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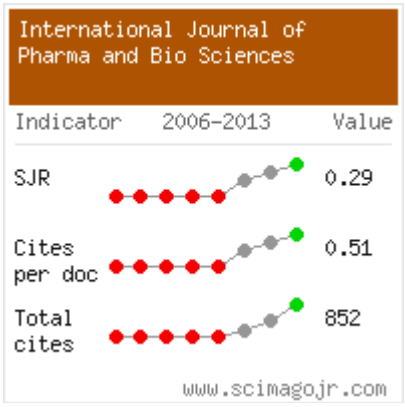

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FOOD FOR ALL- IMPACTS OF BIOTECHNOLOGY ON FOOD SUSTAINABILITY

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ABSTRACT

Environmental Science that is building quality,which is maintained with help of Biotechnology. The term is developing sustainability standards for products that use energy. Biotechnology consist of new processing technology and control systems,Which are not being harmful to the environment or depleting natural resources. Improved farming systems for growing and harvesting food, genetic improvement of food crops, and new techniques for monitoring food safety, and thereby supporting long-term ecological balance . Special care is taken to make sure that genes coding for allergens are not transferred to other species .These are usefull in development of our nation .

KEYWORDS
Sustainability, Biotechnology and ecological balance.

INTRODUCTION

The word "sustainable" comes from the word "sustain" which means to maintain, support, or to endure.[1] People concerned in sustainable agriculture are trying to identify and solve the problems in our current agricultural system in order to provide food and fiber in a healthy environment for people over the long term. Sustainability is defined as a requirement of our generation to manage the resource base such that the average quality of life that we ensure ourselves can potentially be shared by all future generations.[2] Development is sustainable if it involves a non-decreasing average quality of life. Sustainability is the capability to continue a defined behaviour for an indefinite period. The term biotechnology can thus include traditional and local knowledge, organic and agro ecological practices, conventional breeding, the application of tissue culture and genomic techniques, marker-assisted breeding and gene splicing. "Modern biotechnology" is defined on Biosafety[4]and is commonly understood as "the manipulation of genetic material and fusion of cells beyond normal breeding barriers," with the most common example being genetic engineering (GE) in which genes are inserted or deleted through transgenic technologies to create genetically modified (GM) organisms (GMOs).[3] The use of the term "modern" is by con-vention only, and does not in any way suggest that the techniques are more difficult or relevant than other biotechnologies with longer histories.

Biotechnology has made marvellous contributions to agriculture, with some biotechnologies as old as agriculture itself.[5,6] Free-to-the-public technologies and extension ser-vices are important to farmers. In contrast, modern bio-technology [30] has a poor track record of importance to the poor and subsistence farmer [7] and its control by a relatively small number of large worldwide companies means that adopting modern biotechnologies [7] could also require accepting significant

social changes and adopting agricultural models that may not result in poverty reduction or sustainable practices,[1] Hence also increasing the dependency of local farmers on technological exports from the wealthy countries.

MATERIAL AND METHOD

DNA transfers naturally between organisms. Several natural mechanisms allow gene flow across species. These occur in nature on a large scale – for example, it is one mechanism for the development of antibiotic resistance in bacteria. This is facilitated by transposons, retrotransposons, proviruses and other mobile genetic elements that naturally translocate DNA [9,10] to new loci in a genome. Movement occurs over an evolutionary time scale.
The introduction of foreign germplasm into crops has been achieved by traditional crop breeders by overcoming species barriers. Plant tissue culture and deliberate mutations have enabled humans to alter the makeup of plant genomes. Introducing new genes into plants requires a promoter specific to the area where the gene is to be expressed. The codons of the gene must be optimized for the organism .

METHODS

Genetically engineered crops have genes added or removed using genetic engineering techniques, originally including gene guns, electroporation, microinjection and agrobacterium.
1.***Gene guns*** (also known as biolistics) "shoot" (direct high energy particles or radiations against) target genes into plant cells.[8] It is the most common method. DNA is bound to tiny particles of gold or tungsten which were subsequently shot into plant tissue or single plant cells under high pressure. The accelerated particles enter both the cell wall and membranes.[13]The DNA separates from the metal and is included into plant DNA inside the nucleus. This method has been applied successfully for many cultivated crops.The major disadvantage of this procedure is that serious damage can be done to the cellular tissue.
2.***Agrobacterium tumefaciens***-mediated transformation is another common technique. [9] Agrobacteria are natural plant parasites, and their natural capability to transfer genes provides another engineering method. To create a suitable environment for them selves, these Agrobacteria insert their genes into plant hosts, resulting in a propagation of modified plant cells near the soil level (crown gall). When *Agrobacterium* infects [11] a plant, it transfers this T-DNA to a random site in the plant genome. When used in genetic engineering the bacterial T-DNA is removed from the bacterial plasmid and replaced with the desired foreign gene. The bacterium is a vector, enabling transportation of foreign genes into plants. This method works especially well for dicotyledonous plants like potatoes, tomatoes, and tobacco. Agrobacteria infection is less successful in crops like wheat and maize.
3.*Electroporation* is used when the plant tissue does not contain cell walls. [10,12] In this technique, "DNA enters the plant cells through minute pores which are temporarily caused by electric pulses."
4.*Microinjection* directly injects the gene into the DNA.
Plant scientists, backed by results of modern wide-ranging profiling of crop composition,[14] point out that crops modified using GM techniques [10] are less likely to have accidental changes than are typically bred crops.

Biotechnology And Agriculture

Biotechnology is revolutionary for agriculture [11,12] and the food system, because it places control over food production in the genes. Food production, of course, has always been empowered by genes, but we haven't been able to see them, precisely select them, or move them across traditional species barriers.[15] Now we can. And day by day we are learning which traits in crops are

controlled by individual genes, how to turn those genes on and off, how to splice them into the organisms, and how to amplify gene products.

So first, we have an awesome new technology that operates at the genetic level of the food system--the most fundamental level of food characterization.[16] This means that the production and quality commands in the food system begin with the genes and, most importantly,[17] with those who hold the genes and wield the new genetic technologies.

Second, coupled with the new genetic technologies [18] is the legal power to own genes. Those who have been following the legal developments in the biological empire over the last 6 years or so know that genes can now be unproved, as can certain techniques used in genetic strategy [29] (U.S. Supreme Court, 1980). This means that discoverer or for profit interest can have a property right in genetic material.

Third, in the area of food and agriculture, there are quite noticeably a lot of genes.[19] There are genes that control yield in crop.

Biotechnology And Food Process

Food processing have been among some of the earliest and, recently, most aggressive [20] investors in biotechnology:

1 A contractual relationship with DNA Plant Technology Corporation .

2 To conduct joint research on ways to increase the protein content of cereal grains.

3 General Foods has contracted with investigators to seek to reduce its costs .

4 Foods is seeking new varieties of trees that will produce a lower levels of the naturally occurring bitter flavours .

5 The vegetable products are developed in a tissue culture process, in which natural genetic variation [21] produces mutants that can be selected for particular characteristics.

All the work is for advantage and changes in the nutritional integrity and safety [22] of food crops.

Food Quality And Food Safety

Biotechnology in agricultural production and food processing may affect the quality and safety [23] of food in several direct and indirect ways:

1. By displacing or altering the genes that control the nutritional constituents of food crops
2. By altering the genes that affect the levels of naturally occurring toxins in food crops,
3. By extending certain agricultural production practices, such as the use of pesticides, that lead to chemical residues in food and water.

The point here is that capital momentum in biotechnology research,[24] even at this very early stage, could determine what traits are pursued, which ones remain together, and which ones are not pursued. And in this process, the public interest in food safety and food quality may not be satisfactorily represented by the action of the market. But is the current regulatory apparatus adequate for ensuring that all genetically altered crops [25] will not suffer nutritional fluctuations or toxicant changes.

RESULT AND DISCUSSION

Now increasing attention has been paid to the development of sustainable agriculture in which the high productivities [8,26] of plants ensured using their natural adaptive potentials, with a minimal disturbance of the environment.

It is our view that the most promising strategy to reach this goal is to substitute dangerous agrochemicals (mineral fertilizers, pesticides) with environment-friendly preparations of symbiotic microbes, which could improve the nutrition of crops [27] and livestock, as well as their protection from biotic (pathogens, pests) and abiotic (including pollution and climatic change) stresses (Yang

et al., 2009).With recent advances in biology, materials, computing, and engineering, environmental biotechnologists [26] now are able to use microbial communities for a wealth of services to society.

CONCLUSION

There are major different points of view have been expressed ,on one hand, recombinant DNA technology [8,13] is seen as a potent tool for enhancing crop productivity (first generation GMOs) [27] and food quality (second generation GMOs) or "drug factories", for the production of vaccines and/or curative medicines (third generation GMOs). GMO supporters point to evidence that GMOs must be considered essential for promoting sustainable agriculture. [28]

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Effect of Gymnemic acid on fungus pathogens- *Gymnema sylvestre* R. Br.

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ABSTRACT

The petroleum benzene, ethanol, and aqueous extract of anti diabetic medicinal plant *Gymnema sylvestre* was assayed *in vitro* searching for antifungal activity against human pathogenic microorganism (*Candida albicanse and Aspergillus niger)* by using the disk diffusion method. Soxlated extract of *Gymnema* leaf on different solvents was assayed by *in vitro* screening for antimicrobial activity. Standardized micro-organisms obtained from Microbiology Department Barkatullah University Bhopal. Organisms were growing individually and homogeneously grow in PDA plate. Inhibition halos were evaluated and controlled by the use of the fluoroquinolone ciprofloxacin. The aqueous extract of such medicinal plant showed the best zone of inhibition 2.5-3mm against the organism. A maximum zone of inhibition was obtained *Candida albicans* (3.25mm) on aqueous in comparison to *Aspergillus niger* (2.1mm). The petroleum benzene extracts that showed activity against microorganisms had at least of antimicrobial activity when compared to halo inhibition produced by the commercial antibiotic ciprofloxacin utilized as a control. On the other hand ethanol extract had performed comparatively the best inhibition on both organisms. A tiny amount of data is presented, as the preliminary antimicrobial properties of the medicinal plant, under the urgent necessity of new antibiotics in the market and in the face of the increased resistance of infectious microorganisms to antimicrobials.

KEYWORDS- *Antimicrobials; Candida albicanse; Aspergillus niger; petroleum benzene; ethanol; Gymnema sylvestre and aqueous.*

INTRODUCTION

According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies, the early civilization in China, India and the near east, but it is doubtless an art as old as mankind. Now a days, the use of phytochemicals for pharmaceutical purpose has gradually increased in many countries, these plants are still widely used in ethno-medicine around the world. [4] Medicinal plants represent a rich source of antimicrobial agents and powerful drugs.[13] The use of crude extracts of plants parts and phytochemicals, of known antimicrobial properties, can be of great significance in the therapeutic treatments, which are due to the secondary metabolites synthesized by the plants. These products are known by their active substances like, alkaloids, phenolic compounds which are part of the essential oils, as well as in tanning. Plant produces a wide variety of secondary metabolites which are used either directly as precursors

or as lead compounds in the pharmaceutical industry. This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants.[1] In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection-fighting strategies to control microbial infections.[12] *Gymnema sylvestre* R. Br. Belonging to the family Asclepiadaceae, use of plants as medicine is as old as human civilization. It is a very effective medicinal plant use in the treatment of asthma, eye complaints, inflammations, family planning and snake bite.[2,11,15] It is a potent antidiabetic plant and used in folk, Ayurvedic and Homeopathic system of medicine, studied by Kapoor, (1977), Ravi and Wahi, (1995), Mitra et al, (1995). Property of leaves of this plant, to appreciate the taste of sugar has been tested by Mr. D. Hooper in (1887). The aim of this study was to evaluate the antifungal activity of *Gymnema sylvestre* R.Br. used in Ayurveda and traditional medicinal system for treatment of diabetes and manifestations caused by microorganisms. Therefore, leaf extracts of the following solvents were tested for their potential activity against fungal pathogens like, *Candida albicand* and *Aspergillus niger*. Here, we investigated the antifungal activity of medicinal plant- *Gymnema sylvestre* R.Br. currently used by native plant to treat their ailments. Leaves were collected in field growing plant in Bhopal, in the Madhya Pradesh state India.

MATERIAL AND METHODS -

PLANT MATERIALS –

The leaves of *Gymnema sylvestre* used in Ayurveda and traditional systems of medicine were collected from its natural habitat from Kasturi Herbal farm Misrod, Bhopal in the month of July. The plant was authenticated from Laghu Vanupaj Prasannskarn & Anusandhan Kendra Barkheda Pathani, Bhopal (MP). The collected plant materials were washed thrice in tap water and twice with distilled water to remove the adhesive contaminants and dust particles, then plant material were dried in shade.

PREPARATION OF PLANT EXTRACTS -

The powdered plant materials were extracted successively with different solvent non-polar to polar (petroleum benzene, ethanol and distilled water to afford shoxlet extractor (Hoopers,s method). Solvents were evaporated under reduced pressure and stored at °C for use.

EXTRACTION WITH PETROLIUM ETHER -

200gm of dry leaf powder was packed into a clean soxhlet extraction unit and 1 liters of petroleum ether (30-40^{0}C) was added and extracted for 24-36 hours till all the components are soluble in petroleum. Petroleum extract is collected and distilled in a distillation unit. Then a net weight of 15 gm of petroleum ether extracts was obtained. Petroleum ether extraction was used for defatting dried leaf power.

EXTRACTION WITH ETHANOL-

The plant material is then extracted with ethanol (50-60^{0}C). Ethanol was added and the extraction was carried out for 24-36 hours till the total ethanol soluble extract was obtained. The ethanol soluble extract was distilled and finally 30gm of the thick paste were obtained.

EXTRACTION WITH DISTILLED WATER –

The dry leaves part is also extracted with distilled water (80-90^{0}C). Water was poured and the extraction was carried out approx 24-36 hours till the total water soluble extract was obtained, leaves become colorless. The aqueous extract was distilled and finally 65gm of the dry extract were obtained.

MICRO-ORGANISMS CULTURE AND MAINTENANCE -

Clinical isolates of the microorganisms were used along with the standard strains. Quality control strains of *Aspergillus niger* and *Candida albicans* obtained from, Department of microbiology Barkatullah University Bhopal M.P. The funguses were maintained on Potato dextrose broth and cultured on Potato dextrose agar (PDA) at 28°C.

ANTIFUNGAL SCREENING -

Antifungal activities of the extracts were determined by the disk diffusion method as described by Taylor, R.S.L., 1995. Each purified extracts were dissolved in DMSO with dilution series (10µg/ml, 20µg/ml, 50µg/ml, 100µg/ml and 250µg/ml), sterilized by filtration using sintered glass filter, and stored at 4°C. PDA plates were inoculated with each fungal culture by pour plate method. The filter paper discs (5mm in diameter) impregnated with concentrations of the extracts was placed on test organism-seeded plates. DMSO was

used to dissolve the extract and was completely evaporated before application on test organism-seeded plates. For the determination of zone of inhibition fungal strains were taken as a standard antibiotic for comparison of the results. Proper controls experiment was carried out under similar condition by using nystatin and griseofulvin for antifungal activity as standard drugs. The activity was determined after 24h of incubation at 27 ^{0}C. The diameters of the inhibition zones were measured in mm. summit ion

RESULTS AND DISCUSSION:

The results obtained in the present study relieved that the tested *Gymnema Sylvestre* leaf extracts posses' potential antifungal activity against *Aspergillus negier* and *Candida albicans* on different solvent (non polar – polar) extract dissolve in DMSO. Antifungal activity was evaluated by filter Paper Disc Diffusion method and compare with nystatin and griseofulvin as a Standard Drugand DMSO as a control sample. Remarkable antifungal activities were recorded with *Candida albicans* (3.25mm) (Fig. F) on high concentration of aqueous extract (250µg/ml). According to this work petroleum extract on high concentration was show good result in comparison to low concentration on both fungal species, but *Candida* (1.40) (Fig . D) represent significant zone of inhibition then *Aspergillus* (1.10mm).[7] (Table- 1, 2 . Fig. A) On the other hand low to high concentration of ethanol leaf extract shows positive result incorporation in compare to petroleum benzene extract against *Candida* (1.76mm) (Fig. E) and *Aspergillus* (1.50mm)(Fig. B). Comparatively the aqueous leaf extract with concentration show significantly maximum zone of inhibition result against both type of pathogen fungus specious.[3] (Table- 1, 2. Fig.A-F) Low concentration of all types of extract show no zone of inhibition against both pathogens. Low to high concentration of extract literally show small to large inhibition zone on both fungus, but the highest zone of inhibition obtain 250µg/ml aqueous extract on *candida albicans* after 24 h on 27^{0}C tem. (Table- 1, 2) Same as hydro alcohol extracts effective at low concentration against S. aureus, S. mitis, S. Mutansi and C. albicans.[9] Large scale isolation of secondary metabolites from *G. sylvestre* can be predicted to remain an essential component in the search for new secondary metabolites and its pharmacological activities. Its leaf extracts exhibit broad spectra of antimicrobial activity. However, further studies needed to isolation and identification of compounds responsibility for antifungal activity.

Table 1

Antifungal activity of Gymnema sylvestre leaf extract in various solvents on different concentrations and standard control (10 µg/ml) against Aspergillus negier species tested by disc diffusion method.

	Zone of inhibition (mm) on various concentration and control				
Leaf extract	10µg/ml	20µg/ml	50µg/ml	100µg/ml	250µg/ml
Petroleum benzene	—	—	—	0.60	1.10
Ehanolic	—	—	0.41	0.80	1.50
Aqueous	—	0.40	0.70	1.30	2.10
Control	1.4	2.00	2.40	2.76	3.00

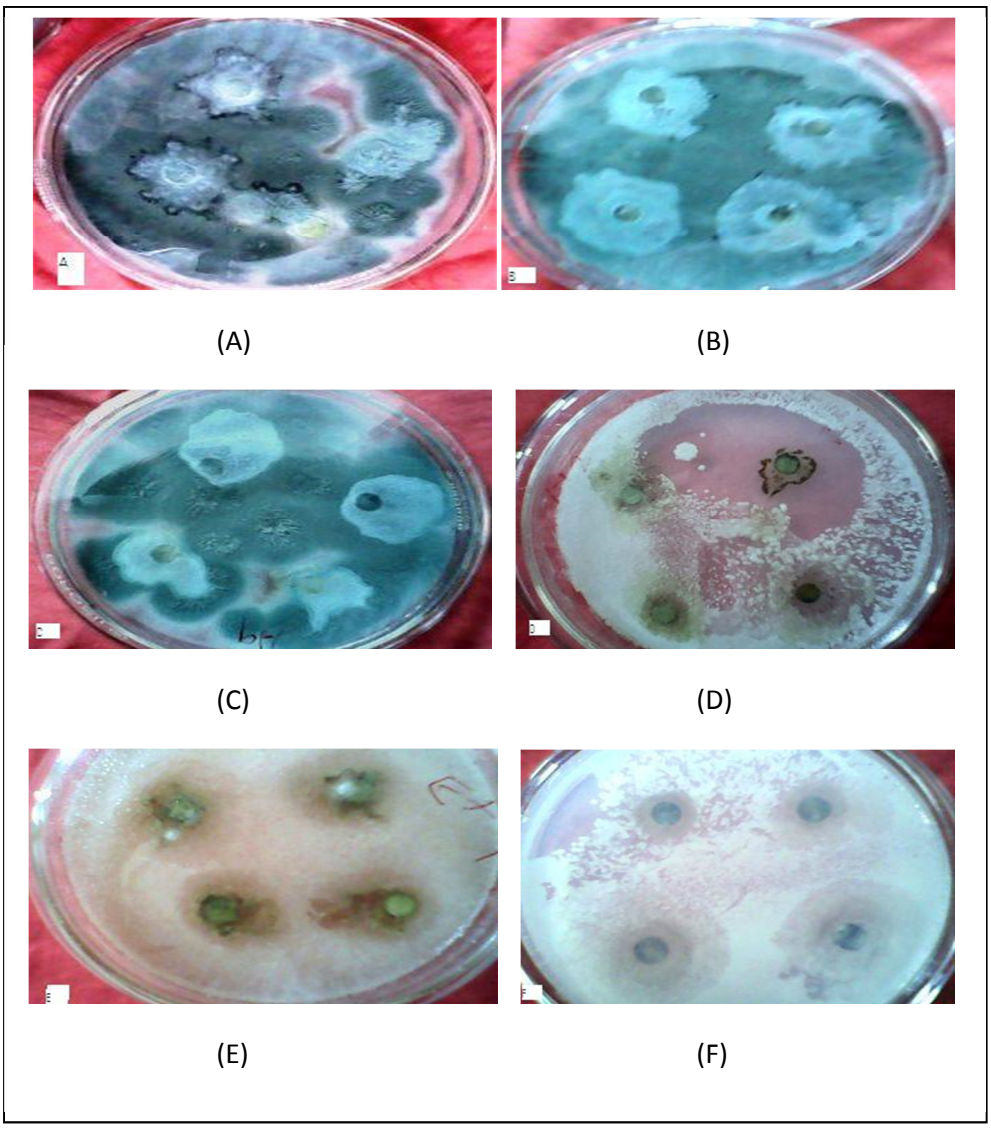
Table 2

Antifungal activity of Gymnema sylvestre leaf extract in various solvents on different concentrations and standard control (10 µg/ml) against Candida albicans species tested by disc diffusion method.

	Zone of inhibition (mm) on various concentration and control				
Leaf extract	10µg/ml	20µg/ml	50µg/ml	100µg/ml	250µg/ml
Petroleum benzene	—	—	0.32	1.00	1.40
Ehanolic	—	—	0.50	1.23	1.76
Aqueous	—	0.56	0.86	2.57	3.25
Control	1.4	2.00	2.50	2.90	3.50

Figure

Antifungal activity of leaf extract of various solvents against Aspergillus negier and Candida albicans: A. effect of petroleum benzene extract on A. negier, B. effect of ethanol extract on A. negier, C. effect of aqueous extract on A. negier, D.effect of petroleum benzene extract on Candida albican,s E. effect of ethanol extract on Candida albicans, F. effect of aqueous extract on Candida albicans.



(A) (B)

(C) (D)

(E) (F)

CONCLUSION

The result of present investigation clearly indicates the antifungal activity of leaves and ascertains the value of this plant used in ayurveda, which could be of considerable interest to the development of new drugs. The result of this study supports the use of plants as therapeutic agents for the treatment of several diseases caused by the pathogenic bacterial and fungal populations.

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MAKE IN INDIA: THEME TO IMPROVE THE HEALTH ISSUE RELATED TO REGULAR CONTACT WITH MICROORGANISM ON BODY AND TREATED WITH NORMAL HOUSE HOLD HERBS

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ABSTRACT

Candida albicans is a common member of human gut flora, and 40% healthy adult's GIT tract contains these. It is normally non pathogenic but become pathogenic in immuno-competent individuals under a variety of conditions. Ancient time herbal medicine was the only sources of treatment. There are many herbs are in market that were recovery the microorganism related problem. Herbal treatments were not containing any serious side effect on the body so it was safe to use. In our daily routine several herbs are use in our house hold activities. Systemic Candidiasis was the serious problem for female, immunity suffers person and surgery taken patient. So with the help of Make in India concept we introduced the Herbal Preclinical study. In our present investigation *Coriander sativum* seeds were shows that it was able to reduced the systemic Candidiasis. In the present study treated with the extract there was significant ($P<0.001$) decline in kidney burden as compared to vehicle treated animals and the percentage survival was found to be 80.19% till 14th day.Furthermore allopathic medicines were costly as compare to herbal medicine.

KEYWORDS:*Corianderum sativum***,** Candidiasis,*Candida albicans,* gastrointestinal tract etc.

INTRODUCTION

Medicinal plantsare valuable natural resourcesand regardedaspotentially safedrugs. Theyhavebeen playing animportant role in alleviatinghuman sufferingbycontributingherbalmedicines in the primary health caresystems or rural andremotehillyareas where more than 70%ofpopulation depends on folklore andtraditional systemofmedicines.Medicinal plantshave been testedfor biological, antimicrobial and hypoglycaemicactivity.[1,2]

Theeconomic burden associatedwith empirictreatment, formularydecision-making should bebasednot onlyonefficacyandsafetyoftheavailable antifungalagents but also onassociatedcosts. Randomized controlledtrials(RCTs),alone, oftendo not provide sufficient informationfor decisions relatedto reimbursementor budg*et al*location, specificallyifmedicalresource consumptionis not measured.Model-based health economicstudiesare well accepted for the assessmentofthe cost-effectiveness of treatment. Theyallowthe integration of efficacy and safety data fromclinical trials andmedical resource consumption fromother data sources.Thesemodels also takeintoaccounttheuncertainties thatareentailed in such acombinationof information.[3]

In the recentpast therehasbeen agrowinginterest inTraditional medicine/Complementaryand AlternativeMedicine (TCAM) andtheir relevance to public health both in developed anddeveloping countries.[4]

Around 80%ofthe population continues tousetraditionalmedicine in Africa.Asiaand Latin America and many governments inthese regions have incorporated traditionalmedicine practicestohelp meet their primaryhealth care needs.In industrialized countries, almost halfthepopulation nowregularlyusesomeform of TCAM (United States,42%;Australia, 48%;France, 49%;Canada, 70%),andconsiderable useexists in many developingcountries (China, 40%;India, 70%;Chile,71%; Colombia,40%; upto80% inAfricancountries).[5]

Countries such as India and China have purposively sought to develop the traditional medicine sector in order to strengthen their traditional medical heritage and at the same time also enable cost-efficacy in health care delivery to their people. It is also a response to capitalize on the economic opportunity arising from an increasing global demand for herbal products.[6]

Heavy burden of communicable diseases such as HIV, malaria and other parasitic diseases, pneumonia, diarrhea, tuberculosis, coupled with chronic diseases such as diabetes, ischemic heart diseases etc., (a situation often referred as double burden), persistently torment lives in these countries. High maternal and child mortality, rapid demographic changes and urbanization, under utilization of public healthcare, ineffective health support systems for poor population, increasing privatization of health facilities, migration of medical professionals, environmental changes and related epidemics are some other major public health concerns in such economies.In many regions of the world where modern healthcare is not readily available or affordable,

public continue to rely on traditional medicines which are based on locally available natural resources and cultural knowledge. In a public health context, availability, accessibility, affordability, utility, quality, efficiency and equity have relevance in respective order in promotion of traditional medicine.[7]

Among the several hundred species of microorganisms in the oral cavity, yeasts, especially members of the genus Candida, are representative of the few fungi considered to be commensally oral flora.[8]*Candida albicans* is the most common species isolated from the human oral cavity. The term Candida originates from Latin word candid, meaning white.[9, 10]Oral candidiasis, which is frequently caused by *Candida albicans*, is one of the most common fungal opportunistic infections in immune-compromised patients.[11]

The clinical significance of the oral candidiasis, which is not life-threatening but causes significant morbidity in patients, is increasing with time.[12, 13]Oral candidiasis is usually treated by topical antifungal agents, which include nystatin, miconazole, fluconazole, itraconazole and amphotericin B. However, the management of Candida infections faces a number of problems including; limited number of effective antifungal agents.[14,15]Oral candidiasis is the most common human fungal infection [16, 17]especially in early and later life. In the general population, carriage rates have been reported to range from 20% to 75% without any symptoms. The incidence of *Candida albicans* isolated from the oral cavity has been reported to be 45% in neonates, 45%–65% of healthy children, 30%–45% of healthy adults, 50%–65% of people who wear removable dentures, 65%–88% in those residing in acute and long term care facilities, 90% of patients with acute leukaemia undergoing chemotherapy, and 95% of patients with HIV. *Candida albicans* is a normal commensal of the mouth and generally causes no problems in healthy people.[18, 19]

Plants constitute an important source of natural products which differ widely in their structures, biological properties and mechanism of action.[20]Various phytochemical components especially polyphenols, flavonoids, phenolic acids etc. were responsible for the free radical scavenging and antioxidant activity of the plants. Traditional medicinal plants are often cheaper, locally available and easily consumable, raw or as simple medicinal preparations. Considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics has been carried out on Ayurvedic medicinal plants in order to establish the scientific basis of their therapeutic potentials.[21]

Corianderum sativum L. is an important spice crop and occupies a prime position in flavouring substances. *Corianderum sativum* is well known for its antioxidant properties and some of its active components have been identified. *Corianderum sativum* contains active phenolic acid compounds, including caffeic and chlorogenic acid. The flavonoids include quercetin, keampferol, rhamnetin and apigenin. Most of these compounds are known to inhibit free radicals generated in the cellar system, when they are obtained through the diet. While there is still limited understanding of the mechanisms through which they act, initial research indicates that *Corianderum sativum* is effective as both a treatment and preventive agent for several chronic diseases.[22]

The mature fruits have a fresh and pleasant flavour and are largely used all over the world in ground or volatile isolate form for flavouring sweets, beverages, tobacco products and baked goods and as a basic ingredient for curry powder. The essential oil obtained from its fruits at amounts ranging from approximately 0.5 to 2.5% is used both in flavours and in the manufacture of perfumes and soaps. It is cultivated as a domestic plant. [23]Hence the present study was to improve the health issue related to regular contact with microorganism on body and treated with normal house hold herbs.

MATERIAL AND METHOD

Plant material

Corianderum sativum Seeds were purchase from local shop of P&T Square, Bhopal city of Madhya Pradesh India. The plant was identified by Dr. Zia-Ul-Hassan from the Department of Botany at the Safia college of Science Peer Gate, Bhopal, India, and the voucher specimen (525/Botany/Safia/15) has been deposited at the Herbarium of the Safia college of Science Peer Gate, Bhopal.

Preparation of the plant extracts

The seeds of *Corianderum sativum* were dried in shade and powdered. The hydroalcoholic extract of *Corianderum sativum* Seedwas obtained by maceration in 80% Methanolic solution for 48 to 72 hours at room temperature, and this procedure was repeated twice. The hydro-alcoholic extract of*Corianderum sativum* Seed was concentrated on a rotary evaporator and then dried with a spray dryer. The yield of the hydro-alcoholic extract of*Corianderum sativum* was 2.4%.[24, 25]

Phytochemical investigation

The Phytochemical screening of the plant extract was carried out by following methods[26, 27, 28], to detect the presence or absence of certain bioactive compounds.

Microbial strain

The standard strain of *Candida albicans* (MTCC 227) obtained from the Pinnacle Biomedical research Institute, Bhopal, (M.P.) India. The studied strain was maintained on Potato Dextrose agar slants at 4°C temperature.

Antimicrobial sensitivity activity

The antimicrobial assay was performed by agar well diffusion method. [29, 30] The media (Sabouraud Dextrose Agar Media), along with the inoculums (1x10^8 CFU/ml), was poured into the Petri plate (Hi Media). For the agar well diffusion method, a well was prepared in the plates with a cup-borer (0.85 cm) and 50 µl of the test samples were pipette directly into the well. The plates were incubated for 48 hours at 28°C. Antifungal activity was determined by measuring the diameter of the zone of inhibition surrounding fungal growth. For fungal strain, controls were included that comprised pure solvents instead of the extract.[31] The control zones were subtracted from the test zones and the resulting zone diameter is shown in the Table 1. The experiments were repeated three times and the mean values are presented with ± Standard Deviation (SD).

Minimum Inhibitory Concentration Determination (MIC)

The MIC is the lowest concentration of a substance that inhibits the growth of fungi within a defined period of time. Broth micro-dilution MICs were determined in 96-well round-bottom microtiter plates. Spore suspensions were prepared in potato dextrose medium and adjusted to a final inoculum concentration of 0.4 × 10^4 to 5×10^4 CFU/ml. The plates were incubated at 28°C and read after 48 hours. The MIC of antifungal agent was defined as the lowest concentration at which there was complete inhibition of growth compared with that of the drug-free controls.[32]

IN VIVOSTUDY

Animals

The four week old *Albino wistar* Rats (150 to 200gm) with a background were obtained from **Pinnacle Biomedical Research Institute, Bhopal (M. P)**. The *Albino wistar* Rats were kept by in-house breeding. The animals had a conventional microbiological status and were housed in individually ventilated cage racks (6 Rats/cage). Rats were fed a Standard pellets (supplied by Golden Feeds, New Delhi) and acidified water, *ad libitum*. Animals were housed in relatively humidity of 30.7% At 22 ± 2° C and 12:12 light and dark cycle. All animal experiments were conducted and approved by the Institutional Animals Ethics Committee (IAEC) of PBRI, Bhopal (CPCSEA Reg. No. 1283/PO/c/09/CPCSEA, Protocol Approval No. PBRI/IAEC/PN-417).

Evaluation of acute oral toxicity of the Corianderum sativum seed extract

The acute oral toxicity study was carried out according to OECD (Organization For Economic Co-operation and development) 423 guideline which is base on a stepwise procedure with the use of minimum number of this study and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The hydro-alcoholic extract of *Corianderum sativum* (5-2000 mg/kg body weight) was orally administered to a group of Rats, both male and female. The behaviour parameters observed after administration were convulsion, hyperactivity, sedation, grooming, and increased or decreased respiration during a period of seven days. Food and water were provided *ad libitum*. The doses used in the present study were selected based on previous studies.[33, 34]

Systemic infection by Candida albicans

To produce infection, the rats were induced by the intra-peritoneal with suitable inoculums in a volume of 0.2 to 0.25 ml. After infection, the rats were observed twice daily, and animals exhibiting profound inanition or an inability to reach food and water were sacrificed.

The experimental design involved administration of each of the three test agents by daily oral dosing for a period of 7 days. dosing regimens were started on days -1,-2, -3, 0, 1, 2, 3 and relative to the day of challenge (day 0) with 2×10^4 CFU of *Candida albicans*/ ml. Before and after the challenged day animals were treated with 200 mg/kg body weight and 400 mg/kg body weight*Corianderum sativum* seed extract and 5 mg/kg body weight Fluconazole respectively. Survival was monitored for all experimental groups till 14 day. These conditions were in accordance with those of previously described method[35,] with slight modification. The pathological status of the rat was determined by visual examination of internal organs after their death or sacrifice at the completion of the experiment. All surviving rats were killed by cervical dislocation on 15th day determination of the numbers of CFU of *Candida albicans* per gram from the kidney.[36, 37] This determination was made by aseptically removing and weighing both kidneys, homogenizing kidneys in 5 to 10 ml of saline with a High Speed Homogeniezer (Remi RQ-124A), and Kidney burden was determined by culturing of homogenates in physiological saline followed by plating 0.1 ml aliquots onto Sabouraud dextrose agar plates. The plates were incubated at 28°C, and the number of colonies was enumerated after 48 h of growth.[38]All

animal care procedures were supervised and approved by the Institutional Animals Ethics Committee (IAEC) of PBRI, Bhopal.

Histology

For histology, some part of kidney was deep in 10% formaldehyde and smear were examined microscopically after stating with 1% methylene blue for the presence of fungal elements.

Biostatical interpretation

All data are presented in Mean ± SD. Data were analyzed by One Way ANOVA followed by Bonferroni t-test. P<0.050 and P<0.001 were considered as level of significance (n=6).

RESULTS

Phytochemical investigation

The phytochemical investigation was carried out by previously described procedure. The test for glycosides (Borntrager's test and Legal's test), alkaloids (hager's and Wager's test), carbohydrates (Molish and Benedict's test), reducing sugar, flavonoids (Lead acetate test,Alkaline reagent test and Shinoda test) were positive. The hydroalcoholic extract of *Coriander sativum* seed have positive potential for the tests of Treterpenoids and steroids (Salkowski's Test and Libbermann Burchard's Test), protiens Tannin and phenolics (Ferric Chhloride Test, Lead Acetate Test,Dilute Iodine Solution Test and Gelatin Test), and saponins (Froth's test).

In vitro antimicrobial activity

Corianderum sativum seed extract and Fluconazole at 0.781mg/ml and 0.039 µg/ml was found to complete inhibit the growth of *Candida albicans* in culture respectively. Table no. 1, 2 and 3 provides a presentation of the inhibitory effect.

Table 1

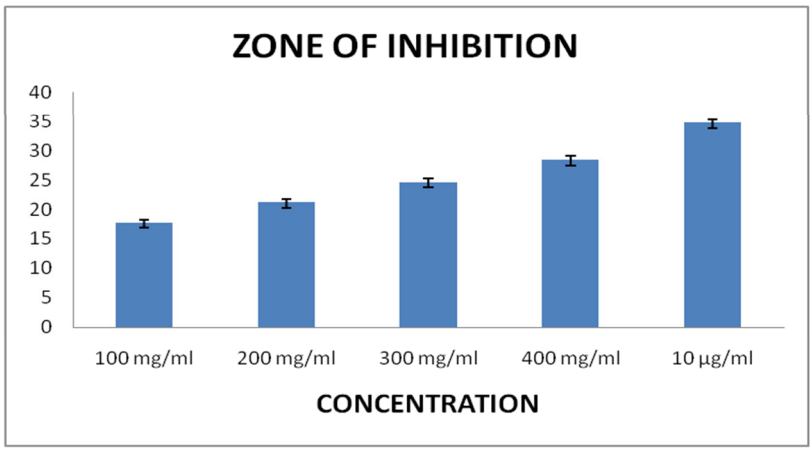
In vitro antimicrobial activity (well diffusion assay)

Extract used	100mg/ml	200mg/ml	300mg/ml	400mg/ml	Standard (10µg/ml)
Hydroalcholic (Methanol 80%)	17.87±0.472	21.25±0.500	24.75±0.500	28.50±0.577	35.87±0.497

All values arepresent in Mean±SD

Graph 1

Zone of inhibition of hydro-alcoholic extract of Corriandrum sativum



All values are present in Mean±SD

Table 2

MIC of Hydro-alcoholic Extract of Corianderum sativum seed

S. NO.	CONCENTRATION	ABSORBANCE AT 660 nm
1.	100mg/ml	2.031
2.	50mg/ml	1.965
3.	25mg/ml	1.876
4.	12.5mg/ml	1.776
5.	6.25mg/ml	1.543
6.	3.125mg/ml	1.498
7.	1.562mg/ml	1.387
8.	0.781mg/ml	1.215
9.	0.390mg/ml	1.412
10.	0.195mg/ml	1.533

Graph 2

Minimum Inhibitory Concentration of Corianderum sativum seed Extract

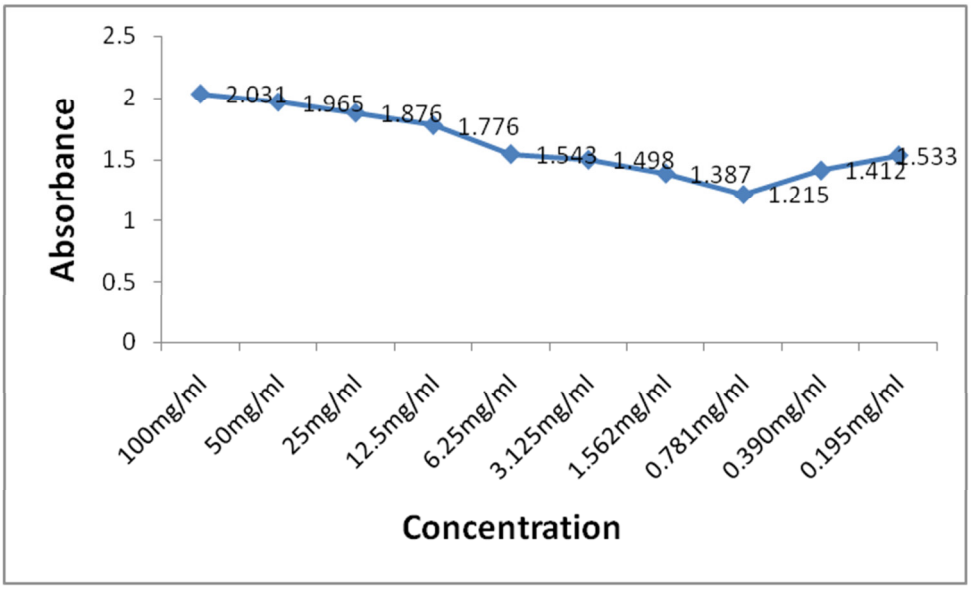


Table 3

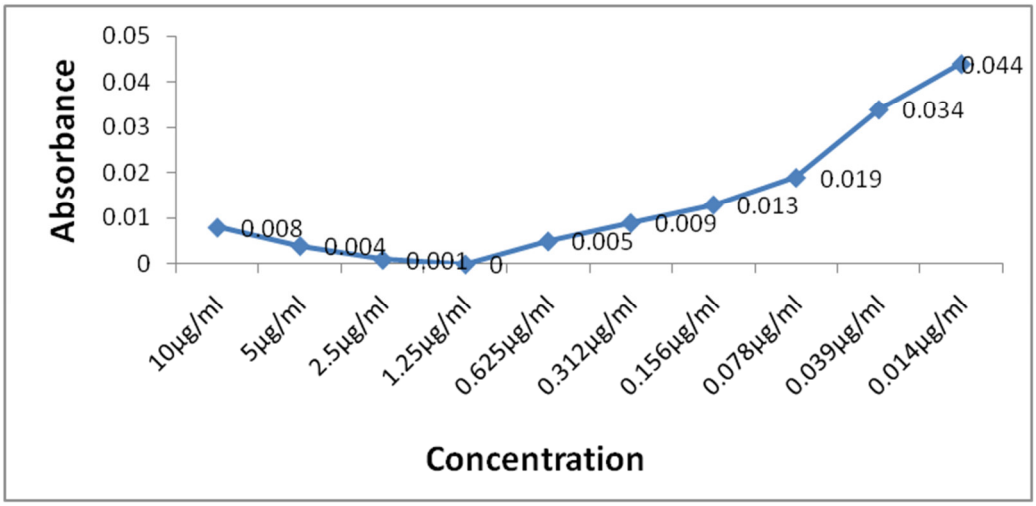
Minimum Inhibitory Concentration of Fluconazole

S. NO.	CONCENTRATION	ABSORBANCE AT 660 nm
1.	10µg/ml	0.019
2.	5µg/ml	0.014
3.	2.5µg/ml	0.012
4.	1.25µg/ml	0.009
5.	0.625µg/ml	0.008
6.	0.312µg/ml	0.006

7.	0.156µg/ml	0.004
8.	0.078µg/ml	0.001
9.	0.039µg/ml	0.000
10.	0.014µg/ml	0.000

Graph 3

Minimum Inhibitory Concentration of Fluconazole



Corianderum sativum seed extract can protect rat from systemic Candidiasis:

Table 4

Evaluation of Acute oral toxicity of the Corianderum sativum seed extract

Group	Dose (mg/kg)	Weight (gm)	No. of rat (Tested/ Survived)
A	5 mg/kg	194	6/6
B	50 mg/kg	190	6/6
C	200 mg/kg	185	6/6
D	400 mg/kg	189	6/6
E	2000 mg/ml	182	6/6

Up to 2000 mg/kg body weight no morality was observed; hence it was considered as Not Observed Adverse Effect Limit. Its $1/10^{th}$ and $1/5^{th}$, i.e. 200 mg/kg and 400 mg/kg were selected as dose for further in vivo investigations.

Table 5

Percent survival in different group of animals infected

Groups	Days of survival rate (%)									
	D 1	D 3	D 5	D 7	D 9	D10	D11	D12	D13	D14
G I	100	100	90	83.33	54.17	16.66	12.34	10.12	10.12	8.00
G II	100	100	100	100	100	90.00	83.15	80.00	80.00	72.00
G III	100	100	100	100	100	100	100	93.76	90.13	80.19
G IV	100	100	100	100	100	100	100	100	100	100

Graph 4

Percent survival in different group of animals infected

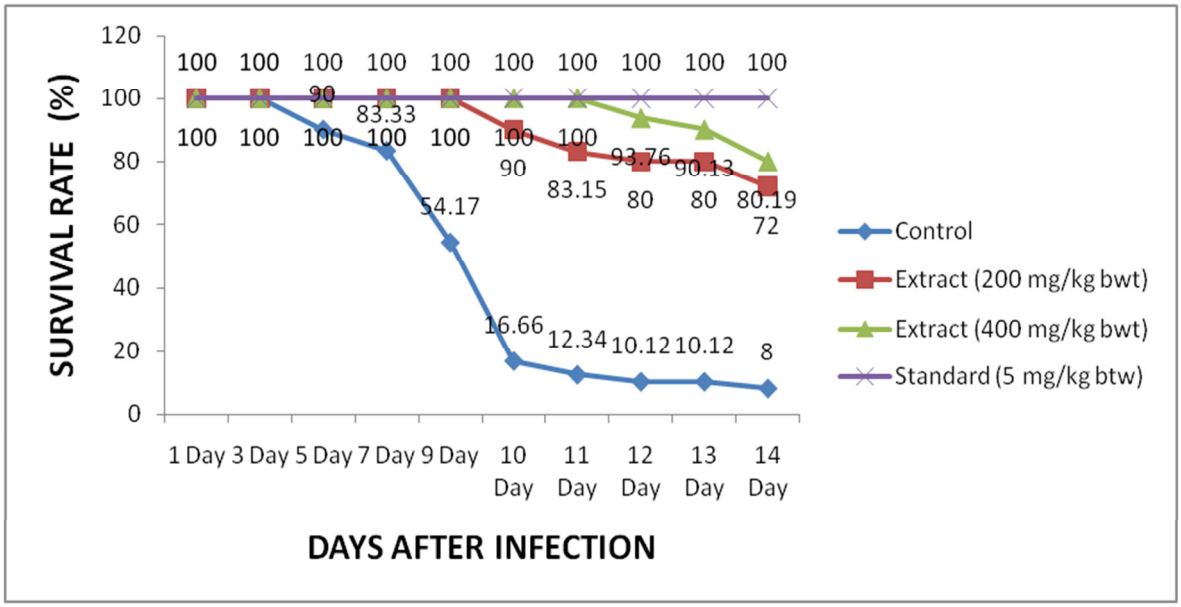


Table 6

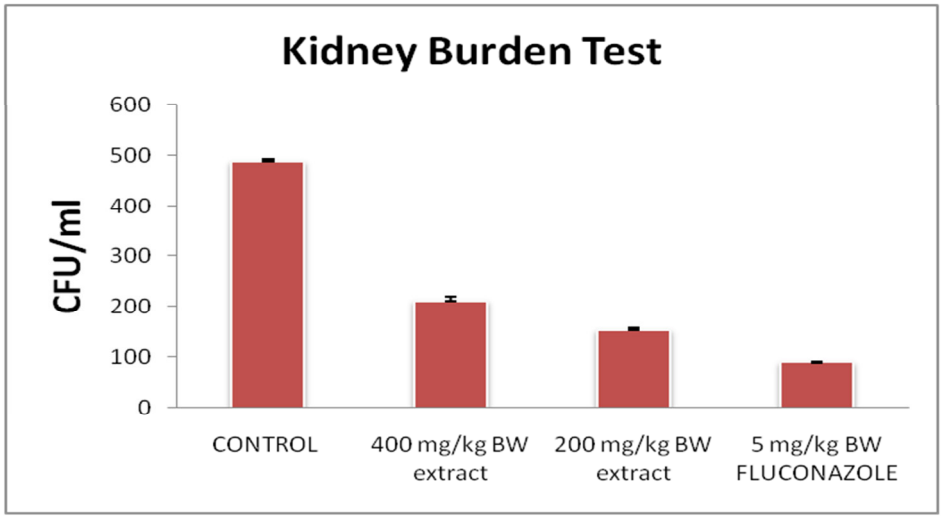
Kidney Burden Test (CFU/g)

S. NO.	GROUPS	CFU/gm of KIDNEY
1.	Control+Candida	489.00±5.797
2.	Extract (200mg/kg bw)	213.00±20.640**
3.	Extract (400mg/kg bw)	155.00±8.295 **
4.	Fluconazole	91.00±2.608**

All data are presented in Mean ± SD. Data were analyzed by One Way ANOVA followed by Barfferoni's test. P<0.005 was considered as level of significance (n=6) when comparing with control group. There is P<0.001 presented by **, and P<0.050 is presented by *.

Graph 5

Kidney Burden test



Histology of Kidney

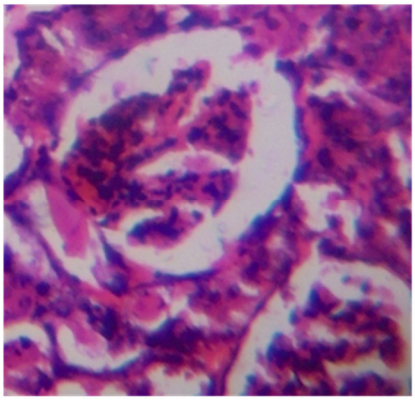


Figure 1: Control Group (Showing Kidney burden)

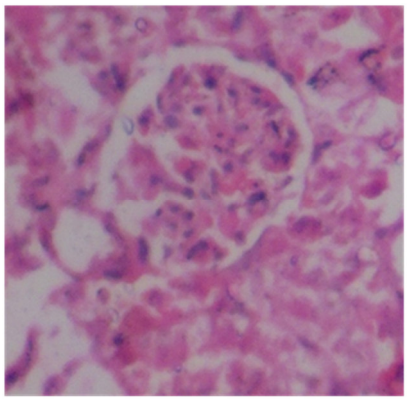


Figure 2: Treatment Group (After Kidney Burden)

DISCUSSION

Large scale community interventions like home herbal gardens in India have demonstrated that many simple primary health care problems like fever, upper respiratory tract infections, gastro-intestinal problems such diarrhoea, dysentery, worm infestations, hepatitis, anaemia, arthritic conditions, and certain gynaecological conditions can be managed at household level through simple herbal home remedies and early identification and interventions. Reproductive health and nutrition forms two important aspects of household care. Considerable health cost saving has been found through this program apart from health and nutrition benefits.[39]

Today in India there are over 9,000 registered pharmaceutical industries of various Indian systems of medicine.

It is an accepted fact that TCAM is playing an important role in care of such chronic diseases. Systematic studies and wide dissemination of potentials of traditional medicine are required for further popularization of such methods.

These herbs used for cooking and flavouring are caressingly claimed to have broad spectrum antimicrobial activity. *Corianderum sativum* has been suggested to have potent antimicrobial activity, including anthelmintic properties, due to its phenolic, alcoholic and terpenoid constituents.[40] The objective of the present study was to assess the antimicrobial properties of *Corianderum sativum* against *Candida albicans* in both *in vitro* and *in vivo*.

Until recently, fungi were not recognized as important pathogens because the annual death rate due to candidiasis was steady from 1950 to 1970.[41, 42] Since 1970, this rate increased significantly due to more widespread use of immuno-suppressive therapies, indiscriminate use of broad- spectrum antibacterial agents, the common use indwelling intravenous devices and immuno-suppressive viral infections such as AIDS, these developments and the associated increase in fungal infections. [43]

In this paper we have demonstrated, that *Corianderum sativum* effectively inhibits the *in vitro* growth of *Candida albicans*.

Antimicrobial activity of test sample was ascertained against *Candida albicans* using well diffusion assay. Zone of inhibition of hydro-alcoholic extract was 17.87±0.472, 21.25±0.500, 24.75±0.500, 28.50±0.577, 35.85±0.087 at the concentration ranges used for present investigation were 100 mg/ml, 200 mg/ml, 300 mg/ml and 400 mg/ml respectively. For the standard drug using Fluconazole showed the zone of inhibition 35.85±0.087 at the concentration of 10 µg/ml. As if was decided to administer test sample through oral route, and if any component is intended to be administered inside the body, its toxicity testing is an important requirement. In this concern OECD 423 guidelines was used to investigate acute oral toxicity of hydro-alcoholic extract of *Corianderum sativum* Seed.

In systemic Candidiasis no mortality was observed till 4th day in either of treatment group. Afterward percentage survival in vehicle treated animals was 16.66% on 10th day. In extract (200 mg/kg btw) treated animals no mortality was observed till day 9. Afterward mortality occurred and hence percentage survival was found to be 72.00% till 14th day. And in extract (400 mg/kg btw) treated animals no mortality was observed till day 10. Afterward mortality occurred and hence percentage survival was found to be 80.19% till 14th day. In standard antifungal no mortality was observed throughout the treatment schedule. This confirmed that extract was having protective potential against *Candida albicans* induced mortality in systemic infection. Afterward kidney burden in kidney homogenate was also confirmed. In standard antifungal drug treated animals on observation day no kidney burden was observed, this confirmed complete eradication of fungal growth in kidney. In extract treated animals there was significant ($P<0.001$) decline in kidney burden as compared to vehicle treated animals in all dilution of kidney homogenate. Analysis was done with keeping number of animals same as to that of survival, i.e. four animals for vehicle treated group, five animals for extract treated group, six animals for standard drug treated group.

Thus daily oral administration of hydro-alcoholic extract of *Corianderum sativum* seeds may be highly effective in the prevention and treatment of Candidiasis.

CONCLUSION

From the present study we concluded that thisherbal extract having strong property to inhibit the fungal infection in systemic Candidiasis. Many antifungal drugs have side effect like high blood pressure, skin rashes, digestion issue etc. but our extract was not. With the recovery of fungal infection it's also helpful for the better digestion, anaemia, menstrual disorders etc. Because of its cost-efficiency and easy availability in market this can be the best herbal medicine with no side effects. And these types of homemade remedies will be the future of self employment of small scale employment.

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ANALYSIS OF LEAVES LENGTH OF RICE GENOTYPE PERCENTAGE DUE TO THE EFFECT OF MICRONUTRIENTS AND THEIR INTERACTION

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ABSTRACT

Rice is the most important grain with regard to human nutrition and caloric intake, providing more than one fifth of the calories consumed worldwide by humans. In present studies The leaf length of two genotype of rice IR-36 and JR-345 were studied against the various micronutrient and found that in case of IR-36 grown with Multiplex +$ZnSO_4$ +FYM Measuring highest leaf length(27.00 cm),being significantly higher to all the rest of the interactions.JR-3-45 with control resulted in the lowest value(26.55 cm).

Key words: *Oryza sativa*, micronutrient, genotype, leaf length

INTRODUCTION

Increasing food production and water saving are the major challenges for rice growers at micronutrient deficient soil in India. The role of micronutrients in various physiological and biochemical processes in plant is well known, which enables a rapid change in the physiology of plant within one season to achieve desirable results.[1] Rice is one of the most consumed cereal in the world as a food product. Rice is the seed of the grass species *Oryza sativa* (Asian rice) or *Oryza glaberrima* (African rice). As a cereal grain, it is the most widely consumed staple food for a large part of the world's human population. Leaves are the most important organs for plants. Without leaves, plants cannot capture light energy or synthesize organic compounds via photosynthesis. Without leaves, plants would be unable perceive diverse environmental conditions, particularly those relating to light quality/quantity. [2]

MATERIAL AND METHOD

The present experiment was conducted at the regional Agricultural research station, Kuthulia Rewa (M.P.). The field experiments carry out during rainy season. Firstly soil sample were collected randomly through a soil auger up to 15cm depth from 10 different spots of the experimental plot and mixed them to form composite sample. The field experiment laid out in split –plot design during both the seasons. The treatments comprised of three genotype of rice and eight micronutrient levels thus forming twenty four treatment combinations. These treatments were randomly arranged in each replication, keeping in all three replications. The genotype were taken in the main-plots, and the micronutrient levels in the layout plan as depicted

Treatments	**Symbol**
Main-plots treatments (Genotypes 2)	
IR-36	G1
JR -345	G2

Sub-plot treatment (Micronutrient levels 8)

No Micronutrient (Control) M0
25 Kg Zn SO_4/ha (Soil application) M1
FYM@10 tones/ha M2
FYM+25kg $ZnSO_4$/ ha M3
Multiplex (Foliar spray thrice) M4
Multiplex+ $ZnSO_4$ M5
Multiplex+FYM M6
Multiplex+ $ZnSO_4$+ FYM M7

Treatments' combinations: 24

1. G1M0	9. G2M0
2. G1M1	10. G2M1
3. G1M2	11. G2M2
4. G1M3	12. G2M3
5. G1M4	13. G2M4
6. G1M5	14. G2M5
7. G1M6	15. G2M6
8. G1M7	16. G2M7

Details of layout in the field
Experiment design: Split plot
Replications: three
No. of plot in one replications: 24
Gross plot size: 3.4m ×4.5m= 15.3 m^2
Net plot size: 4.0m × 3.0m= 12m2
Row and plant spacing: 20cm and 10cm
No. of rows per plot: 10
Replication border: 1.0m
Main plot and subplot border: 0.5m
Total no. of plot: 72

Micronutrient level (M)	**Genotype (G)**		**Mean**
	IR-36	**JR-345**	
Control	21.89	18.11	**20.00**
$ZnSO_4$ (25kg/ha)	23.33	21.22	**22.27**
FYM(10 t/ha)	24.66	22.77	**23.71**
$ZnSO_4$+ FYM	26.00	24.44	**25.22**
Multiplex spray (thrice)	23.00	20.11	**21.55**
Multiplex+ $ZnSO_4$	24.22	23.89	**24.05**
Multiplex+ FYM	26.55	25.77	**26.16**
Multiplex+ $ZnSO_4$ +FYM	27.00	26.55	**26.77**
Mean	**24.58**	**22.86**	

Table (1) : Leaf length (cm) as influenced by rice genotypes and micronutrients and their interactions

RESULT & DISCUSSION

The leaf length was found significantly higher in case of IR-36 grown with Multiplex +$ZnSO_4$ +FYM Measuring highest leaf length(27.00 cm),being significantly higher to all the rest of the interactions.JR-3-45 with control resulted in the lowest value(26.55 cm). plant grows taller there are better possibilities to have more number of effective's leaves. Sulphur and zinc are known for precursor of growth as they play greater and crucial role during early phase of plant life. Zinc role is as multifaceted as the interface that reduces its availability.[3] Wheat and its Leaves Average data of two years (2012-2014) showed that flag leaf chlorophyll content increased significantly (p=0.05) due to foliar application of micronutrient (Fe, B and Fe+B) at individual growth stages (ZGS21 or ZGS41) and at both stages of growth (ZGS21+ZGS41). Wheat leaf was a significant influence of Zn treatment on flag leaf area which might have negatively affected disease severity. In the case of Mn and B treatments there was no significant influence on flag leaf area. [4] Maize Leaf area growth and nitrogen concentration per unit leaf area, N_a (g m^{-2} N) are two options plants can use to adapt to nitrogen limitation. Previous work indicated that potato (*Solanum tuberosum* L.) adapts the size of leaves to maintain N_a and photosynthetic capacity per unit leaf area.[5]The Genotype and Micronutrients interaction, the best interaction was PAC-708 applied with the combined levels of Multiplex +Znso4 +FYM over all the remaining interactions.[6]

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Community Study of Plant Species in Van-Vihar National Park Bhopal M.P with special reference to Eco-sustenance of life in near future

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Abstract: *The present paper reflect vegetation cover along with plant species of different kinds in the van vihar national park district Bhopal. It consists of 78 species under 61 genera and 41 families of angiosperms. The four dominant species as per the (R .A) are Mimosaceae 14.7, Ramnaceae 8.7, Apocynace 7.6, Fabaceae 5.6 Asteraceae 9.6. The family mimosaceae with species having Abduance value 14.7 (Total), but only the species Acacia leucophloea showed 8.0 RA which is highest for a single species of all the members of Mimosaceae family in the said area. This species is dominant in the said area and found as a soil and rock binder. Majority of the plant species found there are natural kinds but few of them are agriculture type and some plants are introduced type for various types. Some exotic elements of ecological importance have also been recorded from the sites with stress conditions need further study. These are lantana, bombexceibapithecelloibumdulcie exotic all the plants have made the influence on the said habitat. Other species forming large vegetation of the said area boosts huge protection for the eco-habitat and change the habitat best suited for new comer. As the soil is nitrogen prone and stressed in condition, need elaborate work to advocate the requisites to a stress tolerant condition for sustenance of life by eco-restoration programme.*

Key words: *van vihar national park, district Bhopal, check list of species, phytodiversity conservation –management, eco-restoration*

Introduction:

The environment in the van vihar national park is characterized by mild winds;high humidity and limited availability of macronutrients. Plants have evolved special strategies for survival in the Harish environment. The most important factor is physicochemical characteristics that effect the distribution of plant communities. The site is play a crucial role to adjust as a transition zone but today's activities imposed by man in such a way that the eco-system is much hampered due

anthropogenic activities[11]. Generally, the people break the rules and regulations imposed by development authority and destroying vegetation havoc day by day .the good example is national park van vihar were the people destroying vegetation like Gamphrenacelosioides, Blumealacera, vernoniacinerea .the negative activities imposed by tourists cause grate loss biodiversity of locial kind.

Therefore, study on such vegetation and monitoring of the vegetation is essential to restore the ecosystem in pristine as soon as possible by the help of forest development authority. the present study is therefore a study of vegetation and initial steps of monitoring the same habit to revive the habitat from eco-degradation and a mile stone for eco-restoration.

Area Under Study:-Study area fall under the state capital Bhopal. It lies between Vindhyan ranges in the shallow hills to the south west the area measuring is 445.21 hectare. It was declared as a national park on January, 26. 1983 and was named van vihar. the soil of this area is filled with trees, shrubs, herbs, climbers,animals of different types and having varied ecological conditions. The soil condition also varies from site to site with low amount of macronutrients including soil organic matter. The study taken from the entire site above 5 kms of range.

Photo plate showing the pictures of Van-Vihar during the field work.

Soil and Climate:– The soil of this area are black (vert sol), red (invert sol) the vert sol soil is found in the right from entering SairSapata gate this is the fertile soil for cotton and different grasses. The red soil (invert sol) is found on entering from the main gate of lack view in the hilly hocks. It is the fertile soil for different vegetation. The climate of this Area is characterized by a dry and increasingly hot season fromMarch tomild June. October and November consists the post monsoon season. The temperature of march- June is max. 45^0C and min. 26.6^0C, night temperature 25.8-10.3^0C. The relative humidity varies from 10% to 91% (April-May) humidity is above 70%and in some places it is 20%.[11]

Materials and methods:-

Extensive field visits were carried out to different places of the study site. Quadrants as well as transects were taken for monitoring the vegetation in late summer monsoon and winter as per the latest the ecological methods for eco-restoration study, vegetation monitoring was done following the concept of Greipsson[6] parameter taken for stability study and concept of structure and function of elements in the ecosystem along with dynamics of vegetation idea of Dash and Dash[4] was taken. Books journals and magazines plant specimen from field were also collected and processed for presentation as herbarium specimen and for identification using botanical and ecological standard.The study site contains 78 species which fall under 61 genera 41 family.

In dry season, the number of species reduced in a great extent and in monsoon it increase so that there is always a degree of fall which need to study in a later phase to know the actual status of vegetation in a lucid but more conspicuous. The table shows the list of species available in the said area with relative abundance of species which have made in terms of density of species of the study area.

Name	**Family**	**Density in Community**
Acacia nilotica (L.) willd. Ex Dellite	*Mimosaceae*	*1.0*
Acacia leucophloeaRoxb	*Mimosaceae*	*2.2*
Aegle marmeloL.	*Rutaceae*	*0.3*
Annona squamosaL.	*Annonaceae*	*0.1*
AlstoniascholarisL.	*Apocynaceae*	*0.1*
AlangiumsalviifoliumL.f	*Carnaceae*	*0.2*
Apludavaria L.	*Poaceae*	*0.5*
AilanthusexcelsaRoxb	*Simaroubaceae*	*0.9*
AlternantherasessilisL.	*Amaranthaceae*	*0.5*
. *AlbiziaproceraRoxb.*	*Mimosaceae*	*1.2*
. *AchyranthesasperaL.*	*Amaranthaceae*	*1.5*

. *BombaxceibaL.*	*Bombacaceae*	*9.4*
. *Butea monospermaLam.*	*Fabaceae*	*0.3*
. *BlumealaceraBrum.f.*	*Asteraceae*	*0.2*
. *Bauhinia malabaricaRoxb.*	*caesalpiniaceae*	*0.4*
. *BixaorellanaL.*	*Bixaceae*	*0.1*
. *Cassia fistula L.*	*caesalpiniaceae*	*1.5*
. *CacculushirsutusL.*	*Menispermaceae*	*1.1*
. *CapparisspinosaL.*	*Capparaceae*	*0.5*
. *CachlospermumreligiosumL.*	*Cachlospermaceae*	*1*
. *Celosia argenteaL.*	*Amaranthaceae*	*1.3*
. *Coldeniaprocumbens L.*	*Boraginaceae*	*0.6*
. *DalbergialanceolariaL.f.*	*Papillonaceae*	*1.2*
. *DalbergialatifoliaRoxb.*	*Papillonaceae*	*1.1*
. *DiospyrousmelanoxylonRoxb.*	*Ebenaceae*	*0.3*
. *DiospyrouscardifoliaRoxb.*	*Ebenaceae*	*0.2*
. *DalbergiasissooRoxb.*	*Fabaceae*	*2.1*
. *Eucalyptus globulusL.*	*Myrtaceae*	*1.3*
. *EhretialaevisRoxb.*	*Boraginaceae*	*0.3*
. *FicusbenghalensisL.*	*Moraceae*	*1.3*
. *FicusracemosaL.*	*Moraceae*	*1.0*
. *GamphrenacelosioidesL.*	*Amaranthaceae*	*2.2*
. *Grevillea robustaA.cunn*	*Proteaceae*	*0.5*

. *HolopteleaintergrifoliaRoxb.*	*Ulmaceace*	*0.1*
. *HeteropagoncontortusL.*	*Poaceae*	*1.4*
. *HolarrhenaantidysentericaBuch.Ham*	*Apocynaceae*	*0.2*
. *HyptissuaveolensL.*	*Lamiaceae*	*0.7*
. *Ipomoea fistulosaJacq.*	*Convolvulaceae*	*2.4*
. *Lagerstroemia parvifloraRoxb.*	*Lythracaeae*	*0.3*
. *LeucaenaleucocephalaLam.*	*Mimosaceae*	*0.1*
. *Lantana indica L.*	*Verbenaceae*	*1.1*
. *LanneacaromandelicaHott.*	*Anacardiaceae*	*1.5*
. *Melia azedarachL.*	*Meliaceae*	*1*
. *MadhucalatifoliaRoxb.*	*Sapotaceae*	*0.2*
. *MumasopshexandraRoxb.*	*Sapotaceae*	*1.1*
. *NymphoidesindicumL.*	*Menyanthaceae*	*0.1*
. *NymphoidescristataRoxb.*	*Menyanthaceae*	*0.1*
. *OroxylumindicumL.*	*Bignoniaceae*	*0.3*
. *Phoenix sylvestrisL.*	*Aracaceae*	*9.6*
. *Phoenix acaulisRoxb.exBuch*	*Aracaceae*	*6.4*
. *PongamiapinnataL.*	*Fabaceae*	*1.3*
. *PterospermumaccrifoliumL.*	*Sterculiaceae*	*2.1*
. *Pistia stratiotes L.*	*Aracaceae*	*0.1*
. *PolygonumglabrumWilld*	*Polygonaceae*	*3.2*

. PutranjivaroxburghiiWall.	*Euphorbiaceae*	*0.2*
. PeltophorumferruginumBenth.	*caesalpiniaceae*	*1.6*
. PlumeriarubraL.	*Apocynaceae*	*0.5*
. Sidacordifolia L.	*Malvaceae*	*1.5*
. SidarhombifoliaL.	*Malvaceae*	*1.1*
. StenolobiumstansL.	*Bignoniaceae*	*0.3*
. SaccharummunjaRoxb.	*Poaceae*	*1.3*
. SyzygiumcuminiL.	*Myrtaceae*	*2.2*
. ScirpuslitoralisL.	*Cyperaceae*	*1.2*
. TamarindusindicaL.	*caesalpiniaceae*	*3.3*
. Tridax procumbent L.	*Asteraceae*	*1.6*
. Terminalia chebulaRetz.obs.But	*Combretaceae*	*0.2*
. Terminalia bellirica (Gaertn).Roxb	*Combretaceae*	*1.1*
. Vernoniacinerea	*Asteraceae*	*2*
. VentilagocalyculataGaertn.	*Rhamnaceae*	*0.7*
. WrightiatinctoriaR.Br.	*Apocynaceae*	*1.5*
. WoodfordiafruticosaL.	*Lythracaeae*	*0.3*
. ZiziphusnummulariaBurn.f.	*Rhamnaceae*	*0.9*
. ZiziphusoenopliaL.	*Rhamnaceae*	*1.4*
. ziziphusjujubalam.	*Rhamnaceae*	*5.5*
species reported from van vihar 74	*Total=*	*95.3*

Results and Discussion:-

In the present study five dominant families were observed from the study area the families were Mimosaceae (6 species) Rhamnaceae (5 species) Apocynace (5 species) Fabaceae (4 Species) Asteraceae (4 specie) found. These are heterogeneous group. Other families with low number of species showed a less degree of distribution as they were homogenous found in a habitat of specified kind. So, study of homogeneity to heterogeneity may be a new parameter to study the actual status of vegetation in the same site for study and survey in an eco-restoration programme[11].This need based study requires study of nutrients in soil for actual kind of group vegetation study particularly at ecotone area.The studies also focus on diversity and dominance of a group of species in same site by considering families rather than species. This may be regarded as association parameter of species in a particular eco-habitat. This also amplifying the documentation of record species and their density at a glance which may be under threat or may be threat under different physiochemical factors need elaborate studies from plant physiology and the environmental studies to make the species either fit in the habitat or not. This guideline may be the guidelines for resources studies in the near future for a need based way to record the modern day research and extension activities in the said area. some versatile tree species are found in this said area.Asfor example, Pongamiapinnta,Azadirachtaindica ,Butea monosperma, Cassia fistula, These species are used as medicinal plants and have grate economic importance ,so forest department should take initiative in a large scale to raise these species .Another important medicinal plant, Plumeriarubra (Apocynaceae)is found in van vihar national park with low frequency and abundance .The species are found in Srilanka ,China .All the species are competitors in the said habitat and used naturally to oscillate the vegetation and bring change in succession. So, studies of species of the individual type on a community are essential to know the functional attributes of each kind before going to climax.

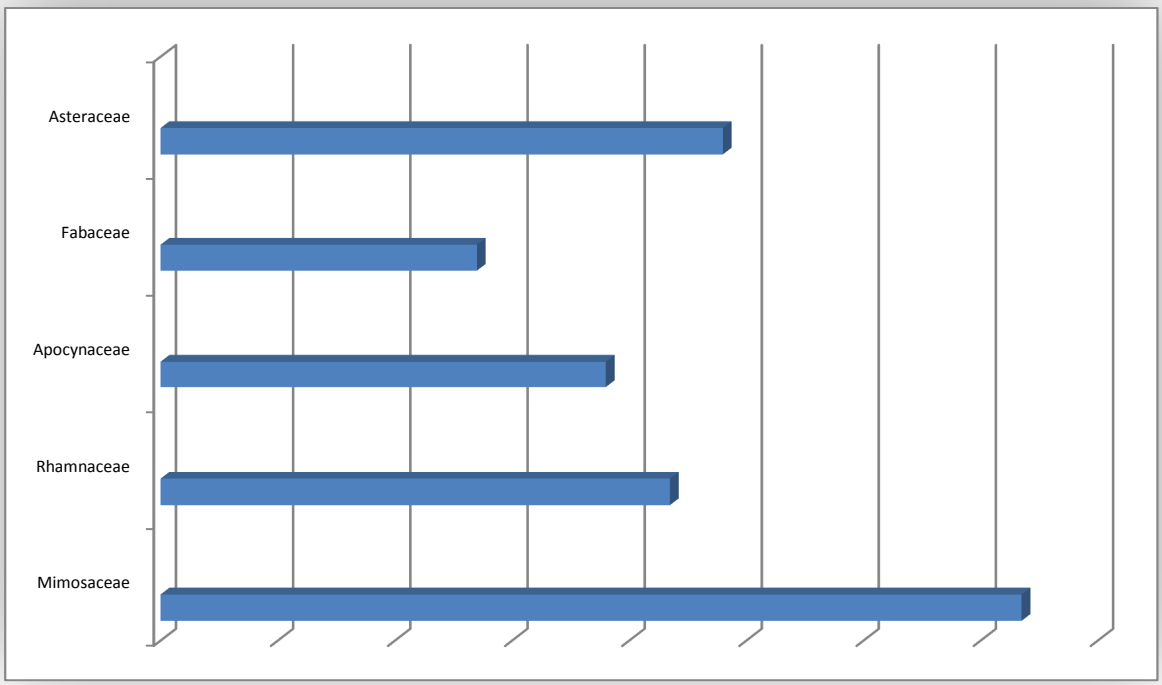

Fig 1. Bar Graph showing relative Abundance (RA) of five dominant families in the study site.

Note that,family Mimosaceae with 14.7, Rhamnaceae with 8.7, Apocynaceae with 7.6, fabaceae with 5.4, Asteraceae with 9.6 abudance value,though the poaceae with four species having a total 10.5 abundnce value,here Acacia leucophloea is a dominant with 8.0 RA value which is the highest for single species of dicot in the said area. The species is dominant in this area as soil showed significant ecological value. Some species like Scirpuslitoralis and Ailanthus excels species are found in least. Such a way these species posses least abundance and for uniformity of result their value are not included here.

All the species are the competitors in the said habit and used naturally to oscillate the vegetation and bring change in the succession. So studies of species of individual type on a community are essential to know the functional attributes of each kind before going to climax.Bestelmeyer et al.,[2] proposed, six types of mechanisms driving vegetation change includes (1)stability, (2) size oscillation of plant (3) loss and re-establishment of plants within functional group (4) loss of one plant function group and replaced with another (5) spatial reorganization of vegetation patchesand (6) cascading transitions that spread from small to broad scale. In the present study, vegetation and soil is also going to change due to different catastrophic changes. Man made cause is also going to change in addition to the natural type.[9]

Eco-stress of people is another important factor that can suppress the area and vegetation and can increase the loss of species and composition structure as well as loss nutrients through the gradual change of nutrients[10].

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Conclusion:

Check list of species which are vanished from this area due to lack of physio-chemical characteristics. It also shows the eco-stress of people which is the important factor that can suppress the area and vegetation and can increase the loss of species and composition structure as well as loss of nutrients through the gradual change of nutrients. It also shows the dominant species on the said area due to suitable physio-chemical characters. It also highlights importance of proper management and care for eco-restoration and proper site for exotic species.

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SAFFRON AS AN ANTI- CANCER HERB AND ITS PHARMACOLOGICAL IMPORTANCE

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ABSTRACT:

Saffron is known as one of the most majestic Natural medicine which is considered essential for drug development because it is reported to have pharmacological activity in Asia, Middle East especially China, Spain and in major parts of India Jammu and Kashmir. In tradional system of medicine saffron is also used for the relief of pain and for the treatment of cancer. Saffron belongs to family Iridaceae and it is derived from the dried red stigmas of the *Crocus Sativus* L.flower. It is also used in cooking, baking and in cosmetics. Saffron possesses numerous applications; it is used as an anti-oxidant agent, antitoxic, and anti-cancer due to its secondary metabolites and their main derivatives (Safranal, crocin, crocetin, picrocrocin, dimethyl crocetin). It has been reported from the data that the major derivatives of saffron especially crocin and crocetin will significantly affect the growth of certain cancer cells while not affecting normal cancer cells. It has been demonstrated by modern pharmacological studies that saffron extract has an anti-tumor effect, radical scavenging property, and hypolipidemic effect. In this review we have tried to focus important biological properties of *Crocus Sativus* which are pharmacologically very essential.

KEYWORDS:

Crocus Sativus, Pharmacology and medicinal property of saffron, Crocin, Picro-crocin.

INTRODUCTION:

Cancer is one of the most dreadful diseases of the 20th century spreading further with continuance and increasing incidence in 21st century[1]. Saffron is considered as one of the most expensive spices

of the world which is derived from the dried red stigmas of the *Crocus Sativus* a member of the Iridaceae family. Saffron (*Crocus Sativus* L.) is mostly cultivated in Iran, Spain Greece, China and in major parts of India Jammu and Kashmir.

Fig. (1a)- Saffron Flower

Fig (1b)- Saffron Stigma

In folklore medicine saffron is used as an anti-spasmodic, eupeptic, gingival sedative and edematogenic remedy. Saffron is also applied in the form of paste to treat skin diseases like acne. It is also used to treat insomnia and in the treatment of measles, dysentery, jaundice, cholera etc. The powdered form of saffron stigma is used as a drug in the treatment of cataracts, nightblindnes and poor vision. Sushruta used it as a blood purifier and to treat skin eruptions internally. Saffron is also used as a nerve sedative, in the treatment of fever, and enlargement of liver. Saffron is considered as a protective agent against chromosomal damage and the best modulator of lipid peroxidation for reducing blood pressure and in the treatment of psoriasis. Saffron has been regarded tradionally as the highly valued medicinal plant to treat a wide variety of ailments such as depression, respiratory problems, colds, asthama and heart diseases[2].

The nutritional supplement value of saffron was provided by Pars Bioscience LLC in powdered form and it was analysed to show the following contents NL-Proximate (moisture, ash, protein, carbohydrate, fat, calories). The results of these analyses are given in Table No. (1). Vitamins' (vitamin A, C, and folic acid) and minerals (calcium, copper, iron, magnesium, phosphorus, sodium and zinc). The details are given in table No.(2). Some of the important biological functions of saffron and its main derivatives are mentioned in table no (3). In the

treatment of variety of cancers several studies have been performed including colorectal cancer cells (HCT-116,SW-480 and HT-29), non-small cell lung cancer (NSCLCS) cells [3], Breast cancer cells (MCF-7) [4], Lung fibroblast cells (WI-38), VA-13 cells (WI-38 cells transformed in-vitro by SV40 tumor virus[5], lung cancer bearing mice [6], Skin carcinogenesis in mice [7]. Saffron contains more than 150 volatile and aroma yielding compounds. It also contains many non-volatile compounds many of which are carotenoids including zeaxanthine, lycopene and various α and β caroteines.

The golden yellow orange colour of saffron is primarily due to α crocin. This crocin is trans-crocetin di- (β –D gentiobiosyl ester), it bears the systemic IUPAC name 8, 8-diapo 8, 8 carotenic acid. This means that the crocin underlying saffron aroma is a digentiobios ester of the carotenoid crocetin[8]. Crocin is hydrophobic and is a conjugated polyene dicarboxylic acid, and is oil soluble. The picrocrocin is responsible for the bitter taste of saffron and its systematic name is 4-(β –D-glycopyranosyloxy) 2, 6, 6 trimethycyclohexane-1-ene-1carboxaldehyde) is a union of the aldehyde sub element known as safranal.

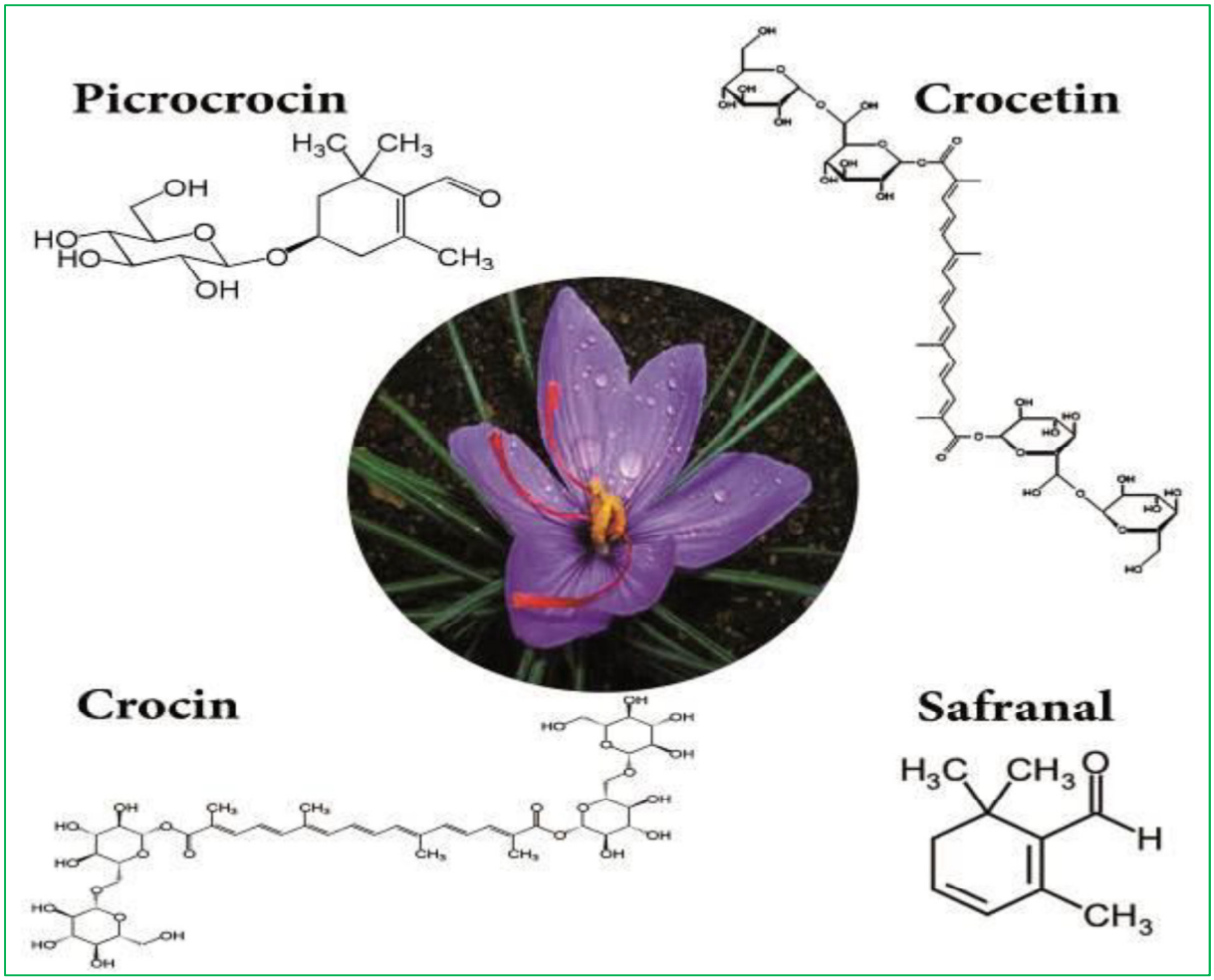
Table No.1: Showing nutritional supplement (NL-Proximate) and analysis of saffron

Analysis of Saffron	Results (Per 100g serving size)
Moisture content	7.7g
Ash	4.6g
Protein	15.6g
Total carbohydrate	69.6g
Calories	363 Calories
Calories from fat	22.1Cal

Table 2: Showing Nutritional vitamins and minerals of saffron

Vitamins	
Vitamin A	< 100 IU
Vitamin C	<1.0mg
Folic Acid	800mcg
Minerals	-
Calcium	124mg
Copper	0.908mg
Iron	23.7mg
Magnesium	154mg
Phosphorus	404mg
Potassium	1750mg

Sodium	39.0mg
Zinc	4.15mg
Manganese	2.44mg



The four major bio-active constituents in saffron are Crocin ($C_{44}H_{64}O_{24}$), Picro-crocin ($C_{16}H_{26}O_7$) Crocetin ($C_{20}H_{24}O_4$), and Safranal ($C_{10}H_{14}O$). The chemical structure of crocin consists of crocetin as a central core and two sugars that are responsible for the colour of the compound. The another constituent of saffron is picro-crocin and it is responsible for the bitter taste of saffron. Safranal is the metabolite of picro-crocin and it gives aroma and ordour of saffron[9-10].

Table No (3) Showing important biological functions of saffron and its main derivatives

Activity	**Saffron Constituents Tested**	**Reference**

Prevention of gastric disorder	Saffron crude extract, Ethanolic saffron extract	11 12
Prevention of stomach ulcer	Crocin	13
Digestive enhancement	Aqueous saffron extract	14
Anti-cancer function and Cyto-toxic effect on tumor cells	Ethanolic saffron extract Crocin, Safranal, Crocetin, and Picrocrocin	15 16 17
Tumor inhibition	Crocin, Saffron, Crocetin.	18
Prevention of insulin resistance	Crocetin	19
Anti-depression activities	Capsulated Ethanolic saffron extract, Saffron petal extract	20 21
Premenstrual Syndrome treatment (PMS)	Capsulated Ethanolic saffron extract	22
Ditrimental health effects vomiting , uterus bleeding abortion	Saffron	23 24

Pharmacological properties of saffron:

(1) Anti-oxidant Activity: - Medicinal properties of saffron have indicated that saffron has a potential anti-oxidant activity, which is mostly due to the presence of crocin as a unique carotenoid. The synergetic effects of all the bioactive constituents gave saffron a significant anti-oxidant activity. High radical scavenging activity of these compounds is probable due to their ability to donate a hydrogen atom to the DPPH radical[25].

(2) Anti-cancer Activity: - Saffron and its characteristic compounds possess anti-tumor and anti-carcinogenic activities *in-vivo* and *in-vitro*. Oral administration of saffron extract suppressed the growth of DLA and S-180 tumor cells but there was no growth of EAC tumor cells in mice [26].

(3) Radical scavenging activity: - Radical scavenging activity is involved in aging processes, anti-inflammatory, anticancer and wound healing activity. Hence, in the present study the DPPH radical scavenging activity of a natural product that possesses biological properties, an extract of saffron, and some of its bioactive constituents (crocin, safranal) was studied. It was shown that a methanolic extract of saffron exhibited high antioxidant activity, although it contains several active and inactive constituents due to the ability to donate a hydrogen atom to the DPPH radical[27].

(4) Antityrosinase Activity :- A common flavonol, kaempferol isolated from the fresh flower petals of *Crocus sativus* was found to inhibit the oxidation of L-3, 4- dihydroxyphenylalanin (L-DOPA) catalysed by mushroom tyrosinase with an ID (50) of 67microgram/ml (0.23mM)[28].

(5) Effect on Uterus and Estrus Cycle: - *Crocus sativus* has hot and dry qualities, stimulant or inebriant depending on dosage, sun dried filaments ingested strengthen the uterus and treats menstrual problems, stimulates sexual desires for women[29-30].

(6) Glutathione S-transferase (GST) Activity: - The treatment of mice with aq. extract of saffron can significantly inhibit genotoxicity produce by cisplatin, cyclophosphamide, mitomycin and urethane. These genotoxins alone can inhibit glutathione S-transferase (GST)

activity. It was also observes that saffron pre-treatment attenuated the inhibitory effects of genotoxins on GST activity[30].

(7) Anti-Spasmodic Effects :- The anti-spasmodic effects of saffron is a double blind clinical trial by using 40 patients and proved efficacy of saffron as an herbal drug to treat mild to moderate depression. In today's society Depression is a serious disorder and the estimates of lifetime prevalence are very high more than 21% of the general population in some developed countries. Further research involving much larger patient population could uncover more details about the anti-depressive effects of saffron. However this clinical study gives scientific basis for safe and effective traditional usage of saffron for the treatment of depression[31].

(8) Pain Relieving Effect: - The pharmacological studies done on saffron prove therapeutic efficacy of safranal isolated from *Crocus sativus* as a pain killer through inhibition of pain signalling nociceptive receptors. The opioid agonist Naloxone did not inhibit the anti-nociceptive effect that is why this pain relieving effect is not mediated through opioid receptors but by

inhibition of synthesis or action of prostaglandins. This work scientifically supports the traditional usage of *Crocus sativus* as abdominal pain killer[32].

(9) Effect on sexual behaviour: - The aphrodisiac activities of *C. sativus* stigma aqueous extract and its constituents, safranal and crocin, were evaluated in male rats. The aqueous extract (80, 160, and 320 mg/kg body wt.), crocin (100, 200, and 400 mg/kg body wt.), safranal (0.1, 0.2, and 0.4 ml/kg), sildenafil (60 mg/kg body wt. as a positive control), and saline were administered intraperitoneally to male rats. Mounting frequency (MF), mount latency (ML), intromission latency (IL), and ejaculation latency (EL) were the factors evaluated during the sexual behaviour study. Crocin, at all doses, and the extract, especially at doses 160 and 320 mg/kg b.w. increased MF, IF, and EF behaviours' and reduced EL, IL, and ML parameters. Safranal did not show aphrodisiac effects. This study exhibited an aphrodisiac activity of saffron aqueous extract and its constituent crocin[33].

(10) Protective effect on kidney and urinary disorders:-Traditional medicine of clod desert Ladakh has large potential to treat various ailments among tribal communities inhabited in the remotest region of Indian subcontinent. This study was conducted to document the new ethno medico botanical information and traditional use of medicinal plants

against kidney and urinary disorders, and thus to conserve the rapidly disappearing traditional Knowledge system of Amchis of Ladakh. The information was collected from 105 villages of Leh and Kargil districts of Ladakh region by involving 47 Amchis (the herbalists), village heads and old aged persons including women population through on spot interview and repeated queries among other interviewees over a period of 3 years from 2004–2006. The use *crocus sativus* with 68 medicinal plants belonging to 29 families and 58 genera of clod desert was documented against the treatment of problem in urine discharge, burning sensation and painful urination, inflammation and bleeding in the kidney, irritable condition of bladder, haemorrhage of kidney and removal of blocked urine and kidney stone were the frequently reported disorders in the tribal communities of Ladakh region in India[34]**.**

(11) Anti- secretory and antiulcer activity: - An aqueous suspension of saffron was subjected for evaluating gastric antiulcer activity induced by pylorus ligation (Shay rats), indomethacin and various necrotizing agents including (80% ethanol, 0.2 M NaOH and 25%NaCl) in rats. Gastric wall mucus and non-protein sulfhydryl contents were also estimated in rats. Histopathological assessment of rat stomach was carried out. The saffron aqueous suspension at doses (250 and 500 mg/kg) exhibited decrease in basal gastric secretion and ulcer index in Shay rats and

indomethacin treated groups. Gastric wall mucus elevation was observed. No significant histopathological changes were noted. A large margin of safety was observed in animals after acute and chronic treatment.) Saffron exhibited significant anti-secretory and antiulcer activities without causing any deleterious effects on acute and chronic toxicity in rodents[35].

(12) Cellular and molecular effects:

It has been found that crocin posseses antiapoptotic effects on non-cancerous cells. Crocin suppresses cell death induced by tumour necrosis factor-alpha (TNF-α), cysteine protease mRNAs and simultaneously restores[36]

Other important applications of Saffron:

Glucose Uptake Regulatory Effect of Saffron

Kang et al., (2012) recently elucidated mechanism of the hypoglycaemic actions of saffron through investigating its signalling pathways associated with glucose metabolism in skeletal muscle cells. They found that saffron strongly enhanced glucose uptake and the phosphorylation of AMPK (AMP-activated protein kinase)/ACC (acetyl-CoA carboxylase) and MAPKs (mitogen-activated protein kinases), but not PI 3-kinase (Phosphatidylinositol 3-kinase)/Akt. According to their results, the co-treatment of saffron and insulin further improved the insulin sensitivity via both insulin-independent (AMPK/ACC and MAPKs) and insulin-dependent (PI 3-kinase/Akt and mTOR) pathways. In line with the findings of GLUT4 translocation, it was also suggested that there is interference between the two signalling pathways of glucose metabolism in skeletal muscle cells. Overall, AMPK plays a key role in the effects of saffron on glucose uptake and insulin sensitivity in skeletal muscle cells.

Anti-Depressant and Mood Improving Effects of Saffron

Crocin and ethanolic extracts of saffron are known to show antidepressant impact on rodents. crocin reduced immobility time and increased climbing time at dose 50–600 mg/kg may be via individual uptake inhibition of dopamine and norepinephrine**.** In another study, it was found that saffron supplementation statistically improved the moods of people compared with the placebo group after receiving 30 mg/day of saffron for six weeks evaluated based on the Hamilton

Depression Rating Scale (HAM-D). In the similar study done by Noorbala and colleagues it had been determined that saffron extracts were very effective in treating mild to moderate depression similar to fluoxetine (the antidepressant, Prozac) after 30 mg/day intake for six weeks[38].

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CONCLUSION:

Our planet earth is gifted with millions of species of the plants which are scientifically very important. One among them being *Crocus sativus* is a boon to the human society. Therefore the herbs are to be used for the treatment of many diseases and proper researches are to be carried out to prove it less harmful than the allopathic medicines. Also the main carotenoids of saffron especially crocin, safranel and picro-crocin have the potential to treat and to prevent various forms of cancer. So *Crocus sativus* should be taken as a viable option in the treatment of skin, breast, lung, liver and other forms of cancers. In this review paper we have mainly focussed some of the important applications and properties of *Crocus sativus* which are very essential and should be taken under consideration.

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Dermatoglyphic Patterns in Breast Cancer Patients

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Abstract

Aim: Breast cancer is one of the most prevalent cancers in women worldwide and there is a need to look for cost effective and non-invasive diagnostic screening methods for its early and effective diagnosis. The present study is an attempt to study the role of dermatoglyphics, which is the study of epidermal ridge configuration on palms, soles and finger tips, as a potential diagnostic screening technique for breast cancer. **Study Design**: Seventy Breast Cancer females were selected from the hospital registry of JLNCH&RC at random and "atd" angles or axial triradius angles were measured in them along with seventy healthy control after taking their informed consent and other details such as Medical and Ob/Gyn History and making a Pedigree Chart. All the cases belonged to age group of 18-66 years. **Result**: Dermatoglyphic patterns of breast cancer patients enrolled in the present study were found to be similar. The angles were found to be a little at the higher end **>42º** in case of breast cancer patients. The dermatoglyphics of cancer patients were also compared with the healthy controls. The "atd" angles of healthy controls were found to be mostly at the lower end, <42º as compared to the breast cancer patients. The mean "atd" angle of breast cancer patient was found to be **48º** and that of Healthy Control was found to be **39º** as shown in **Table 1. Conclusion**: On the basis of this report, we can conclude that dermatoglyphics has the potential to help in the identification of woman at increased risk for the development of breast cancer, and the earliest possible diagnosis of breast cancer would improve the results of breast cancer treatment. Long term studies and large sample size is required to come to a firm conclusion.

Introduction

Breast cancer is one of the most prevalent cancers in women and accounts for nearly 23% of all cancers worldwide. It is common between the ages of 35-54. Only 5-10% occurs in women with a clearly defined genetic predisposition. The presentation includes a lump in the breast or clear fluid discharge from nipple and skin changes. Factors disposing to carcinoma breast are genetic predisposition, hormonal factors, parity (nulliparous), virus (murine mammary tumor virus),

ionizing radiation, immunologic incompetence and personal demographic factors [1-2]. It has been increasing at an alarming speed and there is a need for searching for new and better diagnostic tools for its early and effective diagnosis. The current diagnostic tools include mammography, ultrasonography, and FNAC. Mammography has also resulted in a lot of false negative cases and the anxiety resulted due to this understandable. Hence, there is a need to look for newer screening methods which are easier to perform and noninvasive. In view of the increasing incidence of this deadly disease, there is a need for effective early diagnosis of this disease. In this regard, dermatoglyphics can be proved as a powerful tool to identify women with high risk of developing breast cancer. The relevance of dermatoglyphics is to the identification of people with genetic predisposition to develop certain diseases. Here lays the importance of dermatoglyphics in diseases and their practical application. If a meaningful association can be established, it may be of use in screening inexpensively populations at risk so that anticipation and early detection of symptoms can help in averting the disease or complications associated with the disease [3]. Thus, Dermatoglyphics can serve as a cost effective tool for sorting out women at risk and thus decreasing the economic burden on screening mammography in a developing country like ours.

Dermatoglyphic patterns of breast cancer patients observed in the present study are a basic data that can be useful for further studies. There is a possible genetic influence on the dermatologyphic patterns of breast cancer patients as found by the current study.

Material and Method

Selection of subjects: Seventy Breast Cancer females were selected from the hospital registry of JLNCH&RC at random. Informed consent was taken from each individual participating in the research work along with other details such as Medical History, Family History, and Ob/Gyn History. Pedigree Analyses were also done of the chosen subjects to look for any family history of Breast Cancer and other risk factors. The research work has been approved by the Institutional Human Ethical Committee (**(IEC/01/06/18.5.13)**. All the cases belonged to age group of 18-66 years. Seventy age-matched Healthy Controls were also enrolled for comparison studies.

Dermatoglyphics

The method adopted for printing palm was modified ink method by **Purvis Smith** (1969). The materials used were printers, duplicating ink from, Cardboard roller, gauze pads and sheets of paper.

The patient and controls were asked to wash their hands with soap and water to remove grease and dirt and dried. Then ink was applied over the palm and fingers with a gauze piece and smeared

thoroughly in light strokes. A sheet of paper was kept at the edge of the table. The finger ridges were printed starting from thumb to little finger in the same order. The fingertips were rolled manually to ensure the full prints of the ridges, then the palm was rolled on cardboard roller with paper taking care that the cupped regions of the palm were printed properly. Once the prints were obtained, "atd" angles were drawn with the help of protractor and angles measured.

Method of "atd" angle measurement

In an arch: The triradius is the core and hence the count is zero.

Atd, adt, dat angles: A line was drawn from axial triradius 't' to the digital triradii 'a' and 'd' and all the three angles in the triangle were measured using a protractor.

Parameters observed: "atd" angle.

Result:

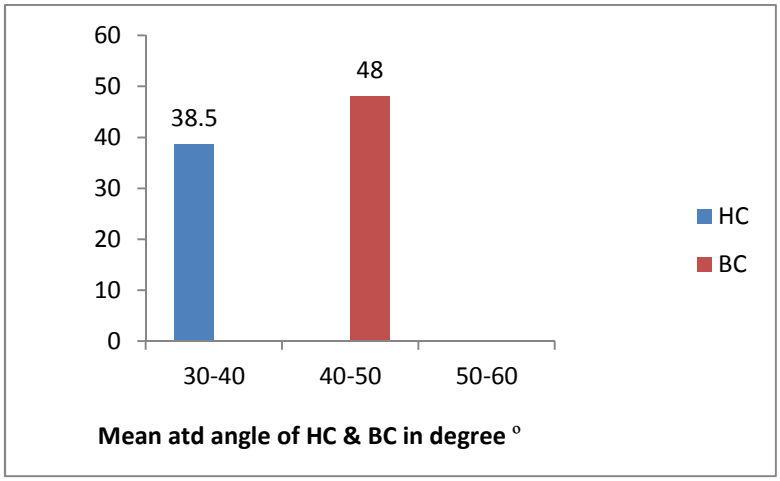
Dermatoglyphic patterns of breast cancer patients enrolled in the present study were found to be a little on the higher side. The angles were found to be a little at the higher end **>42º** in case of breast cancer patients. The dermatoglyphics of cancer patients were also compared with the healthy controls. The "atd" angles of healthy controls were found to be mostly at the lower end, <42º as compared to the breast cancer patients. The mean "atd" angle of breast cancer patient was found to be 48 º and that of Healthy Control was found to be 39 º as shown in **Table 1.**

Table 1: Mean "atd" angle of Healthy Control and Breast Cancer females

Code no	Mean "atd" angle
HC (N=70)	**39°**
BC (N=70)	**48°**

HC: Healthy Control Females, BC: Breast Cancer females

Graph 1: Mean "atd" angles of Breast Cancer and Healthy Control females.



HC: Healthy Control Females, BC: Breast Cancer females

Figures:

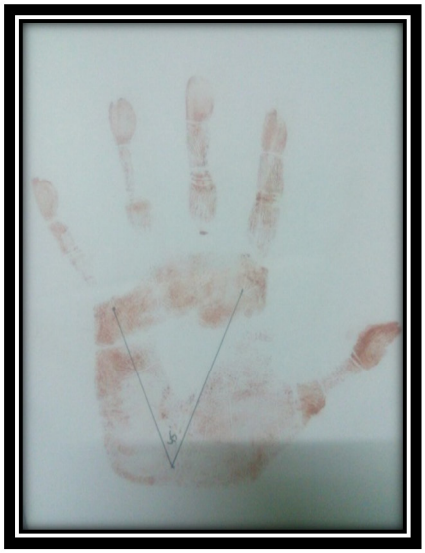


Fig 1: 'atd' angle of 35° in HC female

Fig 2: 'atd' angle of 38 ° in HC female

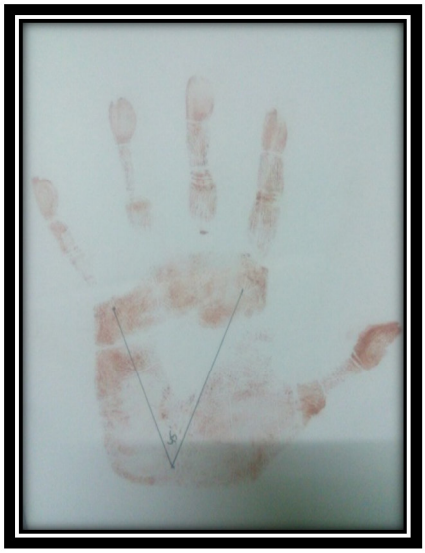


Fig 3: 'atd' angle of 45º in BC patient

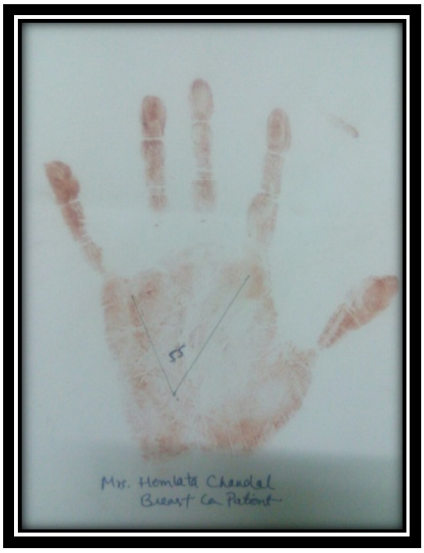


Fig 4: 'atd' angle of 55 º in BC patient

Conclusion

Dermatoglyphic is utmost importance for the clinicians to diagnose the genetic disorders especially congenital malformations and syndromes properly and dermatoglyphic tool is clinically established tool to look for congenital malformations[4-6]. Thus, the potential of this tool in the screening for other diseases cannot be ruled out and the present study has attempted to look for dermatoglyphic marker of Breast Cancer. Dermatoglyphic patterns of Breast Cancer patients observed in the present study is a basic data that can be useful for further studies. There is a possible genetic influence on the dermatoglyphic patterns of breast cancer patients. Long term studies and large sample size is required to come to a firm conclusion.

The anthropometric marker developed by this research work by using dermatoglyphic tool of "atd" angle measurement has huge potential as a diagnostic screening tool and it is very easy, cost effective and non-invasive parameter to screen high risk females of developing Breast Cancer as shown by the result. It is a much simpler method of screening and reduces the anxiety faced by females while opting for mammography and hence can aid the diagnostic procedure in huge way. Our work is supported by a similar work done by **P.E. Natekar *et al.*, 2006** and **Shivaji B. Sukre *et al.*, 2012** in which they performed different parameters of dermatoglyphics including "atd" angle on Breast Cancer patients and came to the same conclusion as ours of Breast Cancer patients having wider "atd" angle [7-8].

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Novel Tumor Markers for Breast Cancer - A Review

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Breast cancer is one of the most prevalent cancer known in women and accounts for about 23% of all cancers worldwide. It has been increasing at an alarming rate and during the last two decades has increased dramatically. Mammography and Histopathological Examinations remains the cornerstone of breast Cancer Diagnosis but it has also resulted in overdiagnosis and a number of false negative cases thus increasing the anxiety of women suspecting breast cancer. Hence, there is a need to look for newer avenues which are not only efficient but also cost effective and to establish new high-risk categories so that only the high risk group should go for screening. Other tests like genetic testing, hormonal receptor assay for ER and PR status and some other antigen studies like CA 15-3, CA-125 and CEA assay are important tools for treatment plan and prognostic assessment. A number of research works is being carried out worldwide to look for novel tumor markers for early and effective diagnosis of Breast Cancer and for screening females with high risk. Out of which most of the markers are not clinically useful yet and some are still on the research stage. In this particular review, we have tried to find out some useful biomarkers that hold promise in screening this very dreadful and unfortunately ever increasing disease in females worldwide. The literatures that were relevant to tumor markers in breast cancer was reviewed and then assessed for their potentiality in clinical use.

Keywords: Breast Cancer screening, biomarker, early diagnosis, prognosis, tumor marker

POTENTIAL NOVEL TUMOR MARKERS FOR BREAST CANCER DIAGNOSIS AND SCREENING

A. SERUM TUMOR MARKERS

A number of serum tumor markers have been described for breast cancer, such as CA 15-3, , carcinoembryonic antigen (CEA), BR 27.29 (CA27.29), tissue polypeptide specific antigen, tissue

polypeptide antigen, and HER-2. The clinically established out of these serum markers are CA 15-3 and CEA and even these two biomarkers do not have the specificity and sensitivity required for their use in early diagnosis of Breast Cancer. The less widely used serum biomarkers are CA27.29, tissue polypeptide specific antigen (TPS), tissue polypeptide antigen (TPA), and HER-2 [1-2]. The usefulness of serum markers in breast cancer, however, lies in aiding in early diagnosis in combination with other established clinical tests, in determining the prognosis of disease, in predicting treatment response or resistance to specific treatments, surveillance after primary surgery, and monitoring disease progression in patients with advanced stage disease. By far out of the above-mentioned tumor markers only CA 15-3 has the potential in predicting metastasis, and it is very useful in determining prognosis with a high level of preoperative CA 15-3 indicating poor prognosis [3]. As far as its use in early diagnosis is concerned its not that useful as it lacks both sensitivity and specificity. For example, according to American Society of Clinical Oncology (ASCO) Expert Panel, a 5 to 10 fold increase in CA 15-3 serum level above the reference limit could predict metastasis but a lack of increase does not rule out metastasis either. Similarly, increase in CA 15-3 is also sometimes found in apparently healthy individuals (~5%); in patients with liver disease; and in patients with other types of advanced adenocarcinomas [4].

CA 15-3 alongwith CEA are the most widely used markers in monitoring chemotherapy in patients with advanced breast cancer.

MOLECULAR TUMOR MARKERS

The most well-established molecular tumor markers for breast cancer are hormone receptors (ER and PR), HER-2 oncogene, Ki-67, and p53 proteins, and the genes for hereditary breast cancer. Some other potential molecular markers that are promising tools for future development in effective diagnosis and treatment plan include miRNA, caveolin, CXCR4, and FOXP3 and these markers also show lower toxicity.

The status of estrogen receptor (ER), progesterone receptor (PR), and HER-2 has been successfully used in identifying a high-risk group in Breast Cancer and for most effective treatment plan in this group of patients [5].

The most useful out of these markers are HER-2 receptor overexpression in some breast cancer and this is very useful diagnostic marker in breast cancer patients and also in monitoring treatment response in patients undergoing treatment with Herceptin. Herceptin is a humanized monoclonal

antibody directed against the extracellular domain of HER-2 and it has been successfully used in the treatment of patients with HER-2 positive advanced breast cancer [6].

KI 67 ANTIGEN

The Ki-67 antigen is a labile, nonhistone nuclear protein that is linked to the cell cycle and is expressed in mid-G1, S, G2, and M phases of proliferating cells but not in quiescent or resting cells of the G0 and early G1 phases. Ki-67 score is frequently measured by IHC methodology and is scored as the percentage of stained invasive carcinoma cells [7]. Vielh *et al*., demonstrated a strong correlation between phase S and Ki-67 and they verified that quantitative evaluation of Ki-67 can offer a precise estimation of tumor proliferation index [8].

Ki-67 has both prognostic as well as predictive value as evaluated by Luporsi ***et al.,*** (**2012**) but there is a need for better standardization of techniques and scoring methods for its better use in every day practice [9].

TUMOR PROTEIN P53

The p53 is involved in the critical pathways of cell cycle arrest, apoptosis, DNA repair, and cellular senescence, which are essential for normal cellular function and integrity. Alteration in TP53 gene can alter its response to cellular stress ultimately leading to cancer [10]. Thus study of TP52 gene mutation and expression of mutant p53 protein is a very useful molecular marker in predicting the risk of cancer as well as disease progression. In breast cancer, approximately 30% of patients show TP53 gene mutation and their expression, mutation freqency and mutation types vary according to the subyupe of breast tumor involved [11-12] and hence this marker has the potential to identify the subtype of breast cancer.

BREAST CANCER SUSCEPTIBILITY GENES (BRCA1 AND BRCA2)

The most useful and successful genetic testings in predicting familial breastcancer has been the BRCA1 and BRCA 2 genes as around 80% of the cases related to familial breast cancer are associated with these two particular genes, BRCA 1 for breast and BRCA 2 for ovarian cancer, BRCA1 and BRCA2. The BRCA genes have been classified as tumour-suppressor genes. BRCA proteins play important roles in different cellular processes, including activation and transcriptional regulation, repair of DNA damage, beyond the control of cell cycle, cellular

proliferation, and differentiation [13]. Mutation of these genes also vary in their frequency and type of mutation between variuos ethnic group and geographical regions owing to interaction between genetic and environmental characteristics.

BRCA 1 and BRCA 2 mutation testing has greatly contributed to the genetic counselling and genetic testing of families with breast cancer history by predicting the high risk of first degree females of breast cancer patients and thus in preventing the occurrence of disease in these high risk category altogether and thus this genetic testing is widely popular and useful.

Besides these two genes, according to Apostolou and Fostira [14] more susceptible genes have been discovered and these include rare germline mutations in other high penetrant genes; the most important between them include TP53 mutations, PTEN (phosphatase and tensin homolog on chromosome ten) mutations, STK11 (serine/threonine kinase 11) mutations, CHEK2 (checkpoint kinase 2), ATM (ataxia telangiectasia mutated), PALB2 (partner and localizer of BRCA2), and BRIP1 (BRCA1-interacting protein C-terminal helicase 1) to name a few.

POTENTIAL NOVEL TUMOR BIOMARKER

CYTOGENETIC TUMOR MARKER OF BREAST CANCER

A number of cytogenetic analyses have been done to look for novel and efficient tumor markers for effective and early diagnosis of breast cancer and these are continuously being updated in the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer.

According to a review of 508 cytogenetically analyzed breast cancers, the most common chromosomal aberrations involved chromosome arms 1q and 6q [15]. Cytogenetic analysis has a great potential to be used as a tumor marker for screening high risk females as most of the cancer show chromosomal damage and the specific chromosomal aberration pattern will be an indication of breast cancer.

BIOCHEMICAL TUMOR MARKER OF BREAST CANCER

A number of biochemical analysis are routinely being done clinically in monitoring breast cancer patients like complete blood profile, alkaline phosphatase, γ-glutamyltransferase, aspartate

transaminase, bilirubin, calcium, and creatinine but these have very limited value as far as detecting metastasis after treatment for operable breast cancer is concerned [16] . In this regard Plasma protein pattern analysis by electrophoretic methods can be useful to see any alteration in their pattern in Breast Cancer patients and also in high risk females and compared with the pattern of healthy females for screening purpose. This is one avenue that needs to be explored.

ANTHROPOMETRIC TUMOR MARKER OF BREAST CANCER

Dermatoglyphics is a branch of anthropometry that deals with the study of epidermal ridges and patterns in our palm and soles and it is an useful tool in diagnosing patients with congenital malformations and in fact it is considered as a window of congenital abnormalities and is a sensitive indicator of intrauterine anomalies. Currently several dermatoglyphic researches are being carried out to look for its usefulness in correlating dermatoglyhphic tool with cancer prediction and their prognostic ability from the hand features. As far as its role in breast cancer is concerned certain studies have proved its potential as an important non-invasive and cost effective diagnostic tool [17-18]. In humans, the development of the mammary buds begins as well as dermal ridges of the volar pads develop during the 6th week of gestation and here lies the correlation between breast development and epidermal palm patterns as the genetic message contained in the genome - normal or abnormal - is deciphered during this period and is also reflected by dermatoglyphics. Hence these features may serve as markers of altered early development in the breast.

FUTURE DIRECTIONS

In light of the knowledge of breast cancer and the alarming rate at which it is increasing, it is very essential to look for new effective tumor markers which are not only specific and sensitive but also cost effective and preferably non-invasive for both screening purposes and for monitoring treatment in already diagnosed breast cancer patients. It will definitely benefit the medical world in the form of novel methods of diagnostic screening for breast cancer and a new risk category to focus on so that more effective method of treatment could be tailored made for specific patients. These high risk categories can then be counseled to go for further mammographic screening and other methods of breast cancer diagnosis. Mammography is the cornerstone for diagnostic screening for breast cancer so far but this has also resulted in over-diagnosis and a lot of false negative cases. The establishment of a high-risk category and cost effective and preferably non-invasive method of diagnostic screening will definitely aid in the overall diagnosis and screening procedures for breast cancer.

CONCLUSION

It can be concluded from the present review that the main disadvantage of current tumor markers include the lack of specificity and sensitivity in case of serum tumor markers and the high cost involved in the genetic testings and thus there is a need to look for cost effective and more sensitive and specific markers like dermatoglyphic, cytogenetic and plasma protein pattern study.

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GENETIC, ANTHROPOMETRICAL, AND ENVIRONMENTAL CAUSES OF HUMAN PRIMARY AND SECONDARY INFERTILITY IN MALES: A REVIEW

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Abstract

Advanced nations are facing one of the most serious problems in the form of Infertility, whether it is Primary or Secondary infertility in males as well as in females. Generally, in 50% of cases, the male partner is responsible for the cause of infertility. In the modern scientific world, Researchers and Medical practitioners developed various treatment techniques for male Infertility and the results are producing steadily. However, still, there is no uniform pattern of treatment for patients facing the problem of obstructive azoospermia, for those having zero percent of mature sperms in the testes. Even though on the basis of various evidence, it is suggested that genetic predisposition is also the cause of this serious issue of male infertility, but in the vast majority of patients, the cause has not been elucidated. In this review article, we will discuss some factors which are generally ignored but are likely to be involved in male infertility like Less anogenital Distance (<2 inches),

Environmental factors, Lifestyle, and the genes that have been shown to be involved in humans male infertility including the recent findings of our research work.

Keyword Infertility, Anogenital Distance (AGD), Azoospermia, Semen,

1. INTRODUCTION

Declining birth rate is one of the most serious problems faced by the developing countries, although it is not confirmed that it is because of the rise in the cases of infertile couples .While both environmental (i.e. global warming and pollution) as well as social (i.e. increase in the marriage age, Lifestyles, and Stress) factors are behind the rise in the number of infertile patients, approximately a half case of infertility are contributed by male partner only. In the modern world, various techniques like, *in vitro* fertilization (IVF), intracytoplasmic injection (ICSI) and so-called TESE ICSI (testicular sperm extraction) for the harvesting of sperm from the testes developed for infertile males are developed to counter infertility issue. Although by these methods are producing results gradually, no effective technique has been proven for the patients having non-obstructive azoospermia, in which the mature sperm is absent in the testes. It is suggested by some evidence that genetic predisposition is there in the patients with azoospermia, but in the majority of the cases, this has not been elucidated [1].

Conversely, some recent studies linked many genes to spermatogenesis, the mechanisms is yet to be currently clarified. These findings on animals have yet to be confirmed in human trials for further animal findings. This is because a retrograde genetic approach is required to identify the affected Genes in humans, and because of the knockout phenotype of the mouse not always reproduced authentically in humans. This paper will discuss male infertility caused by the involvement of various environmental factors and the genes responsible for it including the current research findings.

2. ENVIRONMENTAL FACTORS AND MALE INFERTILITY

The debate is very long on male reproductive ability whether it is because of the environmental factors present in the area of residence or in the workplaces. A sensational report was given by the Whorton *et al.,* published in 1977 which revealed that out 25 male workers 14 were diagnosed with Azoospermic or oligospermia who used to work in producing insecticide dibromo-3-chloropropane (DBCP). [2]

Carlsen *et al.*, in 1992 reported that there is a significantly marked decrease in the sperm count from last 50 years [3]. Brake and Krause in the same year reported that during the period since 1970 the Scottish Male population faced the problem of decreased sperm

count of approximately 25% compared with the period prior to 1959 annual mean rate of 2.1% [4]

Clinicians and researchers have asserted that in advanced countries the progress in the societal areas decline in the natural has likely to be the cause of human male infertility. Reported risk factors include high temperatures in working places [5], noise associated by Industries [6], radiation exposure [7] electromagnetic waves [8], and a mixture of chemical substances [9]. Several studies have compared subjects with male infertility (azoospermia or oligospermia) to healthy subjects (normal sperm count). On the contrary, many scientific studies indicate the absence of a correlation between male infertility and environmental factors [10, 11]. For this discrepancy, one reason is the insufficient sample size to determine the significant difference statistically. Less the 100 subjects were included in these studies in nearly all cases.

One more reason is that almost all are survey studies using questionnaires, with no objective tests like measuring concentrations of blood. Consequently selecting a subject as a healthy individual is very problematic because the level of exposure is very confusing. Recently many studies on the basis of semen finding include subjects as a healthy individual.

3. MOBILE PHONES AND INFERTILITY

In 2011 Ashok Agarwal *et al.,* [12] concluded in their scientific research work that people are using cell phones vigorously; it has become the need of the hour. They are becoming a vital part of the day to day life. However, the health risk associated with the usage is often overlooked. Recently evidence from the several studies supports a growing claim that cell phone usage may have a harmful effect on sperm parameters leading to decreased male fertility.

4. ANOGENITAL DISTANCE AND MALE INFERTILITY

Eisenberg ML *et al.,* 2012 [13] in their research work revealed that a marker for genital development, Anogenital distance (AGD) has been examined in both humans and animals as a marker for genital development. They also found that AGD shows the suggestive relationships between the functions of genital tract development, sperm count, and testosterone. On the other hand, the cause of diverse AGDs between men remains unsure. Some rodent studies suggest that *in-utero* androgen signaling throughout the masculinization

programming window determines adult AGD with minimum influence from postnatal exposures to androgen This correlation implies that prenatal androgen and estrogen exposures and AGD may reflect fetal determinants in the adult. The AGD less than 2 inches in males may lead towards infertility or low sperm count and in females, it leads to low breast growth.

5. TESTICULAR DYSGENESIS SYNDROME (TDS)

It was suggested by Skakkebaek *et al*., 2001 [14] that Cryptorchidism, reduced semen quality, testicular cancer and Hypospadias are symptoms of a common underlying cause known as Testicular dysgenesis syndrome (TDS), which already arises in the foetal life due to Leydig cell and Sertoli dysfunction. In the same year **Toppari *et al.,*** [15] published in their research paper and found that features of (TDS) testicular dysgenesis have been found in men with Hypospadias, poor semen quality, contralateral testis in patient with unilateral testicular cancer or Cryptorchidism.The aetiology of TDS in the clinical components remains unknown.

6. GENETIC CAUSES

Chromosomal Anomalies

Approximately 5% of infertility male infertility accounts due to the chromosomal abnormalities, and the incidence increases to 15% in azoospermic male population [16]. Y chromosome abnormality, like microdeletios, is the major cause of azoospermia (ejaculate without sperm) and oligospermic sever cases (>20 million sperms /ml (WHO 2010) Therefore, errors in chromosomal level are a relevant area of research to determine the factors responsible for male infertility and the role of genetics.

The quick references are shown in the **table 1** provided by O'Brien and O'Flynn in 2010 on chromosomal abnormalities. Incorrect chromosome number or Aneuploidy, in infertile men is the most common chromosomal abnormalities error in infertile males [17]**.** Human male with non-obstructive azoospermia have mainly high incidence of aneuploidy [18]**,** especially in their sex chromosomes [19]**.**

Even though the genetic material is of altered amount in the aneuploid sperms, occasionally oocyte gets the chance to fertilize successfully and the incorrect number of chromosomes is passed to their offsprings [20]**.**

Common chromosomal anomalies linked with male infertility, Phenotypes and its Prevalence

Table 1.

Genetic abnormality	Phenotype	Prevalence, %**age**
Y-Chromosome microdeletions	Azoospermia to oligozoospermia	10–15 (azoospermic); 5–10 (oligozoospermic)
Chromosomal abnormalities	Azoospermia to Normozoospermia	5 (total infertile population); 15 (azoospermic)
Robertsonian translocation	Azoospermia to Normozoospermia	0.8 (total infertile population); 1.6 (oligozoospermic); 0.09 (azoospermic)
Klinefelter syndrome	Azoospermia to severe oligozoospermia	5 (severe oligozoospermia); 10 (azoospermic)
AZFc deletion	Severe oligozoospermia to non-obstructive Azoospermia	6–12
AZFb deletion	Azoospermia, spermatogenic arrest	0.5–1.0 (2)
Partial AZF-c deletions	From azoospermia to Normozoospermia	3–5 (2)
AZFa deletion	Azoospermia, Sertoli cell–only syndrome	0.5–1.0 (2)

O'Brien and O'Flynn. Genetic causes of MF infertility. Fertil Steril 2010.

Semen development during puberty

Bablok, L and Janczewski, Z. 1985 in their study revealed that after the onset of puberty twelve months later first ejaculation in males often occurs [21].

Time after first ejaculation (months)	Liquefaction time	Average Semen volume (mL)	Average sperm Concentration in an ejaculate

			(million/mL)
0 months	No liquification time	0.5 ml	0 million/ ml
06 months	No liquification time	1.00 ml	20 million/ ml
12 months	No/Yes	2.5 ml	50 million/ ml
18 months	Yes	3.00 ml	70 million/ ml
24 months	Yes	3.5 ml	300 million/ ml

Data obtained from http://en.wikipedia.org/wiki/Ejaculation

FUTURE PROSPECTUS

Fertility is the boon in the society of mammalian because motherhood has been Flourished, welcomed and given special position in the family as well in the society. Infertility is not a curse rather it is a medico-social event which should be handled in such a way so that the sentiments and ethics should not be hurt of an individual. Tremendous investigations and scientific work have been stepped in to save the couple who were infertile. But still, there is no pattern of investigation as an advice given by a consultant to the infertile couple, which fully supports the real cause of such issues. It was observed that patients having undescended testis, AGD distance less than 2 inches and having abnormal sperm morphology, less sperm count, motility, viscosity, azoospermia, and oligospermia are more susceptible to infertility. Such intensive study not only helps the consultant for proper medication but also helps an individual to know the problem and remedy if any i.e. (IUI/IVF/ICS).

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ISOLATION OF MICROFLORA ON THE INGUINAL SURFACE AND THE ROLE OF BACTERIAL INFECTION IN THE SPERM QUALITY OF INFERTILE MALES OF INDIAN POPULATION

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ABSTRACT

The present study of Microflora in the inguinal region of infertile males gives an insight about the prevalence of a number of microbes like *S. aureus, Streptococcus, E. coli, Klebsiella Spp.* and *Candida albicans* in this region and these could potentially affect a male infertility damaging sperm resulting in their poor quality. Swab sample was collected from the inguinal surface of different fertile and infertile subjects. To investigate the microorganism found in the lower genital tract like Thigh (Th) Testis (Ts) Penis (P), base of the penis (Bp), and Other pubic area (O). The sample was collected within duration of Two years from 2014 to June 2016 from 200 different subjects in Jawahar Lal Nehru Cancer Hospital and Research Centre, Idgah Hills, Bhopal and analyzed. Four different types of microbes were found in all the sample cultured *Staphylococcus aureus* (23%), *Escherichia coli* (5.5%), *Streptococcus* (13.5%), *Candida Albicans* (6.5%) , *Klebsiella Spp.* (11.5%) and no growth was found in 40%. Most of the microorganisms were found in subjects having azoospermia, oligospermia, Asthenozoospermia, Necrozoospermia, and in infertile ones, which reveals that microbes are mainly prevalent in these areas and may have a potential role in the poor quality of sperm or low sperm quality.

KEY WORDS

Male infertility, Microorganism, Bacterial infection, *Streptococcus,* Azoospermia.

INTRODUCTION

Genitourinary tract infection affects about 15%cases of the male infertility[1]. The quantitative and qualitative sperm alterations are due to the severe and chronic infection and inflammation in the

male reproductive system which cooperate the spermatogenetic processes and sperm cell function[2,3,4]. It is always controversial that bacterial infection plays a main role in male infertility it is due to the lack of important analysis tools to study specimens of seminal fluids as a result of which these infectious processes lead to the decline of spermatogenesis and obstruction of the seminal tract[5]. Urologists are usually the specialists who are initially responsible for assessing the male partner if male infertility is suspected. However, infertility can be a multifactorial condition. The male infertility guideline's panel consists of endocrinologists and urologists with experience in the diagnosis and treatment of male infertility and special training in Andrology. In most of the couples, reproduction (conceiving a baby) is a natural experience. However, for some couples it is very not easy to conceive. Male infertility is diagnosed when reproductive problems have been found in male only after testing both partners[6]**.** Infertility is the inability to conceive naturally after at least one year of unprotected intercourse[7]**.** It is estimated that as many as 15% of couples worldwide, whom seeks children suffer from infertility[8].

In primary infertility, Couples are unable to conceive once after the regular unprotected intercourse for one year while, on the other hand, secondary infertility is difficult to carry a pregnancy second time or had a miscarriage. Male causes of infertility are found in about 50% of infertile couples[9]**.** Male infertility has multifactorial etiologies. This could be physical abnormalities, genetic, drugs, injuries, genital tract infection, toxins, radiation, or unexplained. In male infertility Erectile dysfunction (ED) is also a contributory factor.

MATERIAL AND METHOD

Over a period of two years (2014-2016) swab samples of two hundred different subjects (Normal and Infertile) was investigated from the inguinal surface. The research work was conducted in the Department of Research, JNCH&RC Bhopal. Institutional Ethical Committee, approve the research work (IEC 546/16.03.16). All infertile patients who were referred from different hospitals for treatment to the Department of Research, JNCH & RC,

were taken as subjects. All the cases belonged to age group of 18-45 years of age, and the healthy volunteers were from the local area or attendants who accompanied the subjects. Sixty five (65%) registered subjects were having the history of Infertility, and 35% subjects were healthy with normal semen analysis.

Collection of swab sample for bacterial culture

The samples were collected from the inguinal surface of infertile males with the help of swab stick. Subjects were asked to fill the informed consent and all other questionnaires mandate for the research work and pedigree analysis was also done to know the family medical history of at least three generation[10]. Before the sample collection, the patient was asked to remove the pubic hairs and wash thoroughly the inguinal region with clean water only and stay away from any antiseptic that might potentially infect the specimen. **Culture Method**

After the sample collection in the sterilized condition, the sample was cultured using blood agar, Chocolate agar and MacConkey agar in autoclaved petri plates (Borosil) and incubated at 37^{o}C for 24-48 hours. The cultures were examined for growth, isolation and identification of bacterial isolates were carried out in accordance with Bergy's Manual textbook of microbiology (Anathanarayan and Panikers)[11]. The infective microorganisms were identified by preparing slide smear followed by Gram staining[12], biochemical testing and cultivation on media of the cultivable microorganisms. Significant cultures were tested with antibiotics to check for their sensitivity pattern using the antibiotic disc methods. The antibiotic used, and their zone of inhibitions is given in **Table 3 & 4.** The microorganism was identified by using gram staining technique biochemical reaction, i.e. Coagulase, Catalase and Indole tests and Antibiotic sensitivity range[13,14].

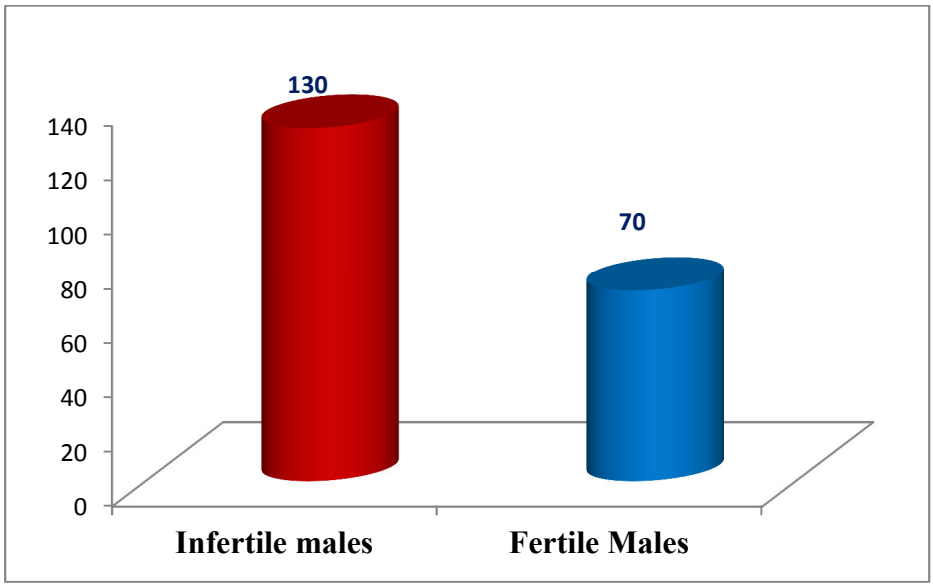
Semen Sample Analysis

Semen sample was collected in a sterile container by masturbation method in a sample collection room of the hospital by keeping abstinence of 3-5 days. Before the sample collection, the subject was asked to pass the urine and wash hands and penis with soap and rinse with clean water. Semen analysis was done for semen volume, sperm concentration, motility and morphology for both the groups as per the WHO guidelines of 2010[15].

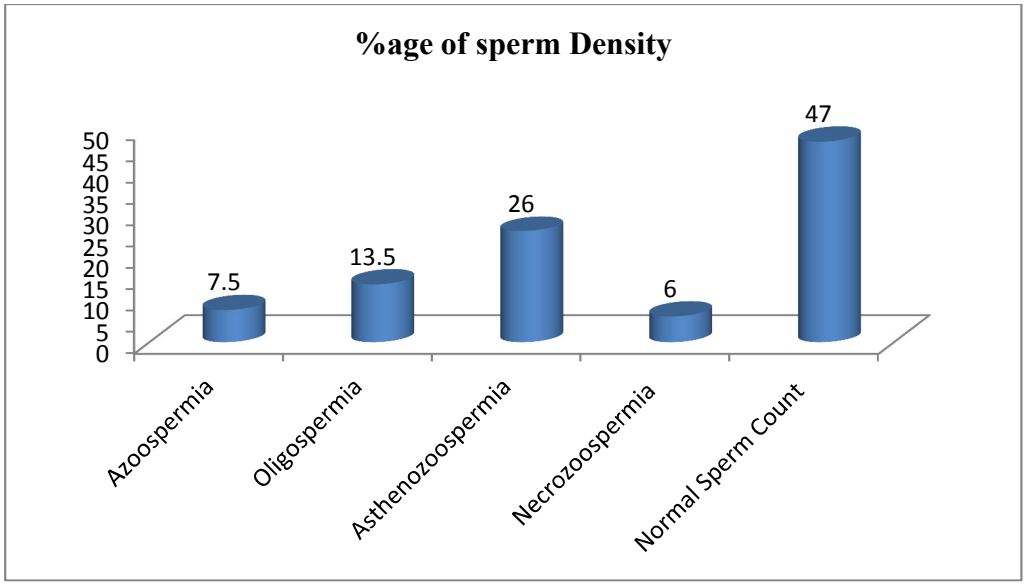
RESULT AND OBSERVATIONS

Out of 200 different swab culture samples collected from the men with the help of a swab sticks from the inguinal surface, i.e., Penis, testis, thighs, Glans of the penis and other areas of the same region 65% subjects were reported to be infertile with abnormal semen profile, sperm morphology and sperm density. Data obtained from this experimental work in **Graph 2** revealed that 7.5% cases were Azoospermic, 13.5% Oligospermia, 26% Asthenozoospermia, 6% Necrozzoospermia and 47 % were of normal sperm count.

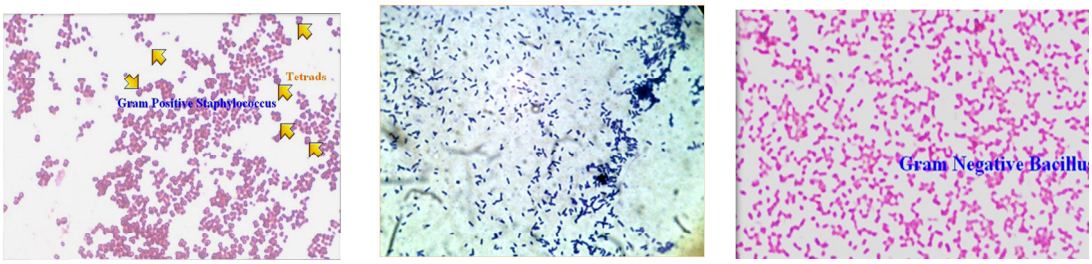
Based on the antibiotic tests and biochemical reactions it was revealed that 60% subjects were positive for pathogenic bacteria as shown in **Graph 3**. Among them *Staphylococcus aureus* was (23%), *Escherichia coli* (5.5%), *Streptococcus* (13.5%), *Candida Albicans* (6.5%) , *Klebsiella Spp.* (11.5%)**.**



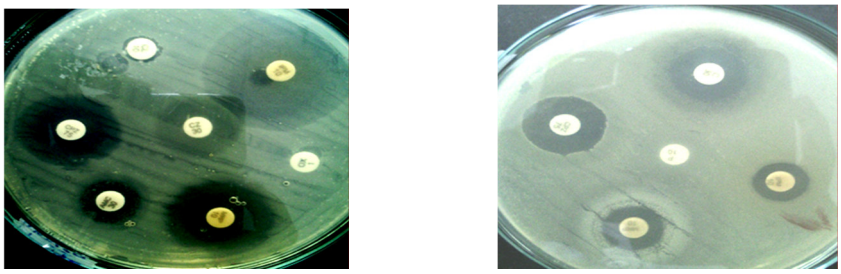
Graph 1: Showing Number of Fertile and Infertile males



Graph 2: Showing type and %age of sperm density.



Gram +ve *Staphylococcus aureus* Gram +ve *Streptococcus* Gram-negative *E. coli*

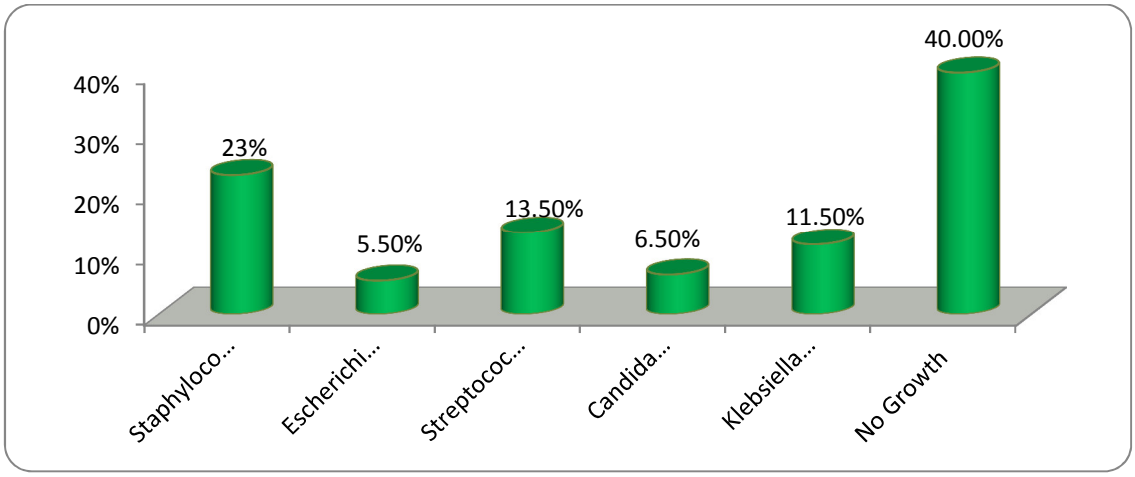


Antibiotic sensitivity test for Gram- Positive and Gram-negative bacteria

Figure 1: Showing various type of bacteria present on the inguinal surface of infertile males

GROUP	Microflora	Frequency	Percentage
A	*Staphylococcus aureus*	46	23%
B	*Escherichia coli*	11	5.5 %
C	*Streptococcus*	27	13.5%
D	*Candida albicans*	13	6.5%
E	*Klebsiella Spp.*	23	11.5
F	No Growth	80	40

Table 1: Shoeing Frequency of bacterial isolates



Graph 3: Showing the type and %age of Bacterial infection

Group	Microflora	Biochemical Test			
		Coagulase	Catalase	Oxidase	Indole
A	*Staphylococcus aureus*	Positive	Positive	Negative	Negative
B	*Escherichia coli*	Negative	Negative	Negative	Positive
C	*Streptococcus*	Negative	Negative	Negative	Negative
D	*Candida albicans*	Negative	Negative	Negative	Negative

Table 2:Biochemical Test for identification/ Isolation of Bacteria.

S.No.	Antibiotic	Zone of inhibition (cm)	Remark
1	Norfloxacin	2.3 cm	Highly Sensitive
2	Amoxyclav	2 cm	Highly Sensitive
3	Imipenem	3.5 1cm	Highly Sensitive
4	Meropenem	3.2 cm	Highly Sensitive
5	Ofloxacin	1.6 cm	Highly Sensitive
6	Oxacillin	1.9 cm	Highly Sensitive

Table 3: Zone of inhibition of Gram-Negative antibiotics

S.No.	Antibiotic	Zone of inhibition(cm)	Remark
1	Tetracycline	2.4 cm	Highly Sensitive
2	Imipenem	3.5 cm	Highly Sensitive
3	Meropenem	3.0 cm	Highly Sensitive
4	Penicillin G	1.2	Sensitive
5	Ampicillin	2.6 cm	Highly Sensitive
6	Chloramphenicol	2.2	Highly Sensitive

Table 4: Zone of inhibition of Gram-Positive antibiotics

DISCUSSION AND CONCLUSION

The impact of semen contamination in males and genital tract infection has examined by many studies, but it is still controversial the detrimental bacteria affect on the sperm quality[22].

Sanocka–Maciejewska *et al*., [23] in his study revealed that frequently have reported that the bacteria found in the genitourinary tracts of men have no adverse effect on

normozoospermic males and their semen quality; however, in patients facing the problem of infertility with pathological semen parameters usually bacteria diminished the antioxidant capacity of sperm.

The present study of Microflora in the inguinal region of infertile males gives an insight about the prevalence of a number of microbes like *S. aureus, Streptococcus, E. coli,* and *Candida albicans* in this region, and these could potentially affect a male's fertility damaging sperm resulting in their poor quality.

It is important for all healthcare professionals to have a clear understanding of the many causes, risk factors, treatments and physiological impacts of infertility in order to provide the best possible care to their patients promoting positive behavior as part of preventive care and timely diagnosis will help to ensure that patient's quality of life is maintained, and that stress during this difficult time is minimized. In this regard, the present study will help in understanding the microbial population prevalence in the inguinal region of infertile males that should be checked in order to minimize their hazardous effect on fertility of these males.

The non-specific seminal tract infection can be an important cause of male infertility, and these infections may affect fertility in several ways by damaging sperm, hampering their motility, by producing an inflammatory structure in the urinary tract or changing the chemical composition of the seminal fluid. Infection of seminal fluid could also be the cause of the chronicity,of urinary tract infection by acting as the reservoir of infection, (**Mogra, N. *et al.,* 1981)[16].** Thus, the present study aimed to look for microbial infections in inguinal surfaces like Thigh (Th) Testis (Ts) Penis (P), base of the penis (Bp), and Other (O) areas of infertile males who could be the cause of their infertility, and it was found that *Staphylococcus aureus, Streptococcus, E. coli, Candida albicans* were mainly prevalent in these areas and may have a potential role in the poor quality of sperm or low sperm quality. Similar study has been done by **Sanovka Macleiewska *et al.,* 2005[17]**, in which the negative

influence of those microbes towards sperm reproductive potential has been revealed in cases of infection with *Staphylococcus aureus and E. coli.* In another study carried out by **Auroux *et al.,* 1991**[18] indicated that it was probable that the presence of *E. coli* in semen decreases sperm motility, but that this depends on sperm bacteria, /semen ratio/ml. Our study is also supported by a research carried out by **Huwe *et al.,* 1998**[19] that revealed that a variety of pathogenic microbes, including *Candida albicans* exert a significant inhibitory effect on spermatozoa motility. The study done by **Abbas Al-Janabi *et al.*,2013**[24] also revealed that the presence of the pathogenic bacteria of different species in the seminal fluid culture of the infertile subjects effect the sperm quality. Hence, it can be concluded that microbial infection in the inguinal region has a major role to play in causing infertility, and aggressive measures should be taken to prevent infection.

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DECLARATION OF INTEREST

The authors declare that there is no conflict of interest regarding this paper submission.

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Bryophytes: Indicators of Environment Pollution

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ABSTRACT

Environmental pollution is increasing day by day, posing a exceedingly serious problem for the flora and fauna. Bryophyte proves to be a potential bio-indicator of environment pollution. The habitat diversity, totipotency, structural simplicity, rapid rate of duplication and high metal accumulation capacity make bryophytes an ideal flora for pollution studies. The decline and lack of bryophyte populations especially epiphytes is a phenomenon primarily induced by environmental pollution caused by gaseous and particulate pollutants. Bryophytes are reliable indicator of environment pollution as they are easy to grip and show a huge range of specific sensitivity and visible symptoms to pollutants greatly exceeding that of higher plants.

Keywords: Bryophyte, Bio-indicator, Environment pollution, Pollutants.

INTRODUCTION

Bryophytes grow up in a variety of habitats particularly in moist places on rock, soil, trunks and branches of trees and fallen log. They take nutrients directly from substances dissolved in ambient humidity. Bryophytes are used as reliable indicators of air pollution (LeBlanc,F.and Rao,D.N.1975). They either individually or mutually with lichens can be valuable organisms in developing an index of atmospheric purity (IAP) which is based on the number, frequency and resistance factor of genus. This index can provide a fair picture of the wide range effects of pollution in a given area (Rao, 1982). There are two categories of bryophytes in response to

pollution which are very susceptible to pollution and show observable symptoms of injury still in the presence of minute quantities of pollutants. This serve as good indicators of pollution. These plants trap and prevent recycling of such pollutants in the ecosystem for different periods of time. Analysis of such plants gives a fair plan about the degree of metal pollution. Bryophytes have been disappearing from urban industrial environments because of their sensitivity to polluted air.

METHODOLOGY

1. Survey method:

Periodic surveys are made on the inhabitant bryophytes in different sites. The number, frequency and plenty of inhabitant species and dominance of the growth form can be compared with the past records, reports and periodic herbarium collection. Vanishing of already reported species (sensitive) and appearance of new species (tolerant) indicate the stress conditions in the sites.

2. Transplantation method:

Transplanted species show the adapted pattern of development of shoots and branching and deposition of wax on the plant surface. The common symptoms are plasmolysis and chlorophyll removal in the leaf cells and finally the plants drop the capacity to regenerate. Transplantation can be done by following three ways.

(a).Transplantation in the soil Bryophytes grown in tiny plots, prepared in the ground on selected sites of pollution and some plots of nonpolluted site are established as control.

(c).Bryometer it is an impoverished box in which bryophytes are grown in humid chamber with their original substrata. The box has transparent sides made with lean glass with hole so that air and light can pass through the plants within the box. The boxes are kept in different locations of the polluted area. The periodic observations on the development and survival rate of the bryophytes specify the trend of pollution in the site.

3. Ecophysiological method:

In this method the exposure of pollutants can be given to the survive plants in the field by fumigation or plants can be cultured in the medium having different concentrations of pollutant and heavy metals. Observation on the survival rate, injury, chloroplast degradation, or other kind of unusual growth of the protonema and the mature plants indicate the

toxicity of pollutants. This method is useful in determining toxicity level of pollutants and the tolerance levels of different species.

PLANT USED AS POLLUTION INDICATORS:

Though all types of sensitive species can be used in monitoring pollution, most useful and commonly used plants include lichens, mosses, algae, aquatic ferns etc. Mosses, lichens, ferns, algae and aquatic plants are generally more valuable in pollution monitoring because their range of pollutant specificity is usually much higher than that of higher vascular plants. Examples of some common types of plants valuable as pollution monitors are given below.

In freshwater bodies: spread of Sphagnum moss indicates increased water acidity. Specific changes in the aquatic flora can indicate the pH of the water quite correctly.

In terrestrial areas: Decrease in the populations of mosses (Sphagnum, Bryum) generally indicates air pollution by SO_2, NO_2, fluorides and HCl. Absence of most bryophytes, mainly Sphagnum indicates atmospheric SO_2 pollution of 0.17 ppm or more. Poikilohydrous mosses are useful as pollution indicators.

Pollutants

pollutant may be gaseous such as carbon monoxide (CO), fluorides, hydrocarbons (HC), hydrogen sulphide (H_2S), nitrogen oxides (NO), Ozone (O_3), sulphur dioxide (SO_2), aldehydes, lead and automobile exhaust fumes These pollutant either in a gaseous state mixed with air or in a liquid state affected by dew, rain, or snow, will be noxious to bryophytes attached to the bark.

EFFECT OF POLLUTION ON BRYOPHYTES

Bryophytes have been vanishing from metropolitan industrial environments because of their sensitivity to polluted air. Species diversity in a polluted area varies not only with the distance from the cause of pollution but also with the type of substrate. Air pollution inhibits gametangial formation and sexual reproduction in bryophytes. They also reduce photosynthesis by degrading chlorophyll and growth of plants and finally cause their death. Metals and metalloid are also inhibit the growth activity. When the metal enters the cell, it inhibits the photosynthetic activity. Enzymes are poisoned when a heavy metal gains to the cell interiors. Mercury is particularly toxic low concentration greatly inhibited photosynthesis, temporarily improved respiration, reduced chlorophyll levels and caused loss of intracellular $K^+$from Rhtidiadelphus squarrosus(brown and whitehead 1986). when the pollution level goes down, the percentage frequency of species goes up, which subsequently increases the fertility percentage. These situations vary from species to species depending on the prevailing climatic conditions in the area.

BIO-INDICATORS OF POLLUTION.

Bioindicators refers to all organisms that explain the quality of the environment on the basis of changes in morphology; physiology etc. but biomonitors provide both qualitative and quantitative information. The use of organisms as monitors of the environment is known as biomonitoring and the organisms are known as biomonitors .The accumulated pollutants are simply measured by the analysis of organisms that give the information about the level of pollutant deposition. Mosses and lichens are considered as the most appropriate plant material to study the atmospheric deposition of heavy metals. Bryophytes indicate the existence of element and their concentration gradient in the respective substrata. The unique qualities of bryophytes to collect the elements are due to their wide distribution, capability to grow on variety of habitats, large surface area, and lack of cuticle and stomata and evergreen and ectohydric nature of plants.

CONCLUSION

The accumulation and retention of pollutants by bryophytes has helped in the interpretation of heavy metal emission pattern. There is a great need to extend observations on mineral location and effect to a much wider range of species. More species should be recognized to inhibit the chemical envirnment. The role of morphological features in trapping particulate material is an unstudied but vital research field.

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