Journal of Agriculture and Rural Development in the Tropics and Subtropics
Vol. 118 No. 1 (2017) 141–148

urn:nbn:de:hebis:34-2017031552255 ISSN: 2363-6033 (online); 1612-9830 (print) – website: www.jarts.info

Effects of drying and storage management on fungi (Aflatoxin B1) accumulation and rice quality in Cambodia

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Abstract

Rice postharvest practices of farmers incur losses that limit supply and affect global production. Aside from physical losses, quality can be affected, leading to a possible accumulation of aflatoxin B1 (AFB1) that is harmful to humans when ingested. This is particularly important for countries like Cambodia that aim for both food security and rice exports. The objective of the research was to determine the effects of different field drying and storage practices on AFB1 accumulation and milled rice quality in Cambodia. The study had four drying treatments and four storage treatments, in a randomized complete block (RCB) design. Tests were done for moisture content (MC), milling quality, germination rate, and AFB1 accumulation. High-performance liquid chromatography (HPLC) method was used to determine AFB1 contamination and one-way analysis of variance (ANOVA) was performed using CropStat 7.2. No significant AFB1 content was detected. Different field drying treatments used, as well as duration and type of storage also had no significant effect on the accumulation of AFB1 in rice. Milled rice quality was higher with limited or no field drying ($P < 0.01$). Storing in IRRI-Superbag at 14 % MC resulted in higher germination ($P < 0.01$) than in other treatments. Storing in IRRI-Superbag at 16 % MC, however, resulted in lower head rice recovery than in the other three treatments. Reducing field drying and storing hermetically at 14 % MC could therefore potentially reduce rice postharvest losses. Field drying practices of 12 days or less can keep AFB1 contamination at bay.

Keywords: field drying, germination, hermetic storage, milling quality, moisture content, postharvest, rice

1 Introduction

The growing population of the world demands a high and stable supply of rice, with about 900 million poor people being dependent on rice as producers and consumers (Pandey *et al.*, 2010). This dependence on rice puts increasing pressure on the agricultural sector to meet supply and quality requirements. Global per capita consumption has consistently increased since the 1960s, with 88 % of the total amount consumed in Asia (Timmer, 2010). In Cambodia, per capita consumption is 143 kg per year while the population is 14.9 million and growing at a rate of 1.7 % (MAFF, 2010; IndexMundi, 2013).

The Cambodian government, which seeks to re-establish the country as a major exporter, cannot stop by focusing on supply alone. Like that of other crops, rice quality is affected by variety and conditions in pre- and postharvest handling (Gummert *et al.*, 2010). Aside

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from spills during handling, rice is easily contaminated by fungi and insects, in particular if the un-threshed rice panicles and grains are left in the field for several days (field drying). This is a common practice in Cambodia which is to pre-dry the grains, as farmers wait for available threshers. Potentially, the fungi contaminate the rice more easily when it has cracks or broken grains. Relative humidity, particularly in the rainy season, rises to levels that cause the moisture content of rice in storage to increase, resulting in cracks in the milling process. Physical losses have been estimated to be 15–25 % throughout the different postharvest activities (*ibid.*).

Moreover, climate conditions in Cambodia, as in other countries in Asia with temperatures of 26–39 °C and relative humidity of 67–98 %, are conducive to fungi growth and contamination in rice (Sales & Yoshizawa, 2005). Previous studies have detected fungi of the genera *Aspergillus*, *Penicillium*, and *Rhizopus* in stored rice (Phillips *et al.*, 1988). Mycotoxins are found to result from the secondary metabolites of fungi, and can cause illness and death to humans (Bennett & Klich, 2003). Fungi such as *Aspergillus*, *Fusarium*, and *Penicillium* genera produce aflatoxins, of which *Aspergillus flavus* is one that can seriously harm humans (Moss, 1991). Of the four main types of aflatoxin, aflatoxin B1 (AFB1), AFB2, AFG1, and AFG2, it is AFB1 that has been strongly linked to causing cancer in people (Coker, 1994). Recent studies from different countries have detected aflatoxin B1, B2, G1, and G2 levels in rice that were above the regulatory limits in samples tested (Rahmani *et al.*, 2011; Firdous *et al.*, 2012). The worldwide regulations for mycotoxin content in foodstuffs have been set to 4 ppb of AFB1 and 8 ppb of total aflatoxins (B1+B2+G1+G2) as the maximum tolerance level (Van Egmond, 1999; Dohlman, 2003). The European Union (EU) standard is 2 ppb for AFB1 and 4 ppb for total aflatoxins (EC, 1999). The United States, however, have set 20 ppb for total aflatoxins as tolerance level (FAO, 2004).

The 5–10 % physical losses in storage (Gummert *et al.*, 2010) can be reduced by using hermetic storage. This is a new technology in which the grains are enclosed in a hermetically sealed container that prevents them from absorbing water from the ambient air, kills insects, and prevents new infestation. A study comparing open and hermetic storage systems and their effects on the quality of milled rice for 8 months of storage have been done in Vietnam (Diep *et al.*, 2006). Milled rice recovery from paddy stored under open conditions dropped 2.9 % compared with initial conditions while in hermetic storage (such as Super Bags developed by the International Rice Research Institute, IRRI) milled rice recovery dropped by only 0.76 % (*ibid.*). However, such studies have documented losses under laboratory conditions, but not quantified the effects of practices of farmers, such as long field drying periods and different storage techniques, on physical and quality losses, as well as on the accumulation of mycotoxins.

This study aimed at establishing whether AFB1 is present in rice grains from sub-optimal postharvest systems such as those in Cambodia. It also aimed at quantifying the amount of mycotoxins accumulated in different treatments. We hypothesized that field drying will affect AFB1 contamination in rice. For drying, we examined the effects of different field drying periods on AFB1 accumulation and milled rice quality. For storage, we tested whether different methods, moisture contents, and duration of storage have an effect on AFB1 accumulation and milled rice quality.

2 Materials and methods

2.1 Drying experiments

The drying experiments were executed on farmers' rice fields in Thnong Khang Kerth Village, Smorng Cherng Commune, Kamchaymear District, Prey Veng Province. This allowed similar conditions as in the farmers' practice of field drying, with some control against cows and birds. An improved traditional rice variety, Phka Romduol, was selected and three rice fields were used. In each rice field, 12 plots were used for four treatments with three replications per treatment, providing a total of 36 plots. A randomized complete block (RCB) design was used in the experiments. Experiments were carried out in November-December 2011 (end of the rainy season), with ambient temperatures of 25–36 °C, and relative humidity of 45–85 %.

The field drying study used four treatments: (1) No FD (no field drying, the manually cut crop was immediately threshed); (2) 4-day FD, the manually cut crop was left for field drying for 4 days; (3) 8-day FD, with field drying for 8 days; and (4) 12-day FD, with field drying for 12 days. For each treatment, the cut crop was threshed using a mechanical thresher and 30 kg of the obtained paddy was sun-dried to 13–14 % moisture content (MC) in case the MC was higher than 14 %.

From the primary sample, a secondary sample of 5 kg per replication was obtained for AFB1 analysis. Another secondary sample of 1 kg per replication was obtained for milled rice quality assessment.

2.2 Storage experiments

The storage experiments were done at the Ministry of Agriculture, Forestry and Fisheries (MAFF) in an area where birds and rats can be controlled. It was implemented in December 2011 to May 2012 (dry season). This storage experiment included two different storage methods, and two initial moisture contents: (1) IRRI-Super Bag at 14 % MC (SB-14 %), (2) polypropylene bag at 14 % MC (PPB-14 %), (3) IRRI-Super Bag at 16 % MC (SB-16 %), and (4) polypropylene bag at 16 % MC (PPB-16 %). The four treatments were stored either for 2, 4 or 6 months. All treatments had three replications. These were compared with initial samples that had either 14 % MC or 16 % MC. The IRRI-Super Bag is a type of hermetic or airtight storage that minimizes gas and moisture transfer from the ambient air (Gummert *et al.*, 2010). The polypropylene bag is the woven plastic material commonly used by farmers for grain storage. It is permeable to relative humidity, water, and insects.

The rice used for this experiment was bought from a farmer (total of 1,400 kg; Phka Romduol variety). The farmer's rice field was harvested using a combine harvester and the paddy was sun-dried to reduce the MC from 22 % to either 16 % or 14 % for the different treatments. After sun-drying, samples for each treatment were re-cleaned using a mechanical cleaner, and then mixed. After mixing, nine bags (30 kg per bag) were obtained for each of the treatments (three bags for each treatment and storage period).

From each 30-kg bag per replication, a sample of 5 kg was obtained and sent for AFB1 analysis. Furthermore, another sample of 1 kg per bag was obtained for milled rice quality assessment after 2, 4 and 6-month period and for assessment of the germination rate after 6 month storage period.

2.3 Aflatoxin analysis

To test for AFB1 contamination from each 5-kg sample, 1-kg sub-samples were obtained and crushed. The AFB1 concentrations for calibration curve were eluted by methanol and quantified using high-performance liquid chromatography (HPLC). Then we used Methanol : Acetonitrile : Water = 1:1:3 (Merck), and LC–10AVP HPLC (Shimadzu, Japan) plus LC-10ATVP Pump. All analyses (36 samples for the field drying experiment and 42 samples for the storage experiment) were executed at the Southern Sub-Institute of Agricultural Engineering and Postharvest Technology (SIAEP) laboratory in Vietnam.

2.4 Rice quality analysis

A total of 36 samples for the field drying experiment and another 42 samples for the storage experiment (1 kg per sample) were used to test the germination rate, milled rice quality, and head rice recovery (percentage of whole milled rice plus broken milled rice that have retained >80 % of the whole) by using a laboratory rice mill.

2.5 Statistical analysis

One-way analysis of variance (ANOVA) was performed on experimental data collected using CropStat 7.2. Separation of treatment means was done using LSD at the 5 % level of significance.

3 Results

3.1 Effects of drying practices on aflatoxin content and grain quality

3.1.1 Aflatoxin content

AFB1 was detected in all samples but no significant differences between the treatments were found and the mean AFB1 content (Table 1) was much lower than the current EU limit of 2 ppb for cereals.

Table 1: *Mean Aflatoxin B1 content (in parts per billion) for the field drying (FD) treatments.*

Treatment	*AFB1 content* (ppb)	*Standard deviation*
No FD	0.392	0.08
4-day FD	0.437	0.06
8-day FD	0.297	0.14
12-day FD	0.160	0.13

3.1.2 Moisture content (%)

The MC of paddy dropped from 22 % to 15.3 % after 4 days of field drying, 11.8 % after 8 days, and 11.5 % after 12 days. Hence, treatments 1 and 2 had to be dried further before milling to achieve 13–14 % MC required. Moreover, there was a significant difference ($P < 0.05$) in MC at milling between treatments 2 and 3 as well as between 2 and 4 (Table 2). The reduction of moisture content during the first 4 days of drying was similar among treatments 2, 3 and 4. The rate of MC reduction, decreased however over time. Between days 5–8 (treatments 3 and 4), the average MC reduction was 0.87 per day, and between days 9–12 (treatment 4), it was only 0.07 per day.

Table 2: *Moisture content (MC) at milling.*

Treatment (days of field drying)	*MC at milling*	*SD*
No FD	13.1	1.1
4-day FD	13.2	0.4
8-day FD	11.8	0.7
12-day FD	11.5	0.4

3.1.3 Percentage of rice recovery

After milling, the No FD treatment obtained the highest milled rice recovery (65.6 %), with a decreasing, but non-significant, trend as the field drying period became longer (Fig. 1).

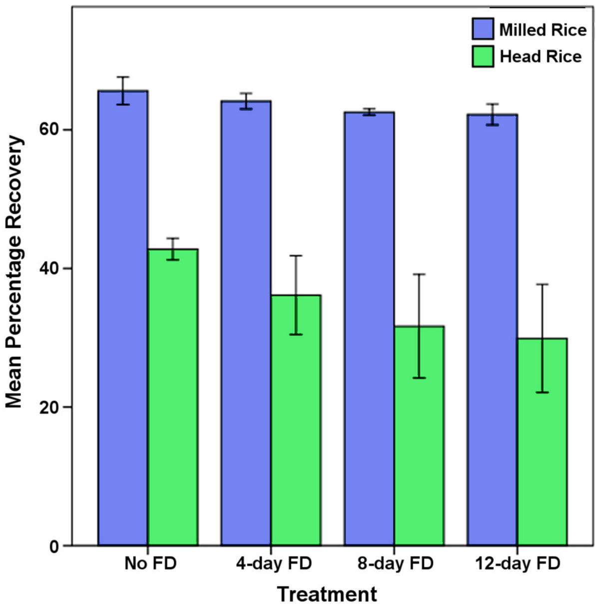

Fig. 1: *Mean milled rice and head rice recovery (%) for different field (FD) drying treatments.*

The No FD treatment obtained also the highest quality, with 43 % head rice recovery (Fig. 1). With increasing field drying period head rice recovery decreased significantly as compared to the control ($P < 0.01$). No FD had a 7 %, 11.1 % and 12.9 % higher head rice recovery ($P < 0.01$) as compared to 4-day FD, 8-day FD and 12-day FD, respectively.

3.2 Effects of storage practices on aflatoxin content and grain quality

3.2.1 Aflatoxin (AFB1) content

AFB1 was detected in 92 % of the paddy samples; however, the content in the samples stored for 2, 4, and 6 months was lower than the EU limit of 2 ppb (Table 3). No significant differences were found between the treatments.

3.2.2 Moisture content (%)

The changing moisture content of paddy during storage is given in Table 4. Paddy stored hermetically (IRRI Super Bag (SB) treatments) showed less fluctuation in MC than paddy stored in polypropylene bags where air and moisture could penetrate (PPB treatments). Paddy stored with 16 % initial MC fluctuated more than paddy stored with 14 % initial MC. Of these, the SB treatment fluctuated less, with a maximum of 0.5.

3.2.3 Germination rate after 6 month period

The SB-14 % treatment had a significantly higher germination rate ($P < 0.01$) than the other three treatments (Fig. 2). The germination rate of SB-16 % was even significantly lower than the two PPB treatments ($P < 0.01$). These two PPB treatments did not differ from each other. The hermetic storage (SB) results in higher germination rates than storage in polypropylene bags if grains are stored at an initial MC of around 14 %. Under airtight conditions, higher moisture contents reduce germination.

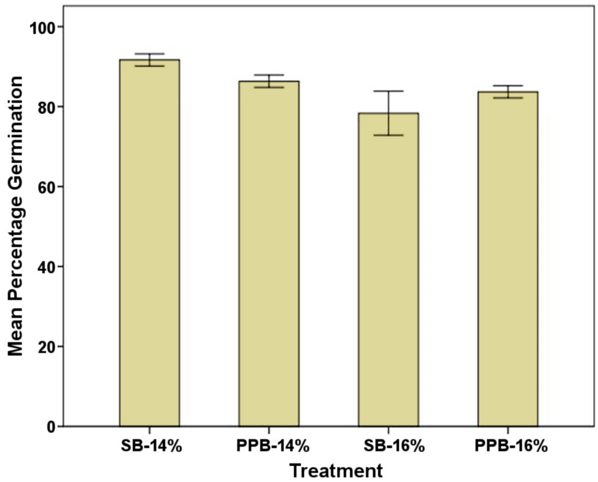

Fig. 2: *Comparison of germination percentage for two storage methods after 6 months of storage (SB: IRRI Super Bag; PPB: polypropylene bag; 14 % and 16 % initial paddy moisture content).*

3.2.4 Percentage rice recovery after 2 to 6 months of storage

Before storage, milled rice recovery was 64.5 % for the 14 % MC treatments, and 60 % for the 16 % treatments (Table 5). Milled rice recovery of PPB-16 %, rose to 62 %, 64 %, and 65 % after 2, 4, and 6 months of storage because its MC dropped to between 14.1–14.5 %, making it more suitable for milling. For SB-14 % and PPB-14 %, milled rice recovery was not significantly different.

Table 3: *Aflatoxin B1 (AFB1) content in parts per billion (ppb) found in paddy samples for two storage methods and three storage periods at two initial moisture contents (SD in brackets).*

Treatment	*Initial*	*After 2 months*	*After 4 months*	*After 6 months*
SB-14 %	0.09 (0.02)	0.65 (0.48)	0.54 (0.02)	0.56 (0.10)
PPB-14 %	0.09 (0.02)	0.44 (0.12)	0.64 (0.09)	0.72 (0.16)
SB-16 %	0.68 (0.20)	0.60 (0.65)	0.74 (0.17)	0.55 (0.12)
PPB-16 %	0.68 (0.20)	0.51 (0.09)	0.80 (0.20)	0.60 (0.17)

SB: IRRI Super Bag; PPB: polypropylene bag; 14 % and 16 % initial paddy moisture content

Table 4: *Comparison of moisture content (%) of paddy for two storage methods and three storage periods.*

Treatment	*Initial*	*After 2 months*	*After 4 months*	*After 6 months*
SB-14 %	14.0	14.2	14.0	14.3
PPB-14 %	14.0	13.9	13.3	14.4
SB-16 %	15.9	16.5	16.2	16.1
PPB-16 %	15.9	14.3	14.1	14.5

SB: IRRI Super Bag; PPB: polypropylene bag; 14 % and 16 % initial paddy moisture content

Table 5: *Comparison of milled and head rice recovery (%) for two storage methods and three storage periods, compared to the initial paddy before storage, with LSD and CV values.*

Treatment	*Initial* (control)	*After 2 months*	*After 4 months*	*After 6 months*
		Milled rice recovery		
SB-14 %	64.51	62.83	62.35	65.50
PPB-14 %	64.51	61.84	59.63	64.28
SB-16 %	60.18	56.81	54.65	61.07
PPB-16 %	60.18	62.27	63.75	65.50
LSD	1.46	1.33	1.40	0.75
CV (%)	1.30	1.20	1.30	0.60
		Head rice recovery		
SB-14 %	44.53	43.53	42.80	42.08
PPB-14 %	44.53	41.07	38.74	36.06
SB-16 %	33.95	33.47	32.90	32.43
PPB-16 %	33.95	40.96	39.95	37.11
LSD	1.62	1.0	0.76	0.92
CV (%)	2.20	1.3	1.10	1.30

SB: IRRI Super Bag; PPB: polypropylene bag; 14 % and 16 % initial paddy moisture content

Head rice recovery differed between treatments as well as between storage durations, especially compared with initial values representing optimal conditions for milling (Table 5). At the start of the experiment, head rice recovery for 14 % MC treatments was 44.5 % and for 16 % MC treatments 34.0 %.

The head rice recovery of paddy in the SB-14 % treatments dropped less (around 2 % after 6 months) compared to the PPB-14 % treatment (around 8 %) (Table 5). For PPB-16 %, head rice recovery went up to 41 % after 2 months because its MC dropped to 14.3 %, making it more suitable for milling. After 4 months, however, its head rice recovery started to decline. Head rice recovery for both SB treatments had the least fluctuation.

4 Discussion

This study explored whether there would be AFB1 contamination in rice grains from different field drying and storage treatments in Cambodia. The mean AFB1 contaminations found were not higher than 1 ppb and therefore much lower than the current standard EU limitations of 2 ppb (Tables 1 and 3). A study in India for example, showed that one out of 35 samples was contaminated with AFB1 and B2 at levels of 15–30 ppb (Siruguri *et al.*, 2012). In Vietnam, a study also found AFB1 contamination in rice (Nguyen *et al.*, 2007). These two studies however used samples that were either rain-damaged grains or milled rice samples from markets (stored as milled rice). Nguyen *et al.* (2007) showed that AFB1 contamination is higher when there is high ambient moisture content such as in the rainy season. In the current study the samples tested on AFB1 were fresh grains bought from a farmer and stored during dry season months. This study provided preliminary data for Cambodian conditions, wherein local field drying practices combined with non-rainy (dry) conditions did not result to AFB1 contamination in rice.

The different field drying periods as well as duration and type of storage had no significant effect on the presence of AFB1 in rice. With enough sunlight resulting in favourable high temperatures (mean of 35.6 °C) and a relative humidity of around 75 %, the moisture content of paddy dropped to about 15.3 % within 4 days of field drying. Furthermore, the AFB1 analysis in the current study was on samples that included the rice husk. A study in China by Liu *et al.* (2006) found that AFB1 content could be reduced by removing the husk. The experiment should therefore be repeated during the wet-season, and AFB1 content analysis to include samples without husk.

Although the field drying method did not affect AFB1 content, it affected the rice quality. Lengthening the field drying period reduced head rice recovery significantly even if the milled rice recovery was not affected. Meas (2012) documented that, already after 24 hours of storing wet rice grains, its quality started to deteriorate. Hence, quality can be optimised by reducing grain MC to 14 % immediately after harvest.

The low head rice recovery for 8- and 12-day FD is due to its low MC at threshing and milling (<12 % MC), where 13–14 % MC is seen as optimal (IRRI, 2009). Also, longer field drying periods increase paddy cracking due to the fact that grains absorb dew at night and dry again during daytime, resulting in lower head rice recovery.

Storing paddy at 14 % MC in Super Bags gave the best quality in terms of germination rate and head rice recovery compared to the other storage treatments. This is the case even with prolonged periods of storage (up till 6 months). Our findings concur with those of Sim (2010) that the drop in head rice recovery is lower (4 %) in hermetic storage than in open storage (15 %) after 8 months. The moisture content of paddy under hermetic storage fluctuated less than with PPB storage. These results concur with other studies looking at rice storage. In a study done in Vietnam, the MC in hermetic storage (comparable to the SB treatments) fluctuated less compared with storage where moisture exchange with surrounding air could happen (Diep *et al.*, 2006). Other studies found that the MC of paddy stored hermetically fluctuated up to 0.2 % while paddy stored in storage such as PPB fluctuated up to 1.2 % (Diep *et al.*, 2006; Sim, 2010; Ouk, 2011).

However, it is not advisable to store paddy hermetically at high MC of 16 % because it has a negative effect on germination rate, as well as on milled rice and head rice recovery. These findings support the current recommendation for storing paddy at 13–14 % MC using hermetic storage to maintain rice quality for 6–12 months (IRRI, 2009). The preservation of quality is indicated by germination rate. On this note, it was found previously that there is a 15 % difference in germination between paddy stored hermetically and in open storage (Sim, 2010; Ouk, 2011).

5 Conclusion

Studies on aflatoxin in rice commonly analysed damaged grains, parboiled rice, rice bran as well as brown rice; this study looked into Cambodian conditions where rice is stored as paddy and milled as white rice. Our findings demonstrate that the practice of Cambodian farmers of field drying for 12 days or less after crop harvest, as well as different duration and type of storage during dry season had no significant effect on the presence of AFB1 in rice. AFB1 content detected was much lower than current standard EU limitations. Field drying however, had significant effect on rice quality especially head rice recovery. Future studies relating to aflatoxins on rice storage could examine the effects of other common practices of farmers such as keeping wet grains over different periods, piling bundles of cut crop for different periods (not only over the dry-season harvest but also for the monsoon-crop harvest). Also, the effect of storage at higher MC such as 18 % or 20 %, for rice stored during rainy season, on AFB1 accumulation could be explored.

Acknowledgements

The authors would like to thank

– The Asian Development Bank and the International Rice Research Institute through the Postharvest Project for funding of this research
– Ms. Rica Joy Flor for editing help and comments on the paper,
– Ms. Jennifer Niem from IRRI, Dr. Pham Van Tan, and Ms. Tram Anh San from the Southern Sub-Institute of Agricultural Engineering and Postharvest Technology (SIAEP) laboratory for insights on mycotoxin study and analysis.

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