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EFFECT OF LEVEL OF RELATIVE HUMIDITY ON FUNGI AND GERMINATION CAPACITY OF PADDY *(ORYZA SATIVA* L.) GRAINS IN EGYPT

By

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تأثير مستوى الرطوبة النسبية على فطريات وانبات حبوب الارز في مصر

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تمت دراسة التغيرات التي تحدث في فطريات الحبوب المحمولة وانبات الحبوب ومحتواها المائي عند تخزين حبوب الارز عند مستويات مختلفة من الرطوبة النسبية (تقريباً صفر ، ٥٥٪ ، ٧٦٪ ، ٩٢٪ و ١٠٠٪) والتحضين عند ٢٨°م ولمدة ١ ، ٢ ، ٣ ، ٤ شهور .

وجد أن التعداد الكلي للفطريات يزداد زيادة معنوية بزيادة المحتوى المائي للبذور وفترات التخزين وسجل أعلى تعداد للفطريات عند ٩٢٪ و ١٠٠٪ رطوبة نسبية بعد ٤ ، ٣ شهور علي التوالي . وترجع هذه الزيادة إلى ازدهار وزيادة أعداد فطريات عديدة مثل : اسبيرجيلس كانديدس ، اسبيرجيلس سيدوي ، اسبيرجيلس تامرياي ، اسبيرجيلس فيرسيكلر ، اسبيرجليس فلافس ، بنيسيليوم كريزوجينم ، فيوزاريوم اوكسيسبورم وكلادوسبوريم كلادوسبوريدس .

وجد أن جميع مستويات الرطوبة النسبية (عدا صفر) تؤدي إلى تناقص معدلات انبات الحبوب مع زيادة فترات التحضين ، وتزداد معدلات التناقص في الانبات سريعاً مع زيادة الرطوبة النسبية التي تؤدي إلى زيادة المحتوى المائي للبذور ، وسجل موت جميع الحبوب عند ٩٢٪ و ١٠٠٪ رطوبة نسبية والتحضين لمدة ٤ و ٣ شهور حيث ارتفع المحتوى المائي للحبوب إلى ٢١,٦٪ و ٢٢,٦٪ على التوالي .

Key Words: Relative humidity, paddy grains-borne fungi, germination capacity, moisture contents, storage fungi

ABSTRACT

The changes in the composition of paddy grain-borne fungi in the germination capacity, and in the moisture content of paddy grains at various levels of relative humidity (r.h.) (~ 0-100%) stored at 20°C for 1, 2, 3 and 4 months, were investigated. The total count of fungi significantly increased with the increase of moisture content and storage periods and the highest counts were estimated at 92% and 100% r.h. after 4 and 3 months, respectively. The preceding increases up in the total counts were mainly related to the flourishing of numerous species such as. *Aspergillus candidus, A. sydowii, A. tamarii, A. versicolor, A. flavus, Penicillium chrysogenum, Fusarium oxysporum* and *Cladosporium cladosporioides.*

At all levels, except at ~ 0%, of relative humidity the germination capacity of grain decreased slowly or rapidly with the increase of the storage period and the rate of decrease was enhanced with the rise of relative humidity which increased the moisture contents of grains, and complete mortality (100%) was recorded at 92% r.h. after 4 months and at 100% after 3 months, when the moisture contents of paddy grains rose to 21.6% and 22.6% respectively.

INTRODUCTION

In a previous paper Abdel-Hafez *et al.* (1992) studied the effect of conditioning paddy grain to certain levels of moisture content (11.5%, 17%, 22.5% and 28%) and stored for 8 months at 8°, 18° and 28°C, on the germination capacity and composition of paddy grain borne fungi. The present investigation was aimed to study and follow up the changes in paddy grain-borne fungi, in the moisture content in the germination capacity of paddy grain, stored at different levels of relative humidity.

MATERIALS AND METHODS

Samples of Egyptian paddy (*Oryza sativa* L.) (rice in the husk) were collected from different Governorates in Egypt, characteristic for rice cultivation, carried to the laboratory and thoroughly mixed under aseptic conditions and the composite sample was stored at 3-5°C.

Storage of Paddy grain at various levels of relative humidity.

Numerous levels of relative humidity were maintained by placing saturated solutions of some salts in closed desiccators as described by Tomkins (1932). The grain (1.5 kg for each level of relative humidity) was placed in muslin bags and was hanged above the solutions and the desiccators were incubated at 20°C. Four levels of relative humidity were used: 55%, 76%, 92% and 100%. Using saturated solutions of $Ca(NO_3)_2$ $4H_{20}$, NaCl, $Na_2Co_3.1OH_{20}$ and distilled water respectively. A very low level of relative humidity (presumably zero) was maintained by keeping the grain in a desiccator over anhydrous calcium chloride. At intervals (1, 2, 3 and 4 months) the fungi were assayed by the dilution-plate method. Also the moisture content and the germinalility of the grains were determined as percentage.

Determination of paddy grain-borne fungi

Paddy grain-borne fungi were estimated by using the dilution plate method as described by Christensen (1963) and employed by Moubasher *et al.* (1972). Glucose- (10 g/l) Czapek's agar +rose bengal (1/15000) as a bacteriostatic agent (Smith, Dawson 1944) was used as isolation medium. Six plates were used for each treatment; hey were incubated at 28°C for 7-10 days and the developing fungi were identified, counted and calculated per mg dry paddy grains.

The colonies of slow-growing fungi were transferred to slants or plates to ensure precise counting. Other agar media such as potato-dextrose, malt extract agar and Czapek's agar + 0.05 yeast extract were used for the growth and the identification of fungi.

Determination of paddy grain germination:

Paddy grain was first surface-sterilized by shaking in 2% sodium hypochloride solution for 5 minutes, rinsed in three changes of sterile distilled water. Thereafter it was incubated at 25°C over a pad of moist sterile filter paper, placed in a sterile Petri-dish for 7-10 days. The grain with healthy roots and plumules was counted and expressed as percentage.

Determination of moisture content of paddy grains:

Replicate samples of paddy grain were ground in a blender and a weighed portion of their flour was dried in an oven for 24 h. at 105°C then cooled in a desiccator and re-weighed to a constant weight. The moisture content was then calculated at percent on an oven-dry basis.

RESULTS

A: Mycoflora of paddy grain (recovered at 28°C)

The results in Table (1) show clearly that the total count of fungi was mostly consistent and similar to the control sample at ~0% r.h., but at 55% and 76% r.h. the count was slightly increased with the increase of storage period. At 92% and 100% r.h. the count of fungi sharply increased and the highest counts were estimated after 4 and 3 months, respectively.

Aspergillus significantly flourished at 92% and 100% r.h. and the best counts were estimated after 4 and 3 months, respectively. The significant increase of *Aspergillus* was related to the behavior and flourishing of some *Aspergillus* species such as *A. candidus, A. sydowii, A. tamarii* and *A. versicolor.* Also, numerous *Aspergillus* species significantly promoted such as *A. fumigatus* at 92% and 100% r.h. after 4 months; *A. flavus* and *A. niger* at 100% r.h. after 3 and 4 months; and *A. amstelodami* at 76% after 3 and 4 months.

Penicillium significantly increased at 92% r.h. after 4 months, and at 100% r.h. after 2, 3 and 4 months. The count of *P. chrysogenum* was parallel to that of the genus count. *P. citrinum* was significantly increased at 100% r.h. after 3 months only.

Fusarium regularly increased with the increase of the level of relative humidity and storage period, but the increase were significant at 92% r.h. after 2, 3 and 4 months, and at 100% r.h. after all periods of storage. Two species were identified, namely *F. oxysporum* and *F. moniliforme.* The count of *F. oxysporum* was parallel to that of the genus count. *F. moniliforme* significantly flourished at 92% r.h. after 4 months and at 100% r.h. after 1 and 2 month.

Cladosporium significantly developed at 55% and 92% r.h. after 1 and 2 months; at 76% r.h. after 2 months; and at 100% r.h. after 1 month. It was represented by three species namely, *C. cladosporioides, C. herbarum* and *C. sphaerospermum* and their counts were almost parallel to that of the genus count.

Paecilomyces terricola irregularly flourished at some treatments and the best count was recorded at 100% r.h. after 1 month. *Alternaria alternata* and *Trichothecium roseum* were significantly promoted in treatments 2 and 3, respectively.

B: Moisture content of paddy grain:

The results in Table (2) show that the initial moisture content of paddy grains was 5.75%. When the grain was stored over anhydrous calcium chloride (~ 0% r.h.), the moisture content decreased rapidly to 1.1% after 1 month to a minimum level (0.4% m.c.) after 4 months. At 55% and 76 r.h. the moisture content of the grain slowly rose to reach 10.2% and 12% after 4 months, respectively. At high levels or relative humidity 92% and 100%, the moisture content of the grain increased rapidly with the lapse of the storage period and reached 21.6% and 25% after 4 months, respectively.

C: Germination capacity of paddy grain:

The results in Table (2) reveal that the initial percentage of germination (100%) of the grain was almost unaffected (92-98%) by the levels of relative humidity ~ 0%, 55% and 76 after 4 months. But, at high levels 92% and 100% r.h. the loss of germination capacity was serious and complete mortality (100%) was recorded after 4 and 3 months, respectively.

Table 1

Counts (calculated per 1 mg of dry grain) of common fungal genera and species (recovered on glucose-Czapek's agar at 28°C) of paddy grain stored at different levels of relative humidity (~0%, 55%, 76%, 96% and 100%) after a, 2, 3 and 4 months at 20°C.

% Relative humidity Genera and species	Control	~ 0 %				55 %				76 %				92 %				100 %			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Alternaria alternata	1.3	1.0	1.3	1.2	1.2	0.2	1.4	1.2	1.2	1.8	1.6	0	1.4	1.4	0	1	0	1.2	0	0	0
Aspergillus	0.8	0	1.0	2.2	3.0	0.8	2.0	7.2	11.6	2.4	5.2	11.0	16.2	4.4	290	720	2880	24.8	816	3092	2480
A. amstelodami	0	0	0	0	0	0	0	0.4	0	0	0	1.8	6.0	0	0	0	0	0	0	0	0
A. candidus	0.1	0	0.8	0.3	1.1	0	1.8	2.0	3.4	0.4	1.6	3.4	4.4	0	7	195	600	0.4	71	300	360
A. flavus	0.2	0	0	0.2	0.1	0	0.2	0	0.4	0	0.8	0	0	0	0	0	0	2.0	0	52	20
A. fumigatus	0.1	0	0	0.2	0.6	0.6	0	1.0	0.4	1.2	1.6	1.6	1.4	1.2	1	4	64	1.2	0	0	80
A. niger	0.2	0	0	0.2	0	0.2	0	1.4	2.0	0	0	0.2	0	0	1	0	0	1.2	0	80	52
A. sydowii	0.1	0	0	1.1	1.2	0	0	1.4	0.2	0.8	0.4	2.0	2.8	3.2	244	498	1686	17.2	602	2016	1408
A. tamarii	0	0	0	0	0	0	0	0.4	2.6	0	0	0.6	1.6	0	0	6.7	48	2	21	24	24
A. versicolor	0.1	0	0.2	0	0	0	0	0.6	0	0	0	0	0	0	1	17	414	0	122	644	416
Cladosporium	4.3	5.6	5.4	4.1	3.9	9.8	8.8	5.6	1.2	6.2	9.2	0	4.8	9.4	15	3	0	15.6	0	0	0
C. cladosporioides	3.5	4.7	3.8	2.9	2.4	6.4	6.4	4.2	3.4	4.4	6	0	3.4	6.6	11	2	0	10.4	0	0	0
C. herbarum	0.6	1.3	1.2	0.8	1	2.4	1.8	1	2.2	1.8	2.6	0	1	2	3	1	0	4	0	0	0
C. sphaerosperum	0.2	0.6	0.4	0.4	0.5	1	0.6	0.4	0.8	0	0.6	0	0.4	0.8	1	0	0	1.2	0	0	0
Fusarium	1.9	1.6	1	0.6	1.2	2.6	3.4	3.6	0.4	2.6	3.4	4.8	6.2	5.2	14	16	56	8.4	21	40	44
F. moniliforme	0.2	0.1	0	0	0.1	0	0	0.4	4	0.2	0	0.4	0.4	0	0	0	4	1.2	2	0	0.2
F. oxysporum	1.7	1.5	1	0.6	1.1	2.6	3.4	2.2	0.4	2.4	3.4	4.4	5.8	5.2	14	16	52	7.2	19	40	42
Paecilomyces terricola	0.8	1.6	1.6	1.6	1.2	1	1	0.8	3.6	1	3	1.8	1.4	1.8	0	0	0	6	0	0	0
Penicillium	1	0.5	0.2	0.5	0.4	0.8	1.4	1.2	0.4	1	1.2	2.4	2.4	0.2	29	31	168	2.4	122	468	168
P. chrysogenum	0.7	0.4	0.2	0.2	0.2	0.4	1.4	0.8	1	0.8	0.8	1.2	1.6	0.2	25	31	168	1.6	122	428	168
P. citrinum	0.1	0	0	0	0	0	0	0	0.4	0	0	0.4	0.2	0	4	0	0	0	0	40	0
Trichothecium roseum	0	0	0	0	0.2	0	0	0.8	1	0.1	0	0	0.4	0	0	0	0	0	0	0	0
Gross total count	11	11.6	12.1	1.6	11.3	15.8	21	23.2	27	16.8	23.2	28.4	33.4	22.8	338	771	3088	59.6	959	3652	3044

The least significant difference analysis (LSD) was employed for statistical analysis of the results.
* Denotes significant increase of count comparable with the control count.
Non-significant species are omitted from the table, but their counts are included in the total count of fungi.

Table 2

Changes in the percentages of moisture content and the germination of paddy grain, stored at five levels of relative humidity for 1, 2, 3 and 4 months at 20°C (~0 = presumable value kept over anhydrous calcium chloride).

Relative humidity (%)	Months of storage			
	1	2	3	4
	Moisture content (%)			
~0 %	1.1	0.92	0.8	0.4
55 %	8.1	8.6	9.2	10.2
76 %	9.4	10.1	10.8	12.0
92 %	12.8	15.8	18.6	21.6
100 %	16.1	19.2	22.6	25.0
	Germination capacity (%)			
~0 %	100	100	100	98
55 %	100	98	96	96
76 %	98	98	96	92
92 %	94	86	54	0
100 %	92	56	0	0

Initial moisture content = 5.75% (Control).
Initial germination capacity = 100 %

Compared between the effect of relative humidity and moisture content (Abdel-Hafez *et al.* 1989) on paddy grain-borne fungi and germination capacity of these grain the following observations were obtained: 1. There were basic similarities between the effect of high levels of relative humidity and moisture content on paddy grain-borne fungi. 2. High levels of relative humidity (92% and 100%) and moisture content (22.5% and 28%) had a very serious effect on the germination capacity of paddy grain due to the significant promotion of some members of *Aspergillus, Cladosporium, Fusarium* and *Penicillium* at these levels. 3. *Cladosporium* spp. which behaved as field fungi significantly increased at high levels of moisture contents (22.5% and 28%) thereby their abundance was significantly promoted at moderate levels of relative humidity (55% and 76%) after 1 or 2 months where the moisture content of paddy grain rose to 8.1-1.8.6% and 9.4-10.1% respectively.

DISCUSSION

When the paddy grain is stored at different levels of relative humidity (55-100%), the moisture content is consequently increased at a rate proportional to the level of relative humidity. But, when the paddy grain is stored over anhydrous calcium chloride (~ 0% r.h.) the moisture content decreased to

reach 0.4% after 4 months. A similar observations were obtained by El-Hissy *et al.* (1981) and Mazen *et al.* (1992) when peanut and cotton seeds were stored at various levels of relative humidity (40-100%) & (75-100%) respectively.

The mycoflora significantly flourished slowly or rapidly, according to the level of relative humidity. A ~ 0% r.h. the total count of fungi was almost consistent. At 55% and 76% r.h. the total count of filamentous fungi was slightly increased with the increase of the incubation period, but at 92% and 100% r.h. the counts were sharply increased and the maxima were reached after 4 and 3 months, respectively. As a result of the flourishing fungal flora the rate of mortality of grain embryos increased with the increased of relative humidity and the germination capacity of the grains was completely lost (0%) after 4 and 3 months at 92% and 100% r.h. where the moisture content of paddy grain rose to 21.6% and 22.6%, respectively. Ghosh (1951) found that fungal growth occurred between 100% and 65% r.h., below 75% r.h. growth being slower, with stored rice grain at 30° ±1°C. Biswas & Sircar (1976) reported that storage of rice seeds at ambient temperature and low humidity gave the best germination after 1 year. Ghosh *et al.* (1981) kept wheat grain under 75%, 80% and 85% r.h. and at temperatures of 20°±2°C for 1 year and found that increase in grains infection by fungi with decrease in grain germination capacity was more evident with longer storage, higher temperature and higher relative humidity. *Paecilomyces terricola, Trichothecium roseum* and *Aspergillus amstelodami,* were slightly increased at 55% or 76% r.h. after some storage periods, where the moisture content of paddy grain slowly rose. On the other hand, Christensen and Kaufmann (1965) reported that most of these above species did need relatively lower limits of moisture content for invasion of starchy grains at 92% and 100% r.h. and after most of storage periods numerous fungal species sharply increased up to the increase of moisture content of paddy grain (21.6% and 25% m.c. after 4 months, respectively). These species were *Aspergillus candidus, A. sydowii, A. tamarii, A. versicolor, Penicillium chrysogenum, Fusarium oxysporum, Cladosporium cladosporioides* and *C. herbarum.* Abdel-Hafez (1971) reported that *Aspergillus* species appeared to be good colonizers of some Egyptian cereal grains (Wheat, sorghum and corn) at 80% r.h. during the second (91-180 days) or third period (181-300 days) of storage. Also *Penicillium* could considerably increase its population in wheat, corn and sorghum grains stored at 80% r.h. during the first period (1-90 days), but it was then overgrown by *Aspergillus.*

In conclusion, paddy grain must be stored at low level of relative humidity and with anhydrous calcium chloride in elevators to decrease the moisture content of grain and the relative humidity of the environment to a lower limit which is unfavorable for the activity of paddy grain-borne fungi.

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