7. SUMMARY

The annual legume groundnut (*Arachis hypogea*) is the fifth most important world’s principle oilseed crop, grown in 109 countries of tropical, subtropical and warm temperate climates and is the primary edible oil source of India. Edible oils and fats are indispensable ingredients for a healthy and balanced diet and are very important stuff of bulk consumption. Groundnut oil constitutes a major component of daily diet consumption throughout India, which is obtained from the high aflatoxin risk agricultural product groundnut. Number of aflatoxicosis outbreaks reported India implicates either the raw material groundnut or its by-product groundnut cake as a causative agent. The current research was undertaken to ascertain the aflatoxicogenic contamination of groundnut oil marketed and search into the traditional food preparation for their reduction and reports the finding.

During this research, twenty different popular marketed oil and local brands were collected and were subjected to physico-chemical analysis like acid value, saponification value, iodine value and peroxide value to reveal their quality. From the collected oil samples inoculation in Sabouraud’s dextrose agar 113 isolates were isolated, of which 17 were found to be *A. flavus* and 7 were *A. niger*. These isolates were screened for their aflatoxicogenic nature using AFPA, coconut agar and Hara medium and their aflatoxin was quantitated using HPLC. The isolate *A. flavus* MTCC 10680 was found to be highly aflatoxicogenic and xerophilic in nature. Earlier the isolates were identified using morphological, cultural and molecular characterization. Their phylogenetic relationship was also determined.

Groundnut samples were artificially contaminated with the selected *A. flavus* MTCC 10680, the oil was expelled from them and used in the further toxin removal studies. The aflatoxins content of the oil was determined by TLC and quantitated by HPLC. The culinary plants *A. sativum*, *Z. officinale*, *A. cepa* L. *A. cepa* var. *aggregatum* and *M. koenigii* selected for antiaflatoxicogenic activity evaluation, their bioactive compounds were extracted and used. Marked reduction in the aflatoxins content of the groundnut oil was observed when heat-treated with culinary plants. *A. sativum* reduced 79.5% and 86.9% of AFB1 and AFB2 respectively, *Z. officinale* reduced aflatoxin B1 and aflatoxin B2 to about 86% and 87.8%
respectively. *A. cepa* L. showed 80% and 82.7% of aflatoxin B₁ and aflatoxin B₂ reduction, *A. cepa* var. *aggregatum* showed 76% of AFB₁ and 78.18% of AFB₂ reduction and *M. koenigii* showed 72% of reduction of aflatoxin B₁ and 73.58% reduction of aflatoxin B₂.

*A. sativum* exhibited highest mycelial inhibition activity against *A. flavus* MTCC 10680 among all the culinary plants used. It analysed 500µl inhibited 90.5% of mycelial biomass and 99.62% aflatoxin production. *Z. officinale* biomass is also a dose dependent one with the highest inhibited 97.68% of aflatoxin B₁ and 97.85% of aflatoxin B₂ production. *A. cepa* L. extract showed 67.21% of AFB₁ inhibition and 87.70% of AFB₂ inhibition where as *A. cepa* var. *aggregatum* extract showed inhibition of aflatoxin B₁ and B₂ to about 48.66% and 82.31%. Methanol extract of *M. koenigii* inhibited 89.6% of mycelial biomass, 34% of AFB₁ and 99.6% of AFB₂ at 2500µl extracts concentration. The mycelial biomass inhibition was found to be 47.17% (acetone), 88.9% (acetonitrile) and 74.6%( chloroform) extract at the same concentration. *A. sativum* extract exhibited zone of inhibition at the lowest concentrations of 20 µl, 40 µl, 60 µl and 80 µl, the zone of inhibition is 8mm 12mm, 18mm and 18mm respectively when extract were analysed using well diffusion method.

GC-FID analysis of fatty acid in groundnut oil treated with *A. sativum* showed the presence of 13.56 g of palmitic acid, 4.11 g of stearic acid, 40.54 g of oleic acid, 35.31 g of linoleic acid, 1.88 g of gamma linolenic acid, 4.24 g of 8,1,14 – eicosatrienoic acid, 17.66 g of saturated fat, 40.54 g of mono unsaturated fat, 41.44 g of poly unsaturated fat.

Groundnut oil treated with *Z. officinale* showed the presence of 12.61 g of palmitic acid, 4.29 g of stearic acid, 40.86 g of oleic acid, 35.45 g of linoleic acid, 1.96 g of gamma linolenic acid, 4.48 g of 8,1,14 – eicosatrienoic acid, 16.90 g of saturated fat, 40.86 g of mono unsaturated fat, 41.90 g of poly unsaturated fat.

Groundnut oil treated with *A. cepa* L. and *A. cepa* var. *aggregatum* showed the presence of 13.75 g and 13.64 g of palmitic acid, 4.09 gand 4.04 g of stearic acid, 40.35 g and 40.45 g of oleic acid, 35.06 and 35.62 g of linoleic acid, 1.81 g and 1.83 g of gamma linolenic acid, 4.06 g and 4.11 g of 8,1,14 – eicosatrienoic acid, 17.84 g
and 17.68 g of saturated fat, 40.35 g and 40.45 g of mono unsaturated fat, 41.48 g and 41.55 g of poly unsaturated fat respectively.

Groundnut oil treated with *M. koenigii* revealed the presence of 13.26 g of palmitic acid, 4.12 g of stearic acid, 40.74 g of oleic acid, 35.39 g of linoleic acid, 1.86 g of gamma linolenic acid, 4.31 g of 8,1,14 – eicosatrienoic acid, 17.37 g of saturated fat, 40.74 g of mono unsaturated fat, 41.57 g of poly unsaturated fat.

Fatty acids in groundnut oil after boiling without spice extract is 14.41 g of palmitic acid, 4.25 g of stearic acid, 42.09 g of oleic acid, 37.03g of linoleic acid, 1.89 g of gamma linolenic acid, 8,1,14 – eicosatrienoic acid is nil, 18. g of saturated fat, 420.074 g of mono unsaturated fat, 38.91 g of poly unsaturated fat.

The oil quality parameter changes in terms of physic-chemical analysis like acid value, iodine value, peroxide value and saponification value of groundnut oil heat treated with culinary plants *A. sativum*, *Z. officinale*, *A. cepa L.*, *A. cepa* var. *aggregatum* as well as *M. koenigii* were determined. No significant changes were observed.

Phytochemical analysis of *A. sativum* extract by GC-MS showed 28 compounds, among which 2-Ethenyl-1,3-Thia-Cyclohex-5-Ene in high level. *Z. officinale*, *A. cepa L.*, and *A. cepa* var. *aggregatum* extracts revealed the presence of 80, 16 and 9 compounds respectively. (-)-Zingiberene is present at the percentage of 16.57 in *Z. officinale* extract. 2-Furancarboxaldehyde showed the highest percentage in *A. cepa* L. extract. 1,3-Propanediol, 2-(Hydroxymethyl)-2-Nitro- is present in high percentage in *A. cepa* var. *aggregatum* extract. Acetone, acetonitrile and chloroform extract of *M. koenigii* showed 30,32 and 27 compounds respectively. Caryophyllene is the major bioactive compound present in acetone, acetonitrile and chloroform extract of *M. koenigii*. Methanol extracts of *M. koenigii* showed 21 compounds, 1-Methyl-pyrrolidine-2-carboxylic acid is the major compound.

Among 12 bioactive compounds of *A. sativum* 5-Octanoyl-2,4,6(1H,3H,5H)-pyrimidinetrione have high docking score of 59.421 with verl gene protein. In *Z. officinale*, gingerol and zingerone have docking score of about 75.425 and 53.271 respectively. Guanosine of *A. cepa L.* have high docking score of 48.63.
Hexadecanoic acid of *A. cepa* var. *aggregatum* have docking score of 67.351. Isophytol of *M. koenigii* showed have docking score of 72.925.

Hence, it can be concluded that the culinary plants may provide safe natural antifungal preservatives protecting the culinary oils from pathogenic and saprophytic storage fungi mediated spoilage. It also established the storage fungi associated threats of culinary groundnut oil.