

## Original Research Article

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## Isolation of *Aspergillus parasiticus* and Detection of Aflatoxin B1 on Local Peanut (*Arachis hypogaea* L.) Varieties in Bali

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### ABSTRACT

This study was aimed to determine the contamination of *Aspergillus parasiticus* and aflatoxin B1 on Local varieties of peanuts in Bali. A total of 18 Local varieties of peanut were taken at several farmers in the field on each district in Bali. This study used descriptive analysis that showed in the form of figure and table. Samples were isolated using PDA (Potato Dextro Agar) during the incubation period 5 days (120 hours) at room temperature (29 °C) and further purified by the media DRBC (Dichloran Rose Bengal Chloramphenicol) and Aflatoxin B1 was analyzed using the Enzyme Linked Immunosorbent Assay (ELISA) [Chinaphuti, 2003]. The results showed *A. parasiticus* contaminated Local varieties of peanuts are the lowest in Bali on the sample Amlapura Local 1, Gianyar Local 1, Mangupura Local 1 and Tabanan Local 1 were  $1.0 \times 10^4$  cfu ml<sup>-1</sup>, and the highest population present in the sample Singaraja Local 1 was  $1.0 \times 10^5$  cfu ml<sup>-1</sup>. Aflatoxin B1 content of the peanut contaminating Local varieties ranged from  $2.4 \pm 0.57$  ppb – 100 ppb. Local varieties of peanuts for consumption according to Codex standards maximal 15 ppb is 94.4 % (ranged from 0-15 ppb)

#### Keywords

Local peanuts, *A. parasiticus*, Aflatoxin B1

#### Article Info

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### Introduction

In the early 1960s, many laboratories focused their attention on an outbreak of hepatotoxic disease in turkeys and other poultry (turkey X disease), a problem affecting animals worldwide (Anonymous, 2004).

Turkey X disease was attributed to contamination of peanut meal with aflatoxins, toxic metabolites produced by some strains of *Aspergillus flavus*, *A. parasiticus*, and *A. nomius* (Boutrip, 1977; Chinaphuti, 2003). Strains of *A. flavus* and *A. parasiticus* can produce aflatoxins B1, B2, G1, G2 and M

(Cullen and Newberne, 1994), contaminating a number of agricultural products such as peanuts, corn, and cereal grains (Dharmaputra, 1999). Aflatoxin contamination occurs by colonization of the fungus on susceptible crops, or may arise during harvesting, drying, storage, or processing.

Peanut is a legume commodity product in Indonesia, which are mostly used for the purpose of consumption. The production of peanuts in Bali reached 11.582 tons (1). The figure shows the highest production compared to soy production reached 5.555 tons and a total of 753 tons of green beans.

The top position peanuts as a source of cash income for farmers in Indonesia (Kasno, 2005). However, the presence of impurities of boletus in peanuts cause losses not least, because the percentage of boletus are infecting peanut in Indonesia is quite high. The main species that contaminate peanut *A. flavus* and *A. parasiticus* (Pitt *et al.*, 1998), with the resulting toxin called aflatoxin.

Crops contaminate most nuts and is a major problem in the world. In Australia the major crops has been a problem in the peanut industry since 20 years ago, but has now become a major issue for the industry food safety (Johnson, 1997). Aflatoxin poisoning does not lead to acute, but in chronic liver organ disorders cause. However until now in Indonesia such issues have not been a serious attention from various parties, to control aflatoxin contamination problems and resolve them.

The presence of contamination of boletus in peanuts cause high economic losses among other occur automatic detention (imprisonment) against Indonesia in agricultural commodities in the world market, the loss of agricultural products that are quite high (cannot be consumed/sold) (Kozakiewics, 1995).

Aflatoxin contamination in peanut seed is an important issue to the quality of food around the world. The percentage of samples contaminated peanut aflatoxin and aflatoxin total deposits increased from 35% to 55%, from range 19.4-39.8 ppb become 10.1- 88.5 ppb. Two types of aflatoxin are found which are B1 and B2, which types of crops which are most frequently found is B1.

Based on the research done the isolation of *A. parasiticus* and the detection of aflatoxin B1 of local peanuts (*Arachis hypogaea* L.) varieties in Bali.

## Materials and Methods

### Sample preparation

The materials used in the study include peanut varieties obtained from local farmers in Bali, media Potato dextrose agar (PDA), media DRBC (Dichloran Rose Bengal Chloramphenicol) consisting of 10 g of glucose, 5 g of peptone, 1 g of KH<sub>2</sub>PO<sub>4</sub>, 0.05gram MgSO<sub>4</sub>7H<sub>2</sub>O, 0.05g rose Bengal, 0.01 g of chloramphenicol, 0.2 dichloran in ethanol 1 ml of sterile water, pH 5.6 (King *et al.*, 1979), Whattman No 2, methanol, alcohol 96%, Test Kit of aflatoxin B1 (Chinaphuti, 2003).

This research carried out in the laboratory of Microbiology Laboratory, Faculty of agricultural technology, Laboratory of Veterinary of Province of Bali. Sampling was done randomly at centre of peanut production, where each sample specified taken to more farmers. A total of 18 Local varieties of peanut were taken at several farmers in the field on each district in Bali. 500-1000 g of sample of peanut was taken ranges between of material chosen at random. This research used descriptive analysis that showed in the form of figure and table. The description of the 18 samples were obtained from each Center for agriculture in the 9 districts in Bali can be seen in table 1.

### Isolation and identification

*A. parasiticus* was isolated from 18 samples of peanut Varieties in 9 Local district in Bali. Peanuts are cleaned first with water, then crushed with a sterile blender. 10 g of the sample taken and entered into 90 ml of sterile water in an Erlenmeyer flask. Then as much as 1 ml inoculated in the Petri dish was filled with media PDA. Cultures incubated in room temperature for 5 days (120 hours), therefore was done identification.

The isolates *A. parasiticus* was isolated using cork borer to be inoculated in DRBC medium and then stored for 5 days (120 hours) in room temperature. Identification is done by observing isolates *A. parasiticus* in microscope

### Detection of Aflatoxin B1

20 g of samples weighed and put into blender add 100 ml of methanol 70 on a sample, then the most high speed blender for 2-3 minutes. After samples destroyed filtered using filter paper Whatman No. 4 and strain samples to extract a clear look. The filtrate is accommodated as many as 1.0 ml with microtubule and added 1 ml of sterile water.

A standard solution of aflatoxin (0; 0.2; 0.5; 1, 2 and 5 (ng/ml)) and a sample of 50 µl put into the micro wells, incorporated into the respective wells. Wells that already contain standard and sample each added enzyme conjugate to each well 50 µl and wells incubated for 30 min at room temperature and dark spaces. The liquid in the micro wells subsequently disposed outside on tissue paper clean and washed with sterile water. The work was repeated four times.

A substrate contains 3.3 ' 5, 5 ' - Tetramethylbenzidine 0.4 g/l in alkaline organic and substrate B contains 0.02 H<sub>2</sub>O<sub>2</sub> in citric acid buffer. Substrates A and B mixed 30 minutes earlier. Later added as much as 100 µl into wells is blended well and incubated for 5 minutes at room temperature and dark spaces.

The solution contains 0.01 M stop reaction of phosphate acid as much as 100 µl added into respective wells. Further reading absorbance on the activities of the micro wells by ELISA Reader at a wavelength of 450 nm. OW to calculate number of aflatoxin in peanuts is

$$\% \text{ absorbansi} = \frac{B}{B0} \times 100$$

Description

B: the value of absorbance of each sample  
B0 standard: the value of absorbance in 0

A Total of aflatoxin contained in each sample can be calculated using paper millimeters. Where X is a line of standard aflatoxin B1 concentration while the maximum absorbance indicates each Y sample (Chinaphuti, 2003).

### Results and Discussion

#### Characteristic of *A. parasiticus*

The research was conducted on 18 samples of local peanut varieties obtained from each district in Bali. Isolation of *A. parasiticus* using media DRBC (Dichloran Rose Bengal Chloramphenicol) and medium PDA (potato dextro to) local varieties of peanuts during the incubation period of 5 days can be seen in Figure 1.

Figure 1 can be seen *A. parasiticus* color green darkness colonies commonly called ivy green in colour (King *et al.*, 1979). *A. parasiticus* can germinate on surface wounds from crops (corn and beans). Then penetrate against the growth of the embryo. Observations with the microscope showed morphological forms of *A. parasiticus* as in Figure 2.

Description:

A = mold *Aspergillus parasiticus*: stigmata

B = vesicles of the mold *Aspergillus parasiticus*

C = mold *Aspergillus parasiticus konidia*

D = konidiofor mold *Aspergillus parasiticus*

According to (Pitt and Hocking, 1997), *A. parasiticus* has conidiophore that emerges from the surface of the Harare International Festival of the -500 length 250  $\mu\text{m}$ , colorless or light brown and smooth-walled; spherical vesicles with diameter 20  $\mu\text{m}$  and -35 only 3/4 of the surface of the vesicles in the fertile (fertile); vesicles form the phialide-11  $\mu\text{m}$  long, 7 conidia producing spherical, usually 4-6  $\mu\text{m}$  in diameter, rough-shaped cell walls and usually forms the head of the conidia of the round (King *et al.*, 1979; Pitt and Hocking, 1997). *A. parasiticus* is growing at between 12-40 ° C with an optimum growth temperature of 30 ° C; the value of aw minimum for growth was 0.82 at a temperature of 25 ° C, 0.81 at a temperature 30 ° C and at a temperature of 37 ° 0.80 C and pH between 1.5-10.5 on the temperature of the third (Pitt and Hocking, 1997).

### **Population of *A. parasiticus***

Research results of the 18 samples of local peanut varieties obtained from local farmers in each district in Bali, showed a variation of *A. parasiticus* on local varieties of peanuts. In Table 3 can be seen the population of *A. parasiticus*, which contaminate local peanut varieties.

The Table 3 can be seen that the population of *A. parasiticus*, which contaminate local peanut varieties was vary. The smallest population is  $10 \times 10^3 \text{ cfu.ml}^{-1}$  -  $10.2 \times 10^4 \text{ cfu.ml}^{-1}$ . The population of *A. parasiticus* is lowest in samples of AL1, GL1, ML1 and TL1 was  $10 \times 10^3 \text{ cfu.ml}^{-1}$ . While the highest populations are present in a sample of SL2 are  $10.2 \times 10^4 \text{ cfu.ml}^{-1}$ . This is caused by *A. parasiticus* growth ability on optimum temperature 32 ° C growth; the value of the minimum for the growth is the aw 0.82 at a temperature of 25 ° C; 0.81 at a temperature of 30 ° C and at a

temperature of 37 ° 0.80 C and pH between 1.5-10.5 on the temperature of the third (King *et al.*, 1979; Pitt and Hocking, 1997). According to (King *et al.*, 1979; Kuswantoro and Sudarmadji, 1988). Since in the soil, during drying and storage can be covered by the mold *A. parasiticus* and *A. flavus*. Suitable Habitat for the growth of the mold is the moisture content of 14-30 and a temperature between 21-37°C. In the closed storage and aeration conditions less encourage mold growth.

### **Aflatoksin B1 content of peanut**

Results of the analysis of the content of aflatoxin B1 on the 18 samples of local peanut varieties taken of farmers in each district in Bali can be seen in Table 4.

In the Table 4 can be seen that there are variations in contamination AFB1 of local peanut varieties. Contamination AFB1 in local peanut varieties ranges from 1.5 – 14 ppb ppb, while samples of the DL2 in excess of 100 ppb

Variation of aflatoxin content in peanuts can be caused by several factors, namely crops can be produced by *A. parasiticus* at between 12-40 ° C, the water activity (Aw) 0.86 and pH 3-8 (13). According to (Jay, 2000), formation of aflatoxin pad peanuts occurred at optimum 0.93-0.98 aw and RH 83% or higher at a temperature of 30 ° c. the ability of mold to form and store crops depends on several factors including genetic potential mold requirements-environmental requirements (substrate, temperature, humidity, and pH) and duration of contact between the mould and the substrate.

Aflatoxin production is strongly influenced by the presence of mold, interaction between the substrate and the environmental conditions (Saad, 2001; Duniaji, 2009).

**Table.1** Providing the code in samples obtained from each district in Bali

Sample	Sample at Distric of Bali
SL1	Farmer at Singaraja 1
AL1	Farmer at Amlapura 1
KL1	Farmer at Klungkung 1
GL1	Farmer at Gianyar 1
BL1	Farmer at Bangli 1
DL1	Farmersat Denpasar 1
ML1	Farmersat Mangunpura 1
TL1	Farmer at Tabanan 1
NL1	Farmer at Negara 1
Sample	Sample at Distric of Bali
SL2	Farmer at Singaraja 2
AL2	Farmer at Amlapura 2
KL2	Farmer at Klungkung 2
GL2	Farmer at Gianyar 2
BL2	Farmer at Bangli 2
DL2	Farmer at Denpasar 2
ML2	Farmer at Mangunpura 2
TL2	Farmer at Tabanan 2
NL2	Farmer at Negara 2

**Table.2** Population of *A. parasiticus* that contaminated local peanuts varieties in Bali (cfu.ml<sup>-1</sup>)

Sample	<i>A. parasiticus</i> (cfu.ml <sup>-1</sup> )	Other fungi	Sampel	<i>A. parasiticus</i> (cfu.ml <sup>-1</sup> )	Other fungi
SL1	50 x 10 <sup>3</sup>	84 x 10 <sup>2</sup>	SL2	10,2 x 10 <sup>4</sup>	58 x 10 <sup>2</sup>
AL1	10 x 10 <sup>3</sup>	20 x 10 <sup>3</sup>	AL2	-	40 x 10 <sup>3</sup>
KL1	25 x 10 <sup>2</sup>	95 x 10 <sup>2</sup>	KL2	20 x 10 <sup>3</sup>	20 x 10 <sup>2</sup>
GL1	10 x 10 <sup>3</sup>	90 x 10 <sup>3</sup>	GL2	20 x 10 <sup>3</sup>	90 x 10 <sup>3</sup>
BL1	50 x 10 <sup>3</sup>	28 x 10 <sup>2</sup>	BL2	10 x 10 <sup>2</sup>	37 x 10 <sup>2</sup>
DL1	-	30 x 10 <sup>3</sup>	DL2	20 x 10 <sup>3</sup>	90 x 10 <sup>3</sup>
ML1	10 x 10 <sup>3</sup>	10 x 10 <sup>3</sup>	ML2	40 x 10 <sup>3</sup>	17 x 10 <sup>2</sup>
TL1	10 x 10 <sup>3</sup>	40 x 10 <sup>3</sup>	TL2	40 x 10 <sup>3</sup>	90 x 10 <sup>3</sup>
NL1	90 x 10 <sup>3</sup>	30 x 10 <sup>3</sup>	NL2	12 x 10 <sup>2</sup>	40 x 10 <sup>3</sup>

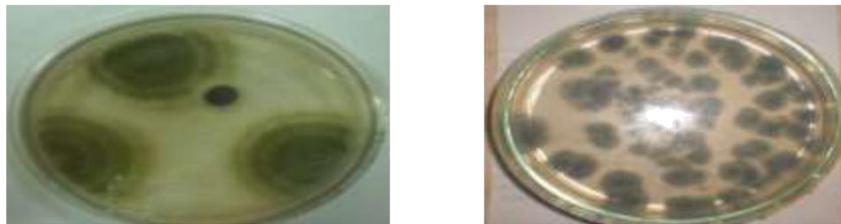
**Table.3** Aflatoxin B1 content in peanut of local varieties in Bali

Sample	Aflatoxin B1 (ppb)	Sample	Aflatoxin B1 (ppb)
SL1	10.4 ± 0.14	SL2	4.8 ± 0.57
AL1	10.8 ± 0.28	AL2	8.4 ± 0.28
KL1	8.2 ± 0.28	KL2	4.8 ± 0.28
GL1	8.0 ± 0.28	GL2	7.4 ± 0.57
BL1	9.2 ± 0.57	BL2	6.6 ± 0.28
DL1	4.0 ± 0.28	DL2	100
ML1	8.4 ± 0.28	ML2	14.0 ± 0.28
TL1	3.6 ± 0.28	TL2	5.6 ± 0.28
NL1	8.0 ± 0.57	NL2	2.4 ± 0.57

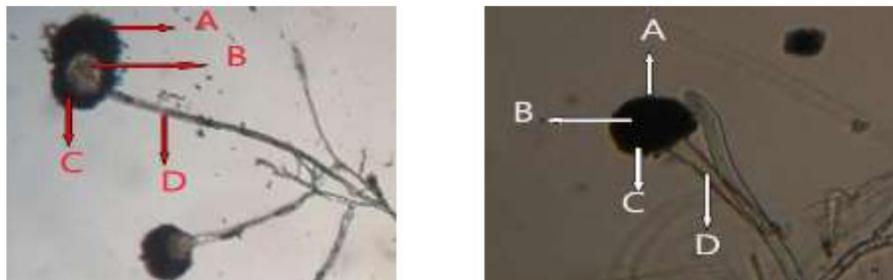
**Table.4** The proportion of AFB1 on local varieties of peanut

Content of AFB1(ppb)	Proportion (%)
0-5	27,78
5-10	44,44
10-15	22,22
100	5,56

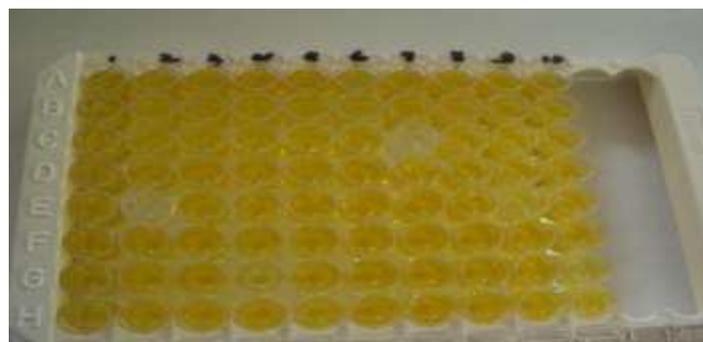
**Fig.1** *A. parasiticus* in media the DRBC incubated for 5 days (120 hours) at room temperature



**Fig.2** *A. parasiticus* is observed with a microscope with a magnification of 400x



**Fig.3** Test results ELISA AFB1 in ground nuts local varieties



Aflatoxin is produced by *A. flavus* and *A. parasiticus* on groceries will be spurred its creation in the depressed mold condition (stress), due to the limited nutrition and environmental temperature is high (Boutrip, 1977; Dharmaputra *et al.*, 2005). A high

concentration of aflatoxin can also occur as a result of poor post-harvest handling especially during storage under conditions of high humidity, which also occurred in field before harvest (Bankole and Mabekoje, 2004). According to Kozakiewics (1995), Duniaji

(2009). States the drought (water stress), high temperature and insect destroyer host plants is a major factor and the production of aflatoxin mould infestations.

ELISA test results showed a discoloration of the substrate on the samples examined. Yellow color on the test substrate ELISA shows a lower content of AFB1 compared to the clear color on the ELISA test. In Figure 3 the ELISA test results can be seen in the sample the local varieties of peanuts.

Figure 3 indicates that the yellow color is obtained after using stopping reactions on substrates before reading absorbance at micro wells with an ELISA Reader at a wavelength of 450 nm. The comparison of the color yellow shows more the low content of AFB1. Instead the more nodes the higher content of AFB1.

Requirements/standards of the Codex alimentary Commission the maximum content of AFB1 in ground nuts is 15 ppb. Based on it can be noted that the proportion of local varieties of peanuts aflatoxin B1 contaminated in Bali. Can be seen in Table 5.

Table 5 can be seen that the proportion of AFB1 was found 27, 78 percent at 5 samples (DL1, TL1, SL2, KL2, NL2) range 0-5 ppb. It was found 44.44 percent of 9 samples (KL1, GL1, BL1, ML1, NL1, AL2, GL2, BL2, TL2) Ranging 5-10 ppb and 22.22 percent was found at 3 sample (SL1, AL1, ML2) range 10-15 ppb., while the range more than 100 ppb sample (DL2) is 5,56 percent and these samples was not recommended for consumable. Aflatoxin was found in the highest sample DL2 which is more than 100 ppb, whereas the lowest aflatoxin content of the sample NL2 is 4 ppb. While the standard FDA United States (USFDA) and the Food Drug Administration (FDA) maximum of AFB1 Indonesia on peanuts is 20 ppb

Sample (DL2) is unfit for consumption due to the aflatoxins content more than 100 ppb that is in excess of the standards. According to (Chinaphuti, 2003; Loosmore and Marksman, 1961; Wilson and Payne, 1994), the standards of the Codex Alimentary Commission recommends the content of aflatoxin 15 ppb (AFB1, AFB2, AFG1, AFG2) on peanuts and aflatoxin M1 0,05 ppb in the milk. According to FDA standards established Indonesia since 9 September 2004 namely the content of aflatoxin B1 and total aflatoxin in peanuts processed by 20 and 35 ppb (Busby and Wogan, 1984)

*A. parasiticus* contaminated local varieties of peanuts are the lowest in Bali on the sample Amlapura Local 1, Gianyar Local 1, Mangupura Local 1 and Tabanan Local 1 was  $1.0 \times 10^4$  cfu ml<sup>-1</sup>, and the highest population present in the sample Singaraja Local 1 is  $1.0 \times 10^5$  cfu ml<sup>-1</sup>. Aflatoxin B1 content of the peanut contaminating local peanut varieties ranged from 2.4 ppb – 14.0 ppb. Local varieties of peanuts for consumption according to Codex standards maximal 15 ppb is 94.4 %.

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