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Storage of mungbean seed *(Vigna radiata* (L) Wilczek*)* inoculated with four species of Aspergilli

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RINGKASAN

Empat spesies Aspergilli iaitu Aspergillus flavus, A. niger, A. fumigatus *dan* A. parasiticus *digunakan di dalam kajian ini. Biji-biji kacang hijau disuntik dengan kulat-kulat diatas dan kejangkitan kulat dan peratus percambahan hingga ke minggu 18 masa simpanan pada kelembapan bandingan (RH) 30%, 50% dan 95% diuji. Kejangkitan yang disebabkan oleh* A. flavus, A. fumigatus *dan* A. parasiticus *adalah lebeh daripada 80% selepas 18 minggu simpanan pada tahap kelembapan bandingan 95%. Kejangkitan tertinggi (90%) oleh* A. niger *didapati selepas 9 minggu simpanan pada 95% RH tetapi peratus kejangkitan pada kemudiannya adalah berkurangan. Pada kelembapan bandingan 30% dan 50%, kejangkitan* A. fumigatus *adalah yang paling rendah dan kejangkitan oleh* A. flavus *adalah paling tinggi selepas 18 minggu simpanan. Biji-biji yang disimpan pada kelembapan bandingan 30% dan 50% tidak mengurangkan percambahan walaupun biji telah disimpan selama 18 minggu. Tetapi biji-biji yang disimpan pada kelembapan bandingan 95% menunjukkan kekurangan percambahan selepas biji disimpan selama 12 minggu.*

SUMMARY

Four species of Aspergilli namely Aspergillus flavus, A. niger, A. fumigatus *and* A. parasiticus *were used in the study. Mungbean seeds were inoculated with the the above fungi; mould invasion and percent germination up to 18 weeks of storage at 30%, 50% and 95% RH were evaluated. The infection due to* A. flavus, A. fumigatus *and* A. parasiticus *was greater than 80% after 18 weeks of storage at 95% relative himidity (RH). Maximum infection of 90% by* A. niger *was obtained after 9 weeks of storage at 95% RH but thereafter, the percentage infection decreased. At 30% and 50% RH, infection by* A. fumigatus *was lowest while that of* A. flavus *was highest after 18 weeks of storage. Seeds stored at 30% and 50% RH did not show any decline in germination even after the 18th week of storage. However, seed stored at 95% RH showed a decline in germination after the 12th week of storage.*

INTRODUCTION

According to Christensen (1973), fungi that invade seeds can be divided into two general groups, field fungi and storage fungi. Field fungi are those that invade seeds as they are developing on the plants in the field or after the seeds have matured but before they are harvested. Field fungi may discolour seeds, weaken or kill the embryos, cause prolonged dormancy and incite various blights and root rots in the plants grown from seeds. Storage fungi are those that grow on seeds or other kinds of materials while in storage. They have the ability to grow without free water. They are made up of several group of species of *Aspergillus* and a few of *Penicillium*. Certain storage fungi, primarily, *Aspergillus* spp. are serious pathogens of seeds (Christensen and Kaufmann, 1969).

Aspergillus species have been reported to invade and destroy stored seeds including cereal grains, cocoa beans, cotton seeds and legume seeds (Christensen and Kaufmann, 1969; Rocadori *et al.* 1972; Harman and Grannet, 1972; Saharan and Gupta, 1973).

Seeds by nature are hygroscopic and tend to be influenced by the surrounding atmosphere. At

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a high moisture content they heat up rapidly in storage. The respiration of the sample is accelerated. Coupled with this, associated micro-organisms also play an important role in the high respiratory processes. Therefore, good storage conditions are required to prevent seed deterioration thus increasing the storage life of the seed and maintaining good seed viability.

This study was conducted to determine the pathogenicity of various *Aspergillus* isolates to seeds of mungbean under a short duration of storage.

MATERIALS AND METHODS

Source of seed

Mungbean seed (*Vigna radiata* (L) Wilczek) of local variety was used for the study. The seed had a germination percentage of 95%, and moisture content of 8.0%.

Source of inoculum

Four species of Aspergilli were used in the study, viz, *Aspergillus flavus, A. fumigatus, A. niger* and *A. parasiticus. A. flavus* and *A. niger* were isolated from padi seeds and *A. parasiticus* was obtained from the Faculty of Veterinary Medicine (an isolate from stored feed stuff). The inoculum was prepared by growing the isolates on Czapeks agar for two weeks at room temperature. Seeds were inoculated with the spores of the test fungi by rolling the seeds on a two-week old culture (Tervet, 1944; Saharan and Gupta, 1973). The seeds were inoculated with individual species of Aspergillus. Inoculated and uninoculated seeds were stored at 30%, 50% and 95% RH in kilner jars for a period of 18 weeks (Wexler and Brombaker, 1951). Sampling for germination, mold invasion and moisture content was done every three weeks.

Determination of fungus invasion

The percentage of seeds infected with the various isolates of Aspergillus was determined after surface disinfection with 1% solution of sodium hypochlorite for 10 minutes (Fields and King, 1962). The seeds were then plated on malt-salt agar containing 5.0% sodium chloride, 2.0% malt extract and 2.0% agar (Christensen, 1957); W. Zainun Nik and Parbery, 1977). The seeds were incubated at room temperature for eight days and fungi that grew out of the seeds were identified.

Seed Germination

The percentage germination of the seed was determined by growing the seed on moist paper towels in germination boxes measuring 15 x 15 cm. A total of 100 seeds in four replicates of 25 each were used. The seeds were not surface sterilised before germination. The boxes were left at room temperature for a week. Germination was considered normal if the radicle and epicotyl were well developed (Field and King, 1982).

Determination of moisture content

The moisture content of the seed was determined by the ovem method on a wet weight basis. The seeds were dried at 105°C for 16 hours (Anonymous, 1966).

RESULTS AND DISCUSSIONS

Aspergillus infection of mungbean seeds stored for 18 weeks at 20°C and 30%, 50% & 95% RH are given in figures 2, 4 and 6. Infection of *A. flavus, A. fumigatus* and *A parasiticus* was greater than 80% after 18 weeks of storage at 95% RH. However, the infection due to *A. niger* at 95% RH after 18 weeks of storage was onlu 20%. Tervet (1944) found that *A. niger* when inoculated on soybean seeds produced normal seedlings in contrast to *A. flavus.* Maximum infection of 90% by *A. niger* was obtained after nine weeks of storage, after which there was a gradual reduction

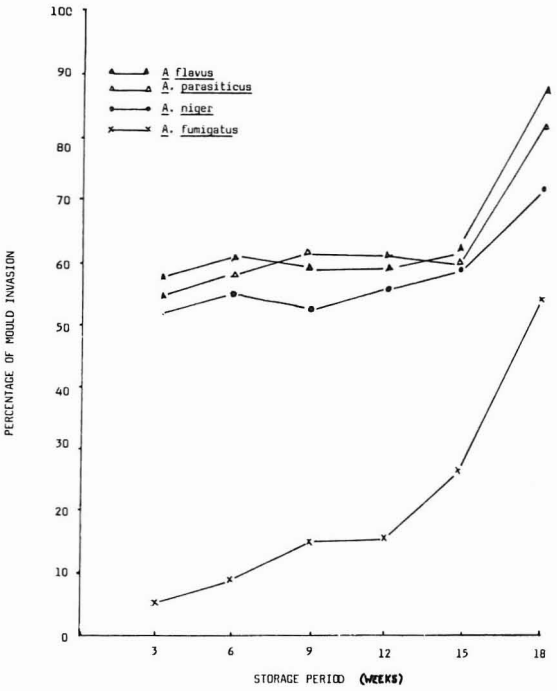

Fig. 2. The effect of Aspergillus *spp on mould invasion of* Vigna radiata *seed stored at 20 ± 2° C and at 30% RH.*

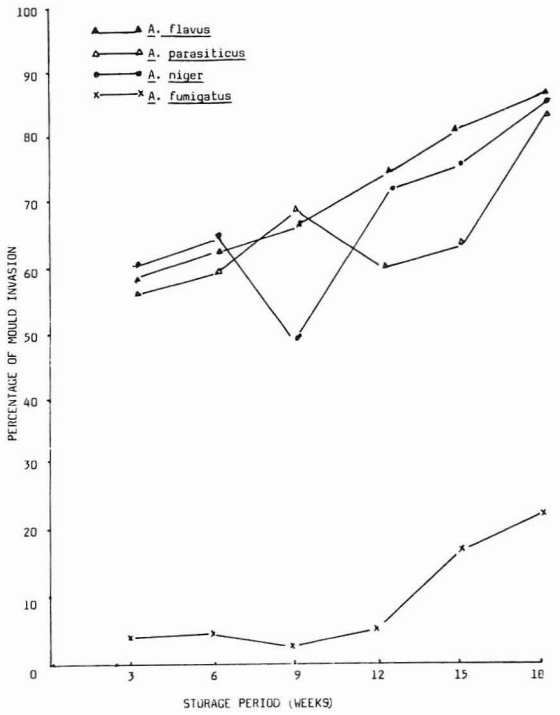

Fig. 4. The effect of Aspergillus *spp on mould invasion of* Vigna radiata *seed stored at 20 ± 2°C and at 50% RH.*

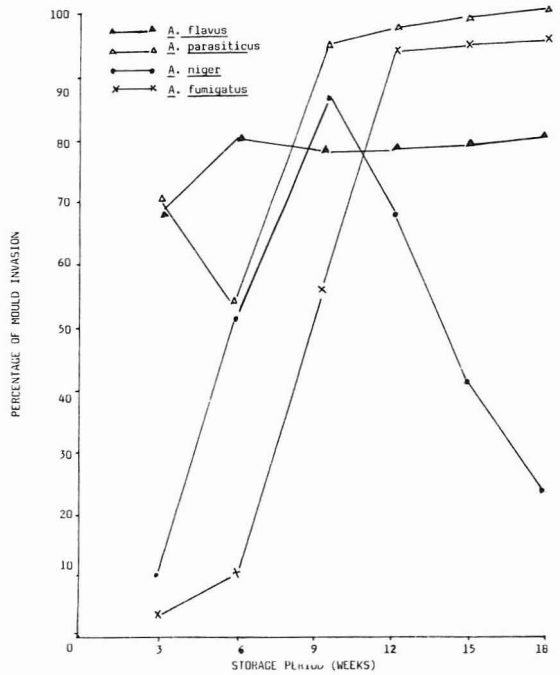

Fig. 6. The effect of Aspergillus *spp on mould invasion of* Vigna radiata *seed stored at 20 ± 2°C and at 95% RH.*

up to 18th week of storage (*Fig. 6*). A gradual reduction in the infection of *A. niger* after 9th week of storage at 95% RH could be due to bacterial antagonism. Christensen (1974) stated that bacteria can grow at moisture content in equilibrium with RH of 100%. This is achieved after the fungi have raised the temperature of spoiling grain to about 55°C and in the process have raised the moisture content to the point where water is available to bacteria. The moisture contents of seeds throughout the storage period at various RH are given in Table 1. Moisture contents of seed stored at 30% RH were slightly lower at the 18th week of storage while those seed samples stored under 50% RH maintained a more constant moisture content. However, there was an increase in moisture contents of seed stored at 95% starting from the 3rd week up to the 18th week of storage. This was to be expected since the seeds were achieving their equilibrium moisture content with the surrounding. The moisture content of Ayerst (1969) gave 78% RH as the absolute lower limit that permits germination of spores of *A. flavus* and that RH of 95% was required for germination.

Lopez and Christensen (1967) believed that members of the *A. flavus* group require a moisture content of 18.0 – 18.5% in equilibrium with RH of 83 – 85% to invade starchy cereal grains such as maize. Tervet (1944) showed that soybean seeds with a high moisture content, 13 percent and above, become mouldy when stored at room temperature and many seeds are infected with *Aspergillus* spp. This study showed that mungbean seeds with 8% moisture content also become mouldy even at RH of 30%. The infection by *A. flavus* was at its maximum at 30% and 50% RH after 18 weeks of storage. The infection by *A. parasiticus* was highest at 95% RH at the 18th week of storage. However, at the 6th week of storage the infection percentage was just above 50%.

Effect of Aspergillus species on germination and infection.

The effect of seed infection by *Aspergillus* species on subsequent seed germination are given in Figures 1, 3 and 5 and in Table 2. Significant differences in the germination of the control seeds compared to the inoculated seeds are shown in Table 2. Loss in seed germination in this case may not be due to *Aspergillus* infection alone but may be affected by storage at different RH which can also result in differential loss in seed germination (Table 2 and Fig. 5). At 95% RH and at 18 weeks of storage, significant differences in seed germination between the *Aspergillus* – treated seeds and the control were obtained. For instance, seeds which were treated with *Aspergillus parasiticus* gave

TABLE 2
Percentage germination of mungbean seed inoculated with four species of Aspergilli and stored at three relative humidities

	3 wks	6 wks	9 wks	12 wks	15 wks	18 wks
30% RH						
A. flavus	86	86	87	87	89	94
A. niger	83	87	81	81	83	91
A. fumigatus	84	91	87	86	87	96
A. parasiticus	86	87	88	80	76	96
Control	83	88	88	90	86	96
LSD 5%	0.70	1.26	0.58	1.14	0.49	0.61
LSD 1%	1.03	1.86	0.85	1.68	0.72	1.58
50% RH						
A. flavus	93	93	90	91	89	99
A. niger	96	90	93	90	95	93
A. fumigatus	93	90	92	93	89	98
A. parasiticus	90	90	94	94	93	99
Control	96	97	90	90	87	96
LSD 5%	1.54	0.91	1.34	1.45	1.03	1.06
LSD 1%	2.27	2.14	2.14	2.14	1.52	1.58
95% RH						
A. flavus	95	90	94	96	50	29
A. niger	96	86	93	95	65	31
A. fumigatus	94	92	95	83	48	16
A. parasiticus	94	93	93	96	35	8
Control	95	95	98	93	72	48
LSD 5%	1.05	1.414	1.03	1.50	1.47	17.72
LSD 1%	1.55	2.092	1.52	2.22	2.17	26.22

for the control and the Aspergilli inoculated seed. However, highly significant decrease in seed germination between the control and the Aspergilli treated seeds was obtained for the seeds stored at 95% RH after the 12th week of storage (Table 2). This shows that all the four species of Aspergilli contribute to the loss in germination of soybean seeds during storage at high relative humidities after a certain duration of storage.

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