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Seed Health and Quality of Rice Seeds Produced by Farmer and Growing in Cho Moi District, An Giang Province, Vietnam

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A B S T R A C T

This study was conducted at Laboratory in An Giang University; the varieties examined were collected from farmer households in 2018 – they were varieties that the farmer stored for the next crop (Winter-Spring) from Autumn-Winter crop in Cho Moi district, An Giang Province, Vietnam. The research evaluated the quality of seed rice, which is provided and planted by the farmer. As a result, this study showed that almost all of the varieties of seed rice from farmer households indicated the ratio of filled-grain, the germination, and the determination of seedling healthy, which are lower than certified variation. Besides, the samples of seed rice from farmer household have more infected fungal; especially the samples with higher in a filled-grain ratio is less infected by fungi, there were 12 types of seed borne-fungi in rice varieties from the farmer (in Cho Moi district), included: *Alternaria padwickii*, *Rhizoctonia solani*, *Aspergillus oryzae*, *Bipolaris oryzae*, *Cercospora janseana*, *Curvularia lunata*, *Fusarium moniliforme*, *Pyricularia oryzae*, *Sarocladium oryzae*, *Tilletia barclayana*, *Fusarium graminearum*, *and Ustilaginoidea virens.*

INTRODUCTION

Rice is the main crop in the world, especially in Asia countries. Mekong Delta in Vietnam has a rich potential of rice genetic diversity and one of the most important rice production areas in Vietnam. The Mekong Delta's rice productions constituted around 52% of the entire country and contribute more than 90% of rice export per year (Hoa, 2006). An Giang province is the one that has the most extensive rice cultivar area in the Mekong Delta region with 13.46% (more than 620.000 Ha) (Department of Agriculture and Rural Development of An Giang Province, 2014). Belong to An Giang province, Cho Moi district has large rice cultivars which have a part in rice production total of An Giang province.

Nowadays, the intensive rice production situation in Mekong Delta, especially in An Giang province. Besides that, there are threats from natural disasters, pest damages are increasing and more perilous. That is why the selection of rice seeds for cultivating is essential for each

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region. Moreover, using healthy and quality rice seeds is the key to maintaining yields and productivity, intensifying farmers' income.

Reports of Thuy *et al.* (2012) and Trung *et al.* (2001) showed that 26 kinds of fungi were on the rice varieties in 7 provinces in Mekong Delta. In the south of Nigeria in 2008, Utopo *et al.* (2011) reported nine kinds of fungi on seed rice. They were *Trichoconis padwickii* (about 37%), *Helminthosporium oryzae* (about 17%), *Fusarium moniliforme* (about 14%), *Rhizopus oryzae*, *Aspergillus niger*, *Curvularia lunata*, *Penicillium* sp., *Alternaria oryzae*, and và *Pyricularia oryzae*. In Pakistan, on rice samples stored in stock, only four species of fungi were recorded, they were *Fusarium moniliforme*, *Aternaria* sp., *Helminthosporium* sp. và *Curvularia* sp. (Butt *et al.*, 2011)

MATERIALS AND METHODS

Collection of seed samples

The samples of rice seeds collected were based on Vietnam Standard 8548:2011 (Vietnam Standard, 2011). Firstly, 27 seed specimens were collected at 27 farmer households which belong to 9 villages of Cho Moi district in An Giang province. The rice varieties are OM 6979, IR 50404, and Jasmine, which all are popular rice ones at Cho Moi district. The farmers stored the rice seeds after harvesting of Autumn-Winter season, and they could use them to sow on the next crop. In each location of farmer households, rice seeds' specimen was a randomized selection with the plot of rice seeds.

This study used nine rice seed samples from the farmer household (named ND1 to ND9) to compare with a control sample (name Control) certified by a company.

Assessment of seed purity

The experiment was conducted in a completely randomized design (CRD) with ten treatments (ND1 to ND9 and a Control) and four replications. The method was based on a procedure of Vietnam Standard 8548:2011 (Vietnam Standard, 2011). Briefly, 100 seeds of each replica were randomly selected, classified the shapes (seed of weeds, weedy rice seed, or others), then calculated the percentage.

Assessment of filled-grain ratio of rice seeds

The filled-grain ratio assessment experiment was carried out in CRD with ten treatments and four replications for 100 rice seeds/one-replication. The procedure followed steps of Vietnam Standard Method 8548:2011 (Vietnam Standard, 2011)

Assessment of germination ratio

The experiment for assessing the germination ratio was performed in CRD with ten treatments and four replications for 25 seeds/one-replication. This experiment used the Top Paper method based on the Vietnam Standard Method 8548:2011 (Vietnam Standard, 2011) and (ISTA-International Seed Testing Association, 1996). The rice seeds were put in Petri dishes containing three layers of sterilized papers. After four days, the germination of seeds was recorded. The results were expressed in percentage.

Assessment of Ratio of Normal Seedlings

The experiment for assessing the Normal Seedlings ratio was carried out in CRD with ten treatments and four replications for 100 seedlings/one-replication. The data was checked based on a Textbook of modules for studying the quality of certified rice seeds from the Ministry of Agriculture and Rural Development of Vietnam. After eight days of sowing, the ratio of normal seedlings was recorded.

Detection of seed-borne fungi

The experiment was performed in CRD with ten treatments and four replications for 100 seeds/one-replication. The study procedure followed the Blotter Method of ISTA (International Seed Testing Association, 1996), according to Characteristic Description of Fungi from Barnett & Barry (1998) and Mathur & Kongsdal (2000). Shortly, three layers of papers were taken, soaked with distilled water, and put into a Petri dish. Then, 25 rice seeds were placed in the petri dish, with one seed in the center of the petri dish, eight seeds in a circle, and 16 seeds in the next circle outside. The space between every seed and each circle line is similar. Petri dishes were placed in growth cabinet conditions, including 22^{o}C of temperature, 70-85% of relative humidity, 12/12 h for light/darkness. After six days of incubation, the seed-borne fungi were detected under a microscope. The ratio of seed-borne fungi and frequency of fungal infection were calculated using the formulae (1) and (2).

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The ratio of seed-borne fungi (%)

$$= \frac{Quantity\ of\ seed - infected\ fungi}{Total\ seed}\ x\ 100\%$$

Infection frequency of fungus X (%) = $\frac{Quantity\ of\ seed\ infected\ by\ fungi\ X}{Total\ seed}\ x\ 100\%$

RESULTS AND DISCUSSION

The percentage of purity seed and filled-grain

The quality of rice seeds is affected by a lot of factors. The percentage of purity seed and filled-grain are important factors of all.

Parameter of purity seeds is influenced by weeds seed and others seed, leading to high uniformity of seedlings when growing. In Table 1, the result of testing purity seeds showed it is relatively different between each treatment. The control treatment had the lowest purity seeds of 3.75%, non-significant differences of statistics with the other treatments, except ND2 treatment, which had 15.25% of purity seed – the highest one. This result proved that the control treatment (certified rice seeds of a seed company) is qualified for seed requirements.

Table 1. The percentage of seed purity and filled-grain

Treatments	Seed purity percentage (%)[1]	Filled-grain percentage (%)[1]
ND1	11.50 ab	96.50 ab
ND2	15.25 c	93.50 bc
ND3	10.25 ab	93.25 bc
ND4	11.00 ab	94.50 bc
ND5	10.00 ab	91.00 c
ND6	8.75 a	94.25 bc
ND7	7.25 a	93.25 bc
ND8	8.50 a	95.00 b
ND9	9.75 ab	93.25 bc
Control	3.75 a	99.25 a
Sig.	***	**
CV (%)	21.7	2.4

*[1] Means followed by a similar letter in the column are significantly different according Duncan; ** significantly different of statistics at 1%; *** significantly different at 0.1%.*

Besides, almost of rice seeds from farmer households are good and undifferentiated with control rice seeds.

The percentage of filled-grain rice is also an important factor, which is the determinant of the germination ratio and rice seeds' health. Generally, the varieties have a high percentage of filled-grain that is also high of germination ratio or inverse. According to the results in Table 1, the percentage of filled-grain is significantly different at 1%.

Figure 1. Normal and abnormal seedlings.

The highest ratio of filled-grain showed at control treatment (99.25%) which is statistically higher than the other treatments, except ND1 treatment (96.0% of filled-grain). The lowest one showed in ND5 (91.0%). The others including ND2 (93,5%), ND3 (93,25%), ND4 (94,5%), ND6 (94,25%), ND7 (93,25%), ND9 (93,25%) are also lower than and significant different of statistics to the control treatment. The result indicated that in the stored condition of farmer households, cultivation techniques, nutrition during the growing time, and rice seeds production process resulted in low in-filled-grain quantity.

The germination and normal seedling

The parameters of seed germination and normal seedling are the important target of seed research. Table 2 shows the experiment's result using the rolled paper method for testing the vigor of seeds and seedlings. The best seed germination was in the control treatment (95.25 %), but this value is non-significant different from statistics which are comparing with treatments of ND4 (87.5 %), ND7 (86%), ND8 (88.5 %), and ND9 (88.75 %). Contrarily, the other treatments are differently significant compared to the control one. They concluded ND1, ND3, ND4, ND6 with 83.75%, 80%, 77.5% and 78.25%, respectively. According to Vietnam Criterion 01 – 54: 2011, the minimum seed germination is 80 %, so almost all rice seeds provided by the farmer are satisfied with this requirement.

The normal seedling (Figure 1) is the one that can continue development to become the normal plant after

germinating; the requirement of the selection of normal seedling is based on Vietnam Standard 8548:2011. The result of normal seedling was showed in Table 2, and it was taken after eight days of the germinating stage. According to Table 2, the best of healthy seedlings is the control (92.25%). Seedlings of this treatment are better than the others. In addition, treatments of ND2 (83.5%), ND4 (83.75%), ND7 (86.25%), ND8 (81.5%) and ND9 (86.75 %) have normal seedlings and not different with the other treatments. However, ND1, ND3, ND5, and ND6 with low at the value of normal seedling are 78%, 74.25%, 75.75%, and 75.5% are significantly different compared with control one.

Table 3. The ratio of germination and normal seedling of each rice seeds.

Treatment	Seed Germination (%)[1]	Normal Seedling (%)[1]
ND1	83.75 bc	78.00 bc
ND2	83.75 bc	83.50 abc
ND3	80.00 bc	74.25 c
ND4	87.50 abc	83.75 abc
ND5	77.50 c	75.75 bc
ND6	78.25 c	75.50 bc
ND7	86.00 abc	86.25 ab
ND8	88.50 ab	81.50 abc
ND9	88.75 ab	86.75 ab
Control	95.25 a	92.25 a
Sig.	*	*
CV (%)	7.2	8.4

*[1] Means followed by a similar letter in the column are significantly different according Duncan; * significantly different of statistics at 5%.*

The infected fungal ratio on rice seeds

On agricultural production in general, rice cultivation, mainly the health of seeds, is the one important factor. When fungi infect the seeds, they have an intimate relationship with the diseased paddy after growing. Thus, determining the level of fungal infection on rice seeds is important to appreciate seed health and treat rice seeds before growing.

Table 3, which was infected fungal testing in rice seeds using the Blotter method, indicated that control treatment had 59.38 % of fungal infection (with the fair level infected by fungi). However, it is the lowest when comparing with the others and significant difference in the statistic. The highest fungal infection is ND8 (97.5 %). This value is not significantly different with other treatments including ND9 (96.88 %), ND6 (94.38%), ND5 (93.75%), ND4 (92.5), ND7 (92.25%). The other treatments are ND1, ND2, ND3 (85.63 %), which have high infected fungi and a significant statistical difference. The results are in line with (Tuat *et al.*, 2004). These authors reported that all breeder seeds, foundation seeds, registered seeds, and certified seeds from farmer households are infected with fungi.

Table 2. The infected fungal ratio (%) on rice seed

Treatments	Fungal infected (%)[1]
ND1	85.63 b
ND2	85.63 b
ND3	85.63 b
ND4	92.5 ab
ND5	93.75 ab
ND6	94.38 ab
ND7	91.25 ab
ND8	97.5 a
ND9	96.88 a
Control	59.38 c
Sig	***
CV (%)	6.1

*[1] Means followed by a similar letter in the column are significantly different according Duncan; *** significantly different of statistics at 0.1%.*

The Correlation between filled–grain ratio and the infected fungal ratio of rice seeds

Figure 2 showed that it had the opposite between the infected fungal ratio and filled–grain one. Particularly, an increase of filled–grain related to a reduction of fungal infection on seed and opposite. Because fungi are usually infected unfilled–grain or half-filled, the fungal infected rate is low when the filled–grain ratio is high. The varieties which have more unfilled–grain is high of fungal infected on seeds and opposite. Unfilled–grain or

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half-filled is also an important factor of some diseases on grains. The same report was indicated by (Thuy, 2011). These authors confirmed discoloration or unfilled grain agent is from 11 kinds of different parasitic fungi (Thuy, 2011).

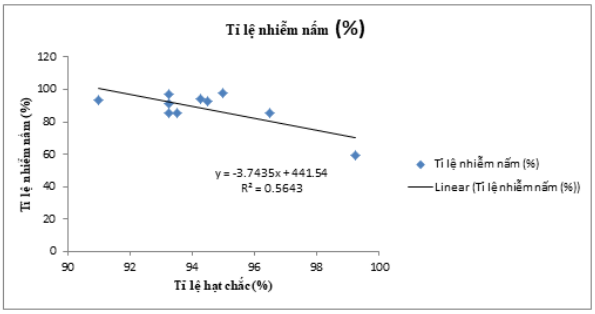

Figure 2. The Correlation between filled-grain ratio and infected fungal ratio

The fungal component on rice seeds

The Blotter method was used to determine what kind of fungi have on rice seeds. Table 4 showed that 12 kinds of fungi were on the rice varieties which the farmers in Cho Moi stored for sowing in the next crop. They were *Alternaria padwickii, Rhizoctonia solani, Aspergillus oryzae, Bipolaris oryzae, Cercospora janseana, Curvularia lunata, Fusarium moniliforme, Pyricularia oryzae, Sarocladium oryzae, Tilletia barclayana, Fusarium graminearum,* and *Ustilaginoidea virens.*

The result is similar to the report of Mew & Misra (1994). They have also identified that the fungi on rice seeds are: *Alternaria padwickii, Bipolaris oryzae, Cercospora janseana, Curvularia lunata, Ephelis oryzae, Fusarium moniliforme, Microdochium oryzae, Nakataea sigmoidea, Pyricularia oryzae, Rhizoctonia solani, Sarocladium oryzae, Tilletia barclayana, and Ustilaginoidea virens.* Besides, My K.T.N. *et al.* (2017) also showed that Jasmine 85 (in An Giang province) also has seven fungal infected on seed: *Alternaria padwickii, Bipolaris oryzae, Sarocladium oryzae, Aspergillus sp., Fusarium moniliforme, Mucor sp.* and *Penicillium sp.*

Infection frequency of fungi on rice seeds

In addition to testing and confirming the fungal component on seeds, the frequency of fungi appeared on seeds is also important to definite common fungi on each seed sample.

Table 4. Component of fungi on rice seeds in Cho MOi district, An Giang Province.

Science name	Family	Order
Alternaria padwickii	Dematiaceae	Moniliales
Rhizoctonia solani	Dematiaceae	Moniliales
Aspergillus oryzae	Trichocomaceae	Eurotiales
Bipolaris oryzae	Dematiaceae	Moniliales
Cercospora janseana	Mycosphaerellaceae	Mycosphaerellales
Curvularia lunata	Dematiaceae	Moniliales
Fusarium moniliforme	Nectriaceae	Hypocreales
Pyricularia oryzae	Moniliaceae	Moniliales
Sarocladium oryzae	Moniliaceae	Moniliales
Tilletia barclayana	Tilletiaceae	Ustilagiales
Fusarium graminearum	Nectriaceae	Hypocreales
Ustilaginoides virens	Ustilaginaceae	Ustilaginales

Table 5. The infection frequency of fungi

Science name	ND1	ND2	ND3	ND4	ND5	ND6	ND7	ND8	ND9	ĐC
Alternaria padwickii	+++	+++	+++	+++	+++	+++	+++	+++	+++	+
Rhizotocnia solani	+	++	+	++	++	+++	+	++	++	-
Bipolaris oryzae	-	++	-	-	+	-	-	+	+	-
Cercospora janseana	-	++	+++	+++	++	+++	+++	+	++	+
Curvularia lunata	+	+	+	+++	++	+	-	++	+	+
Fusarium moniliforme	-	-	-	+	+	+	-	+	+	-
Pyricularia oryzae	-	-	-	-	-	+	-	-	+	-
Sarocladium oryzae	-	-	-	+	-	-	-	-	-	-
Tilletia barclayana	++	+++	+++	+++	+++	+++	++	+++	+++	+
Ustilaginoides virens	+++	+++	+++	+++	+++	+++	+++	+++	+++	+
Aspergillus oryzae	+	+++	+++	++	+++	+++	+++	+++	+++	++
Fusarium graminearum	-	-	-	-	+	+	-	-	+	-

Note: + less appearance during sample analysis (5 – 15 %); ++ much appearance during sample analysis (15 – 25%); +++ frequently appearance during sample analysis (>25%)

The observed results in Table 5 showed that the composition and frequency of fungal species appearing on ten rice samples were different. The fungi appearing common on ten treatments are *Alternaria padwickii*, *Rhizoctonia solani*, *Cercospora janseana*, *Curvularia lunata*, *Tilletia barclayana*, *Ustilaginoidea virens*, *Ustilaginoidea virens*, and *Aspergillus oryzae*. Especially, *Tilletia barclayana* and *Ustilaginoidea virens* appear on all of the treatments in this research. Treatments of ND4, ND6, ND9 are more infected of fungi than treatments of ND1, ND2, ND3, ND7. The control treatment is less infected of fungal than was comparing with the other treatments.

The result of this study's analysis is similar to the report from Mew and Gonzales (2002) when they subdivided samples from many different regions of the world. The appearance frequency of fungus *Alternaria padwickii* is the most, with around 40 – 100 % in 1989–1997. The *Alternaria padwickii* appeared on all of the locations of sampling.

This study showed the popularity of both *Alternaria padwickii* and *Aspergillus oryzae* on all of the samples. This finding is similar to the results of Nguyen Thi Kieu My *et al.* (2017). They isolated fungal from the samples that were collected from many districts of An Giang province (Chau Doc, Thoai Son, Tinh Bien, Tri Ton, An Phu, Chau Thanh, Chau Phu, Long Xuyen, and Cho Moi).

Besides, the mold, such as *Aspergillus* sp. caused damage during storage; there were similar reports from Zafar *et al*. (2014) when they isolated fungi on the seeds. Almost all samples have *Aspergillus* sp.

Thus, the ratio of seed-infected is high of each sample is very high from the rice samples of the farmer. The seed with a high rate of filled–grain is less infected fungal. Totally, there have 12 kinds of fungi on rice seeds.

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CONCLUSIONS

Almost all seed samples harvested and stored by the farmers are lower at filled–grain, lower at seed germination and seedlings than the company's registered certified rice seeds. However, the majority of seeds from the farmers are still meet the requirements of germinating ratio following to (Vietnam Standard, 2011) (the rate of germination > 80 %).

The seed samples collected from farmer households are high of infected fungal ratio. Besides, 12 types of fungi exist on seeds from farmer households. Therefore, if farmers use storage seeds for breeding from the previous crop, it is necessary to apply some measures to screen seeds and remove unfilled–grain or half-filled grain before sowing.

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