CHAPTER 3

MATERIALS AND METHODS

Seed sample

Rice seed sample cv Khao Dawk Mali 105 (KDML 105) was obtained from Chiang Mai Rice Seed Center, Bureau of Rice Seed, Rice Department. Their initial qualities assessed were as follows: the seed moisture content was 11.30%, the seed germination was 88%, the seed viability was 97%, the speed of germination 15.03 seedling/day, the seedling growth rate was 6.03 mg/seedling/7 days and germination by cold test was 95 %. The percentage of seed-borne invasion was 87.50, mainly 69.63% was *Alternaria padwickii* and 3.63% was *Fusarium moniliforme*.

Ozone generator

Ozone was produced electrostatically by ozone generator model WAO-2501 (Asiatech Industry IN). The unit had one generator head, AC power 220 Volt, electrical frequency 50 Hertz, electrical power 18 Watts, it could be operated to the rate of ozone production at 250 mg/hr.

Figure 1.1 Ozone generator

Ozone chamber

Ozone chamber was constructed of stainless-steel (8.5 cm dia. × 16.5 cm). A perforated steel floor was welded 1 cm from the bottom of the chamber to form a plenum.

Figure 1.2 Ozone chamber

Evaluation on the ozone efficacy against rice seed-borne fungal and the effect of ozone application on rice seed quality

The experiment on the evaluation of the ozone efficacy against seed borne fungi was arranged in split plot design with 4 replications, The main plot is ozonation times: 0 (T1), 2 (T2), 4 (T3), 6 (T4) and 8 hours (T5). The sub-plot is seed conditions: dry (11% MC) and wet (18% MC, sprayed with sterilized distilled water 20 ml) seeds. The ozone generator was ducted to the top of an ozone chamber containing 200 g of rice seed cv. Khao Dawk Mali 105 (KDML 105), ozone doses of 1.25 mg/g rice seed/hr was used. Rice seeds from each treatment were sampled to evaluate seed borne infection and seed qualities. The data record are follow as:

Evaluation rice seed-borne fungal infection

Potato dextrose agar (PDA) was used for the incubation of seeds. The seeds were surface disinfected by soaking in 1% sodium hypochrorite for 3 minutes and washed in sterilized distilled water for 3 times before plating them in PDA. In each petri-dish, 20 seeds were placed totalling 400 seeds per treatment. The dishes were incubated at room temperature for 7 days in alternating cycles of 12 hr darkness and 12 hr light. The light was supplied by daylight fluorescent tubes (Philips TLD 18W/54). After 7 days, the identification of the different colonies were done visually and then examined under

stereo-binocular microscope followed by an examination of the fruiting structures under a compound microscope for observing the infection of seed-borne fungus.

Evaluation rice seed quality

Seed moisture content : The moisture content (MC) of the rice seed was determined by hot-air oven drying method. The percentage of MC was expressed on wet weight basis investigated following International Rules for Seed Testing (ISTA, 2006).

The percentage of seed germination: The percentage of seed germination was determined by the between paper method (BT) according to standard germination (ISTA, 2006). Four replication of 100 seeds were placed into between papers. After that, incubated in the germination chamber at 25°C for 14 days. The result of the germination test is calculated as the average of four replicates of 100 seeds, and expressed as a percentage by number of normal seedlings with out decimal.

Seed viability : Tetrazolium test (TZ test) was used to as a viability test. A viable seed should show staining in all those tissues whose viability is necessary for normal seedling development. For the purpose of the test, a viable seed should show by its biochemical activity the potential to produce a normal seedling (AOSA, 2002). First, the 200 seeds were soaked in the distilled water to let the seeds imbibe inflate for 8-16 hrs at the temperature of 25°C. After that, the imbibed seeds were sectioned though the embryo and then soaked in the solution of 0.1% Triphenyl tetrazolium chloride for 2 hrs at 30°C in the dark. The viability of seeds was determined by observing the red staining area of the embryo.

Seed vigor : (AOSA, 2002). The objective of vigor tests is to assess the potential of seeds to produce normal seedlings under a wide range of field conditions. The following parameters were evaluated:

- **Speed of germination test:** High speed of germination is an indication of vigorous seed lot. Four replications of 100 seeds were placed into between papers. Normal germinated seedlings with 3 cm radicles emerged and 2 cm plumules. Number of germinated seeds were counted daily from the first day and the cumulative index was made by the formula

$$N = n1/1 + n2/2 + n3/3 + \ldots + nx/x$$

n1 … nx : are the number of seed germination on day 1 to day x

1 … x : are the number of days.

- **Seedling Growth rate**: Seedling Growth rate was determined by rolled-towel germinaton of 4 replications. Each roll consisted of three standard weight paper towels, two below the seed and one covering the seed. Fifty seeds per towel were oriented with 25 seeds per row, radicle end pointed toward the bottom of the towel and embryo side up. The seeds were covered and the three towels were loosely rolled. The rolled towels were placed in a dark germinator at 25°C for seven days. At the end of seven days, the towels were removed, a germination count was made. The normal seedlings were cut free from their kernels and placed in an envelope for drying. Seedling were dried at 80°C for 24 hours, then weighed to the nearest mg and the total dry weight of the normal seedlings per towel was divided by the number of seedlings, then a seedling growth rate of mg/ seedling was obtained.
- **Cold test :** A rolled towel contain four replicates of 50 seeds each. For each replicate the seeds were placed on saturated towel in two 25-seed rows. The top towel was rolled back and the seed were planted on the second towel. The planted seeds were slightly covered with soil substrate, the top towel was replaced and the two towels containing the seed and soil substrate were loosely rolled. The rolled towels were placed in plastic boxes to prevent loss of moisture. The plastic boxes were placed in a seed germinator at 10°C for 7 days before transfer to a warm chamber at 25°C for 7 days. After which they were evaluated. The criteria for seedling evaluation were the same as applied to the standard germination test.

Evaluation on the ozone efficacy against insects

Adults of Rice weevil, *Sitophilus oryzae* were used as experimental insects in a completely randomized design (CRD) with 4 replication. Five ozonation times: 0, 1, 2, 3 and 4 hours, was used to evaluate the insect control. Fifty adult Rice weevil were placed into a separate (5 cm dia. × 5 cm) cage containing 55 g of rice seed. The separate cage are placed in a ozone chamber containing 200 g of rice seed cv. Khao Dawk Mali 105 (KDML 105). The ozone generator was ducted to the top of an ozone chamber, ozone doses of 1.25 mg/g rice seed/hr was used. After the treatment, the numbers of live and dead insects were determined.

Statistical analysis

Statistic analytical determination was conducted in 4 replication. For statistical analysis of results between treatments, a two-way analysis of variance (ANOVA), treatment means separated by LSD comparison at 5 % level. Statistical analysis was carried out with SX version 8 software.