.9 Seedling growth rate (mg/seedling/7 days) of rice seed in dry and wet seed treated with ozone 1.25 mg/g rice seed/hr at various times

Table 4.9 Percentage of rice seed germination by cold test in dry and wet seed treated with ozone 1.25 mg/g rice seed/hr at various times

Ozonation time (hr)	Germination (%)		Average
	Dry seed (11% MC)	Wet seed (18% MC)	
0 (Control)	95a	92ab	94a
2	75de	83bc	79b
4	69ef	80cd	74bc
6	65f	74de	70cd
8	65f	65f	65d
Average	74b	79a	
LSD .05 (Ozonation time) 6.64			
LSD .05 (Seed condition) 3.37			
LSD .05 (Ozonation time*Seed condition) 7.53			
CV (Ozonation time) 7.98%			
CV (Seed condition) 6.54%			

* The different letters in the same row and column indicate the statistically significant by LSD at 5% level

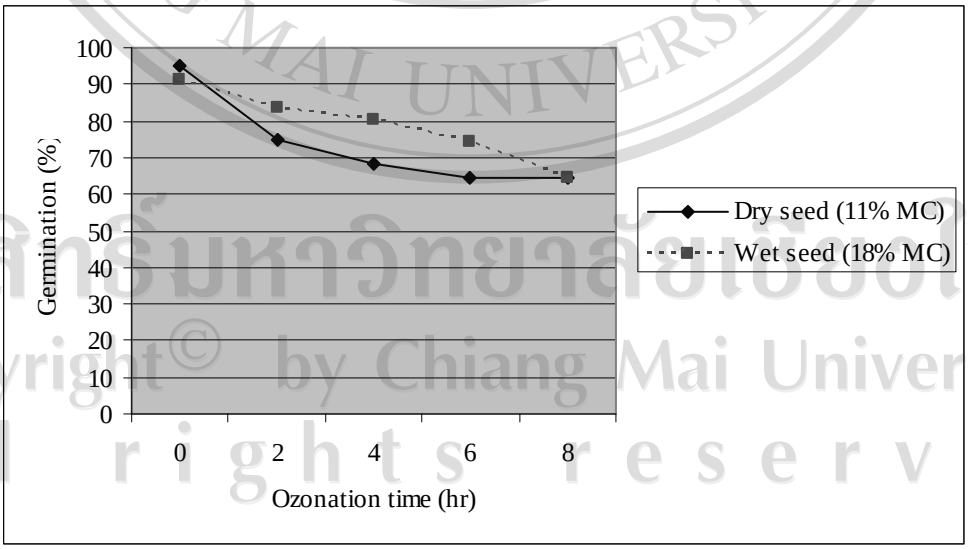

Figure 4.10 Percentage of rice seed germination by cold test in dry and wet seed treated with ozone 1.25 mg/g rice seed/hr at various times

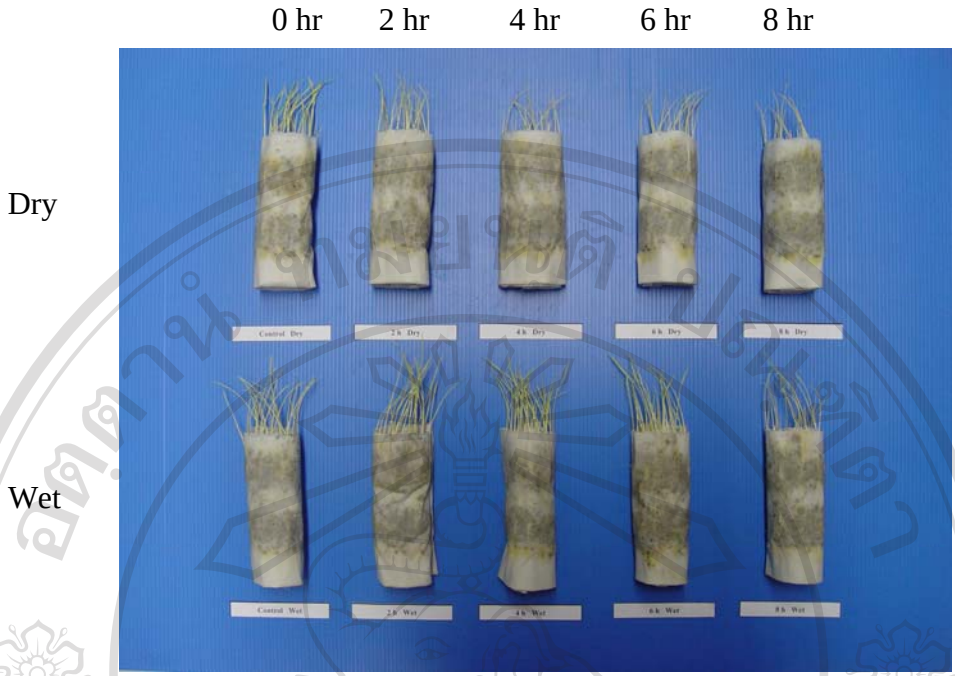

Figure 4.11 Growth of rice seedling by cold test in dry (A) and wet (B) seed treated with ozone 1.25 mg/g rice seed/hr for 0, 2, 4, 6 and 8 hr.

Effect of ozonation to control *Sitophilus oryzae* (rice weevil) adult in rice seed.

The effect of ozone to control adult rice weevil by the percentage of insect mortality was examined after ozone treatment with 1.25 mg/g rice seed/hr at various time: 0, 2, 4, 6 and 8 hrs. The results showed that the percentages of rice weevil mortality were related with the increase of ozonation times. The ozonation time increase significantly increased mortality as compared with the control (Table 4.10). The mortality levels of 100% were observed for rice weevil in rice seed treated which ozonation times 3 and 4 hr. While at the ozonation time for 1 and 2 hrs controlled rice weevil only 59 and 81% respective. The result are consistent with Stephen *et al*. (2001) project, which reported that ozone fumigation treatments at 50 ppm. for 3 days, and 25 ppm. for 5 days significantly increased mortality of Indian meal moth larvae, maize weevil adults and red flour beetle adults. Under the ozonation times 1 and 2 hrs, the minority of surviving adult rice weevils after ozonation lost their ability to control the movement, that are accordant to Stephen *et al*. (2001) who reported that after ozonation under condition 50 ppm. For 3 days, the majority of maize weevil adults surviving displayed behaviors, for examples: more than one pair of legs failing to move, or a lack of coordinated movement in all legs.

Strail (1998) also observed similar behavioral effects as well as delayed mortality after the insects survived initial ozone treatment.

Table 4.10 Percentage of adult *Sitophilus oryzae* (rice weevil) mortality in rice seed treated with ozone 1.25 mg/g rice seed/hr at various time

Ozonation time (hr)	% Mortality
0	0d
1	59c
2	81b
3	100a
4	100a
LSD .05 (Ozonation time) 5.50	
CV (Ozonation time) 5.37%	

* The different letters in the same column indicate the statistically significant by LSD at 5% level

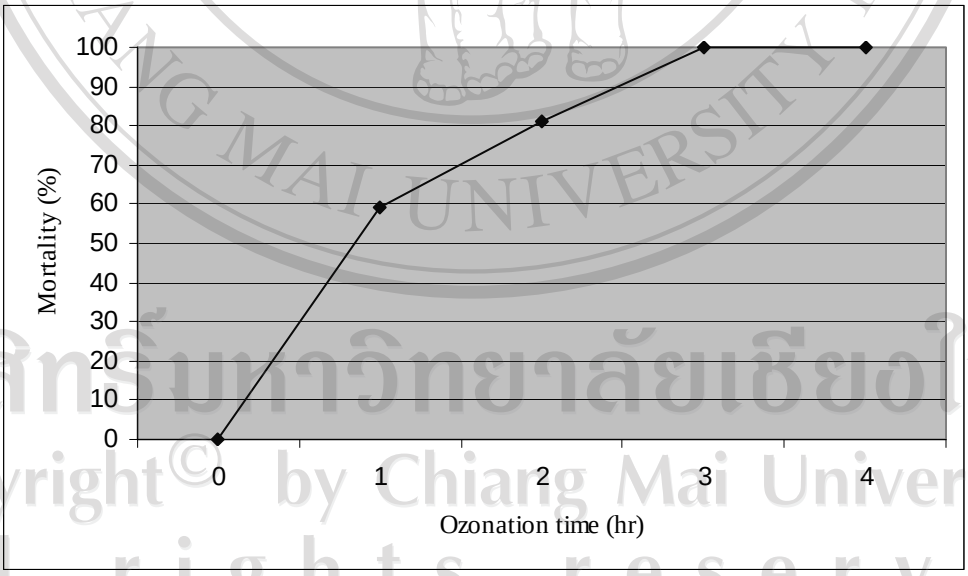

Figure 4.12 Percentage of adult *Sitophilus oryzae* (rice weevil) mortality in rice seed treated with ozone 1.25 mg/g rice seed/hr at various time