SCREENING AND CHARACTERIZATION OF GROUNDNUT GENOTYPES FOR LOW AFLATOXIN (AFB_1) PRODUCTION

By

K.VENKATA RAMANAMMA B.Sc.(Ag).

THESIS SUBMITTED TO THE
ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE
(IN THE FACULTY OF AGRICULTURE)

DEPARTMENT OF PLANT PATHOLOGY
SRI VENKATESWARA AGRICULTURAL COLLEGE
ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
TIRUPATI - 517 502 (A.P.)

AUGUST, 2003

CERTIFICATE

This is to certify that **Ms.K.VENKATA RAMANAMMA**, has satisfactorily prosecuted the course of research and that the thesis entitled "**SCREENING AND CHARACTERIZATION OF GROUNDNUT GENOTYPES FOR LOW AFLATOXIN (AFB_1) PRODUCTION**" submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any University.

Date :

Place : Tirupati

(Dr. N.P.ESWARA REDDY)
(Major Advisor)

DECLARATION

I, ***Ms.K.VENKATA RAMANAMMA*** *hereby declare that the thesis entitled "****SCREENING AND CHARACTERIZATION OF GROUNDNUT GENOTYPES FOR LOW AFLATOXIN (AFB$_1$) PRODUCTION****" submitted to Acharya N.G. Ranga Agricultural University, Hyderabad for the degree of* ***MASTER OF SCIENCE IN AGRICULTURE*** *is the result of original research work done by me. I also declare that the material contained in this thesis has not been published earlier.*

Date : **(K.VENKATA RAMANAMMA)**

CERTIFICATE

This is to certify that the thesis entitled ***"SCREENING AND CHARACTERIZATION OF GROUNDNUT GENOTYPES FOR LOW AFLATOXIN (AFB_1) PRODUCTION"*** submitted in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** of the Acharya N.G.Ranga Agricultural University, Hyderabad is a record of the bonafide research work carried out by ***K.VENKATA RAMANAMMA*** under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All the assistance and help received during the course of investigation have been duly acknowledged by the author of the thesis.

(Dr. N.P.ESWARA REDDY)
Chairman of the Advisory Committee

Thesis approved by the Student's Advisory Committee

Chairman : **(Dr.N.P.ESWARA REDDY)** _____
Associate Professor
Dept. of Plant Pathology
S.V.Agricultural College
Tirupati - 517 502

Member : **(Dr. T.V.CHALAM)** _____
Professor
Department of Plant Pathology
S.V.Agricultural College
Tirupati

Member : **(Dr. P. HARINATH NAIDU)** _____
Senior Scientist
Department of Plant Pathology
Regional Agricultural Research Station
Tirupati

Member : **(Dr. P. SUDHAKAR)** _____
Scientist
Department of Plant Physiology
Regional Agricultural Research Station
Tirupati

CONTENTS

Chapter No.	Title	Page No.
I.	INTRODUCTION	
II.	REVIEW OF LITERATURE	
III.	MATERIALS AND METHODS	
IV.	RESULTS	
V.	DISCUSSION	
VI.	SUMMARY	
	LITERATURE CITED	

LIST OF TABLES

Sl.No.	Title	Page No.
1.	Aflatoxin (AFB_1) content in different groundnut genotypes after *in vitro* screening	
2.	Aflatoxin content (AFB_1) in seeds of different groundnut genotypes after *in vivo* screening	
3.	Percentage of seed infection and aflatoxin content in different groundnut genotypes after *in vivo* screening	
4.	*Aspergillus flavus* population at different days after inoculation under Drought and irrigated conditions	
5.	Total phenols and aflatoxin content in different groundnut genotypes after harvesting	
6.	Total sugars and aflatoxin content in the different groundnut genotypes after harvesting	
7.	Total seed proteins and aflatoxin content in different groundnut genotypes after harvesting	
8.	Shell wall thickness in different groundnut genotypes after harvesting	
9.	Shell wall integrity in different groundnut genotypes at the time of harvesting	
10.	Percentage of kernel moisture, pod wall moisture and aflatoxin content of different groundnut genotypes at the time of harvesting	

LIST OF ILLUSTRATIONS

Sl.No.	Title	Page No.
1.	Aflatoxin content (AFB_1) in seeds of different groundnut genotypes after *in vivo* screening	
2.	Percentage of natural seed infection in different groundnut genotypes after harvesting	
3.	Total phenol content in different groundnut genotypes after harvesting	
4.	Relationship between aflatoxin content and total phenols under drought conditions	
5.	Total sugars in different groundnut genotypes after harvesting	
6.	Total seed protein content in different groundnut genotypes after harvesting	
7.	Shell wall integrity in different groundnut genotypes after harvesting	
8.	Relationship between aflatoxin and shell wall integrity	
9.	Percentage of kernel moisture in different groundnut genotypes after harvesting	
10.	Percentage of pod wall moisture in different groundnut genotypes at the time of harvesting	

LIST OF PLATES

Sl.No.	Title	Page No.
1.	Pure culture of *Aspergillus flavus*	
2.	Mass multiplication of *Aspergillus flavus*	
3.	General view of field experiment conducted at S.V.Agricultural College dryland farm	
4.	Artificial seed infection of groundnut seeds by *Aspergillus flavus*	
5.	Natural seed infection of groundnut genotypes under drought conditions	
6.	Natural seed infection of groundnut seeds under irrigated conditions	
7.	Natural seed infection in Narayani under drought and irrigated conditions	
8.	Natural seed infection in Kalahasti under drought and irrigated conditions	
9.	Colony forming units of *Aspergillus flavus* on AFPA medium	
10.	Protein profiles of different groundnut genotypes	
11.	Peroxidase isozyme profiles of different groundnut genotypes	
12.	Esterase isozyme profiles of different groundnut genotypes	

Plate 1. Pure culture of *Aspergillus flavus*

Plate 2. Mass multiplication of *Aspergillus flavus*

Plate 3. General view of field experiment conducted at S.V.Agricultural College dryland farm

Plate 4. Artificial seed infection of groundnut seeds by *Aspergillus flavus*

Plate 5. Natural seed infection of groundnut genotypes under drought conditions

Plate 6. Natural seed infection of groundnut seeds under irrigated conditions

Plate 7. Natural seed infection in Narayani under drought and irrigated conditions

Plate 8. Natural seed infection in Kalahasti under drought and irrigated conditions

Plate 9. Colony forming units of *Aspergillus flavus* on AFPA medium

Plate 10. Protein profiles of different groundnut genotypes

Plate 11. Peroxidase isozyme profiles of different groundnut genotypes

Plate 12. Esterase isozyme profiles of different groundnut genotypes

LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Per cent
°C	:	degree celsius
µg	:	microgram
µl	:	microlitre
AFPA	:	*Aspergillus flavus / Parasiticus* Agar medium
CD	:	Critical difference
c.f.u	:	Colony forming units
Cm	:	Centimetre
Cv	:	Coefficient of variation
DAI	:	Days after inoculation
DAS	:	Days after sowing
EC	:	Electrical conductivity
Fig	:	Figure
g	:	gram
h	:	hours
ha	:	hectares
KD	:	Kilo dalton
kg	:	Kilogram
lt	:	litre
m	:	metre
M	:	Molar
m. tonnes	:	million tonnes
mg	:	milligram
min.	:	minutes
ml	:	milliliter
mm	:	millimeter
N	:	normality
ng	:	Nanogram
OD	:	Optical density
PEO	:	Peroxidase
Pg	:	Picogram
ppb	:	parts per billion
ppm	:	parts per million
PPO	:	Polyphenol oxidase
Psi	:	Per square inch
RH	:	Relative humidity
rpm	:	revolutions per minute
Sec.	:	seconds
SEM ±	:	Standard error of mean
Spp.	:	Species (Plural)
t	:	tonnes
var.	:	variety

ACKNOWLEDGEMENTS

*It is only by grace and blessings of **Lord Srirama** that I have been able to complete my studies successfully hither to and present humble piece of work for which I am eternally indebted.*

*It gives me immense pleasure to express my gratitude to **Dr.N.P.Eswara Reddy**, major advisor, Associate Professor, Department of Plant Pathology, S.V.Agricultural college, Tirupati for insightful guidance, incessant cooperation, benign attitude, concrete suggestions during my course of study.*

*I wish to express my genuine thanks to **Dr.P.V.Reddy,** Principal investigator of AICAR aflatoxin project, Department of Plant Physiology, R.A.R.S, Tirupati for providing this interested and enthusiastic problem work.*

*I express my sincere thanks to **Dr.P.Sudhakar**, Scientist, Department of Plant Physiology, RARS, Tirupati, member of my advisory committee for his kind cooperation, valuable suggestions and timely help during the period of my research work.*

*I respectively acknowledge my sincere feelings of gratitude to **Dr.P.Harinath Naidu**, Senior Scientist, Department of Plant Pathology, RARS, Tirupati, member of my advisory committee for meticulous guidance and valuable suggestions during the course of this investigation.*

*I owe my effusive thanks to **Dr.T.V.Chalam**, Professor, Department of plant pathology, S.V. Agricultural College, Tirupati, member of my advisory committee for his valuable suggestions and encouragement during the course of study.*

*It is with love, I wish to express my gratitude to **Dr.S.V.Ramakrishna Rao**, Principal Scientist, AICRP on tropical fruits (citrus) for his unbound affection, cheerful assistance, meticulous guidance and blessings during my course of study.*

*I express my profound sense of gratitude to **Dr.K.Chandrasekhar Rao,** Associate Dean, S.V. Agricultural College, Tirupati for his valuable advices and encouragement throughout the period of investigation.*

*I convey my sincere thanks to **Dr.K.Seetharami Reddy,** Professor and Head, **Dr.M.Reddy Kumar**, Assistant Professor, Department of plant pathology, **Sri.M.Hemanth Kumar**, Assistant Professor, Department of Genetics and Plant Breeding, **Dr.K.Sreenivasulu Reddy,** Associate Professor, **Dr.N.Ramavatharam**, Professor and Head, Department of Soil Science, **Dr.N.Venugopal Rao,** Professor and Head, Department of Entomology, **Dr.G.Mohan Naidu,** Department of Statistics for their stimulating suggestions, timely help and persistent encouragement in pursuit of my research work.*

*I wish to express my sincere thanks to **K.Bayyapu Reddy**, Research Associate, National Seed Project, Hyderabad for his timely help during the period of my research work.*

*I wish to express my unboundful gratitude and deep sense of honour to my parents **Smt. Lakshmidevi** and **Konda Reddy** for their unlimited love and affection which helped me a lot in shaping my life.*

*I express my gratitude and special regards to my beloved brothers **Venugopal Reddy, Vishnu Vardhan Reddy**, my affectionate sister **Ramadevi,** cousin **Ramarangeswara Reddy**, my aunt **Rameswari** and uncles **Maheshwara Reddy** and **Tirupama Reddy**.*

*I am in dearth of words to express my deep feeling to my dear most amiable friends **Viswanath, Lalitha, Syam Sundara Reddy, Sudhakar** and **Lavanya** for their unparallel affection, moral support and love which will go a long way throughout my life.*

*My heartful thanks to colleagues **Arunasri, Haritha** and **Madhusudhan**, my Juniors **Mohanmurali, Durai, Kiran, Rajani** my seniors **Rajalakshmi, Prasad** and **Kavitha** for their help during the period of my study and investigation.*

*I am thankful to my senior **M.K.Jyosthna**, for her timely help, constant encouragement and valuable suggestions during my research work.*

*I am highly grateful to Research associates **Babita, Vijaya Kumar, Latha** and **Sundaram**, Senior Research Fellows, Department of Plant Physiology and Plant Pathology, R.A.R.S, Tirupati.*

I wish to thank my friends ***Mohan, Suneetha, Arunaakka, Trivikram Reddy, Sree Laskhmi, Venumadhav Reddy, Geetha, Rajasekhar Reddy, Usha, Sreevani, Revati, Sreenu, Jyoshna, Uma, Swetha, Sangeeta*** *and* ***Seetala.***

I place a great owe to ***Narasimha Reddy, Ventakalakshmi, Ravi*** *and* ***Bhaskar*** *non teaching staff of Department of plant physiology, RARS and* ***Subramanyam,*** *Record Assistant, Department of Soil Science, Agricultural College, Tirupati for their sustained help and cooperation during my research work.*

Finally A thank to ***Hemanth, Ramu*** *and* ***Ravi (Smart Centre)*** *who have taken keen interest in neat execution of the thesis.*

I greatfully acknowledge the ***Acharya N.G.Ranga Agricultural University*** *for the financial assistance given in the form of stipend.*

I record my deep sincere thanks to all those who have helped me directly and indirectly to bring out this work in the present form.

(K. VENKATA RAMANAMMA)..

Name of the Author	:	**K.VENKATA RAMANAMMA**
Title of the thesis	:	**"SCREENING AND CHARACTERIZATION OF GROUNDNUT GENOTYPES FOR LOW AFLATOXIN (AFB_1) PRODUCTION"**
Submitted for the award of degree	:	Master of Agriculture Science.
Faculty	:	Agriculture
Department	:	Plant Pathology
Major Advisor	:	**Dr.N.P.Eswara Reddy**
University	:	Acharya N.G.Ranga Agricultural University
Year	:	August, 2003

ABSTRACT

Aflatoxin is one of the major problems in groundnut produced by *Aspergillus flavus* and *Aspergillus parasiticus,* which hinders the export of groundnut. Host plant resistance is one of the feasible and economic way of controlling the production of aflatoxin.

Twelve groundnut genotypes *viz.*, Kalahasti, Narayani, TMV-2, Tirupati-3, Tirupati-4, K-3, JL-24, J-11, K-4, K-5, TCGP-6 and K-134 were screened against aflatoxin production under *in vitro* and *in vivo* conditions. None of the seeds of 12 genotypes were tolerant to *A. flavus* infection and aflatoxin production under *in vitro* conditions.

To maintain sufficient fungal load in experimental soil, *A. flavus* inoculum was applied at 30 and 60 days after sowing.

The soil population was increased in soil after the first inoculum application. More *Aspergillus flavus* populations was observed under drought conditions compared to irrigated conditions.

The genotypes were tested for seed infection and aflatoxin (AFB_1) content after harvest. Percentage of seed infection and aflatoxin content was high under imposed drought conditions compared to irrigated conditions. Percentage of seed infection and aflatoxin content had no correlation. Among the genotypes tested J-11 and K-4 recorded low aflatoxin content under irrigated conditions. Whereas under drought conditions, Tirupati-4 showed low aflatoxin contamination.

Among the biochemical characteristics studied total sugar content increased under drought conditions compared to irrigated conditions, whereas phenols and proteins accumulation had no influence. However under drought conditions total phenols had negative relation with aflatoxin contamination.

Among the pod characteristics studied the genotype Tirupati-4 under drought conditions and J -11 under irrigated conditions maintained relatively high shell wall integrity and high pod and kernel moisture had low aflatoxin contamination specially under drought conditions. It was found that shell wall thickness had no relation with aflatoxin production for all the genotypes.

The SDS-PAGE analysis of total seed proteins showed that all genotypes varied significantly in protein profiles. However, Tirupati-3 showed more qualitative difference in protein profiles when compared to other genotypes. Significant difference was not observed in peroxidase isozyme activity among tested genotypes. Esterase isozyme analysis revealed that presence of E_2 band in J-11 may be responsible for its low aflatoxin production.

As the international regulations for minimum standards for aflatoxin contamination is becoming stringent (5 ppb) the genotypes Tirupati-4 and J-11, can be recommended for cultivation in drought prone areas of *kharif* and *rabi* seasons respectively in Andhra Pradesh.

CHAPTER - II

REVIEW OF LITERATURE

A review of the earlier studies on screening methods, biochemical and molecular characterization of *Aspergillus flavus* resistant and susceptible groundnut genotypes and other crop disease systems are presented in this chapter. The review is presented under the following heads.

2.1. ECONOMIC IMPORTANCE OF AFLATOXINS

2.2. SCREENING STUDIES

2.3. ESTIMATION OF ASPERGILLUS FLAVUS POPULATION IN SOIL

2.4. AFLATOXIN ESTIMATION BY ELISA

2.5. BIOCHEMICAL STUDIES

2.6. MOLECULAR STUDIES-TOTAL SEED PROTEINS AND ISOZYMES

2.1. ECONOMIC IMPORTANCE OF AFLATOXINS

A variety of contaminants are found naturally occurring in foods. Of these, mycotoxins are the major contaminants and 25 per cent of foods are contaminated with mycotoxins. Among them aflatoxins are the major

mycotoxins produced by toxigenic strains of *A.flavus* and *A.parasiticus* in the suitable environment. Aflatoxins are the secondary metabolites produced by these fungi. These are hepatocarcinogens, mutagens, and toxins (Wogan, 1965). Aflatoxins cause economic and trade problems at almost every stage of marketing of groundnut specially during export. Earlier reports indicated that over a decade, the export of groundnut productions from India has declined from 550 metric tonns (valued at US$ 42.5 million) to 265 metric tonns (valued at US$32.5 million) due to the presence of aflatoxins. Importing countries have prescribed the standards for groundnut. Australia set maximum permissible limit for groundnut at 15 ppb per kg and USA at 20 ppb (Sellschop *et al.*,1965). In India permissible level for aflatoxin in groundnut is 30ppb per kg (ICAR,1987). According to Indian council of medical research (ICMR), Lucknow 21% of groundnut and maize samples in India are unfit for human consumption due to aflatoxin contamination. In order to protect the international trade, in 29th meeting, codex committee on food additives and contaminants (CCFAC) a draft level of 15microgram per kg for total aflatoxin in peanuts intended for further processing was proposed as the maximum level. If this level is applied 37 per cent of our groundnut samples are rejected. In Andhra Pradesh groundnut samples contain 15-19 per cent excess aflatoxin than the permissible level.

Various surveys conducted in different parts of India have shown a range of aflatoxin levels in peanut food products including raw peanut kernels (0.8 to 2200 µg/kg), edible flour (0 to 200 µg/kg), unrefined oil (upto 786µg/kg) and peanut cake (27 to 1122µg/kg) depending upon the agroclimatic location and storage conditions (Ghewande,1997).

Due to the contamination of aflatoxins, the peanut is considered as a high risk commodity. The problem of aflatoxin contamination is worldwide but in India, the poor harvesting practices, high temperature, high moisture levels and post harvest practices are conducive for fungal growth, proliferation and aflatoxin contamination. *A. flavus* grows at a relative humidity of 85 per cent or more (Ayerst and Budd 1960). If other conditions are favourable, aflatoxin can be produced at temperature ranging from 11 to 40°C although 25 to 30°C is the optimal range (Diener and Davis, 1987). Aflatoxins are produced under drought conditions in preharvest peanut crop.

To manage the aflatoxin problem in groundnut, identification of resistant crop varieties is the best approach, but none of the varieties screened so far are completely immune to aflatoxin production.

Description of the Pathogen

A.flavus is a saprophyte or facultative parasite. It produces green coloured conidia on artificial medium. The sterigmata are typically biseriate, conidia conspicuously echinulate, conidiophores heavy walled, coarsely roughened and sclerotia may be produced.

2.2. SCREENING STUDIES

Mixon and Rogers (1973) developed a new *in vitro* seed colonization procedure for screening the groundnut genotypes against *A. flavus*. Their results indicated that Valencia type genotypes viz., PI337394F and PI337409 were resistant to two toxin producing strains of the fungus.

Priyadarshini and Tulpule (1978) studied the reaction of different varieties of maize and groundnuts and stated that there is no direct correlation between fungal growth and aflatoxin production, suggesting that the genotypes produced different amounts of aflatoxin per unit growth of the fungus.

Bartz *et al.*, (1978) performed an *in vitro* experiment of seed colonization of fifteen cured and hand shelled peanut genotypes and showed that

florunner was the most tolerant cultivar and Tifspan was the most susceptible to seed colonization by *A. flavus.*

Mehan *et al.,* (1980) reported that inoculation of seeds of seven groundnut cultivars with three different toxigenic strains of *A.flavus* showed marked differences in invasion potential between cultivars. Among them, J-11, PI 337409 and PI 337394 were found to be resistant to invasion and colonization by all three strains and the strain NRRL 3000 was less virulent than other two on all the cultivars.

Mehan *et al.,* (1981) screened eleven groundnut genotypes and found one Indian commercial cultivar J-11 and two groundnut lines PI 337409 and PI 337334 were resistant to invasion and colonization by *A. flavus.*

Levels of *A. flavus* infection and aflatoxin contamination are related primarily to environmental conditions especially to drought stress during pod maturation. Hence, the levels of *A. flavus* seed infection can not be directly correlated to the aflatoxin production (Davidson *et al.,* 1982).

Mehan *et al.,* (1982) screened nine groundnut genotypes for seed colonization by *A.flavus*. The genotype J-11 showed complete resistance,

PI 337394, PI 337409 showed less seed colonization and internal invasion by *A.flavus*. Among the 3 fungal strains, AF 8-3-2A produced highest level of aflatoxin B_1, while AFS-3 produced the least, NRRL 3000 being intermediate. They further found that there is no correlation between seed resistance to *A.flavus* colonization and aflatoxin production when the seeds were infected.

Kisyombe *et al.*, (1985) evaluated 14 peanut genotypes in rain shaded field microplots against to *A. Parasiticus* and found that genotypes J-11 and Lampang were resistant to this fungus under both dry and moist field conditions. They also evaluated 34 genotypes for dry seed resistance in laboratory and found that there was no correlation between genotype for resistance to dry seed infection and resistance under field conditions.

Blankenship *et al.*, (1985) evaluated groundnut genotypes to *A.flavus* infection under laboratory conditions and found that all were resistant. However, these genotypes when evaluated under field conditions by imposing the drought and temperature conditions were found to be susceptible.

Mehan *et al.*, (1986) evaluated 502 mature, undamaged peanut genotypes against *A.flavus* under lab conditions and found that all genotypes

supported production of aflatoxin B_1 but significant genotypic differences were found in levels of aflatoxin B_1 production.

Mixon (1986) studied peanut genotypes resistant to seed invasion by *A.flavus* and *A.parasiticus*. All resistant lines showed reduced contamination of aflatoxin of seed immediately after harvesting even though environmental conditions were conducive to *A.flavus* and *A. parasiticus* infection.

Resistance to aflatoxin contamination in peanut operates at three levels, resistance to fungal invasion at the pod wall, at the seed coat and resistance to aflatoxin production in the cotyledinary tissue.(Mixon ,1986)

Mehan *et al.,* (1986) evaluated several groundnut genotypes by *in vitro* seed colonization by *A.flavus* (IVSCAF) method and found that IVSCAF resistant genotypes showed lower levels of seed infection than susceptible genotypes.

Mehan *et al.,* (1988) evaluated 11 peanut genotypes by IVSCAF and showed that six were resistant and five susceptible. They were evaluated under field conditions in seven environments in South India.. Five of the IVSCAF resistant genotypes had significantly greater resistance to infection

of seed by *A.flavus* and had lower aflatoxin contamination than the susceptible genotypes.

Kiran-kalia *et al.,* (1988) evaluated 53 groundnut cultivars and found that high yielding lines were susceptible to invasion by *A.flavus* and aflatoxin contamination. These results also indicated that line OG 35-1 showed highest resistance with low yield potential and J-11 showed resistance to aflatoxin production and moderately susceptible to *A.flavus* invasion.

Waliyar and Bocklee-morvan (1989) reported significant varietal differences in levels of seed invasion by *A.flavus* at harvest. They also showed that under field conditions, resistance was positively correlated with *in vitro* seed colonization. The commercially grown CVS 55-437, 73-30 and 73-33 exhibited moderate to high levels of resistance to infection. The cultivar resistant to seed invasion had lower frequency of *A.flavus* counts in their rhizosphere compared to those of susceptible cultivars.

Naguib *et al.*, (1990) reported that all 21 cultivars of groundnut genotypes from ICRISAT and four from Egypt supported the production of aflatoxin B_1 and B_2 in seed when inoculated with *A.flavus*.

Mehan *et al.,* (1991) evaluated 1107 groundnut genotypes for natural seed infection by *A.flavus* and found that two were resistant. He also reported some genotypes susceptible to *in vitro* seed colonization by *A.flavus* showed resistance to seed infection in the field.

Desai *et al.,* (1991) tested 39 different groundnut varieties and breeding lines to *A.flavus* infection and found that tested groundnuts were significantly differed in infection and aflatoxin production, infection and seed colonization were strongly correlated and no correlation was found between infection and aflatoxin content.

Ghewande *et al.,* (1993) screened 38 groundnut genotypes, under artificially inoculated conditions and reported that there was a significant correlation between infection, colonization and aflatoxin content. However , there was no correlation between sugar content and infection, colonization and aflatoxin content.

Sanders *et al.,* (1993) investigated effect of root stress and pod stress on aflatoxin production separately. He showed that aflatoxin were consistently found in pods although roots were well watered and no toxin production in pods which were watered.

Waliyar *et al.,* (1994) evaluated 25 groundnut lines and reported that 55-437, J-11 and PI337394 were least infected, ICGV-87084, ICGV-87094, ICGV-87110 were resistant and var-29 showed a high percentage of infection with low aflatoxin contamination.

Anderson *et al.,* (1995) evaluated 12 potentially resistant genotypes for preharvest aflatoxin contamination and found that none of the genotypes were more resistant ($P \leq 0.05$) to pre-harvest aflatoxin contamination than the genotype florunner.

Anderson *et al.,* (1996) developed an effective procedure i.e., *in vitro* seed colonization for screening the individual plants for resistance to invasion by *A.flavus*.

Nahdi (1996) screened 4 groundnut genotypes TMV-2, NcAc 17090, Robut 33-1 and EC 76446 in two seasons by creating early and midseason drought, and found increased infection of seeds by *A.flavus*. Aflatoxin contamination was found only in second season.

Chiou *et al.,* (1999) studied mould infection and aflatoxin contamination of crops by *A.flavus* and *A.niger* inoculation either with *A.niger* alone or combined with *A.flavus* resulted in various levels of seed and seedling mortality and lower yields of peanut pods than yields of other inoculation treatments. He also showed that colonization and aflatoxin content were independent of artificial inoculation.

Holbrook *et al.,* (2000) evaluated 20 genotypes of groundnut having drought tolerance and susceptibility. These results indicated that susceptible genoptypes had greater preharvest aflatoxin contamination and drought tolerant genotypes had less preharvest aflatoxin contamination.

Mohan *et al.,* (2003) screened 13 confectionary groundnut genotypes against *A. flavus* seed colonization. None of the genotypes of cultivated groundnut showed stable resistance to *A. flavus* although there is certain degree of resistance to seed colonization in the genotypes studied.

2.3 ESTIMATION OF *Aspergillus flavus* POPULATION IN SOIL

Pitt (1980) estimated *A.flavus* population from 300 soil samples of Australia by using AFPA medium. Nearly 90 per cent of the samples from

soil in which groundnuts had grown showed in 100 to 5000 spores per g. of soil. Among 30 virgin soil samples, only 3 samples contained *A.flavus*, one sample contained 100 spores per gm of soil. Thus it is concluded that groundnut cultivation increases the fungal population in soil.

Okazaki *et al.*, (1992) estimated the population of *Aspergillus flavus* 30±9.7 and 11±2.3 propagules /gm of soil in kumamoto and miyakonojo areas respectively and also found that the fungal populations widely distributed in fields.

Lee and Chuang (1993) estimated aflatoxin producing strains of *Aspergillus flavus* in soil by using the differential medium i.e., aflatoxin producing agar (APA).The highest frequency of *A.flavus* population was found at a depth of 5-10 cm followed by 10-15 cm, but population was low at upper layers (0-5 cm) and least in deeper layers (15 -20 cm). Aflatoxin producing strains were in the range of 45-72 per cent of the total *A.flavus* population of the soil and more strains were isolated from soil than pod shells and kernels.

Karam *et al.*, (1996) estimated pathogenic fungal population in 128 soil samples from Egypt. Among the 40 isolated *Aspergillus* spp. showed dominance. *A.niger* showed highest population density i.e., 8358 c.f.u (colony

forming units) per gm of soil. *A.flavus* showed population density of 3573 c.f.u per gm of soil.

Gracia *et al.,*(1995) conducted field plot studies by using two treatments, one with low level of *A.flavus* population and other with high fungal population. Initial sampling of soil showed great variability in *Aspergillus* propagule counts with in the field. Highest infection and contamination was observed in the treatment which was basally inoculated with the highest level of inoculum, indicating a direct relationship between soil population and extent of infection and aflatoxin contamination in pre harvest maize. The extent of infection and aflatoxin contamination were lower during the wet than dry season.

2.4 AFLATOXIN ESTIMATION BY ELISA

Nakib *et al.,* (1981) compared the aflatoxin B_1 in corn, wheat and peanut butter by ELISA that gave more consistent data at relatively lower standard deviation than Radio Immuno Assay. Moreover, the ELISA results were comparable to those obtained by other established chemical methods.

Fan *et al.,* (1984) used indirect ELISA technique for the estimation of aflatoxin B_1 in corn and peanut butter.

Ram *et al.,* (1986a) detected aflatoxin B_1 in maize and cotton seed by ELISA and found that the toxin is in the range of 7 to 422 µg/kg and 7 to 3258 µg/kg respectively.

Ram *et al.,* (1986b) analysed the aflatoxin B_1 in groundnut butter samples which were artificially added with AFB_1. Recovery of AFB_1 added to groundnut butter samples ranged from 85 to 112per cent. By this procedure they had detectable level of aflatoxin (>5µg per kg) in 3 samples out of 63 commercial samples of groundnut butter .

Anjaiah *et al.,* (1989) used competitive direct ELISA for estimation of aflatoxin B_1 in naturally contaminated groundnut seed samples and concluded that this assay is more rapid and less expensive than physicochemical methods. Moreover, it can be used to detect as low as 50 pg of aflatoxin B_1.

Zhu *et al.,* (1987) analyzed aflatoxin B_1 in 253 samples of maize and groundnut oils from high liver cancer incidence areas and analyzed aflatoxin M_1 in their urine samples by direct ELISA. A good correlation was observed between total dietary AFB_1 intake and total AFM_1 excretion in humans.

Candlish *et al.,* (1987) estimated the aflatoxin content in groundnut kernels, groundnut butter and maize by enzyme immunosorbent assay (EIA) and Thin layer chromatography (TLC) and observed the positive correlation between EIA and TLC.

Cole *et al.,* (1988) estimated the aflatoxin content in 152 groundnut grade samples by ELISA and HPLC (High performance liquid chromato-graphy) and found that 41 per cent of the samples contained 26-2542 µg/kg aflatoxin. The results of ELISA and HPLC agreed in 98.6 per cent of the composite lot analysis with the detection of 20 µg/kg or greater.

Mortimer *et al.,* (1988) analysed the aflatoxin content in groundnut butters (129 samples) by ELISA and these results showed that 6.2 per cent of samples contained aflatoxin over 10 µg per kg, 8 per cent contained 2.5 to 10 µg per kg and in the remainder (86 per cent) does not contain aflatoxin. These results concluded that ELISA is a faster than conventional approaches.

Chu *et al.,* (1988) analyzed aflatoxin content in groundnuts and groundnut products by Radio immuno assay (RIA), which had sensitivity in the range of 0.1-0.5 ng, whereas ELISA, had sensitivity in the range of 2.5-25pg per assay. Simple and quick immunoassay (ELISA) protocols for

monitoring aflatoxin B_1 in groundnuts was developed that require less than 1h to complete and detect 5 to 10µg/ kg product.

Park *et al.,* (1989) estimated aflatoxin B_1 in 12 raw and roasted groundnuts and maize containing natural aflatoxins and also supplemented with aflatoxin B_1. Overall correlation was good between ELISA and TLC results for maize and roasted groundnut products. It is concluded that the ELISA method is approved interim official first action due to its simplicity and fast determine the presence or absence of aflatoxin B_1 at a concentration of ≥20 ng/g in maize and roasted groundnuts.

Park *et al.,* (1989) recommended the use of ELISA as a screening method to determine the presence or absence of aflatoxin B_1 at a concentration of ≥ 15 mg per g in cotton seed products and mixed feed.

Figuiera *et al.,* (1990) estimated the aflatoxin B_1 in groundnuts, Brazilnuts, Almonds, Hazelnuts and Walnuts by ELISA and recommended an alternate method to the already adopted TLC method .

Smart *et al.,* (1990) detected aflatoxin B_1 from 21 maize grains collected from different wound inoculation sites by *A.flavus* and showed that

no two grains were contain average toxin levels and nearly 80 per cent of samples contained aflatoxin B_1.

Azer and Cooper (1991) used ELISA system and HPLC method simultaneously to analyse 178 samples of foodstuffs for total aflatoxins. High correlation coefficient values obtained between results of two methods with nuts, nut products, groundnuts and poor correlation for cereals and grain samples.

Abouzied *et al.*, (1991) assessed the contamination of aflatoxin B_1, zearalenone and vomitoxin in 92 grain food samples by ELISA. Out of these only one sample (buckwheat flour) contained aflatoxin B_1 (19 ng/gm) and 50 per cent of samples contained vomitoxin (1µg).

Lim *et al.*, (1991) analyzed 743 samples of groundnut commodities in Singapore, the samples 34, 13, 0 and 1 were contaminated with AFB_1, B_2, G_1 and G_2, respectively. Among 121 maize samples 71, 20 and 2 samples are positive for AFB_1, B_2 and G_1 respectively.

Mehan *et al.,* (1992) reported the significant differences in the production of AFB_1 aflatoxin between the accessions of wild *Arachis* spp. and 5 of groundnut samples.

Pollet *et al.,* (1992) found that traditionally stored groundnut seeds were less contaminated than those from the markets. A correlation was found between aflatoxin contamination and damage by insects, fungi and rainfall parameters.

Patey *et al.,* (1992) used ELISA technique for the quantification of aflatoxin content in peanut butter.

Ramakrishna and Mehan (1993) utilized indirect and direct ELISA for the determination of aflatoxin B_1 in groundnut and detected as low as 20 pg per well. It is also concluded that both direct and indirect methods are useful for routine analysis of aflatoxinB_1 in groundnuts.

Aldao *et al.,* (1995) quantified the aflatoxin B_1 by an indirect ELISA in groundnut samples and observed the cross reactivity of antibodies with aflatoxin B_2,G_1 and G_2.

Wang *et al.*, (1995) collected 32 maize samples and estimated trichothecenes (nivalenol and deoxynivalenol), fuminosins (FB_1, FB_2, FB_3) by HPLC and aflatoxin B_1 by ELISA using monoclonal antibody. Five of these samples contained AFB_1, FB_1, FB_2, FB_3 and nivalenol and six of these samples were positive for above toxins including deoxynivalenol.

Sylos *et al.*, (1996) estimated aflatoxin content in 10 samples of groundnut and 9 samples of maize by ELISA and minicolumn chromatography and detected >20 µg/ kg toxin in 50 per cent groundnut seeds and none in maize samples and also ELISA has taken less time to complete than minicolumn chromatography.

Muhlemann *et al.*, (1997) collected different samples for estimation of aflatoxin by ELISA and TLC. Regression analysis showed that there is high correlation between these two methods. Moderate levels of aflatoxin contamination were found in rice, soft maize and bean (4.5, 4, 3.7 ng/g respectively) and very high levels were found in groundnuts and hard endosperm maize (698 and 110 ng/g respectively). In groundnut, high aflatoxin contamination levels appeared to be variety dependent and influenced by bad post harvest conditions. Recoveries from artificially contaminated samples ranged from 71 per cent in milk to 112 per cent in

beans, using ELISA and from 77 per cent in maize to 82 per cent in beans using TLC.

Zhang *et al.,* (1997) collected 246 samples of corn from different areas and analyzed for aflatoxins and fuminosins by ELISA and more toxins were detected in areas of high oesophageal cancer (HEC) and low toxins were detected in HEC low risk areas.

Reddy *et al.,* (2001) estimated AFB_1 in different grades of chilli samples by indirect ELISA and showed that 59 per cent of samples were contaminated with AFB_1 and 18 per cent contained the toxin at non permissible levels. Maximum percentage of chilli pods showing AFB_1 levels higher than 30 µg per kg in grade 3.

2.5 BIOCHEMICAL CHARACTERS

Total phenols, Total proteins , Sugars

Bhatia *et al.,* (1972) concluded that ability of tomato plants to resist infection by *Alternaria solani* depend on the quality of phenolics in the leaf , stem and roots of the plants. Higher amounts of total phenolics were found in the resistant variety than in susceptible variety.

Deshpande and Pancholy (1979) evaluated five genotypes against *A.flavus* and found that two of them are highly resistant to colonization than others and noted a reduction in oil and protein content and rapid increase in free fatty acids and changes in aminoacid composition.

Chattopadhyay and Bera (1980) found an increase in phenolics and phenol oxidase activity in resistant variety of rice leaves following infection with bacteria and fungi. Polyphenol oxidase converts phenols to Quinones, which may be responsible for general resistance in higher plants towards bacteria and fungi.

Mishra *et al.,* (1980) reported that the total phenols and O-dihydric phenols were higher in *C. graminicola* resistant sorghum varieties than susceptible ones.

Bilgrami *et al.,* (1983) conducted an experiment on chemical changes in dry fruits due to aflatoxin elaboration by *A.flavus* and found that significant loss in the quantity of total, reducing and non-reducing sugars as well as ascorbic acid level. An increase in total protein and phenol content was also observed in *A. flavus* infected coconut almond, cashewnut, walnut and mekhana.

Anahosur *et al.,* (1985) found that higher levels of sugars and phenolics were present in sorghum genotypes resistant sorghum to *Macrophomina phaseolina* than in susceptible ones.

The results of Shree and Reddy (1986) indicated the large amount of total phenols, reducing sugars, free aminoacids and proteins in resistant (CSH6 and 148) lines of sorghum against *Helminthosporium turcicum.*

Basha and Pancholy *et al.,* (1986) inoculated the peanut seeds with 4 different *Aspergillus* lines and infected seeds showed decrease in oil, iodine value, soluble carbohydrates and protein content. Two dimensional gel electrophoresis showed gradual disappearence of a high molecular weight polypeptide of 70KD. Several polypeptides with molecular weights between 16KD and 34KD were also appeared 9 days after inoculation.

Gupta *et al.,* (1987) analysed the leaf extract from sesame varieties susceptible (Till No.1 and HT-1) and resistant (RT-4-6,HT-24) to *Alternaria sesami* for total phenols. These results indicated that in all varieties, the total phenols were decreased. Phenols were higher in resistant cultivars than susceptible one depending on age of plants and variety.

Yadav and Mishra (1987) observed that there was increase in total phenols, soluble proteins and peroxidase enzyme activity in rice tungro virus infected TN 1 (susceptible) and IR-8 (tolerant) rice varieties, while in saket-4 (resistant) variety, it remained unaltered.

Raguchander *et al.,* (1988) studied the resistance mechanism of triticale cultivars against leaf blight fungus *Bipolaris sorokiniana*. They observed lower levels of phenols, sugars and proteins in susceptible cultivars than in resistant cultivars.

Chattopadhyay (1989) observed high levels of phenols in resistant rapeseed varieties i.e., RC 781 and YRT3 than susceptible Benoy, composite varuna. The total sugar content decreased more rapidly in susceptible cultivars than the resistant cultivars against *Alternaria* blight.

Preamalathasingh *et al.,* (1990) evaluated 38 different cultivars of pulses and found that two of them were highly resistant against aflatoxin elaboration. Moreover, the analysis of healthy seeds showed that the amount of total phenols and proteins were greater in resistant variety, while the amount of total sugar was more in susceptible varieties.

Gupta *et al.,* (1990) reported that total phenols increased initially and then decreased with age of plant in RC-781, RH-8113 (tolerant) and RH-3 (susceptible) cultivars of mustard, when infected with *Alternaria brassicae.*

Balasubramanian and Narayanaswamy (1991) observed significant increase in total phenolics in groundnut when infected with *Phoma microspora*, leaf blight pathogen in highly susceptible groundnut cultivar,OSN-2 compared to others.

Malhotra (1993) investigated biochemical constituents in tomato genotypes differing in resistance against *Fusarium* wilt. The crude protein was higher in healthy susceptible varieties. However, the increase in protein content was observed in resistant plants after infection.

Mandavia and Parameswaran (1993) reported higher levels of aminoacids and phenolics in the resistant cultivar (PLJ-1) of Limabean infected with stem rot.

Bhatia and Takur (1994) noted an increase in protein content of pearlmillet in leaves and stem but decrease in roots at all stages of growth in susceptible cultivar in relation to downy mildew pathogen.

Chowdhury *et al.,* (1995) recorded higher levels of phenolics, proteins and peroxidase and polyphenol oxidase activities in IAA treated groundnut plants against *Puccinia arachidis* compared to untreated plants.

Rajivkumar and Singh (1996) observed that the polyphenol content in healthy sunflower leaves was higher and increased at 40 and 70 days after inoculation with *Alternaria*.

Sindhan *et al.,* (1996) observed that total phenols and orthodihydroxy phenols were higher in flag smut resistant healthy wheat lines incomparision to susceptible varieties. However the concentration was reduced in infected leaves of all the varieties .

The results of Bhite *et al.,* (1997) revealed that resistant cultivars contained significantly higher amount of total phenolics and chlorogenic acid in leaves compared to susceptible cultivars of pigeonpea infected with sterility mosaic virus.

Bhavani *et al.,* (1998) reported high protein content in mosaic virus infected sunflower leaves compared to healthy leaves.

Rathi *et al.,* (1998) observed higher amounts of total phenols in powderymildew resistant pea leaves than susceptible varieties. After infection, the phenolic contents were more in resistant varieties than in susceptible ones.

Grewal *et al.,* (1999) reported the higher concentration of total sugars, total phenols and proteins in resistant varieties of wheat compared with the susceptible varieties against *Neovossia indica* causing kernal bunt.

Maintenance of high protein and phenolic contents in resistant genotypes of groundnuts when infected with *Cercospora* was reported by Bera *et al.,* (1999).

Kernel moisture and podwall moisture

Cole *et al.,* (1985) suggested that after seed invasion by *A. flavus*, growth of the fungus and aflatoxin production did not occur until the natural resistance mechanisms in the kernel had broken down as a result of environmental (water and temperature) stresses.

Wotton and Strange (1987) suggested that phytoalexin production, under high moisture conditions may provide a resistance mechanism to prevent spore germination and hyphae extension of *A. flavus*.

Cole *et al.,* (1993) found that enhanced resistance of peanut genotypes was partially associated with improved drought tolerance as measured by the ability to maintain high kernel moisture under extended drought conditions.

Holbrook *et al.,* (1994) evaluated pre-harvest aflatoxin resistance in a set of groundnut genotypes that had reputed variations in drought conditions.

Cole *et al.,* (1995) found that the susceptibility of peanuts to aflatoxin contamination is related to kernel moisture content and temperatures during pre-harvest and post-harvest conditions.

Nageswara Rao *et al.,* (2001) has suggested that management of drought, by either escape tolerance or avoidance mechanisms may therefore have a significant impact on a genotype ability to reduce aflatoxin contamination.

2.6 ELECTROPHORETICAL ANALYSIS OF TOTAL SEED PROTEINS BY SODIUM DODECYL SULPHATE POLY-ACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

Cherry *et al.,* (1966) analysed the protein banding pattern of different groundnut cultivars by disc electrophoresis. Examination of a large number of

Virginia 56 R seeds showed much intravarietal qualitative and quantitative protein electrophoretic variation. Some of these variations occurred within all both intra and inter geographical locations. This consistency of the protein variation within and between the different cultivars made it difficult to clearly distinguish them electrophoretically. Some minor qualitative and quantitative variations in protein banding patterns partially distinguished a few of the different cultivars within and between peanut types grown in the different regions, but these variations were not consistent between the geographical location.

Cherry *et al.*, (1975) analysed the proteins of groundnut seeds seven days after infection with *A. pasasiticus* and compared with uninoculated seeds by gel electrophoresis. They reported that soluble extracts from inoculated peanuts showed that proteins were hydrolyzed to many small molecular weight components, which eventually disappeared as fungal growth progressed. A corresponding increase in quantity of most free aminoacids was observed shortly after the inoculation of the peanut. It was concluded that infection of fungus initiated a sequence of events where by proteins were hydrolyzed first to small polypeptides and (or) insoluble components, then to free aminoacids.

Cherry *et al.,* (1976) examined proteins and total aminoacids in peanuts inoculated with *Aspergillus oryzae* at various time intervals over an 18 day test period. Aquarius buffer soluble proteins were declined first and increased rapidly during test period. Gel electrophoresis showed that proteins were converted to aminoacids. These aminoacid profiles distinguished from inoculated and uninoculated seeds because of increased levels of aminoacids in inoculated seeds than uninoculated seeds.

Bianchi-Hall *et al.,* (1979) analysed fifty-eight accessions of wild peanuts for seed storage proteins by SDS-PAGE. Many dark and lightly stained bands were observed, only the major bands corresponding to the acidic and basic proteins were compared. One to five bands were observed with regard to acidic arachin and 2 to 5 bands were observed with regard to basic arachin proteins. It helps in clear assessment of the large amount of variability in protein composition in peanuts and should aid in defining phylogenetic relationships in arachis.

Krishna *et al.,* (1986) observed the protein profiles of six diploid spp. of *Arachis monticola* and five accessions of *Arachis hypogea* and found highly conserved nature of "arachis" polypeptide in all the accessions.

Singh *et al.,* (1991) reported the protein profiles of nine spp, ten tetraploid accessions, two synthetic amphidiploids and two autotriploids of *Arachis dutanensis* was identified as the probable donor of a genome as against *A. cardanasii* of *A. villosa* because of number of similar bands.

Lanham *et al.,* (1994) screened 72 accessions representing 22 spp of *Arachis* for seed storage protein variability. They detected variation among section, genome types and spp. They could differentiate the two sub spp. of *A. hypogea* (fastigate and hypogaea) based on presence or absence of 44 KD or 42 KD polypeptides. The 44 KD band was found in spp. fastigated, while 42 KD band in spp. hypogea only.

Lavon *et al.,* (1999) determined the SDS-PAGE pattern of soluble and chloroplast membrane proteins in rough lemon (*Citrus volkameriana*) under K, Mg and Ca deficiencies. The SDS-PAGE patterns of soluble and chloroplast membrane proteins did not reveal major quantitative changes and they concluded that the data do not demonstrate a general close link between chlorosis minerally deficient citrus leaves and nitrogen metabolism.

Naik and Kole (2001) analysed the total seed proteins of 37 mungbean genotypes by SDS-PAGE. These results showed the protein bands of varying

intensity and heterogeneous molecular weights over five zones i.e., A to C within a molecular range of 17.4 to 75.0 KD.

ISOZYMES

The occurrence of dissimilar isozyme electrophoretic phenotypes among cultivars of same kind represents intervarietal variation. The number of polymorphic bands (qualitative variation) detectable largely determines the potential value of isozyme systems for varietal identification.

Brim *et al.,* (1969) observed the large differences in peroxidase zymograms among different soyabean tissues and also reported three quantitative genotypic differences involving peroxidase isozymes.

Wood (1971) compared the relative intensities of the principal isoperoxidase zymograms of extracts from systematically infected cucumber leaves by a wild strain of cucumber mosaic virus with healthy leaves. The susceptible cultivars Ashley and Marker showed greater intensities of the principal isoperoxidase bands in comparision with resistance cultivar China.

Seevers *et al.,* (1971) separated peroxidase isozymes from healthy and inoculated lines of wheat near isogenic lines for resistance and susceptibility

to race 56 of *Puccinia graminis tritici* by gel electrophoresis. Among the 14 isozymes detected in both healthy and infected leaves, increase in only one (isozyme 9) were associated with the development of resistant disease reaction at 20°C.

Cherry *et al.,* (1974) analysed 7 isozymes viz., esterase, peroxidase, catalase, leucine aminopeptidase, acid phosphatase, alcohol dehydrogenase and an achromatically detected oxidase of aflatoxin contaminated groundnut seeds, cotyledons and axial tissues. These results showed the same sequence of changes from 0 to 5 days after contamination, distinguished from as standard profile of uninoculated peanuts. These results also indicated the changes in composition of enzymes, such as the depletion of alcohol dehydrogenase and acid phosphatase, intensification of esterase and peroxidase and induction of new isozymes of esterase, peroxidase, leucine amino peptidase, catalase and oxidase.

Cherry *et al.,* (1978) analysed the different isozymes like esterases, leucine aminopeptidase, gluconase, alcohol dehydrogenase and alkaline and acid phosphatase pattern in extracts of groundnut seeds infected with aflatoxicogenic strains. These results indicated that the zymograms did not

differ significantly from patterns of seeds infected with non aflatoxicogenic strains.

Hammerschmidt *et al.,* (1982) observed the enhancement of peroxidase activity in second leaf of cucumber, when first leaf was inoculated with *Colletotrichum legenarum.* This increased activity was supposed to be partially associated with the fastest moving acidic isozymes observed when separated by PAGE.

Lima (1982) observed considerable polymorphism among esterases in citrus by electrophoretic studies with leaves of young shoots of 161 taxa.

Arora and Bajaj (1985) recorded the variability in peroxidase enzyme banding pattern in hypocotyls of mungbean after infection with *Rhizoctonia solani*.

Wehling (1986) studied esterase, peroxidase, glutamate oxaloacetate, transaminase, acid phosphatase, beta glycosidase, aconitase and 6-phospho-glyconate dehydrogenase. A total of 32 polymorphic isozyme loci were identified, of which atleast 22 were suitable for use as genetic markers.

Puchalski *et al.,* (1986) observed the induction of three peroxidase isozyme in rye seedlings after inoculation with *Fusarium nivale* and suggested that the isozyme band P x 4 could be used as a maker in identifying resistant genotypes.

Chahal *et al.,* (1988) investigated the peroxidase isozyme pattern among downy mildew resistant and susceptible pearl millet lines and healthy plants, which indicated the involvement of C_5, C_6 and C_9 isoperoxidases in developing resistance.

Liao (1988) analysed the esterase isozymes from seeds and seedlings of IA citrus species using PAGE. They found that the zymograms might be used to detect biochemical and genetic relationship between species.

Uta Gireshammer and Wynne (1990) surveyed 61 US cultivars, one breeding and 6 exotic lines of groundnut for 25 enzyme systems using horizontal gel electrophoresis. Polymorphism was consistently observed with glutamate oxaloacetate transaminase (GOT), phosphohexoisomarase (PHI) and isocitrate dehydrogenase (IDH) indicating limited variability.

Gillikin and Graham (1991) reported that majority of the peroxidase activity in soybean seeds was localized in the seed coat. It is having isoelectric point (PI) and molecular weight of 37 KD similar to the properties of glycoprotein.

Lacks and Stalker (1993) evaluated 33 South American groundnut accessions from six countries for 18 isozymes against early leaf spot. Out of these, the polymorphism was observed among glutamate oxaloacetate transaminase (GOT), phospho-hexoisomerase (PHI) and isocitrate dehydrogenase (IDB). A specific PHI band was observed in all three hybrid lines with early leaf spot resistance.

Candela *et al.,* (1994) performed isoelectric focusing in case of *Capsicum annuum* infected with cucumber mosaic virus. These results indicated the presence of two acidic and one basic isoperoxidases.

Subash Chandrabose and Ranjan (2000) analysed the banding pattern of peroxidase isozyme in 45 and 60 days old tomato leaf samples against *Ralstonia Solanacearum* (bacterial wilt) by using PAGE. They reported that the zymograms of PR x -7 (Rm = 0.361) and PR x -8 (Rm =0.381) in 45 days old samples and PR x -5 (Rm =0.297) in 60 days old samples could be used as a markers to identify resistant and moderately resistant varieties.

CHAPTER - III

MATERIALS AND METHODS

3.1 LOCATION OF WORK

All the experiments were conducted during the year 2002-2003 in the Department of Plant Pathology, S.V.Agricultural College and Regional Agricultural Research Station, Tirupati, Chittoor District in Andhra Pradesh. Field experiments were carried out in dryland, S.V. Agricultural College Farm, Tirupati. The experimental site is located at 13° North latitude and 79° East latitude with an altitude of 182.9 m. above mean sea level in the tropical belt of South India.

3.2 CLIMATE

Tirupati comes under the Southern Agroclimatic zone of Andhra Pradesh. This zone is characterized by fairly hot summer and rainfall is received in two spells *viz.,* South-West monsoon (June-September) and North-East monsoon (October-January) periods. This zone receives more rainfall during North-East monsoon period. The average annual rainfall of Tirupati is about 1000 mm.

3.3 GLASSWARE USED

The glassware used in the experimental work are petriplates, test tubes micropipettes, pipettes, beakers, volumetric flasks and conical flasks.

3.4 CLEANING OF GLASSWARE

The glassware was thoroughly washed with a detergent followed by tap water and then placing them in a cleaning solution, which consisted of potassium dichromate (K_2Cr_2,O_7) 60g, concentrated sulphuric acid ($H_2\ SO_4$) 60 ml per 1000 ml of distilled water. The glassware was kept in cleaning solution for 24 hours and then rinsed thrice with distilled water before use.

3.5 STERILIZATION

After drying, the glassware was sterilized in hot air oven at 160°C for two hours for further use. Media, water blanks, blotting papers and distilled water were sterilized at 121°C for 15 minutes in an autoclave.

3.6 PATHOGEN

The virulent strain of *A. flavus* was obtained from Department of Plant Pathology, Regional Agricultural Research Station, Tirupati.

3.7 MEDIA USED

The following media were used throughout the experiment.

1. Potato Dextrose Agar (PDA) medium was used for maintaining the *Aspergillus flavus* culture throughout the experimental period.

Composition of PDA

Peeled potato slices	:	200 g
Dextrose	:	20 g
Agar	:	20 g
Distilled water	:	1000 ml.

The medium was sterilized in an autoclave at 15 psi for 15 minutes.

2. The *Aspergillus flavus* / *parasiticus* Agar (AFPA) medium was used for estimation of the *Aspergillus flavus* population in soil

Composition of AFPA medium (Pitt, 1982)

Bacteriological peptone	:	10 g
Yeast extract	:	20 g
Ferric ammonium citrate	:	0.5 g
Chloramphenical	:	0.2 g
Agar	:	15 g
Dichloran	:	2 mg
Distilled water	:	1 lt.

After the addition of all the ingredients, the medium was sterilized in an autoclave at 121°C for 15 minutes. Chloramphenical was added after sterilization.

3.8 MASS MULTIPLICATION OF PATHOGEN

The fungus was multiplied by organic-matrix method as per the modified procedure given by Will *et al.,* 1994.

Cracked Bajra seeds was soaked in water for overnight. After that seeds were taken out and sterilized in autoclave at 121°C for 15 minutes. A 5 mm disc of *A. flavus* from actively growing 10 day old culture was transferred to sterilized seed and incubated at 25-30°C for 7 days.

3.9 PLANT MATERIAL

Twelve groundnut genotypes were used in screening for low aflatoxin production. They were Narayani, Kalahasti, TCGP-6, JL-24, J-11, TMV-2, TPT-4, TPT-3, K-134 obtained from Regional Agricultural Research Station, Tirupati. The remaining genotypes *viz.,* Kadiri-3, Kadiri-4 and Kadiri-5 were obtained from Agricultural Research Station, Kadiri.

3.10 *IN VITRO* SCREENING

In vitro screening of different groundnut genotypes for seed colonization by *Aspergillus flavus* has done according to the procedure given by Mixon and Rogers, 1973.

Twenty grams seeds were placed in a 250 ml beaker, soaked for 15 to 20 minutes in sterile demineralised water using two changes of approximately 100 ml each in the soaking process. After the second change, the water was drained from the seed, and the sample was inoculated with a 1 ml of spore suspension (4 x 10^6 spores/ml) obtained from 10 days old culture of *Aspergillus flavus* multiplied on AFPA medium. Seeds were placed in petriplates and sterile water was added to adjust the seed moisture to 30 per cent. Incubated in a humid chamber for 7 days at 25°C, 98% relative humidity. The percentage of seed infection was recorded based on number and colour of the spots on the seed.

3.11 *IN VIVO* SCREENING FOR AFLATOXIN

The experiment was conducted during *Rabi* season with two main treatments and 12 sub treatments and 6 replications with factorial RBD design. The two treatments were drought and irrigated conditions each with 3 replications in mini plots with the size of 1.8 x 2.5 m^2.

Sowing

The field was prepared by ploughing thrice and then levelled by harrowing. The seeds were sown in the plots as per the layout given below along with recommended dose of fertilizers. JL-24 (susceptible genotype) was used as a check.

Field layout for screening different groundnut genotypes against *Aspergillus flavus.*

Drought			**Irrigation**		
R_1	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**
T_1	T_{12}	T_7	T_4	T_7	T_{11}
T_2	T_{11}	T_6	T_5	T_8	T_{12}
T_3	T_{10}	T_5	T_6	T_9	T_1
T_4	T_9	T_4	T_7	T_{10}	T_2
T_5	T_8	T_3	T_8	T_{11}	T_3
T_6	T_7	T_2	T_9	T_{12}	T_4
T_7	T_6	T_1	T_{10}	T_1	T_5
T_8	T_5	T_{12}	T_{11}	T_2	T_6
T_9	T_4	T_{11}	T_{12}	T_3	T_7
T_{10}	T_3	T_{10}	T_3	T_4	T_8
T_{11}	T_2	T_9	T_1	T_5	T_9
T_{12}	T_1	T_8	T_2	T_6	T_{10}

Plot size	:	1.8x 2.5m^2
Season	:	*Rabi* (2002-2003)
Date of sowing	:	18.12.2002
Spacing	:	30 x 10cm
Design	:	Factorial RBD.
Number of main treatments	:	2
Number of replications	:	6
Number of sub-treatments	:	12

T_1 - Kalahasti (TCGS-320)

T_2 - Narayani (TCGS-29)

T_3 - TMV-2

T_4 - TPT-3

T_5 - TPT-4

T_6 - K-3

T_7 - JL-24

T_8 - J-11

T_9 - K-4

T_{10} - K-5

T_{11} - TCGP-6

T_{12} - K-134.

The mass multiplied inoculum of *A. flavus* was applied at 30 days after sowing (DAS) and second time at 60 DAS. Initially slight furrow was made at one side of the row and then pathogen was applied @ 2.5 g. per one meter

length of the plot and then the furrow was closed. Irrigation was given just two days before the inoculum application in order to allow the multiplication of the pathogen in the soil.

A total of sixteen irrigations were given to the crop in the irrigation treatment (R_1, R_2 & R_3) at an interval of 7 to 10 days. The crop under drought treatment (R_4, R_5 & R_6) was given regular irrigations (7 to 10 days interval) upto 60 days after sowing. 60 DAS the interval between irrigations were upto 18 days for imposing the stress conditions. The total number of irrigations given to the crop in this treatment are reduced to nine.

The crop was harvested after 120 days and then the genotypes were evaluated for aflatoxin by ELISA test.

3.12. NATURAL SEED COLONIZATION

After harvesting the groundnut seeds were kept for seed colonization.

About 50 seeds were collected from each genotype in each replication. They were surface sterilized with 2 per cent sodium hypochlorite solution for 2 minutes and then washed three times with sterile distilled water. Sterilized circular blotting papers were placed on each sterilized petriplate and moistened with sterile distilled water. Then 10 seeds were kept in each petriplate, so that each harvested plot replicated five times and they were incubated for 7 days at 25°C.The fungus infected seeds produced green

coloured conidia on their surface. The percentage of the seed colonization was calculated with the following formula :

$$\text{Percentage of seed colonization} = \frac{\text{Number of seeds infected}}{\text{Total number of seeds}} \times 100$$

3.13 ESTIMATION OF *Aspergillus flavus* POPULATION IN SOIL

The fungal population from the rhizosphere was estimated at four times *viz.,* before sowing, ten days after first inoculation, 10 days after second inoculation and at the time of harvesting by serial dilution method as per the procedure given by Johnson and Curl (1972).

3.13.1 Soil sample collection

In each plot soil sample was collected in the following manner. More than ½ kg of the soil was collected from each corner and centre of the plot at 5-10 cm depth in root zone. Then all the soil samples were pooled and mixed. The soil was levelled and made in square shape. It was divided into four equal parts. Then opposite two parts were discarded. This procedure was repeated until a representative sample of 1/2 kg was obtained and it was used for estimation of fungal population. Soil samples were collected under dry conditions.

3.13.2 Serial dilution method :

The collected soil sample was shade dried for 3 days. Then it was sieved through 1mm sieve 10 g of soil was taken and added to 90 ml of sterile distilled water and shaken thoroughly. From this 1ml of soil solution was taken and added to 9 ml of sterile distilled water. This process was continued upto the dilution of 10^{-4}. Then one ml thoroughly shaked soil solution was added to sterile peteriplates containing AFPA medium. *A. flavus* grown rapidly and produced brilliant orange yellow colony colors on the reverse side of the petriplate after 4 days incubation at 25 ±1°C. The number of colonies per plate were recorded and represented as number of colony forming units per gram of soil.

Fungal population in soil = Number of colony forming units per plate. X 10^4 /gm soil

3.14 ESTIMATION OF AFLATOXIN CONTENT BY INDIRECT COMPETITIVE ELISA (ENZYME LINKED IMMUNOSOR-BENT ASSAY)

Aflatoxin content in groundnut kernels was estimated as per the procedure given by Reddy *et al.*, (1988).

Sample preparation

100 g of harvested groundnut seeds were collected and powdered. From that 20 g of powder was taken and wet grinded in 70 per cent methanol containing 0.5 per cent Kcl. The extract was transformed to a conical flask and shaked it for 30 min at 300 rpm. The extract was filtered through the Whatman No.41 filter paper and collected in glass test tubes.

Procedure

Coating :

a. ELISA plates were coated with 150µl of AFB_1-BSA conjugate (1µl AFB_1-BSA in 10 ml of 0.2 per cent carbonate buffer).
b. Incubated it overnight in refrigerator or incubator at 37°C for 1 hour.
c. Washed the plate thrice with PBS-T 20 (phosphate buffer saline-tween) for 3 min.

Blocking :

a. Then 160µl of 0.2 per cent BSA (Bovine serum albumin) added and incubated it at 37°C for one hour.
b. Washed the plate thrice with PBS-T 20.
c. Dilution of antiserum was prepared in a ratio of 1:2000 in a test tube and incubated at 37°C.

Competition :

AFB_1 standards ranging from 0.1 to 50 ng /ml were prepared in groundnut extracts (diluted to 10 %) not containing any aflatoxin. About 20 g of healthy groundnut kernels free of aflatoxin were powdered and extracted with 100ml of 70 per cent methanol containing 0.5 per cent Kcl. The extract was filtered and diluted to 1:10 in PBST -BSA. This was used as a diluent for preparing aflatoxin standards.

a. Simultaneously prepared pure toxin (AFB_1) by diluting with above prepared Healthy groundnut (HGN) extract in a test tube.
b. 100µl of AFB_1(50ng/ml) was added to first two columns of first 2 hours.
c. Then 100 µl of diluted HGN extract was added to remaining wells of first two rows.
d. The remaining wells were loaded with 90µl of BSA + 10µl of sample extract to be analysed.
e. 50µl of incubated antiserum was loaded to each well of ELISA plate and kept in shaker for 10 min.
f. Incubated the plate for one hour at 37°C to facilitate reaction between toxin and antibody and washed the plate thrice with PBS-T20.

Conjugation

a. 150 µl of goat antirabbit IgG with labelled enzyme was loaded to all wells.

b. Incubated the plate for 1 hr at 37°C.

c. Washed the plate thrice with PBS-T20.

Substrate

a. 150µl of substrate buffer [PNPP (P-Nitro Phenyl Phosphate) in 10 per cent diethylene amine] was added to each well.

b. Simultaneously substrate was added to top left corner well as blank.

c. Incubated at normal temperature in dark for colour development at 15 min. interval.

d. Absorbance was measured at 405 nm in ELISA reader. AFB_1 content was estimated by the following formula :

$$\frac{\text{AFB1 conc. (mg/ml) in sample extract X a dilution with buffer X extract solvent volume used (ml)}}{\text{Sample weight.}} = \text{PPb/kg seed}$$

3.15 DETERMINATION OF BIOCHEMICAL CHARACTERS

3.15.1 Estimation of phenols

Total phenols were estimated in harvested seeds according to the procedure of Sadasivam and Manickam (1996).

About 0.5 g of each seed sample was weighed and ground with a pestle and mortar in ten times volume of 80 per cent absolute ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was collected. Reextracted the residue with five times the volume of 80 per cent

ethanol, centrifuged at 10,000 rpm for 10 min. Both the supernatants were pooled. The supernatnt was evaporated to dryness. Dissolved the residue in a known volume of distilled water (5 ml). Different aliquots (0.2 to 2 ml) were pipetted out into test tubes and made up the volume in each tube to 3 ml with sterile distilled water. To this 0.5 ml of folinciocalteau reagent was added immediately after 2 ml of 20 per cent, Sodiumcarbonate solution was added after 3 minutes. The contents were mixed thoroughly and placed the tubes in a boiling water for one minute, cooled and the absorbance was measured by spectronic-20 at 650 nm against a reagent blank. The standard curve was prepared with different concentrations of catechol.

Preparation of reagents

a. Eighty per cent ethanol was prepared by adding the 80 ml of absolute alcohol and making upto 100 ml with distilled water.

b. Twenty per cent sodiumcarbonate was prepared by dissolving 20 g of sodiumcarbonate in 100 ml of distilled water.

3.15.2 Estimation of total sugars

The total sugar content in harvested groundnut seeds was estimated by anthrone method given by Hedge and Hofrieter (1962).

One hundred mg of the sample was taken in a boiling tube.It was hydrolyzed it by keeping in a boiling water bath for 3h with 5ml of 2.5 N-HCl and cooled at room temperature. It was then neutralized with solid sodium carbonate until the effervescence ceased. Then made up the solution in each tube to 100ml and centrifuged at 3000 rpm for 15 min. The supernatant was collected. Aliquots of 0.5 and 1ml were pipetted into separate test tubes. Standards were prepared by taking 0,0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard into different test tubes '0' served as a blank. In all the test tubes the volume was made to 1 ml by adding distilled water and then 4ml of anthrone reagent was added to each tube. All the test tubes were heated in a boiling water bath for 8 min. Test tubes were cooled rapidly and green to dark green colour was measured at 630 nm by spectronic-20. A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. Amount of sugars present in the sample tube was calculated from the graph.

Preparation of reagents

1. 200 ml of 2.5 N HCl was prepared by adding 43.1 ml of HCl to 100 ml of distilled water and finally made upto 200 ml.
2. Anthrone reagent : It was prepared by dissolving 200 mg anthrone in 100 ml of ice cold 95 per cent H_2SO_4. Prepared fresh before use.
3. Standard glucose:

Stock : It was prepared by adding the 0.1 g of glucose to 100 ml of distilled water. Working standard was prepared by diluting the 10 ml of stock 10 times with distilled water.

3.15.3 Estimation of total proteins

Total protein content in harvested groundnut seeds was estimated as per the procedure given by Lowry *et al.,* 1951.

One gram of groundnut seeds was taken and washed thoroughly with tap water followed by distilled water and dried in between folder papers. The seeds were homogenized in a mortar (0.1 M Tris-HCl, PH-8.3; 0.5 M sucrose and 0.5 per cent β-mercaptoethanol) @ 2 ml/g. The homogenate was squeezed through muslin cloth and centrifuged at 10000rpm for 10 minutes. The supernatant was collected and equal volume of 20 per cent Trichloro Acetic Acid (TCA) was added to each sample and kept for 2 hours at 4°C. The TCA precipitate was collected by centrifugation at 10,000 rpm for 10 minutes. The pellet was washed twice with 5 per cent TCA and thrice with ice cold solvent ether. The final protein pellet was dried under vacuum and solubilised in a minimal known volume of 0.1 N-HCl solution. Then the suspension was centrifuged at 3000 rpm for 10 min. and the supernatant was collected.

Twenty microlitres of protein extract obtained from different samples was taken and to each sample 5 ml of freshly prepared alkaline copper sulphate reagent (Reagent C) was added. The samples were mixed thoroughly and the solution was allowed to stand for 10 minutes at room temperature to which 0.5 ml of Folin-Ciocalteau reagent was added to each sample, mixed thoroughly and incubated for 30 minutes. The absorbance of the sample was read at 660 nm by using spectronic-20. The amount of protein (mg g^{-1} of seed) was calculated by using bovine serum albumin (BSA) standard curve.

Preparation of reagents

i. Alkaline $CuSO_4$ reagent

Reagent A :

It was prepared by adding 2 gm of sodium carbonate to 100 ml of 0.1N sodium hydroxide.

Reagent B:

Prepared by adding 0.5 gm of copper sulphate ($CuSO_4.5H_2O$) to 100 ml of 1 per cent of potassium sodium tartrate.

Reagent C:

Prepared freshly just before use by mixing 50 ml reagent of A and 1 ml of reagent B.

ii. Folin -Ciocalteau reagent

Folin ciocalteau reagent and distilled water were mixed in 1:1 ratio and prepared freshly.

3.15.4 Finding of shell wall thickness and shell wall integrity

Shell wall thickness

It was measured by screwgauge. After complete drying, the shells were collected and measured thickness in the centre and the side of the shell wall by screwgauge as per the procedure given by Vasanthi and Raja Reddy, 1995.

Shell wall integrity

Shell wall integrity was measured at the time of harvesting as per the procedure given by Reddy *et al.*, (2003).

At the time of harvesting 5 mature pods were collected from each genotype. The pods were washed thrice with distilled water. The pods were taken into plastic vials and 20 ml of distilled water was added. All plastic vials were kept in a mechanical shaker for 2 hours. After that initial electrical conductivity was measured by Electrical conductivity meter. Then they kept in refrigerator for overnight. The vials were freezed by dipping them in liquid nitrogen for half an hour and the vials were kept in a mechanical shaker for 2

hours. Electrical conductivity was measured and it was considered as final EC value.

$$\text{Shell wall integrity (\%)} = \frac{\text{Initial electrical conductivity}}{\text{Final electrical conductivity}} \times 100.$$

3.15.5 Determination of kernel moisture and pod wall moisture

Kernel moisture and pod wall moisture were measured at the time of harvesting (Cole *et al.,* 1983).

At the time of harvesting 5 mature pods were collected from each genotype. After that shells were separated from kernels and fresh weight has taken separately for each genotypes. After wards shells and kernels were kept separately in butter papers and oven dried. Then dry weight was taken, kernel moisture and pod wall moisture were calculated and expressed in percentage.

Kernel moisture = Fresh wt. of kernels - Dry wt. of kernels X 100

Pod wall moisture = Fresh wt. of shells - Dry wt. of shells x 100

3.16 ELECTROPHORETICAL ANALYSIS OF TOTAL SEED PROTEINS BY SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE):

Total proteins from the seeds of twelve groundnut genotypes were analyzed according to the procedure given by Laemmli (1970) .

Preparation of protein sample

Decoated ten seeds of each genotype, powdered and defatted using defatting solution (chloroform, methanol and acetone in 2:1:1). Defatting was performed for 24 hours with three solvent changes and later the ground material was air dried, transformed into 1.5 ml microfuge tubes and 0.4 ml of sample buffer was added to 100 mg of seed powder and kept overnight at room temperature. The samples were centrifuged at 15000 rpm for10 minutes and supernatant was collected. To this 20µl of sodium dodecyl sulphate-mercaptoethanol mixture, [prepared by mixing the 750µl of SDS, (1g SDS in 14 ml distilled water) and 250 µl of mercaptoethanol 10 µl of bromophenol blue and one or two pellets of sucrose were added to the 50µl of supernatant]. The samples were boiled for 5 minutes and cooled and then used for electrophoretic analysis.

Preparation of gel

Vertical slab gel unit in the casting mould with 1mm spacers was used. The separations were performed on 12.5 per cent of separating gel and 4.5 per cent stacking gel.

12.5 per cent of separating gel (18 ml) was prepared by mixing the following :

4.5 ml of 1.5 M Tris HCl (pH 8.8)

7.5 ml of Acrylamide-bisacrylamide (29.2 : 0.8)

1.5 ml of glycerine

4.5 ml of distilled water

80µl of 10 per cent Ammonium Per Sulphate (APS) (Prepared fresh before use)

10 µl of TEMED (N,N,N^1,N^1-Tetramethyl ethylene diamine)

4.5 per cent stacking gel (6 ml) was prepared in the following manner.

1.5 ml of Tris buffer (1.5M Tris-HCl, PH-8.8)

0.9 ml of 30 per cent stacking gel acrylamide.

3.6 ml of distilled water.

20 µl of 10 per cent APS

*10 µl of TEMED.

*TEMED was added just before pouring the gel mixture.

The resolving solution was mixed well and poured between the plates of the cassette to a level of 2 cm from the top. Care was taken to avoid air bubbles to be trapped in the gel solution. Thin layer of distilled water was added gently along the surface of the gel and then allowed the gel for polymerization. After polymerization, the water on the resolving gel was poured off and wiped off with filter paper. Stacking gel was prepared and over

layed on the resolving gel. The comb was inserted into the stacking gel and allowed for polymerization.

Sample loading

Comb was gently removed from the gel after polymerization. The lower and upper chamber was filled with 1X electrode buffer (Tris base-6g, glycine-14.4 g, SDS-1g and distilled water 1000ml, pH-8.3). Any air bubbles in the wells were removed by rinsing the wells with distilled water. Protein samples were loaded into wells. The electrode unit was connected to power pack and run the gel at 100 V with 30 mA. The electrophoresis was stopped when the dye reached to the bottom of the gel.

Comassie-Brilliant blue staining

The gel mould was carefully removed after electrophoresis and the gel was immersed in glass tray containing the staining solution (2.5 g commassie brilliant blue -250g , 500 ml methanol, 100 ml of Acetic acid and then distilled water was added till the final volume of 2000 ml and filtered) and it was kept overnight. Later, the gel was placed in destaining solution (250 ml of methanol, 70 ml of Acetic acid and distilled water was added to the final volume of 1000 ml). The destaining solution was changed until the visible protein bands were seen.

3.16.1 Isozyme analysis by polyacrylamide gel electr-ophoresis

Extraction of isozyme

About 0.1 g of 12 groundnut genotype seeds under the study were taken and ground in 0.5 M, Tris-HCl buffer (PH-6.8) at 4°C using pre chilled mortar and pestle. These extracts were centrifuged at 10000rpm at 4°C for 15 minutes.

All the isozymes were analyzed by native polyacrylamide gel electrophoresis similar to SDS-PAGE but without SDS. 60µl of extract was loaded on polyacrylamide gel. The slab gel consisted of stacking portion of 4 per cent acrylamide in 0.5M Tris-HCl (PH-6.8) and resolving gel consists of 7.5 per cent acrylamide in 1.5M Tris-HCl (PH-8.8). 1 x electrode buffer (Tris-glycine, PH-8.3) was used according to Shaw and Prasad (1970). The quantity of solutions were prepared according to the requirement. The electrophoresis was carried out at constant current of 30 mA at 4°C. The electrophoresis was stopped when the dye reached to the bottom of the gel. Likewise the different gels were run for esterases and peroxidases and stained with appropriate staining solution.

Peroxidase (PEO)

After electrophoresis the gel was stained by the following procedure.

Staining

A. Sodium acetate buffer (0.2 M PH-5.6)- 100 ml.

B. Benzidine - 100 mg

C. Hydrogen peroxide - 0.1 ml.

Solution A was boiled with B, cooled and filtered, hydrogen peroxide was added just before incubation. Gel was incubated in dark till brown bands appeared and rinsed with 7 per cent acetic acid. Gel was stored in 2 per cent acetic acid.

Esterases

After electrophoresis gel was incubated for 30-45 min in a solution of 0.1M sodium phosphate buffer (PH 7.2) containing 0.1 per cent fast blue RR salt and ∝-naphthyl acetate dissolved in 50 per cent acetone. The gel was distained with water : methanol : acetic acid (15:4:1). The position of the isozyme bands in the gel was expressed by measuring the distance migrated by the particular band from the cathode end. Band frequency was calculated for all the genotypes.

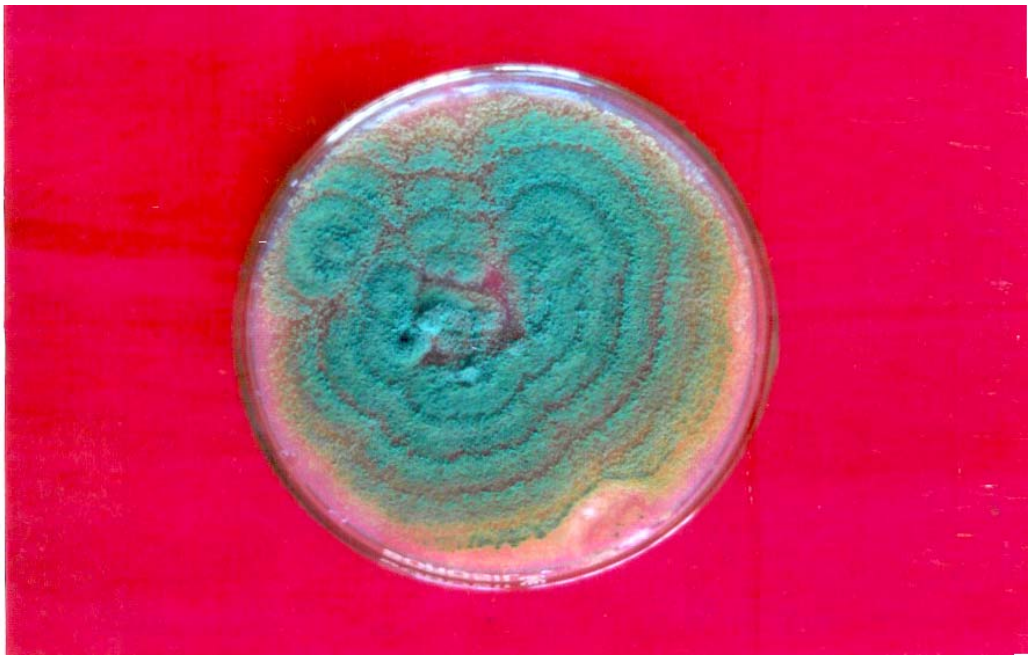


Plate 1. Pure culture of *Aspergillus flavus*

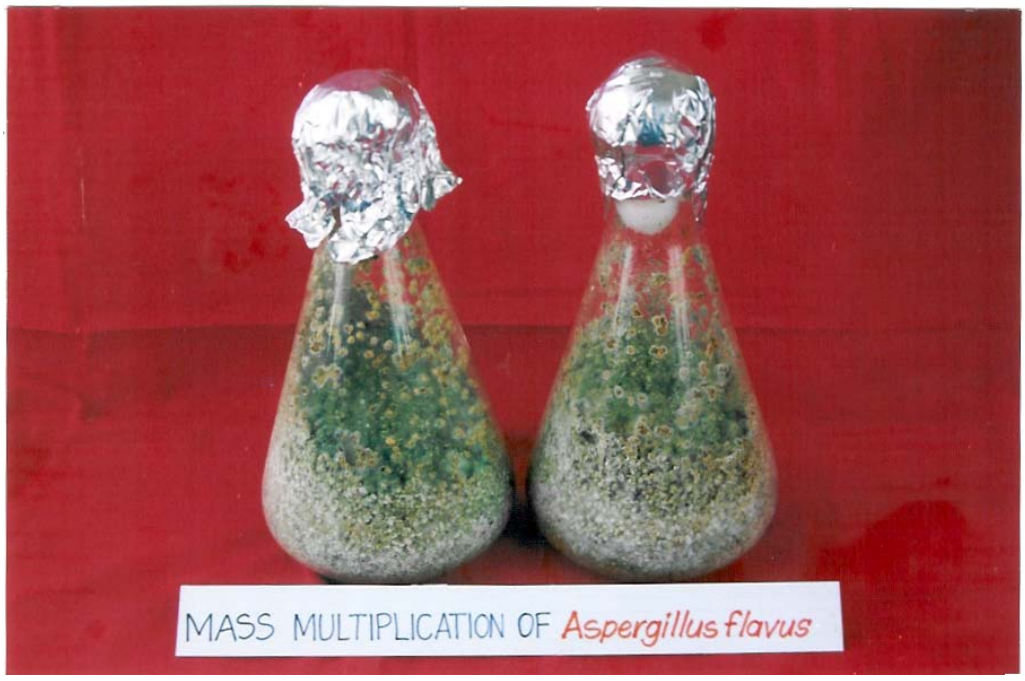


Plate 2. Mass multiplication of *Aspergillus flavus*

Plate 3. General view of field experiment conducted at S.V.Agricultural College dryland farm

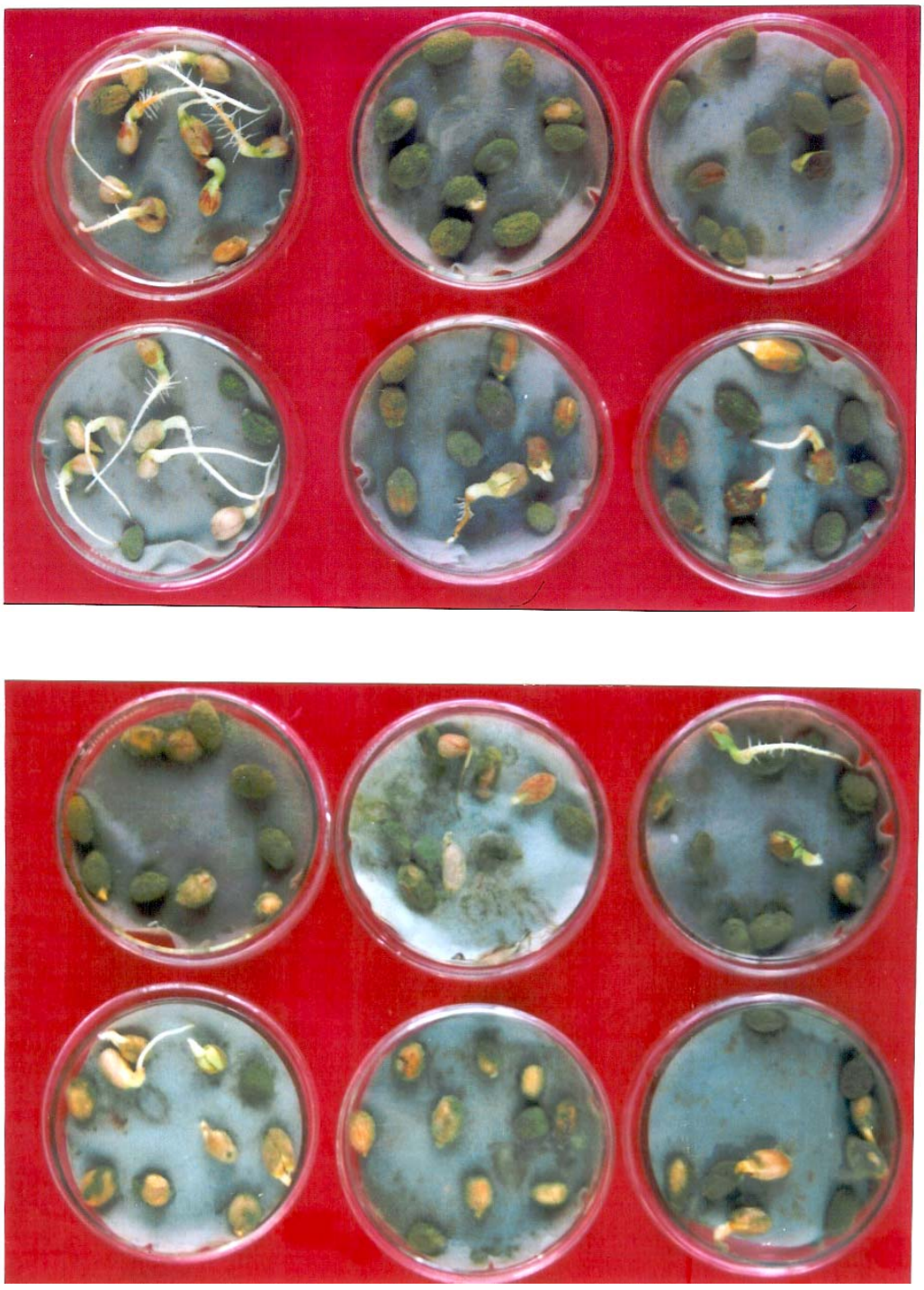
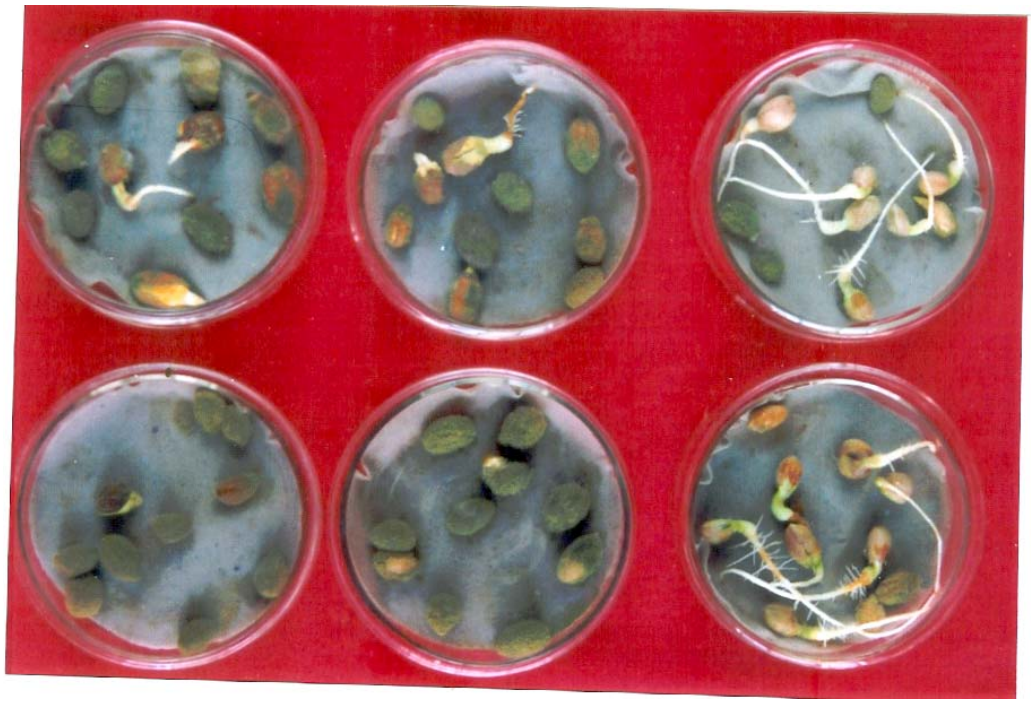


Plate 4. Artificial seed infection of groundnut seeds by *Aspergillus flavus*



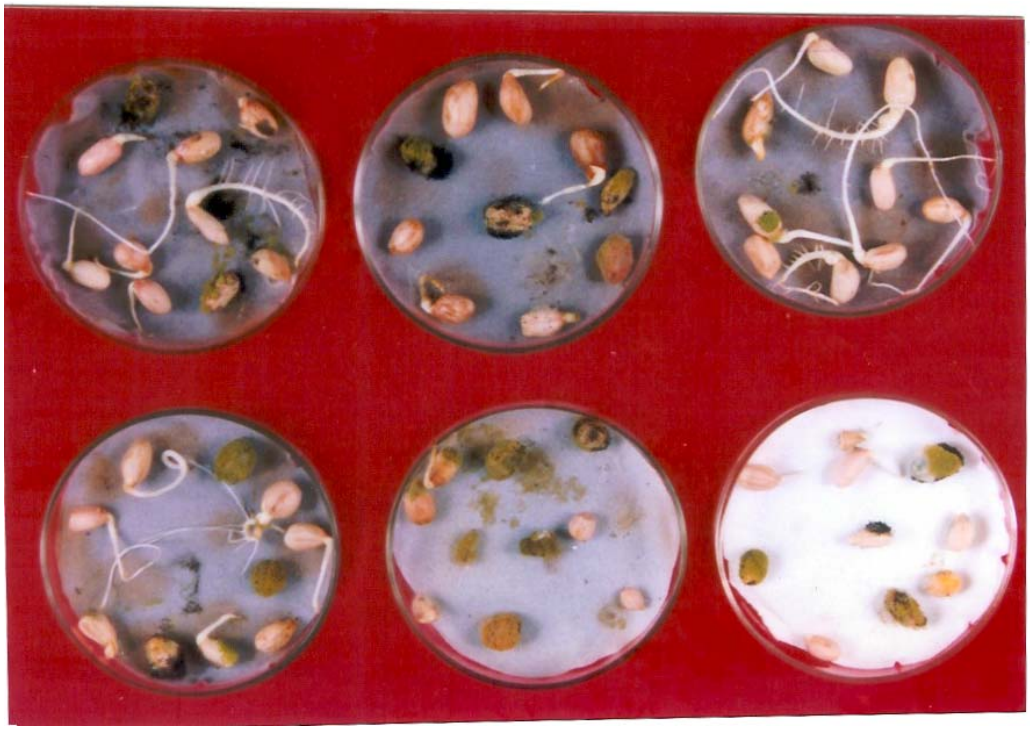


Plate 5. Natural seed infection of groundnut genotypes under drought conditions

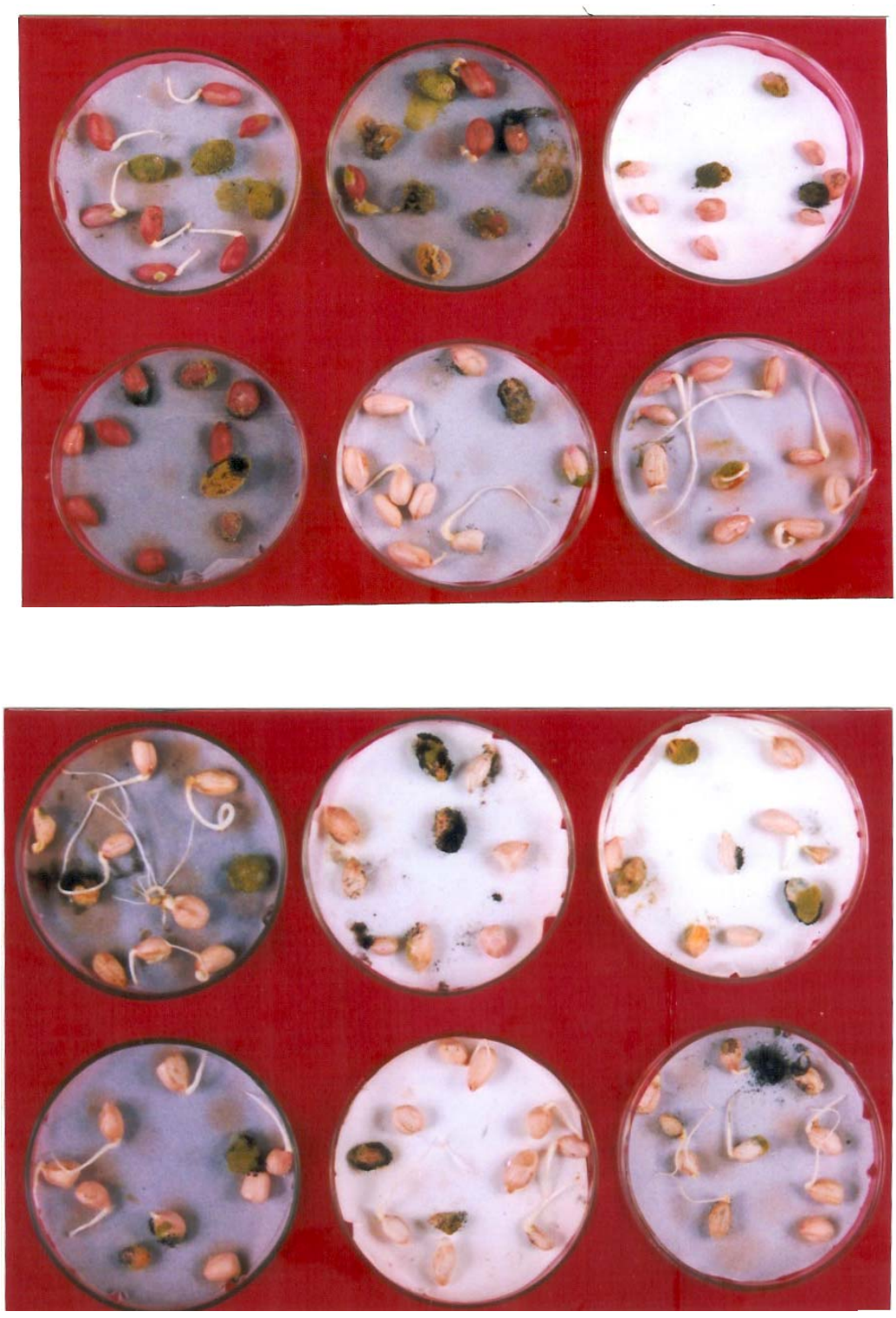


Plate 6. Natural seed infection of groundnut seeds under irrigated conditions

Plate 7. Natural seed infection in Narayani under drought and irrigated conditions

Plate 8. Natural seed infection in Kalahasti under drought and irrigated conditions

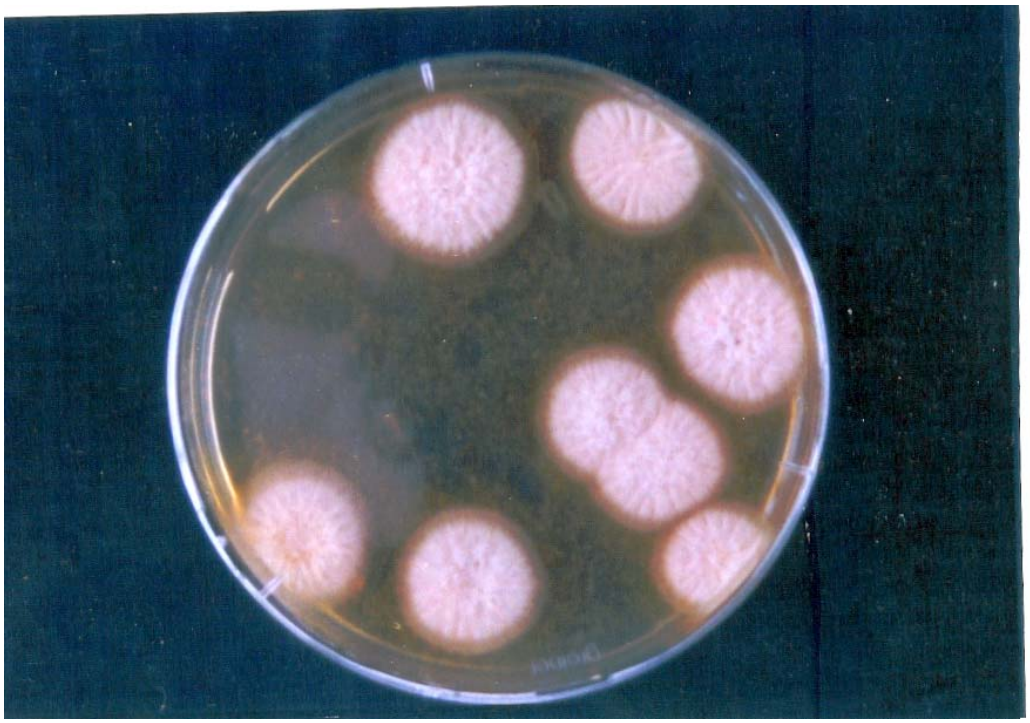


Plate 9. Colony forming units of *Aspergillus flavus* on AFPA medium

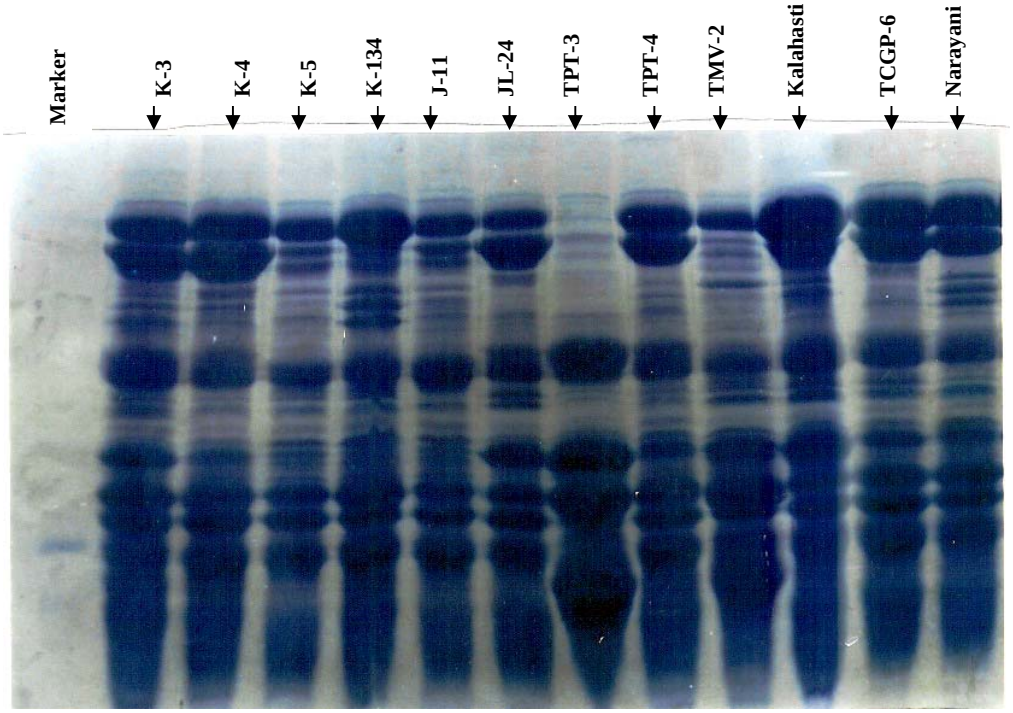


Plate 10. Protein profiles of different groundnut genotypes

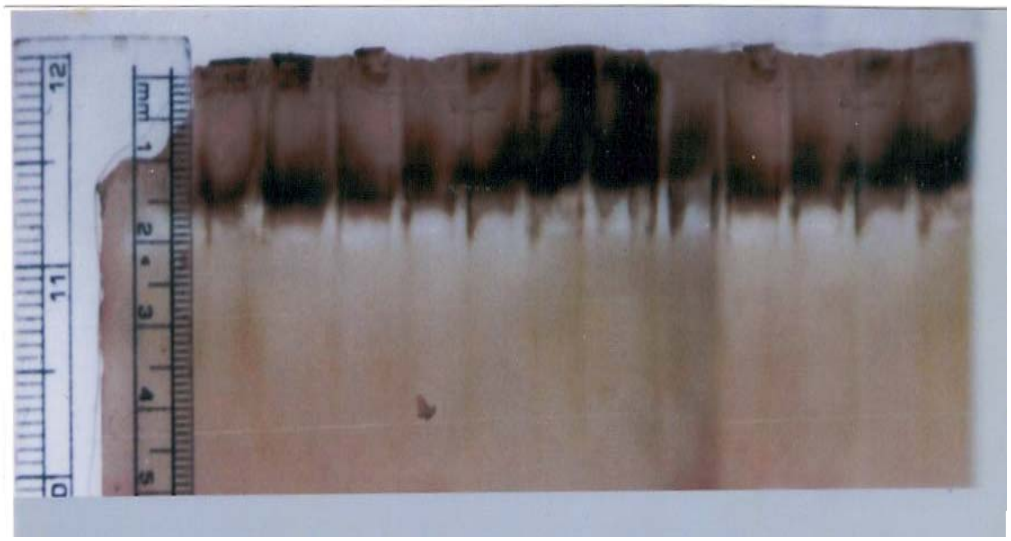


Plate 11. Peroxidase isozyme profiles of different groundnut genotypes

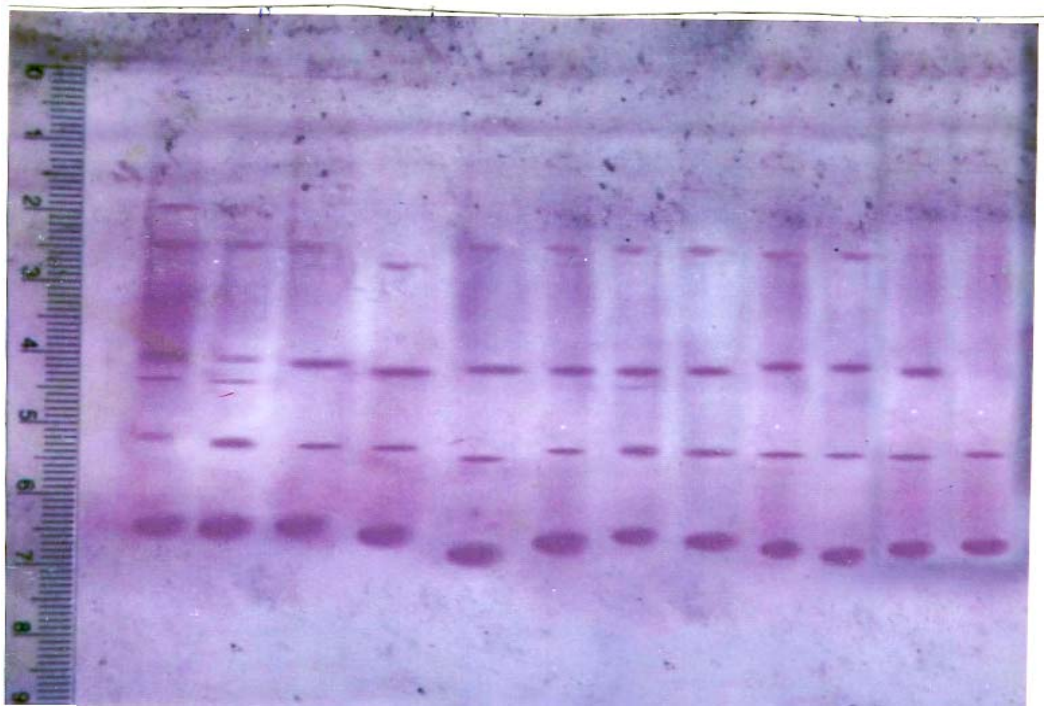


Plate 12. Esterase isozyme profiles of different groundnut genotypes

CHAPTER - IV

RESULTS

The aflatoxin content was estimated by indirect ELISA throughout the experiment as mentioned in section 3.14.

4.1 SCREENING STUDIES

4.1.1 In vitro screening of different groundnut genotypes for aflatoxin production

The *in vitro* screening experimental results were presented in Table 1 (Plate 4).

All the genotypes were susceptible to *Aspergillus flavus* infection and the seeds of all the genotypes appeared visually green coloured due to green coloured conidia of *Aspergillus flavus*. Aflatoxin production was observed in all genotypes and significant difference was observed among genotypes. Highest toxin content was reported in Narayani and TCGP-6 genotypes (>5000 ppb per kg) and lowest in TPT-4 and K-3 (2800 ppb per kg).

4.1.2. *In vivo* screening of different groundnut genotypes for aflatoxin production

The experimental results were presented in Table 2 and Fig.1

Twelve groundnut genotypes were screened under field by artificial application of *A. flavus* to the soil.

The results indicated that there was significant difference in aflatoxin content for both treatments except TPT-4, K-5 and K-134. Higher aflatoxin content was observed under drought conditions compared to irrigated conditions. Genotypic differences were non significant under both stress and irrigated conditions. TPT-3 (2507.3 ppb per kg kernel) and k-4 (2503.4 ppb per kg) recorded high aflatoxin content and TPT-4 (5.1 ppb per kg) recorded low aflatoxin content under drought conditions. Under irrigated conditions K-3 (85 ppb per kg) was observed with the high aflatoxin contamination where as J-11 and k-4 (5 ppb per kg) was recorded the low. The interaction between genotypes and treatments was also non significant.

4.1.3. Natural seed colonization

The experimental results of natural seed colonization were presented in Table 3 and Fig.2 (Plate 5, 6, 7, 8). These experimental results revealed that there is significant difference in seed colonization between the two treatments except for TPT-3 and TPT-4. Seed infection was observed more under drought

conditions compared to irrigated conditions. It was more in Narayani (60%) and less in K-134 (13.3%) under irrigated conditions. Under drought conditions less seed infection was observed in K-3 (20%) and more in Kalahasti (85%). The observed differences were non significant among genotypes under irrigated and drought conditions and the genotypes Kalahasti, TMV-2, TPT-3 having on par values with each other under irrigated conditions. The interaction was also non significant between the genotypes and treatments.

4.2. ESTIMATION OF *Aspergillus flavus* POPULATION IN SOIL

The results on *A.flavus* population in soil were presented in Table 4 (Plate 9).

Before sowing *A. flavus* population was 1.26×10^4 c.f.u per gm soil before sowing both in drought and irrigated conditions. The fungal population was 13.35×10^4 c.f.u and 12.02×10^4 c.f.u per gm of soil under drought and irrigated respectively 10 days after the first application of inoculum. Fungal population of 15.39×10^4 c.f.u and 14.86×10^4 c.f.u was observed per gm of soil under stress and irrigated conditions respectively 10 days after second application of inoculum. At the time of harvesting the fungal population was

observed as 16.62 x 10^4 c.f.u and 15.89 x 10^4 c.f.u per gm of soil under stress and irrigated conditions respectively.

4.3 DETERMINATION OF BIOCHEMICAL CHARACTERS

4.3.1.Estimation of total phenols

The experimental results on total phenols in groundnut seeds after harvesting under drought and irrigated conditions were presented in Table 5 and Fig. 3 & 4.

These results indicated that there is a non-significant difference in total phenolic content in harvested groundnut kernels under irrigated conditions except K-5, TPT-4 and JL-24. The genotypic differences were non-significant under drought conditions for all genotypes except for TPT-3, TCGP-6, K-134, J-11 and K-5. Among the genotypes tested K-5 recorded highest phenols (2.9 mg per gm of seed),where as TMV-2 and K-134 recorded lowest(1.95mg per gm of seed) under irrigated conditions. When stress was imposed Kalahasti reported highest phenolic content (2.82 mg per gm) followed by J-11 (2.67 mg per gm) followed by TPT-4(2.57 mg per g)and TMV-2 showed lowest (1.25 mg per gm) compared to all other genotypes. The interaction between genotypes and treatments was non-significant.

4.3.2.Estimation of total sugars

The sugar content in harvested kernels is presented in the Table 6 and Fig. 5.

The data on sugar content in the harvested groundnut kernels revealed that under drought conditions sugar content was significantly higher in all the genotypes compared to irrigated treatment. The genotypic differences were significant under irrigated and drought conditions except TPT-3 and Narayani. Under irrigated conditions, the total sugars were highest in J-11 (106.3 mg/g) and lowest in TPT-3 (57.5 mg/g).

Under drought conditions highest sugar content was reported in K-4 (166.3 mg/g) and the lowest in Kalahasti (81.3 mg/g) compared to all other genotypes. The interaction between genotypes and treatments was also significant.

4.3.3. Estimation of total proteins

The results of total protein content in harvested kernels was presented in Table 7 and Fig 6. The data on protein content revealed that total proteins were more under irrigated conditions. The genotypic differences were significant under irrigated conditions except TPT-4, J-11, Narayani and K-5.

The genotypic differences were non-significant under drought conditions except K-4 and TMV-2.

Highest proteins were reported high in Kalahasti (185 mg g^{-1}) and low in K-3 (156 mg g^{-1}) under irrigated conditions. Highest proteins were reported in Kalahasti (165 mg g^{-1}) and lowest in K-3 (147.2 mg g^{-1}).

4.3.4. Shellwall Thickness

The results of shellwall thickness of different genotypes were presented in Table 8.

The results revealed that there was no significant difference in shellwall thickness between the two treatments. It was observed that the genotypic differences were also non-significant among genotypes under irrigated and drought conditions except that the genotypes K-3,TMV-2 and Kalahasti. The interaction between genotypes and environment was also non-significant.

4.3.5. Shellwall Integrity

The experimental results were presented in Table 9 and Fig. 7&8. These results indicated that there is a significant difference in shellwall integrity in groundnut kernels at the time of harvesting between irrigated and drought conditions. Shellwall integrity was more under irrigated conditions

when compared to drought conditions. Under irrigated conditions it was highest in the genotype J-11 (94.6 per cent) followed by K-5 (92.6 per cent) and it was lowest in Narayani (70.4 per cent). Under drought conditions also the J-11 showed maximum (88.4 per cent) followed by minimum (64.8 per cent) values respectively. It also indicated that genotypes differed significantly under irrigated and drought conditions. The genotypes TMV-2, TPT-3, K-5 and K-134 showed non-significant difference under irrigated conditions. TPT-4 and K-4 are on par with each other in shell wall integrity under drought conditions. The interaction between genotypes and treatments was also significant.

4.3.6. Kernel Moisture

The data on kernel moisture were presented in Table 10 and Fig. 9. In irrigation treatment kernel moisture was significantly higher in all the genotypes compared to drought conditions. The genotypic differences were non-significant for all genotypes except K-3 and JL-24 under irrigated conditions. Under drought conditions no significant differences were found among genotypes whereas K-3 and K-134 have shown on par values.

Among the genotypes tested, kernel moisture was highest in K-5 (58.8 per cent) followed by relatively J-11 (55.9 per cent) and lowest in K-3 (46.2

per cent) under irrigated conditions. Under drought conditions, the highest kernel moisture was observed in TPT-4 (47.9 per cent) and lowest in TMV-2 (32 per cent). The interaction between genotypes and treatments was also significant.

4.3.7. Podwall Moisture

The data regarding podwall moisture in groundnut seeds during harvesting were presented in Table 10 and Fig. 10. The results showed that, there is significant difference in Podwall moisture between irrigated and drought conditions. Under irrigated conditions, Tirupati-4 showed highest podwall moisture (50.8 per cent) among all genotypes and K-3 showed lowest podwall moisture (41.2 per cent). Under irrigated conditions among all genotypes, Tirupati-4 (50.8 per cent) and J-11 (50.2%) showed high podwall moisture and K-3 (41.2 per cent) showed lowest moisture content. Under drought conditions padwall moisture was high in Tirupati-4 (44.7 per cent) followed by K-5 (39.7 per cent) followed by J-11 (38.2 per cent) and least in Tirupati-3 (27.3 per cent). There is no significant difference among all genotypes under irrigated and drought conditions. However, the genotypes TPT-3 and K-134 having on par values with each other for pod wall moisture under irrigated conditions and K-4, TCGP-6 having on par values under drought conditions.

4.4 QUALITATIVE ANALYSIS OF TOTAL SEED PROTEINS

4.4.1 Electrophoretical analysis of total seed proteins by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

The seed proteins from 12 groundnut genotypes were analysed by SDS-PAGE and presented in Plate 10.

The total proteins are heterogeneous in size ranging in molecular weight from 12 KD to more than 100 KD. Most of the proteins were present in all the genotypes. In Tirupati-3 mainly 4 major bands were observed. However, a significant difference was observed in protein banding profiles among all genotypes under the study. Protein band having a molecular weight of 97.4 KD was present in all genotypes except in Tirupati-3. A protein band of 80 KD was observed in all genotypes and which is less represented in K-5, J-11, TMV-2 and very less in Tirupati-3. The protein bands in a region between 55 KD and 70 KD molecular weight differs qualitatively among genotypes and well expressed in K-134 and less expressed in K-4, K-3, Kalahasti, Narayani, TPT-4 and completely absent in TPT-3. A molecular weight of 43 KD and 30 KD protein was observed in almost all genotypes and well expressed in Tirupati-3. In all genotypes 22 KD protein band was observed but well expressed in TPT-3. A low molecular weight protein having a molecular weight of 12 KD was observed only in TPT-3.

4.4.2 ISOZYME STUDIES

4.4.1. Peroxidases

The banding profiles of peroxidases in different groundnut genotypes are presented in Plate No.11. These results revealed that there is no significant difference in peroxidase bands among the genotypes. A common band at a distance of 1.6 cm from the cathode end was present in all the genotypes. However in J-11 and Tirupati-4 genotypes, intensity of the band appears to be more compared to other genotypes.

4.4.2. Esterases

The esterase banding profiles of all the groundnut genotypes are presented in Plate No.12.

Maximum of six bands designated as E_1 to E_6 were observed in genotypes K-4 and K-5 compared to other genotypes.

The E_1 band at a distance of 1.9 cm from cathode end was seen only in K-4 and K-5. The E_2 band at a distance of 2.3 cm from cathode end was seen in all the genotypes except in K-134 and TMV-2 and in J-11 it is with a distance of 2.8 cm from cathode end. E_3 band at a distance of 4.1 cm from cathode end was present in all genotypes except in TMV-2. The E_4 band at a distance of 4.4 cm from cathode end was presented only in K-4 and K-5. The esterase band E_5 and E_6 was seen in all the genotypes with a small migrational difference in TPT-3.

Table 10 : Percentage of kernel moisture, pod wall moisture and aflatoxin content in different groundnut genotypes at the time of harvesting (expressed in percentage)

S.No.	Genotypes	Kernel moisture		Pod wall moisture		Aflatoxin (ppb)	
		Irrigated	Drought	Irrigated	Drought	Irrigated	Drought
1.	Kalahasti	53.2 (46.8)	42.5 (40.7)	48.6 (44.2)	38.5 (38.3)	22.5 (0..89)	9.5 (1.29)
2.	Narayani	52.3 (46.2)	34.7 (36.1)	46.5 (43.0)	30.7 (33.6)	12.0 (0.74)	1696.3 (1.91)
3.	TMV-2	55.9 (48.4)	32.0 (34.4)	49.6 (44.8)	30.0 (33.2)	11.5 (0.37)	1263.5 (2.15)
4.	TPT-3	52.0 (46.1)	34.1 (35.7)	48.5 (44.1)	27.3 (31.4)	10.5 (0.64)	2507.3 (1.45)
5.	TPT-4	55.3 (48.1)	47.9 (43.2)	50.8 (45.5)	44.7 (41.9)	13.8 (0.58)	5.1 (0.69)
6.	K-3	46.2 (42.8)	39.0 (38.6)	41.2 (39.9)	32.7 (34.9)	85.0 (0.40)	6.8 (0.67)
7.	JL-24	51.0 (45.6)	34.0 (35.7)	44.3 (41.7)	30.1 (33.3)	33.0 (0.86)	400.5 (1.24)
8.	J-11	58.3 (49.3)	42.1 (38.1)	50.2 (45.1)	38.2 (35.8)	5.0 (0.27)	32.0 (0.67)
9.	K-4	52.6 (52.3)	36.3 (37.0)	45.7 (42.3)	30.2 (33.3)	5.0 (0.77)	2503.4 (1.13)
10.	K-5	58.8 (49.9)	47.3 (43.5)	49.3 (44.6)	39.7 (39.0)	22.5 (0.64)	25.5 (1.40)
11.	TCGP-6	54.6 (47.6)	32.3 (34.6)	45.6 (42.5)	30.2 (33.2)	23.0 (0.83)	689.3 (1.23)
12.	K-134	57.5 (49.3)	39.0 (38.6)	49.6 (44.8)	38.0 (38.0)	8.0 (0.60)	15.5 (1.07)
	Mean	54.5 (47.6)	38.3 (38.0)	48.3 (44.0)	34.2 (35.5)	21.0 (0.61)	773.6 (1.24)

Figures in parentheses are angular transformed values for kernel moisture and Pod wall moisture
Figures in parentheses are log aflatoxin values

	Kernel moisture		Angular transformed values		Pod wall moisture		Angular transformed values	
	SEM	**CD (5%)**	**SEM**	**CD (5%)**	**SEM**	**CD (5%)**	**SEM**	**CD (5%)**
Treatments	0.57	1.76	0.34	0.96	0.65	1.85	0.39	1.1
Genotypes	1.41	4.02	0.83	2.35	1.59	4.53	0.59	2.7
Interaction	1.99	5.68	1.17	3.32	2.25	6.41	1.34	3.8
CV (%)	7.5				6.3			

Table 3 : Percentage of seed infection and aflatoxin content in different groundnut genotypes after *in vivo* screening

S.No.	Genotypes	Seed infection		Aflatoxin (ppb)	
		Irrigated	Drought	Irrigated	Drought
1.	Kalahasti	36.5 (36.9)	85.0 (71.8)	22.5 (0..89)	9.5 (1.29)
2.	Narayani	60.0 (46.8)	75.0 (61.8)	12.0 (0.74)	1696.3 (1.91)
3.	TMV-2	36.5 (36.9)	41.5 (39.2)	11.5 (0.37)	1263.5 (2.15)
4.	TPT-3	36.5 (40.1)	38.0 (38.2)	10.5 (0.64)	2507.3 (1.45)
5.	TPT-4	41.5 (40.0)	40.0 (39.1)	13.8 (0.58)	5.1 (0.69)
6.	K-3	15.0 (22.8)	20.0 (21.1)	85.0 (0.40)	6.8 (0.67)
7.	JL-24	27.5 (38.0)	46.5 (43.1)	33.0 (0.86)	400.5 (1.24)
8.	J-11	40.0 (25.4)	75.0 (28.2)	5.0 (0.27)	32.0 (0.67)
9.	K-4	26.5 (30.9)	48.3 (44.0)	5.0 (0.77)	2503.4 (1.13)
10.	K-5	45.3 (42.1)	32.5 (30.2)	22.5 (0.64)	25.5 (1.40)
11.	TCGP-6	25.0 (29.9)	68.3 (55.9)	23.0 (0.83)	689.3 (1.23)
12.	K-134	13.3 (23.4)	60.0 (51.1)	8.0 (0.60)	15.5 (1.07)
	Mean	33.6 (34.4)	52.5 (43.6)	21.0 (0.61)	773.6 (1.24)

Figures in parentheses are angular transformed values for seed infection
Figures in parentheses are log aflatoxin values

	Seed infection values		Angular transformed values	
	SEM	**CD (5%)**	**SEM**	**CD (5%)**
Treatments	2.92	8.32	1.91	5.44
Genotypes	7.16	20.4	4.68	13.32
Interaction	10.13	28.83	6.62	18.84
CV (%) 43.6				

Table 5 : Total phenols and aflatoxin content in different groundnut genotypes after harvesting (expressed in mg/gm of seed)

S.No.	Genotypes	Total phenols under		Aflatoxin (ppb)	
		Irrigated	Drought	Irrigated	Drought
1.	Kalahasti	2.05	2.83	22.5 (0..89)	9.5 (1.29)
2.	Narayani	2.15	1.50	12.0 (0.74)	1696.3 (1.91)
3.	TMV-2	1.95	1.25	11.5 (0.37)	1263.5 (2.15)
4.	TPT-3	2.26	1.80	10.5 (0.64)	2507.3 (1.45)
5.	TPT-4	2.70	2.57	13.8 (0.58)	5.1 (0.69)
6.	K-3	2.12	2.02	85.0 (0.40)	6.8 (0.67)
7.	JL-24	2.45	2.30	33.0 (0.86)	400.5 (1.24)
8.	J-11	2.17	2.67	5.0 (0.27)	32.0 (0.67)
9.	K-4	2.25	2.45	5.0 (0.77)	2503.4 (1.13)
10.	K-5	2.90	2.60	22.5 (0.64)	25.5 (1.40)
11.	TCGP-6	2.33	1.85	23.0 (0.83)	689.3 (1.23)
12.	K-134	1.95	1.95	8.0 (0.60)	15.5 (1.07)
	Mean	2.26	2.15	21.0 (0.61)	773.6 (1.24)

Figures in parentheses are log aflatoxin values

	SEM	CD (5%)
Treatments	0.021	0.597
Genotypes	0.0514	0.146
Interaction	0.072	0.206
CV (%) 5.7		

Table 6 : Total sugars and aflatoxin content in the different groundnut genotypes after harvesting (expressed in mg/gm of seed)

S.No.	Genotypes	Treatments		Aflatoxin (ppb)	
		Irrigated	Drought	Irrigated	Drought
1.	Kalahasti	69.5	77.5	22.5 (0..885)	9.5 (1.290)
2.	Narayani	58.8	105.0	12.0 (0.737)	1696.3 (1.912)
3.	TMV-2	81.3	125.0	11.5 (0.366)	1263.5 (2.149)
4.	TPT-3	57.5	85.0	10.5 (0.641)	2507.3 (1.448)
5.	TPT-4	81.6	107.5	13.8 (0.581)	5.1 (0.690)
6.	K-3	69.4	116.5	85.0 (0.401)	6.8 (0.673)
7.	JL-24	75.6	90.0	33.0 (0.864)	400.5 (1.239)
8.	J-11	106.3	126.3	5.0 (0.271)	32.0 (0.648)
9.	K-4	63.4	166.3	5.0 (0.77)	2503.4 (1.135)
10.	K-5	90.0	111.3	22.5 (0.648)	25.5 (1.405)
11.	TCGP-6	85.5	92.5	23.0 (0.837)	689.3 (1.230)
12.	K-134	62.5	95.0	8.0 (0.60)	15.5 (1.074)
	Mean	78.7	104.5	21.0 (0.619)	773.6 (1.241)

Figures in parentheses are log aflatoxin values

	SEM	CD (5%)
Treatments	0.36	1.027
Genotypes	0.88	2.516
Interaction	1.25	3.558
CV (%)	2.4	

Table 7 : Total seed protein and aflatoxin content in different groundnut genotypes after harvesting (expressed in mg/gm of seed)

S.No.	Genotypes	Treatments		Aflatoxin (ppb)	
		Irrigated	Drought	Irrigated	Drought
1.	Kalahasti	185.0	165.0	22.5 (0..89)	9.5 (1.29)
2.	Narayani	176.0	155.0	12.0 (0.74)	1696.3 (1.91)
3.	TMV-2	182.0	162.0	11.5 (0.37)	1263.5 (2.15)
4.	TPT-3	163.0	148.6	10.5 (0.64)	2507.3 (1.45)
5.	TPT-4	172.0	151.0	13.8 (0.58)	5.1 (0.69)
6.	K-3	156.0	147.2	85.0 (0.40)	6.8 (0.67)
7.	JL-24	165.0	142.0	33.0 (0.86)	400.5 (1.24)
8.	J-11	173.0	154.0	5.0 (0.27)	32.0 (0.67)
9.	K-4	184.0	162.0	5.0 (0.77)	2503.4 (1.13)
10.	K-5	176.0	154.0	22.5 (0.64)	25.5 (1.40)
11.	TCGP-6	179.0	150.0	23.0 (0.83)	689.3 (1.23)
12.	K-134	180.0	152.0	8.0 (0.60)	15.5 (1.07)
	Mean	174.2	153.5	21.0 (0.61)	773.6 (1.24)

Figures in parentheses are log aflatoxin values

	SEM	CD (5%)
Treatments	0.2859	0.8257
Genotypes	0.7003	2.0225
Interaction	0.9904	2.8602
CV (%) 4.4		

Table 9 : Shell wall integrity in different groundnut genotypes at the time of harvesting (expressed in percentage)

S.No.	Genotypes	Treatments		Aflatoxin (ppb)	
		Irrigated	Drought	Irrigated	Drought
1.	Kalahasti	84.5 (66.8)	79.3 (62.9)	22.5 (0..89)	9.5 (1.29)
2.	Narayani	70.4 (57.0)	64.8 (59.8)	12.0 (0.74)	1696.3 (1.91)
3.	TMV-2	78.2 (62.1)	76.3 (60.8)	11.5 (0.37)	1263.5 (2.15)
4.	TPT-3	78.4 (62.3)	72.4 (58.3)	10.5 (0.64)	2507.3 (1.45)
5.	TPT-4	81.5 (64.5)	80.2 (63.5)	13.8 (0.58)	5.1 (0.69)
6.	K-3	83.4 (65.9)	78.4 (62.3)	85.0 (0.40)	6.8 (0.67)
7.	JL-24	85.2 (67.3)	75.4 (60.2)	33.0 (0.86)	400.5 (1.24)
8.	J-11	94.6 (76.5)	88.4 (70.0)	5.0 (0.27)	32.0 (0.67)
9.	K-4	89.4 (71.0)	80.2 (63.5)	5.0 (0.77)	2503.4 (1.13)
10.	K-5	92.6 (74.2)	84.3 (66.6)	22.5 (0.64)	25.5 (1.40)
11.	TCGP-6	79.8 (63.2)	72.3 (58.2)	23.0 (0.83)	689.3 (1.23)
12.	K-134	92.2 (73.7)	86.4 (68.3)	8.0 (0.60)	15.5 (1.07)
	Mean	84.41 (67.04)	78.2 (62.8)	21.0 (0.61)	773.6 (1.24)

Figures in parentheses are log aflatoxin values

	Shell wall integrity		Angular transformed values	
	SEM	**CD (5%)**	**SEM**	**CD (5%)**
Treatments	0.177	0.505	0.135	0.358
Genotypes	0.435	1.237	0.332	0.944
Interaction	0.615	1.75	0.469	1.335
CV (%) 5.6				

Table 8 : Shell wall thickness in different groundnut genotypes after harvesting (expressed in mm)

S.No.	Genotypes	Drought	Irrigation
1.	Kalahasti	0.95	0.97
2.	Naryani	0.56	0.55
3.	TMV-2	0.7	0.7
4.	TPT-3	1.12	1.12
5.	TPT-4	0.55	0.56
6.	K-3	0.45	0.46
7.	JL-24	1.22	1.22
8.	J-11	0.57	0.59
9.	K-4	0.55	0.55
10.	K-5	0.55	0.56
l1.	TCGP-6	1.08	1.10
12.	K-134	1.22	1.2

		SEM	CD (5%)
Treatments		0.003	Non-significant
Genotype		0.007	0.021
Interaction		0.011	Non-significant
CV (%)	4.3		

Table 2 : Aflatoxin content (AFB_1) in seeds of different groundnut genotypes after *in vivo* screening

S.No.	Genotypes	Aflatoxin (ppb per kg)	
		Irrigated	Drought
1.	Kalahasti	22.5 (0..89)	9.5 (1.29)
2.	Narayani	12.0 (0.74)	1696.3 (1.91)
3.	TMV-2	11.5 (0.37)	1263.5 (2.15)
4.	TPT-3	10.5 (0.64)	2507.3 (1.45)
5.	TPT-4	13.8 (0.58)	5.1 (0.69)
6.	K-3	85.0 (0.40)	6.8 (0.67)
7.	JL-24	33.0 (0.86)	400.5 (1.24)
8.	J-11	5.0 (0.27)	32.0 (0.67)
9.	K-4	5.0 (0.77)	2503.4 (1.13)
10.	K-5	22.5 (0.64)	25.5 (1.40)
11.	TCGP-6	23.0 (0.83)	689.3 (1.23)
12.	K-134	8.0 (0.60)	15.5 (1.07)
	Mean	21.0 (0.61)	773.6 (1.24)

Figures in parentheses are log aflatoxin values

	Aflatoxin values		Log aflatoxin values	
	SEM	**CD (5%)**	**SEM**	**CD (5%)**
Treatments	42.2	12.03	0.136	Non-significant
Genotypes	103.5	Non-significant	0.334	Non-significant
Interaction	146.4	Non-significant	0.472	Non-significant
CV (%)	320		87.9	

Table 4 : ***Aspergillus flavus*** **population at different days after inoculation under Drought and irrigated condition (c.f.u x 10^4 / gm. of soil)**

	Drought	**Irrigated**
Before sowing	1.26	1.26
After first inoculation (40 DAS)	13.35	12.20
After second inoculation (70 DAS)	15.39	14.86
At the time of harvesting	16.62	15.89

DAS : Days after sowing.

Table 1 : Aflatoxin (AFB_1) content in different groundnut genotypes after *in vitro* screening (expressed in ppb per kg)

S.No.	Genotypes	Aflatoxin(ppb)
1.	Kalahasti	3333
2.	Narayani	5000
3.	TMV-2	4200
4.	TPT-3	3600
5.	TPT-4	2800
6.	K-3	2800
7.	JL-24	4200
8.	J-11	3667
9.	K-4	4200
10.	K-5	4200
11.	TCGP-6	5000
12.	K-134	3100
	Mean	3833.6

	SEM	CD (5%)
Genotypes	82.35	240.4
CV (%)	18.8	

CHAPTER - V

DISCUSSION

Groundnut (*Arachis hypogea L.*) is an important oil seed crop, grown widely in India. It is mainly used as source of edible oil. In India groundnut is grown in an area of about 8.3 million ha with a production of 6.4 million tonnes. The crop is affected by several diseases. Aflatoxins produced by *Aspergillus flavus* is one of the major problems. These are highly carcinogenic, immuno suppressive agents. It is hindering the groundnut export into other countries because of highly toxic nature.

The available management practices are not economical and no effective method has been found to reduce the aflatoxin production in groundnut. Management of aflatoxin contamination is possible through host plant resistance. In the present study, twelve groundnut genotypes against aflatoxin production were screened and the nature of biochemical and molecular mechanisms were also studied.

Twelve groundnut genotypes were screened against aflatoxin production both under *in vitro* and *in vivo* conditions. Aflatoxin B_1 (AFB_1) was quantified in groundnut kernels by using indirect ELISA method. Anjaiah *et al.,* (1989) also estimated AFB_1 toxin, upto 50 picogram in different

groundnut samples through ELISA method. All the groundnut genotypes under the study were found to be highly susceptible to *A.flavus* infection and aflatoxin production under *in vitro* conditions. In all the genotypes infection was 100 per cent and aflatoxin content was above 2,800 ppb (Table 1). It is evident from the results that all the 12 genotypes prone to severe infection and aflatoxin production, when groundnut kernels were artificially inoculated with *A. flavus*. Mehan *et al.,* (1986) evaluated 502 mature, undamaged peanut genotypes against *A.flavus* infection under *in vitro* conditions and also found them susceptible. Naguib *et al.,* (1990) evaluated 21 groundnut cultivars from ICRISAT and four from Egypt against to *A.flavus* infection and found them susceptible and also observed aflatoxin B_1 and B_2 in all the cultivars.

Under *in vivo* conditions TPT-3 and K-4 (2507.3 ppb per kg kernel) recorded high aflatoxin content and TPT-4 (5.1 ppb) recorded low aflatoxin under drought conditions. Under irrigated treatment K-3 (85 ppb) with high aflatoxin content and J-11 and K-4 (5 ppb) recorded low aflatoxin content. Significant treatmental difference in level of aflatoxin content was observed by all the 12 genotypes except TPT-4, K-5 and K-134. It clearly shows that all the 12 genotypes except TPT-4, K-5 and K-134 have responded to the imposed treatments and significantly different levels of aflatoxin was produced by each genotype in two different treatments. Level of *A. flavus*

infection and aflatoxin contamination are related primarily to environmental conditions especially to drought stress during pod maturation, soil insect pod damage, leaf drop due to foliar diseases and antagonistic mycoflora present in the soil. Hence, the levels of *A. flavus* seed infection can not be directly correlated to the aflatoxin production (Davidson et al., 1982).

In the present studies the *A. flavus* seed infection is not reflecting the levels of aflatoxin contamination since, the aflatoxin production depends on many other environmental factors besides *A. flavus* infection.

Under irrigated conditions Narayani showed high (60%) and K-134 showed low (13.3%) seed infection in natural seed colonization. Whereas under imposed drought conditions Kalahasti produced high (85%) and K-3 produced low (15%) seed infection. However natural seed colonization has no correlation with aflatoxin production in the genotypes. Similar results were reported by Priyadarshini and Tulpule (1978) who studied the reaction of different varieties of maize and groundnut against Aspergillus flavus and stated that there was no correlation between fungal infection and aflatoxin production and fungus produced different amounts of aflatoxin per unit of fungal growth.

These results revealed that despite of pathogen invasion in the groundnut kernels, aflatoxin is not necessarily produced. Probably biochemical constituents of kernel and genotypic, environmental conditions are playing role in provocating fungus to produce toxin. However it needs further investigation. Desai *et al.*, (1991) evaluated the 39 different groundnut varieties, breeding lines against *A.flavus* infection and observed significant differences in infection and aflatoxin production.

In the present investigation high natural seed infection was observed under simulated drought conditions compared to irrigated conditions probably under drought conditions groundnut pods secretes leachets which attract the fungus for infection. However it needs in depth investigation to know the biochemical nature of secretions.

To maintain sufficient *Aspergillus flavus* populations in field experiment, inoculum was applied to soil at 30 and 60 DAS. The quantitative measurements were made to confirm the load. The initial fungal population was 1.2×10^4 c.f.u per g of soil under drought and irrigated conditions. The fungal population increased to 13.35×10^4 c.f.u and 12.02×10^4 c.f.u under drought and irrigated conditions respectively at 10 days after the first application of inoculum to the soil. However, slight increase in fungal

population was observed both under drought and irrigated conditions 10 days after second application of inoculum. The fungal population at the time of harvesting was 16.62 x 10^4 c.f.u and 15.89 x 10^4 c.f.u per g of soil under drought and irrigated conditions respectively. Further, these results indicated that single application of *A. flavus* results may be adequate for *in vivo* screening. Since there is no significant increase in *A. flavus* population between first and second application.

Pitt *et al.*, (1980) estimated *A.flavus* population by using AFPA medium in Australia from 300 soil samples. Nearly 90 per cent of the samples from soil in which groundnut had grown showed 100 to 5000 spores per g. of soil. Among 30 virgin soil samples, only 3 samples contained *A.flavus.* In the present studies also AFPA medium was used for the estimation of *A. flavus* population.

Phenols have long been associated with passive and active defence responses of plants. Because of their accumulation in both compatible and incompatible interactions, the relative contribution of any group or class of phenols to expression of resistance or class of phenols or ultimate reaction of pathogen development in compatible reactions remains in question (Nicholson and Hammerschmidt, 1992). High phenolic content in K-5 (2.900 mg/g,) and

low in TMV-2 and K-134 (1.950 mg/gm) were observed under irrigated conditions. Under drought conditions high phenol content in Kalahasti (2.825 mg/kg) followed by J-11,K-5 and TPT-4 and low phenols in TMV-2 (1.250 mg/kg) were observed. Negative correlation between phenolic content and aflatoxin production particularly under drought conditions was observed in majority of the genotypes.

These results are in agreement with the findings of Premalathasingh *et al.*,(1990) who evaluated 38 different cultivars of pulses and found negative correlation between aflatoxin production and total phenol content.

Several workers implicated phenols as resistant factor (Mahadevan 1966 and Sridhar 1972). They are highly reactive on oxidation and may result in the formation of substances highly toxic to pathogens (Patil and Dimond, 1967). This stimulation of active defence reaction by oxidation of phenols may be feable in susceptible variety.

Thus, in the present study a positive correlation between the phenolic content and disease resistance under drought conditions could be observed in majority of the resistant genotypes .

In the present investigation, high total protein content was observed in Kalahasti (185 mg/g) and low in K-3 (156 mg/g) under irrigated conditions. Under drought conditions also Kalahasti and K-3 reported maximum (165 mg/g) and minimum (147.2 mg/g) protein content. Reduction in protein quantity was observed in all genotypes. It may be due to proteins were degraded into low molecular weight compounds and finally into carbon and nitrogen components which favours fungal growth..These results are in agreement with Cherry *et al.*, (1978) reported that the lower quantity of total soluble proteins in *A.flavus* infected seeds of groundnut at 4 DAI compared to control under *in vitro* conditions.

The present study results indicated that there is no relationship between total protein content and aflatoxin production.

In the present study more quantity of sugars are found in K-4 (166.3 mg/g of seed) and less quantity in Kalahasti (77.5 mg/g of seed) under drought conditions. Under irrigated conditions more amount of sugars were found in J-11 (106.3 mg/g of seed) and lowest in Tirupati-3 (57.5mg/g of seed). Under simulated drought conditions high sugar content in kernels was recorded irrespective of genotypes, compared to irrigated conditions. This indicated the possibility of attracting fungus to invade and infect under such

conditions. Our results are in agreement with Ghewande *et al.,* (1993) showing no correlation between sugars and aflatoxin production.

Podwall moisture and kernel moisture were observed high in genotype K-5, followed by relatively J-11 and TPT-4 and less in K-3 under irrigated conditions and under drought conditions Tirupati-4 and J-11 maintained high kernel moisture and TMV-2 had low kernel moisture. The results revealed that under imposed drought conditions the genotypes which maintained high pod and kernel moisture had low aflatoxin contamination. Cole *et al.,* 1993 reported similar results that under extended drought conditions groundnut genotypes which are having the ability to maintain high kernel moisture showed enhanced resistance and produced low aflatoxin. Rucker *et al.,* 1995 demonstrated a good correlation between a range of drought tolerance characteristics and aflatoxin contamination. Cole *et al.,* 1995 reported that kernel moisture content and temperature are related to aflatoxin production in susceptible groundnut genotypes during pre-harvest and post-harvest conditions.

Because of the high correlation between kernel moisture and preharvest aflatoxin contamination (Dorner *et al.,* 1989), there is scope to exploit the relationship to reduce or possibly eliminate, preharvest afaltoxin

contamination by identifying germplasm with capacity to maintain high kernel water activity during severe drought. In the present study, the genotype Tirupati-4 contained high kernel moisture under drought conditions with lowest aflatoxin production. The Tirupati-4 genotype could be a candidate to include in breeding programme in evolving drought tolerant varieties.

In the present study, shell wall thickness has no correlation with aflatoxin contamination. Because genotypes with high shell wall thickness also showed high aflatoxin content. It indicated that high shell wall thickness will not resist the entry of fungus.

However, results of shell wall integrity showed weak negative correlation with aflatoxin production in most of the genotypes. J-11 and Naryani showed maximum and minimum shell wall integrity values under both irrigated and imposed drought conditions respectively. Aflatoxin content in kernels was quantified accordingly under both conditions. These results indicated that the genotype which maintain high shell wall integrity, especially under drought conditions, can withstand aflatoxin contamination to some extent. However, the mechanism of invasion of pathogen into shell wall does not known, it needs further investigation.

The total seed proteins from all the genotypes under the study were analysed by SDS-PAGE in order to observe the qualitative differences in protein content in anticipation that it may be correlated to aflatoxin production.

In the present investigation, qualitative and quantitative differences were observed in protein profiles of different genotypes. However, a protein having molecular weight of 97.4 KD was absent but 22KD protein was expressed in genotype Tirupati-3. Moreover, most of the proteins are absent in Tirupati-3 in region between 55 to 70 KD. The aflatoxin analysis results indicates that Tirupati-3 is highly susceptible under drought conditions (Table). The presence of 22 KD and 12 KD protein may be used as a marker in identification of susceptible varieties.

In the present study, the variety Tirupati-4 contained high kernel moisture under drought condition with lowest aflatoxin production. The Tirupati-4 genotype could be a candidate to include in breeding programme in evolving drought tolerant varieties.

In the present study, J-11 followed by K-5 showed maximum shell wall integrity and Narayani showed minimum shell wall integrity under both

drought and irrigated conditions. There was no significant correlation between the shell wall integrity, shell wall thickness and aflatoxin production among the varieties under the study.

Bianchi-Hall *et al.,* (1979) reported a large amount of variability in protein composition in peanuts and should aid in defining polygenic relationship in Arachis.

It was observed that there is no significant difference in peroxidase activity among genotypes. Polymorphism for esterase was observed among genotypes indicating the genetic variability. However in J-11, E_2 band at a distance of 2.8 cm may be responsible for its low aflatoxin production. Whereas these isozyme banding pattern can not be linked to resistance or susceptibility to *A. flavus* infection and aflatoxin production.

The present investigation revealed that under imposed drought conditions, seed infection and aflatoxin production in groundnut kernels was high compared to irrigated conditions in most of the genotypes under drought conditions, total sugar content was high in all the genotypes. Since most of the fungal pathogens get attracted to sugar substrates *Aspergillus flavus* may also

preferring this condition for invasion. Similarly, during drought conditions, pod wall and kernel moisture were reduced compared to irrigated conditions. During end of season drought conditions, kernel moisture content is decreased and protection from natural defence mechanisms is lost, making the kernel vulnerable to colonization by the *A.flavus* fungus and aflatoxin contamination. Under high moisture conditions phytoalexin production may provide a resistance mechanism to prevent spore germination and hypha extension of *A.flavus.*

Among the genotypes screened J-11 and TPT-4, K-5 maintained relatively low aflatoxin content in kernels under irrigated and drought conditions respectively. This can be supported with the findings that these genotypes maintained high phenol content for resistance mechanism and relatively high shell wall integrity and high pod kernel moisture content.

From the results Tirupati-4 is the most suitable genotype for *kharif* cultivation while J-11 is the ideal genotype for *rabi* groundnut areas with low aflatoxin risk.

CHAPTER - VI

SUMMARY

Groundnut is a major oil seed and food crop of the semi arid tropics. Aflatoxins produced by *Aspergillus flavus* and *A.parasiticus* is the major problem hindering export of groundnut. These are also carcinogenic and immunosuppressive agents in humans and livestock. No germplasm source is available that is completely immune to this disease so far in the world. *A. flavus* is a soil born pathogen and aflatoxin production is affected by several factors. All the strains of *A.flavus* do not produce aflatoxin. The available management practices are not economical to control the disease. The only economic and ecofriendly way is the host plant resistance.

Severe strain of *A.flavus* was obtained from Regional Agricultural Research Station, Tirupati and subcultured on PDA and used throughout the experiment.

Twelve groundnut genotypes viz., Kalahasti, Narayani, TMV-2, Tirupati-3, Tirupati-4, K-3, JL-24, J-11, K-4, K-5, TCGP-6 and K-134 were screened against aflatoxin production under *in vitro* and *in vivo* conditions.

Under *in vitro* conditions all the genotypes were highly susceptible to fungal infection and high aflatoxin contamination.

Sufficient *Aspergillus flavus* spore load was maintained in the soil to infect the groundnut seeds by artificial inoculation. The *Aspergillus flavus* population was more in drought when compared to irrigated.

The 12 genotypes were tested for seed infection and aflatoxin (AFB_1) content after harvest. Percentage of seed infection was high under imposed drought conditions compared to irrigated conditions. It was observed that genotypes K-3 and K-134 showed low seed infection under drought and irrigated conditions respectively. Aflatoxin content was low in J-11 and Tirupati-4 under irrigated and drought conditions. However, percentage of seed infection and aflatoxin content in kernels had no correlation.

Among biochemical characters studied, phenols accumulation was not influenced by drought or irrigated conditions. Under imposed drought conditions phenols had negative relation with aflatoxin content i.e. those genotypes accumulated higher phenol content had low aflatoxin contamination. Possibly due to increased disease resistance mechanism. Total sugars accumulated high under drought conditions in all the genotypes and

which may be attracting the fungus for invasion.. Among the pod characteristics studied shell wall thickness had no correlation in aflatoxin production among the genotypes. Shell wall integrity, pod wall moisture kernel moisture had week negative correlation with aflatoxin content specially under drought conditions.

J-11 had high shell wall integrity and low aflatoxin content under irrigated conditions, whereas TPT-4 maintained relatively higher shell wall integrity and high shell and kernel moisture had low aflatoxin contamination under drought conditions.

The SDS-PAGE analysis of total seed proteins indicated presence of most of the proteins in all the genotypes. However, significant differences were observed in protein profiles in different genotypes under the study and Tirupati-3 was significantly differed from all the genotypes. High molecular weight protein having molecular weight of 97.4 KD and the protein bands in the region of 55 to 70 KD was absent in TPT-3.A low molecular weight of protein 12 KD was absent in all the genotypes and it is very well expressed in Tirupati-3. No significant differences were observed for peroxidase isozyme among the genotypes. Significant differences were observed among the

genotypes. The presence of E_2 band in J-11 may be responsible for its low aflatoxin production.

Finally, among genotypes tested Tirupati-4 showed low aflatoxin content under drought conditions and J-11, K-4 showed low aflatoxin content under irrigated conditions. However, K-4 was highly susceptible to aflatoxin production under drought conditions. These results can be well supported with the findings that these genotypes (J-11 and Tirupati-4) maintained high phenol content for disease resistance mechanism and relatively high shell wall integrity and high kernel and podwall moisture content.

TPT-4 can be recommended in drought prone areas of *kharif*, whereas J-11 is ideal for irrigated *rabi* area of Andhra Pradesh, to reduce the aflatoxin risk.

LITERATURE CITED

Abouzied M M, Azcona J I, Braselton W E and Pestka J J 1991 Immunochemical assessment of mycotoxins in 1989 Grain foods: evidence of deoxynivalenol (Vomitoxin) contamination. Applied and Environmental Microbiology **57 (3):** 672-677.

Aldao M A J, Carpinella M C, Corelli M and Herrero G G 1995 Competitive ELISA for quantifying small amounts of aflatoxin B_1 Food and Agricultural Immunology **7(4):** 307-314.

Anahosur K H, Patil S H and Hedge R K 1985 Relationship of salt sugars and phenols with charcoal rot of sorghum. Indian Phytopathology **38:** 335-337.

Anderson W F, Holbrook C C and Wilson D M 1996 Development of greenhouse screening for resistance to *Aspergillus parasiticus* infection and pre-harvest aflatoxin contamination in peanut. Mycopathologia **135:** 115-118.

Anderson W F, Holbrook C C, Wilson D M and Matheron M E 1995 Evaluation of pre-harvest aflatoxin contamination in several potentially resistant peanut genotypes. Peanut Science **22:** 29-32.

Anjaiah V, Mehan V K, Jayanthi S, Reddy D V R and Mc Donald D 1989 Enzyme linked immunosorbent assay (ELISA) for aflatoxin B_1 estimation in groundnuts. Aflatoxin contamination of groundnut. Proceedings of the international workshop, ICRISAT Center, Patancheru-502 324, Andhra Pradesh, India pp.183-189.

Arora Y K and Bajaj K L 1985 Peroxidase and Polyphenol oxidase associated with induced resistance of mungbean to *Rhizoctonia solani* kuhn. Phytopathology **114:** 325-331.

Ayerst G and Budd D B 1960 Effect of moisture content on the storage of Brazil nuts. Journal of Science of Food and Agriculture **11:** 390-396.

Azer M and Cooper C 1991 Determination of aflatoxins in foodstuffs by HPLC and immuno sorbent assay system. Journal of Food Protection **54(4):** 291-294.

Balasubramanian P and Narayanaswamy P 1991 Post infectional changes in groundnut leaves induced by groundnut blight pathogen. Madras Agricultural Journal **78:** 44-48.

Bartz Z A, Norden A J, Lapnade J C and Demuynk T J 1978 Seed tolerance in peanut *(Arachis hypogea* L.) to members of the *Aspergillus flavus* group of fungi. Peanut Science **5:** 53-56.

Basha S M and Pancholy S K 1986 Qualitative and quantitative changes in the protein composition of peanut (*Arachis hypogea* L.) seed following infestation with *Aspergillus* spp. differing in aflatoxin production. Journal of agriculture and food chemistry. **34:** 638-643.

Bhatia I S, Uppal D S and Bajat K C 1972 Study of phenolic contents of resistant and susceptible varieties of tomato *Lycopersicon esculentum* in relation to early blight diseases. Indian Phytopathology **25:** 231-235.

Bhatia J N and Takur D P 1994 Biochemical components of pearl millet in relation to downy mildew disease. Indian Journal of Mycology and Plant Pathology **24:** 216-219.

Bhavani U, Venkatasubbiah K, Sudhakara Rao A and Sai Gopal D V R 1998 Studies on mosaic disease of sunflower: Biochemical changes and growth parameter. Indian Phytopathology **51:** 357-358.

Bhite B R, Chavan J K and Kachara D P 1997 A biochemical marker for resistant to sterility mosaic disease in pigeon pea. Journal of Maharashtra Agricultural University **22:** 340-341.

Bianchi-hall and Thomson R C 1979 Protein deterioration during seed storage of peanuts. Electophoretic analysis of qualitative and quantitative variations. Journal of Agriculture and Food Chemistry **27 (1):**112-115.

Bilgrami K S, sinha K K and Anjana Singh 1983 Chemical changes in dry fruits during aflatoxin elaboration by *Aspergillus flavus* Link. Ex. fries. Current Science **52 (20):** 960-963.

Blankenship P D, Cole R J and Sanders T H 1985 Comparative susceptibility of four experimental peanut lines and florunner cultivar to preharvest aflatoxin contamination. Peanut Science **12:** 70-72.

Brim C A, Ushanis S A and Tester C F 1969 Organ specificity and genotypic differences in isoperoxidases of soyabean. Crop Science **9:** 843-845.

Candela M E, Munoz R, Alcazar M D and Espin A 1994 Isoperoxidase involvement in the resistance of *Capsicum annum* to infection by cucumber mosaic virus. Journal of Plant Physiology **143:** 213-217.

Candlsih A A G, Stimson W H and Smtih J E 1987 The detection of aflatoxin B_1 in peanut kernels, peanut butter and maize using a monoclonal antibody based enzyme immunoassay. Food Microbiology **4(2):** 147-153.

Chahal S S, Kumar R, Sidhu J S and Minocha J L 1988 Peroxidase isoenzyme pattern in pearl millet lines resistant and susceptible to downy mildew. Plant Breeding **101:** 256-259.

Chattopadhyay A K 1989 Relationship of phenols and sugars in *Alternaria* blight resistance of rapeseed and mustard. Indian Journal of Mycological Research **27:** 195-199.

Chattopadhyay S B and Bera A K 1980 Phenols and polyphenol oxidase activity in rice leaves infected with *Helminthosporium oryzae.* Phytopathology **98:** 59-63.

Cherry J P, Beuchat L R and Kochler P E 1978 Soluble proteins and enzymes as indicators of change in peanuts infected with *Aspergillus flavus.* Journal of Agricultural and Food Chemistry **26(1):** 242-245.

Cherry J P, Beuchat L R and Young C T 1976 Protein and aminoacid changes in peanut (*Arachis hypogaea* L.) seeds infected with *Aspergillus oryzae*. Journal of Agriculture and Food Chemistry **24(1):** 79-85.

Cherry J P, Mayne R Y and Ory R L 1974 Proteins and enzymes from seeds of *Arachis hypogaea* L. XI Electrophoretically detected changes in 15 peanut cultivars grown in different areas after inoculation with *Aspergillus parasitcus.* Physiological Plant Pathology **4:** 425-434.

Cherry J P, Neucere N J, Ory R L 1966 Comparison of proteins of peanuts grown in different areas 1. Disc electrophoretic analysis of qualitative and quantitative variations. Nature **202:** 1305-1308.

Cherry J P, Young C T and Beuchat L R 1975 Changes in proteins and total amino acids of peanuts (*Arachis hypogaea*) infected with *Aspergillus parasisticus.* Canadian Journal of Botany **53:** 2639-2649.

Chiou R Y, Wen Y Y, Ferng S and Lean S P 1999 Mould infection and aflatoxin contamination of the peanut kernels harvested from spring and fall crops as affected by artificial inoculation of the seeded kernels with *Aspergillus flavus* and *Aspergillus niger*. Journal of the Science of Food and Agriculture. **79:** 1414-1422.

Chowdhury A K 1995 Biochemical changes associated with induction of resistance in groundnut plants to *Puccinia arachidis* by seed treatment with non conventional chemicals. Indian Journal of Mycology and Plant Pathology **25:** 231-234.

Chu F S, Lee R C, Trucksess M W and Park D L 1988 Evaluation by ELISA of cleanup for TLC of aflatoxin B_1 in corn, peanuts and peanut butter. Journal of Association of Official Analytical Chemists **71(5):**955-956.

Cole R J, Dorner J W and Holl brook C C 1995 Advances in mycotoxin elimination and resistance. In advances in peanut science. American Peanut Research and Education Society 13 : 456-474.

Cole R J, Dorner J W, Kirtsey J W and Dowell F E 1988 Comparision of visual, ELISA screening and HPLC methods in detecting aflatoxin in farmers stock peanut grade samples. Peanut Science **15 (2):** 61-63.

Cole R J, Sanders T H and Dorner J W 1985 Mean geocarpospehre temperatures that induce aflatoxin contamination of peanuts under drought. Mycopathologia **91:** 41-46.

Cole R J, Sobolev V S and Dorner J W 1993 Potentially important sources of resistance to prevention of preharvest aflatoxin contamination in peanuts. Proceedings of American Research and Education Society 78 (Abstract).

Davidson J I, Hill R A, Cole R A, Mixon A C and Henning R J 1982 Field performance of two peanut cultivars relative to resistance to invasion by *A. flavus* and subsequent aflatoxin contamination. Proceedings of the American Peanut Research and Education Society **14 (1)**: 74-78

Desai S , Ghewande M P, Nagaraj G, Naryan P, Chauhan S and Singh H 1991 Screening for resistance to *Aspergillus flavus* and aflatoxin production in groundnut. Mycotoxin Research **(7) 2:** 79-84.

Deshpande A S and Pancholy S K 1979 Colonization and Biochemical changes in peanut seeds infected with *Aspergillus flavus*. Peanut Science **6:** 102-105.

Diener U L and Davis N D 1987 Biology of *A. flavus* and *A. Pogasiticus.* In aflatoxin in maize. A proceeding of the workshop (Zuber M S, Lillehoj M S and Renfro B L (ed.,) CIMMYT, pp.33-40.

Diener U L, Cole R J, Sanders T H, Payne G A, Lee L S and Klich M A 1987 Epidemiology of aflatoxin formation by *A. flavus.* Annual review of Phytopathology **25:** 249-270.

Dorner J W, Cole R J, Sanders T H and Blankenship P D 1991 Interrelationship of kernel water activity, soil temperature, maturity and phytoalexin production in preharvest aflatoxin contamination of drought stressed peanuts. Mycopathologia **105:** 117-128.

Nakib O E, James J, Pestka J, Fun S and Chu U 1981 Determination of Aflatoxin B_1 in corn, wheat and peanut butter by enzymes linked, immunosorbent assay and solid phase radio immunoassay. Journal of Association of Official Analytical Chemistry **64(5):** 1077-1082.

Fan T S L and Chu F S 1984 Indirect enzyme linked immunosorbent assay for detection of aflatoxin B_1 in corn and peanut butter. Journal of Food Protection **47(4):** 263-266.

FAO 2002 FAO quarterly bulletin of statistics, Food and Agricultural Organization of the United Nations, Rome.

Figuiera A C, Taylor K D A and Bartow P J 1990 ELISA determination of aflatoxin levels in whole nuts. Food and Agricultural Immunology **2(3):** 125-134.

Ghewande M P 1997 Aflatoxin contamination of groundnut and its management in India. Aflatoxin contamination problems in groundnut in Asia: Proceedings of the first Asia working group meeting, 27-29 May 1996; Ministry of Agricultural and Rural Development Hanoi Vietnam (Mehan V K and Gowde C L L (ed,)) International Crops Research Institute per semiarid tropics, Patancheru 502 304, Andhra Pradesh, India.

Ghewande M P, Nagaraj G, Desai S and Narayan P 1993 Screening of groundnut bold seeded genotypes for resistance to *A. flavus* seed colonization and less aflatoxin production. Seed Science and Technology **21(1):** 45-51.

Gillikin J W and Graham J S 1991 Purification and developmental analysis of the major anionic peroxidase from the seed coat of *Glycine max.* Plant Physiology **96:** 214-220.

Johnson G I 1996 Relationship between soil populations under field conditions in the Philippines. In: Mycotoxin contamination in grains. (ed. Gracia R P,Cotty P J,Angle J S and Barrios J A).17th ASEAN Technical Seminar on grain post harvest Technology, ACIAR-Technical reports series. **(37):** 54-60.

Grewal T S, Indu Sharma, Areja J S, Aujla S S and Sharma I 1999 Biochemical basis of resistance to Kernal bunt in wheat. Crop Improvement 26: 56-62.

Gupta S K, Gupta P P, Kaushik C D and Saharam G S 1987 Biochemical changes in leaf surface extract and total chlorophyll content of sesame relation to *Alternaria* leaf spot disease. Indian Journal of Mycology and Plant Pathology **17:** 165-168.

Gupta S K, Gupta P P, Yadava T P and Kaushik C D 1990 Metabolic changes in mustard due to *Alternaria* leaf blight. Indian Phytopathology 43: 64-69.

Hammerschmidt R, Nuckles E M and Koc J 1982 Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenaria.* Physiological Plant Pathology 20: 73-82.

Hedge J E and Hofrieter B T 1962 In: Methods in carbohydrate chemistry (eds Whistler R L and Be Miller J N). Academic Press, New York.

Holbrook C C, Matheron M E, Wilson D M, Anderson W F, Will M E and Norden A J 1994 Development of a large scale field system for screening peanut for resistance to pre-harvest aflatoxin contamination. Peanut Science **21(1):** 20-22.

Holbrook C C, Kvien C K, Rucker K S, Wilson D M, Hook J E and Matheron M M 2000 Pre-harvest aflatoxin contamination in drought tolerant and drought intolerant peanut genotypes. Peanut Science **27:** 45-48.

ICAR 1987 Aflatoxins in groundnut. Technologies for better crop bulletin No.33. Indian Council of Agricultural Research. New Delhi.

Johnson L F and Curl E A 1972 Methods for research on the ecology of soil borne plant pathogen. Burgess Publishing Company. Minnesota. pp.6-8.

Karam-el-Din-AA, Youssef Y A and Zaki S 1996 Distribution of pathogenic and potentially pathogenic fungi among soil fungal flora in Egypt. African Journal of Mycology and Biotechnology **4(2):** 23-39.

Katiyar S, Dash B C, Thakur V, Guptan R C, Sarin S K and Das B C 2000 Tumor suppressor gene mutations in hepatocellular carcinoma, patients in India. Cancer **88(7):** 1565-1573.

Kiran Kalia, Desai H M, Chakraborty M K and Kalia K 1988 Resistance of Groundnut (*Arachis hypogaea*) to aflatoxin. Indian Journal of Agricultural Sciences **58(2):** 121-123.

Kisyombe C T Beute M K and Payne G A 1985 Field evaluation of peanut genotypes for resistance to infection by *A parasiticus.* Peanut Science **12:** 12-17.

Krishna T G, Pawar S E and Mitra 1986 Variation and inheritance of Arachin polypeptides of groundnut. Theoretical and Applied Genetics, 73: 82-87.

Lacks G D and Stalker H T 1993 Isozyme analysis of *Arachis* species and inter-specific hybrids. Peanut Science **20 :** 76-81.

Laemmli U K 1970 Cleavage of structural proteins during the assembly of the heads of bacteriophage T_4. Nature **227:** 680-685.

Lanham P G, Foster B P and Nicol M C 1994 Seed storage protein variation in *Arachis* species. Genome **37:** 487-496.

Lavon R, Salomon R and Goldschmidt E E 1999 Effect of potassium, magnesium and calcium deficiencies on nitrogen constituents and chloroplast components in citrus leaves. Journal of the American Society for Horticultural Science **124:** 158-162.

Lee H C and Chuang T Y 1993 Comparison of *A. flavus* and aflatoxin contamination among various types of peanut harvest at penghu. Plant Pathology bulletin **2(2):** 88-97.

Liao Y F 1988 A study of esterase isozymes from related citrus plants. Acta Botanica Science **30:** 163-168.

Lim S K, Bay-Yeo T K and Lock-Lee-Sl 1991 Aflatoxin levels in raw commodities. Singapore Journal of Primary Industries **19(1):** 59-63.

Lima H 1982 Enzymatic polymorphism in citrus. Cienica-Tenica-en-la-agricultura-citricos-y-otros-furtales **5:** 79-97.

Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with the folin phenol reagent. Journal of Biological Chemistry **193:** 265-275.

Mahadevan A 1966 Biochemistry of infection and resistance. Phytopathology **57:**96-99.

Malhotra S K 1993 Biochemical components of tomato genotypes in relation to *Fusarium* wilt. Indian Journal of Mycology and Plant Pathology **23:** 302-304.

Mandavia M K and Parameswaram M 1993 Changes in amino acids and phenols in limabean (*Phaseolus lunatus*) varieties resistant and to stem rot disease (*Macrophomina phaseolina*). Gujarat Agricultural University Journal **18:** 19-23.

Mehan V K, Mc Donald D and Ramakrishna N 1986 Varietal resistance in peanut to aflatoxin production. Peanut Science **13:** 7-10.

Mehan V K, Mc Donald D and Ramakrishna N 1988 Effects of adding inoculum of *Aspergillus flavus* to pod zone soil on seed infection and aflatoxin contamination of peanut genotypes. Oleagineux **43(1):** 21-28.

Mehan V K, Mc Donald D, Haeavu L J and Jayanthi S 1991 The groundnut aflatoxin problem. Review and Literature Database. Vi+. 387 pp.

Mehan V K, Mc Donald D, Nigam S N and Lalitha B 1981 Groundnut cultivars with seed resistance to invasion by *Aspergillus flavus*. Oleagineux **36(10):** 501-505.

Mehan V K, McDonald D, Ramakrishna N and Williams J H 1986 Effect of genotype and date of harvest on infection of peanut seed by *A. flavus* and subsequent contamination with aflatoxin. Peanut Science 13(2): 46-50.

Mehan V K, Reddy S V, Nahdi S, Mc Donald D and Jaynthi S 1995 Aflatoxin producing potential of various strains of *A. flavus* from groundnut fields in different soil types. International Arachis News Letter **15:** 42-43.

Mehan V K,Mc Donald D,Singh A K and Moss J P 1992Aflatoxin production in seeds of wild *Arachis* species.Oleagineux 47(2):87-89.

Mishra A, Siradbana B S and Shirpuri A 1980 Phenols in relation to resistance of sorghum to anthracnose. Philippine Agriculture **63:** 71-73.

Mixon A C 1979 Developing groundnut lines with resistance to seed colonization by toxin producing strains of *Aspergillus* species. Pest artic. News sum. **25:** 394-400.

Mixon A C 1986 Reducing *Aspergillus* species infection of peanut seed using resistant genotypes. Journal of Environmental Quality **15:** 101-103.

Mixon A C and Rogers K M 1973 Peanut accessions resistant to seed infection by *Aspergillus flavus.* Agronomy Journal **65:** 560-562.

Mohan G, Bentur K G, Parameshwarappa and Appa Rao 2003 *In vitro* screening of confectionary groundnut genotypes for seed colonization by *A. flavus.* ISOR National Seminar: Stress Management in Oilseeds. January 28-30, pp.328-329.

Mortimer D N, Shepherd M T, Gilbert J and Morgan M R A 1988 A survey of the occurrence linked immunosorbent assay of cleanup for thin layer chromatography of aflatoxin B_1 in corn, peanuts and peanut butter. Journal of Association of Official Analytical Chemists **71(5):** 953-956.

Muhlemann M, Luthy J and Hubner P 1997 Mycotoxin contamination of food in ecquador. Milteilungen-aus-dem-gebiette der-Le bensmitttelun-ter suchung-und-hygiene **88(4):** 474-496.

Nageswara Rao Rachaputi, Graeme Wright, Steven Krosch and Jeff Tatnell 2001. Improved agronomic practices to sustain peanut yield and reduce aflatoxin contamination in water limited environments in Southern Queenland. 10th Australian Agronomy Conference 28 January-1 February. Hobart Tasmania (in press).

Naguib K, Naguib M M,Daib M M,Sahab A F and Amrah H 1990 Occurrence of aflatoxins in several groundnut cultivars infected with strains of *A.flavus*.Egyptial journal of phytopathology.20(2):99-106.

Nahdi S 1996 Population of *Aspergillus flavus* in field and pod zone soil in groundnut fields. Indian Phytopathology **49:** 57-61.

Naik B S and Kole C 2001 Electrophoretic studies on seed protein profile of mungbean. Legume Research **24:** 169-173.

Nicholson R L and Hammerschmidt R 1992 Phenolic compounds and their role in diseases resistance. Annual review of Phytopathology **30:** 369-389.

Okazaki H, Saito M and Tsuruta O 1992 Population levels of *A. flavus* and *A. parasiticus* in field soils in two areas of Kyushu district. Annals of the Phytopathological Society of Japan 58(2): 208-213.

Park D L, Miller B M, Hart L P, Yang G, Mcvey J L, Page S W, Pestka J J and Brown L H 1989 Enzyme Linked immunosorbent assay for screening aflatoxin B_1 is cotton seed products and mixed feed collaborative study. Journal of Association of Official Analytical Chemists **72(2):** 326-332.

Patey A L, Sharman M and Gilbert J 1992 Determination of total aflatoxin levels in peanut butter by enzyme linked imunosorbent assay: collaborative study. Journal of AOAC International **75(4):** 693-697.

Patil S S and Dimond A E 1967 Inhibition of *Verticillium* polygalacturonase by xoidation products of polyphenols. Phytopathology **57:** 492-496.

Pitt J I 1980 Field studies on *A. flavus* and aflatoxins in Australian groundnuts ICRISAT, 1989. Aflatoxin contamination of groundnut. Proceedings of the International Workshop. Mc Donald D an Mehar V K (ed.) 6-9 Oct. 1986, ICRISAT Center, Patancheru.

Pitt J I 1982 An improved medium for the detection of *A. flavus* and *A.parasiticus*. Journal of Applied Bacteriology 54: 109-114.

Pollet A, Declert C, Wiegandt W, Harkema J, Lisdork-E-Van-de, Van-de-Lisdonk 1992 Three years studies on relationships between traditional groundnut storage and aflatoxin problems in cote-d' Ivoire. Oleagineux **47 (2):** 71-85.

Premalatha Singh, Sita Bhagat and Syed Khalid Ahmad 1990. Aflatoxin elaboration and nutritional deterioration in some pulse cultivars during infestation with *A. flavus.* Journal of Food Science and Technology **27(1):** 60-62.

Priyadarshini E and Tulpule P G 1978 Relationship between fungal growth and aflatoxin production in varieties of maize and groundnut. Journal of Agriculture and Food Chemistry **26(1):** 249-252.

Puchalski J, Klim P and Buczyynsk A B 1986 Application of isozyme markers for selection of ryes resistant to pink show mould (*Fusarium nivale*). In Eucarpia meeting of the cereal section in rye. 11-13 June, 1985, Sweden. Proceedings Part-II, Svalor, Sweden.

Raghuchander T, Srikant Kul Karni and Hedge R K 1988 Studies on leaf blight of triticale caused by *Bipolaris Sorokiniana* (Sacc.) Shoem. Plant Pathology Newsletter **6:** 45.

Rajivkumar and Singh S B 1996 Changes in biochemical constituents of sunflower leaves in relation to *Alternaria* blight development. Indian Journal of Mycology and Plant Pathology **26 :** 234-236.

Ram B P, Hart L P, Cole R J and Pestka J J 1986a Application of ELISA to retail survey of aflatoxin B_1 in peanut butter. Journal of Food Protection **49(10):** 792-795.

Ram B P, Hart P, Shot Well OL and Pestika J J 1986b Enzyme linked immunosorbent assay of aflatoxin B_1 in naturally contaminated corn and cotton seed. Journal of the Association of Official Analytical Chemists **69(5):** 904-907.

Ramakrishna N and Mehan V K 1993 Direct and indirect competitive monoclonal antibody based ELISA of aflatoxin B_1 in groundnut. Mycotoxin Research **9(1):** 53-63.

Rathi A S, Parashar R D and Sindhan G S 1998 Biochemical changes in pea leaves due to powdery mildew infection. Journal of Mycology and Plant Pathology **28:** 330-333.

Reddy D V R, Nambiar P T C, Rajeswari R, Mehan V K, Anjaiah V and Mc Donald D 1988 Potential of enzyme linked immunosorbent assay for detecting viruses, fungi, bacteria, mycoplasma like organisms, mycotoxins and hormones. Bio-technology in tropical crop improvement, proceedings in tropical crop improvement, proceedings of international biotechnology workshop, 12-15 January 1987, International crops research institute for the Semiarid tropics Patancheru 502324, Andhra Pradesh, India pp.43-49.

Reddy P V, Sudhakar P, Sudhakar Rao A, Harinath Naidu P, Sujatha D and Vijayakumar B 2003 Selection for groundnut varieties with low aflatoxin risk Stress management in oil seeds for aflatoxin risk. Stress management in oil seeds of attaining self reliance in vegetable oils January (28-30) pp. 327-328.

Reddy S V, Kiranmayi D, Uma Reddy M, Tirumala Devi K and Reddy D V R 2001 Aflatoxin B_1 in different grades of chillies (*Capsicum annum* L.) in India as determined by indirect competitive ELISA. Food additives and Contaminants **18:** 553-558.

Rucker K S, Kvien C J, Holbrook C C and Hook J E 1995 Identification of peanut genotypes with improved drought avoidance traits. Peanut Science **22 :** 14-16.

Sadasivam S and Manickam A 1996 Biochemical methods. New age International (P) Limited, Publishers-II (eds), New Delhi : 193-194.

Sanders T H, Cole R J, Blankenship P D and Dorner J W 1993 Aflatoxin contamination of peanuts from plants drought stressed or pod and root zones. Peanut Science **20:** 5-8.

Sargeant K, Sheridan A, O'Kelly J and Carnaghan R B A 1961 Toxicity associates with certain samples of groundnuts. Nature **192:** 1096-1097.

Seevers P M, Daly J M and Catedral F F 1971 The role of peroxidase isozymes in resistance to wheat stem rust disease. Plant Physiology **48:** 353-360.

Sellschop J P F, Kriek N P J and Dupreez J C G 1965 Distribution and degree of occurrence of aflatoxin in groundnuts and groundnut products. Symp. Mycotoxins foodstuffs Agriculture, aspects, Pretoria, South Africa, pp.9-17.

Shaw C R and Prasad 1970 Starch gel electrophoresis of enzymes, a compilation of recipes. Biochemistry and genetics **4:** 297-320.

Shree M P and Reddy C M 1986 Effect of *Helminthos Poriose* infection on certain biochemical constituents in the resistance and susceptible varieties of sorghum. Indian Journal of Mycology and Plant Pathology **4:** 46-52.

Sindhan G S, Prashar R D and Indra Hooda 1996 Relationship between biochemical parameters and flag smut resistance in wheat. Indian Journal of Mycology and Plant Pathology **26:** 291-293.

Singh A K, Sivaramakrishna S, Malak H, Mengesha and Ramaiah C D 1991 Phylogenetic relationship in section arachis based on seed protein profile. Theoritical and applied genetics **82:** 593-597.

Smart M G, Shot Well O L and Caldwell R W 1990 Pathogenesis in *Aspergillus* ear rot of maize aflatoxin B_1 levels in grains around wound inoculation sites. Phytopathology **80(12):** 1283-1286.

Sridhar R 1972 Influence of nitrogen fertilization and *Pyricularia oryzae* development in some oxidases, their substrate and respiration of rice plants. Acta Phytopathology Academic Science **7:** 57-70.

Statistical Abstract, 2001. Statistical organization. Dept. of Statistics and Progrmame implementation, Government of India, New Delhi.

Subashchandra Bose S and Ranjan S 2000 Peroxidase isozyme as marker for bacterial wilt resistance in tomato (*Lycopersicon esculentum* mill) Vegetable Science **27:** 136-141.

Sylos C M D E, Rodriguez Amaya D, Pinto C and Desylos C M 1996 Comparison of immunoassay and minicolumn chromatography for the screening of aflatoxins in groundnut and maize Alimentos-e-Nutricao 7: 7-14.

Uta Grieshammer and Wynne J L 1990 Isozyme variability in mature seeds of US peanut cultivars and collections. Peanut Science **17:** 72-75.

Vasanthi R P and Raja Reddy C 1995 Research publications : Inheritance of Pod size (length and width) and shell thickness in Groundnut / Arachis hypogaea L. Genetic Research and Education : Current trends and next

50 years; Proceedings of Golden Jubilee symposium of Indian Society of Genetics and Plant Breeding 967-909.

Vasanthi S and Bhat R V 1998 Mycotoxins in food occurrence, health and economic significance and food control measures. Indian Journal of Medical Research **108 :** 212-224.

Waliyar F and Bockeiee- Mor Van A 1989 Resistance of groundnut varieties to *A. flavus* in faction. Pages 305-310 in Aflatoxin contamination of groundnut : Proceedings of the international cost shop, 6-9 October 1987, ICRISAT Centre, India (Mc Donald, D and Mehan V K eds) Patancheru-502324, Andhra Pradesh, India: International Crops Research Institute for semiarid tropics.

Waliyar F, Ba A, Haesn H, Bonkoungou S and Bose J P 1994 Sources of resistance to *A. flavus* and aflatoxin contamination in groundnut in West Africa. Plant disease **78:** 704-708.

Wang D S, Liang X X, Nguyen Thuy Chau, Le-Doan-Dien, Tanaka T and Ueno Y 1995 Natural occurence of *Fusarium* toxins and aflatoxin B_1 in corn for feed in north Vietnam Natural Toxins **3 (6):** 445-449.

Wehling P 1986 Electrophoretic analysis of 10 enzymes systems in rye: linkage relationships and chromosomal location of isozyme loci. Eucarpia meeting of the cereal section on rye; 11-13 June 1985, Svalov, Sweden Proceedings, Part-I 101-125.

Will M E, Holbrook C C and Wilson D M 1994 Evaluation of field inoculation techniques for screening. Peanut genotypes for reaction to pre-harvest *A. flavus* group infection and aflatoxin contamination. Peanut Science **21:** 122-125.

Wogan G N 1965 Aflatoxin contamination of peanuts and other commodities. Nutrition document: Aflatoxin 13, (WHO / FAO /UN / EF), July 1965 Meeting - Rome, Processed, 4 pp.

Wood J R 1971 Peroxidase isozymes in leaves of cucumber (*Cucumis sativus* L.) cultivars systematically infected with the W strain of cucumber mosaic virus. Physiological Plant Pathology **11:** 133-140.

Wotton H R and Strange R N 1987 Increased susceptibility and reduced phytoalexin accumulation in drought stressed peanut kernels challenged with *Aspergillus flavus.* Applied Environmental Microbiology **53:** 270-273.

Yadav B P and Mishra M D 1987 Metabolic changes induced by rice tungro virus in rice cultivars. Indian Phytopathology **40:** 139-148.

Zhang H, Nagashima H and Goto T 1997 Natural occurrence of mycotoxins in corn, samples from high and low risk areas for human oesophagal cancer in china. Mycotoxins **44:** 29-35.

Zhu J Q, Zhang L S, Hu X, Xiao Y, Chen J S, Xu-Yc, Fremy J and Chu F S 1987 Correlation of dietary aflatoxin B_1 levels with excretion of aflatoxin M_1 in human urine. Cancer Research **47(7):** 1848-1852.