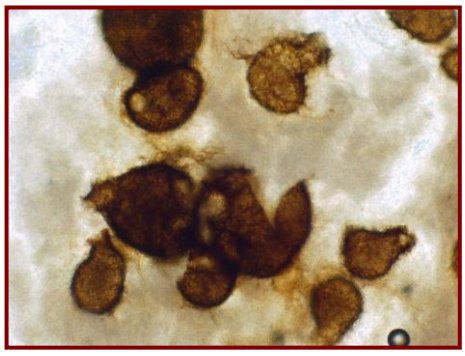
FINAL TECHNICAL REPORT

(01.08.07 to 31.08.10)

Germplasm Conservation / Microbial Diversity Preservation



Submitted

To

MADHYA PRADESH BIOTECHNOLOGY COUNCIL,
26, KISAN BHAVAN, III FLOOR,
JAIL ROAD, ARERA HILLS, BHOPAL

By

Dr. A.K. Pandey
Chairman,
M.P. Private Universities Regulatory Commission,
Gyan Vatika, Valmi Road, Bhopal (M.P.)

Dr. Karuna S.Verma
Professor,
Department of Biological Sciences,
R.D. University, Jabalpur- 482001(M.P.)

- : INDEX: -

FINAL TECHNICAL REPORT

(01.08.07 to 31.07.10)

Title of the project/scheme	: **Germplasm Conservation /**
	: Microbial Diversity Preservation
Period for which sanctioned	: **Three Years**
Principal Investigator (Former) (1-8-07 to 1-10-09)	: **Prof. A.K.Pandey**
Principal Investigator (Present)	: **Prof. Karuna S. Verma**
Amount Sanctioned for the project	: Rs. **22, 32,000**
Amount received in first year	: Rs. **19, 44,000**
Amount received in Second year	: Rs. **1, 44,000**
Amount received in Third year	: Rs. **1, 44,000**
Fellowship	**:** Rs**. 1, 44,000**
Equipments	: **NIL**
Name of the Organization	**: Department of Biological Sciences**
	R. D. University, Jabalpur

Madhya Pradesh Biotechnology Council sanction vide letter no: **338/A-5/2007**

Date of sanctioning the project	**: 27.06.07**
Date of joining of Project Staff	: **01.08.07**
Staff sanctioned for the project	: **One Junior Research Fellow**
	One Technical Assistant
Problem under study	: **Conservation of vanishing**
	Germplasm

ACKNOWLEDGEMENT

It is a momentous opportunity and privilege to express my deep sense of gratitude to all those who have helped me to accomplish the project entitled "**Germplasm Conservation\Microbial Diversity Preservation".** PI is grateful to Dr. Ram Rajesh Mishra, Hon'ble Vice Chancellor, R.D.University, Jabalpur for his guidance and academic as well as administrative support during this work. PI is also thankful to the Registrar and other staff of R.D. University for their administrative support during the pursuit. PI also thankful to Dr. A.K.Pandey, Chairman, M.P. Private University Regulatory Commission, former PI of this project for their kind cooperation and support. PI offers deep sense of gratitude to the Madhya Pradesh Biotechnology Council (MPBC), Bhopal for the financial support. PI acknowledges her heartfelt thanks to venerable Dr. Moni Mathur, Advisor, MPBC for her contact advice and encouragement. PI is also thankful to Mr. S.K.Panwar, Chief Executive Officer and other staff of Madhya Pradesh Biotechnology Council for their help in various ways. Sincere thanks to Prof. S.P.Gautam, Chairman, Central Pollution Control Board, New Delhi and Prof. S.S. Sandhu, Director, CSRD, Peoples Group, Bhopal for their help in various ways. PI is also grateful to Prof P.K. Singhal, Head, Department of Biological Sciences, R.D.University, Jabalpur for providing laboratory facilities during this academic pursuit. PI also gives heartfelt thanks to Dr. Jamaluddin, Emeritus Scientist, CSIR for his valuable suggestions time to time. Great pleasure is encountered while expressing heartfelt gratitude to Mr. Mithilesh Kumar Jaiswal, JRF and Ms. Ritu Panjwani, TA in this project for being actively involved in completion of this project. PI is also thankful to the research scholars of Mycology Research Lab and Aerobiological Research Lab, Department of Biological Sciences, R.D.University, Jabalpur for their help and support.

MAJOR OBJECTIVES

The present project has the following objectives:

- Development of infra structure for storage of Fungi & Yeast.
- Collection, Isolation & Purification of fungal cultures.
- Identification of fungi on morphological basis.
- Standardization of short/ long-term storage.
- Preparation of a checklist and pictorial guide.
- Characterization of Biomolecules: Herbicidal potential, Antimicrobials & Enzymes.
- Finally establishment of Regional Culture Collection & Herbarium.
- Training/workshop for students, teachers & scientists.

Work Completed:

- Development of infra structure for storage of Fungi & Yeast.
- Collection, Isolation & Purification of fungal cultures.
- Identification of fungi on morphological basis.
- Standardization of short/ long-term storage.
- Preparation of a checklist and pictorial guide.
- Characterization of Biomolecules: Herbicidal potential, Antimicrobials & Enzymes.
- Finally establishment of Regional Culture Collection & Herbarium.
- Training/workshop for students, teachers & scientists.

INTRODUCTION

Madhya Pradesh is located in the geographic heart of India, is a land of varied topography characterized by extraordinary vegetation diversity nurtured by extremely varied environmental conditions. From hilly areas, dramatic ravines and forested plains to river basins, the place clasps myriad facets of nature in its lap. In summer, weather remains extremely hot and humid as it is located at a considerable distance from the sea. The mean maximum temperature rises to around 42.5^{o}C in the northern region. However, in most of the places, the mean maximum temperature remains around 35^{o}C to 40^{o}C. In Monsoon, Maximum downpour happens between the months of June and September, with a little rainfall during December and January, due to the low pressure build up in the atmosphere. The average rainfall however varies from region to region. The severity of the winters is generally experienced more in the northern regions of the state. The average winter temperature in the state ranges from 10^{o}C to 27^{o}C. Overall we can say that the vegetation of Madhya Pradesh is very rich in Biodiversity. This particular climate is very suitable for the diversity of Microorganism and hence rich floras of microorganism are found in Madhya Pradesh.

The international Convention on Biological Diversity (CBD) defines biodiversity as "the variability among living organisms from all sources including, *inter alia*, terrestrial, marine, and other aquatic ecosystems and the ecological complexes of which they are part".

Microdiversity/Biodiversity is an additional sum of the studies on genetic, taxonomic, commercial and ecosystem aspects of living systems. Germplasm obtained from the vast biodiversity provides a major source of biological materials for the development of medicines, vaccines, pharmaceutical products, for improved crops, animal varieties and for other biotechnology applications.

Fungi and yeasts are important organisms in the environment as they play an essential role in sustainable growth and sustenance of human population. They are considered as a potential source of biotechnological product *viz.* food, medicines, solvents, enzymes, agrochemicals etc. Besides the beneficial uses, they are also involved in food spoilage and pathogenic to plants and animals. Proper exploitation, conservation and screening may provide some more biotechnological potential strains for beneficial utilization. Regular training will generate local expertise for sustainable use of mycodiversity in central India.

They form a major component of tropical ecosystems and are involved innumerable interactions with plants, animals and man as saprophytes, parasites and symbionts. The variety of terrestrial habitats occupied by fungi is extremely large, since fungi are notorious versatile opportunists, and almost

anything that can decompose to yield energy, will find some fungi able to colonize it. More than about 1 lakh species of fungi have been named or recognized but new species and genera are still being described from allover the world especially form the tropics including India. It is generally assumed that one third of fungal diversity of the globe exists in India .Out of 1.5 million fungi, only 50 % are identified and remaining 50%needs to be identified. Unfortunately, around 5-10% of fungi are cultured artificially. Many earlier describe species of fungi either at the verge of extinction or facing threat.

Due to overexploitation, the diversity of living organisms including fungi is vanishing. The 1992, Earth summit at Rio Janerio, was culmination of international concern. Fungi, critical component of biodiversity diminishing at accelerated trace, in spite of international geosphere and biosphere programme and convention on biological diversity.

The systematic exploitation of potentially unexplored habitats will undoubtedly lead to revise and add the prospective wealth of fungal and yeast diversity potentially in geographies in tropical regions. Thus, a regional culture collection (repository *in vivo*) and with preservation of corresponding exsiccate specimens in herbarium are of vital importance for conservation of vanishing germplasm. The enormity of the geography and resultant fungal and yeast diversity in targeted area requires region specific efforts for conservation of fungal germplasm in addition to the existent.

Environmental conditions prevailing in the state also provide an excellent opportunity to survive and multiply a large variety of weeds. A weed is a plant with a competitive and aggressive behavior, which is persistence and resistance to control and is useless, unwanted and undesirable. Weeds compete for water, nutrients and sunlight and reduce the vigor and growth of ornamental landscape plants. They are usually plants that are very prolific, invasive, competitive, harmful, destructive or difficult to control. Weeds detract from the appearance of landscapes and can be hosts for disease causing organisms and insects. Weeds adversely affect health, comfort and work efficiency of men.

The realization that fungi are important natural enemies of weeds is not new but their organized use in biological control is now gaining importance. Biological control is the use of natural enemies to reduce the damage caused by weed population. A wide number of fungal candidates are being exploited and explored for their biorationals properties and many fungal metabolites are being patented and commercialized for weed management.

Weed management

Biological control especially thorough microorganisms have shown excellent potential in weed management. Virtually all plants are affected by diseases, and weeds are no exception. Plant pathogens may be fungi, bacteria, viruses, nematodes, viroides, protozoa, mycoplasma etc which exhibit levels of parasitism ranging from obligate to saprophytism. They may be either highly host specific or have a wide host range. They can be disseminated entirely by passive means or have obligatory relationships with insects or nematodes. The environmental requirement of plant pathogens are also varied ranging from those with narrow infection requirements that complete one or two disease cycle per season.

Efforts made by the scientists reached to a point where following strategies for exploitation of fungal strains have been clearly defined-

1.Classical strategy

2.Mycoherbicidal

3.Integrated Management

4.Biorationals

Biotechnological applications of different Fungi:

Fungi as a Mycoherbicide:

Invasive weeds are emerging as another major threat to agricultural and natural areas and to the long-term health and biodiversity of our nation's land and water resources. Weed problems become more complicated in natural and urban areas, where economic, environmental or human-health risks may entirely preclude the use of chemical herbicides. Development of newer weed-management agents and technologies including biologically based approaches is of greater importance now than ever before.

Mycoherbicide or fungi based herbicides are defined as plant pathogenic fungi developed and used in an undative strategy to control weeds in the way as a living product that controls specific weeds in agriculture as effectively as chemicals. The initial commercialization of Devine and Collego has led to patenting and deployment of a large number of fungal pathogens. It has been proved to be one of the important alternative methods for sustainable management of several weeds.

Phytotoxic Metabolites from Fungi:

Plant pathogens have long been considered to produce toxic substances that play a major role in pathogenesis. Microbial toxins pose minimal to no risk to the environment due to their chemical make-up, rapid degradation or the small amounts required for effective control (Walker and Templeton 1978).

Mushrooms:

Mushrooms occupy a place in ancient Greek and Roman literature, Salvak, Scandinavian, Yoruba, Himachal, Kashmiri and Ainu folk force and beliefs. Mushrooms are known for its nutritional and therapeutic value. It is a rich source of protein, vitamins and minerals. It may be consumed for their palatability and nutritional value. Palatability can be judged by colour, texture, flavour and taste but the determination of nutritional value requires thorough biochemical analysis. They also have various health benefits such as antioxidative, antitumor and hypercholesterolemia effects. Therefore, edible mushrooms are regarded as an ideal health food.

Yeast

Yeasts are a growth form of eukaryotic microorganisms classified in the kingdom of Fungi. The useful physiological properties of yeast have led to their use in the field of biotechnology. Fermentation of sugars by yeast is the oldest and largest application of this technology. Many types of yeasts are used for making many foods: Baker's yeast in bread production, brewer's yeast in beer fermentation, yeast in wine fermentation and for xylitol production.

DETAILS OF METHODOLOGY

Collection, Isolation & Purification of fungal cultures:

Field Survey and Sampling Details:

Frequent surveys of different habitats in Madhya Pradesh and Chhattishgarh were conducted during all the respective three years of the project. These include-Jabalpur, Katni, Umaria, Satna, Sidhi, Rewa, Shahdol, Amarkantak, Dindori, Mandla, Balaghat, Seoni, Chhindwara, Narshinghpur, Hoshangabad, Betul, Ambikapur, Bilaspur, Kundam, Khandari, Singrawli, Beohari, Singrauli, Bhopal, Indore, Shivpuri, Rewa, Katni etc.Survey was made atleast once during all the three seasons. Intensity of survey was more during post monsoon season. Necessary equipment like hand lens, polythene bags, knives, scissors and field notebook were carried during the visit.

Collection and Primary Processing of samples:

Infected / infested samples were placed separately in polythene bags/Paper Packets for the detail examination in the laboratory. For the purpose of correct identification of the samples of their flowers, fruits, leaves, stems and roots were also collected and kept in specimen bags for confirming their identity later. The specimens were then transferred to the envelopes. Collection number, name of the fungi, family of the host, locality, date of collection and the name of the collector were noted on the fore flap of the envelopes. The fully dried and pressed materials were also be deposited in Herbarium of Dept. of Biological Sciences, R.D. University, Jabalpur.

Laboratory Processing and Preliminary observations:

In the laboratory, the completely dried specimens were disinfected before hand and sprayed with 0.1% alcoholic solution of $HgCl_2$, each infected leaf was first examined carefully with the naked eye thereafter scrapped mounts of the infected tissue were prepared in water. The slides were studied carefully under a compound microscope. Fungi were identified. For routine microscopic study in the laboratory temporary slides were prepared in different type of stains and mounts according to the nature of fungi. Then the slides were sealed with DPX or good quality commerce wax or nail polish and ready for study or storage for further use.

- **Lactophenol**

Phenol (crystals)	: 20.0 gms
Lactic Acid	: 20.0 gms
Glycerol	: 40.0 ml
Distilled Water	: 20.0 ml

- **Lacto Phenol & Cotton Blue solution**

Phenol (crystals)	: 20.0 gms
Lactic Acid	: 20.0 gms
Cooton Blue	: 0.05 gms
Glycerol	: 40.0 ml
Distilled Water	: 20.0 ml

(Purvis *et al*., 1964, 1966)

- **Lactofuschin** (a new media for mounting fungi)

Formulation

Acid fuschin	: 0.1gm
85% Lactic Acid	: 100ml

Isolation of Fungi:

Fungi were isolated from disease parts such as rhizosphere, phyllosphere, phylloplane and seeds employed following methods-

(A) From infected parts of the weed

Lesion containing tissues were cleaned in running water and blot dried on sterile filter paper. Infected parts of the weeds were cut into small pieces and surface sterilized with 0.05% NaOCl for 3 min, then rinsed with sterile water. The surface disinfected pieces were placed in petridishes containing PDA with Rose Bengal and chloramphenicol / Streptomycin to avoid bacterial growth **(Martin, 1950)** and incubated in a BOD incubator at 28±1°C. Colonies appeared on the surface were transferred to the slants **(Agarwal & Hasija, 1986)** for subculturing.

(B) From rhizospheric soil:

i. **Warcup's soil plate method**: one gram of oven dried soil was taken in presterilized petridishes and PDA medium was poured into it these were incubated at 28±1°C in a BOD incubator and then transferred to PDA slants **(Warcup, 1950)**.

ii. **Pour plate method**: 10 gms of each soil sample was oven dried and grinded in pestle mortar, these soil samples were dissolved in 100 ml of sterile distilled water and thoroughly shaken to obtain 1:10 dilution and then serial dilutions were prepared by adding sterile distilled water. 1.0 ml of each sample were pipettes aseptically in presterized petridishes and PDA medium was poured these were incubated in a BOD incubator at 28±1°C and as soon as colonies appeared they were transferred to PDA slants **(Agarwal & Hasija, 1986).**

(C) From seeds:

i. Washing method

Randomly selected 10 gm seeds were transferred to 150 ml Erlenmeyer flasks containing 100 ml of sterile distilled water, shaken thoroughly for 20 min in water bath shaker at room temperature to separate the spores adhering to the seed surface. After shaking suspension was centrifuged for 5 min and then serial dilution were prepared, which was employed for isolation.

ii.Blotter Method

Ten seeds obtained from washing method were surface sterilized with (NaOCl) placed in sterilized plastic petridish containing three layered moistened blotters, the seeds were incubated at 28±1ºC (RH 90%) with 12/12 has light and dark period and observed regularly after 3rd day of incubation. As soon as fungal colonies developed on the surface, they were transferred to PDA slants.

iii.Agar Plate Method

Ten seeds were surface sterilized and placed in petridishes containing PDA medium supplemented with Chloramphenicol (0.75 ml/L) then the seeds were incubated at 28±1ºC and examined microscopically after 2nd day of incubation colonies developed were transferred to PDA slants. Slants to slants transfer were made after 2-3 months **(Booth, 1971 b & c)**.

Potato dextrose Agar (Agarwal and Hasija, 1986)

Thinly sliced, peeled white potato	: 200 gm
Agar	: 20 gm
Dextrose	: 20gm
Distilled water	: 100 ml

PURIFICATION OF CULTURES

The cultures grown on a petridish were purified by Loop Streak method. Loop Streak method: Heavy suspension of spores was prepared in sterile distilled water in a watch glass. Loopful of spores was taken in a platinum loop needle and streaking was done in a zig zag manner over the surface of the solidified agar plate. When these spores germinate the developing colonies were observed to be discrete and then were transferred to fresh medium.

IDENTIFICATION OF CULTURES

For microscopic studies various temporary and permanent slides were prepared in different types of stains and mounts according to the nature of fungal forms involved.

Slide Culture technique (Agarwal & Hasija, 1986)

This technique was also adopted for the identification to observe the conidial ontogeny (Hughes, 1953 b). It allows for the examination of the colony in various stages of development and improves the chances of observing the natural configuration of the spores and conidia on the sporulating structures.

Identification of moulds was based almost entirely on the morphological structures like colony surface, textures, hyphal pigments, exudates, margin shape and growth rayes, hyphae, spore bearing fruiting bodies and the spores themselves. Fruiting body structures (both macroscopic and microscopic) were used to classify or identify fungi. Sporulating cultures identification was also based on the anamorph teleomorph stage.

The identification and description of fungi were made with the help of various books, monographs and reviews published in standard journals and books as Ainsworth *et al*. (1973); Von Arx (1981); Barron (1968); Barnett and Hunter (1972); Carmichael *et al*. (1980); Sutton (1980); Subramanian (1956, 1977, 1982, 1986), Alexopolous and Mims (1979), Smith and Onions (1994) were very useful in identification of pathogenic group.

STANDARDIZATION OF SHORT/ LONG-TERM STORAGE

Preservation of isolated fungi:

It is important that microorganism resources are preserved in a physiologically and genetically stable state. Attempts are also made to maintain and preserve the valuable fungal cultures by applying certain short term storage method (Agrawal & Hasija, 1986) and long term storage methods (Carmichael, 1956; Hwang, 1966; Danial & Chandler, 2000, Pasarell and McGinnis1992).

I. SHORT TERM PRESERVATION: -

It involves the maintenance of cultures for up to 1 year. Most Fungal cultures can be maintained for that period by serial transfer. Inoculum's transferred from an actively growing fungus culture to test tubes (Screw cap or Plugged with cotton or foam) or Petri dishes (wrapped with Para film to reduce drying) containing an agar medium of choice (Nakasone et al., 2005), but Sub culturing may also lead to contamination and also loosing their viability.

II. LONG-TERM PRESERVATION:-

Microorganisms require special preservation known as Long-term preservation method (AFFR & NIAS 1987; Kirsop & Doyle 1991) is essential for their in-depth study; however, both the viability and the stability of living cells should be ensured during the preservation period. It includes:

A.Immersion in Distilled Water: - Fungal isolates are usually preserved in water at room temperature (10), an easy, inexpensive, low maintenance and economical procedure introduced for fungi by Castellani in 1939 (5). Apparently, the water suppresses morphological changes in most fungi. Disks are transferred to sterile, screw - cap vials (30ml), filled with sterile distilled water. Test tubes (loosely capped and wrapped with Para film) are stored at room temperature; tightly capped tubes and vials are stored at 4^0C. Disks are removed aseptically and transferred to fresh agar medium to retrieve cultures (McGinnis *et al.* 1974).

"Many fungi will survive for a decade or more in sterile water."

B.Storage in Glycerol - PDA slants are overlaid with sterile glycerin at a 25 – 50 % concentration and stored at 4^0 C.

C. Lyophilization: It's a low cost form of a permanent preservation. An agar slant with medium that supports good growth and sporulation is inoculated with the organism, which is allowed to grow until it reaches the resting phase. About 1.5-2.0 ml sterile menstruum is added to an agar slant; spores are suspended in Non fate dry milk powder (Sterile 5% or 10% solution) and filtered sterilized bovine serum by gently scrapping the agar surface with pasture pipette. Tubes are plugged loosely with cotton and of the glass are lubricated with the caster oil, and the tubes are placed on the vaccum manifold. The manifold is lowered until the lyophilization tubes are immersed in a dry ice and ethylene glycol bath that is maintained between 40º C and - 50º C while the contents of each tube freezes for about 5 minutes. A vaccum is applied to the system for about 30 minutes while the bath warms to about 0ºC. The manifold then is raised to remove the tubes from the solvent bath. Drying of the lyophilization preparation continues at room temperature until the pressure in the system which is about 30 milliTorrs. Evaporative cooling keeps the sample frozen during the drying process. The tubes than are sealed under vaccum using a gas-oxygen torch. Finished lyophilization ampoules are stored in numbered plastic boxes or sealed plastic bags in a 4 ºC in a refrigerator. The purity and the viability of the preparation in one lyophilization vial should be checked one to two weeks after preservation (Schipper, M. A. A., and J. Bekker-Holtman. 1976).

D. Lyophilization skimmed milk:

Prepare a 20 % solution of skim milk and autoclave at 116 ºC for 20 minutes in 10 ml tubes, Prepare the spore suspension by slowly introducing about 2 ml of milk into the culture tubes or plates while gently scraping the surface of the culture with pipette. Dispense 0.2 ml of suspension into each vial and lyophilize. Store the lyophilized tubes at 4 ºC in a refrigerator.

E. Stored in Liquid Nitrogen:

Storage in liquid nitrogen vapor (above the liquid at __130°C) is a more convenient and less expensive alternative for long-term storage of living cells. Storage above the liquid nitrogen prevents leakage of the liquid nitrogen into the vials (Stalpers *et al.* 1987)

F. Cryopreservation and revival of frozen cells

Cryopreservation of fungi and Oomycetes and revival of the frozen cells were described previously 7, 9. Briefly, discs (6 mm diam.) were cut out of an agar plate on which mycelia were growing. Five or ten discs were transferred into a plastic vial with a screw cap containing1 mL of 10% (w/w) glycerol. After cold-hardening in are frigerator at 5ºC for two to three days and freezing in a deep-freezer at –70ºC for two to three days, the vial was moved to an atmosphere of liquid nitrogen at –165ºC.For reviving frozen cells, a vial containing frozen cells was thawed quickly in a water bath at 30 to 37ºC.The discs were put on an appropriate agar plate medium, such as potato dextrose agar or V8 agar, and were incubated. Visible growth of fungal colonies from 80% or more of the discs on agar plate medium was assessed as successful preservation (Meyer, E. 1955).

G.Freeze-drying method and revival of freeze-dried cells

A freeze-drying technique was employed for preservation of bacteria, Actinomycetes and yeasts. The suspension was dispensed to Pyrex ampoules, frozen at –40ºC overnight and freeze-dried under a vacuum lower than 10 mT with a vacuum freeze dryer. After freeze-drying, ampoules were sealed and cut with a gas burner. Ampoules keeping a vacuum were preserved at 5ºC.For reviving freeze-dried cells; they were re-suspended in 100 µL of water. The cell suspension was transferred onto an appropriate agar plate medium, such as standard agar or potato peptone glucose agar, and was incubated under conditions where the cells could grow well. Visible growth of the microbial colonies on the plate was assessed as successful preservation.

Characterization of Biomolecules: Herbicidal potential of Fungi

Screening

Screening is very important step in the selection of suitable agent to be developed for mass production and formulation of phytotoxic compounds. It is mainly because the ability of synthesis of specific compounds is not the property of a genus but varies significantly even in strains or species. Therefore, a screening was undertaken to select potential strain.

Strains for Screening Purpose

To select potential strains, which were able to produce broad-spectrum herbicidal compounds, screening was done. 27 isolates belonging to 7 genera were screened for herbicidal potential and are listed in Table A.

Table A: Fungi employed for assessing phytotoxic potential

Isolate No.	Name of fungus	Weeds	Place of collection
FGCCW#02	*Alternaria alternata*	*Parthenium hysterophorus*	Dindori
FGCCW#11	*Alternaria alternata*	*Parthenium hysterophorus*	Dindori
FGCCW#32	*Alternaria alternata*	*Lantana camara*	Panagar
FGCCW#25	*Curvularia lunata*	*Parthenium hysterophorus*	Narsingpur
FGCCW#33	*Curvularia lunata*	*Lantana camara*	Sagar
FGCCW#39	*Curvularia palliscence*	*Lantana camara*	Shahpura
FGCCW#36	*Curvularia senegalensis*	*Lantana camara*	Panagar
FGCCW#56	*Curvularia lunata*	*Hyptis suaveolens*	Bargi
FGCCW#07	*Phoma harbarum*	*Parthenium hysterophorus*	Maharajpur
FGCCW#18	*Phoma harbarum*	*Parthenium hysterophorus*	Patan
FGCCW#38	*Phoma multirostrate*	*Lantana camara*	Jabalpur
FGCCW#34	*Phoma glomerata*	*Lantana camara*	Bhopal
FGCCW#54	*Phoma harbarum*	*Hyptis suaveolens*	Jabalpur
FGCCW#70	*Phoma harbarum*	*Hyptis suaveolens*	Jabalpur
FGCCW#06	*Pestalotia indica*	*Parthenium hysterophorus*	Panagar
FGCCW#08	*Sclerotium rolfsii*	*Parthenium hysterophorus*	Mandla
FGCCW#23	*Sclerotium rolfsii*	*Parthenium hysterophorus*	Gwalior
FGCCW#09	*Colletotrichum dematium*	*Parthenium hysterophorus*	Dindori

FGCCW#16	*Fusarium roseum*	*Parthenium hysterophorus*	Narsingpur
FGCCW#01	*Fusarium oxysporum*	*Parthenium hysterophorus*	Jabalpur
FGCCW#13	*Fusarium nivale*	*Parthenium hysterophorus*	Narsinghpur
FGCCW#41	*Fusarium oxysporum*	*Parthenium hysterophorus*	Shahpura
FGCCW#43	*Fusarium moniliforme*	*Lantana camara*	Jabalpur
FGCCW#63	*Fusarium roseum*	*Hyptis suaveolens*	Patan
FGCCW#65	*Fusarium oxysporum*	*Lemna gibba*	Jabalpur
FGCCW#55	*Fusarium solani*	*Hyptis suaveolens*	Jabalpur
FGCCW#60	*Fusarium monilliforme*	*Pistia stratiotes*	Seoni

Taking this into consideration, here in this part screening of phytotoxin of collected fungi during survey that attacks weeds has been discussed. It was performed by two screening process- Primary screening and Secondary Screening by using CFCF.

General Bioassays:

Following bioassays were performed to assess the herbicidal potential of CFCF of recovered isolates:

Shoot cut bioassay:

Shoots of the target weeds from 30-35 days old seedlings grown in pots containing soil: sand: peat (1 : 1 : 1) inside a plant growth chamber (Yorco, India) .They were then dipped in different dilutions of the CFCF in glass vials to assess the effect of toxin on shoots. These were incubated and the effects of the toxic metabolites were observed on the shoots after 12, 24 and 48h at room temperature 28±2°C (Sharma and Sharma, 1969 and Chiang *et al*., 1989). Phytotoxic damage was recorded visually on a five-point rating scale of 0-5.

Where,

0-0.99 = slight curling and wilting;

1-1.99 = slight chlorosis;

2-2.99 = marked chlorosis, slight necrosis;

3-3.99 = high necrosis and marked chlorosis;

4-4.99 = acute necrosis and marked chlorosis;

5.00 = acute chlorosis & acute necrosis leading to death of the shoots.

(b) Detached leaf bioassay:

Detached leaf bioassay technique was employed to test the phytotoxicity (Chiang *et al*., 1989). Healthy young leaves from the pre-flowering stage of weeds (15-20 days old) in the field were picked up and surface sterilized in 0.02% NaOCl solution for 5 minutes, then rinsed thrice in sterile distilled water and placed on moist cotton filter paper (pre-sterilized) bed in 9 cm diameter plastic petri-plates. The toxin was applied to the excised leaves and incubated for 4 days at $27/22^0$C day/night temperature in plant growth chamber (Yorco, India). The potential of these were rated as:

0-0.99 = slight wilting and curling;
1-1.99 = 1-20% LAD;
2-2.99 = 21-40% LAD;
3-3.99 = 41-60% LAD;
4-4.99 = 61-80% LAD;
5.00 = 81-100% LAD (Leaf Area Damage)

In all the bioassays, sterilized non-metabolized growth medium were used for control and sterilized distilled water served as control over control.

(c) Seedling bioassay: (Templeton, 1972)

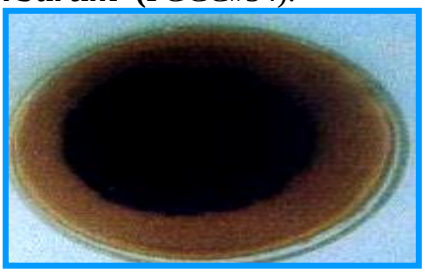
Seedlings of 3-4 weeks old raised in plastic pots were sprayed to runoff with CFCF. Observations regarding phytotoxicity were made regularly as mentioned earlier.

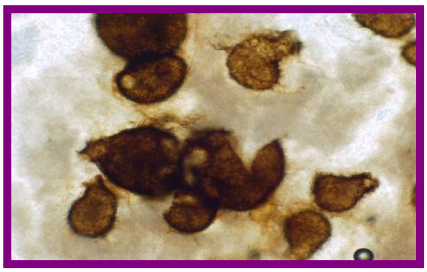
RESULTS OF THE EXPERIMENTS

During Screening a total of 27 isolates belonging to 7 genera recovered earlier from infected/infested parts of weeds collected from different habitats of Madhya Pradesh were subjected to phytotoxicity testing (**Table 1).**

The phytotoxicity of secondary metabolites produced by different species varied greatly. Fungal strain viz., *Phoma* herbarum (FGCC#54) showed maximum toxicity against all the weeds treated with CFCF obtained from 21 days old fermented broth. This was followed by strains FGCC# 18 effective against the entire test weeds except *Parthenium* and FGCC#25 effective against all the test weeds except *Lantana*. It is evident that phytotoxicity of CFCF varied significantly with the concentration of toxins and organism produced. Fungal strains of *Phoma* spp. *viz*., FGCC#18 and FGCC#54, induced significant toxicity even at lower concentration. Toxicity gradually increased with increase in concentration. It was also recorded that impact on the weeds was rapid on higher concentration. But only one strain i.e. *Phoma* herbarum (FGGCC#54) out of the three isolated species, was responsible for considerable damage to the all the four target weeds. It was found to be broad spectrum. On the basis of herbicidal potential, *Phoma* herbarum (FGCC#54) strain was selected for further investigations.

Phoma herbarum **(**FGCC#54).





Description:

Mycelium immersed, branched, septate, hyaline or grayish green. Conidiomata pycnidial, immersed, unilocular, brown, globose, separate or aggregated, thin walled, ostiole single to each pycnidium, central, 70-160 µm in diameter. Condiophores very short. Conidiogenous cells enteroblastic, phialidic, integrated, hyaline, smooth. Conidia hyaline, subglobose to ellipsoid, aseptate, thin walled, cylindrical or fusiform, 3.7-7.4 x 1.5-3.5 µm.

Phoma spp are known to have very high phytopathogenic potential not only in economically important crop plants but also attacks weed severely (Vikrant *et al.*, 2006).

STANDARDIZATION OF PHYSICO-CHEMICAL CONDITIONS FOR PHYTOTOXIN PRODUCTION OF SELECTED STRAINS

I. EFFECT OF CULTURE MEDIA

Various nutritional media were tested to select the best medium for phytotoxin production by *Phoma* herbaru (FGCC#54). Data recorded in **Table 2** indicates that Richard's Broth induced maximum phytotoxin production by the test fungal strain followed by Czapeck Medium and Pferrer's Medium.

II. EFFECT OF HYDROGEN ION CONCENTRATION

Data presented in **Table 3** clearly reveals that the test strain could produce maximum biomass at pH level 4.0 followed by 5, 6, 7, 8, 9, 10 and 11. Least biomass production was seen at pH 12.

III. EFECT OF INCUBATION TEMPERATURE

There was a gradual increase in toxin production from 0°C-25°C and maximum toxin production occurred at 28±1°C. After which there was a significant reduction in metabolite production with gradual increase in incubation temperature. Maximum phytotoxicity was observed for the weed *Parthenium* amongst other test weeds at 48 hpt. **Table 4.**

IV. EFFECT OF INCUBATION PERIOD ON PHYTOTOXIN PRODUCTION

As shown in **Table 5,** maximum phytotoxic damage could be attributed to 21 days followed by 28 days, 14 days and 7days old Fermented broth damaging *Parthenium* shoots to maximum extent.

V. EFFECT OF DIFFERENT CONCENTRATIONS OF PHYTOTOXINS

As evident from data presented in **Table 6** shoots of test weeds were immersed in different concentrations of 21 days old CFCF of the test fungal strain and phytotoxic damage was observed by employing shoot cut bioassay. 75% along with 100% exhibited maximum phytotoxic damage to the weed shoots after 48hpt followed by 50% and 25% CFCF.

VI. EFFECT OF DIFFERENT CARBON AND NITROGEN SOURCES ON PHYTOTOXIN PRODUCTION

As depicted in **Table 7 and Table 8,** it is clear that sucrose is the best carbon source and potassium nitrate was selected as the best nitrogen source for phytotoxin production by the test fungal strain *Phoma* herbarum (FGCC# 54). Maximum phytotoxic damage was obtained at 48hpt followed by 24 hpt and 12 hpt and 4C:6N ratio was selected for further studies (Jackson & Bothast 1990).

PURIFICATION:

Phytotoxins were extracted from 21 days old fermented broth of *Phoma* herbaum (FCGG#54) grown as stationary culture (28 ± 1^0C) on Richard's medium. The CFCF was obtained as described earlier and concentrated to 1/50 folds of the original volume. This was further subjected to extraction procedures.

Extraction of Phytotoxic metabolites was done by two types of methods:

a) Solid Phase Extraction (I Phytotoxic metabolite).

b) Organic Solvent Extraction (II Phytotoxic metabolite).

SOLID PHASE EXTRACTION:

SPE were obtained viz., the aqueous or the inorganic phase and solid phase extract or the organic is also extensively used for extraction of material from biological systems. On solid Phase Extraction, two fractions phase.

The data represented in **Table 9** clearly indicates that phytotoxic damage was found to be maximum for *Parthenium* with the organic or the solid phase extracted fraction. The inorganic or the aqueous phase exhibited lesser phytotoxicity to the test weeds indicating the presence of the bioactive moiety in the organic phase.

Solvent extracted fractions: All the layers were subjected to *in vacuo* desiccation at 40^0C in a rotary vacuum evaporator (Buchi R-300 Rotavapor, Buchi Co. Germany,) to remove any traces of solvents and to obtain residues.

The test residues were prepared as stocks using distilled water (100,000 µg/ml) and were tested for their phytotoxic activity by detached leaf method (Strobel, 1973; Sugawara *et al*., 1985). The phytotoxic fraction was further tested by shoot cut bioassay. Distilled water served as control.

SOLVENT EXTRACTION

Solvent extraction of solid materials is the method can be used for the extraction and preconcentration of a wide range of non-volatile or semi volatile species.

The fractions so obtained were named alphabetically as obtained viz.

- Fraction A (Carbon tetrachloride)
- Fraction B (Chloroform fraction)
- Fraction C (Ethyl Acetate fraction)
- Fraction D (Butanol fraction)

after the solvent extraction were vacuum evaporated at 40ºC and their residues tested using detached leaf bioassay to detect and isolate the phytotoxic moiety. It was found that fraction B (ethyl acetate) induced phytotoxic damage after 72 hours post treatment employing detached leaf bioassay. Other fractions A, C and D did not induce any phytotoxic symptoms **Table 10.**

Column Chromatography:

Column chromatography was used to isolate phytotoxin from solvent extracted fraction. Reactivated (at 110°C for 2 hours) column chromatography grade silica gel (60-120 mesh) slurry was applied on a glass column (bed size 45 x 1.5 cm) and the column was packed. The column was washed with solvent Chloroform .Glass wool was placed above the silica gel bed. Solvent extracted fraction (ethyl acetate) was mixed with silica gel and kept overnight at room temperature for evaporation. When it becomes free flowing it was loaded onto the column. The free floating silica adsorbed sample was loaded onto the column. The column was eluted with $CHCl_3$: Methanol (90:10 to 0:100). Ten fractions, 5ml each were collected**.** The impurities remained adsorbed to the silica bed on the top of the column bed. The fractions were assessed for their phytotoxic activity by detached leaf bioassay and the active fractions so obtained were further analyzed by thin layer chromatography and subjected to further experimentation.

S. No.	Fraction No.	Solvent System $CHCl_3$:CH_3OH
1.	1-4	90:10
2.	5-8	80:20
3.	9-12	70:30
4.	13-16	60:40
5.	17-20	50:50
6.	21-24	40:60
7.	25-28	30:70
8.	29-32	20:80
9.	33-36	10:90
10	37-40	0:100

Data in **Table 11** show **(21-24)** pooled fraction exhibited maximum phytotoxicity to *Parthenium* followed by *Lantana, Hyptis* and *Sida* detached leaves. While significant phytotoxicity was shown by **(25-28)** pooled fraction. **Fractions (17-20) and (29-32)** exhibited mild phytotoxicity to the test weeds.

Thin- layer Chromatography

The fractions collected from columns were analyzed by thin layer chromatography on reactivated silica gel –G, 0.15 mm analytical plates. Ethyl Acetate: Chloroform (70:30 v/v) was used for phytotoxin. The fractions containing similar components were pooled and the solvent was evaporated. The fractions showing two components were pooled evaporated, dissolved in a small quantity of distilled water and were subjected to phytotoxicity tests.

Detection of compounds on thin layer chromatogram:

The compounds separated on the thin layer chromatographic plates were detected by observing the colour development by keeping them in iodine chamber. The appearance of colour after impregnation of the plates in iodine was used as a marker for Phytotoxin detection. The spot was separated by TLC, was scooped out and dried in an oven at 40^0C for 6 hours. It was assessed for phytotoxicity.

The TLC of the ethyl acetate extracted fraction on silica gel G plates in **Ethyl Acetate: Chloroform (70:30 v/v)** solvent system gave a single spot with Rf value **0.65**.

PREPARATION OF A CHECKLIST AND PICTORIAL GUIDE.

About 483 fungal isolates belonging to 61 genera have been collected from different habitats of Central India and maintained for their further use. As identification upto species level is a tome taken process identification put species level is still underway for the isolated fungal strains. The project work undertaken during the tenure has resulted in the discovery of two new species. Some of the fungal strains have been corrected identified and proposed as sp. Nova. However others are still compared with other species.

The new species are:-

1.*Acrodictys steviae*; Host- stevia rebaudiana leaves; place of collection –SFRI, Jabalpur.

2.*Bispora aegle*; host-Aegle marmelos leaves; Place of collection- Shivpuri.

3.*Cercosporidium zizyphi*- leaves of Z*izyphus zizuba.* Place of collection-Manegaw, Jabalpur.

FUNGI ISOLATED FROM NON-AGRICULTURAL WEEDS

S No.	FGCC#	Place	Weed	Name of Fungi
1.	01	Jabalpur	*Parthenium hysterophorus*	*Fusarium oxysporum*
2.	02	Dindori	*Parthenium hysterophorus*	*Alternaria alternata*
3.	03	Shahpura	*Parthenium hysterophorus*	*Chaetomium globosum*
4.	04	Sihora	*Parthenium hysterophorus*	*Helminthosporium oryzae*
5.	05	Bhedaghat	*Parthenium hysterophorus*	*Myrothecium roridum*
6.	06	Panagar	*Parthenium hysterophorus*	*Pestalotia* indica
7.	07	Maharajpur	*Parthenium hysterophorus*	*Phoma herbarum*
8.	08	Mandla	*Parthenium hysterophorus*	*Sclerotium rolfsii*
9.	09	Dindori	*Parthenium hysterophorus*	*Colletotrichum dematium*
10.	10	Panagar	*Parthenium hysterophorus*	*Colletotrichum dematium*
11.	11	Dindori	*Parthenium hysterophorus*	*Alternaria alternata*
12.	12	Panagar	*Parthenium hysterophorus*	*Paecilomyces* varotii
13.	13	Narsinghpur	*Parthenium hysterophorus*	*Fusarium nivale*
14.	14	Panagar	*Hyptis suaveolens*	*Acremonium zonatum*
15.	15	Sagar	*Lantana camara*	*Drechslera indica*
16.	16	Narsinghpur	*Parthenium hysterophorus*	*Fusarium roseum*
17.	17	Bargi	*Parthenium hysterophorus*	*Drechslera indica*
18.	18	Patan	*Parthenium hysterophorus*	*Phoma herbarum*
19.	19	Shahpura	*Lantana camara*	*Aspergillus terrus*
20.	20	Bhitauni	*Lantana camara*	*Dreschelera indica*
21.	21	Bhitauni	*Lantana camara*	*Colletotrichum gleosporoides*
22.	22	Panagar	*Lantana camara*	*Aspergillus niger*
23.	23	Gwalior	*Parthenium hysterophorus*	*Sclerotium rolfsii*
24.	24	Barela	*Hyptis suaveolens*	*Nigrospora oryzae*
25.	25	Narsinghpur	*Parthenium hysterophorus*	*Curvularia lunata*
26.	26	Panagar	*Lantana camara*	*Cladosporium herbarum*

27.	27	Satna	*Lantana camara*	*Helminthosporium oryzae*
28.	28	Satna	*Lantana camara*	*Colletotrichum gleosporioides*
29.	29	Jabalpur	*Lantana camara*	*Pestalotia brassicicola*
30.	30	Jabalpur	*Lantana camara*	*Haplosporella lantane*
31.	31	Shihora	*Parthenium hysterophorus*	*Curvularia lunata*
32.	32	Panagar	*Lantana camara*	*Alternaria alternata*
33.	33	Sagar	*Lantana camara*	*Curvularia* lunata
34.	34	Bhopal	*Lantana camara*	*Phoma glomerata*
35.	35	Mandla	*Hyptis suaveolens*	*Alternaria alternata*
36.	36	Panagar	*Lantana camara*	*Curvularia snegalensis*
37.	37	Bargi	*Cenatora*	*Alternaria solini*
38.	38	Jabalpur	*Lantana camara*	*Phoma multirostrata*
39.	39	Shahpura	*Lantana camara*	*Curvularia pallescens*
40.	40	Shahpura	*Parthenium hysterophorus*	*Chaetomium globosum*
41.	41	Shahpura	*Parthenium hysterophorus*	*Fusarium oxysporum*
42.	42	Belkheda	Seeds of *Parthenium hysterophorus*	*Colletotrichum dematium*
43.	43	Jabalpur	*Lantana camara*	*Fusarium moniliforme*
44.	44	Rajmarg	Stems of *Parthenium hysterophorus*	*Colletotrichum gleosporioides*
45.	45	Katni	*Lantana camara*	*Fusarium roseum*
46.	46	Singrauli	*Parthenium hysterophorus*	*Myrothecium roridum*
47.	47	Jabalpur	*Eicchornia crassiapes*	*Curvularia lunata*
48.	48	Mandla	*Pistia stratiotes*	*Alternaria alternata*
49.	49	Raipur	*Hyptis suaveolens*	*Trichoderma viride*
50.	50	Narsinghpur	*Eicchornia crassiapes*	*Aspergillus niger*
51.	51	Gwalior	*Parthenium hysterophorus*	*Pestalotia brassicicola*
52.	52	Bina	*Parthenium hysterophorus*	*Cladosporium* oxysporum
53.	53	Ujjain	*Cenatora*	*Cercosporidium punctum*

54.	54	Jabalpur	*Hyptis suaveolens*	*Phoma herbarum.*
55.	55	Jabalpur	*Hyptis suaveolens*	*Fusarium solani*
56.	56	Bargi	*Hyptis suaveolens*	*Curvularia lunata*
57.	57	Bilaspur	*Parthenium hysterophorus*	*Sclerotium rolfsii*
58.	58	Jabalpur	*Eicchornia crassiapes*	*Alternaria alternata*
59.	59	Sagar	*Sorghum halepense*	*Monodicticys putrivenses*
60.	60	Seoni	*Pistia stratiotes*	*Fusarium monilliforme*
61.	61	Rewa	*Lantana camara*	*Torula herbarum*
62.	62	Beohari	*Echinocloa colonum*	*Fusarium roseum*
63.	63	Patan	*Hyptis suaveolens*	*Fusarium roseum*
64.	64	Shivpuri	*Parthenium hysterphorus*	*Myrothecium verrucaria*
65.	65	Jabalpur	*Lemna gibba*	*Fusarium oxysporum*
66.	66	Indore	*Lantana camara*	*Phoma herbarum.*
67.	67	Jabalpur	Seeds of *Parthenium hysterophorus*	*Helminthosporium solani*
68.	68	Narsinghpur	*Cenatora*	*Cercospora personata*
69.	69	Narsinghpur	*Cenatora*	*Alternaria sesami*
70.	70	Jabalpur	*Hyptis suaveolens*	*Phoma herbarum*

FUNGI ISOLATED FROM AGRICULTURAL WEEDS

S No.	**FGCC#**	**Place**	**Weed**	**Name of Fungi**
1	71.	Jabalpur	*Euphorbia pulcherrima*	*Trichoderma viride*
2	72.	Jabalpur	*Euphorbia pulcherrima*	*Fusarium solani*
3	73.	Bhitauni	*Euphorbia pulcherrima*	*Fusarium moniliforme*
4	74.	Bhitauni	*Euphorbia pulcherrima*	*Curvularia lunata*
5	75.	Bhitauni	*Euphorbia pulcherrima*	*Trichoderma solani*

6	76.	Maharajpur	*Phyllanthus niruri*	*Penicillum notatum*
7	77.	Maharajpur	*Digera muricata*	*Fusarium roseum*
8	78.	Maharajpur	*Digera muricata*	*Trichoderma solani*
9	79.	Maharajpur	*Digera muricata*	*Arthinium japoniaum*
10	80.	Shahpura	*Digera muricata*	*Aspergillus terreus*
11	81.	Bhitauni	*Digera muricata*	*Chaetomium perlucidum*
12	82.	Shahpura	*Lucas cepholotus*	*Curvularia spicifera*
13	83.	Sihora	*Lucas cepholotus*	*Aspergillus fumigatus*
14	84.	Bhedaghat	*Lucas cepholotus*	*Fusarium oxysporum*
15	85.	Bargi	*Lucas cepholotus*	*Curvularia lunata*
16	86.	Panagar	*Lucas cepholotus*	*Paecilomyces fumosoroseus*
17	87.	Jhansighat	*Coix sp.*	*Fusarium nivale*
18	88.	Bargi	*Coix sp.*	*Drechslera maybeanus*
19	89.	Patan	*Coix sp.*	*Alternaria alternata*
20	90.	Barela	*Coix sp.*	*Diplococcum resinae*
21	91.	Panagar	*Coix sp.*	*Trichoderma virens*
22	92.	Panagar	*Xanthium strumarium*	*Sclerotium rolfsii*
23	93.	Panagar	*Xanthium strumarium*	*Fusarium oxysporum*
24	94.	Jhansighat	*Xanthium strumarium*	*Drechelera biseptata*
25	95.	Jhansighat	*Xanthium strumarium*	*Aspergillus flavus*
26	96.	Shahpura	*Euphorbia hirta*	*Fusarium oxysporum*
27	97.	Shahpura	*Euphorbia pulcherrima*	*Aspergillus oxyzae*
28	98.	Bhitauni	*Euphorbia pulcherrima*	*Mucor mucedo*
29	99.	Bhitauni	*Euphorbia pulcherrima*	*Trichoderma reesei*
30	100.	Panagar	*Euphorbia pulcherrima*	*Aspergillus fumigatus*
31	101.	Panagar	*Commelina caroliniana*	*Aspergillus niger*
32	102.	Panagar	*Commelina caroliniana*	*Dreschelera ostraliensis*
33	103.	Jhansighat	*Commelina caroliniana*	*Drechelera specifera*
34	104.	Jhansighat	*Commelina caroliniana*	*Aspergillus terreus*
35	105.	Jabalpur	*Psoratea corylitotia*	*Emerciella nidulans*

36	106.	Jabalpur	*Psoratea corylitotia*	*Colletotrichum gleosporoides*
37	107.	Jabalpur	*Psoratea corylitotia*	*Curvularia lunata*
38	108.	Adhartal	*Psoratea corylitotia*	*Drechelera rostrata*
39	109.	Adhartal	*Corchorus olitonius*	*Rhizopus oryzae*
40	110.	Patan	*Corchorus olitonius*	*Cladosporium oxysporum*
41	111.	Patan	*Corchorus olitonius*	*Acremonium alternatum*
42	112.	Barela	*Corchorus olitonius*	*Helminthosporium oryzae*
43	113.	Barela	*Alysicarous monilitor*	*Curvularia spicifera*
44	114.	Barela	*Alysicarpus monilitor*	*Emericella astellata*
45	115.	Bhitauni	*Alysicarpus monilitor*	*Cladosporium oxysporum*
46	116.	Bhitauni	*Alysicarpus monilitor*	*Rhizoctonia solani*
47	117.	Bargi	*Cyperus Rotundus*	*Penicillium claviforme*
48	118.	Bargi	*Cyperus Rotundus*	*Colletotrichum dematium*
49	119.	Bargi	*Cyperus Rotundus*	*Aspergillus flavus*
50	120.	Shahpura	*Cyperus Rotundus*	*Curvularia lunata*

FUNGI ISOLATED FROM MEDICINAL PLANTS

S No.	**FGCC#**	**Place**	**Plant**	**Name of Fungi**
1	121.	Jabalpur	*Embelica officinalis*	*Pestalotia guenipi*
2	122.	Bargi Forest	*Asparagus racemosus*	*Chaetomium globoum*
3	123.	Gwalior	*Zizyphus zizuba*	*Nigrospora sphaerica*
4	124.	Gwalior	*Zizyphus zizuba*	*Acremonium alternatum*
5	125.	Gwalior	*Jatropha gosipifolia*	*Fusarium nivale*
6	126.	Jabalpur	*Stevia rebaudiana*	*Curvularia lunata*

7	127.	Jabalpur	*Stevia rebaudiana*	*Helminthosporium victoriae*
8	128.	Jabalpur	*Zizyphus zizuba*	*Dreschslera biseptata*
9	129.	Jabalpur	*Aloe vera*	*Sclerotium rolfsii*
10	130.	Guna	*Withania somnifera*	*Alternaria alternata*
11	131.	Barela	*Asparagus racemosus*	*Alysidium resinae*
12	132.	Gwalior	*Asparagus racemosus*	*Phoma multirostrata*
13	133.	Guna	*Butea monosperma*	*Cladosporium herbarum*
14	134.	Guna	*Citrus lemon*	*Gloeosporium album*
15	135.	Jabalpur	*Curculigo orchioides*	*Phoma tropica*
16	136.	Jabalpur	*Chlorophytum boriviallianum*	*Fusarium solani*
17	137.	Jabalpur	*Acorus calamus*	*Helminthosporium microsorum*
18	138.	Jabalpur	*Oscimum sanctum*	*Curvularia oscimi*
19	139.	Jabalpur	*Rauwolfia serpentine*	*Colletotrichum dematium*
20	140.	Jabalpur	*Stevia rebaudiana*	*Alternaria alternata*

LIST OF YEAST ISOLATED

S.No	**FGGY#**	**Place**	**Yeast**
1	1	Antartica	*Kluyveromyces marxianus*
2	2	Antartica	*Pichia anomala*
3	3	Antartica	*Torulapora delbrueckii*
4	4	Antartica	*Rhodotorula minuta*
5	5	Antartica	*Tremelle brasiliensis*
6	6	Antartica	*Trichosporon brigelli*
7	7	Antartica	*Saccharomyces cerevisiae*
8	8	Antartica	*Saccharomyces cerevisiae*

9	9	Antartica	*Saccharomyces uvarum*
10	10	Antartica	*Endomycopsella vinii*
11	11	Antartica	*Candida mucilagina*
12	12	Antartica	*Dekkera intermedia*
13	13	Antartica	*Brettanomyces clausenii*
14	14	Antartica	*Rhodotorula aurantiaca*
15	15	Antartica	*Rhodotorula gracilis*
16	16	Antartica	*Rhodotorula rubra*
17	17	Antartica	*Rhodotorula minuta*

LIST OF MUSHROOM ISOLATED

S.No.	FGCCM#	Mushrooms	Host	Place
1.	1	*Ganoderma lucidum*	Sal tree	Jabalpur
	2	*Ganoderma lucidum*	Neem tree	Mandla
	3	*Ganoderma lucidum*	Eucalyptus tree	Kalpi
	4	*Ganoderma lucidum*	Mango tree	Jabalpur
	5	*Ganoderma lucidum*	Dead log	Barela
2.	6	*Ganoderma applenatum*	Tamarandous tree	Jabalpur
3.	7	*Lentinus cladopus*	Dead log	Kalpi
	8	*Lentinus cladopus*	Dead log of Mango tree	Jabalpur
	9	*Lentinus cladopus*	Dead log of wood	Katni
	10	*Lentinus cladopus*	Dead log of wood	bargi
4.	11	*Macrolepiota procera*	Grassland Soil	Mandla
5.	12	*Xyleria polymorpha*	Dead log	Jabalpur
6.	13	*Pleurotus sajorcaju*	Mango tree	Dindori
7.	14	*Pleurotus florida*	Grassland	Jabalpur

8.	15	*Tricholoma gignatum*	Peepal Tree	Barela
9.	16	*Schizophyllum commune*	Dead log of tree	Jabalpur
10.	17	*Termitomyces hemii*	Termites zone	Mandla
11.	18	*Scleroderma texsens*	Grassland Ground	Bedaghat
12.	19	*Cantharellus cibarium*	Bamboo	Balaghat
13.	20	*Volvariella volvacea*	Dump of house waste	Mandla
14.	21	*Lycoperdon pyriforme*	Mixed Sal plant	Kalpi
15.	22	*Agaricus bisporus*	Grassland Ground	Jabalpur
16.	23	*Lepiota procera*	Grassland Ground	Jabalpur

REFERENCES

- Agrawal, G. P. & S. K. Hasija (1986). *Microorganisms in the laboratory. A laboratory guide for Mycology, Microbiology & Plant Pathology*. Print House Lucknow (India), pp. 155
- Agriculture, Forestry and Fisheries Research Council &National Institute of Agro-environmental Sciences (1987). Biseibutsu no chouki hozon hou [Long-term preservationof microorganisms]. Agriculture, Forestry andFisheries Research Council & National Institute of Agro-Environmental Sciences, Tsukuba, Japan, pp.183 [In Japanese].
- Ainsworth, G.C., Sparrow, F.K. and Sussman, A.S. (1973 a). The Fungi: An advanced Treatise. Vol. IVa and IVb. Academic Press, New York.
- Ainsworth, G.C., Sparrow, F.K. and Sussman, A.S. (1973 b). Taxonomic reviews with keys: Ascomycetes and Fungi imperfecti. IV A., Academic press, New York and London. 592 pp.
- Alexopolous, C.J. and Mims, C.W. (1979). Introductory Mycology (3rd Eds.). John Wiley and Sons, New York. 632 pp.
- Arx, J.A.Von (1981). The genera of fungi sporulating in pure culture. (3rdEds.). (eds. J.Cramer, FL-9490 vaduz). 411 pp.
- Barnett, H.L. and Hunter, B.B. (1972). Illustrated genera of Imperfect Fungi. Burgess Publishing Company. 241 pp.

- Barron, G.L. (1968). The Genera of Hypomycetes from Soil, Baltimore, Maryland, Williams and Wilkins.
- Booth, C. (1971a). The genus Fusarium. CMI, Kew, Surrey, England. 237 pp.
- Booth, C. (1971b). Introduction to General Methods. In: Methods in Microbiology (eds.Booth,C.) Acad. Pr. New York.79-110 pp.
- Carmichael, J.W. (1956). Frozen storage for stock cultures of fungi. *Mycologia* 54 : 378-381.
- Carmichael, J.W., Kendrick, W.B., Conners, I.L. and Sigler, L. (1980). Genera of Hypomycetes. Edmonton. The University of Alberta Pr. Alberta, Canada. 386 pp.
- Castellani, A. **(**1939). The viability of some pathogenic fungi in sterile distilledwater. J. Trop. Med. Hyg. **42:**225–226.
- Chiang, M.Y., C.G. Van Dyke and K.J. Leonard (1989). Evaluation of endemic foliar fungi for potential biological control of Johnson grass *Sorghum halepense*: Screening and mist range test. *Plant Dis*. 73: 459-464.

- Chiang, M.Y., C.G. Van Dyke and K.J. Leonard (1989). Evaluation of endemic foliar fungi for potential biological control of Johnson grass *Sorghum halepense*: Screening and mist range test. *Plant Dis*. 73: 459-464.

- Daniel E. Legard and C.K.Chandler (2000). Cryopreservation of Strawberry pathogens in a -95°C Mechanical Ultra – low Temperature Freezer .*Hort Sciences* 35 (35) : 1357.
- Hughes, S.J. (1953b). Conidiophores, Conidia and Classification. Can. J. Bot. 31: 577-659.
- Jackson, M.A. and R.J. Bothast (1990). Carbon concentration and carbon to nitrogen ratio influence submerged-culture conidiation by the potential bioherbicide *Colletotrichum truncatum* NRRL 13737. *Appl. Environ. Microbiol*. 3435-3438.

- Jackson, M.A. and R.J. Bothast (1990). Carbon concentration and carbon to nitrogen ratio influence submerged-culture conidiation by the potential bioherbicide *Colletotrichum truncatum* NRRL 13737. *Appl. Environ. Microbiol*. 3435-3438.

- Kirsop, B. E. & Doyle, A. (1991). Maintenance of microorganisms and culture cells: A manual of laboratorymethods, 2nd ed., Academic Press, London, pp.308.
- Martin, J.P. (1950). Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soc. Sc. 69: 215-232.

- McGinnis, M. R., A. A. Padhye, and L. Ajello **(**1974). Storage of stock culturesof filamentous fungi, yeasts, and some aerobic actinomycetes in sterile distilled water. Appl. Microbiol. **28:**218–222.
- Meyer, E. **(**1955). The preservation of dermatophytes at subfreezing temperatures.Mycologia **47:**664–668.
- Pasarell, L., and M. R. McGinnis **(**1992). Viability of fungal cultures maintained
- Schipper, M. A. A., and J. Bekker-Holtman **(**1976). Viability of lyophilizedfungal cultures. Antonie Leeuwenhoek J. Microbiol. **42:**325–328.
- Sharma, M.C. and B.C. Sharma (1969). Toxic metabolites production of *Colletotrichum gloeosporioides* causing citrus die back in India. *Indian Phytopathol*.22: 74-76.
- Sharma, M.C. and B.C. Sharma (1969). Toxic metabolites production of *Colletotrichum gloeosporioides* causing citrus die back in India. *Indian Phytopathol*.22: 74-76.
- Smith, D. & Onions, A. H. S. (1994) The preservation and maintenance of living fungi, 2nd ed., CAB International, Oxfordshire, pp.122.
- Stalpers, J. A., A. De Hoog, and I. J. Vlug **(**1987). Improvement of the strawtechnique for the preservation of fungi in liquid nitrogen. Mycologia **79:**82–89.
- Strobel, G.A. (1973).The Helmnthosporoside binding protein of sugarcane. *J. Biol. Chem.* 248:1321-1328.
- Strobel, G.A. (1973).The Helmnthosporoside binding protein of sugarcane. *J. Biol. Chem.* 248:1321-1328.
- Subramanian, C.V. (1956). Fungi imperfecti from Madras VIII. Proc. Ind. Acad. Sci. 42(B): 283-292.
- Subramanian, C.V. (1956). Hyphomycetes-I & II. J. Indian. Bot. Soc. 5: 53-91.
- Subramanian, C.V. (1977). Revisions of Hyphomycetes I. Kavaka 5: 93-98.
- Subramanian, C.V. (1982). Tropical Mycology, its future needs and development. Curr. Sci. 51: 321-325.
- Subramanian, C.V. (1986). The progress and status of mycology in India. Proc. Indian Acad. Sci. (Pl. Sci.) 96: 379-392.

- Sugawara, F., Strobel, G., Fishcher, L.E., Van, Dyke, G.D. and Clardy,J.(1985). Bipolaroxin: A selective phytotoxin produced by *Bipolaris cyanodontis*. *Proc. Nat. Acad. Sci. USA* **82**: 8291-8294.
- Sugawara, F., Strobel, G., Fishcher, L.E., Van, Dyke, G.D. and Clardy, J.(1985). Bipolaroxin: A selective phytotoxin produced by *Bipolaris cyanodontis*. *Proc. Nat. Acad. Sci. USA* **82**: 8291-8294.
- Sutton, B.C. (1980). The Coelomycetes, Fungi Imperfecti with pycnidia, acervuli and stromata. C. A. B. International Mycological Research Institute, Kew, Survey, England
- Templeton, G.E. (1972). Alternaria toxins related to pathogenesis in plants. In: *Microbial toxins: fungal toxins* VIII (Ceigler, Kadis. and Ajil eds). Academic Press New York. London.
- Templeton, G.E. (1972). Alternaria toxins related to pathogenesis in plants. In: *Microbial toxins: fungal toxins* VIII (Ceigler, Kadis. and Ajil eds). Academic Press New York. London.
- Vikrant, P., K.K. Verma, R.C. Rajak and A.K.Pandey (2006). Characterization of a phytotoxin from *Phoma herbarum* for management of *Parthenium hysterophorus*. *J. Phytopathol*. 154: 1-8.
- Vikrant, P., K.K. Verma, R.C. Rajak and A.K.Pandey (2006). Characterization of a phytotoxin from *Phoma herbarum* for management of *Parthenium hysterophorus*. *J. Phytopathol*. 154: 1-8.
- Walker, H.L. and Templeton, G. E. (1978). *In vitro* production of phytotoxic metabolites by *Colletotrichum gloeosporioides* f sp *aeschynomene*. *Plant Sci Lett.*, 13, 91-99.
- Walker, H.L. and Templeton, G. E. (1978). *In vitro* production of phytotoxic metabolites by *Colletotrichum gloeosporioides* f sp *aeschynomene*. *Plant Sci Lett.*, 13, 91-99.
- Warcup, J.H. (1950). The soil plate method for isolation of fungi from soil. Netura 166: 117.

Significance of the Data:

Collection of various fungi were done during the tenure of project, subsequent exploitation for their biotechnological potential have also been done. Characterization of important biomolecules is also conducted simultaneously. Also providing the facility of identification of fungal species to the colleges, Univerties and Institutes of Madhya Pradesh. The infra structure for storage of important fungi and Yeast have been developed and finally there is an establishment of Regional Culture Collection & Herbarium.

Identification services:-

Identification of fungi on morphotaxonomic grounds was offered to various Universities, agencies and colleges.

1. APS University, Rewa.
2. Madhya Pradesh council of Science and technology, Bhopal.
3. Holy Cross Women College, Ambikapur
4. Mata Gujri College Jabalpur.
5. St. Alloysius College, Jabalpur.
6. Govt. Home Science College, Jabalpur.

Abstract of the research work done during the period:

Fungal cultures were collected from different parts of Central India. Screening for their biotechnological application is conducted for characterizing some bioactive compounds and also because of their biotechnology aspects.

Articles/ research papers published in scientific journals during the reporting period related to the project:

1. Sadaf Quereshi, Ritu Panjwani, A.K. Pandey and Ajay K. Singh (2008). Formulation of Phytotoxins of *Phoma* sp. FGCC#18 for the management of *Parthenium hysterophorus* L *Archives of Phytopathology and Plant Protection*. (Accepted).
2. Nikita Banerjee, Jaya Singh, Sadaf Quereshi and A.K.Pandey (2009). Mass production, Formulation and Plot Trials of Phytotoxins from *Colletotrichum dematium* FGCC#20 effective against *Parthenium hysterophorus* L. Electronic *Journal of Environmental, Agricultural and Food Chemistry*. 8(6): 416-424.
3. Noor Afshan Khan, Sadaf Quereshi, Akhilesh Pandey and Ashutosh Srivastava. Antibacterial Activity of Commercial and Wild *Lathyrus* Species Seed Extracts. Turkish Journal of Biology. (Accepted).
4. Ritu Panjwani and A.K.Pandey (2009). Invitro antioxidant activity of *Coprinus comatus* extracts. *J. Basic & Appl. Mycol*. Vol. 8(I & II):37-40.
5. Mithilesh Jaiswal, A.K. Pandey and Jamaluddin. Pathogenic ability of different Mycoflora against excised *Parthenium* leaves. *J. of Tropical Forestry* (Accepted).
6. Mithilesh Jaiswal, A.K. Pandey and Jamaluddin. Virulence of different Indigenous Fungal Pathogens against *Parthenium.*. *J. Basic & Appl. Mycol* (Accepted).

Significant Conference/ Meeting / Seminar in which data was presented during the period

i. Oral presentation on –"Ecofriendly agrochemicals from Fungi" National seminar on: Microbiology-opportunities, challenges and skills", organized by Department of Botany, D.N. Jain College, Jabalpur.20-21 Feb'2009.

ii. Oral Presentation on "*Phoma* spp: Potential sources of herbicidal secondary metabolites" Microtech-2008,organized by organized by Society for Basic & Applied Mycology, SBAM, Dept. of Biological Sciences, R.D. University, Jabalpur (M.P.), 21 Nov'2008.

iii. Oral Presentation on "Mass production and formulation of secondary metabolites of *Phoma* sp. FGCC#54", National Science Day Celebration organized by Society for Basic & Applied Mycology, SBAM, Dept. of Biological Sciences, R.D. University, Jabalpur (M.P.) and sponsored by Madhya Pradesh Council of Science and Technology, Bhopal. 2 Feb-1 March'2009.

iv. Poster Presentation on Invitro antioxidant activity of *Coprinus comatus* extracts on 2nd Bhartiya Vigyan Sammelan & Expo 2009, 27th Nov. to 3rd December.

v. Paper Presentation on "Invitro antioxidant activity of *Agaricus bisporus* and *Coprinus comatus* in liquid media supplemented with agrowaste" on the 97th Indian science Congress held at University of Kerala, Thiruvananthapuram, Jan 3 to 7, 2010.

List of books, journals, reprints procured during the period for the research work on the project: Several reprints from various journals and authors related to the studies were obtained during this period. Departmental Library, Central Library, Rani Durgavati University, Jabalpur was consulted in this regard.

List of Equipments/ instruments purchased from council funds with cost and stock book page of the institution and the year of purchase:

S.No	Instrument	Cost	Stock Book page	Year of purchase
1	Digital Microscope (Motic)	3,62,700.00	28	2008
2	Rotary Vacuum Evaporator (Jindal)	1,09,913.00	30	2008
3	BOD Incubator (Remi)	53,438.00	31	2008
4	Generator (Kirloskar)	5,31,703.00	32	2008
5	Cold Room (Blue Star)	7,36,875.00	33	2008

Human Resource Development Programme:

Under this programme following students were trained for their M.Phil or M.Sc dissertations**. (2008).**

Ph.D.

1. Dr. Sadaf Qureshi , awarded (2009)

2. Mr. Shyamji Shukla, Registered

3. Ms. Ritu Panjwani, Registered

4. Mr. Mithilesh Jaiswal, Registered

M.Phil

1. ***Javed Iqbal (2008).*** Antimicrobial activities of some selected Indian mushrooms.

2. ***Vandana Saxena (2008).*** Taxonomic studies of fungi associated with medicinal plants.

3.***Rashmi Hanote (2008).*** Standardization of nutritional conditions, mass production and formulation of herbicidal secondary metabolites of *Streptomyces* sp.

M.Sc

1. ***Shweta Dubey*** *(2008).* Mycoflora of wild plants of Jabalpur
2. ***Hema Bangari*** *(2008).*Mycoflora associated with *Jatropha curcas*, a potential source of biofuel
3. ***Richa Tripathi*** *(2008*). Diversity of keratinophilic fungi at Sihora
4. ***Manisha Sharma*** *(2008).*Studies on diversity of fungi associated with floricultural plants of Jabalpur.
5. ***Abhishek Kumar Awasthi (2010)*** **Diversity of fungi in various sugar industries of Madhya Pradesh.**
6. ***Ajay Gond (2010).*** **Diversity of Mycorrhizal fungi associated with oil yielding plants.**
7. ***Amit Kumar Pandey (2010).*** **Evaluation of Antimicrobial activity of agaricus bisporus (Button Mushroom) of Jabalpur.**
8. ***Varun Tripathi (2010).*** **Study of Endophytic fungi from some important medicinal plants.**

B.Sc. (Botany)

1. **Akansha Tiwari** (2009-10). Basidiomycestes of Madhya Pradesh: Diversity and its application.

Table-I: screening of fungal strain.

S. No.	Name of fungus	Incubation period	Herbicidal Potential									
			Seedling Bioassay					Shoot cut Bioassay				
			P	L	H	X	C	P	L	H	X	C
1	*Alternaria alternata* FGCCW#02 *(Parthenium hysterophorus*)	Control	0	-	0	0	0	0	0	0	0	-
		7	1	-	1	1	1	1	1	2	1	-
		14	2	-	2	2	1	2	2	2	1	-
		21	3	-	1	1	1	3	3	2	1	-
		28	3	-	2	2	1	3	3	3	1	-
	Alternaria alternata FGCCW#11 *(Parthenium hysterophorus)*	Control	0	-	0	0	0	0	0	0	0	-
		7	1	-	0	0	1	1	2	1	1	-
		14	1	-	1	1	1	2	1	2	2	-
		21	1	-	1	1	1	3	2	1	1	-
		28	1	-	1	1	1	3	3	2	2	-
	Alternaria alternata FGCCW#32 *(Lantana camara)*	Control	0	-	0	0	0	0	0	0	0	-
		7	1	-	2	1	1	1	2	1	1	-
		14	2	-	2	1	1	2	1	2	2	-
		21	3	-	2	1	2	3	2	1	1	-
		28	3	-	3	1	3	3	3	2	2	-
2	*Curvularia* lunata FGCCW#25 *(Parthenium hysterophorus)*	Control	0	-	0	0	0	0	0	0	0	-
		7	1	-	1	1	2	1	1	0	0	-
		14	2	-	2	1	1	1	2	1	1	-
		21	3	-	2	1	2	1	1	1	1	-
		28	3	-	3	1	3	1	1	1	1	-
	Curvularia lunata FGCCW#33 *(Lantana camara)*	Control	0	-	0	0	0	0	0	0	0	-
		7	1	-	1	1	2	1	1	0	0	-
		14	2	-	2	2	3	1	2	1	1	-
		21	3	-	1	1	3	1	1	1	1	-
		28	3	-	2	2	3	1	1	1	1	-
	Curvularia pallescens FGCCW#39 *(Lantana camara)*	Control	0	-	0	0	0	0	0	0	0	-
		7	1	-	1	1	2	1	2	1	1	-
		14	2	-	2	2	3	2	1	2	2	-
		21	3	-	1	1	3	3	2	1	1	-
		28	3	-	2	2	3	3	3	2	2	-
	Curvularia senegalensis FGCCW#36 *(Lantana camara)*	Control	0	-	0	0	0	0	0	0	0	-
		7	1	-	1	1	2	1	2	1	1	-
		14	2	-	2	2	3	2	1	2	2	-
		21	3	-	1	1	3	3	2	1	1	-
		28	3	-	2	2	3	3	3	2	2	-
	Curvularia lunata FGCCW#56 *(Hyptis suaveolens)*	Control	0	-	0	0	0	0	0	0	0	-
		7	1	-	1	1	2	1	2	1	1	-
		14	2	-	2	2	3	2	1	2	2	-
		21	3	-	1	1	3	3	2	1	1	-
		28	3	-	2	2	3	3	3	2	2	-

3	*Phoma herbarum* FGCCW#07 *(Parthenium hysterophorus*)	Control	0	-	0	0	0	0	0	0	0	0	-
		7	1	-	1	1	2	1	2	1	1	-	
		14	2	-	2	2	3	2	1	2	2	-	
		21	3	-	1	1	3	3	2	1	1	-	
		28	3	-	2	2	3	3	3	2	2	-	
	Phoma herbarum *FGCCW#18 *(Parthenium hysterophorus*)	Control	0	-	0	0	0	0	0	0	0	-	
		7	2	-	2	2	2	2	2	2	2	-	
		14	3	-	3	3	3	3	3	3	3	-	
		21	4	-	4	4	3	4	4	4	4	-	
		28	5	-	5	5	5	5	5	5	5	-	
	Phoma multirostrata FGCCW#38 *(Lantana camara)*	Control	0	-	0	0	0	0	0	0	0	-	
		7	1	-	1	1	2	1	2	1	1	-	
		14	2	-	2	2	3	2	1	2	2	-	
		21	3	-	1	1	3	3	2	1	1	-	
		28	3	-	2	2	3	3	3	2	2	-	
	Phoma glomerata *FGCCW#34 *(Lantana camara)*	Control	0	-	0	0	0	0	0	0	0	-	
		7	2	-	2	2	2	2	2	2	2	-	
		14	3	-	3	3	3	3	3	3	3	-	
		21	4	-	4	4	3	4	4	4	4	-	
		28	5	-	5	5	5	5	5	5	5	-	
	Phoma herbarum *FGCCW#54 *(Hyptis suaveolens)*	Control	0	-	0	0	0	0	0	0	0	-	
		7	2	-	2	2	2	2	2	2	2	-	
		14	3	-	3	3	3	3	3	3	3	-	
		21	4	-	4	4	3	4	4	4	3	-	
		28	4	-	4	4	3	5	5	5	5	-	
	Phoma herbarum FGCCW#70 *(Hyptis suaveolens*)	Control	0	-	0	0	0	0	0	0	0	-	
		7	1	-	1	1	2	1	2	1	1	-	
		14	2	-	2	2	3	2	1	2	2	-	
		21	3	-	1	1	3	3	2	1	1	-	
		28	3	-	2	2	3	3	3	2	2	-	
4	*Pestalotia indica* FGCCW#06 *(Parthenium hysterophorus*)	Control	0	-	0	0	0	0	0	0	0	-	
		7	1	-	1	1	2	1	2	1	1	-	
		14	2	-	2	2	3	2	1	2	2	-	
		21	3	-	1	1	3	3	2	1	1	-	
		28	3	-	2	2	3	3	3	2	2	-	
5	*Sclerotium rolfsii* FGCCW#08 *(Parthenium hysterophorus*)	Control	0	-	0	0	0	0	0	0	0	-	
		7	1	-	1	1	2	1	2	1	1	-	
		14	2	-	2	2	3	2	1	2	2	-	
		21	3	-	1	1	3	3	2	1	1	-	
		28	3	-	2	2	3	3	3	2	2	-	
	Sclerotium rolfsii *FGCCW#23 (Parthenium hysterophorus*)	Control	0	-	0	0	0	0	0	0	0	-	
		7	1	-	1	1	2	1	2	1	1	-	
		14	2	-	2	2	3	2	1	2	2	-	
		21	3	-	1	1	3	3	2	1	1	-	
		28	3	-	2	2	3	3	3	2	2	-	
6	*Colletotrichum dematium* FGCCW#09 *(Parthenium hysterophorus*)	Control	0	-	0	0	0	0	0	0	0	-	
		7	1	-	1	1	2	1	2	1	1	-	
		14	2	-	2	2	3	2	1	2	2	-	
		21	3	-	1	1	3	3	2	1	1	-	
		28	3	-	2	2	3	3	3	2	2	-	

7	*Fusarium roseum* *****FGCCW#16 *(Parthenium hysterophorus*)	Control	0	-	0	0	0	0	0	0	0	-
		7	2	-	2	2	2	2	2	2	2	-
		14	3	-	3	3	3	3	3	3	3	-
		21	4	-	4	4	3	4	4	4	4	-
		28	4	-	4	4	3	4	4	4	4	-
	Fusarium oxysporum *FGCCW#01 *(Parthenium hysterophorus*)	Control	0	-	0	0	0	0	0	0	0	-
		7	2	-	2	2	2	2	2	2	2	-
		14	3	-	3	3	3	3	3	3	3	-
		21	4	-	4	4	3	4	4	4	4	-
		28	4	-	4	4	3	4	4	4	4	-
	Fusarium nivale FGCCW#13 *(Parthenium hysterophorus*)	Control	0	-	0	0	0	0	0	0	0	-
		7	1	-	1	1	2	1	2	1	1	-
		14	2	-	2	2	3	2	1	2	2	-
		21	3	-	1	1	3	3	2	1	1	-
		28	3	-	2	2	3	3	3	2	2	-
	Fusarium roseum *FGCCW#45 *(Lantana camara)*	Control	0	-	0	0	0	0	0	0	0	-
		7	2	-	2	2	2	2	2	2	2	-
		14	3	-	3	3	3	3	3	3	3	-
		21	4	-	4	4	3	4	4	4	4	-
		28	5	-	5	5	5	5	5	5	5	-
	Fusarium moniliforme *FGCCW#43 *(Lantana camara)*	Control	0	-	0	0	0	0	0	0	0	-
		7	2	-	2	2	2	2	2	2	2	-
		14	3	-	3	3	3	3	3	3	3	-
		21	4	-	4	4	3	4	4	4	4	-
		28	5	-	5	5	5	5	5	5	5	-
	Fusarium roseum *FGCCW#63 (*Hyptis suaveolens*)	Control	0	-	0	0	0	0	0	0	0	-
		7	2	-	2	2	2	2	2	2	2	-
		14	3	-	3	3	3	3	3	3	3	-
		21	4	-	4	4	3	4	4	4	4	-
		28	5	-	5	5	5	5	5	5	5	-
	Fusarium oxysporum FGCCW#65 (*Hyptis suaveolens*)	Control	0	-	0	0	0	0	0	0	0	-
		7	1	-	1	1	2	1	2	1	1	-
		14	2	-	2	2	3	2	1	2	2	-
		21	3	-	1	1	3	3	2	1	1	-
		28	3	-	2	2	3	3	3	2	2	-
	Fusarium solani *FGCCW#55 (*Hyptis suaveolens*)	Control	0	-	0	0	0	0	0	0	0	-
		7	2	-	2	2	2	2	2	2	2	-
		14	3	-	3	3	3	3	3	3	3	-
		21	4	-	4	4	3	4	4	4	4	-
		28	5	-	5	5	5	5	5	5	5	-
	Fusarium moniliforme FGCCW#60 *(Hyptis suaveolens*)	Control	0	-	0	0	0	0	0	0	0	-
		7	1	-	1	1	2	1	2	1	1	-
		14	2	-	2	2	3	2	1	2	2	-
		21	3	-	1	1	3	3	2	1	1	-
		28	3	-	2	2	3	3	3	2	2	-

ter
W#55, FGCCW#63)

Table2: Assessment of Phytotoxic damage in target weeds by phytotoxin produced in different media. (Shoot Cut bioassay)

S. No	Media	*Parthenium* (PDR)			*Lantana* (PDR)			*Hyptis* (PDR)			*Sida* (PDR)		
		12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt
1	Control a	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	Control b	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	FPDB	3.36±0.03	3.50±0.04	3.93±0.05	3.15±0.02	3.26±0.03	3.61±0.04	3.06±0.03	3.13±0.03	3.39±0.02	2.84±0.03	3.05±0.03	3.33±0.02
4	SNB	2.90±0.03	3.25±0.03	3.62±0.03	2.81±0.03	2.96±0.03	3.26±0.04	2.65±0.04	2.88±0.02	3.11±0.03	2.61±0.04	2.77±0.03	3.00±0.04
5	MPB	2.86±0.03	3.01±0.04	3.29±0.03	2.63±0.02	2.94±0.03	3.13±0.03	2.40±0.03	2.75±0.02	2.92±0.03	2.35±0.03	2.72±0.03	2.95±0.04
6	Martin's Broth	3.07±0.04	3.21±0.02	3.34±0.04	2.83±0.03	2.92±0.04	3.26±0.04	2.73±0.03	2.85±0.03	3.08±0.04	2.72±0.03	2.80±0.02	2.94±0.03
7	SaDB	2.10±0.03	2.21±0.02	2.39±0.03	1.94±0.03	2.26±0.04	2.60±0.03	1.85±0.02	1.99±0.04	2.20±0.04	1.39±0.06	1.77±0.03	2.26±0.03
8	Miller's Broth	1.58±0.02	1.77±0.05	2.14±0.04	1.62±0.04	1.83±0.03	2.05±0.03	1.13±0.02	1.37±0.04	1.73±0.03	1.10±0.01	1.33±0.04	1.72±0.03
9	Cohn's Medium	2.80±0.05	3.19±0.03	3.47±0.04	2.79±0.05	2.94±0.03	3.26±0.03	2.69±0.03	2.86±0.03	3.05±0.06	2.64±0.03	2.73±0.03	2.92±0.05
10	Fermi's Medium	3.25±0.03	3.57±0.04	3.80±0.02	3.18±0.02	3.35±0.03	3.68±0.3	3.05±0.04	3.19±0.03	3.41±0.02	2.93±0.03	3.09±0.04	3.32±0.04
11	Pferrer's Medium	3.53±0.04	3.67±0.02	3.87±0.05	3.42±0.02	3.53±0.04	3.84±0.04	1.89±0.04	2.13±0.02	2.47±0.06	1.88±0.04	2.04±0.04	2.33±0.04
12	A & H Medium	0.58±0.05	0.65±0.02	0.70±0.05	0.34±0.03	0.51±0.05	0.57±0.05	0.28±0.03	0.36±0.03	0.54±0.03	0.25±0.03	0.34±0.03	0.45±0.03
13	Coon's Medium	0.65±0.04	0.77±0.03	0.90±0.04	0.43±0.04	0.64±0.03	0.90±0.02	0.40±0.02	0.64±0.02	0.78±0.04	0.32±0.03	0.57±0.03	0.68±0.02
14	Czapeck Medium	4.28±0.04	4.38±0.04	4.60±0.04	4.08±0.04	4.38±0.03	4.54±0.02	3.89±0.02	4.07±0.04	4.34±0.03	3.63±0.02	3.88±0.03	4.18±0.03
15	Richard's Medium	4.38±0.04	4.62±0.03	4.77±0.03	4.17±0.02	4.37±0.03	4.65±0.04	4.06±0.04	4.31±0.03	4.50±0.01	3.82±0.04	4.03±0.05	4.16±0.05
	SEm±	0.05	0.04	0.06	0.04	0.05	0.04	0.05	0.03	0.04	0.04	0.05	0.03
	$LSD_{5\%}$	0.16	0.35	0.22	0.19	0.38	0.48	0.33	0.27	0.34	0.43	0.21	0.15

Values are Means ± SEM of three observations; Control a- Unmetabolised growth medium

Control b- Sterilized Distilled Water; Amount of Toxin employed=15 ml/shoot; RH-85%

PDR - 0-0.99= slight curling & wilting; 1-1.99=slight chlorosis; 2-2.99=marked chlorosis, slight necrosis; 3-3.99=high necrosis and marked chlorosis; 4-4.99=acute necrosis and marked chlorosis; 5=acute chlorosis and acute necrosis leading to death of shoots.

Table 3: Assessment of Phytotoxic damage in target weeds by phytotoxin produced at different pH levels by *Phoma herbarum (*FGCC#54). (Shoot Cut Bioassay)

S. No	pH	*Parthenium* (PDR)			*Lantana* (PDR)			*Hyptis* (PDR)			*Sida* (PDR)		
		12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt
1	Control a	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	Control b	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	3.0	3.30±0.03	3.48±0.02	3.76±0.05	3.29±0.04	3.38±0.04	3.77±0.02	3.13±0.02	3.27±0.03	3.67±0.04	3.05±0.02	3.29±0.04	3.57±0.05
4	4.0	4.49±0.03	4.74±0.03	4.85±0.03	4.36±0.04	4.58±0.04	4.75±0.02	4.07±0.03	4.28±0.05	4.46±0.03	3.83±0.04	4.10±0.04	4.41±0.04
5	5.0	4.34±0.02	4.45±0.03	4.63±0.02	4.28±0.04	4.39±0.02	4.54±0.03	4.18±0.02	4.23±0.03	4.32±0.02	3.69±0.03	3.89±0.02	3.99±0.04
6	6.0	3.93±0.03	4.10±0.03	4.24±0.02	4.22±0.03	4.33±0.03	4.38±0.04	4.05±0.03	4.14±0.02	4.29±0.03	3.58±0.03	3.60±0.03	3.71±0.02
7	7.0	3.63±0.03	3.74±0.02	3.84±0.03	4.12±0.02	4.20±0.02	4.27±0.04	3.95±0.02	4.03±0.03	4.14±0.03	3.33±0.03	3.40±0.03	3.44±0.03
8	8.0	3.57±0.03	3.65±0.02	3.71±0.02	3.60±0.04	3.72±0.03	3.92±0.04	3.43±0.03	3.61±0.04	3.80±0.03	3.04±0.03	3.15±0.02	3.29±0.02
9	9.0	2.30±0.02	2.44±0.03	2.52±0.03	3.19±0.04	3.27±0.03	3.50±0.04	3.23±0.03	3.33±0.03	3.41±0.03	2.07±0.03	2.27±0.03	2.37±0.04
10	10.0	2.12±0.02	2.21±0.03	2.34±0.03	1.19±0.03	1.32±0.02	1.41±0.03	1.13±0.02	1.25±0.03	1.30±0.03	1.21±0.03	1.30±0.04	1.47±0.03
11	11.0	0.61±0.03	0.74±0.03	0.93±0.04	0.72±0.02	0.81±0.03	0.93±0.04	0.60±0.30	0.65±0.03	0.78±0.02	0.30±0.02	0.49±0.03	0.60±0.03
12	12.0	0.37±0.04	0.58±0.03	0.79±0.03	0.37±0.03	0.49±0.03	0.66±0.03	0.40±0.02	0.59±0.03	0.76±0.03	0.15±0.03	0.22±0.03	0.29±0.02
	SEm±	0.04	0.03	0.14	0.18	0.15	0.04	0.04	0.13	0.12	0.04	0.14	0.03
	$LSD_{5\%}$	0.12	0.20	0.26	0.35	0.24	0.19	0.22	0.30	0.28	0.17	0.33	0.24

Values are Means ± SEM of three observations; Control a- Unmetabolised growth medium

Control b- Sterilized Distilled Water; Amount of Toxin employed=15 ml/shoot; RH-85%

PDR - 0-0.99= slight curling & wilting; 1-1.99=slight chlorosis; 2-2.99=marked chlorosis, slight necrosis; 3-3.99= high necrosis and marked chlorosis; 4-4.99=acute necrosis and marked chlorosis; 5=acute chlorosis and acute necrosis leading to death of shoots.

Table 4: Assessment of Phytotoxic damage in target weeds by phytotoxin produced at different temperatures by *Phoma herbarum* (FGCC#54). (Shoot Cut Bioassay)

S. No	Incubation Temperature (°C)	*Parthenium* (PDR)			*Lantana* (PDR)			*Hyptis* (PDR)			*Sida* (PDR)		
		12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt
1	Control a	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	Control b	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4	5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
5	10	0.11±0.02	0.25±0.03	0.34±0.03	0.15±0.02	0.23±0.02	0.31±0.03	0.12±0.02	0.20±0.02	0.26±0.02	0.10±0.02	0.15±0.02	0.23±0.02
6	15	0.32±0.03	0.38±0.02	0.47±0.03	0.30±0.04	0.36±0.03	0.46±0.03	0.28±0.04	0.34±0.02	0.40±0.02	0.25±0.03	0.27±0.01	0.33±0.03
7	20	2.57±0.04	2.93±0.04	3.11±0.04	2.59±0.02	2.91±0.03	3.12±0.01	2.43±0.03	2.78±0.03	2.92±0.04	2.36±0.04	2.68±0.03	2.85±0.03
8	25	3.44±0.03	3.73±0.03	3.86±0.03	3.38±0.03	3.61±0.04	3.75±0.02	3.28±0.04	3.43±0.03	3.55±0.02	3.19±0.02	3.35±0.01	3.49±0.04
9	28	4.14±0.02	4.38±0.03	4.81±0.03	4.08±0.02	4.40±0.03	4.75±0.03	3.93±0.03	4.30±0.02	4.49±0.01	3.34±0.03	4.15±0.03	4.31±0.03
10	30	4.05±0.03	4.28±0.03	4.58±0.02	4.03±0.02	4.26±0.03	4.51±0.03	3.89±0.04	4.23±0.03	4.35±0.01	3.79±0.03	4.11±0.02	4.19±0.03
11	35	4.01±0.02	4.22±0.02	4.51±0.03	3.83±0.03	4.13±0.02	4.38±0.03	3.71±0.02	4.06±0.02	4.15±0.03	3.58±0.03	3.92±0.03	4.09±0.02
12	40	1.72±0.03	1.89±0.02	2.10±0.04	1.65±0.04	1.82±0.04	2.09±0.01	1.46±0.04	1.75±0.03	1.94±0.03	1.32±0.04	1.67±0.02	1.84±0.03
	SEm±	0.18	0.05	0.04	0.09	0.04	0.12	0.10	0.05	0.03	0.04	0.06	0.14
	$LSD_{5\%}$	0.54	0.38	0.16	0.27	0.25	0.31	0.23	0.21	0.11	0.15	0.18	0.36

Values are Means ± SEM of three observations; Control a- Unmetabolised growth medium;

Control b- Sterilized Distilled Water; Amount of Toxin employed=15 ml/shoot; RH-85%

PDR - 0-0.99= slight curling & wilting; 1-1.99=slight chlorosis; 2-2.99=marked chlorosis, slight necrosis; 3-3.99=high necrosis and marked chlorosis; 4-4.99=acute necrosis and marked chlorosis; 5=acute chlorosis and acute necrosis leading to death of shoots.

Table :5 Assessment of Phytotoxic damage on target weeds by phytotoxin produced at different incubation periods by *Phoma herbarum (FGCC#54)* (Shoot Cut Bioassay)

S. No	Incubation days	*Parthenium* (PDR)			*Lantana* (PDR)			*Hyptis* (PDR)			*Sida* (PDR)		
		12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt
1	Control a	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	Control b	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	7	2.90±0.05	3.28±0.04	3.60±0.04	2.67±0.06	3.23±0.04	3.53±0.02	2.54±0.04	3.25±0.03	3.49±0.03	2.49±0.02	2.89±0.03	3.39±0.03
4	14	3.95±0.04	4.17±0.04	4.43±0.02	3.84±0.05	4.11±0.06	4.33±0.04	3.73±0.02	3.91±0.04	4.24±0.02	3.67±0.01	3.95±0.04	4.12±0.02
5	21	4.35±0.03	4.53±0.04	4.74±0.04	4.25±0.03	4.51±0.05	4.72±0.02	4.19±0.03	4.45±0.04	4.71±0.02	4.12±0.01	4.41±0.03	4.60±0.01
6	28	4.19±0.04	4.45±0.05	4.64±0.03	4.14±0.04	4.48±0.03	4.56±0.04	4.20±0.03	4.38±0.03	4.51±0.02	3.89±0.02	4.20±0.03	4.38±0.04
	SEm±	0.03	0.06	0.05	0.03	0.12	0.04	0.05	0.05	0.06	0.03	0.10	0.11
	$LSD_{5\%}$	0.11	0.23	0.18	0.15	0.34	0.21	0.15	0.26	0.37	0.18	0.37	0.32

Values are Means ± SEM of three observations; Control a- Unmetabolised growth medium

Control b- Sterilized Distilled Water; Amount of Toxin employed=15 ml/shoot; RH-85%

PDR - 0-0.99= slight curling & wilting; 1-1.99=slight chlorosis; 2-2.99=marked chlorosis, slight necrosis;

3-3.99=high necrosis and marked chlorosis; 4-4.99=acute necrosis and marked chlorosis; 5=acute chlorosis and acute necrosis leading to death of shoots.

Table 6: Assessment of Phytotoxic damage on different weeds by different concentrations of 21days old phytotoxins (Shoot Cut Bioassay)

S. No	Weed	Concentration	PDR	PDR	PDR
			12 hpt	24 hpt	48 hpt
1	*Parthenium hysterophorus*	Control a	0.00±0.00	0.00±0.00	0.00±0.00
2	*Parthenium hysterophorus*	Control b	0.00±0.00	0.00±0.00	0.00±0.00
3	*Parthenium hysterophorus*	25	3.84±0.05	4.11±0.05	4.19±0.03
4	*Parthenium hysterophorus*	50	3.95±0.04	4.17±0.04	4.43±0.04
5	*Parthenium hysterophorus*	75	4.35±0.03	4.53±0.04	4.74±0.04
6	*Parthenium hysterophorus*	100	4.37±0.05	4.55±0.04	4.75±0.05
7	*Lantana camara*	Control a	0.00±0.00	0.00±0.00	0.00±0.00
8	*Lantana camara*	Control b	0.00±0.00	0.00±0.00	0.00±0.00
9	*Lantana camara*	25	3.68±0.05	3.94±0.03	4.15±0.05
10	*Lantana camara*	50	3.84±0.05	4.11±0.05	4.33±0.04
11	*Lantana camara*	75	4.25±0.03	4.51±0.05	4.66±0.04
12	*Lantana camara*	100	4.26±0.03	4.53±0.05	4.68±0.03
13	*Hyptis Suaveolens*	Control a	0.00±0.00	0.00±0.00	0.00±0.00
14	*Hyptis suaveolens*	Control b	0.00±0.00	0.00±0.00	0.00±0.00
15	*Hyptis Suaveolens*	25	3.74±0.05	3.95±0.04	4.14±0.03
16	*Hyptis suaveolens*	50	3.87±0.04	4.20±0.03	4.38±0.04
17	*Hyptis suaveolens*	75	4.19±0.03	4.45±0.04	4.58±0.04
18	*Hyptis suaveolens*	100	4.20±0.03	4.46±0.03	4.60±0.05
19	*Sida acuta*	Control a	0.00±0.00	0.00±0.00	0.00±0.00
20	*Sida acuta*	Control b	0.00±0.00	0.00±0.00	0.00±0.00
21	*Sida acuta*	25	3.59±0.03	3.83±0.03	3.99±0.05
22	*Sida acuta*	50	3.65±0.03	3.76±0.03	4.08±0.02
23	*Sida acuta*	75	3.94±0.03	4.14±0.03	4.27±0.04
24	*Sida acuta*	100	3.95±0.03	4.18±0.03	4.29±0.04
	SEm±		0.04	0.06	0.05
	$LSD_{5\%}$		0.10	0.23	0.18

Values are Means ± SEM of three observations

Control a- Unmetabolised growth medium

Control b- Sterilized Distilled Water

Amount of Toxin employed=15 ml/shoot RH-85%

PDR - 0-0.99= slight curling & wilting; 1-1.99=slight chlorosis; 2-2.99=marked chlorosis, slight necrosis; 3-3.99=high necrosis and marked chlorosis; 4-4.99=acute necrosis and marked chlorosis; 5=acute chlorosis and acute necrosis leading to death of shoots.

Table 7: Assessment of Phytotoxic damage on target weeds by phytotoxin produced on different carbon sources by *Phoma herbarum (* FGCC#54) (Shoot Cut Bioassay)

S. No	Carbon Sources	*Parthenium* (PDR)			*Lantana* (PDR)			*Hyptis* (PDR)			*Sida* (PDR)		
		12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt
1	Control a	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	Control b	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	Mannose	3.87±0.03	4.17±0.04	4.28±0.03	3.94±0.02	4.09±0.02	4.21±0.03	3.86±0.03	3.96±0.03	4.14±0.02	3.79±0.04	3.93±0.03	4.10±0.02
4	Fructose	4.06±0.02	4.18±0.03	4.32±0.02	4.03±0.04	4.17±0.03	4.23±0.04	3.96±0.03	4.06±0.02	4.18±0.02	3.70±0.03	3.80±0.04	4.04±0.03
5	Glucose	4.14±0.04	4.24±0.03	4.38±0.04	3.96±0.04	4.07±0.04	4.30±0.03	3.91±0.02	4.20±0.04	4.22±0.03	3.84±0.03	4.09±0.02	4.14±0.03
6	Sucrose	4.46±0.04	4.70±0.03	4.86±0.03	4.42±0.02	4.65±0.02	4.74±0.02	4.29±0.04	4.35±0.03	4.61±0.02	4.07±0.03	4.20±0.02	4.38±0.03
7	Maltose	4.10±0.03	4.30±0.03	4.47±0.02	4.06±0.03	4.24±0.03	4.48±0.04	4.08±0.04	4.18±0.04	4.28±0.03	3.99±0.03	4.13±0.03	4.23±0.02
8	Lactose	4.29±0.02	4.52±0.04	4.76±0.04	4.16±0.02	4.43±0.03	4.65±0.03	4.13±0.03	4.20±0.03	4.44±0.03	4.00±0.04	4.29±0.02	4.41±0.02
9	Sorbitol	3.48±0.02	3.57±0.04	3.78±0.04	3.65±0.03	3.76±0.03	4.08±0.02	3.65±0.03	3.81±0.02	4.00±0.04	3.51±0.04	3.68±0.03	3.91±0.03
10	Citric acid	3.36±0.03	3.50±0.03	3.70±0.03	3.21±0.03	3.37±0.03	3.54±0.03	3.15±0.03	3.36±0.03	3.54±0.03	3.29±0.03	3.31±0.02	3.49±0.02
11	Starch	3.74±0.04	4.06±0.02	4.20±0.02	3.73±0.02	3.86±0.04	4.07±0.04	3.78±0.04	3.85±0.04	4.07±0.03	3.69±0.03	3.80±0.04	3.95±0.03
12	Dextrin	3.59±0.03	3.83±0.03	4.00±0.02	3.60±0.02	3.75±0.02	3.95±0.02	3.50±0.02	3.66±0.04	3.93±0.03	3.30±0.04	3.70±0.02	3.80±0.03
13	No Carbon	3.46±0.02	3.66±0.04	3.78±0.04	3.39±0.03	3.57±0.02	3.67±0.03	3.33±0.02	3.41±0.03	3.55±0.04	3.21±0.03	3.37±0.03	3.51±0.03
	SEm±	0.28	0.20	0.15	0.23	0.25	0.16	0.25	0.27	0.18	0.11	0.08	0.23
	$LSD_{5\%}$	0.82	0.64	0.80	0.59	0.43	0.39	0.54	0.60	0.39	0.45	0.53	0.59

Values are Means ± SEM of three observations; Control a- Unmetabolised growth medium
Control b- Sterilized Distilled Water; Amount of Toxin employed=15 ml/shoot; RH-85%
PDR - 0-0.99= slight curling & wilting; 1-1.99=slight chlorosis; 2-2.99=marked chlorosis, slight necrosis; 3-3.99=high necrosis and marked chlorosis; 4-4.99=acute necrosis and marked chlorosis; 5=acute chlorosis and acute necrosis leading to death of shoots.

Table 8: Assessment of Phytotoxic damage on target weeds by phytotoxin produced on different nitrogen sources by *Phoma herbarum (* FGCC#54) (Shoot Cut Bioassay)

S. No	Nitrogen sources	*Parthenium* (PDR)			*Lantana* (PDR)			*Hyptis* (PDR)			*Sida* (PDR)		
		12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt	12 hpt	24 hpt	48 hpt
1	Control a	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	Control b	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	Potassium Nitrate	4.55±0.02	4.72±0.03	4.90±0.02	4.41±0.03	4.54±0.03	4.68±0.03	4.37±0.04	4.48±0.03	4.64±0.02	4.13±0.04	4.25±0.04	4.48±0.04
4	Sodium Nitrate	4.45±0.03	4.60±0.02	4.71±0.04	4.15±0.03	4.21±0.02	4.33±0.02	4.23±0.03	4.44±0.02	4.55±0.03	4.06±0.03	4.11±0.02	4.34±0.03
5	Ammonium Nitrate	3.92±0.03	4.10±0.04	4.29±0.04	3.59±0.03	3.69±0.04	3.92±0.02	3.88±0.03	4.04±0.03	4.17±0.03	3.75±0.02	3.97±0.03	4.13±0.02
6	Ammonium Sulphate	3.73±0.03	3.94±0.03	4.10±0.02	3.61±0.02	3.75±0.02	3.83±0.02	3.50±0.02	3.69±0.03	3.87±0.03	3.37±0.04	3.53±0.02	3.67±0.04
7	Ammonium Oxalate	3.04±0.04	3.16±0.03	3.35±0.03	2.95±0.02	3.08±0.03	3.27±0.04	3.02±0.02	3.15±0.03	3.22±0.03	2.95±0.02	3.11±0.03	3.20±0.02
8	Ammonium Tartarate	3.45±0.03	3.71±0.02	3.85±0.03	3.47±0.03	3.61±0.02	3.76±0.03	3.41±0.03	3.59±0.02	3.71±0.02	3.36±0.04	3.52±0.02	3.66±0.02
9	Ammonium Chloride	3.12±0.04	3.28±0.02	3.49±0.04	3.04±0.03	3.22±0.04	3.36±0.03	3.14±0.03	3.26±0.02	3.33±0.04	2.89±0.03	3.14±0.03	3.27±0.03
10	Urea	2.93±0.03	3.09±0.03	3.22±0.03	2.74±0.03	2.90±0.04	3.08±0.03	2.86±0.02	2.89±0.02	3.02±0.03	2.65±0.03	2.80±0.03	2.95±0.02
11	Peptone	4.37±0.03	4.53±0.03	4.66±0.03	3.95±0.02	4.16±0.03	4.45±0.03	4.25±0.02	4.32±0.03	4.39±0.03	4.25±0.02	4.32±0.02	4.38±0.03
12	Yeast Extract	4.21±0.04	4.30±0.02	4.38±0.02	3.78±0.04	4.06±0.04	4.18±0.03	3.94±0.03	4.09±0.04	4.13±0.03	3.85±0.03	3.93±0.04	4.10±0.02
13	No Nitrogen	3.30±0.03	3.46±0.04	3.68±0.04	3.15±0.03	3.37±0.03	3.55±0.04	3.31±0.02	3.44±0.02	3.54±0.03	3.11±0.02	3.26±0.03	3.48±0.03
	SEm±	0.04	0.08	0.06	0.04	0.06	0.05	0.04	0.10	0.08	0.06	0.10	0.05
	$LSD_{5\%}$	0.10	0.34	0.25	0.42	0.38	0.29	0.20	0.31	0.36	0.25	0.34	0.19

Values are Means ± SEM of three observations; Control a- Unmetabolised growth medium

Control b- Sterilized Distilled Water; Amount of Toxin employed =15 ml/shoot; RH-85%

PDR- 0-0.99= slight curling & wilting; 1-1.99=slight chlorosis; 2-2.99=marked chlorosis, slight necrosis; 3-3.99=high necrosis and marked chlorosis; 4-4.99=acute necrosis and marked chlorosis; 5=acute chlorosis and acute necrosis leading to death of shoots.

Table 9 . Phytotoxic assessment of solid phase extracted fractions on test weeds (Detached Leaf Bioassay)

S. No	Weed	Solid Phase Extract	PDR	PDR	PDR
			36 hpt	48 hpt	72 hpt
1	*Parthenium hysterophorus*	Control a	0.00±0.00	0.00±0.00	0.00±0.00
2	*Parthenium hysterophorus*	Control b	0.00±0.00	0.00±0.00	0.00±0.00
3	*Parthenium hysterophorus*	Inorganic	0.75±0.05	0.83±0.04	0.96±0.04
4	*Parthenium hysterophorus*	Organic	4.40±0.03	4.58±0.04	4.70±0.03
5	*Lantana camara*	Control a	0.00±0.00	0.00±0.00	0.00±0.00
6	*Lantana camara*	Control b	0.00±0.00	0.00±0.00	0.00±0.00
7	*Lantana camara*	Inorganic	0.67±0.05	0.79±0.05	0.89±0.03
8	*Lantana camara*	Organic	4.05±0.02	4.20±0.04	4.45±0.03
9	*Hyptis suaveolens*	Control a	0.00±0.00	0.00±0.00	0.00±0.00
10	*Hyptis suaveolens*	Control b	0.00±0.00	0.00±0.00	0.00±0.00
11	*Hyptis suaveolens*	Inorganic	0.27±0.02	0.45±0.02	0.73±0.02
12	*Hyptis suaveolens*	Organic	3.70±0.04	3.94±0.03	4.10±0.04
13	*Sida acuta*	Control a	0.00±0.00	0.00±0.00	0.00±0.00
14	*Sida acuta*	Control b	0.00±0.00	0.00±0.00	0.00±0.00
15	*Sida acuta*	Inorganic	0.11±0.02	0.15±0.02	0.22±0.03
16	*Sida acuta*	Organic	3.56±0.03	3.78±0.02	3.93±0.03
	SEm±		0.06	0.03	0.02
	$LSD_{5\%}$		0.17	0.08	0.07

Values are Means ± SEM of three observations; Control a- Unmetabolised growth medium
Control b- Sterilized Distilled Water; Amount of solvent extracted fraction employed=5 ml/leaf; RH-85%
PDR - 0-0.99 = slight curling & wilting; 1-1.99 = 1-20%LAD; 2-2.99=21-40% LAD; 3-3.99 = 41-60% LAD;
4-4.99=61-80%LAD; 5=81-100%LAD (leaf area damage)

Table 10: Effect of different solvent extracted fractions of *Phoma herbarum* (FGCC#54.) (Detached Leaf bioassay)

S. No	Weed	Fractions	PDR	PDR	PDR
			24 hpt	36 hpt	72 hpt
2	*Parthenium hysterophorus*	DW	0.00±0.00	0.00±0.00	0.00±0.00
3	*Parthenium hysterophorus*	Carbon tetra chloride	0.66±0.05	0.82±0.04	0.92±0.05
5	*Parthenium hysterophorus*	DW	0.00±0.00	0.00±0.00	0.00±0.00
6	*Parthenium hysterophorus*	Chloroform	0.70±0.04	0.87±0.04	1.01±0.03
8	*Parthenium hysterophorus*	DW	0.00±0.00	0.00±0.00	0.00±0.00
9	*Parthenium hysterophorus*	Ethyl acetate	4.46±0.04	4.70±0.03	4.86±0.03
11	*Parthenium hysterophorus*	DW	0.00±0.00	0.00±0.00	0.00±0.00
12	*Parthenium hysterophorus*	Butanol	0.80±0.05	0.96±0.04	1.07±0.04
14	*Lantana camara*	DW	0.00±0.00	0.00±0.00	0.00±0.00
15	*Lantana camara*	Carbon tetra chloride	0.55±0.04	0.73±0.03	0.73±0.03
17	*Lantana camara*	DW	0.00±0.00	0.00±0.00	0.00±0.00
18	*Lantana camara*	Chloroform	0.58±0.04	0.75±0.04	0.78±0.05
20	*Lantana camara*	DW	0.00±0.00	0.00±0.00	0.00±0.00
21	*Lantana camara*	Ethyl acetate	4.13±0.03	4.20±0.03	4.44±0.03
23	*Lantana camara*	DW	0.00±0.00	0.00±0.00	0.00±0.00
24	*Lantana camara*	Butanol	0.61±0.05	0.75±0.04	0.91±0.04
26	*Hyptis suaveolens*	DW	0.00±0.00	0.00±0.00	0.00±0.00
27	*Hyptis suaveolens*	Carbon tetra chloride	0.34±0.03	0.37±0.05	0.52±0.04
29	*Hyptis suaveolens*	DW	0.00±0.00	0.00±0.00	0.00±0.00
30	*Hyptis suaveolens*	Chloroform	0.52±0.03	0.63±0.04	0.79±0.03
32	*Hyptis suaveolens*	DW	0.00±0.00	0.00±0.00	0.00±0.00
33	*Hyptis suaveolens*	Ethyl acetate	3.99±0.03	4.13±0.03	4.27±0.05
35	*Hyptis suaveolens*	DW	0.00±0.00	0.00±0.00	0.00±0.00
36	*Hyptis suaveolens*	Butanol	0.60±0.04	0.74±0.03	0.78±0.05
38	*Sida acuta*	DW	0.00±0.00	0.00±0.00	0.00±0.00
39	*Sida acuta*	Carbon tetra chloride	0.20±0.05	0.33±0.03	0.34±0.05
41	*Sida acuta*	DW	0.00±0.00	0.00±0.00	0.00±0.00
42	*Sida acuta*	Chloroform	0.46±0.05	0.51±0.05	0.70±0.04
44	*Sida acuta*	DW	0.00±0.00	0.00±0.00	0.00±0.00
45	*Sida acuta*	Ethyl acetate	3.70±0.03	3.80±0.04	4.04±0.03
47	*Sida acuta*	DW	0.00±0.00	0.00±0.00	0.00±0.00
48	*Sida acuta*	Butanol	0.47±0.05	0.55±0.04	0.79±0.03
	SEm±		0.02	0.06	0.04
	$LSD_{5\%}$		0.07	0.15	0.09

Values are Means ± SEM of three observations; Control a- Unmetabolised growth medium
Control b- Sterilized Distilled Water; Amount of solvent extracted fraction employed=5 ml/leaf RH-85%;
PDR - 0-0.99= slight curling & wilting; 1-1.99=1-20%LAD; 2-2.99=21-40% LAD; 3-3.99=41-60% LAD; 4-4.99=61-80%LAD; 5 = 81-100%LAD (leaf area damage)

Table 11: Phytotoxicity of Column purified fractions by Detached Leaf bioassay (Phytotoxin)

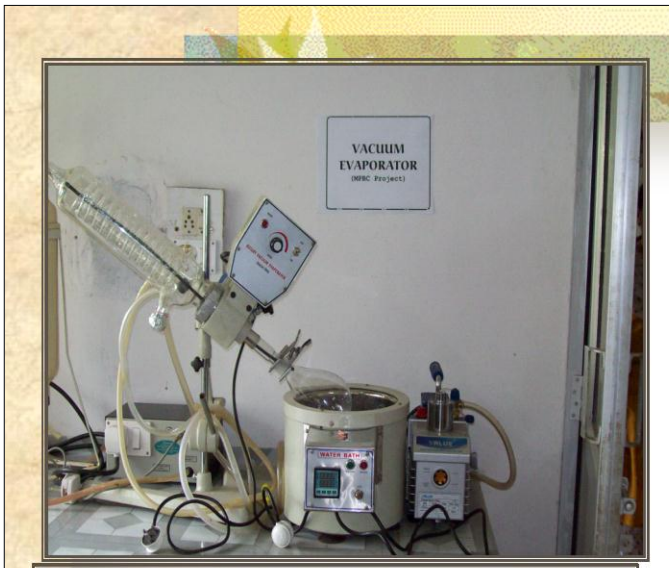
Column purified Fractions	Phytotoxic Damage Rating (72 hpt)			
	Parthenium	***Lantana***	***Hyptis***	***Sida***
Control a	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Control b	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1-4	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
5-8	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
9-12	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
13-16	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
17-20	0.93□0.03	0.83□0.03	0.74□0.03	0.51□0.03
21-24	4.81□0.04	4.70□0.04	4.39□0.03	3.73□0.02
25-28	1.75□0.03	1.68□0.04	1.62□0.03	0.66□0.03
29-32	0.73□0.03	0.54□0.03	0.45□0.03	0.34□0.02
33-36	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
37-40	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
SEm±	0.02	0.04	0.05	0.03
$LSD_{5\%}$	0.05	0.12	0.09	0.08

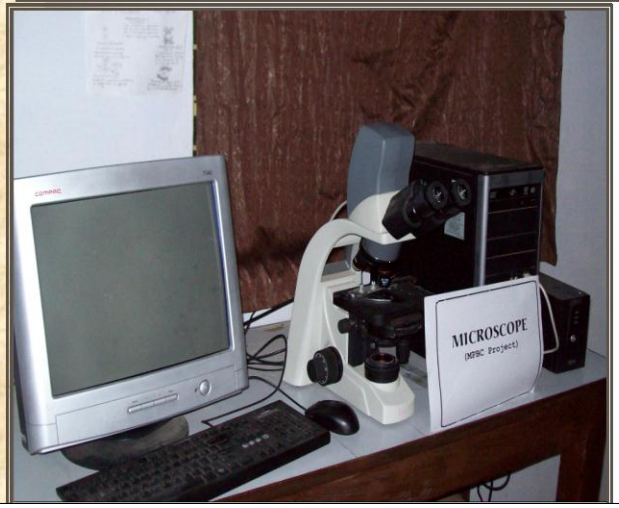
Values are Means ± SEM of three observations; Control a- Unmetabolised growth medium

Control b- Sterilized Distilled Water; Amount of column purified fraction employed=5 ml/leaf; RH-85%

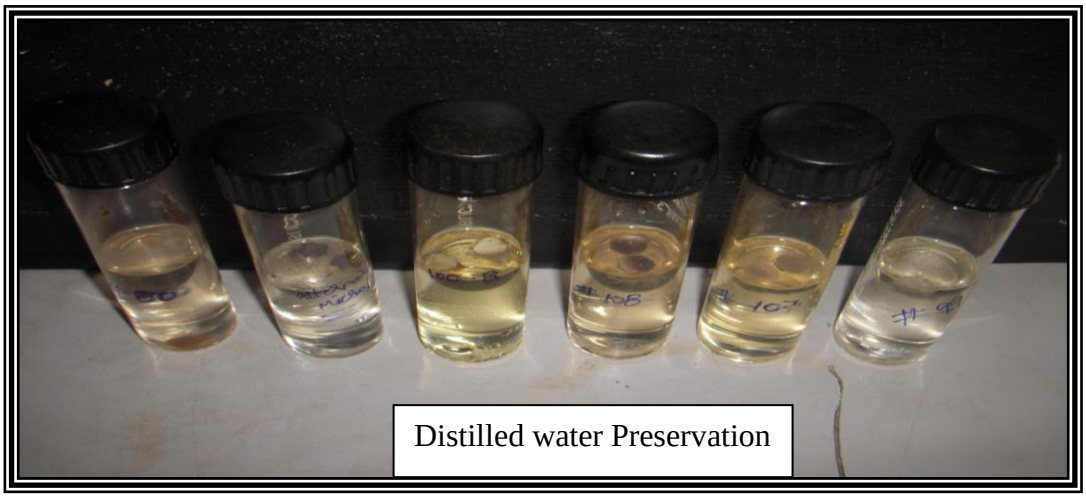
PDR - 0-0.99=slight curling & wilting; 1-1.99=1-20%LAD; 2-2.99=21-40% LAD; 3-3.99 =41-60% LAD; 4-4.99=61-80%LAD; 5=81-100%LAD (leaf area damage)

INSTRUMENTS SECTIONED BY MPBC

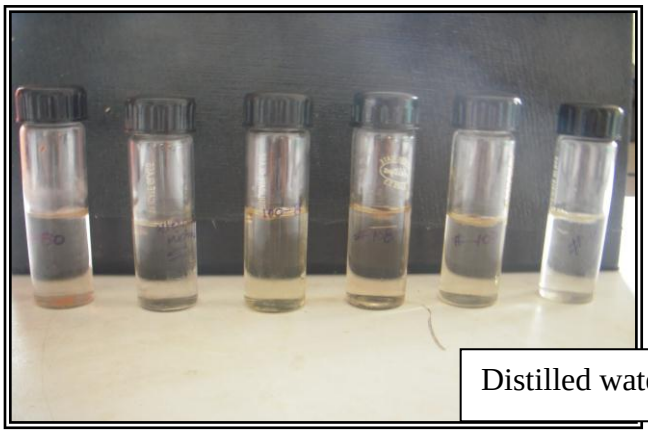


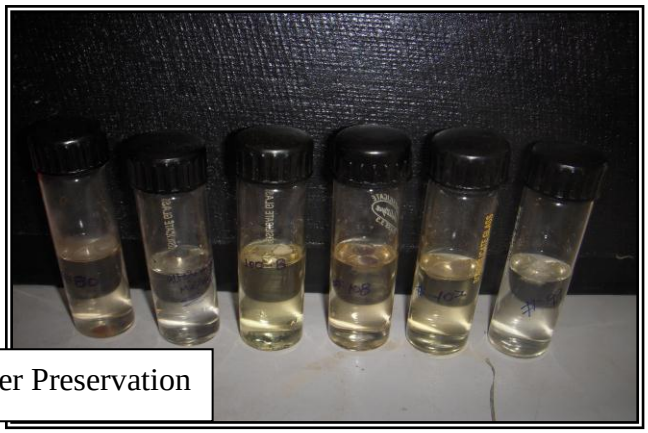


CULTURE PRESERVATION

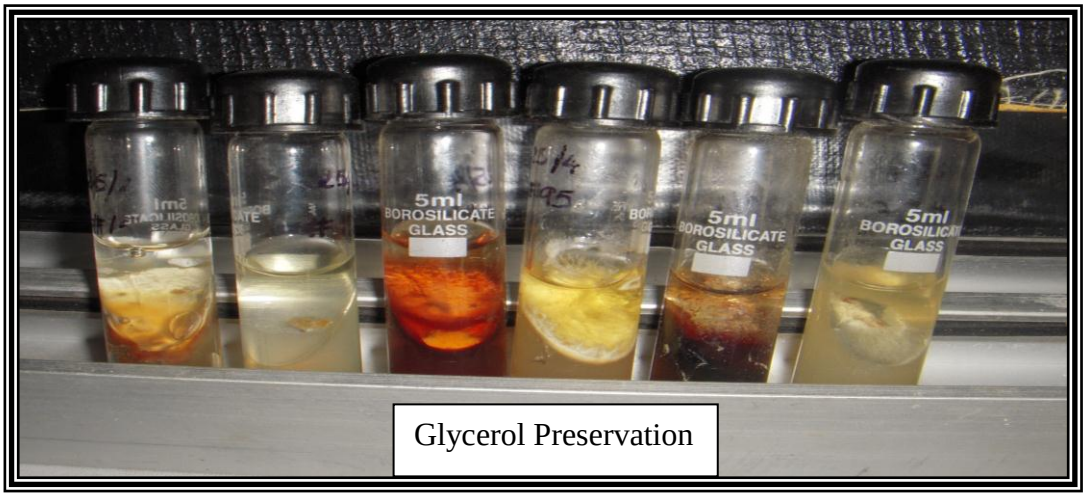


Distilled water Preservation





Distilled water Preservation



Glycerol Preservation

WORKSHOPS AND TRAININGS

SESSION 2007-2008

SESSION 2008- 2009

SESSION 2009- 2010