



Research article

Effect of infrared ray drying on seed infection and seed germination of paddy

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Abstract

The effect was studied of infrared ray (IR) drying as an alternative method for seed disinfection of freshly harvested paddy cultivar RD47, collected from Suphan Buri province, Thailand. Paddy seeds were treated using a laboratory gas-fired infrared dryer at IR temperatures of 40–45°C, 60–65°C and 80–85°C on the surface of the paddy seed. Afterward spore germination was tested of four fungi: *Alternaria padwickii*, *Bipolaris oryzae*, *Curvularia lunata* and *Fusarium semitectum*. An *in vitro* test of IR treatment at 80–85°C was able to inhibit spore germination of the four fungal pathogens of paddy while IR treatment at 40–45°C and 60–65°C did not have an effect on treated spores. In the *in vivo* test, the effect of IR on seed infection and the germination of paddy was investigated compared with shade drying (control). The artificially inoculated paddy with the four fungal pathogens and non inoculated paddy were treated with IR at 40–45°C and 65–75°C. The results revealed that IR drying reduced seed infection of *A. padwickii*, *B. oryzae* and *F. semitectum*. The germination rate of inoculated paddy treated with 40–45°C IR was 91.0–94.8% which was higher than for 65–70°C IR and the control. The results of the *in vivo* test showed that IR significantly decreased natural infection by *A. alternata* and *B. oryzae* after IR drying at 40–45°C. Seed infection of *A. padwickii*, *C. lunata* and *F. semitectum* rapidly decreased after IR drying at 65–70°C. However, IR drying at 40–45°C and 65–70°C of paddy reduced seed germination to 85.1 and 79.5%, respectively.

Introduction

Rice (*Oryza sativa*) is a staple food for over half the world's people and has the second largest cereal production after maize with over 685 million tonnes recorded in 2008 (FAOSTAT) (Lantin, 1999). In 2015, total rice exports from Thailand were 8.56 million tonnes valued at THB 136,803 million (Thai Rice Export Association, 2016). Estimated rice postharvest losses in Southeast Asia are 15–25%, while the drying and storage processes have losses of about 11–15%.

(FAO, 2006; Gummert, 2013) Problems due to insects and fungi with rice seed storage lead to reduced quality and quantity of seed production. A high moisture content in rice causes discoloration (Dillahunty et al, 2001), high respiration (Dillahunty et al, 2000), deterioration by fungi (Christensen and Kaufmann, 1965; Farrell et al, 2002), damaged by insects (Farrell et al, 2002), decreased seed germination (Paderes et al., 1996) and high mycotoxin contamination (Trung et al., 2001). Most of the major diseases of rice are seed borne (Mew and Gonzales, 2002). Dirty panicle disease in rice is usually

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associated with seed infection and causes yield reduction, quality deterioration and poor germination (Haque et al., 2007; Ora et al., 2011). As such, it is becoming a major obstacle to rice production in Thailand. A preliminary field survey showed that *Bipolaris oryzae*, *Curvularia lunata* and *Fusarium semitectum* were frequently isolated species (Sawatsuk et al., 2015). Germination and the germination index of spotted seed was generally not different from in clean seed, whereas fully blackened seed had significant decreases with germination being less than 80% (Thawong, 2002). This disease reduces the quality of paddy by reducing seed germination and causes economic loss with rice crops. Thus, drying management is very important after harvest. The moisture content in seed is an important factor that enhances microorganism infection in stacked paddy (Zuxun et al., 2004; Farrell et al., 2002). A drying process is required to reduce the moisture content to a safe level before storage to inhibit the growth of storage fungi and insects and to reduce the respiration of paddy (Desai, 2004).

Infrared radiation (IR) is light in the frequency range 1011–1014 Hz with a wavelength of 700–1000 nm (Barr, 1961; Ozisik and Siewert, 1982). IR has recently been of interest for drying agricultural products and has been reported to be beneficial where a high moisture content material is involved as this affects the quality of products such as rice (Maity et al., 2004; Pan et al., 2008; Laohavanich and Wongpichet, 2008), wheat (Savas and Basman, 2016), green pea (Barzegar et al., 2015), blueberries (Shi et al., 2008), red ginseng (Ning et al., 2015), jujube (Chen et al., 2015) and carrot (Togrul, 2006). In Thailand, two methods are used for paddy drying: traditional drying systems using sun drying and mechanical drying systems using heated-air drying, low-temperature drying and flash drying (Soponronnarit, 1997). Drying using IR is one process of interest for drying rice. The advantages of IR in current food and agricultural processing methods is its quick moisture removal, higher heat-transfer capacity, instant heating because of direct heat penetration, energy saving, faster heat treatment preservation of vitamins and lower cost (Skjoldebrand, 2001; Das et al., 2009; Jittanit et al., 2010). IR heating has been studied on control plant pathogens. The sterilization effect of IR (2.0 kW for 30 s) was examined on wheat inoculated with *Aspergillus niger* (Hamanaka et al., 2000). Far-infrared (FIR) heating at a surface temperature of 72°C was applied to inactivate spores of *A. niger* and *F. proliferatum* in corn meal for 6 min (Jun and Irudayaraj, 2003). However, the application was limited because heat has an effect on seed quality. The objective of the current research was to study the effect of IR drying to decrease seed moisture content and as an alternative method of seed sterilization to reduce contamination by field fungi and storage fungi without any reduction in seed germination

Materials and Methods

Fungal isolation and identification

Newly harvested rice (*Oryza sativa*) cv. RD47 samples were collected in 2012 from the Rice Research Center, Suphan Buri, Thailand. The paddy samples (50 kg each) were randomly reduced to 500 g

according to International Seed Testing Association (1996) using a Boerner divider. Each sample was then further reduced to 400 seeds. For fungal identification, each paddy sample was surface sterilized in 1% sodium hypochlorite for 5 min and then dried in a laminar flow chamber for 30 min under aseptic conditions. The seed samples (10 seeds per agar plate) were placed on sterile potato dextrose agar (PDA) and incubated at 25°C for 7 d under a near visible ultraviolet (NUV) lamp (Philips black light fluorescent lamp TL40 watt) with 12 hr light and 12 hr darkness. Colonies obtained with distinct morphologies were selected and sub cultured on the PDA to obtain pure cultures. Characteristic growth of mycelia and conidia were identified using International Seed Testing Association (2008). Stock cultures were maintained on PDA slants at 4°C for further study. The fungi were transferred to fresh media and incubated at room temperature before being used in the other experiments.

Preparation of the spore suspension

Colonies of *Alternaria padwickii*, *Curvularia lunata* and *Fusarium semitectum* on PDA incubated under an NUV light lamp with 12 hr light and 12 hr darkness for 7 d were used for preparing spore suspensions. *Bipolaris oryzae* was cultured for 14 d on rabbit food agar (25 g rabbit food, 15 g agar and 1,000 mL distilled water) to induce spore production under the NUV lamp with 12 hr light and 12 hr darkness. Then 20 mL sterile water containing 0.1% (volume per volume Tween 80 was poured into each plate and then the colony was scraped with a sterile triangular-shaped piece of glass to dislodge the spores. Spore were counted using a hemacytometer under a compound microscope and adjusted to 1×10^6 spores/mL by dilution.

In vitro testing, effect of infrared radiation treatment on spore germination

Drying in this research used a laboratory gas-fired infrared dryer at the Faculty of Engineering, Maharakham University, Maha Sarakham province, Thailand and an IR temperature of 800°C (Laohavanich and Wongpichet, 2009) which provided the exhausted hot air for drying. The spore suspension including *A. padwickii*, *B. oryzae*, *C. lunata* and *F. semitectum* at concentrations of 1×10^6 spore/mL were poured onto open-lidded plates. The plates were placed on trays under an IR burner at temperature ranges of 40–45°C, 60–65°C and 80–85°C for 3, 6 and 9 min, respectively. The heating temperature on each tray at 40–45°C, 60–65°C and 80–85°C was maintained by adjusting the distance between the IR burner and the plate to 20, 15 and 13 cm, respectively. The temperature of each tray was checked using an infrared thermometer. The infrared burner was set to radiate IR at a peak wave range of 2.70 μ m. Data of spore germination were observed under a compound microscope at 12 hr after IR using 3 replications (3 plates/replication).

In vivo testing, effect of infrared treatment on artificially inoculated paddy

Paddy RD47 samples (500 g each) harvested in 2012 from the

Rice Research Center, Suphan Buri, Thailand were surface sterilized under ultraviolet light at a wavelength of 253.7 nm for 2 hr. The paddy was inoculated by mixing well with 20 mL of spore suspension at concentrations of 1×10^6 spore/mL of *A. padwickii*, *B. oryzae*, *C. lunata* and *F. semitectum* and incubating at 25°C for 12 hr and an uninoculated control used 20 mL of sterile water. The artificially inoculated paddy samples were placed on trays and subject to IR heating at 800°C using a laboratory gas-fired infrared dryer. The heating temperatures were set at 40–45°C and 65–70°C on the seed surface for 7 min by adjusting the distance between the IR burner and seed sample to 10 and 20 cm, respectively. The initial and final moisture contents of paddy were measured and recorded. After IR 2 d, samples were checked for fungi on the paddy using the blotter method (see *Determination of seed infection* below) and then incubated for 7 d. Any fungi that had developed on seed were examined under a stereo microscope. Seed germination was determined using the between-paper method (see *Determination of seed germination* below).

In vivo testing, effect of infrared treatment on naturally infected paddy

Random samples were taken of newly harvested paddy seed RD47 in 2013 from the Rice Research Center, Suphan Buri, Thailand. The initial and final moisture contents of the paddy were measured and recorded. The paddy samples were placed on trays and subjected to IR drying at 800°C using a laboratory gas-fired infrared dryer. The heating temperatures were set at 40–45°C and 65–70°C on the seed surface for 7 min by adjusting the distance between the IR burner and seed sample to 10 and 20 cm, respectively. Fungal identification and seed germination were determined using the same procedures described for artificially inoculated paddy above.

Determination of seed infection

Fungal infection was determined using the blotter test following International Seed Testing Association (1996) and Mathur and Kongsdal (2003). A sample of 25 seeds was placed on six layers of moist blotter paper using four replications (100 seeds/replication) and incubated for 7 d at 25°C under the NUV lamp with 12 hr light and 12 hr darkness. After incubation, fungal development on each seed was examined under a stereo microscope. The number of seeds infected was counted and calculated as a percentage.

Determination of seed germination

Seed germination was tested using the between-paper method (International Seed Testing Association, 1996). Paddy seeds (100 seeds/replication) were placed between layers of moist paper (15 cm × 30 cm) using four replications. The seeds were covered with another sheet of moist paper towel and the combined papers were rolled loosely from opposite the label end. The rolls were placed upright in a deep-bottomed plastic tray and the paper towel was kept moist by spraying with water during incubation at 25°C for 14 d. The number of seeds germinating was counted and calculated as a percentage.

Moisture content

The moisture content of paddy on a % wet basis was measured using a grain moisture tester (model PM-600; Kett Electric Laboratory).

Experimental design and statistical analysis

The experiment was laid out in a completely randomized design and analysis of variance was undertaken using the Statistical Package for the Social Sciences software (SPSS 11.5 IBM). Differences between the effects of different treatments were analyzed using Duncan's multiple range test and an independent-sample t test. Significance was tested at $p < 0.05$.

Results and Discussion

Isolation and identification the pathogen

Paddy seed RD47 were isolated for *C. lunata*, *A. padwickii* and *F. semitectum* at an initial moisture content of 19.7% (wet basis). *B. oryzae* was found in small amounts only in paddy RD47 (Table 1). *C. lunata* was more common on the paddy (29.3%). A preliminary field survey showed that *B. oryzae*, *C. lunata* and *F. semitectum*, were frequently isolated species (Sawatsuk et al., 2015). The four fungal pathogens *A. padwickii*, *B. oryzae*, *C. lunata* and *F. semitectum* have been identified as causing dirty panicle disease in rice (Bureau of Rice Research and Development, 2016). Study on the effect of grain discoloration severity on seed storability found the most detected fungal pathogens were *A. padwickii*, *Dreschera oryzae*, *B. oryzae*, *Curvularia* sp. in rice varieties KDML105, RD7 and SPR90 and more than 25% disease incidence on paddy has been reported to reduce seed storage (Prasertsak et al, 2009).

Table 1 Occurrence of four fungal species on new harvested paddy seed variety RD47 (blotter method).

Fungus	Fungal detection (%) on paddy seed (initial 19.7% wet basis)
<i>Alternaria padwickii</i>	0.8 ± 0.02 ^a
<i>Bipolaris oryzae</i>	1.3 ± 0.03 ^a
<i>Curvularia lunata</i>	29.3 ± 0.59 ^b
<i>Fusarium semitectum</i>	5.5 ± 0.11 ^a

Mean values within a column superscripted by the same lowercase letter are not significantly different ($p < 0.05$).

In vitro test effect of infrared treatment determination on spore germination

The results showed that spore germination levels of IR-irradiated spores for 3 min, 6 min and 9 min were not significantly different though germination decreased with increasing IR temperature (Table 2). IR treatment at 80–85°C reduced the spore germination of the four fungi. Spore germination of *B. oryzae*, *C. lunata* and *F. semitectum* was inhibited with IR treatment at 80–85°C for each IR irradiation time; *A. padwickii* also showed decreased spore germination.

Table 2 Occurrence of spore germination (mean±SD, %) after treatment with infrared rays (IR) at different temperatures and times

Treatment		Spore germination (%)			
		<i>A. padwickii</i>	<i>B. oryzae</i>	<i>C. lunata</i>	<i>F. semitectum</i>
Untreated		30 ± 0.56 ^{cd}	26 ± 0.60 ^c	18 ± 0.65 ^c	35 ± 1.27 ^d
IR 40–45°C	3 min	34 ± 0.64 ^d	23 ± 0.53 ^{bc}	35 ± 1.27 ^d	0 ^a
	6 min	27 ± 0.51 ^{cd}	18 ± 0.42 ^{bc}	2 ± 0.07 ^{ab}	2 ± 0.07 ^{ab}
	9 min	29 ± 0.55 ^{cd}	14 ± 0.32 ^b	0 ^a	8 ± 0.29 ^{bc}
IR 60–65°C	3 min	28 ± 0.53 ^{cd}	24 ± 0.55 ^{bc}	8 ± 0.29 ^b	8 ± 0.29 ^{bc}
	6 min	21 ± 0.39 ^{bc}	28 ± 0.65 ^c	0 ^a	10 ± 0.36 ^c
	9 min	25 ± 0.47 ^{cd}	19 ± 0.44 ^{bc}	6 ± 0.22 ^{ab}	8 ± 0.29 ^{bc}
IR 80–85°C	3 min	10 ± 0.19 ^{ab}	3 ± 0.07 ^a	0 ^a	0 ^a
	6 min	14 ^a ± 0.26 ^b	0 ^a	1 ± 0.04 ^{ab}	0 ^a
	9 min	4 ± 0.08 ^a	2 ± 0.05 ^a	1 ± 0.04 ^{ab}	0 ^a

Mean values within a column superscripted by the same lowercase letter are not significantly different ($p < 0.05$).

In vivo test effect of infrared treatment on artificial infected paddy

IR drying at paddy surface temperatures of 40–45°C and 65–70°C for 7 min compared with shade drying of inoculated paddy was studied (Table 3). Inoculated paddy with *A. padwickii*, *B. oryzae* and *F. semitectum* had low levels of seed infection after IR drying at 40–45°C and 65–70°C for 7 min. IR drying at 65–70°C had significantly different seed infection for *A. padwickii* and *B. oryzae* (0.8% and 12.3%, respectively) compared with inoculated, shade-dried paddy (control treatment, 71.0% and 90.0%, respectively). In contrast, the seed infection of the four fungi was 16–41.5% after shade drying. Hamanaka et al. (2000) reported that IR sterilized some microorganisms in agricultural products, thus reducing spoilage. The surface temperature increased rapidly as IR directly heated the surface without any need for conductors. Therefore, the irradiating power of 0.5–2.0 kW resulted in 60°C, 80°C, 125°C and 195°C inside the experimental device, and 45°C, 65°C, 95°C and 120°C,

respectively, on the surface of the wheat stack, resulting in 0.83–1.90 log₁₀ colony forming units/g total bacteria after treatment for 60 s.

Seed germination of paddy was determined after using IR drying at paddy surface temperatures of 40–45°C and 65–70°C for 7 min (Table 4). IR drying at a paddy surface temperature of 40–45°C, where the paddy had been inoculated with the four fungi, resulted in 91.0–94.8% seed germination which was higher than with IR drying at 65–70°C and shade drying. Shade drying of inoculated paddy significantly decreased the germination less than 90%; however, IR drying at 40–45°C maintained the germination rate at more than 90%. IR drying at 65–70°C decreased the germination rate to lower levels (75–78.8%) which was unacceptable for good seed quality. The experimental results were consistent with Palawisut et al. (1998) who reported that rice seed infected with *F. semitectum* had decreased germination (59–86%). *B. oryzae*, *Sarocladium oryzae* and *C. lunata* had percentage seed germination rates of 80–85%, 72–85% and 74–88%, respectively.

Table 3 Seed infection (mean±SD, %) of uninoculated and artificially inoculated paddy after infrared ray (IR) drying at different paddy surface temperatures for 7 min compared with shade drying of paddy at 14% wet basis.

Fungus	Seed infection (%)			
	Uninoculated paddy with shade drying	Shade drying	Inoculated paddy IR 40–45°C	IR 65–70°C
<i>A. padwickii</i>	16.0 ± 0.14 ^b	71.0 ± 0.62 ^d	65.0 ± 0.57 ^c	0.8 ± 0.01 ^a
<i>B. oryzae</i>	27.8 ± 0.23 ^b	90.0 ± 0.73 ^c	32.0 ± 0.26 ^b	12.3 ± 0.10 ^a
<i>C. lunata</i>	34.0 ± 0.12 ^a	100.0 ± 0.35 ^b	100.0 ± 0.35 ^b	85.8 ± 0.30 ^b
<i>F. semitectum</i>	41.5 ± 0.14 ^a	100.0 ± 0.34 ^d	91.0 ± 0.31 ^c	80.0 ± 0.27 ^b

Mean values within a row superscripted by the same lowercase letter are not significantly different ($p < 0.05$).

Table 4 Seed germination (mean±SD, %) of uninoculated and artificially inoculated paddy after infrared ray (IR) drying at different paddy surface temperatures for 7 min compared with shade drying of paddy at 14% wet basis.

Fungus	Uninoculated paddy with shade drying	Inoculated paddy		
		Shade drying	IR 40–45°C	IR 65–70°C
<i>A. padwickii</i>	95.8 ^a	78.0 ± 0.09 ^b	91.0 ± 0.10 ^a	78.8 ± 0.09 ^b
<i>B. oryzae</i>	96.8 ^a	89.0 ± 0.10 ^b	94.8 ± 0.10 ^a	75.0 ± 0.08 ^c
<i>C. lunata</i>	95.5 ^a	87.8 ± 0.08 ^b	92.3 ± 0.08 ^a	77.3 ± 0.07 ^c
<i>F. semitectum</i>	96.8 ^a	89.0 ± 0.11 ^b	94.8 ± 0.11 ^a	73.5 ± 0.09 ^c

Mean values within a row superscripted by the same lowercase letter are not significantly different ($p < 0.05$).

In vivo test effect of infrared treatment on natural infected paddy

Newly harvested paddy (without artificial inoculation) after IR drying at paddy surface temperatures of 40–45°C and 65–70°C for 7 min and shade drying was studied to investigate controlling naturally infection (Table 5). Disthaporn et al. (1996) surveyed and analyzed rice seed cleaning from several farms in Thailand and reported that the central region of Thailand including Chachoengsao, Suphan Buri and Chainat provinces, had main fungal contamination on paddy by *A. padwickii* and *C. lunata*. The current results showed different decreases for *A. alternata* and *B. oryzae* infection at IR 40–45°C, while *A. padwickii*, *C. lunata* and *F. semitectum* showed decreases at IR 65–70°C. These results showed the potential of IR drying which could quickly reduce moisture content of seed in 7 min compared to shade drying which required 1–2 hr. Furthermore, it took 36 hr to decrease the moisture content to 11.5% (wet basis) using shade drying (Table 6), while IR drying at 40–45°C and 65–70°C for 7 min reduced the seed moisture content to 12.8% (wet basis) and 12.3% (wet basis), respectively. However, IR drying paddy at 40–45°C and 65–70°C reduced the seed germination rate to 85.1% and 79.5%, respectively (Table 6). Thus, using high temperature drying led to reduced seed germination, whereas using IR drying at 40–45°C resulted in a seed germination rate of more than 80%. Kanjana et al. (2012) investigated the effect of a high temperature oven (12 t continuous-flow drier) to determine a suitable temperature for seed drying. They started drying at a low temperature (45°C) followed by drying at 55°C when the moisture content of the rice was 12% and then cooling in ambient air for 1 hr. If the initial moisture content is more than 20%, they recommended initial drying at 45°C, whereas for an initial moisture content of less than 16%, drying should start at 55°C.

IR drying was associated with a reduced risk of developing seed infection through inhibiting the spore germination of *A. padwickii*, *B. oryzae*, *C. lunata* and *F. semitectum* which can cause panicle disease in rice. IR drying at a paddy seed surface temperature of 40–45°C maintained the germination rate at more than 90%. Drying using IR provided many advantages over conventional heating including shortened processing time, quick moisture removal, a rapid heat transfer rate and low cost. IR Drying could also replace the chemical method of using methyl bromide for disinfesting rice. Drying with IR completely killed storage fungi, contamination fungi, storage insects and improved the quality of rice. The current results could be practically applied for further research to find suitable practice using IR drying for paddy in order to decrease seed infection and moisture content. Thus, further study should investigate improved drying conditions by reducing the temperature and increasing the drying time.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Table 5 Seed infection (mean $\pm$ SD, %) on naturally infected paddy after drying by infrared ray (IR) at different paddy surface temperatures for 7 min compared to shade drying.

Fungus	Seed infection (%)		
	Shade drying	Infrared drying 40–45°C	Infrared drying 65–70°C
<i>A. alternata</i>	5.3 $\pm$ 0.07 ^b	2.0 $\pm$ 0.02 ^a	4.8 $\pm$ 0.06 ^b
<i>A. padwickii</i>	34.8 $\pm$ 0.27 ^b	25.3 $\pm$ 0.20 ^b	20.5 $\pm$ 0.16 ^a
<i>B. oryzae</i>	41.3 $\pm$ 0.23 ^b	32.8 $\pm$ 0.18 ^a	40.5 $\pm$ 0.23 ^b
<i>C. lunata</i>	52.3 $\pm$ 0.17 ^b	61.8 $\pm$ 0.20 ^c	43.0 $\pm$ 0.14 ^a
<i>F. semitectum</i>	34.5 $\pm$ 0.18 ^a	57.5 $\pm$ 0.30 ^b	28.8 $\pm$ 0.15 ^a

Mean values within a row superscripted by the same lowercase letter are not significantly different ($p < 0.05$).

Table 6 Moisture content (mean $\pm$ SD, % wet basis) and seed germination (%) of naturally infected paddy after drying using infrared ray (IR) at different paddy surface temperatures for 7 min compared to shade drying.

Treatment	Moisture content of paddy (% wet basis)		Seed germination (%)
	Initial	After drying	
Shade drying	22.2 ^a	11.5 ^a	98.7 $\pm$ 0.11 ^a
IR 40–45°C	21.3 ^a	12.8 ^a	85.1 $\pm$ 0.09 ^b
IR 65–70°C	21.9 ^a	12.3 ^a	79.5 $\pm$ 0.09 ^c

Mean values within a column superscripted by the same lowercase letter are not significantly different ($p < 0.05$).

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