DISCUSSION

In this study, the fungal extracts (PEI and PES)were prepared by extraction of the moldy rice with chloroform and methylene chloride:methanol (97:3, v:v). The productions of both PEI and PES seemed to be very similar, but the stability of the toxic substances in these fungal extracts may be different. This was found in the case of cytochalasin E. Cytochalasin E was less stable in chloroform than methylene chloride (Glinsukon et al, 1974). Therefore, it is likely that methylene chloride may be a solvent of choice for extraction of the moldy substrates. The production of PES by various strains of A. *niger* and other species was varied a great deal. This may be associated with the individual capability in producing oil rather than the different in the solvents of extraction.

The determination of known mycotoxins produced by *Aspergillus* species such as aflatoxins, ochratoxins and sterigmatocystin is very important in a secreening program for toxigenic fungi. It was found that 6 out of 30 strains of A. *niger* produced aflatoxins B_1 and B_2. Aflatoxin B_1 contents in the PEI was rather low when compared to aflatoxin B_1 contents in the PEI produced by A. *fumigatus* in this study. However, some of A. *niger* strains isolated from market foods and foodstuffs in Bangkok are capable in producing aflatoxin B_1 similar to another part of the world (Kulik and Holiday, 1967). Unfortunately, other known mycotoxins, ochratoxins and sterigmatocystin were not determined. They may be produced by some of these fungal isolates.

Oxalate was reported to be produced by the strains of A. *niger* (Wilson, 1961). Oxalate contents found in the crude toxin of this study was rather small amounts. This might be attributed to the capability of A. *niger* in producing oxalate or oxalate was present but it was not dissolved in the solvents of extraction. The second reason seems to be the case. However, oxalate may be present in the higher amount in the moldy rice if it is determined directly by extracted with aqueous solution, or the fungi are grown on liquid media. This problem is subjected for further study in this laboratory. Therefore, it suggests that the amount of the oxalate present in the crude toxin should not be enough to cause death to the tested animals. However, this point needs further study. In the comparison of the LD_{50} of the crude toxin from A. *niger* (AN-A30-75) and potassium oxalate (Fig. 11), it was found that LD_{50} of potassium oxalate was 196.0 mg/kg BW when administered ip to weanling female rats. It needs at least 6.24 mg/rat to kill 50% of the rats. The amount of oxalate in the crude toxin produced by A. *niger* (AN-022-75) was 170 µg/100 mg. The lethal dose of the crude toxin from this strain was found at a dose of 12.0 mg/rat, which contained only 20.4 µg. This amount of oxalate 20.4 µg is far less than 6.24 mg/rat (LD_{50} dose). It, therefore, suggests that oxalate is not involved in the lethality of the rats treated with the crude toxin.

In the acute toxicity tests, the crude toxins produced by various strains of A. *niger* were very toxic to the rats as considered from the dose of 12.5 g moldy rice (38.46%) to the dose usually tested of 50.0 g moldy rice. Histopathologic changes observed in various tissues suggest that, most of these crude toxins

are nephrotoxins. This finding is similar to those found by Angsubhakorn et al, (1977). According to the degenerative lesions in the kidney of the rats treated with the crude toxins, it may further suggest that the toxic substance(s) in these crude toxins should be different in term of toxicological properties and it should be more than one toxic substance. However, it is not known at the present that some of these toxic substances may be possible new mycotoxins. It is of interest to eliminate the possibility of contamination of aflatoxins and oxalate in these crude toxins. Aflatoxin B_1 content in the crude toxins produced by A. *niger* (AN-008-75 and AN-022-75) was too small to cause death to these rats, as it is the same with oxalate mentioned above. Recently, it was reported that malformins A, B and C were produced by A. *niger* (Irichifima and Curtis, 1969; Takeuchi et al, 1967; Anderegg et al, 1976). One strain of A. *niger* that produced malformin C was isolated from cooked glutinous rice in Thailand (Anderegg et al, 1976). It is likely that some of A. *niger* strains in this study may be capable in producing malformin C. However, it is difficult to make this conclusion, because the pure toxic substance of the crude toxins were not isolated to compare the chemical structures. Histopathologic changes in the various organs cannot be used as a criteria either because no informations avaliable from the toxicity of malformins, except the LD_{50} values of malformin C were 0.90 mg/kg in newborn and 0.87 mg/kg in 28-day-old rats given ip (Kobbe et al, 1977).

The toxicity of the crude toxins produced by various strains of other species was also highly toxic. Especially the crude toxins from A. *fumigatus* (AFU-007-75, AFU-025-75, AFU-030-

-75 amd AFU-119-75) were also highly toxic. The crude toxins from AFU-007-75 had no aflatoxin B_1, so it should not be the toxicity of aflatoxin B_1 to the rats. This finding is supported by the fact that there was the kidney damage of marked dilatation of the convoluted tubules which never been seen in aflatoxin B_1 content were detected in the crude toxins produced by other strains of *A. fumigatus*. This amount of aflatoxin B_1 content can cause death to the rats. Interestingly, there was an additional necrosis of the tubular epithelial cells of kidney. It may be probably that the crude toxins contains another toxic substance(s). However, it needs further detailed investigation. Recently, Yamazaki and coworkers (1971) described two tremorgens, fumitremorgins A and B, from *A. fumigatus*. Chemical composition of these tremorgens was given as $C_{33}H_{45}O_6N_3$ and $C_{26}H_{29}O_6H_3$. Administration ip of 5 mg/mouse resulted in sustained trembling with intermittent convulsions and death for about 70% of the animals within 96 hours was observed. Therefore, it is unlikely that these tremorgens were present in the crude toxins produced by *A. fumigatus* in this study.

The acute toxicity of the crude toxin produced by *A. clavatus* (ACLA-005-75). It was very toxic at dose of 6.25 g moldy rice). It is very difficult to determine whether or not it contains a new mycotoxin because there are many mycotoxins produced *A. clavatus*. *A. clavatus* group, *A. clavatus* Desm. and *A. giganteus* produced the mycotoxin, patulin (Katzman et al, 1944; Florey et al, 1944; Broom et al, 1944; Florey et al, 1949). The LD_{50} in mice is 10-15 mg/kg BW when given sc. Histopathologic changes revealed slight congestion and tubular cells degeneration in kidney. Repeated sc injections of patulin in rats results in development of sarcromas

(Dickens and Jones, 1961). In addition, tremorgen, ascladiol and clavatol are toxic mycotoxins produced by A. *clavatus* (Blyth and Gloyd, 1971; Suzuki and coworkers, 1971; Bergel and coworkers, (1943). More recently, cytochalasin E, a highly toxic metabolite was isolated from A. *clavatus* (Originally isolated from cooked glutinous rice in Thailand) (Glinsukon et al, 1974; 1975a and b). This mycotoxin caused plasma effusion from the circulation and death was ensured by a shock. Histopathologic changes revealed necrosis of liver and kidney.

The acute toxicity of PES produced by various strains of A. *niger* and other species was also observed. The acute toxicity of PES, attributed by the same toxic compounds in the crude toxins, is not yet known. It is possible that PES and the crude toxins produced by the same fungi may possibly be the same toxic compounds. It might be because the toxic compound can partially be dissolved and precipitated in the petroleum ether during the separation of the crude toxin and PES. On the other hand, it might because there is different toxic compounds present in the crude toxin and PES. As it was found in the case of the crude toxin and PES produced by A. *candidus* (AC-023-75), in which the crude toxin caused mild necrosis of the epithelium of mostly proximal and distal tubules in the cortex of kidney PES caused severe hemorrhagic necrosis of the epithelium of most convoluted tubules in the corticomedullary zones of kidney and severe necrosis of the hepatic cells in the centrolobular zones of the liver. At the present, the nature of the toxic compound(s) in the PES is not known. This toxic compound(s) must be readily dissolved in oily residue and they might be toxic fatty acids. Recently, it has been shown that some fungi

elaborate naturally occurring fatty acids that are toxic to brine shrimp (Curtis et al, 1974).

The toxic compound isolated from the crude toxin produced by A. *niger* (AN-A30-75) was blue green fluorescent compound. This is not either aflatoxins or oxalate. When the LD_{50} values of the crude toxin and oxalate were compared. The LD_{50} of the oxalate was about 3 times less than that of the crude toxin. The crude toxin caused necrosis of the tubular epithelium of kidney, whereas no significant histopathologic changes were observed in the kidney of the rats treated with oxalate. However, it is not known whether or not it is a new toxic compound.

In summary, there are many toxigenic fungi as shown on the toxicity tests of the crude toxins and PES in weanling female rats, isolated from market foods and foodstuffs. Approximate two-third of the crude toxins produced by various strains of A. *niger* caused different patterns of kidney degeneration.