SUMMARY

PREVALENCE OF TOXIGENIC FUNGI IN COMMON MEDICINAL HERBS, SPICES AND FOOD MATERIALS

- Out of 65 medicinal herbs and spices screened, 60 were contaminated while 5 were free from fungal contamination.
- Analysis revealed that 45.31% of the samples have a fungal load above $1 \times 10^3$ cfu/g which is the permissible limit set by World Health Organization.
- A total of 191 fungi were isolated in which Aspergillus and Penicillium were the predominant microorganism. Among the various fungal isolates 21 were aflatoxin producing Aspergillus sp. and 9 citrinin producing Penicillium sp.
- On further screening for natural contamination with mycotoxins, AFB$_1$ was detected only in one sample i.e. *Arachis hypogaea* (peanut).
- Though toxigenic fungi were isolated from 33% of the samples, mycotoxin was not detected from any of the medicinal herbs.

GROWTH OF *ASPERGILLUS FLAVUS* AND AFLATOXIN B$_1$ PRODUCTION

- The growth of *Aspergillus flavus* and AFB$_1$ production at various pH showed the maximal mycelia growth at lower pH i.e. 3.0. Though fungal growth was lesser at higher pH, AFB$_1$ production at pH 6.0, 7.5 and 8.5 was higher than at pH above 3.0.
- Rice proved to be the better material for mass production of AFB$_1$. The amount of AFB$_1$ obtained from YES medium and rice was 48.25 µg and 668 µg, respectively.

EFFECT OF SPICES ON THE GROWTH OF *ASPERGILLUS FLAVUS* AND AFLATOXIN B$_1$ PRODUCTION

- Cinnamon and clove inhibited *A. flavus* growth completely at 10 mg/ml concentration, whereas cardamom and star anise did not exhibit antifungal or anti-aflatoxigenic activity in YES medium.
• The MIC of cinnamon and clove was recorded as 4 and 2 mg/ml respectively in YES medium. Concentrations of cinnamon and clove below their MIC had enhanced the fungal growth while AFB₁ production was reduced.

• The relationship between growth of A. flavus and AFB₁ production was studied in the presence of 1 mg/ml of clove. The result indicate that clove specifically inhibit AFB₁ synthesis without the concomitant inhibition of fungal growth.

• These spices exhibited anti-aflatoxigenic activity in rice although fungal growth was not inhibited. Clove and cinnamon inhibited AFB₁ synthesis significantly up to 99 and 92 % respectively while star anise and cardamom inhibited AFB₁ by 46 and 30 % respectively in rice.

• Clove not only inhibits AFB₁ production but also exhibited the potential of degrading AFB₁ directly under heat treatment.

INHIBITION MECHANISM OF AFLATOXIN B₁ PRODUCTION BY ASPERGILLUS FLAVUS

• The concentration of xanthine oxidase in aflatoxigenic Aspergillus was higher than that in non-aflatoxigenic Aspergillus.

• The activity of xanthine oxidase enzyme in toxigenic A. flavus decreased in the presence of clove.

• 1 mg/ml of clove decreased the enzyme activity by 74% and AFB₁ synthesis by 99%.

• The possible mechanism could be that clove scavenged the free radicals thereby inhibiting AFB₁ synthesis or that clove may be inhibiting the enzyme directly.

INHIBITORY EFFECT OF CLOVE ON GROWTH OF PENICILLIUM CITRINUM AND CITRININ PRODUCTION

• The MIC of clove against the growth of P. citrinum and citrinin production was recorded as 1.8 mg/ml in YES culture broth. All concentrations of clove inhibited the fungal growth; however citrinin production was inhibited significantly only at higher concentration.

• The relationship between growth of P. citrinum and citrinin production was studied in the presence of 1.6 mg/ml of clove. Clove inhibited the growth of P. citrinum in culture media.
by 60-70%, throughout the observation period of 14 days. Along with the fungal growth inhibition, citrinin production was inhibited significantly.

- In rice, clove delayed the growth of Penicillium by 3 days; however after 5 days of incubation, fungal growth and citrinin production were equivalent to the control.

DEGRADATION OF AFLAOXIN B1 USING HERBAL DRUG

- Liv-52 was used for degrading AFB1 by incubating at 37 °C for 24 and 48 h. The results showed that AFB1 degradation increased with increasing concentration of the drug. Incubation of AFB1 with 100 mg/ml of Liv-52 resulted in 50% degradation; however complete degradation was not obtained.
- The constituent of Liv-52 were further studied for degrading AFB1. Tamarix gallica and Terminalia arjuna were most effective in degrading AFB1, whereas Cichonium intybus and Solanum nigrum did not exhibit any degradation effect.
- Liv-52 did not exhibit any inhibitory effect on the growth of A. flavus and AFB1 production in YES medium. On the contrary, the presence of Liv-52 in culture medium had enhanced A. flavus growth and AFB1 production.

DECOMPOSITION AND DETOXIFICATION OF AFLATOXIN B1 BY LACTIC ACID

- Degradation of AFB1 using organic acids by heat treatment showed that lactic acid was most efficient in degrading AFB1, whereas acetic and citric acid did not show any significant degradation.
- Although complete degradation was not observed, up to 85% degradation of AFB1 was obtained when heated with 1 M lactic acid at 80 °C for 120 min.
- Under these treatment conditions with lactic acid, AFB1 was degraded into two degradation products which were identified as AFB2 and AFB2a. The formation of polylactic acid was also noted.
- The MTT cytotoxicity assay showed reduced toxicity of AFB1 after treatment with lactic acid. The degraded product, AFB2a, exhibited much reduced toxicity on HeLa cells compared to that of AFB1.