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ABSTRACT

INFLUENCE OF RELATIVE HUMIDITY ON IRRADIATED AND UNIRRADIATED *ASPERGILLUS FLAVUS* INFECTED ON GROUND NUTMEG. Influence of relative humidity (RH) on irradiated and unilradiated *Aspergillus flavus* infected on ground nutmeg was carried out. Humidity was adjusted to 97, 92, 85, 75, 63, and 56% RH. Doses employed were 0 and 5 kGy. The initial moisture content of ground nutmeg was 7.0%. The moisture content increased under RH more than 63% so that the growth of natural mold contamination might occur. Nutmeg can be stored safety under 63% RH. Moisture content of ground nutmeg corresponded with the a_W, under RH 97; 92; 85; 75; 63; and 56% the respective a_W was 0.97; 0.92; 0.81; 0.70; 0.56; and 0.46. *A.flavus* grow under RH 92 and 97% or a_W 0.92 and 0.97 but did not grow under RH 85% , or a_W 0.81. *A.flavus* started growing at 3 days and 10 days observations under a_W of 0.97 and 0.92, respectively. *A.flavus* colony grew larger on the substrates under a_W 0.97 than those grew under a_W 0.92. A dose of 5 kGy was effective enough to inhibit *A.flavus* growth under all RH investigated.

ABSTRAK

PENGARUH KELEMBABAN PADA PERTUMBUHAN *ASPERGILLUS FLAVUS* YANG DIIRADIASI DAN YANG TIDAK DIIRADIASI PADA BUBUK BIJI PALA. Telah dilalukan penelitian pengaruh kelembaban pada pertumbuhan *Aspergillus flavus* pada bubuk biji pala yang diiradiasi dan yang tidak diiradiasi. Kelembaban diatur sedemikian rupa sehingga diperoleh kelembaban (RH) 97, 92, 85, 75, 63, dan 56%. Dosis yang dipergunakan ialah 0 dan 5 kGy. Kadar air awal bubuk biji pala sebesar 7%. Kadar air akan naik pada RH di atas 63% sehingga dapat terjadi pertumbuhan kapang alami. Penyimpanan biji pala pada RH 63% cukup aman. Kadar air biji pala berhubungan dengan a_W. Pada RH 97, 92, 85, 75, 63, dan 56% dengan a_W berturut-turut 0,97; 0,92; 0,81; 0,70; 0,56; dan 0,46. *A.flavus* tumbuh pada RH 92 dan 97% atau a_W 0,92 dan 0,97 tetapi tidak dapat tumbuh pada RH 85% atau a_W 0,81. *A.flavus* mulai tumbuh pada hari ketiga pada biji pala dengan a_W 0,97 dan pada hari kesepuluh pada substrat dengan a_W 0,92. Koloni *A.flavus* tumbuh lebih besar pada a_W 0,97 di-

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bandingkan dengan pada a_w 0,92. Dosis 5 kGy cukup efektif untuk mencegah pertumbuhan *A.flavus* pada semua kondisi kelembaban.

INTRODUCTION

Since dried nutmegs are hygroscopic and tend to adjust to ambient relative humidities it is possible that these nuts absorb or desorb moisture at high or low relative humidity during long distant shipment. Under high ambient relative humidities the moisture content of nutmeg increases so that the incidence of mouldiness tends to be high.

SAPUTRA et al.(1) reported that *Aspergillus* spp was found on nutmeg. Considerable attention should be given to such mould because one of its species, *A.flavus* can produce potent carcinogenic aflatoxin (2).

The growth of *A.flavus* on a substrate is more closely related to water activity (a_w) than to its total moisture content (3). HOLMQUIST et al.(4) reported that maximal growth of *A.flavus* occured at 33°C, pH 5.0 and a_w of 0.99, and slight growth was obserced at an a_w of 0.85 at 27°C and 33°C. Furthermore, FRAZIER (5) reviewed that a_w has to be below 0.85 to inhibit the growth of *Aspergillus* spp.

The use of irradiation to reduce mould count on spices with a dose of 5 kGy is quite promising (1). Moreover, irradiation can be used to inhibit the growth of *A.flavus* since such mould is moderately sensitive to gamma energy (6). Combined treatments of heat and irradiation have synergistic effect in inactivating in vitro *A. flavus* spores (7) and on cocoa beans (8).

No report is available either on the effect of irradiation or

on the effect of relative humidities on the growth of *A.flavus* in ground nutmeg. This work was undertaken to investigate the potential use of radiation for decontaminating *A.flavus* on ground nutmeg under different relative humidities.

MATERIALS AND METHODS

Materials. Locally purchased nutmeg were ground into powder and irradiated with 5.0 kGy for decontamination. Distilled water and the following saturated salt solutions give the following relative humidities at 29 $\pm$ 1^{o}C, distilled water : 97% , sodium tartrate : 92 %, potassium chloride : 85%, sodium chloride : 75%, sodium nitrite: 63% and sodium bromide : 56%.

Organisms and Inocula. The strain studied was *A.flavus* obtained from Bandung Institute of Technology, Bandung , Indonesia. Inocula were obtained by adding 5.0 ml of sterile 0.1% solution of tween 80 to 7 days old slants of Difco potato dektrose agar (PDA) and dislodging conidia with a sterile loop under aseptic condition. The suspensions obtained were diluted as necessary with the same solution and plated on PDA to determine the concentration of the stock solution. The concentration used was 1.6×10^{9} per ml. Stock suspension was diluted to prepare inocule.

Inoculation. Plastic dishes of 11.7 mm high and 38.2 mm diameter were filled mouthful with ground nutmeg and covered with a sheet of parafilm. Conidia suspensions, as much as 0.1 ml were dropped om the centre of the sample using sterile disposable syringe. The inoculum

size was 1.6×10^8 per ml. So that each sample consists of $10^6 - 10^7$ conidia. The inoculated substrate were irradiated with doses of 0 and 5 kGy and incubated under different relative humidities (RH).

Equilibration at Contant RH. Saturated salt solutions were placed on the bottom of the dessicators used. Equilibration was done by placing the inoculated and uninoculated dishes on the shelf above the solution. The uninoculated dishes were incubated for 30 days.The dishes were weighed at 48 hr intervals or more and the moisture contents were determined. Equilibration was considered completed when two or more consecutive weighings and moisture content showed no change, and then the curve of sorption isotherm was made. The inoculated dishes were also incubated for 30 days.

Growth of A.flavus Inocula. Growth rate was determined by measuring the diameter of *A.flavus* colony on the surface of ground nutmeg substrates at 48 hr intervals up to 30 days. Should there be no growth of *A.flavus* inoculum the dishes were kept longer i.e. up to 90 days to make sure the observation.

Moisture Determination. Both equilibration moisture content and a_w of the samples were measured with toluene distillation method and Backman Electric Hygroline Recorder, respectively.

Irradiation. Irradiation were done in a cobalt-60 Gamma Cell 220 the dose rate determined by using a Fricke dosimeter was 1.01 kGy per hour.

RESULTS AND DISCUSSIONS

The effect on RH on the moisture content in the ground nutmeg observed during storage up to 30 days is shown in Figure 1. It was noted that the lower the RH the easier the substrate to adjust to the equilibrium moisture, and the higher the RH the more time needed for adjusment.One of the factors that influence the equilibrium moisture is ambient temperatures (4). The surrounding temperatures were $29 \pm 1^{o}C$.

The initial moisture contents of ground nutmeg were $(7.0 \pm 0.1)\%$. An upward trend in the moisture content was observed only above (63 ± 1) RH. The moisture content was constant at $(63 \pm 1)\%$ RH and desorption occured at $(55 \pm 1)\%$ RH. Results of the growth of *A.flavus* inoculated on unirradiated ground nutmeg and incubated under different relative humidities at $29 \pm 1^{o}C$ showed that there was no growth under $(63 \pm 1)\%$ RH. The observation also indicated that a_w of (0.6 ± 0.01) corresponded to moisture content of $(7 \pm 0.1)\%$ when they were stored at $(63 \pm 1)\%$ RH. Figure 2 represent a water sorption isotherm of ground nutmeg after being equilibrated for 30 days at $29 \pm 1^{o}C$, and shows that a_w should be below 0.6 to prevent natural mould growth, which means that ground nutmeg with water content of 7.0% can be stored safely under 63% RH.

The results also show that irradiation dose of 5 kGy inhibited *A.flavus* growth on the substrate under all levels of RH. The absence of *A.flavus* growth was related to the absence of viable spore populations. SHARMA et al.(5) determined D_{10} of *A. flavus* to be 0.4 kGy so with initial contamination of 10^6 there would be no more surviving

spores, on the irradiated substrates.

A.flavus grew on unirradiated ground nutmeg under 97 and 92% RH, but did not grow under 85% RH. The growth was depended on the a_w of the substrates. Under (97 ± 1), (92 ± 1), (85 ± 2), (75 ± 2), (63 ± 1), and (56 ± 1)% RH the respective a_w were (0.97 ± 0.01); (0.92 ± 0.01) ; (0.81 ± 0.04) ; (0.70 ± 0.05) ; (0.56 ± 0.01) ; and (0.46 ± 0.01). Figure 3 shows the growth of *A.flavus* under different a_w's i.e. 0.97 and 0.92 or RH's of 97% and 92%.

A.flavus started growing at 3 and 10 days of observations under a_w of 0.97 and 0.92 respectively. It may be correct to note that a decrease in the a_w results in an increase in the duration of the lag phase. However, such a statement needs further investigation.

The colony diameters taken on the last day of incubation indicated thet culture in the dishes having 0.97 a_w reached larger extent of growth than those in the dishes having 0.92 a_w. It could therefore be interred that the total growth was depended of the a_w.

The fact that there was no *A.flavus* growth under an a_w of 0.81 supports FRAZIER statement (2), however, it is slighty different from TROLLER'S review (7) who ranged minimum a_w for *A.flavus* growth between 0.78 - 0.80. This difference in the growth ability may be caused by the difference in the substrate itself.

CONCLUSION

From the data collected in these experiments two conclusions can be drawn concerning the growth of *A.flavus* during storage :

1. *Aspergillus flavus* cannot grow on ground nutmeg under 85% RH , or a_w 0.81.

2. Irradiation dose of 5.0 kGy can inhibit *A. flavus* growth, with initial contamination about 10^6, on ground nutmeg under different RH's.

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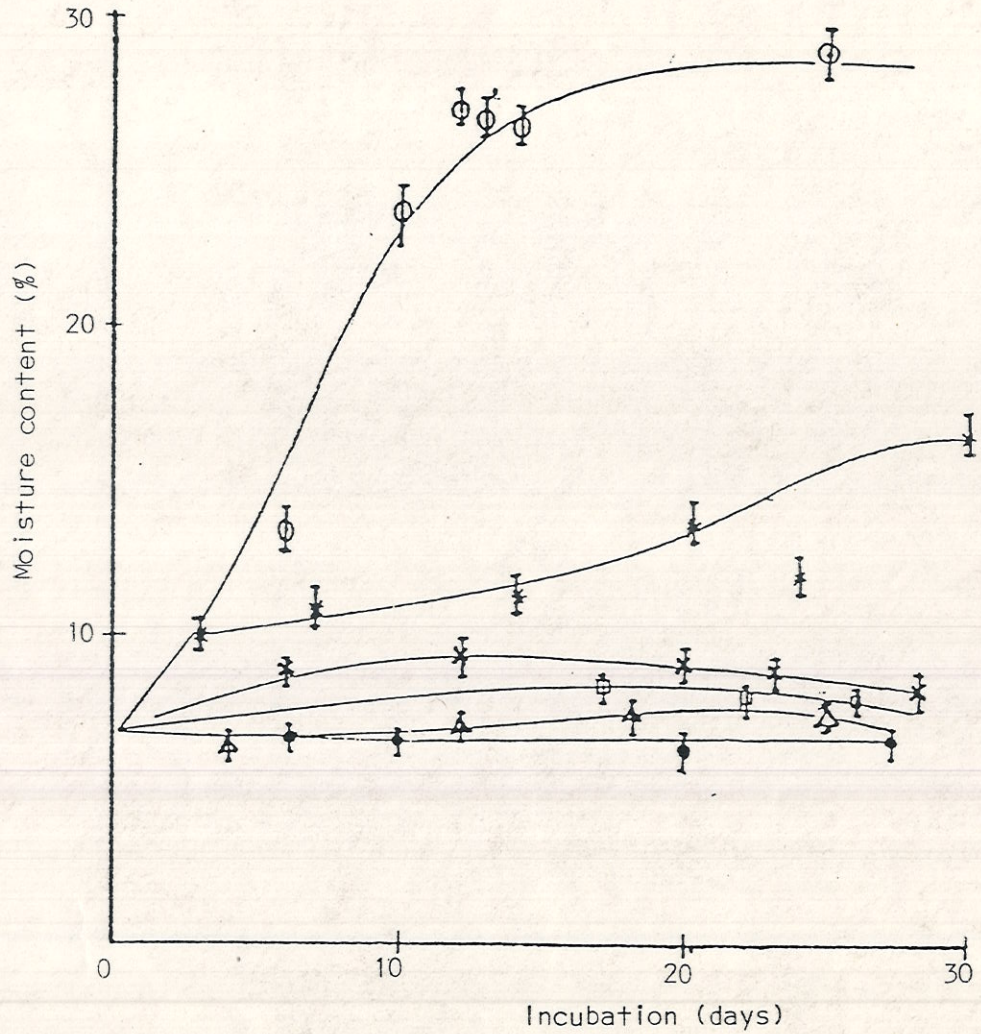

Fig. 1. Relationship between moisture content and storage time.

0 97% ; 92% ; x 85% ; 75% ; Δ 63% ; o 56%.

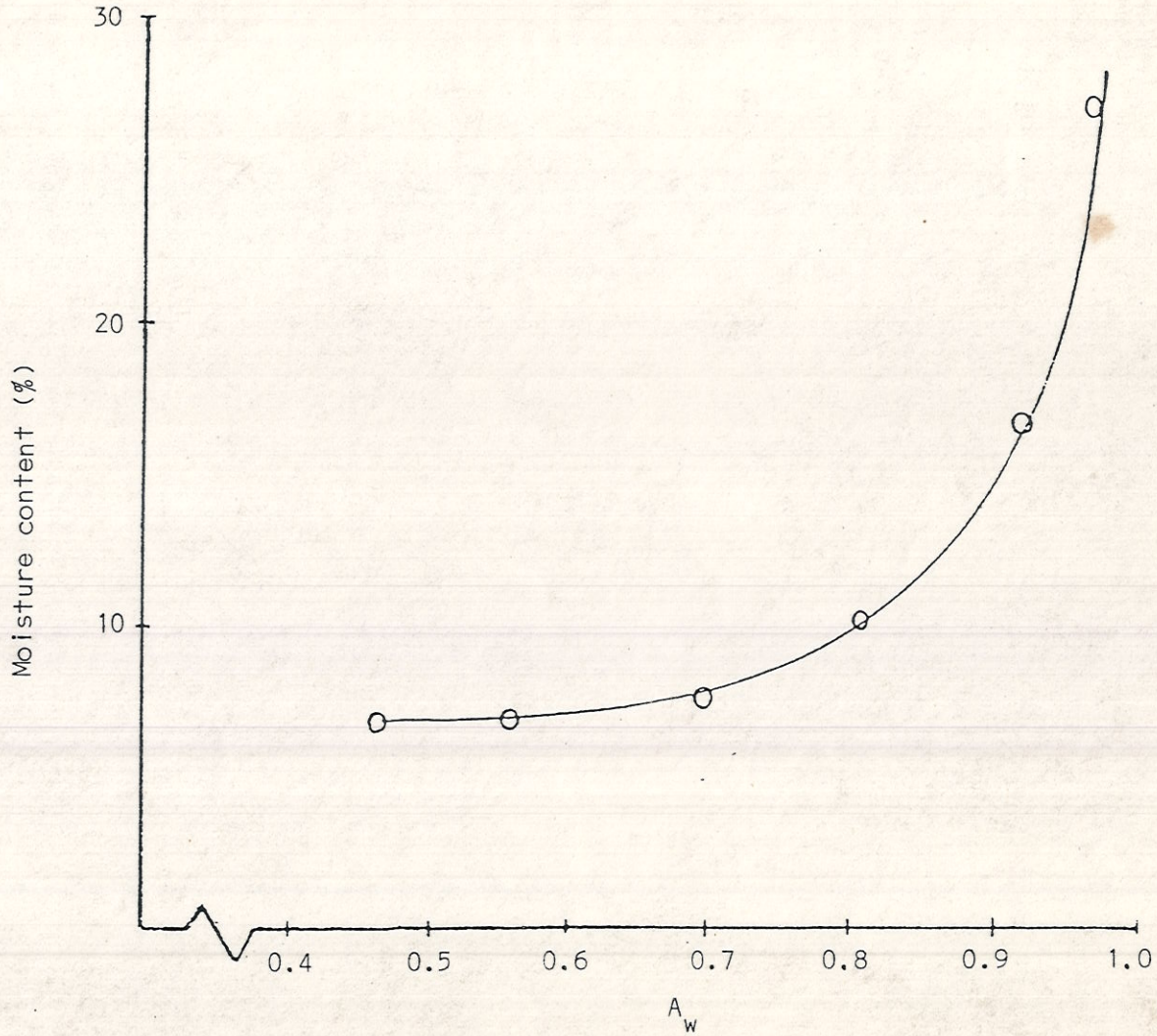

Fig. 2. Sorption isotherm of ground nutmeg after being equilibrated for about 30 days at 29 $\pm$ 1°C.

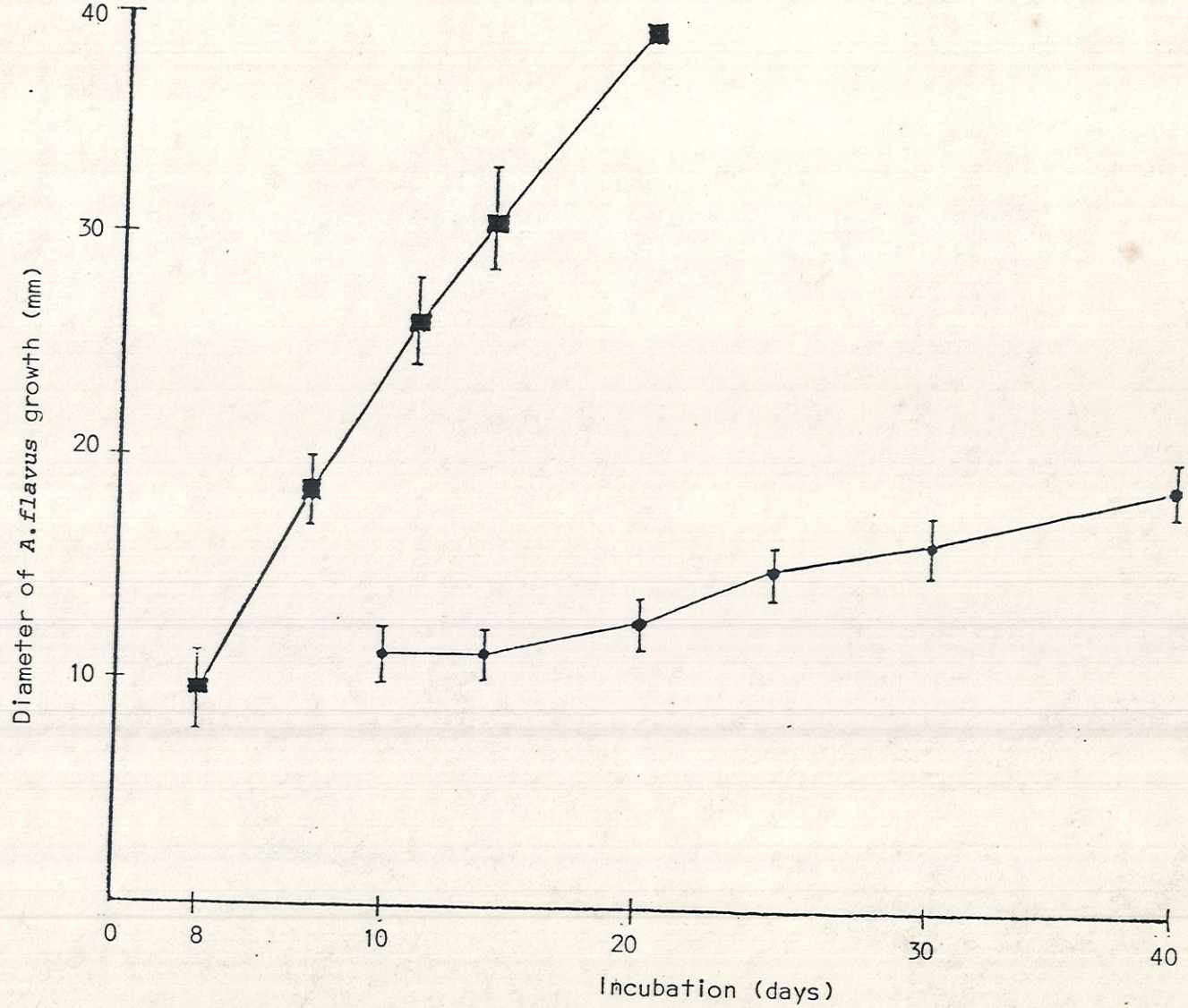

Fig. 3. Growth of *A.flavus* on gorund nutmeg.

Unirradiated, 0.97 a_w ; o Unirradiated, 0.92 a_w.