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BIOPROSPECTING PLANT CELL, TISSUE AND ORGAN CULTURES FOR RESOURCING BIOACTIVE MOLECULES USED AS DRUGS

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ABSTRACT
Pharmaceutically Important bioactive secondary metabolites include alkaloids, glycosides, flavonoids, volatile oils, tannins, resins etc. Most of these secondary metabolites used as bioactive molecules are isolated from wild or cultivated plants because their chemical synthesis is either extremely difficult or economically infeasible. Resourcing these molecules by Biotechnological means utilizing plant cell tissue and organ cultures is an attractive alternative. Some of the recent developments and studies carried out for the production of some of the bioactive secondary metabolites viz: withanolides, bacosides, glychyrhizin, , amarogentin, and terpenoids from tissue cultures of respective plant species showing potential to utilize tissue cultures as alternative potential resource for these molecules are presented. Further, for commercial realization of production of these compounds largely depend upon large scale production using bioreactor cultivation. Bioreactor culture is highly effective means of high-throughput strategy for quality biomass production and production of value added bioactive compounds. A number of bioreactor configurations have been examined for the growth of shoot culture using liquid medium in several plant species. Organ cultures are generally less sensitive to shear stress and more stable in metabolite yields due to their genetic stability. Some case studies where shoot biomass in Growtek, Buchner-flask and Air lift bioreactor systems have been applied provided a useful option to scale-up quality multiple shoots production in case of Bacopamonnieri Swertia chirayita and Chlorophytum borivillium to get consistent high quality biomass for production purpose is discussed. The successes with these plant species to grow these in bioreactor extend practical approach to apply bioreactor systems to other important plants of medicinal value as alternative for resourcing useful bioactive molecules.

1. INTRODUCTION
The resource values of a plant species are measured either in term of the plant itself that provides the product or the derived products that serves as a model for a modification, imitation or otherwise. Discovery is often achieved by considering where the desired product might have evolved naturally. Habitats or a group of species are then identified and explored. Further, combinatorial chemistry and rational drug design are modern approaches for drug discovery. While these have been developed independently of natural products, current thought is that natural products are likely to provide the best lead-molecules in the future

Prospecting Phytodiversity
In recent years, the increasing demand for herbal medicines is being fueled by a growing consumer interest in natural products. Based on the knowledge that many important drugs, such as aspirin were derived from natural products , the industries have at various times invested heavily in the exploration of wild plants in search of commercially profitable bioactive molecules. Some common examples include the discovery of anti-malarial drug quinine from Cinchona sp. alkaloid Diosgenin from Dioscoreadeltoidea used as source for the partial synthesis of cortisone and steroid hormones, hypertensive alkaloid Reserpine from Rauvolfiaserpentina and the analgesic alkaloid aspirin from Filipendulaulmaria, anti-asthmatic alkaloid ephedrine from Ephedra sinica and anti-cancer alkaloid Podophyllotoxin from Podophyllumhexandrum, to mention a few. In addition, a number of new, small molecules, no synthetic chemical entities developed for cancer research are derived from natural products. In ant hypersensitive drug research, 65% of drugs currently synthesized can be traced to natural structures. This emphasizes the important role of many natural products as blueprints rather than the actual end points. Today, number of plant derived pure molecules are used as drugs.

Higher plants are rich source of bioactive constituents or phyto-pharmaceuticals used in pharmaceutical industry. Some of the plant derived natural products include drugs such as morphine, codeine, cocaine, quinine etc; anti-cancer Catharanthus alkaloids, belladonna alkaloids, colchicines, phytostigminine, pilocarpine, reserpine and steroids like diosgenin, digoxin and digitoxin. Many of these pharmaceuticals are still in use today and often no useful synthetic substitutes have been found that possess the same efficacy and pharmacological specificity (1-3). Currently onefourth of all prescribed pharmaceuticals in industrialized countries contain compounds that are directly or indirectly, *via* semi-synthesis, derived from plants. Furthermore, 11% of the 252 drugs considered as basic and essential by WHO are exclusively derived from flowering plants (4). Plant-derived drugs in western countries also represent a huge market value. Prescription drugs containing phytochemicals were valued at more than US$30 billion in 2002 in the USA alone (5). Many plants containing

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high-value compounds are difficult to cultivate or are becoming endangered because of overharvesting (4). Furthermore, the chemical synthesis of plant derived compounds is often not economically feasible because of their highly complex structures and the specific stereochemical requirements of the compounds. The biotechnological production of valuable secondary metabolites in plant cell or organ cultures is an attractive alternative to the extraction of whole plant material. However, the use of plant cell or organ cultures has had only limited commercial success. This is explained by the empirical nature of selecting high-yielding, stable cultures and the lack of understanding of how secondary metabolites are synthesized or how their synthesis is regulated (6, 7). Many biotechnological strategies have been experimented for enhanced production of secondary metabolites from medicinal plants. Some of these include screening of high yielding cell line, media modification, precursor feeding, elicitation, large scale cultivation in bioreactor system, hairy root culture, plant cell immobilization, biotransformation and others (8-10)

Some of the recent developments such as Metabolic engineering of whole plants and plant cell cultures as an effective tool to both increase terpenoid yield and alter terpenoid distribution for desired properties such as enhanced flavor, fragrance or color. definingterpenoid metabolic pathways, particularly in secondary metabolism, enhanced knowledge concerning regulation of accumulation, and application of emerging plant systems biology approaches, have enabled metabolic engineering of secondary metabolites production.(11)

The increased use of genetic engineering tools and an emerging picture of the structure and regulation of pathways for secondary metabolism hasprovided the basis for the production of commercially acceptable levels of product.In view of commercial importance of the secondary metabolites has in recent years resulted in a great interest, in secondary metabolism, and particularly in the possibility to alter the production of bioactive plant metabolites by means of cell culture technology. The advantage of cell culture technology is that it may provide continuous, reliable source of plant pharmaceuticals and could be used for the large-scale production of metabolites. This paper overview some of the recent developments and studies carried out by Author for the production of bioactive secondary metabolites from medicinal plants

2. BIOACTIVE SECONDARY METABOLITES PRODUCED IN THE AUTHOR'S LABORATORY AT IIIM BY TISSUE CULTURE TECHNIQUES

Bacosides- Novel Memory Enhancer molecules

Bacopa monnieri (L.)Wettst.commonly known as Brahmi (Family Scrophulariaceae) is an important Indian medicinal plant known for its memory enhancer molecules. Many active constituents including a number of alkaloids and saponins along with the major constituents bacoside A and B have been attributed to cardiotonic, diuretic, antianxiety, antidepressant and anticonvulsant activities of this plant species The memory enhancing property of *B. monnieri* has led to its over-exploitation from wild and cultivated habitats resulting in its listing as a threatened plant

Current demand and beneficial effects of bacosides encourage research into developing efficient controlled production of methods through *in vitro* cultivation. Bioreactor technology is regarded as a key step for realization of commercial exploitation of plant tissue cultures for propagation and production of phytomedicines In recent years, various bioreactor configures using liquid medium have been used for propagation of number of plant species Bacoside production have been reported in differentiated shoots and plantlets grown *in vitro* of *B. monnieri* by our group Further, the suitability of liquid medium to grow *B. monnieri* shoots have been demonstrated Shoot cultures of *Bacopa monnieri* were cultivated in shake flask (250 ml) and two bioreactor systems: Growtek® and modified bench top air agitated bioreactor. The shoots cultivated in both the systems showed excellent growth. Biomass ~ 169.67g was observed in air agitated bioreactor compared to ~ 23.17g recorded in Growtek® and 15.70 g in shake flask after 4 weeks of incubation. Furthermore, bacoside production expressed as sum of bacoside A_3 and A_2 in shoot cultures grown in the bioreactor system was ~ 3 fold higher as compared to cultures in shake flask. Significant difference in the growth and bacoside biosynthesis suggested usefulness of bioreactor system advantageous for *B. monnieri* shoot proliferation and bacoside production.(12-13)

Withanolides and Glycowithanolides

Withanolides – steroidal lactones, isolated from various *Solanaceous* plants have received considerable attention due to their potential biological activities. Five selected withanolides (withanone, withaferinA, withanolide A, withanolide B, withanolide E) were identified by HPLC-UV (DAD) - positive ion electrospray ionization mass spectroscopy in *Withania somnifera* (L.) Dunal cv. WSR plants and tissues cultured *in vitro* at different developmental phases. Cultures were established from five explants on Murashige and Skoog's medium supplemented with different plant growth regulators. Results suggest that production of withanolides is closely associated with morphological differentiation Phytochemical investigations of multiple shoot cultures of

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selected accessions AGB002 and AGB025 of *Withaniasomnifera* Phytochemical investigations of multiple shoot cultures of selected accessions AGB002 and AGB025 of *Withania somnifera* data established *in vitro* utilizing shoot tip apices cultured on Murashige and Skoog's medium supplemented with BAP (1 mg/L) have been carried out. This has lead to isolation of four glycowithanolides*viz.* Withanoside IV (WSG-3), Withanoside VI (WSG-3A), Physagulin D (WSG-P) and Withastraronolide (WSC-O).The structures of these have been confirmed on the basis of spectroscopy14-15)

In another studies phytochemical investigations of multiple shoot cultures of selected cultivars AGB002 and AGB025 of *Withania somnifera* established i*n vitro* utilizing shoot tip apices cultured on Murashige and Skoog's medium supplemented with BAP (1mg/l) have been carried out. This has lead to isolation of four glycowithanolides *viz.* Withanoside IV (WSG-3), Withanoside VI (WSG-3A), Physagulin D (WSG-P) and Withastraronolide (WSC-O).The structures of these have been confirmed on the basis of spectroscopic data. Multiple shoot cultures could be an alternative renewable resource for production of these biologically active molecules.*(16)*

Glychyrhizin and related Terpenoids
Simultaneous qualitative and quantitative assessment of eight flavonoids and two terpenoids was performed in fourteen *in vitro* raised morphogenic cultures of *Glycyrrhiza glabra.* Our study revealed that the spectrum and production of ten compounds, under investigation, was higher in organized tissue than the undifferentiated mass, however, aerial portions of the *in vitro* raisedplants (leaf and stem) were found to be devoid of glycyrrhizin. Additionally, an interesting correlation was revealed between glycyrrhizin accumulation and various differentiation stages of the plant. We also evaluated cytotoxic effect of the extracts against panel of human cancer cell lines *in vitro*, among which, pancreatic cell line (MIA-PaCa-2) was found to be sensitive to all the fourteen extracts investigated. Notably, extracts with higher glycyrrhizin content displayed cell inhibition activity of the order of 44% against breast cancer cell line. Overall, our findings demonstrated that the metabolite spectrum of varied *in vitro* raised morphogenetic lines, at different stages of maturation, might offer a powerful tool to understand the regulatory aspects of the concerned metabolite pathway and their consequent role in differentiation. Results presented here have revealed that the phytochemical profiling was found associated with the organogenesis (17).

Amarogentin&Amaroswerin
Chemical investigations of various *in vitro* developed morphotypes revealed that proliferating shoot cultures produce bioactive molecules (Amarogentin & Amaroswerin) equal to the parental plants. As the herb is directly being used by the industry without any down stream process of extraction of active principal, the shoot cultures seem to have potential for direct use in the industry. Studies are being carried out to explore possibility for an alternative supply route through biotechnological production of biomass/product using shoot cultures in a bioreactor Present study is aimed at to develop procedure for a. development of shoot cultures of *Swertia chirayita*; b. culturing shoot material in tissue culture under conditions that organogenically produce a proliferating of shoot biomass ; and c. standerdization of the conditions for harvesting said shoots and/or leafy material while at green, actively-growing, non-senescent stage and produce desired amount of amarogentin and amaroswerin(19).

Reserpine and Ajmalicine
*Rauvolfia serpentina*is an erect evergreen, woody perennial shrub and commonly known as Sarpagandha, Major constitutes of sarpagandha roots are reserpine, rescinnamine, deserpidine and yohimbine (Klyshnichenko *et al*., 1995). According to Ayurveda, the roots and whole plants are used for the treatment of cardio vascular disorder, snake bite, rheumatism, hypertension, insanity, epilepsy and hypochondria (Kritikar and Basu, 1993). Infusion, decoction and extract of the roots are employed to increase uterine contraction for expulsion of foetus, to treat painful affection of bowels, diarrhoea, dysentery, cholera and colic (Ghani, 1998). Value of sarpagandha root depends on total alkaloid content and proportion of reserpine and ajmalcine alkaloids present in it. Reserpine has remarkable physiological activities, which have led to its extensive use in the treatment of hypertension, nervous and mental disorders. It is also used in headache and asthma. Ajmalicine has remarkable physiological activities, which have led to its extensive use as anti-hypertensive, anti-bacterial and sedative in drugs.

Experiments were conducted to quantify secondary metabolite production in callus and cell suspension culture of *Rauvolfia serpentina.* Reserpine and ajmalicine were detected in one-month-old callus as well as in cell suspension cultures. Culture medium MSD.5IB (MS+1.0 mgl-1 2,4-D + 0.5 mgl-1 IBA) indicates the highest recovery of reserpine content in both callus and liquid suspension medium of one -month age. Increasing concentration of 2,4-D in liquid medium drastically decreased reserpine content .Linearly decreased Ajmalicine concentration in both callus and cell suspension culture was recorded with increased concentration of 2,4-D

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Embryogenic cell suspension culture of *R. serpentine* may be proved quite useful and convincing tool to improve the yield of secondary metabolites (reserpine and ajmalicine) in *in vitro*. Both alkaloids may be further produced in commercial scale by bioreactor cultivation.

Essential Oils

The biosynthetic capacity of *in vitro* proliferating shoots and regenerated callus clones has been evaluated for essential oil production. On evaluation it was found that the essential oil isolated from foliage of proliferating shoots and regenerated plantlets was a complex mixture with 49 components, 25 of which were identified, corresponding to 80% of the total oil content. The analysis of the identified constituents included monoterpene hydrocarbon (43%), oxygenated monoterpene (31%), sesquiterpene hydrocarbons (7.4%) and oxygenated sesquiterpenes (4.0%). The major constituents were myrcene, limonene, (*E*)-linalool, (*Z*)-□-ocimene and □-caryophyllene oxide.(22)

4. APPLICATION OF BIOREACTOR SYSTEMS FOR HIGH THROUGPUT PROPAGATION OF PLANTS OF MEDICINAL IMPORTANCE

Growing demand for the herbs and beneficial effects of number of medicinal and aromatic plants and need of conservation priority encourage research into developing efficient methods for controlled production through *in vitro* cultivation. Bioreactor cultivation is regarded as a strategy for realization of commercial exploitation of plant tissue cultures for propagation and production of phytomedicines. A number of bioreactor configurations have been examined for the growth of shoot culture using liquid medium in several plant species. Organ cultures are generally less sensitive to shear stress and more stable in metabolite yields due to their genetic stability.

Bioreactor culture is highly effective means of high-throughput propagation and production of value added bioactive compounds In the present investigation shoot multiplication in *Growtek*and *Buchner-flask* and air lift Bioreactor bioreactor systems utilized provide a useful low cost liquid culture option to scale-up quality multiple shoots production for mass cloning of *R. serpentine, Chlorophytum borivilianum, Bacopa monnieri and Swetia chirayita.* The successes with these plant species to grow in bioreactor extend good and practical option to apply bioreactor consideration to other important plants where quality herb production for drug purpose is required. Liquid culture products are improved in quality compared with solid media. Besides being low cost option it enables automation of tissue culture process**.**

5. CONCLUSION & FUTURE NEEDS

The production of chemicals and pharmaceuticals using plant cell cultures has made great strides building on advances in plant science. The use of genetic and rDNA technology tools and regulation of pathways for secondary metabolism have provided the basis for the production of commercially acceptable levels of product production of commercially acceptable levels of products. However.despite progress strategies are still needed to develop information based on a cellular and molecular level for most of the molecules Because of the complex and incompletely understood nature of plant cells in *in vitro* cultures, case-by-case studies have been used to explain the problems occurring in the production of secondary metabolites from cultured plant cells. As such focused approach depending upon nature of compound and resource plant and culture type needs to be taken into consideration for successful application of tissue culture to harvest appreciable level of compound for production at commercial level. Knowledge concerning pathway dissection at molecular level is required to be developed for each compound to harvest the benefit of system biology and metabolic approaches for production at commercial level.

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RADIATION TECHNOLOGIES IN AGRICULTURE AND FOOD SECURITY

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INTRODUCTION

For over 10,000 years, humans have been cultivating plants following selection and breeding of crop plants to suit their needs of increased quality and quantity of produce. Since the Green Revolution during 1960s, intensive and extensive crop cultivation has resulted in higher yields benefiting both farmers and consumers. Ever since, there has been a continued demand for higher agricultural productivity. This has become more and more challenging with the rapidly growing world population and diminishing land and water resources besides environmental extremities with unpredictable consequences. Global food security continues to be the centre stage issue to sustain the food production and meet the demand of ever-growing human population. As per recent United Nations estimate, world's population is going to reach 9.7 billion by 2050 (FAO, 2018). Population expanse in the last century led to a significant rise in food demand. Additionally, climatic change has placed further pressure on both food production and food security. There is a need to develop sustainable solutions to enable plant breeders to develop highly productive crops by using conventional and modern tools. In this context, increasing crop productivity by using various genetic, agronomic and biotech methods to either alter or improve the genetic capacitance appears promising in the present scenario. Improved crop varieties contribute to biodiversity and, are even better as they exert less pressure to expand the area under agriculture because of their high productivity.

Traditionally selection, hybridization and selection have been employed in the improvement of crop varieties. Plant breeding methods have contributed immensely to development of genetically superior crop varieties and these continue to enrich the germplasm base of crop plants by evolving genetically superior varieties for cultivation. Naturally occurring variation being often limited, there is a need to enhance genetic variability by using different genetic and biotechnological techniques. Experimental mutagenesis is considered a major breakthrough in the early 20th century, as an applied research frontier of paving way for plant mutation breeding. Mutation induction by using various physical (X-rays, Gamma rays, alpha particles, fast neutrons, ion beams, UV and cosmic rays) and chemical (sodium azide, ethyl methanesulphonate, methyl methanesulphonate, hydroxylamine and N-methyl-N-nitrosourea) mutagens has become the choice as a new tool in enhancing genetic variability in crop plants. Although different physical and chemical mutagens can be used, gamma rays are the most frequently used agent to develop mutant varieties. The mutagenic action of the physical agents is through the ability to produce reactive ions which in turn cause aberrations at the DNA level to gross chromosomal breakages and re-arrangements. Chemical agents induce point mutations or single base-pair changes, or single-nucleotide polymorphisms. Induced genetic variability in economically important plant species has resulted in the release of 3,218 mutant varieties worldwide in about 180 plant species (Jain, 2005, 2010). Induced mutations have been developed for modification in plant architecture, root characteristics, oil and protein quality, seed size, color and flowering time besides yield. In comparison to crossbreeding methods, induced mutation technique offers modification of single or a few characters in an otherwise promising cultivar without significantly changing the genetic makeup of the crop plant. Currently, induced mutation techniques are being combined with other molecular advances, such as molecular markers and high-throughput mutation screening techniques, thereby becoming more powerful and effective in crop breeding (Shu 2009, Suprasanna et al. 2015).

Several improved mutant varieties have been developed for cultivation in different crop plants and have contributed to economic gain the developing and developed countries (Kharkwal and Shu 2009 ; Jain and Suprasanna 2011). The induced mutants are used either directly or indirectly in a breeding program in the development of improved varieties for cultivation. The International Atomic Energy Agency (FAOIAEA), Vienna, Austria, has been cataloguing identified and released mutant varieties through the mutant variety database (MVD). This database gives comprehensive coverage about crop mutant varieties, mutagen used and improved traits. The mutant varieties have been officially released in 60 countries, among which the top six countries are China, India, the former USSR, Netherlands, Japan and USA (Fig 1). In India, several mutant cultivars of crops, belonging to 56 plant species, have been released for cultivation (Fig 2). Among the mutants, majority is from ornamentals, followed by cereals, legumes and oilseeds (Table 1).

Mutation breeding efforts in cereals, legumes, oil crops, ornamentals and fruit trees has produced significant economic benefits (Bradshaw 2016). The mutant barley varieties 'Golden Promise' and 'Diamant' and other mutant varieties have become important to the brewing industry in Europe (Ahloowalia et al. 2004). Mutant

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durum wheat variety 'Creso' and varieties developed from 'Creso' occupy over half the wheat used for pasta in Italy (Ahloowalia et al. 2004). Rice varieties derived from mutation breeding are grown extensively in Asia and Australia, generating economic benefit to the farmers (Mba 2013). The FAO-IAEA mutant variety database (MVD 2019) has listed around 160 mutant varieties for abiotic stress tolerance with 110 identified for drought tolerance, 36 for salt tolerance and 15 for low temperature tolerance (Suprasanna et al. 2014). Nutritional quality is an area of significance to enhance mineral nutrient and essential aminoacid content, altered protein and fatty acid profiles, physicochemical properties of starch, enhance phytonutrients in fruits and reduced antinutritional factors in staple food grains. Induced mutations could play an important role in inducing mutations for enhancing nutritional quality in crop plants. Of the total mutant varieties developed globally, 776 are identified for improved nutritional quality (Jain and Suprasanna 2011).

The Bhabha Atomic Research Centre, Mumbai has been engaged in the application of radiation technology for the improvement of crop varieties (DAE 2019). More than 42 improved Trombay varieties in oilseeds, pulses, cereals and other crops have been developed and released for commercial cultivation in different parts of the country by the Ministry of Agriculture, Government of India. The Trombay mutant crop varieties have high patronage from the farming community and are extensively grown in different states of the country. At the national level, some of the Trombay varieties are popular as check varieties besides being used as breeding lines for varietal development. The Trombay varieties continue to contribute significantly to the production of different crop plants and ultimately to national food security.

In addition to the currently practiced methods of inducing genetic variability, there is a greater need for developing new methods for enhancing genetic variation. Genetic variability occurring in cell and tissue culture is referred to as 'somaclonal variation' and when combined with mutagenesis, the *in vitro* mutagenesis and selection (IMAS) technology can be useful in crop improvement. Desirable genetic variation has been induced for phenotype alterations, yield potential and fruit size as well as wide range of resistance to abiotic and biotic stresses including diseases and/or pathogens (Suprasanna et al., 2012). Selection of desirable mutants is an important step in any mutation breeding programme. Compared to methods of handling *in vivo* plant material, *in vitro* cultured plant material can be useful for controlled selection (Suprasanna et al., 2012). Tissue culture has a potential for improving effectiveness of mutation induction in several aspects. A wide choice of *in vitro* explant material (in vitro axillary buds, organs, tissues, and cells) can be used for mutagenic treatment. Further, screening performed under *in vitro* offers convenient way of handling of large populations and treatment with selection agents (van Harten 1998). IMAS method is now established for a range of vegetatively propagated crops like banana, sugarcane, potato, cassava, sweet potato, and grapevine (Pathirana 2011, Suprasanna et al. 2012). This technology has been instrumental for undertaking mutation induction based improvement in vegetatively propagated crops like sugarcane and banana.

The genomics interventions have enhanced our understanding of the nature of induced genetic variation. Mutation techniques using ethyl methanesulphonate, T-DNA insertion, transposon tagging and ionizing radiation have provided key information on the nature of mutations which has been useful to explore mutant gene structure, function, spatial and temporal expression and genetic regulation (Suprasanna et al. 2015). High throughput genomics platforms such as cDNA amplifed fragment length polymorphism (AFLP), single strand conformational polymorphism (SSCP), serial analysis of gene expression (SAGE), microarray, differential display, Targeting Induced Local Lesions IN Genome (TILLING), high resolution melt (HRM) analysis have become very useful in rapid and in depth global analysis of mutational events. TILLING technology has been useful for screening EMS induced mutations in mutagenized populations and also with mutant populations developed through gamma and fast neutron irradiation (Till et al., 2007).

Several other applications of radiation technology in agriculture include, soil-water-crop nutrition management, Integrated soil fertility management and insect pest control by sterile insect technique (Mehetre and Venugopalan 2017).

Radiation technologies can also play a seminal role in food security. Food preservation is an essential pre-requisite for ensuring food security and safety. In this regard, strategies for enhancing availability of food and ensuring their quality & safety are important considerations to both the consumer and regulatory authorities. Radiation processing by ionizing radiation is a non-thermal method which can attribute minimal changes in sensory qualities thereby maintaining food quality (Tripathi and Variyar 2015). The technology of radiation processing offers an effective method to chemical based fumigation that has serious consequences on environment and human health. The radiation based method is implemented by exposing food and food products to controlled dose of ionizing radiation aimed to increasing food storage life, reducing post-harvest losses and eliminating food poisoning microorganism (Tripathi and Variyar 2015). The technology can be used

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for disinfestation of food grains and pulses, inhibition of sprouting in bulbs and tubers, extending shelf-life under recommended conditions of storage, ensuring microbiological safety and in overcoming quarantine barriers to international trade. Radiation processing can also have applications for hygienization and sterilization of non-food items including cut-flowers, pet food, cattle feed, aqua feed, ayurvedic herbs and medicines and packaging materials (Tripathi and Variyar 2015). In this regard, BARC has set up a major facility (KRUSHAK) for irradiation of fresh horticultural produce at Lasalgaon, Nashik district, Maharashtra state, India. KRUSHAK has facilitated the irradiation of mangoes for export to USA since 2007. In addition, feasibility studies have been made to extend the technology to other products such as litchi and pomegranate. Another radiation processing facility at Vashi, Navi Mumbai has been operational for processing spices and dry ingredients for microbial decontamination since 2000. A total of 13 irradiation plants have now been set up in the private sector (Tripathi and Variyar 2015).

CONCLUSIONS

Attainment of agricultural and food security has become crucial in the face of increasing population pressure and diminishing natural land and water resources. Sustainable solutions need to be developed to augment research efforts of crop improvement. Induced mutagenesis has made a significant impact with the development of new mutant crop varieties. Several of these mutant varieties have made huge economic benefit. Genomics advances and accessibility to crop genomes will add new dimension to analyze mutational events and mutant traits.

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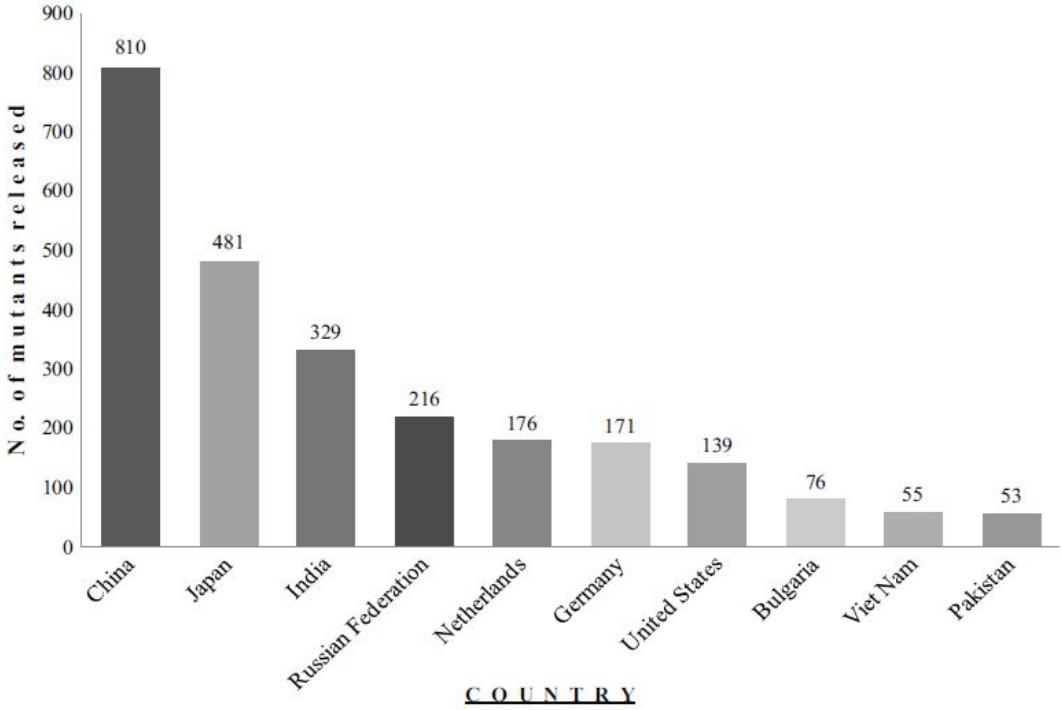

Fig-1: Global status of officially released mutant varieties in top ten countries
(Source: Suprasanna et al. 2015)

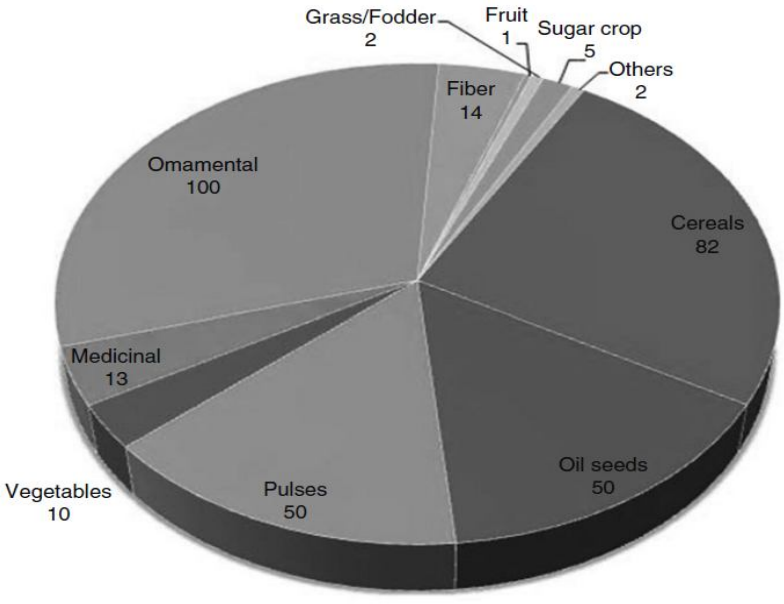

Fig-2: Development of mutant varieties – Indian scenario
(Source: Suprasanna et al. 2015)

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Table-1: Number of released mutant varieties in 57 crop species in India

SN	Latin name	Common name	No. of varieties
1	*Ablemoschus esculentus* L. Moench	Okra	2
2	*Arachis hypogaea* L.	Groundnut	18
3	*Bougainvillea spectabilis* Wild	Bougainvillea	13
4	*Brassica juncea* L.	Mustard	9
5	*Cajanus cajan* L. Millsp.	Pigeonpea	5
6	*Capsicum annum* L.	Green pepper	1
7	*Carica papaya* L.	Papaya	1
8	*Chrysanthemum* sp.	Chrysanthemum	49
9	*Cicer arietinum* L.	Chickpea	8
10	*Corchorus capsularis* L.	White jute	2
11	*Corchorus olitorius* L.	Tossa jute	3
12	*Curcuma domestica* Val.	Turmeric	2
13	*Cymbopogon winterianus* Jowitt.	Citronella	9
14	*Cyamopsis tetragonoloba* L.	Cluster bean	1
15	*Dahlia* sp.	Dahlia	11
16	*Dolichos lablab* L.	Hyacinth bean	2
17	*Eleusine coracana* L.	Finger millet	7
18	*Gladiolus* L.	Gladiolus	2
19	*Glycine max* L. Merr.	Soybean	7
20	*Gossypium arborium* L.	Desi cotton	1
21	*Gossypium hirsutum* L.	American cotton	8
22	*Helianthus annus* L.	Sunflower	1
23	*Hibiscus sinensis* L.	Hibiscus	2
24	*Hordeum vulgare* L.	Barley	13
25	*Hyocyamus niger*	Indian henbane	1
26	*Lantana depressa* L.	Wild sage	3
27	*Lens culinaris* L. Medik.	Lentil	3
28	*Luffa acutangula* Roxb.	Ridged gourd	1
29	*Lycopersicon esculentum* M.	Tomato	4
30	*Matricario cammomilla*	Germen chamomile	1
31	*Mentha spicata*	Spearmint	1
32	*Momordica charantia* L.	Bitter gourd	1
33	*Morus alba* L.	Mulberry	1
34	*Nicotiana tabacum* L.	Tobacco	1
35	*Oryza sativa* L.	Rice	42
36	*Papaver somaniferum* L.	Opium poppy	2
37	*Pennisetium typhoides* L.	Pearl millet	5
38	*Phaseolus vulgaris* L.	French bean	1
39	*Pisum sativum* L.	Pea	1
40	*Plantago ovata* L.	Isabgol	2
41	*Polyanthus tuberosa* L.	Tuberose	2
42	*Portulaca grandiflora* L.	Portulaca	11
43	*Ricinus communis* L.	Castor	4
44	*Rosa* sp.	Rose	16
45	*Sachharum officinarum* L.	Sugarcane	9
46	*Sesamum indicum* L.	Sesame	5
47	*Setaria italica* L.	Foxtail millet	1
48	*Solanum khasianum* Clarke	Khasianum	1
49	*Solanum melongena* L.	Brinjal	1
50	*Solenostemon rotundifolius*	Coleus	1
51	*Trichosanthus anguina* L.	Snake gourd	1
52	*Trifolium alexandrium* L.	Egyptian clover	1
53	*Triticum aestivum* L.	Wheat	4
54	*Vigna aconitifolia* Jacq. M.	Moth bean	5
55	*Vigna mungo* L. Hepper	Blackgram	9
56	*Vigna radiata* L. Wiczeck	Mungbean	15
57	*Vigna unguiculata* L. Walp.	Cowpea	10
		Total	**343**

Source: Kharkwal, M.C., and Q.Y. Shu. 2009

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ROLE OF TISSUE CULTURE IN CONSERVATION OF BIODIVERSITY AND CROP IMPROVEMENT

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ABSTRACT
Biodiversity is the degree of variation of life forms within a given ecosystem, biome or an entire planet. Biodiversity is a measure of the health of ecosystems. Biodiversity is in part a function of climate. Biodiversity is the vast array of all the species of plants, animals, insects and microorganisms inhabiting the earth either in the aquatic or the terrestrial habitats. Global community has experienced major changes as a result of the unfolding and globalization of revolution taking place since years. Ecological degradation and its corollary-biodiversity loss- pose a serious threat to development. 'Ecologically destructive economic activities are inefficient not merely because of the resulting resource misallocation but also because of the (excessive) scale of activity levels; excessive in relation to the limited availability of natural capital when the latter is complementary to human-made capital'. In order to bring about sustainable resource conservation and management, it is essential to adopt several different approaches for managing our forests and biodiversity. Tissue culture has opened exciting frontier in the field of agriculture and offers opportunities for the increase in productivity, profitability, stability and sustainability. Plant tissue culture comprises an array of in vitro techniques, methods and strategies that are part of the group of technologies called plant biotechnology. Plant tissue culture forms an integral part of any plant biotechnological activity. It offers an alternative to conventional vegetative propagation. Tissue culture has been exploited to create genetic variability from which crop plants can be improved, to advance the state of health of the planted material and to increase the number of desirable germplasms accessible to the plant breeder. Tissue culture protocols are available for most crop species, although continued optimization is still required for many crops, especially legumes and woody plants. Tissue culture techniques, in combination with molecular techniques, have been successfully used to incorporate specific traits through gene transfer. In vitro techniques for the culture of protoplasts, anthers, microspores, ovules and embryos have been used to create new genetic variation in the breeding lines, often via haploid production. Cell culture has also produced somaclonal and gametoclonal variants with crop improvement potential. Useful secondary metabolites may be produced by using cell suspension cultures or bioreactor. Thus bio-prospecting of medicinally useful substances is possible though tissue culture. Large scale micropropagation laboratories are providing millions of plants for the commercial ornamental market and the agricultural, clonally-propagated crop market. With selected laboratory material typically taking one or two decades to reach the commercial market through plant breeding, this technology can be expected to have an ever increasing impact on crop improvement as we approach the new millennium.

INTRODUCTION
Food is the necessity for sustainability of human life on this planet. Various crops have been harvested since thousands of years. With the increasing world population, there is relative increased demand of food. Conventional methods are no more adequate to overcome this situation. The field of biotechnology and molecular biology has revolutionized the agriculture and farming methods. With the advent of modern techniques of biotechnological advances and biotechnological tools the production of new plant varieties have made achievable plants resistant against different biotic and biotic stresses tolerant to drought or other harsh environmental conditions had been produced. A number of techniques including tissue culture mutagenesis, transformation have been used. Advance functional genomics studies have given better understanding of plant genome and help in modifying it. RNA interference, next generation sequencing and nano-biotechnology in recent years have shown potential of these techniques in crop improvement according to future need. Although, global food grain production is growing as with increasing population but still 1 billion persons of the world are malnourished because of food insecurity It has been estimated that worldwide food production must be increased by 70% by the year 2050 to fulfill the need of expanding population and growing consumption of food. Plant biotechnology has opened up new horizons in the field of science. It is a viable option, which can provide improved genotypes that can survive under changing climate. Advancements in fields of genomics, stress biology and bioinformatics can help in development of stress tolerant crops.

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ROLE OF TISSUE CULTURE IN CONSERVATION OF BIODIVERSITY

Different techniques in plant tissue culture like micropropagation offer certain advantages over traditional plant breeding techniques. Ornamental, clonally propagated crop industries are working massively and hence dramatically increase the crop cultivars. Number of commercial ventures have been established in India and abroad where technique of micropropagation have been commercially exploited at large. In India more than 200 Commercial units are working for propagation of quality plants. The culture of single cells and meristems can be effectively used to eradicate pathogens from planting material and thereby dramatically improve the yield of established cultivars.

In vitro propagation and conservation strategies of some economically important horticultural plants

Plant genetic resources are the most essential of biological resources for sustaining life on earth as they donate food, dietary and health security for the ever increasing population. Society faces tremendous challenges in the imminent decades. Thus their conservation along with sustainable utilization is of greatest important. Plant Biotechnology is being used as a tool for conservation of natural habitats and their sustainable utilization for ecological balance, technologies for cultivation of plants in different cropping systems and on the problematic soil, development of state-of-art technologies for extraction, characterization and utilization of essential bioactive components, generating scientific and clinical data to support the health claims of botanical drugs, elicitation and enhancing the production of known and novel metabolites using metabolic engineering technology, DNA bar coding : identification and characterization of plant material, design and discovery of newer molecules for human and plant health, development of post-harvest management and handling technologies including establishment of effective partnerships between different stakeholders. In this review we will focus on applications of plant tissue culture in conservation of biodiversity especially work done by our groups.

FRUIT CROPS

Embelica officinalis

Aonla *(Emblica officinalis* syn. *Phyllanthus emblica*) is an important fruit crop of commercial significance due to its high medicinal and nutritional value. Aonla is the richest source of vitamin 'C' among all fruits after Barbados Cherry. The non-availability of desired number of shoots for grafting or budding is bottleneck problem for multiplication, which is labour expensive and economically not viable due to slow rate of multiplication and field survivability. The use of biotechnological approaches especially micropropagation offers a unique alternative for massive multiplication of true to type plantlets.

Mature cotyledons, cotyledonary node and hypocotyls of NA-7 cultivars of *Emblica officinalis* were cultured on MS basal medium with 20 different combinations of auxins and cytokinins in varying concentrations. Considering higher *in vitro* response, culture medium MS5DB (MS + 5.0 $mg.l^{-1}$ 2,4-D + 0.5 $mg.l^{-1}$ BA + 30.0 $g.l^{-1}$ sucrose + 7.5 $g.l^{-1}$ agar) initiated calli in higher frequency (52.44%), morphogenic calli (34.75%) was higher on medium MS4BN (MS + 4.0 $mg.l^{-1}$ BA + 0.5 $mg.l^{-1}$ NAA + 30.0 $g.l^{-1}$ sucrose + 7.5 $g.l^{-1}$ agar) and higher plantlet regeneration frequency (38.62%) was recorded on culture medium MS4B.5N.5G (MS + 4.0 $mg.l^{-1}$ BA + 0.5 $mg.l^{-1}$ NAA + 0.5 $mg.l^{-1}$ GA_3 + 20.0$g.l^{-1}$ sucrose + 7.5 $g.l^{-1}$ agar). Higher *in vitro* rooting response was achieved on MS basal medium supplemented with 2.0 $mg.l^{-1}$IBA and 0.5 $mg.l^{-1}$BA (17.20%). In respect to different explants, higher *in vitro* morphogenic response (callus initiation and morphogenic calli formation) was exhibited by explant cotyledonary nodes followed by mature cotyledons and hypocotyls (Patidar *et al.*,2009). Higher *in vitro* rooting was recorded on MS basal medium supplemented with 2.0 $mg.l^{-1}$IBA and 0.5 $mg.l^{-1}$BA (17.20%). Patidar *et al.* (2010) achieved the best results on basal MS medium fortified with 4.0 $mg.l^{-1}$BAP and 0.5 $mg.l^{-1}$NAA for shoot proliferation efficiency (47.65%), number of shoot(s) per explant (3.20) and average shoot length (1.43) from cultured nodal segments.

Grape

Grape (*Vitis vinifera* L.) is one of the most important fruit crops of the world. Genetic improvement of grapevine is exacerbated by polyploidy, high level of heterozygosity and inbreeding depression. It is also a time-consuming process due to 2-3 years generation cycle. Plant biotechnology could be an attractive means for improving grape however, this requires, as a first step, an efficient and reproducible regeneration system from somatic tissues of mature plants that permits both transformation and regeneration into plantlets. *In vitro* techniques could be used for vine breeding to overcome the difficulties in traditional breeding studies and obtaining new cultivars.

A protocol has been standardized to regenerate three cultivars *namely*: Thompson seedless, Karnet and Local cultivars of *Vitis vinifera* (L.) through indirect and direct *in vitro* organogenesis from nodal segment explants excised from *in vitro* grown regenerants as well as one-year-old plants by Kurmi *et al*. (2011). Induction

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medium MS2D (MS + 2.0 mg.l^{-1} 2, 4 -D + 30.0 g.l^{-1} sucrose + 7.5 g.l^{-1} agar) induced callusing in higher frequencies (84.22%). Culture medium MS3B (MS+3.0 mg.l^{-1} BA + 30.0 g.l^{-1} sucrose + 7.5 g.l^{-1} agar) was found to be more responsive for proliferation of shoots in higher frequencies (80.67%). Shoot (s) per explant in higher numbers (14.08) was attained with culture medium MS.2Td (MS+ 0.2 mg.l^{-1} TDZ + 30.0 g.l^{-1}sucrose + 7.5 g.l^{-1} agar), while nutrient medium MS.2Td.5N (MS+ 0.2 mg. l^{-1} TDZ + 0.5 mg.l^{-1}NAA+ 30.0 g.l^{-1} sucrose + 7.5 g.l^{-1} agar) produced shoot of higher length (8.78cm). Higher *in vitro* rooting response *i.e*. root proliferation ability (89.94%), number (s) of roots (5.78) and root length (7.75 cm) was exhibited by MS rooting medium fortified with 1.0 mg.l^{-1} IBA, 10.0 g.l^{-1} sucrose and 7.5 g.l^{-1} agar. Genotype Thompson Seedless responded better than Karnet and Local for the most of the attributes investigated. The *in vitro* raised plantlets were acclimatized and established successfully in the field.

Subsequently, an *in vitro* regeneration protocol has been developed from 5-8 mm long leaf discs excised from *in vitro* grown regenerants as well as one-year-old plants of three different cultivars of *Vitis vinifera* L *viz*:Thompson seedless, Karnet and Local to find out the best *in vitro* responsive cultivar, culture media combination (especially appropriate auxin/cytokinin ratio) with their interactive effects on frequency of callus induction, formation of morphogenic calli and plantlet regeneration. MS medium fortified with 2.0-3.0 mg.l^{-1} 2, 4-D and 0.5 mg.l^{-1} Kinetin or BA proved more responsive for callus induction, while morphogenic calli as well as plantlets in higher frequencies were investigated with culture medium amended with 2.0 mg.l^{-1} BA in conjunction with 0.5 mg.l^{-1} NAA. In term of genotypic response, genotype Thompson Seedless responded better than Karnet and Local. Higher *in vitro* rooting response *i.e*. root proliferation ability, number (s) of roots and root length was exhibited by MS rooting medium fortified with 2.0 mg.l^{-1} IBA and 15 g.l^{-1} sucrose (Kurmi *et al*.,2010).

Sharma *et al*. (2018) established a simplified procedure for embryogenic cell suspension culture from the friable embryogenic callus of *Vitis vinifera* L. by transferring 6-8 weeks-old embryogenic calli achieved from nodal segment and leaf disc explants in liquid media. The asynchronous embryogenic cultures were swamped with clumps of proliferating embryos of various developmental stages along with modest non-embryogenic tissues. The frequency of embryo proliferation was recorded to determine growth rate of embryogenic tissues under diverse conditions. Modulation of initiation and proliferation of embryogenic cell suspension culture was under regulation of the influence of exogenous plant growth regulators added in culture medium at different concentration and combination. Higher relative growth of embryogenic cell suspension cultures was recorded with combination of culture medium MS+2.0mgl^{-1}2,4-D+0.5mgl^{-1}Kn+30.0gl^{-1} sucrose, whereas, cell clumps/embryoids in higher numbers were attained on culture medium (MS+2.0mgl^{-1}2,4-D+ 0.5mgl^{-1}BA+30.0gl^{-1} sucrose). For subsequent subculturing, reduced level of 2,4-D (1.0 mgl^{-1}) in combination with 0.5 mgl^{-1} BAP accelerated somatic embryogenesis. High frequency plantlet regeneration recorded on regeneration medium supplemented with 0.5 mgl^{-1}, each of BAP, Kn and NAA. In terms of root proliferating ability and root of higher length were documented on rooting medium MS.5IB.5Kn(MS+0.5mgl^{-1}IBA+0.5mgl^{-1}Kn+15.0gl^{-1} sucrose+7.5gl^{-1} agar).Among two genotypes, Thompson seedless was found consistently superior to Karnet for all attributes investigated.

Citrus

Citrus is a common woody subtropical fruits belonging to the family Rutaceae. *Citrus* fruits are not only appetizing and invigorating but also they provide vitamins, minerals and many other essences. Fruits of this family are very admired all over the world due to the taste, aromatic flavor and medicinal properties. Citrus fruits are also used for patients susceptible to health problems such as gastritis, fever and arterial sclerosis. The juice of this species is used in the pharmaceutical industry since it contains a high magnitude of citric acid. There are also reports about positive effects of citrus fruits against cancer of gastrointestinal and upper respiratory tracts. *Citrus* is being propagated through seed and “shield” / “T” budding, but from seed it does not ensure true to type plants because of cross-pollinating nature. Besides, such plants take more time to bear fruits. The less-availability of authentic and disease free planting material and shoots for grafting and budding makes field multiplication difficult to the most of citrus species. Given the immense importance of the *Rutaceae* family in various branches of industry, the micropropagation of *Citrus* has always stimulated great concern among scientists.

Vibhute *et al*. (2012) cultured mature cotyledons of three species of citrus on different fortifications of MS media to assess their *in vitro* response. Induction medium MS5D.5B (MS+ 5.0 mg.l^{-1} 2, 4-D + 0. 5 mg. l^{-1} BAP) proved well for callus initiation (84.18%). Nutrient medium MS4B.5N (MS + 4.0 mg.l^{-1} BAP + 0.5 mg.l^{-1} NAA) performed convincingly for formation of morphogenic calli (31.89%) as well as regeneration of plantlets (79.09%). Higher root proliferating efficiency (77.64%) was recorded on rooting medium MS.5I (MS + 0.5 mg.

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l^{-1} IBA). Roots in higher numbers (3.53) were documented on culture medium MS2I.5Kn (MS + 2. 0 mg. l^{-1} IBA + 0. 5 mg. l^{-1} Kn), while culture media MS.5I.5B (MS + 0.5 mg. l^{-1} IBA + 0. 5 mg. l^{-1} BAP) enhanced mean root length (3.55 cm). In terms of interspecific *in vitro* response, in general, Acid lime followed by Mandarin and Sweet orange responded determinedly for the most of the culture phases. The *in vitro* raised plantlets were acclimatized and established successfully in the field

Consequently hypocotyls of three species of citrus were cultured on different fortifications of MS media to assess their *in vitro* response. Higher *in vitro* morphogenesis (somatic embryogenesis and/or embryogenesis) leading to plantlet regeneration was varied considerably due to species and inoculation medium. Induction medium MS4D.5B (MS+ 4.0 mg.l^{-1} 2, 4-D + 0. 5 mg. l^{-1} BAP) proved well for callus initiation. Nutrient medium MS4B.5N (MS + 4.0 mg.l^{-1} BAP + 0.5 mg.l^{-1} NAA) performed commandingly for formation of morphogenic calli and plantlet regeneration. Higher root proliferating efficiency was recorded on rooting medium MS.5I (MS + 0.5 mg. l^{-1} IBA). Roots in higher numbers were attained from culture medium MS2I.5Kn (MS + 2. 0 mg. l^{-1} IBA + 0. 5 mg. l^{-1} Kn), while culture media MS.5I.5B (MS + 0.5 mg. l^{-1} IBA + 0. 5 mg. l^{-1} BAP) supported for enhancing mean root length. In terms of intrespecific *in vitro* response, in general, Acid lime followed by Mandarin and Sweet orange responded decisively for the most of the culture phases. The *in vitro* raised plantlets were acclimatized and established successfully in the field (Vibhute *et al*., 2009).

Nodal segments excised from *in vitro* germinated seedlings of three citrus *species viz*: *Citrus aurantiafolia*, *Citrus reticulata* and *Citrus sinensis* were cultured on different fortifications of basal MS medium for multiple shoot proliferation. *In vitro* morphogenesis leading to plantlet regeneration was varied considerably among species and with medium composition. In present study, plantlets were regenerated directly *via* auxiliary bud proliferation as well as from callus culture. Induction medium MS5N.5B/MS5N.Kn (MS + 5.0 mgl^{-1} NAA + 0.5 mgl^{-1}BA/Kn) induced callusing in higher frequencies. Culture medium MS.1Td.5N/MS.5B.5N (MS + 0.1 mgl^{-1} TDZ/0.5 BA + 0.5 mgl^{-1} NAA) enhanced shoot proliferating efficiency. Shoot (s) per explant in higher number (s) of higher length were documented on medium MS.1Td or MS.2Td(MS + 0.1/0.2 mgl^{-1} TDZ). Higher root proliferating efficiency was recorded on rooting medium MS.5IB (MS + 0.5 mg l^{-1} IBA). Roots in higher frequency were observed on culture medium MS2IB.5Kn (MS + 2. 0 mg l^{-1} IBA + 0. 5 mg l^{-1} Kn), while culture media MS.5IB.5B (MS + 0.5 mg l^{-1} IBA + 0. 5 mg l^{-1} BAP) enhanced mean root length (Vibhute *et al*., 2017).

MEDICINAL PLANTS

Liquorice

Liquorice (*Glycyrrhiza glabra* L.) is one of the important medicinal plants in Indian system of medicine. In India it is known as "Mulhati". It is widely distributed in the world from 5^0 W to 100^0 E longitudes and 20^0 to 50^0 N latitude. The principle constitute of liquorice is sweet tasting triterpenoidal saponin, glycyrrhetic acid and two molecules of D-glucuronic acid. Glycyrrhetic acid has been shown to possess anti-inflammatory activity and used in treatment of gastric ulcer. Although, traditionally *Glycyrrhiza glabra* is propagated through seed and stolon cuttings, commercial cultivation is restricted due to limited seed set and poor seed viability. Furthermore, the conventional method of propagation is rather very slow and only 40-50% of the cuttings get established in the field. To cater the growing demand for pharmaceutical based industries, it was found essential to multiply this species by adopting tissue culture techniques.

A reliable and reproducible protocol has been developed by Sharma *et al*.(2008) for plant regeneration from 6-8 mm long leaf discs excised from 4 -5 months-old plants of *Glycyrrhiza glabra*. Culture medium B_5D.5Kn (B_5 + 1.0 mg.l^{-1} 2, 4-D + 0.5 mg.l^{-1} kinetin) proved well for callus induction. However, nutrient medium B_5B.5N (B_5 + 1.0 mg.l^{-1} BAP + 0.5 mg.l^{-1} NAA) proved superior for morphogenic calli formation and plantlet regeneration. Higher *in vitro* rooting response (root proliferating efficiency, number of roots and root of higher length) was exhibited by culture medium B_5.5I (B_5 + 0.5 mg.l^{-1} IBA). The *in vitro* raised plantlets were acclimatized and established successfully in the field.

In one of the other studies a protocol has been established to propagate through indirect and direct *in vitro* organogenesis from nodal segment explants excised from 4-5 months-old plants. Induction medium $B_5$2D (B_5 + 2.0 mg. l^{-1} 2, 4 D + 20.0 g.l^{-1} sucrose+7.5 g. l^{-1} agar) induced callusing in higher frequencies (65.93%). Culture medium B_5.5B.5N (B_5 + 0.5 mg. l^{-1} BA + 0.5 mg.l^{-1} NAA + 20.0 g.l^{-1} sucrose + 7.5 g. l^{-1}agar) was found to be more responsive for shoot proliferation (94.12%), shoots per explant (9.32) and mean shoot length (5.20 cm) (Sharma *et al*., 2011).

Rauvolfia serpentina (L)Benth

Rauwolfia serpentina L. Benth. ex. Kurz (family: Apocynaceae) is a woody perennial shrub, commonly known with different names; sarpagandha, snake root plant, chotachand, chandrika, *etc*. and is an indigenous to India

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and other tropical countries of Asia. Only a few drugs have attracted such a worldwide attention as the roots of *Rauvolfia serpentina*. The roots contain 50 indole alkaloids including the therapeutically important reserpine, deserpidine, rescinnamine and yohimbine. According to Ayurveda, the roots and the whole plant are used for the treatment of cardiovascular disorder, snake bite, rheumatism, hypertension, insanity, epilepsy and hypochondria. The plant is vegetatively propogated by root cutting because of poor seed viability, low germination percentage, parthenoocarpy and somatoplast sterility that may be largely ascribed to the presence of cinnamic acid and derivatives in the seed. Hence improvements in plant tissue culture techniques for the mass propagation of *R. serpentina* are highly desirable.

Nodal segments and leaf discs were cultured by Uikey *et al*. (2014) on MS medium amended with diverse auxins and cytokinins in varying concentrations as sole as well as in various combinations. For nodal segment culture, basal MS media supplemented with 2,4-D/ NAA at the concentration of 2.0 $mg.l^{-1}$as sole or in combination with 0.5$mg.l^{-1}$ TDZ were proved significantly superior for callus initiation. Higher shoot proliferating efficiency, number of shoots per explant and mean shoot length were documented on culture media fortified with TDZ in the range of 0.2-0.5 $mg.l^{-1}$ as alone as well as in combination with 0.5$mg.l^{-1}$NAA (more than 92% nodal segments proliferated shoots,>10.5 shoots/explants and mean shoot length>3.85 cm). However, for cultured leaf disc, maximum callus and morphogenic callus formation as well as plantlet regeneration were documented on nutrient media fortified with 2.0 mgl^{-1}auxins: 2,4-D and/or NAA in combination with 0.5$mg.l^{-1}$of a cytokinin BA and/or TDZ (more than 76% leaf discs induced morphogenic calli with >80% regeneration frequencies). Higher *in vitro* rooting response (root proliferating efficiency, number of roots and root of higher length) was exhibited by MS rooting medium amended with 0.1 $mg.l^{-1}$IBA. In another experiments.

Consquently Uikey *et al*. (2016) established embryogenic cell suspension culture from the embryogenic callus culture of *Rauvolfia serpentina* (L.) by transferring 4-6 weeks-old asynchronous embryogenic calli raised from mature embryo and cotyledon explants cultures in liquid media. The cultures obtained were inundated mostly with clumps of proliferating globular embryos with modest non-embryogenic tissues. The number and size of somatic embryos/clumps was recorded to calculate growth of embryogenic tissues under various conditions. Initiation and proliferation of embryogenic suspension culture was influenced by various exogenous plant growth regulators fortified to the culture medium at variable magnitude. For the establishment of suspension cultures, MS medium fortified with 2.0 mgl^{-1} 2,4-D with 0.5 mgl^{-1} BAP was found to be the most effective. For subsequent subculturing, the reduced level of 2,4-D (1.0 mgl^{-1}) in combination with 0.5 mgl^{-1} BAP promoted somatic embryogenesis at a faster rate. Frequent and efficient plantlet regeneration occurred on MS medium supplemented with 0.5 mgl^{-1}, each of BAP, TDZ and NAA.

Sandalwood *(Santalum album* Linn.)

Santalum album L. belongs to the santalaceae family, a medium-sized evergreen hemi root parasitic tree, highly valued for its fragrant heartwood, which contains sandal oil that is used in perfumes, cosmetics, and also in Agarbathi (incense sticks) industries. Conventional breeding of sandalwood for introgression of new genetic information can be an expensive and difficult task because of its long generation time, sexual incompatibility and heterozygous nature. *In vitro* regeneration techniques can be used to encounter difficulties of traditional propagation methods by microcloning of superior lines.

Leaf discs of sandalwood were cultured on different fortifications of MS medium to judge their *in vitro* response. *In vitro* morphogenesis, (somatic embryogenesis and/or organogenesis) leading to plantlet regeneration was influenced considerably by plant growth regulators. Among various medium experimented, medium MSD.5Td (MS + 1.0$mg.l^{-1}$ 2,4-D + 0.5$mg.l^{-1}$ TDZ) supported maximum direct somatic embryogenesis (11.44%), indirect somatic embryogenesis (54.23%) and mean numbers of somatic embryo(s) per explant (160.08), whereas culture medium MS2D.5Td (MS + 2.0$mg.l^{-1}$ 2,4-D + 0.5$mg.l^{-1}$ TDZ) promoted indirect organogenesis (20.38%). Inoculation medium MS2Td.5N (MS + 2.0$mg.l^{-1}$ TDZ + 0.5 $mg.l^{-1}$ NAA) proved superior for direct organogenesis (9.48%) and regeneration of plantlets *via* direct organogenesis (36.69%). MS medium fortified with 2.0 $mg.l^{-1}$ TDZ and 1.0 $mg.l^{-1}$ GA_3 proved superior for plantlet regeneration *via* somatic embryogenesis (163.63%), while regeneration medium MSTd.5GA.5N (MS +1.0 $mg.l^{-1}$ TDZ+ 0.5 $mg.l^{-1}$ GA_3 +0.5 mg. l^{-1} NAA) regenerated plantlets *via* indirect organogenesis (141.25%). The *in vitro* raised plantlets were acclimatized and established successfully in the field (Bele *et al*., 2012).

Tripathi *et al*. (2017a) were cultured three explants *viz*: mature cotyledons, hypocotyls and mature embryos of sandalwood on different fortifications of MS medium to judge their *in vitro* response. *In vitro* morphogenesis (somatic embryogenesis and/or organogenesis) leading to plantlet regeneration was influenced significantly due to different plant growth regulators. Induction media MS2D (MS + 2.0 mgl^{-1} 2,4-D) proved adequate for callus induction. Culture media MS.5D.5B/MSD.5B(MS + 0.5$mg.l^{-1}$ 2,4-D+0.5mgl^{-1}BA/ MS + 1.0 mgl^{-1} 2,4-

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D+0.5mgl^{-1}BA) induced direct somatic embryogenesis and average number of somatic embryo per explant in higher frequencies. Induction media MSD.5B/ MS2D.5B (MS + 1.0 mg.l^{-1} 2,4-D+0.5mgl^{-1}BA/ MS + 2.0 mgl^{-1} 2,4-D+0.5mgl^{-1}BA) enhanced the frequency of indirect somatic embryogenesis. Nutrient media MS2N.5Td (MS + 2.0 mgl^{-1} NAA+0.5mgl^{-1}TDZ) promoted direct organogenesis and plantlet regeneration *via* direct organogenesis and inoculation medium MSN.5Td (MS + 1.0 mgl^{-1}NAA+0.5mgl^{-1}TDZ) supported indirect organogenesis. Regeneration medium MS2TdGA(MS + 2.0 mgl^{-1} TDZ+1.0 mgl^{-1}GA_3) regenerated plantlets in higher frequencies from somatic embryos, while plantlet regeneration *via* indirect organogenic mode was attained in higher ratio on regeneration medium MSTd.5GA.5N (MS +1.0 mgl^{-1} TDZ+1.0 mgl^{-1}GA_3+0.5 NAA) for the most of the explants cultures.

In other experiment, Bele *et al*.(2019) were cultured nodal segments of sandalwood on MS medium amended with different plant growth regulators in varying concentrations to search out higher *in vitro* response leading to plantlet regeneration *via* somatic embryogenesis and/or organogenesis. Higher proportion of direct somatic embryogenesis, number(s) of somatic embryo per explant and plantlet regeneration *via* direct organogenesis were recorded on MS medium supplemented with a moderate concentration of TDZ (1.0 mgl^{-1}) in combination with comparatively a lower concentration of NAA (0.5 mgl^{-1}). A relative higher concentration of BAP (1.0-2.0 mgl^{-1}) in combination with a lower concentration of NAA (0.5 mgl^{-1}) promoted frequency of indirect somatic embryogenesis. Ratio of organ formation directly from surface of cultured explants was recovered from culture medium fortified with a higher concentration of BA at the concentration of 4.0 mgl^{-1} in combination with a lower concentration of NAA (0.5 mgl^{-1}). Maximum plantlets regenerated *via* somatic embryogenesis (direct and/or indirect) on regeneration medium supplemented with 2.0 mgl^{-1}TDZ in combination with 1.0 mg l^{-1}GA_3, while plantlets in higher frequencies *via* indirect organogenesis was attained with regeneration medium amended with comparatively lower concentration of TDZ (1.0 mg l^{-1}) in combination with 0.5 mgl^{-1} GA_3 and 0.5 mg^{l-1} NAA.

***Withania somnifera* (L.) Dunal**

Withania somnifera (L.) Dunal*]*, commonly known as Indian ginseng belongs to the family Solanaceae, having enormous medicinal and aromatic properties and has been included in ancient text of Ayurveda. It is used as an abortifacient, amoebocide, anodyne, bactericide, contraceptive, diuretic and spasmolytic. Biological assays label the plant as having the properties against different diseases like leprosy, nervous disorders, diseases of respiratory and reproductory tract, venereal disorders, rheumatism, inflammation, psoriosis, bronchitis, asthma, consumption, ulcers, scabies, marasmus of children, insomnia, senile debility, alexipharmic, carbuncles, cancer, epilepsy, diabetes *etc*. It can be propagated both by sexual and asexual method. Seed propagation, however is not always satisfactory, since the heterogenetically strain produces a great deal of variation. Again multiplication through cuttings give rise to less ramified plants and is consequently less productive than plants obtained from seeds. Tissue culture techniques can play an important role in the propagation and qualitative improvement of this medicinally important plant.

For higher regeneration potential, mature cotyledon, embryo and hypocotyls explants of two genotypes of *Withania somnifera namely*: JA-20 and MWS-100 were cultured by Jhankare *et al*. (2011a) on MS basal media fortified with different concentrations and combinations of various auxins and cytokinins. Considering higher *in vitro* response, culture medium MS2D.5B (MS + 2.0 mg.l^{-1} 2,4-D + 0.5 mg.l^{-1} BA + 30.0 g.l^{-1} sucrose + 7.5 g.l^{-1} agar) initiated calli in higher frequencies from cultured mature cotyledon and embryo explants, while for hypocotyl explant, induction medium MS2N.5Kn (MS + 2.0 mg.l^{-1} NAA + 0.5 mg.l^{-1} Kn + 30.0 g.l^{-1} sucrose + 7.5 g.l^{-1} agar) was proved superior for callus initiation. For formation of morphogenic calli and plantlet regeneration nutrient medium MS2B.5N (MS + 2.0 mg.l^{-1} BA + 0.5 mg.l^{-1} NAA + 30.0 g.l^{-1} sucrose + 7.5 g.l^{-1} agar) was investigated remarkably superior. Higher *in vitro* rooting response was achieved on rooting medium (MS + 2.0 mg.l^{-1} IBA + 15.0 g.l^{-1} sucrose + 7.5 g.l^{-1} agar). Higher *in vitro* morphogenic response was exhibited by explant mature embryo followed by explants mature cotyledon and hypocotyl. In terms of *in vitro* genotypic response genotype JA-20 was found significantly superior to MWS-100 for the most of the attributes investigated. Regenerated plantlets were established successfully in the field after hardening.

The embryogenic cell suspension culture was established by Jhankare *et al*. (2011b) from the embryogenic callus culture of *Withania somnifera* (L.) by transferring embryogenic callus cultures obtained from mature embryo and hypocotyl cultures in liquid media. The cultures obtained were swamped with clumps of proliferating globular embryos with modest non-embryogenic tissues. The number and size of somatic embryos/clumps was measured to compute growth of embryogenic tissues under various conditions. The suspensions were subcultured every 15 days by adding 10 ml of the old suspension to 40 ml of fresh medium. Initiation and proliferation of such embryogenic suspension culture depended upon the genotypes and various

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exogenous plant growth regulators supplemented to the culture medium at variable magnitude. Culture medium MS2D (MS + 2.0mg.l^{-1} 2, 4-D) was proved superior for callus induction from cultured mature cotyledons and hypocotyl explants. For the establishment of suspension cultures, the liquid MS medium fortified with 1.0 mg.l^{-1} 2, 4-D in combination with 0.5mg.l^{-1}BAP was found to be the most effective. For subsequent subcuturing, the reduced level of 2, 4-D (0.5 mg. l^{-1}) in combination with 0.5mg.l^{-1}BAP promoted faster development of somatic embryos. Frequent and efficient plantlet regeneration occurred on MS medium with 0.5mg.l^{-1} each of NAA,BAP and kinetin. In terms of genotypic effect, genotype JA-20 responded well as compared to MWS-100. Regenerated plants were found to be phenotypically normal and true to the type.

Nodal segment explants of two genotypes of *Withania somnifera viz*: JA-20 and MWS-100 were cultured on MS basal media fortified with different concentrations and combinations of diverse auxins and cytokinins as sole as well as in combinations. Considering higher *in vitro* response, culture medium MS2D (MS+2.0 mg.l^{-1} 2,4-D) initiated calli in higher frequencies (80.36%). Induction medium MS3B (MS + 3.0 mg.l^{-1} BA) initiated shoots in higher proportion (81.74%), while shoot(s) in higher numbers (25.69) and higher length (5.88 cm) were recovered from nutrient medium MS3B.5N (MS + 3.0 mg.l^{-1} BA + 0.5 mg.l^{-1} NAA (Jhankare *et al*., 2013).

Plumbago zeylanica

Plumbago zeylanica L., belongs to family Plumbaginaceae, is a rambling subscandent perennial herb or under shrub. Phytochemical screening of extracts of *Plumbago zeylanica* revealed the presence of several constituents, including plumbagin, linoleic acid, palmitic acid, nonylnonanoate, stigmasterol acetate, lupeol acetate, friedelinol, lupeol, lupanone, sitosterone and stigmasterol. Propagation of chitrak through seed is very difficult due to poor seed setting and quality, erratic germination and seedling mortality as under natural field conditions. Commercial exploration of wild grown plants has led to the rapid decline of this plant in its natural environment necessitating alternate strategies for cultivation and propagation.

Patidar *et al*. (2013a) cultured nodal segment and leaf explants of *Plumbago zeylanica* on three basal media fortified with different concentrations and combinations of diverse auxins and cytokinins as sole as well as in combinations. For cultured nodal segment, culture medium MS2N.5B (MS+2.0mg.l^{-1} NAA+ 0.5mg.l^{-1}BA) proved well for higher degree of callus induction (91.69%), while nutrient medium MS2B (MS+2.0 mg.l^{-1} BA) exhibited higher shoot proliferating efficiency (84.14%). Culture medium MS2B.5N (MS+2.0 mg.l^{-1} BA+0.5 mg.l^{-1}NAA) produced shoot(s) in higher numbers (11.12) as well as shoot of higher length (7.11 cm). Inoculation medium MS2N (MS+ 2.0mg.l^{-1}NAA) initiated callus in higher proportion (91.12%), however enhanced mrophogenic calli formation (45.58%) and plantlets regeneration were achieved on culture medium MSN.5Td (MS+1.0mg.l^{-1}NAA + 0.5 mg.l^{-1} TDZ) from cultured leaf disc. In present study, full strength MS medium supplemented with either of IBA or NAA at the concentration of 0.1 mg.$l^{-1}$was found to be optimum for exhibiting higher *in vitro* rooting response *i.e*. root proliferation, number of roots and root length. Regenerated plantlets were established successfully in the field after hardening with normal phenotypic appearance.

A simplified procedure of embryogenic cell suspension culture were established by Patidar *et al*. (2017) from the friable embryogenic callus of *Plumbago zeylanica* L. by transferring 6-8 weeks-old embryogenic calli achieved from nodal segment and leaf disc explants in liquid media. The cultures obtained were swamped with clumps of proliferating embryos of different developmental stages with modest non-embryogenic tissues. The number and size of somatic embryos/cell clumps was recorded to calculate growth rate of embryogenic tissues under different conditions. Initiation and proliferation of embryogenic cell suspension culture was manipulated by supplementation of diverse exogenous plant growth regulators to culture medium at variable concentration. For the establishment of cell suspension cultures, MS medium fortified with 2.0-3.0 mgl^{-1} 2,4-D with 0.5 mgl^{-1} BAP was found to be the most effective. For subsequent sub culturing, the media containing with 2.0-mgl^{-1} 2,4-D or the reduced level of 2,4-D (1.0 mgl^{-1}) in combination with 0.5 mgl^{-1} BAP supported somatic embryogenesis at a faster rate. Frequent and efficient plantlet regeneration occurred on MS medium amended with 0.5 mgl^{-1}, each of BAP, TDZ and NAA. Full strength MS medium added with either of IBA or NAA at the concentration of 0.1 mg.l^{-1} was found to be optimum for exhibiting higher *in vitro* rooting response *i.e*. root proliferation, number of root(s) and root of higher length. Regenerated plantlets showed normal development and established successfully in the field after hardening with normal phenotypic appearance.

Aloe barbadensis

Aloe vera syn *barbadensis* belongs to the Liliaceae family and is an important medicinal plant. Aloe has been used for pharmaceutical, food, and cosmetic industries. Some of the most important pharmacological activities of *A. vera* are antiseptic anti- tumor anti-inflammatory wound and burn healing effect anti diabetic and as an adjunct to current AIDS therapy. It is xerophytes and can be grown even in dry lands under rain fed conditions.

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Key constituents present in leaves are Anthraquinones (aloin, *Aloe* - emodin), Resins, Tannins, Polysaccharides *etc*. These qualities have prompted industrial and commercial increase in the production of *Aloe vera* worldwide. The main constraint in this production chain is to obtain healthy primary culture for a sustainable supply of the plant materials. *Aloe vera* is mainly propagated by means of suckers or off shoots. There are two drawbacks in the traditional propagation of this plant; which are, slow propagation rate of auxiliary shoots and male sterility. Furthermore, propagation is too slow for commercial plant production. To encounter these problems, several groups have used ***in vitro*** conditions and have had varying success. Therefore, it is an urgent need to develop protocol for fast multiplication of plantlets through micropropagation method to fulfill the increasing demand of planting material for cultivation. The technique of tissue and organ culture is used for rapid multiplication, genetic improvement, obtaining disease-free clones and preserving valuable germplasm.

Uikey *et al*. (2013) cultured stem disc explant of *Aloe* on MS basal medium containing different auxins, cytokinins as sole as well as in combinations. Culture medium MS2D (MS+2.0 mg.l^{-1} 2,4-D) initiated callus in higher proportion. Proliferation medium MS3B (MS+3.0 mg.l^{-1} 2,4-D) demonstrated higher shoot proliferation efficiency (90.41%) as well as number of shoot (s) / responding explant (9.67), while nutrient medium MS3B.5N (MS+3.0 mg.l^{-1} NAA+ 0.5 mg.l^{-1}NAA) produced shoot of higher length (7.04 cm). Higher *in vitro* rooting response viz: root proliferating efficiency (92.88%), number of root (s) (9.75) and mean root length (9.10 cm) was shown by rooting medium MS2IB (MS+ 2.0 mg.l^{-1} IBA). Regenerated plantlets were established successfully in the field after hardening. Phenotypically normal plants were regenerated.

FLOWERING AND ORNAMENTAL PLANTS

Amaryllis

Amaryllis belladonna L. is a perennial bulbous plant that belong to the family Amaryllidaceae. Worldwide the plants have greatest economic value as ornamentals. Plants of amaryllis are suitable for planting in the bed, pot, rockery, shrubbery and greenhouse. In addition huge number of plants are traded for conventional medicines. Africans use bulbs and leaves as poultices and decoctions for treating sores and digestive disorders. A number of species of the genus *Amaryllis* have been used in folk medicine, including *Amaryllis belladonna* L. (also named *Hippeastrum equestre*).Several pharmacological activities are attributed to several typical alkaloids they synthesize. Its *in vivo* propagation can be accomplished by using seeds, offsets and bulblets. However, multiplication of plant from seed showed wide variation in flower color, plant shape and time of flowering. Under such circumstances, the use of biotechnological advances particularly micropropagation proffers an exclusive substitute for mass multiplication of true type plantlets.

An efficient method for rapid propagation of *Amaryllis belladonna* L. has been developed by Sarathe *et al*. (2013) by culturing bulb scales and leaf discs explants on MS medium amended with diverse auxins and cytokinins in varying concentrations as sole as well as in different combinations. For bulb scale culture, culture media MS3N.5B (MS+ 3.0 mg.l^{-1}NAA + 0.5 mg.l^{-1} BA) proved well for callus initiation. Culture media MS3B(MS+ 3.0mg.l^{-1} BA) exhibited higher shoot proliferating efficiency, while number of shoot (s) per explant and shoot of higher length were documented on nutrient medium MSB.5N (MS+ 1.0 mg.l^{-1}BA + 0.5 mg.l^{-1} NAA). For cultured leaf disc, inoculation medium MS2D.5B (MS+ 2.0mg.l^{-1}2,4-D + 0.5 mg.l^{-1} BA) initiated higher degree of callus induction. Elevated mrophogenic calli formation was displayed by culture medium MS2N.5B(MS+ 2.0mg.l^{-1}NAA + 0.5 mg.l^{-1} BA) and plantlets in higher number (s) were regenerated on regeneration medium MS.5Td(MS+ 0.5. mg.l^{-1}TDZ). Higher *in vitro* rooting responses *i.e*. root proliferating efficiency, number of root(s) and root of higher length was recorded on culture medium MS2IB (MS+ 2.0mg.l^{-1}IBA).

Subsequently Veeraballi *et al*. (2017) cultured three explants *viz*: bulb scale, leaf disc and mature embryos on different modifications of MS medium to achieve the best *in vitro* response. For bulb scale culture, nutrient medium MS2N.5B proved well for callus initiation. Culture medium MS.4Td exhibited higher shoot proliferating efficiency, while number of shoot (s) per explants and shoot of higher length were documented on media MS2N.5B/ MS3N.5B. For cultured leaf disc, inoculation medium MS2D.5B/MS3D.5B promoted higher degree of callus induction. Higher morphogenic calli formation was exhibited by culture medium MS2N.5B/MSN.5B. However, plantlets in higher number was recovered from regeneration medium MS.5Td/MSB. For mature embryo culture, culture medium MS2D.5B proved well for callus initiation. Inoculation medium MSN.5B exhibited higher number of shoot proliferating explants. Number of shoots per explant in higher frequencies were obtained on medium MSN.5B/MSB. However, shoot of higher length was recorded on medium MSB. Higher *in vitro* rooting response (root proliferating efficiency, number of roots and mean root length) was exhibited by culture medium MS2IB.

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Gerbera

The Barberton/Transvaal daisy or gerbera which belongs to the family Asteraceae is one of the leading cut flowers and ranks among the top ten cut flowers in the world.The species, a perennial herb and native to South Africa and Asia is grown all over world in a wide range of climatic conditions. The flowers are hardy and stand the rigours of transportation for long. The genus *Gerbera* was named in honour of a German naturalist Traugott Gerber, who travelled Russia in 1743. The genus consists of about forty species. Out of the recorded species, only one species *Gerbera jamesonii* is under cultivation. The flower makes an excellent choice for any gift basket intended to brighten someone's day, or celebrate joyous occasions. The gerbera daisy has long been a symbol of beauty, purity and innocence.Gerbera can be propagated by both sexual and asexual methods. Most of the commercially grown cultivars are propagated through vegetative means to maintain uniformity and genetic purity. Among the vegetative means, multiplication through division of clumps is the most common method used for several decades. Gerbera can also be propagated through cuttings. The plant multiplication by these methods is too slow to be commercially practicable. For commercialization of this crop, planting material is required on large scale which requires the development of an easier, quicker and economically viable method of propagation. The use of biotechnological approaches especially micropropagation offers a unique alternative for mass multiplication of true to type plantlets.

Somatic tissues and seeds were tried for raising reproducible cultures in *Gerbera jamesonii* by Patidar *et al*. (2013b). Mature embryos and leaf sections were used as explants sources and cultured on MS medium supplemented with various auxins and cytokinins in varying concentrations as sole as well as in different combinations. Callusing was observed in both explants however shoot regenerations was obtained only from mature embryo explant. For mature embryo culture, culture media MS3D.5B (MS+3.0 mg.l^{-1}2,4-D+0.5 mg.l^{-1} BA) initiated calli in higher frequencies, while nutrient media MS2N.5B (MS+2.0 mg.l^{-1}NAA+0.5 mg.l^{-1} BA) promoted higher number of shoot proliferating efficiency, number of shoot (s) per responding explant and shoot of higher length. For cultured leaf disc, MS basal medium amended with 2.0-4.0 mg.l^{-1} auxins (2,4-D and/or NAA) promoted higher degree of callus induction, however, regeneration was not attained. Higher *in vitro* rooting response (root proliferating efficiency, number of root (s) and mean root length) was exhibited by rooting medium MS.1IB (MS+0.1mg.l^{-1} IBA).

Bhatt *et al*. (2016) cultured mature embryos on MS medium amended with diverse auxins and cytokinins in varying concentrations as sole as well as in different combinations. Culture media MS3D.5B (MS + 3.0 mg. l^{-1} 2, 4 D + 0.5 mgl^{-1}BA + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar powder) proved well for callus initiation. Culture media MS2N.5iP/MS3N.5ip (MS + 2.0/3.0mg l^{-1} NAA + 0.5 mgl^{-1}2-ip + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) exhibited higher *in vitro* response *i.e.* number of shoot proliferating explants and number of shoot (s) per explant. However, shoot of higher length was recorded on nutrient medium MSB/MS2B (MS + 2.0/3.0 mgl^{-1}BA + 30.0 gl^{-1} sucrose + 7.5 g l^{-1} agar). Higher *in vitro* rooting response (root proliferating efficiency, number of roots and mean root length) was exhibited by rooting medium MS.1IB (MS + 0.1 mgl^{-1}IBA + 15.0gl^{-1} sucrose + 7.5 g l^{-1} agar).

Bhatt *et al*. (2017) were cultured leaf disc on MS medium amended with different auxins and cytokinins in varying concentrations as sole as well as in diverse combinations. Induction medium containing 2.0-3.0 mgl^{-1} auxins (2,4-D or NAA) promoted higher degree of callus induction. Nutrient medium supplemented with 2.0-3.0 mgl^{-1} NAA proved well for formation of morphogenic calli and regeneration medium MSB proved remarkably superior for plantlet regeneration in higher frequency. Higher *in vitro* rooting response (root proliferating efficiency, number of roots and mean root length) was exhibited by rooting medium MS.1IB (MS + 0.1 mgl^{-1}IBA + 15.0gl^{-1} sucrose + 7.5 g l^{-1} agar).

Tuberose

Tuberose (*Polianthes tuberosa* Linn.) originated in Mexico belongs to family Amaryllidaceae is one of the important bulbous ornamental crop in India and being grown in tropical and subtropical areas. Commercially it is being grown in West Bengal, Karnataka, Tamilnadu and Maharashtra and Madhya Pradesh. Among the commercially grown flowers in India, tuberose occupies a prime position owing its popularity as a cut flower, loose flower, to flower loving people because of its prettiness, elegance and sweet pleasant fragrance, for perfumery as well as its potential as source of secondary metabolites. Tuberose can be propagated by seed as well as vegetatively by bulbs and bulblets. However, the requirement of planting material is more in this method because only one plant can be obtained from a bulb and this method is rather slow. Moreover, only single cultivars set seed and seeds are difficult to germinate. To meet the growing demand, massive *in vitro* propagation through tissue culture is the only option.

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Gajbhiye *et al*. (2011) developed a protocol to propagate four cultivars of tuberose *viz*: Phule Rajni, Shringar, Prajwal, and Mexican Single through direct *in vitro* organogenesis from stem disc explants. During the present investigation, culture medium Wh.3Td.5N (Wh+ 0.3 mg. l^{-1} TDZ + 0.5 mg.l^{-1} NAA + 20.0 g.l^{-1} sucrose + 7.5 g. l^{-1} agar) was found to be more responsive for shoot proliferating ability (98.90%) and shoot (s) per explant (11.48). Induction medium WH3N.5Td (Wh+ 3.0 mg.l^{-1} NAA + 0.5 mg.l^{-1} TDZ + 20.0 g.l^{-1} sucrose + 7.5 g. l^{-1} agar) was proved well for enhancing mean shoot length (5.56 cm). In terms of *in vitro* rooting response, culture medium Wh2I (WH+ 2.0 mg.l^{-1} IBA + 10.0 g.l^{-1} sucrose + 7.5 g. l^{-1} agar) was found consistently superior for all culture phases, *i.e*. root proliferating efficiency (96.24%), number of root (s) per responding shootlet (14.20), and average root length (7.56 cm). Among the four cultivars Mexican Single was found significantly superior followed by Prajwal, Phule Rajni and Shringar for the most of the attributes. Regenerated plantlets were established successfully in the field after hardening.

In order to regiment low-cost effective mass *in vitro* propagation protocol it was evident that there is no influential difference in response of cultured stem discs on different culture media, supplemented either with purified or commercial grade sucrose as well as with purified or commercial grade bacto agar. Thus the cost of *in vitro* mass propagation can be reduced significantly by supplementing commercial grade sucrose and bacto agar instead of purified sucrose and agar respectively in culture medium. To search out the responsive culture medium combination with higher regeneration potential, stem disc of *Polianthes tuberosa* was cultured on MS basal medium fortified with different auxins and cytokinins in varying concentrations and combinations. Culture medium Wh4D (Wh + 4.0 mg.l^{-1}2,4-D) proved well for callus initiation. Inoculation medium WhTd.5N (WH + 1.0 mg.l^{-1}TDZ+ 0.5 mg.l^{-1}NAA) exhibited higher shoot proliferating efficiency, while number of shoot (s) per explant in higher umber (s) was attained on culture medium Wh2Td.5N (Wh + 2.0 mg.l^{-1}TDZ + 0.5 mg.l^{-1}NAA). Nutrient medium WH.5B.5N (Wh + 0.5 mg.l^{-1} BA+ 0.5 mg.l^{-1} NAA) produced shootlet of higher length. Higher *in vitro* rooting response (root proliferating efficiency, number of root (s) and root of higher length) was displayed by rooting medium WhI (Wh + 1.0 mg.l^{-1}IBA). **(**Raghuvanshi *et al*.,2013).

Gladiolus

Gladiolus (*Gladiolus hybridus* Hort.) belongs to the family Iridaceae is one of the most important bulbous commercial ornamental plant grown for cut flowers. Due to its magnificent inflorescence with a variety of colors makes it attractive for use in herbaceous borders, beddings, pots and for cut flowers. It has a great economic value and wide market in the country. It propagates either by seed, corm formation or by cormel differentiation. Although, seed is an effective means of gladiolus propagation but seed-raised plants may not produce true type population. Additionally, it takes four seasons for blooming. The propagation by corm and cormel formation is another conventional method of multiplication but it may transmit several viral, fungal and bacterial diseases i.e. *Fusarium* corm rot, *Botrytis* blight, bacterial leaf spot *etc*., thus causing a heavy loss. These low propagation rates hinder the introduction of new varieties or virus-free plants. However, in gladiolus there is a clear scope for further refinement in *in vitro* culture methodology to acquire a higher number of shoots to complement traditional nursery methods. Plant tissue culture offers a potential to deliver large quantities of disease-free, true type healthy stock within a short span of time. The method guaranteed the identical reproduction of the parents tested and selected, and prevented genotypic alteration which would occur after the generative multiplication.

Corm slice explant of gladiolus (*Gladiolus hybridus*) was cultured by Tripathi *et al*. (2017b) on MS medium amended with diverse auxins and cytokinins in varying concentrations as sole as well as in different combinations. Regarding *in vitro* response, induction medium MS2B.5D (MS + 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} 2,4-D + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) proved well for callus initiation. MS medium supplemented with BA in range of 2.0-3.0 mgl^{-1} in combination with 0.5 mgl^{-1} NAA, 30.0 gl^{-1} sucrose and 7.5 gl^{-1} agar exhibited higher shoot proliferating efficiency, number of shoot (s) per explant and shoot of higher length. In respect to *in vitro* rooting response, higher root proliferating efficiency was documented on rooting medium MS.5IB.5Kn (MS + 0.5 mgl^{-1} IBA + 0.5 mgl^{-1} Kinetin + 15.0 gl^{-1} sucrose + 7.5 gl^{-1}agar), while number of root (s) with higher length were recovered on rooting medium MS.5IB (MS+0.5 mgl^{-1} IBA+15.0 gl^{-1} sucrose+ 7.5 gl^{-1} agar).

Cormel explant of gladiolus (*Gladiolus hybridus*) was cultured by Malviya *et al.*(2018). ()on different fortifications of MS medium amended with different auxins and cytokinins in diverge concentrations as alone as well as in different combinations. Regarding *in vitro* response, induction medium MS2D.5B/MS2N.5B 5D (MS + 2.0 mgl^{-1} 2,4-D + 0.5 mgl^{-1} BA + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar/ MS + 2.0 mgl^{-1} NAA + 0.5 mgl^{-1} BA + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) promoted higher degree of callus induction. Nutrient medium MS2N.5B/MSN.5B(MS + 2.0 mgl^{-1} NAA+ 0.5 mgl^{-1} BA+ 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar/ MS + 1.0 mgl^{-1} NAA+ 0.5 mgl^{-1} BA+ 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) exhibited higher shoot proliferating efficiency and shoot

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of higher length, However, higher number of shoot (s) per explant exhibited by regeneration medium MS3B/MS2B (MS + 3.0 mgl^{-1} BA+ 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar/ MS + 2.0 mgl^{-1} BA+ 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar). In terms of *in vitro* rooting, higher root proliferating competence was documented on rooting medium MS.5IB.5Kn (MS + 0.5 mgl^{-1} IBA + 0.5 mgl^{-1} Kinetin + 15.0 gl^{-1} sucrose + 7.5 gl^{-1}agar), while number of root (s) with higher length were recuperated on rooting medium MS.5IB (MS+0.5 mgl^{-1} IBA+15.0 gl^{-1} sucrose+ 7.5 gl^{-1} agar).

ROLE OF TISSUE CULTURE IN BIO-PROSPECTION

Production of secondary metabolites, biotransformation of intermediates into pharmaceutically important bio-molecules and genotype /chemotype designing in selected plant species are the areas where PTC has shown great progress. Phytochemicals, mainly plant secondary metabolites, are reservoirs of huge chemical diversity and unique chemicals, many of which are sources of medicines against several diseases. Nearly 65% of all FDA approved drugs are natural products, their analogues or derivatives. India hosts all major ecosystem–wetlands, mountain, desert, forest, marine, island and mangrove. 12% of world's flora has been identified in India, of which 35% is endemic to India. This has made India as one of the most sought after destination for biochemical and molecular bio-prospecting. However, it is important to take stock and catalogue the existing plant diversity to harness this resource in a fruitful manner. This would also help to establish the demand/supply relationship and decide on future conservation efforts. Cataloguing has to be done at several levels like mapping of bio-resources to geographical regions, preparation of detailed monographs that includes data from published literature and traditional knowledge.

In addition, metabolic profiling of medicinally important taxa is pivotal for identification of commercially acceptable chemotypes. Furthermore, molecular prospection from the selected plants would target at understanding the biosynthesis and regulation of metabolite production. Molecular bio-prospection from these identified plants would eventually pave way for transferring the biosynthetic machinery into a microbe for producing medicinally important phytochemicals them. This would not only help to reduce the costs, but also for bio-diversity conservation.

Uikey *et al*.(2014) quantified secondary metabolites in callus and cell suspension culture of *Rauvolfia serpentina*. Reserpine and ajmalicine were detected in one-month-old callus as well as in cell suspension cultures. Culture medium MSD.5IB (MS+1.0 mgl^{-1} 2,4-D + 0.5 mgl^{-1} IBA) indicates the highest recovery of reserpine content in both callus and liquid suspension medium of one -month age. Increasing concentration of 2,4-D in liquid medium drastically decreased reserpine content .Linearly decreased Ajmalicine concentration in both callus and cell suspension culture was recorded with increased concentration of 2,4-D.

Plumbazin is the major alkaloid responsible for medicinal activities of *Plumbago zeylanica.* Patidar *et al*. (2015) were quantified secondary metabolites in calli obtained from nodal segment and leave disc cultures and cell clumps/embryoid acquired from cell suspension culture of *Plumbago zeylanica*. Higher plumbazin amount was detected in one-month-old friable callus (0.428mg/100gm), cell clumps/embryoids (0.357 $mg.l^{-1}$) as well as in two-months-old rhizogenic calli (1.257 mg per gm) on culture medium MS3D.5IB ((MS+3.0 mgl^{-1} 2,4-D + 0.5 mgl^{-1} IBA). Linearly increased plumbagin concentration in both callus and cell suspension culture filtrate was recorded with increased concentration of 2,4-D.

ROLE OF PLANT TISSUE CULTURE IN CROP IMPROVEMENT

Plant tissue culture is an enabling *in vitro* technology from which many novel techniques have been developed to assist plant breeders. This tool enables us to understand the vast abilities of plants as a totipotent cell. Plant tissue culture has been exploited to create genetic variability from which crop plants can be improved. Tissue culture in association with molecular techniques have been used to transfer desirable both commercially and genetically traits.

The applications of various tissue culture approaches to crop improvement are;

- Development of transgenics against various biotic and a biotic stresses and improvement of quality.
- Somaclonal variation
- *In vitro* selection
- Wide hybridization (embryo rescue)
- Somatic hybridization and cybridization
- Production of double haploids

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Development of transgenics against various biotic and a biotic stresses and improvement of quality

Crop improvement is the prime element of agricultural advancements and there are still many areas to be worked on in the field of crop improvement through biotechnological interventions. When talking about gene transfer or transfer of desirable traits to the target plant, in future there might be an option of complete chromosome transfer *via* microinjection and it can confer multigenic traits.

Gene transfer by genetic engendering

Gene transfer between unrelated species of plants has been playing a very crucial role in crop improvement. By transforming genes many useful traits like resistance to insects, stress and disease has been transferred to many crop varieties from non-cultivated plants. Recombinant DNA methods and many other methods are in use for transformation of genetic information. Genetic engineering is a DNA recombination technique that has made possible gene transfer between dissimilar genera or species Genetic engineering is an exceptional way of breeding as compared to conventional breeding It is a way of extending genetic base. Secondly, as it avoids the problem of linkage drag associated with the conventional breeding it is more effective and it is less time consuming. Till now, many genetic engineered crops have been developed and commercialized that result in improved production efficiency, increased market focus and enhanced environmental conservation. Such crops include longer post-harvest storage tomatoes, insect resistant cotton and maize, virus resistant potato, herbicide resistant soybean and canola, and many other crops.

GENE TRANSFER THROUGH HYBRIDIZATION

Plant breeding and intraspecific gene transfer

In 19th century, plant breeding began with discoveries of how plant traits are inherited. Plant breeding could be carried out by selection of plants with attributes of interest and manipulating into cross fertilization. Improved variety with desired characteristics is formed when a cultivated variety is back crossed with a wild variety. In recent years plant breeders used inter-specific hybridization for gene transfer from a non-cultivated plant species and convertible crop species. For example *Avina sativa* (oat) *and Beta vulgaris* (sugar beet) has been transformed and resulted in increased yields 25-30% and sugar beet nematode resistance respectively.

Gene transfer by non-sexual methods

As plant cells, tissues and organs can be cultured *in vitro* so transfer of genes between plants is possible by non-sexual methods. Non-sexual gene transfer methods depend on ability to produce in certain plant species fully differentiated plants from non-sexual organs and tissues. Stems, pieces of leaves and different undifferentiated clumps of cells in culture can be used as starting material for regeneration. In some species, even a single somatic cell can be used. Cell fusion methods and recombinant DNA techniques for gene transfer have been used from many years. Here we will discuss some gene transfer techniques that are used for crop improvement.

Gene transfer by manipulating DNA directly

In 1940s, methods for transferring DNA directly from one organism to another organism developed as DNA established as a chemical base of genetic inheritance. Non-sexual DNA transfer techniques make possible manipulations that are outside the repertory of breeding and cell fusion techniques. Genes can be obtained from plant, animal, bacterial and viral sources and injected in crops. Tissue specificity, timing and level of gene expression is under control and it can be modified by gene modification into new host. These methods provide the source of diversity and allow controlling the expression of genes.

***Agrobacterium*-mediated gene transfer**

Agrobacterium tumefaciens is a plant-pathogenic bacterium that holds ability to transfer some part of its own genetic material into other plant species by a simple process called transformation. The genes encoded in a region of Ti plasmid called T-DNA. This causes tumorous growth called “crown gall” disease in plants. This bacterium is modified in lab and it transfers gene of interest into plants without causing symptoms of disease. The *Agrobacterium* system is appealing because of the easy protocol that is associated with minimum cost in terms of equipment and also the resulting transgenic plants have simple copy insertion. Many efficient vectors are designed that contain extra copies of virulence genes and are mutated that increases the level of expression of virulence genes For successful results we should test many parameters like feeder cells, infiltration of bacteria, *Agrobacterium* strains *etc*.

By using this method, genes for insect and disease resistance has been transferred. This is the most suitable method of non-sexual gene transfer and there are many useful crops that are tested and are good candidates for agriculture use. By recombinant DNA technique many plant and bacterial genes that encodes enzymes has been engineered that makes crop plants tolerant to broad spectrum and environmentally safer herbicide. For this bacterial gene is engineered in such a way that its enzyme is insensitive to herbicide and then transfer it to plant.

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This can also do by engineering plant so that they express genes that detoxify herbicide. Genes obtained from *Bacillus thuringienesis* has been engineered and transferred to plants that act as insecticides Bt cotton success and exploitation at commercial level in India is well known.

Biolistic transformation

Biolistic transformation is the process of delivery of micro projectiles that are of tungsten or gold coated with DNA and push into the target cells by acceleration. Acceleration provided by electric charge, CO_2, gun powder and by gases and DNA can introduced into and tissue. This method has some limitations *e.g*. it reveals a complex pattern of transgene integration, the delivery of long fragment DNA is challenging and it is more expensive in terms of equipment.

Microinjection

The microinjection technique is a direct physical approach, for introducing substances under microscopic control into defined cells without damaging them. By means of micropipettes, DNA solution is introduced into plant protoplasts. Microinjection can be used with crop species from which whole plant can be obtained from single transformed cells.

Somaclonal variation

Chromosomal variations induced by tissue culture are observed in many crops. Molecular and transposable variations are also present. A number of serious attempts are being made to produce crops by introducing somaclonal variations. Hence a large amount of cytoplasmic and nuclear genetic alterations are made to bring about phenotypic variations. A new type of hybrid plants and clones are being made with the improved traits through somaclonal breeding. This is considered the safest technique to produce plants with desired traits. A large number of clones are required to produce desired results on large scale.

***In vitro* selection**

It is now well known fact that *in vitro* culture of higher plants can be used for selection of mutants. Protoplast, cell suspension and callus cultures are handled like microorganisms to search for biochemical mutants. Selection for resistance is the most straightforward method for mutant selection, whereby resistant cells in a large population can be selected by their ability to grow in the presence of inhibitor while the sensitive cells do not. The differentiated culture (callus) is subjected to selection against inhibitors like antibiotics, amino acid analogues, pathotoxins etc. These compounds are put in the medium at a concentration such that some cell populations survive and can be further grown on a selective medium. Different selection cycles are performed to get tolerant cells/callus cultures that are subsequently regenerated in to plants. These plants are then *in vivo* screened against the inhibitor. If the plants are resistant to the inhibitor, then stable transmission of that character is analyzed in subsequent generations. In this approach, variants for a particular character are selected rather than the general variation obtained in first case where selection is done at the plant level.

It is well recognized that variations occur during cell division and differentiation *in vivo*. Meristem cells, which serve like a 'germ line', are generally immune to such genetic changes. In the normal life cycle of a plant, the mutant somatic cells are eliminated during sexual reproduction and are not passed on the progeny. Such mutant cells, however, have an excellent opportunity to divide and multiply (as do non-mutant cells) when plant tissues are placed in a culture. Imposing selection pressures on cultured cells can result in preferential growth of mutant cells establishing mutant cell lines from which whole plants are recovered. The procedure is useful only for single gene traits, such as resistance/tolerance to certain pathogenic toxins, herbicides antimetabolites, and heavy metals and for screening mutants that over produce useful amino acids.

In vitro selection for salt tolerance is commonly occurring as temporary adaptation. Cells are being able to store extra salts in the vacuoles and survive by adjusting the osmotic pressure. This results in the production of salt tolerant halophytes and well adapted to high salt environment and become unable to grow without salt. Tobacco salt tolerant cell lines are produced. Different other varieties like stress, drought, and heat tolerant varieties are also successfully formed..The various *in vitro* technologies could easily simplify breeding programmes and overcome some important economical and agronomic traits that would never be produced from conventional ways of plant breeding and plant improvement.

WIDE HYBRIDIZATION (EMBRYO RESCUE)

Crops used to produce from tissue culture technology facilitate the interspecific and intergeneric crosses to overcome physiological based self-incompatibility. A vast variety of crops has been recovered through IVF *via* pollination of pistils and self and cross pollination of ovules. Agricultural crops like tobacco, clover, corn, canola, cole, poppy, cotton *etc*. The use of delayed pollination, distant hybridization, pollination with abortive and irradiated pollen and physical and chemical treatment of host ovary have been used to implied haploidy.

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Embryo culture is another kind used to make crops valuable. Orchids, roses, bananas are being formed by embryo culture.

SOMATIC HYBRIDIZATION AND CYBRIDIZATION

Plant protoplasts offer exciting possibilities in the fields of somatic cell genetics and crop improvement. The technique of hybrid production through the fusion of isolated somatic (body), protoplasts under *in vitro* conditions and subsequent development of their product (heterokaryon) to a hybrid plant is known as somatic hybridization. This procedure eliminates sex altogether in hybridization. In the somatic hybridization, the nucleus and cytoplasm of both parents are fused in the hybrid cell. Sometimes, nuclear genomes of only parent but cytoplasmic genes (plastome) from both the parents are present in the fused hybrid, which is known as cybrid or cytoplasmic hybrid. Thus, protoplast fusion technique can be used to overcome the barriers of incompatibility and acts as a method for the genetic manipulations of plant cells.

Methods to prepare large number of single plant cells without their cell walls (protoplast) were developed in 1960s. Fusion could be induced among protoplasts of various plant cells by using electroporation technique and certain chemicals and liposomes. Callus tissue produced from somatic hybrid when grown *in vitro*. In certain species a whole plant can be regenerated from this callus tissue. Sexually incompatible species could have their choromsomes combined by the use of cell fusion method.

PRODUCTION OF DOUBLE HAPLOIDS

The term haploid refers to those plants, which possess a gametophytic number of chromosomes (single set) in their sporophytes. The interest in haploids stems lagely from their considerable potential in plant breeding, especially for the production of homozygous plants and in their studies on the detection of mutations. Ever since, Guha and Maheshwari (1964) developed haploid plants in *Dhatura inoxia* plant breeder have worked intensively to obtain haploids either *in vivo or in vitro*. The success achieved using *in vitro* methods has been spectular and there are reports on haploid being evidenced by anther culture or pollen culture from nearly 250 species and their hybrids. However, their exploitation remained restricted because of low frequency (0.001-0.01) with which they occur in nature.

Anther culture is technique by which the developing anthers at a precise and critical stage are excised aseptically from flower buds and are cultured on a nutrient medium where the microspores within the cultured anther develop into callus tissue and/or embryoids. Such calli and/or embryoids give rise to haploid plantlets either through organogenesis or embryogenesis.

CONCLUSION

The rising commercial demand of horticultural and medicinal plants crossing beyond their sustainable limits of extraction from the wild has alarmingly reduced their natural status and even extirpated habitats. Conservation of biodiversity of horticultural and medicinal plants and keenness to conserve these through biotechnological applications have received considerable attention. Progress in *in vitro* conservation methods, e.g. tissue culture, artificial seeds and cryopreservation, and DNA banks have facilitated conservation of rare and endangered plants of medicinal value suitable for cultivation in suitable areas. Biotechnology and biodiversity of high value medicinal plants can be harnessed together as developmental challenge as well as an economic opportunity in future.

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GENOMICS ASSISTED MOLECULAR BREEDING FOR CROP IMPROVEMENT: STATUS AND PROSPECTS

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ABSTRACT
Functional molecular markers and advances in bioinformatics is generating new tools gradually in genomics research that could increase the efficiency and precision of crop improvement. Eventually, relative values of targeted alleles at specific locus in a segregating population could allow the breeder to improve any genotype for particular trait in silico and to do whole genome selection.Genomics is most powerful tool for deciphering the stress responsiveness of crop species with adaptation traits or to identify underlying genes, alleles or quantitative trait loci. Molecular breeding approaches are most efficient in enhancing the biotic and abiotic stress adaptation of crop plants, and recent advances in high throughput genotyping, sequencing and phenotyping platforms (phenomics) have transformed molecular breeding to genomics assisted breeding (GAB). Most commonly used approaches for genomics assisted breeding are marker assisted selection (MAS) and genomic selection (GS). MAS, includes marker assisted backcrossing, gene pyramiding, mapping for associated targeted traits by specific genes or QTLs, fine mapping of QTL region etc. GS, on the other hand, uses all available marker data for a population to predict breeding value. The development of improved breeding lines for commercial crop cultivation by traditional methods is time consuming and expensive task. With the deployment of genomics assisted breeding, the generation of improved breeding lines has become easier and faster. Although, marker assisted breeding and selection is gradually evolve into genomicsassisted breeding for crop improvement but high costs is major limitation.Despite of that, to meet the demands of the increasing human population and to develop varieties adaptive to changing climatic condition, we must accelerate our current breeding practices and apply genomics based selection approaches.

Keywords: Crop Improvement, Genomics Assisted Breeding, Genomic selection, phenotyping, Marker Assisted Selection

INTRODUCTION
Crops are the major source of food supply, nutrition and industrial raw materials. Global food consumption is increasing day by day as compared to crop yields. Crops improvement for yield and nutrients are major trust are for researchers. Plant biotic and abiotic stresses including diseases, insects, and unpredictable environmental conditions are causing serious yield losses, which, results in shortage of food material for increasing global population.Thus, sustainable crop productivity requires crop breeders tocontinuously release new varieties with high yield potential,high quality, resistance/tolerance to biotic/abiotic stresses,high nutrition-use efficiency, etc.Plant breeding has made great progress in the lastcentury (Zamir 2001). Conventional breeding mostlydepends on phenotypic selection based on breeders'experiences, which resulted in the release of large numbersof high-yielding varieties. The major limitations of conventional breeding is, labor intensive, timeconsuming, less reproducible, and environment dependence, etc. With advances in marker technology, high-throughput genotyping, and Next generation sequencing technology the focus of plantbreeding has gradually switched from phenotype-basedto genotype-based selection. Numerous Marker Assisted Selection (MAS) strategies have been developed for crop improvement: marker-assisted backcrossing, introgression of majorgenes or quantitative trait loci (QTL), selection for quantitativetraits using markers at multiple loci and fine mapping of QTL region to identify targeted trait gene (Hospital *et al.* 1992; Gupta *et al.* 2010). Over thelast two decades, the rapid development of whole-genomesequencing and marker development technologies enabledthe use of high-density single nucleotide polymorphism (SNP) markers to analyze the whole genome at very lowcost.Integration of genomics tools and conventional breedingtriggers new breeding strategies, like gene pyramidingand genome selection (GS), which greatly accelerates thebreeding. In recent years, genomics-assisted breeding (GAB) has become a powerful strategy for plant breeding (Fig.1). GAB enables the integration of genomic tools withhigh throughout phenotyping to assist breeding practicesthrough molecular markers to facilitate the prediction ofphenotype from genotype (Fig. 1). GAB allows breedersto start out with a large population of only genotypicallycharacterized offspring, and then only use a selected subsetfor more expensive phenotypic evaluation. In addition, genotypic evaluation can be done offseason, which helps to speed up breeding. GABis especially useful for the improvement of complex traits dueto its advantages of high accuracy and high selection efficiency. The ultimategoal of GAB is to find the best combinations of alleles (orhaplotypes), optimal gene networks, and specific genomicregions to facilitate crop improvement (Xu *et al.*,

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2012; Carley *et al*., 2017). Genomic tools offer information at bothDNA and transcriptome levels with reduced environmentaleffects. Meanwhile, to better manage genetic resources, the core collection needs to maintain its genetic diversitywith updated genetic information.

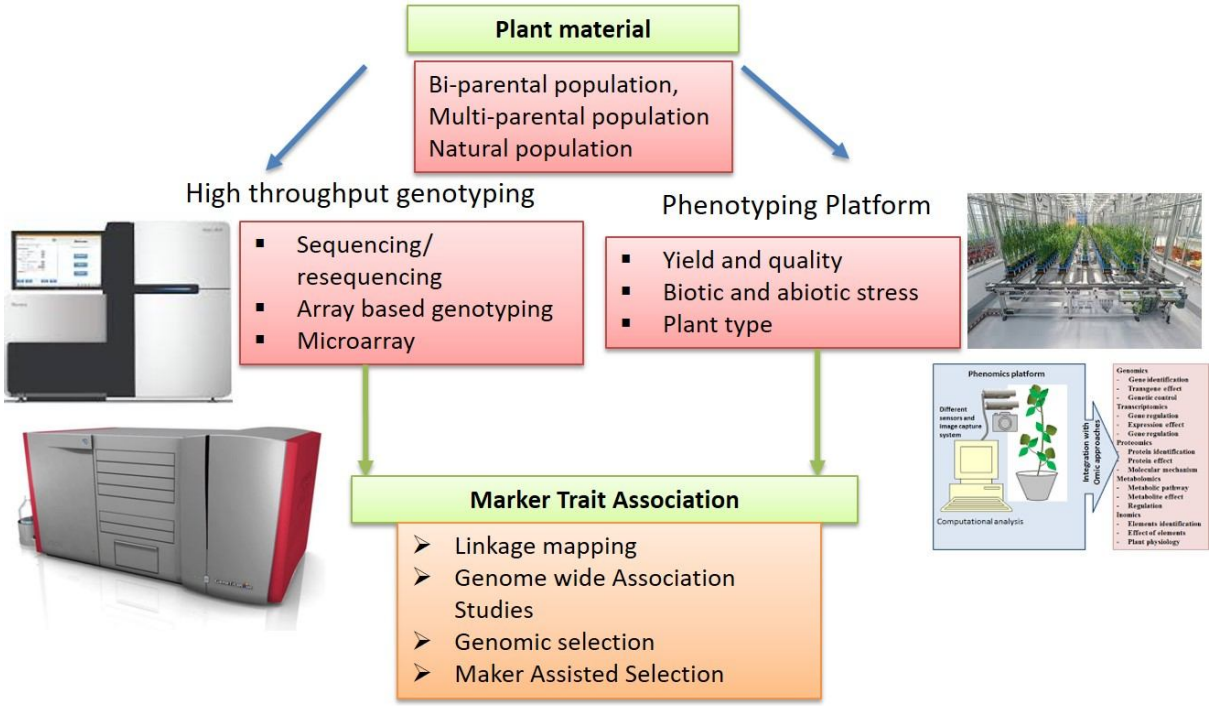

Fig-1: Overview of genomics assisted breeding

MARKER ASSISTED BREEDING

Primarily molecular markers have been used for MAS during backcross programs for the introgression of disease resistance genes. In most such cases, two or more genes have been pyramided to achieve durable resistance to the concerned pathogens of targeted trait. The first variety developed by MAS was a maize hybrid released in the USA for commercial cultivation in 2006 by Monsanto, USA. Since then, several varieties developed by MAS, often improved versions of popular varieties produced through MABC, have been released for commercial cultivation. In India rice varieties, Improved Pusa Basmati 1, Improved Samba Mahsuri and Swarna Sub-1, and maize hybrid Vivek QPM 9 has been released by applying MAS. The molecular markers have enabled detection and mapping of Quantitative Trait Loci, which was not possible with only morphological or even protein-based markers and testing the purity of inbred parents. The markers have made selection independent of the phenotype, the desirable plants can be selected in the seedling stage, and the selected plants can be used for hybridization in the same season. In addition, MAS allows easy pyramiding of oligogenic resistance and combining of horizontal resistance with vertical resistance which is considerably difficult on the basis of disease tests. Closely linked molecular markers have been used for positional cloning of a number of plant genes. Molecular markers have stimulated the development of novel breeding schemes like GS and GAB Schemeto develop varieties with superior adaptation.

HIGH THROUGHPUT PHENOTYPING

A critical component for accelerating the development of new, improved crop varieties is rapid and precise phenotypic assessment of thousands of breeding lines under field conditions. The lack of phenotyping platforms that can efficiently identify individuals exhibiting rare, optimal genotypes from large populations is a major bottleneck for crop improvement. Furthermore, because many agriculturally important traits are influenced by interactions of genotype and environment, field-scale phenotyping is required. Despite technological innovations that characterize genomes quickly and cheaply, and computational methods that continue to improve the analysis of such large data sets, the ability to rapidly and accurately measure plant performance in the field remains a limiting factor in plant breeding and genetics. Field-based high-throughput phenotyping (HTP) is an emerging tool with the potential to accelerate genetic discovery and to identify genetic combinations that will allow more rapid selection of high yielding varieties (Tanger *et al*., 2017; Rouphael *et al*., 2018). These platforms have been successfully deployed in cotton and maize, and the rapid nature of data collection has even enabled analyses of temporally dynamic traits. Recently, an automated method for high-throughput screening of *Arabidopsis* rosette growth in multi-well plates allowing measurement of 11,000 plants

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in less than 2 h (De Diego *et al.* (2017). High-throughput phenotyping technologies are receiving increasing attention for purposes of product screening and development as efficient means to (1) automated, non-destructive online monitoring of several morpho-physiological traits of plants; (2) Observations for the progression of growth, plant performance, and stress responses of individual plants with high accuracy (3) reduced cost, labor, and time for analyses. High-throughput phenotyping technologies have successfully been employed in plant breeding for morpho-physiological characterization of many crops.

HIGH THROUGHPUT GENOTYPING

High throughput genotyping is being applied by using SNP array, which is a relatively cost-efficient, and automatically genotyping assay. It has been widely used in genetic studies of crops, including genome-wide association studies (GWAS), linkage map construction, genomic selection, population structure analysis and gene mapping (Xu *etal.*, 2002). Currently, the most popular high throughput genotyping platforms are the hybridization based SNP array and various NGS enabled genotyping such as GBS and GS (Qian *etal*., 2018, Cooper *etal*., 201; Carley *et al.*, 2017, Fei *etal*., 2017). SNP array is a type of DNA microarray containing designed probes focusing on the SNP positions, which is hybridized with fragmented DNA or cDNA to determine the specific alleles of all SNPs on the array for the hybridized DNA sample of targeted trait. Many SNP arrays have been successfully applied in diploid species genotyping, such as the Apple (480K SNP array), the Maize (600K SNP array), tetraploids peanut (58K), hexaploids wheat (820K), octoploids strawberry (90K), dodecaploids (sugarcane) (345K) and the Rice (700K SNP array). For high-throughput genotyping of crops, SNP array has several advantages over traditional marker based genotyping and NGS approaches. Some of the points includes, SNP array data is relatively easy to analyze compared to data generated using NGS-based methods, labor intensive NGS library preparationand bioinformatics data analysis investment for accurate SNP calling. However, SNP array has its own shortcomings, it required prior genomic information, and in some case manual dosage scoring. Efforts have been taken to reduce theselimitations such as adopting whole genome sequencing with high coverage and updating the markers on the SNP array.For SNP marker selection in development of the array, Illumina and Affymetrix platform are being applied widely. SNP Array selection should include some of the general and important features as SNP depth, SNP types, SNP frequency, additional variations within probe sequence of target SNPs, and probe sequence parameters. Specifically, (1) accuracy of SNPs called can be relate withthe average SNP read depth, or single genotype SNP depth. If the depth is too low, sequence errors could be considered for SNP call. If the depth is too high, the SNPs may be called from repetitive sequences. (2) There are two types of SNPs: transition SNPs (purine/purine or pyramidine/pyramidine i.e., A/G, T/C) and transversion SNPs (purine/pyramidine or pyramidine/purine i.e., A/T, C/G, A/C, and T/G). For SNP array development, the transition SNP type is preferred and transversion SNPs, multiple allelic SNPs and INDELs, are excluded to make array more reproducible (Clarke *et al*., 2016).

MARKER TRAIT ASSOCIATION

For marker and trait association both phenotypic and genotypic data analysis give appropriate results. Linkage mapping is a classical method towards the dissection of the genetic basis of quantitative trait loci for targeted traits.In addition, with the advancement of bioinformatics and massive genetic information, a meta- QTL analysis was developed by integrating published QTL to detect consistent and most effective QTL for trait of interest (Van and McHale 2017).Genome-wide mapping in large populations moved towards the target gene mapping and cloning in crops (Cooper *et al.*, 2014). The availability of high-density SNP markers has opened a way for genomewide association study (GWAS), an approach using natural populations. GWAS could overcome several constraints of conventional linkage mapping and provide a powerful complementary strategy for dissecting complex traits. Genomic selection (GS) predicts genomic estimatedbreeding values of lines by analyzing traits andhigh-density marker scores within an artificially createdpopulation at the whole-genome level (Crossa *et al*. 2017). GS is another promisingbreeding strategy for rapid improvement of complextraits.Although stillcostly, GS has been proved to be superior to markerassistedrecurrent selection for improving complex traits incrops, as it can effectively avoid issues associated with thenumber of QTL that control a trait. Newly developed genetic and genomics tools will enhance, but not replace, conventional breeding and evaluation processes.Although conventional breeding techniques have significantly increased crop production and yield, new approaches are required to further improve crop production in order to meet the global growing demand for food. The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 (CRISPR-associated protein9) genome editing technology has shown great promise for quickly addressing emerging challenges in agriculture and has already been a useful tool for crop improvement including yield and durable resistance. Newly developed genetic and genomics tools will enhance breeding and evaluation processes of crop improvement.

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MAGNETIC FIELD AND ITS IMPACT ON PLANT GROWTH AND DEVELOPMENT

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ABSTRACT
Plant magnetobiology has become an important topic to study and manage biological effects on plants.Studies over the past several years have paved its way asa new advancementfor achieving enhanced growth and development in crop plants including horticultural plants.An emphasis has been made upon the use of this technique with different magnetic field intensity and exposure. The precisemechanism of its action is not known, but biological and chemical cues are being studied as possible mechanisms. This article presents an overview of 'magneto priming' for the enhancement of growth and yield of various plants.

INTRODUCTION
All the living organisms in this ecosystem are under the influence of Earth's geomagnetic field (GMF) and it is believed that all the life forms have evolved in the presence of GMF- whether it be geotaxis in magnetotactic bacteria, cellular responses in humans or stimulation of growth in plants(LefèvreandBazylinski2013).Amagnetic field (MF) is a vector field in the neighborhood of constant magnets or electric currents that is specified by both direction and strength and is characterized by magnetic flux density (measurement in T) and MF strength (measurement in amperes [A]/meter [m]).The magnitude of the Earth's magnetic field at its surface ranges from 25 to 65 μT (0.25 to 0.65 gauss). A German botanist in 1930 Ssawostin first reported faster growth stimulation of wheat coleoptiles under magnetic fields followed by Murphy in 1942 concluding that germination rate of different types of seeds would accelerate underMF treatment.Developments in the area ofmagnetobiology began in the 1960's with the development of space biology and the effects on different life forms separately including studies on migratory birds (Wiltschkoand Wiltschko 1972)and honeybees(Gould 1980). In the subsequent period, magnetoorientation of magnetotactic bacteria (Blackmore 1982)was studied under bacterial magnetotaxis.

Initial studies of MFeffectsonplants were conducted by KrylovandTarakonova(1960)who experimented seed germination under MF treatment and suggested that the effects were mostly anauxin-likeand termed the phenomenon, magnetotropism. There have been several studies on the effects of plants and these have been reviewed (Maffei 2014).To investigate the magneto sensitivity of plants, four different types of magnetic fields have mainly been employed:(1) weak static homogeneous magnetic fields, (2) strong homogeneous magnetic fields, (3) strong in-homogeneous magnetic fields and (4) extremely low frequency (ELF) magnetic fields of low to moderate magnetic flux densities.

Studieshave shown that magnetic fields have significant effect on seed germination, plant growth, development, and yield, depending upon a specific species and on the characteristics of field exposure such as intensity and duration with difference in their growth pattern (Teixeira da Silva and Dobránszki 2016). For example, a significant decrease in the cell numberof onion (*Allium cepa*) meristemshas been observed. Barley (*Hordeum vulgare*) seedlings grown in Helmholtz coils with a 10 nT MF intensity showed a decrease in fresh weight of shoots and roots, as well as dry weight of shoots and roots in comparison with GMF controls. In another study, it was concluded that very low MF was capable of delaying both organ formation and development (Lebedev et al., 1977).Belyavskaya (2004) found that weak electromagnetic fields suppressed the growth of plants, reduced cell division, intensified protein synthesis and disintegration in plant roots. Sunflower (*Helianthus annuus*) seedlings exposed to 20 μT vertical MF showed small, but significant increases in total fresh weights, shoot fresh weights, and root fresh weights, whereas dry weights and germination rates remained unaffected (Fischer et al., 2004). However, the best results have been observed for the plants exposed to Electro magnets.Electromagnetic Fields (EMFs) have magnetic and electrical properties that surround objects with an electrical charge which will interact with other objects within that field. At present, there is a growing tendency to use either strong homogeneous or in-homogeneous field for the treatment of seeds for improving their performance (Shine et al. 2012).

EFFECTS ON GERMINATION RATE AND ROOT AND SHOOT INITIATION
Several studies proved the positive effect of Magnetoprimed seeds with significant andrapid germination and early root and early morphogenesis giving rise to the possibility that magnetism can increase the speed of plant development. Many studies have suggested positive effects in MF treated seeds of agricultural importance or with ornamental value. Groundnut seeds had shown an increase in germination rate and vigorous seedling

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growth, Similar studies on Onion and rice seeds (Vokharia et al., 1991; Alexander and Doijode 1995) have been observed. There are reports sharing that the magnetic field exposure increases germination of extremely low viability seeds and improvement in their quality and sprouting rates (Carbonell et al., 2008; Alexander and Doijode 1995).In case of annual grains like chickpea which is considered financially risky by farmers because of its tendency to face diseases in its early growth stages has also shown response to MF in germination and early growth characteristics(Cakmak et al., 2011) with high survival Percentage. Enhanced photosynthetic efficiency and high growth of crops like wheat and maize has also been reported(De Souza et al., 2008; Shine and Guruprasad 2012). Studies have also demonstrated that the energy involved during germination is increased due to magnetic fields.Germination energy and germination rate of tobacco seeds increased linearly with an induction of 0.15 T at expositions of 10, 20 and 30 min with and without preliminary soaking (Aladjadjiyan and Ylieva 2003).

Based on the treatment dose and type of magnet used, stationary magnets with high magnetic fields demonstrated the best results as compared to WMF and super Weak MF.50 mT and 200 mT for 2 h exposure increased the activities of hydrolyzing enzymes in *Helianthus annuus*, which were responsible for the quick seed germination, improved seedling vigour and better root characteristics of treated seeds in this plant(Vashisth and Nagarajan 2010). By the application of power frequency magnetic fields (100 μT), growth of germinated *Viciafaba*seedlings was enhanced, supported by an increased mitotic index (Rajendra et al., 2005).Exposure of maize seeds to stationary magnetic field strength of 125 or 250 mT enhanced the germination parameters (Zepeda-Bautista et al.,2010). The effects of electromagnetic irradiation on seed vigour of maize hybrids, as well as the response of each genotype were assessed in this study. It was also reported that pre-sowingtreatment increased corn seed vigour through emergence rate, establishment percentage, and dry mass of seedling aerial part, according to the combination of MF intensity, time and the genotype.

There have been several experimental studies on the effect of different MF capacitance on increased growth rates in different species (Table 1). In maize, plants showed higher growth and biomass than control plants, with asignificant increase inmaize plants which were exposed continuously to 125 or 250 mT(Florez et al., 2007). In barley, a stimulating effect on the first stages of seedling growth was observed. Germinating barley seeds were treated with 125 mTMFfor different time periods (1, 10, 20, and 60 min, 24 h, and chronic exposure). Interesting results were obtained with increase in seedling length and seedling weight (Martinez et al., 2002). Pea plants when exposed to 125 or 250 mT stationary MF (1, 10, and 20 min, 1 h and 24 h) and continuous exposure showed better growth in case of continuously exposed plants to the MF compared to control plants(Carbonell et al., 2008).

Several *in vitro* studies have shown that MFs can affect the development of cells and tissues cultured *in vitro*. In soybean, plant regeneration and growth of shoot-tip cultures exposed to MFs (2.9–4.6 mT) for 2.2 and 6.6 s showed an increase in regeneration frequency (87% and 74%, respectively, as compared to 62% in the control (Atak et al. 2003).The root induction also showed similar positive effect of MF with 26% and 36% increase over control. Shoot and root formation rates of Paulownia tissue culture increased when nodes were exposed to external MFs (2.9–4.8 mT for 2.2, 6.6, and 19.8 s during the culture period) compared to the control (Yaycili and Alikamanoglu 2005; Çelik et al., 2008).*In vitro* shoot cultures of

Table-1: Studies on the effects of magnetic field in plants

Crop	Target tissue	Biological effects	MF	Reference
Zea mays	Seed	Improvedgermination and early growth characteristics	200 mT	Sunita et al., 2017
		Improved germination	MF-45	Bilalis et al., 2012
		Increased shoot fresh weight	125,250mT	Florez et al., 2007
Helianthus annuus	Seedlings	Increase in fresh weight	20μT	Fischer et al.,2004
		Increased seedling dry weight, root length, root surface area and root volume	50, 200mT	Vashisth and Nagarajan 2010
Hordeum vulgare	Seedlings	Decrease in fresh weight	10nT	Lebedev et al.,1977
Hordeum vulgare	Seedlings	Increase in length and weight of shoot and root	125mT	Martinez et al.,2000
Arabidopsis	Seed	Delayed flowering	Near null	Xu et al.,

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thaliana		Reproductive growth		2012,2013
Cicer arietinum L	Seed	Increase in root volume and surface area	100 mT	Mridha et al., 2016
Cicer arietinum		Root Promotion of germination Increase in root length, surface area and volume	0–250mT	Vashisth and Nagarajan 2008
Solanum spp.	*In vitro* cultures	Stimulation/inhibition of growth	<GMF	Rakosy-Ticanet et al.,2005
Solanum lycopersicum	Seed Shoots	Promotion of germination Increased mean fruit weight, yield per plant	160–200mT	Souza et al.,2010; Poinapen et al., 2013
Triticumaestivum	Seeds seedlings	Activation of esterases reduction of growth	from 20nT to 0.1mT	Bogatina et al.,1978;Aksenov et al., 2000
Abelmoschusesculentus	Seed	Promotion of germination	99mT	Naz et al.,2012
Betavulgaris	Seedlings	Increased root and leaf yield	5mT	Rochalska 2008 Rochalska2005
Carica papaya	Pollen	Increased pollen germination	>GMF	Alexander and Ganeshan1990
Cryptotaenia japonica	Seed	Promotion of germination	500,750μT	Kobayashi et al.,2004
Dioscoreaoppositа	Seedling	Increased root length and number	2× GMF	Li,2000
Fragariavesca	Plantlets	Increased fruit yield per plant	0.096,0.19and 0.384T	Esitken and Turan,2004
Oryza sativa	Seed	Reduction of germination	125,250mT	Florez et al.,2004
Paulownia fortunei	Tissue cultures	Increased regeneration capability	2.9–4.8mT	Yaycili and Alikamanoglu,2005
Phaseolus vulgaris	Seeds	Promotion of germination Increased chlorophyll emission fluorescence	2 or7mT 3 100,160mT	Sakhnini, 2007; Cakmaket al.,2010
Pisum sativum	Seed Seedlings	Promotion of germination Increased length and weight	60,120,180mT 125,250mT	Iqbal et al.,2012 Carbonell et al.,2011 Polovinkina et al.,2011
Vignaradiata	Seed Seedlings	Promotion of germination	87 to 226mT 600mT	Mahajan and Pandey 2014 Chen et al., 2011

P. tomentosa exposed to a magnetic flux density of 2.9–4.8 mT for 2.2 s recorded a higher growth from 61.9 to 82.5%; with an increase in total chl, chl a, and chl b content and doubled total RNA content of the treated tissues over the control (Çelik et al., 2008). These studies suggest that *in vitro* plant tissues respond to MF exposure in terms of enhanced growth parameters.

In our studies on an important ornamental orchid, we investigated positive influence of high intensity magnetic field (200mT) on *in vitro*gamma irradiated *Dendrobium sonia* cultures. Pre-optmizedmagnetic field strength of 200 mT was applied for different duration (15 min, 30 min, 45min, and 60 min), on freshly isolated protocorm like bodies (Shikha et al.,Unpublished). Our preliminary results suggested that MF treatment significantly altered growth, O^- and OH^- radical level, photosynthetic activities and water use efficiency in irradiated cultures as compared to MF untreatedcontrol cultures(Shikha et al.,Unpublished).

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MECHANISMS OF MF STIMULATION OF PLANT GROWTH AND DEVELOPMENT

Free radical production and photosynthetic efficiency

Every aspect of plant growth requires energy and in cases of stress the total energy produced could be distributed to the defence pathway(Bailey-Serres and Mittler 2006).In general, the electron transport chain (ETC) in chloroplasts operates in an O2 sufficient environment such that if ETC is overloaded it will result in leakage of electrons and generation of free radicals.This free radical produced upon stress is shown to be decreased after Magnetic treatments and alleviating the chlorophyll content and photosynthetic efficiency(Shine and Guruprasad., 2012). The reduction in the free radical content was reported in maize and soybean seedlings after the treatment with 200 mTfor 1 h and 150 mTfor 1 h(Shine and Guruprasad 2011) Photosynthetic parameters were tracked by calculating energy by performance index through three independent functional steps of photosynthesis, the density of reaction centers in the chlorophyll bed (RC/ABS), excitation trapped per photon absorbed (ϕpo) and efficiency with which a trapped exciton can move an electron into the electron transport chain further than QA- (Ψo)(Srivastava et al., 1999). This proved that magnetopriming of soybean seeds enhanced the PI up to 48 and 63% in 150 mT (1 h) and 200 mT (1 h) respectively over control plant, specifically showing enhancement of PI attributable to higher efficiency of ϕpo.Increased Rubisco content and higher light harvesting efficiency in the treated plants leading in an increase in biomass of plants from MFtreatment(Baby et al., 2011).

Water and nutrient uptake

With the most prime requirement of plant of nutrients, supply is only possible through water, and studies have shown that magnetic exposure can enhance intake of these essential nutrients (Ijaz et al., 2012). Even in salt stress these plants could show robust and vigorous growth.The water in the region is heavily salted, which interrupts plant uptake. By exposing the water to magnets, the salt ions change and dissolve, creating purer water that is more easily taken up by the plant.The magnetic field changes water properties due to displacement and polarization of water atoms. Cai et al.(2009)reported that MF caused changes in physicochemical properties of water; these changes included decreasing water surface tension and increase viscosity suggesting an increase of activation energy and water molecule size due to extra hydrogen bond formation. Fundamental to all biological systems is the biochemical activities and the biomolecule (chlorophyll) can be affected by MF as the photochemical activity, respiration ratio and enzyme activity are all influenced under MF (Phirke et al., 1996; Dhawi 2003). In our study on In *Dendrobium sonia,* IRGAbased studies with MH treated PLBs showed that that the water use efficiency was higher in MF treated cultures(Shikha et al., Unpublished).

Modifications at cellular and molecularlevel

Several studies reported that MF affectsatcellular and molecular level leading to plant growth enhancement (Dhawi et al., 2009). The negative charges around the DNA molecule, as any charged entity, increase the potential of MF impact and the theoretical concept of how MF affects the DNA is that MF prolongs free radical ions' lifetime, by inducing the singlet-triplet transition of unpaired electrons leading to oxidative stress (Sahebjameipour et al.,2007).

Several studies reported a decrease in DNA level following low level exposure of magnetic field in arbour seedlings (Racuciu et al., 2008)and date palm (Dhawi and Al-Khayri et al., 2009).The mutagenic effect of MF is indirect because of the limited physical ability of non-ionizing radiation to induce double brake in DNA. Study by Pingpinget al.(2007) suggested that MF increases cell membrane permeability, which may increase uptake of water and nutrition. The effect of magnetic fields can be seen in protein synthesis activation leading to further development of root system (Phirke and Kudbe et a.l, 1996). The MF induced changes in cellular level leading to increase in cell viability, organization and differentiation (Vizcaino et al., 2003). In addition, MF affects cell proliferation and cellular metabolism (Atak et al., 2003) gene expression(Paul et al., 2006) and enzyme activity (Ataket al.,2007).

Magnetoreception

The phenomenon ''ion cyclotron resonance''is suggested to be the mechanism involved in magnetoreception. The movement will be a circulation of ions in a plane perpendicular to an external magnetic field with their lamor frequencies (Galland and Pazur 2005). When this frequency matches with that of the electromagnetic field, there is an absorption of energy from the external field. It is also suggested that exposure to magnetic field can modulate the internal potential energy of a biological tissue which could contribute to improve overall growth and development (Kavi 1983).

Another evidence is that of the Plant cryptochromes being involved in magnetoreception(Ahmad et al. 2007). In Arabidopsis, cryptochromes are encoded by two similar genes, *cry1* and *cry2*. CRY2 protein levels in seedlings decrease rapidly upon illumination by blue light, presumably as a result of protein degradation of the light-

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activated form of the receptor (Ahmad et al., 2007).The expression changes of three Arabidopsis cryptochrome-signaling-related genes (*PHYB, CO and FT*) suggest that the effects of a near-null MF are cryptochrome-related, which may be revealed by a modification of the active state of cryptochrome and the subsequent signaling cascade plant cryptochrome has been suggested to act as a magnetoreceptor (Xu et al., 2012).

Based upon the premise that cryptochromes form radical pairs subsequent to photoexcitation, the MF mediated sensitive responses are suggested to be the result of this radical pair formation (Galland and Pazur2005; Maffei 2014). It is also the most suggested mechanism for magnetic interactions to have an effect on chemical reactions. It is opined that "the kinetics and quantum yields of photo-induced flavin-tryptophan radical pairs in cryptochrome are indeed magnetically sensitive and cryptochrome is a good candidate as a chemical magnetoreceptor" (Maeda et al., 2012). As of now, cryptochromes are considered as the likely mediators of the MF induced biological effects (Maeda et al., 2012).

Magnetoreception is a research frontier with immense application in plants. There is a great amount of information on the physiological effects and biomass accumulation, and cyptochrome and radical-pair based mechanistic view are being established as plausible mechanisms. Molecular studies in this field are needed to elucidate the biological effects. Extensive research is also necessary to extend MF-induced biological effects field-leveldemonstration forpositive outcomeon plant growth and productivity.

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ROLE OF IN VIVO AND IN VITRO PRODUCED STEVIOSIDE ON RESTORING THE LIVER AND PANCREATIC FUNCTIONS IN DIABETIC ANIMALS

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ABSTRACT
Plant cells are totipotent. Plant tissue culture methods are powerful techniques for the propagation, conservation and also for the production of important secondary metabolites of medicinal importance. Stevia rebaudiana Bertoni, produces sweet steviol glycosides and gaining increasing attention due to its zero caloric sugars, as an alternative of white sugar and has been recommended to diabetic patients as a sugar supplement.

The current research was focused on analyzing the antidiabetic potential of steviosides produced in vitro stevia leaf derived callus, in comparison with natural leaves (in vivo) with respect to secondary complications of diabetes on liver and pancreatic efficiency.

Dose of stevia leaf and leaf derived callus in the form of aqueous extract was determined through oral glucose tolerance test (OGTT). Optimized dose (i.e. 400mg/kg leaf and 3.6g/kg callus) was given orally to STZ induced diabetic rats compared to standard drug; glibenclamide. After 21 days, Fasting blood glucose and serological profiling was done as Change in total bilirubin, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxalacetate transaminase (SGOT), α amylase and insulin secretion levels.

The results showed that leaf and callus both gave almost at par with standard drug, in case of FBS, SGPT, SGOT activity, total bilirubin, α- amylase level and insulin production in diabetics induced rats. Our research exertion clearly indicates the anti hyperglycemic, hepatic and pancreas protective action of stevioside in in vitro callus cultures.

INRTODUCTION
Stevia rebaudiana Bertoni, is a medicinally and commercially important plant of Asteraceae family. Leaves of stevia synthesize ent-kaurene type of diterpenoid glycoside, majorly steviosides, having non caloric sweetening property. They, thus, will be good alternative to sugar for diabetic patients. Apart from non-caloric sweetener these glycosides also have pharmacological properties like antitumor or anticancer, antihypertensive, anti-diarrheal, cardiovascular and antimicrobial activity (Brahmachari et al., 2011). Conventional propagation of this crop shows significant drawbacks (Nakamura and Tamura, 1985; Singh and Rao 2005; Oddone, 1997). Hence alternative method, *in vitro* micropropagation, has been applied recently for commercial propagation purposes. *In vitro* techniques are also gained significant attention for their efficiency as alternate source for the production of pharmacologically important metabolites from medicinal plants (Georgiev et al., 2009; Weathers et al., 2010) including steviosides from stevia leaf callus cultures (Neha et al., 2016). Clinical efficacy, however, of the *in vitro* produced metabolites has to be confirmed for their application in the pharmaceutical industries.

In present study we report the functional pharmacological properties of steviosides produced in the stevia leaf callus cultures in comparison to the steviosides produced in natural plants on the basis of their effect on secondary complications of hyperglycaemia i.e. liver and pancreatic function, taking standard antidiabetic drug glibenclamide (gbc) as reference.

MATERIAL AND METHODS
Stevia germplasm that was obtained from IHBT, palampur, Himachal Pradesh, India was used as initial material for the present studies..

Callus was raised *in vitro* cultures in large quantities as per our earlier report Neha et al., (2016) and two months old callus was harvested , oven dried at 50^0C temperature for stevisides extraction.

The leaves collected from field grown plants were shade dried at room temperature. Extract from both the samples (leaf and callus) was prepared using soxhlation method followed drying by rotary evaporation. The concentration steviosides in the samples was estimated by LC-MS method given by Gupta et al (2017).

Pharmacological properties of *in vitro* produced steviosides were studies, in comparision to natural plant leaf extract and the standard drug glibenclamide (gbc), by evaluating the effect on secondary complications- such as

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damage of liver and pancreatic functions, which would arise due to diabetics. Serum glutamate pyruvate transaminase (SGPT), serum glutamate oxalacetate transaminase (SGOT), total bilirubin, α-amylase and insulin levels in the serum were analysed in the streptozotocin (STZ) induced diabetic rats.

ANIMALS STUDIES

All animal experiments and procedures were approved by Institutional Animal Ethical Committee, Jiwaji University, Gwalior, India (approval no. IAEC/JU/09 dt 12.01.2017), constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Five to six months old young adult male albino rats of 170-200 g weight were selected and grown in polypropylene cages and acclimatized to standard environmental conditions i.e. 12 h light and 12 h dark cycle at 25 ± 2 ºC temperature. Before and during the experiments, the rats were fed with standard laboratory pellet diet *ad libitum* and water.

CHEMICALS AND KITS

Streptozotocin from Sigma Aldrich, Glibenclamide from local medical store and all the serologic kits for serum glutamate pyruvate transaminase (SGPT), serum glutamate oxalacetate transaminase (SGOT) , total bilirubin, α-amylase and DRG-insulin ELISA from 'Coral Clinical Systems', Goa, India were used. Accu Check glucometer was used for measuring the fasting glucose level.

OPTIMIZATION OF DOSE VIA ORAL GLUCOSE TOLERANCE TEST

The oral glucose tolerance test (OGTT) was performed on overnight (12h) fasted male rats. Animals were divided into two sets and each set was further divided into six groups (each group consisted of five animals; n=5). Set- I used for the optimization of dose with dry leaf powder and set-II used for optimization of dose with dry callus powder.

The OGTT for optimization of dose was performed with four different concentration of leaf powder (100, 200, 400 and 800 mg/kg b.w.) and dry callus powder (0.9, 1.8, 3.6 and 7.2g/kg b.w.). Group I was served as normal control (NC) and received normal saline (0.9%NaCl; 5ml/kg b.w.), Group II, III, IV & V have received respective dose of dry leaf (100, 200, 400 and 800 mg/kg b.w.) and dry callus (0.9, 1.8, 3.6 and 7.2g/kg b.w.) respectively in the form of aqueous extract and the Group VI has received standard antidiabetic drug glibencamide (600µg/kg b.w.).

Animals in NC were fed with normal saline. Whereas the animals in other groups were fed leaf and callus extracts and standard drug, 30 minutes after the animals were fed with 2g/kg b.w. of Glucose. Blood was withdrawn from tail vein of all the experimental animals after 0, 30, 60, 90 and 120 minutes of glucose administration and blood glucose level was measured using Accu Check Glucometer.

ANALYSIS OF ANT DIABETIC ACTIVITY OF IN VITRO PRODUCED STEVIOSIDES :

In this experiment, diabetes was induced in rats using STZ by the method given by Verspohl, (2002). The study was conducted on 30 rats divided into five different groups of six rats each. The design of experiment was as follows:

Group I: Diabetic control; Diabetic rats without treatment (given normal saline orally), Group II: Diabetic rats were given oral dose of 400 mg/kg stevia leaf in the form of aqueous extract, Group III: Diabetic rats were given oral dose of 3.6 g/kg stevia callus in the form of aqueous extract, Group IV: Diabetic rats were given oral dose of 600 µg/kg glibenclamide, Group V: Non-diabetics control rats.

Body weight and fasting blood glucose (FGB) level were measured weekly. Change in SGOT, SGPT, total bilirubin, α amylase and insulin secretion levels of diabetic rats were evaluated at the end of the treatment of 21 days by using standard kits.

RESULT AND DISCUSSION

Antidiabetic property of any biological or chemical compound is tested by performing the oral glucose tolerance test. In present study aqueous extract of stevia dry leaves (set-I) and callus (set-II) were used to test and determine the dose for the further pharmacological analysis.

Results of blood glucose tolerance test were presented in the Figure 1 a & b. The glucose levels in blood were noticed to increase with time up to 2 hrs in the animals of negative control. While animals in Groups II to V of Set I administrated with different concentration of leaf extract and Set II fed with callus extract and in Group-VI fed with allopathic drug have shown initial increase for first 30 minutes and thereafter, a gradual decrease in blood glucose level was observed. The pattern of reduction in blood sugar levels was similar in these groups. Further, the results clearly show that callus at 3.6 g/kg b.w. and 400 mg/kg b.w. leaf extract have shown

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significant reduction in blood glucose level after 120 minutes of glucose administration in comparison to the standard drug glibenclamide (Figure 1a&b).

PHARMACOLOGICAL STUDIES

(I) Fasting blood sugar (FBS) level

Anti hyperglycaemic effect of crude extract of stevia on artificially induced diabetic animals was evaluated earlier by Bekele et al., (2008), Shukla et al., (2011), Mishra et al., (2011), Sharma et al., (2012), Ray et al., (2013) and Vathsala et al., (2014) and reported similar results as we found (Figure 2)

(II) Evaluation of functional properties of stevioside on liver and pancreatic function

Steviol glycosides are not absorbed by blood but are hydrolyzed into steviol by micro flora of human gut. Steviol is then absorbed and primarily metabolized by liver (Gardana et al., 2003).

SGPT

SGPT and SGOT are the common intracellular enzymes that increased due to the liver damage induced by diabetes (Can et al., 2004).

SGPT enzyme levels in the experimental animals in each group are presented in the Figure 3a. The level of SGPT in the serum was noticed to increase significantly from 25.03 IU/L (in NC) to 56.16 IU/L (DC) on induction of diabetes. When the diabetic animals were treated with allopathic drug, stevia leaf and callus extracts (Group 4, 2 & 3), the level of SGPT decreased significantly to 40.35 IU/L, 43.65 IU/L and 40.75IU/L respectively. The effect of callus, further, was found to be at par with that of allopathic drug (Figure 3a).

SGOT

The results of serum glutamate oxaloacetate transaminase (SGOT) enzyme concentration estimated at the end of the experiment in the animals in Group 1 to 5 are given in the Figure 3a. The healthy male wistar rats in Group 5 (NC) recorded 32.01 IU/L of SGOT and the DC animals (Group 1) recorded 63.73 IU/L activity. When diabetic rats were fed with leaf, callus extracts and allopathic drug, the SGOT amount was observed to decrease to 38.99, 34.63 and 31.51 IU/L levels respectively in Group 2, 3 and 4 animals. The performance of callus extract was noticed to be better than the stevia leaf extract (Figure 3a).

α- amylase

The Figure 3a shows the α-amylas activity results obtained from the 21 days old experimental rats. The level of enzyme increased to 83.01 IU/L in DC control (Group 1) in comparison to normal control (NC; 51.39 IU/L) on induction of diabetes. The pancreatic enzyme activity in the diabetic animals fed with leaf extract (Group 2), and standard drug (Group 4) was noticed to be 71.81IU/L, 72.03 IU/L. While the Group 3, fed with callus extract recorded 56.33IU/L. activity, indicating the regulation of callus extract was better than the leaf extract and standard drug (Figure 3a).

Total Bilirubin

The mean amount of total bilirubin present in all the five groups of animals at the end of experiment i.e. on 21st day, has been presented in Figure 3b. Healthy Group-5 (NC) of animals recorded 0.51 mg/dl and diabetic ones (DC, Group 1) recorded 0.66 mg/dl of total bilirubin. The diabetic rats of group 2, 3 and 4 fed with stevia leaf, callus and standard drug have shown recovery in the levels of total bilirubin and further the performance of stevia leaf and callus (0.57 mg/dl) was same (Figure 3b).

Level of insulin

The insulin level in the experimental animals was estimated using ELISA method and the results obtained are presented in Figure 3c. The normal healthy animals have recorded 5.10 µg/l of insulin in their serum. This level noticed to decrease to 1.03 ±0.17 µg/l on induction of diabetics (Group 1). When these diabetic rats were fed with stevia leaf extract, stevia callus extract and standard drug, the insulin levels recovered to 3.38±0.18 µg/l, 2.88±0.15 µg/l and 3.70µg/l respectively (Figure 3c). Treatment of diabetic animals with stevia callus extract definitely improved the insulin levels in the blood of diabetic rats. The performance, however, was less effective than the other two treatments (Figure 3c).

Antihyperglycemic effect of crude extract of stevia on artificially induced diabetic animals was evaluated earlier by Bekele et al. (2008), Shukla et al. (2011), Mishra et al. (2011), Sharma et al. (2012), Ray et al., (2013) and Vathsala et al., (2014). Present results with Stevia leaf are also in accordance with earlier results. Along with anti hyperglycaemic effect, Shivanna et al, (2013) have analysed and reported the hepatoprotective (through SGPT and SGOT) and renal protective (through γ glutamyl transpeptidase) of stevia in diabetic animals. However, evaluation of *in vitro* Stevia leaf callus produced steviosides function was not reported.

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The present results clearly indicate that callus extracts are good source of pharmaceutically important metabolites and the steviosides performed almost similar to that of natural extracts in the recovery of liver and pancreatic functions in the diabetic rats.

We, therefore, conclude that in vitro culture are good alternate for the production of medicinally important metabolites with functionally similar to the in vivo produced ones. Callus extract has increased insulin level in diabetic rats probably revering the secondary complications raised due to diabetes, similar to the property of allopathic drug and Stevia leaf extract. However, callus extract did not show same level of recovery as that of the other two treatments (Figure 3c).

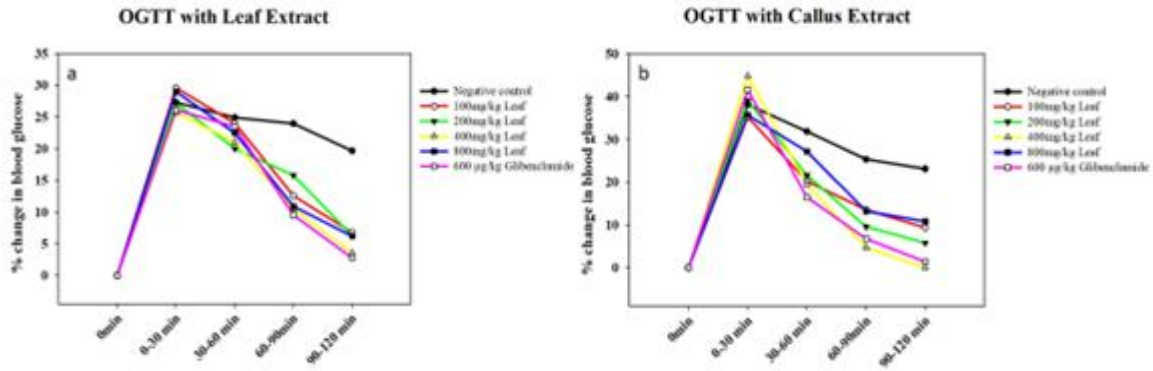

Figure-1: Dose optimization using different concentrations of aqueous extract of Stevia leaf (a) and callus (b) in rats.

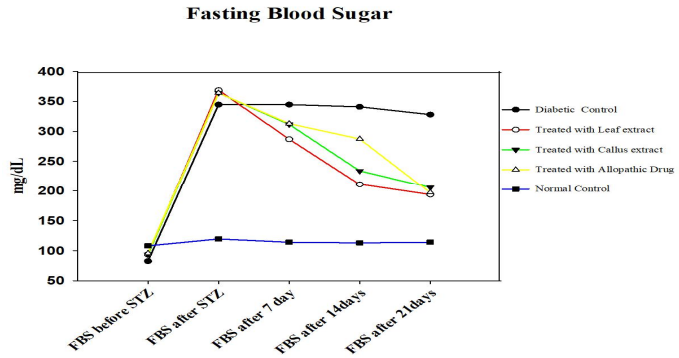

Figure-2: Effect of callus aqueous extract treatments on fasting blood sugar level of diabetic rats in comparison to Stevia leaf extract and allopathic drug.

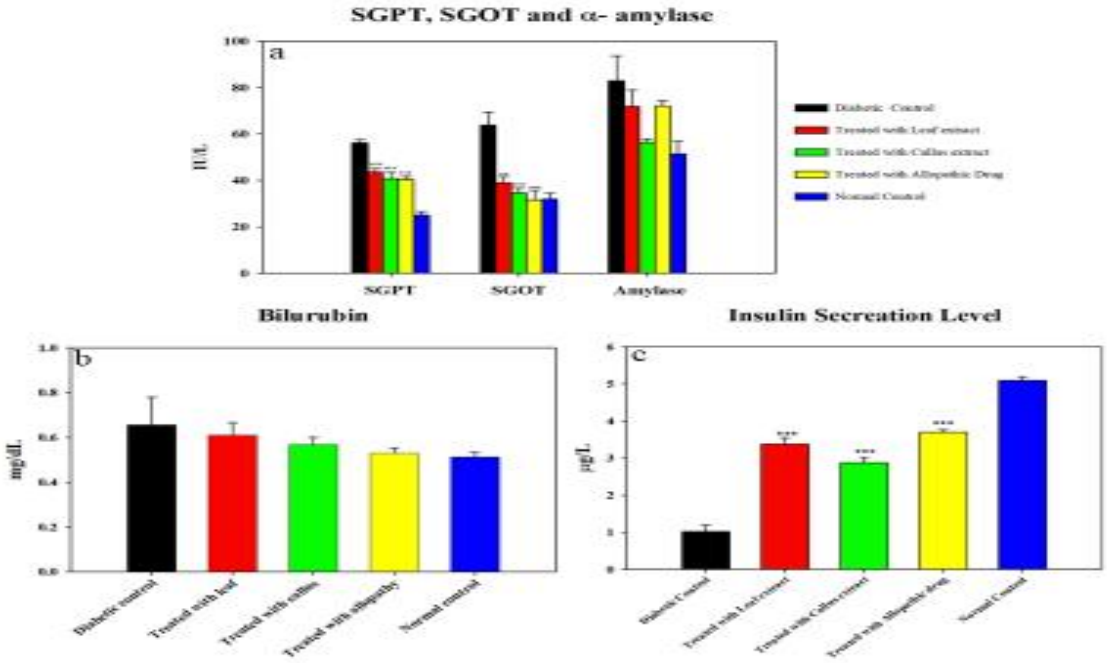

Figure-3: Effect of stevia leaf and callus extract on (a) SGPT (blue bar), SGOT (red bar) and α-amylase (green bar), (b) Bilirubin and (c) insulin levels on STZ induced diabetic rats.

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IMPACT OF ENVIRONMENTAL FACTORS ON INCIDENCE AND MORPHOLOGICAL VARIABILITY OF *FUSARIUM OXYSPORUM F. SP. CICERI* ISOLATES FROM CHICKPEA *(CICER ARIETINUM* L.*)*

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ABSTRACT
The present study is to evaluate effect of environmental factors on chickpea wilt in Bundelkhand Region. Chickpea (Cicer arietinum L.) is one of the major crop of winter season in Bundelkhand Region. Climatic conditions i.e., soil pH, temperature, different nutrition requirement effect disease incidence greatly. H1FOC5 found with highest growth rate is representative of all isolates collected from Hastinapur, Jhansi district of Bundelkhand Region. The effect of environmental factors on isolate H1FOC5 chickpea wilt pathogen Fusarium oxysporum f. sp. ciceri were studied. On different medium, growth pattern of representative Isolate was different i.e., very thin mycelium on Rose Bengal whereas thick cottony on czapek's dox agar. Isolate shows growth ranges from 17 to 65 mm on ninth day after incubation at different temperature on Potato Dextrose Agar medium. Whereas at different pH, growth rate was diferent. Mycelia was white to creamy with pink tinge, margins were slightly lobate or smooth, elevation was raised to umbonate on Potato Dextrose Agar medium. Conidia were curved, typically sickle shaped, with 3-5 septa, chlamydospores were round and oval shaped. Sporulation in all isolates was profuse to moderate. This study shows the role of different media, pH and temperature to understand environmental survival of pathogen of chickpea wilt, which might be helpful in management strategy, laboratory evaluation and prevention from severe incidence of wilt disease in chickpea.

Keywords: Bundelkhand region, Environmental factor, Fusarium oxysporum f. sp. ciceri, pH, Radial Growth, Temperature.

INTRODUCTION
Chickpea *(Cicer arietinum* L.*)* commonly known as Bengal gram is one of the major pulse crop in India and third most important pulse crop in world. This is the second world's most important food legume crop after dry beans. Jukanti *et. al.*, (2012) reported that Chickpea plays a major role in human food and animal feed because of their high content of lysine-rich protein. Its cultivation plays a significant role in farming systems for sustainability of production and maintenance of soil health by fixation of atmospheric nitrogen. Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* (FOC) is one of the serious diseases causes annual loss at 10 percent in yield (Dubey *et. al*., 2007). FOC is quickly dispersed, attacks the root of the plant and causes hydric stress by blocking the xylem vessels and also causes chlorosis, necrosis and abscission (Di Pietro *et al*., 2001; Gupta *et. al*., 2009). Temperature is one of the major factor with 25°C -28°C optimal, among the factors that contribute to pathogenesis for the survival and development of FOC (Bhatti and Kraft, 1992). Chickpea wilt disease is very prevalent in Bundelkhand region because of its environmental condition which are favorable to this disease. Bundelkhand is a rain fed region, environmental factors like high temperature, climatic condition, insufficient underground water, low rainfall and land topography are not support to intensive agricultural development and farmers of the regions takes leguminous crop followed by *kharif*. Because of Continuous cultivation of leguminous crop in same field year by year wilt disease becomes more prominent and causes huge loss to chickpea. The incidence of disease varied with field to field and locality to locality in Bundelkhand. Looking the above facts, present research was planned to see the impact of environmental factors on disease incidence and variability within the isolates isolated from Chickpea from this region. Based on macro-and microscopic characteristics, colonies belonging to FOC were grown on PDA and monospore cultures were grown and then macroscopically characterized by color, appearance and growth rate after 7 day at 25°C.

MATERIALS AND METHOD
Isolation of *Fusarium oxysporum* f. sp. *ciceri* (FOC)
Diseased chickpea plant samples was collected from Hastinapur, Jhansi (Fig 1) of Bundelkhand Region. Diseased plant showing typical wilt symptoms were selected for pathogen isolation. Isolates of *Fusarium oxysporum* f. sp. *Ciceri* was isolated from root and lower part of the stem. The diseased part of sample was cut into small (2-3 mm) pieces and surface sterilization was done with the help of 1% NaClO (Sodium hypochlorite solution) by putting 60 to 80 second. Surface sterilized infected plant parts placed aseptically on Potato Dextrose Agar medium (PDA) after four washing in sterilized water. Inoculated Petri plates incubated for 4-5 days on 26^0 C. Identification of Isolates as *F. oxysporum* f.sp. *ciceri* done by morphological criteria (Leslie and Summerell, 2006).

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Culture of *Fusarium oxysporum* f. sp. *ciceri* further purified by single spore isolation method and maintained by the periodical transfer on PDA. *Fusarium oxysporum* f. sp. *ciceri* was sub cultured on PDA slants and allowed to grow at $26^0 \pm 1^0$C for seven - eight days (Booth, 1975; Nelson et al., 1983). Now these slants were preserved in a refrigerator at 5ºC and revived once in 30 days. Purified isolates were characterized based on microscopic characterization, culture morphology and colony characteristics. In present study, isolate H1FOC5 collected from Hastinapur, Jhansi district of Bundelkhand region, with highest growth rate is representative of all isolated isolates of Hastinapur. The effect of environmental factors on isolate H1FOC5 chickpea wilt pathogen *Fusarium oxysporum f. sp. ciceri* were studied.

Fig-1: Showing wilted chickpea plant

Effect on different media

Six culture media with different composition were used to find out the most suitable one for the mycelial radial growth of H1FOC5. Each culture medium was prepared in 1 litre of water and autoclaved at 121.6ºC at 15 psi for 20 min. These were cooled to 45ºC and then poured aseptically in 90 mm Petri dishes and kept for solidification.

1. Potato Dextrose Agar (PDA) medium - (Peeled and sliced potato 200g, Dextrose 20g, Agar-agar 20g). Final pH (at 25^0c) 5.6 ±0.2.
2. Corn Meal Agar (CMA) medium - Corn Meal Agar infusion from 50 g, dextrose 2.00 g, agar 15.0 g. Final pH (at 25^0c) 6.0 ± 0.2.
3. Czapek's Dox Agar (CDA) medium – Sucrose 30 g, Sodium nitrate 2.00 g, Dipotassium phosphate 1.00 g, Magnesium sulphate 0.50 g, Potassium chloride 0.50 g, Ferrous sulphate 0.01 g, Agar 15.00 g.
4. Oat Meal Agar (OMA) medium – Oat Meal 60.00 g, agar 12.50 g.
5. Modified Czapex dox agar – Sucrose 30.00 g, Sodium nitrate 2.00 g, Magnesium glycerophosphate 0.50 g, Potassium chloride 0.50 g, Dipotassium sulphate 0.35 g, ferrous sulphate 0.01 g, and Agar 12.00 g.
6. Potato Dextrose Rose Bengal (PDRB) – Potato infusion from 200g, Dextrose 20g, Rose Bengal 0.0084 g, Agar 15g final pH (at 250c) 5.6 ± 0.2

Effect of different pH

In this study there were six different pH levels ranging from 4 to 9 with a difference of one were prepared by using pH meter. Different pH was adjusted by using either N/10 HCl for acid and NaOH for basic pH, before autoclaving the PDA medium. For each pH value, three replications were maintained. The Petri plates containing sterilized medium was inoculated with 5mm mycelium disc of H1FOC5 isolates grown on PDA for seven days and incubated at 27 ± 1^0C. At the interval of 24hrs, the linear growth was measured till the petriplate filled completely by pure culture.

Effect of different temperature

The experiments were conducted to find out, the most suitable temperature for mycelial growth. The sterilized poured Petri plates with PDA were inoculated with 5 mm disc of the test pathogen H1FOC5 isolates of seven days old culture. The Petri plates were incubated at 10, 15, 20, 25, 30 and 35^0C temperature, Separate BOD Incubators were arranged and set on required temperature. Three replications were maintained for each treatment and observation for mycelial growth was measured till the petri plate filled completely by pure culture.

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RESULTSAND DISCUSSION

Incidence of chickpea wilt

Chickpea is cultivated in winter season (*Rabi* crop) in Jhansi district of Bundelkhand region. Hastinapur from Jhansi district was selected after survey of the area for the present study. Incidence in selected field was around 20- 25 % in Hastinapur Village of Jhansi district of Bundelkhand region. In present study effect of Environmental factors on incidence and morphological variability was examine by different experiments.

Effect of different media

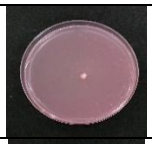
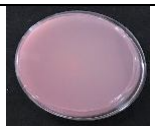
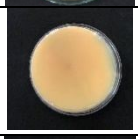
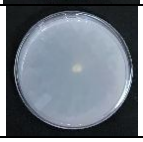
In present study, a total six media were used for study. Performance recorded on each media was given below in table 1. After analysis of the growth rate of the isolate on each media, Czapek's Dox Agar media shows maximum growth rate (max. growth 90 mm with mean 90 mm), Modified Czapek Dox Agar (max. growth 90 mm with mean 87.5 mm), Oat Meal Agar (max. growth 85.5 mm with mean 85.5 mm), Potato Dextrose Agar (max growth 80 mm with mean 80 mm), Corn Meal Agar (max. growth 78 mm with mean 75 mm), whereas Rose Bengal (max growth 52 with mean 56). With highest mean and growth rate Czapek's Dox Agar was found most suitable, Modified Czapek Dox Agar medium was found more favourable for the growth of H1FOC5 isolate, than other Medium. Whereas Rose Bengal medium was less favourable in comparison to all other tested medium. Table 2 Showing morphological characteristics and growth of HIFOC5 on different media.

Table-1: Effect of different media on Growth (Average in mm)

Media*	Colony diameter mm on 7 DAI	Colony diameter mm on 8 DAI	Colony diameter mm on 9 DAI	Sporulation*
RB	39	47	56	+
PDA	61	69	80	+++
OMA	64	75	85.5	+++
CZA	68	77	90	+++
MCA	70	78	87.5	+++
CMA	58	68	75	++

*Sporulation level: + less, ++ moderate, +++ profuse

Table-2: Morphological characteristics and growth of HIFOC5 on different media

S. No.	Media	Front View	Reverse view	Morphology and sporulation
1.	**RB**			Little growth, White very thin Mycelium with very less sporulation.
2.	**PDA**			Medium to high growth rate, White Mycelium with profuse sporulation
3.	**OMA**			Medium to high growth rate, White cottony Mycelium with profuse sporulation
4.	**CZA**			Medium to high growth rate, White Mycelium with profuse sporulation
5.	**MCA**			Medium to high growth rate, White Mycelium with profuse sporulation
6.	**CMA**			Medium to high growth rate, White very thin Mycelium with moderate sporulation

* Media -: RB – Rose Bengal, CZA- Czapek's Dox Agar, PDA - Potato Dextrose Agar, OMA - Oat Meal Agar, MCA- Modified Czapek's Dox Agar, CMA- Corn Meal Agar

Effect of different pH

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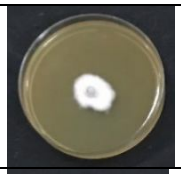
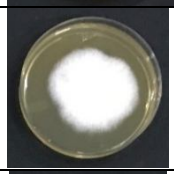
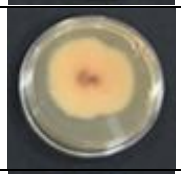
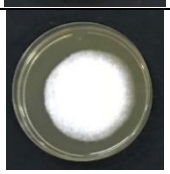
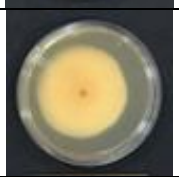
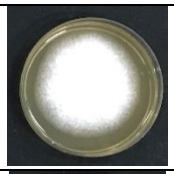
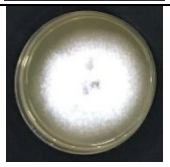
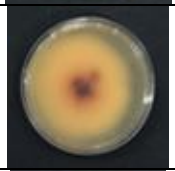
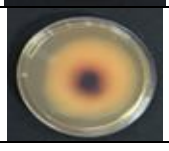
In present study a total six pH (4, 5, 6, 7, 8 and 9) were adjusted on Potato dextrose medium. Performance of selected isolate on different pH was given in table 3 and 4. After the analysis it was found that the pH range from 5 to 8 was found more suitable for the growth of *Fusarium oxysporum* f. sp. *ciceri* H1FOC5 isolate. Sporulation rate is profuse at pH 6 and 7, whereas Sporulation was moderate at alkaline pH range (8, 9). Sporulation was nill at acidic (pH 4) range. For the sporulation of the isolate pH 5 was found not very suitable.

Table-3: Performance of HIFOC5on different pH

Sr no.	pH	Colony diameter avg (mm) on 7 DAI	Colony diameter avg (mm) on 8 DAI	Colony diameter avg (mm) on 9 DAI	Sporulation
1	4	16.25	21.75	28.75	-
2	5	37.00	47.40	53.00	+
3	6	47.40	50.40	60.00	+++
4	7	47.50	54.50	64.00	+++
5	8	51.90	59.50	70.80	++
6	9	45.00	62.00	63.50	++

*Sporulation level -: + less, ++ moderate, +++ profuse

Table-4: Showing growth and sporulation of HIFOC5 on different pH

S. No.	pH	Front View	Reverse view	Morphology and sporulation
1.	pH 4			Little growth, White Mycelium with no sporulation.
2.	pH 5			Low to Medium growth rate, White Mycelium with less sporulation
3.	pH 6			Medium to high growth rate, White Mycelium with profuse sporulation
4.	pH 7			Medium to high growth rate, White Mycelium with profuse sporulation
5.	pH 8			Medium growth rate, White Mycelium with moderate sporulation
6.	pH 9			Medium growth, White Mycelium with moderate sporulation

Effect of different temperature

In present study a total six temperature (10^0C, 15^0C, 20^0C, 25^0C, 30^0C, and 35^0C) were used for study. Performance at different temperature was given below in table 5. After analysis of the growth rate of the isolate at each temperature it was found that 30^0c shows maximum growth rate (max. growth 65 mm with mean 62.5 mm), 20^0C shows highest mean (63.5) but less growth rate (64 mm) in comparison to 30^0C, at 25^0C growth performance was (max growth 61 mean 60.5) whereas at 10^0C and 15^0C (max. growth 19 and 23 mm with mean 18 and 15.5 mm respectively) growth was very low, which indicate less favourable for the mycelial growth. Sporulation was profuse at 25 and 30^0C. Whereas at 10^0C and 15 ^{0}C sporulation was found nill and less respectively.

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Table-5: Effect of different temperature on growth of HIFOC5 of Jhansi district (Average in mm)

Sr no	Temperature	Colony diameter avg (mm) on 7DAI	Colony diameter avg (mm) on 8 DAI	Colony diameter avg (mm) on 9 DAI	Sporulation*
1	**10**	7.5	15.5	18	-
2	**15**	14	18.5	15.5	+
3	**20**	42	51.5	63.5	++
4	**25**	43	50.5	60.5	+++
5	**30**	40.4	45.5	62.3	+++
6	**35**	16.2	17.9	20.3	+

***Sporulation level: + (less), ++ (moderate), +++ (profuse)**

DISCUSSION

This study shows the role of different media, pH and temperature to understand environmental persistence of pathogen of chickpea wilt, which might be helpful in management strategy and laboratory evaluation. Present study support the findings of Khilare *et al.,* (2012) studied that czapek's dox agar and potato dextrose agar were best medium for growth of *Fusarium oxysporum f. sp. Ciceri*. Optimum growth and sporulation on czapek's dox agar was also reported by Jamaria (1972). The incidence of fusarium wilt increases in acidic condition and it is inhibited by alkalinity. Arvayo-Ortiz *et al.,* (2011) suggested the adaptation of FOC to neutral to slightly alkaline pH. Martinez *et. al*., (1996) reported that the chickpea crop grows best at a pH of 6, it can grow when pH is 2 to 12 but the ideal pH for chickpea wilt is lower than 4.5. Vujanovic et al., (2006) isolated FOC from soils at pH 5.3-6.3. Anuar *et. al.,* (2008) reported long-term applications of N decreased the soil pH from 4.2 to 3.7 and caused leaching of K to the lower depth.Khilare *et al.,* (2012) also studied the effect of different temperature on the growth of *Fusarium oxysporum* f. sp. *Ciceri* according to him growth rate mean at 9 DAI was higest at 30^0C. Whereas growth rate mean was lowest at 10^0C with no sporulation. Jamaria (1972) reported that *Fusarium oxysporum* f. sp. *nivium* indicated that increase or decrease in pH level from optimum effect the growth. Imran khan *et al.,* (2011) also studied effect of pH, studies indicated that range from 6.5 to 7.0 showed optimum growth for *Fusarium oxysporum* f. sp. *Ciceri*, Anjaneyareddy (2002) studied that growth of the pathogen was differed in different temperature requirements, ranges between 20^0C to 35 ^{0}C. Landa *et al*., (2001) studied effect of different temperature on *Fusarium oxysporum* f. sp. *Ciceri*. Who found that disease development was higest at 25^0C comparative to others.The aim of this work was to find out the effect of different media, pH and temperature on the chickpea wilt pathogen *Fusarium oxysporum* f. sp. *ciceri*. High Incidence of disease, high growth rate and morphological variability of *Fusarium oxysporum* f. sp.ciceri isolates from chickpea (*Cicer arietinum L.)* might be correlated to pathogenicity level and environmental factors.

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BIOPROSPECTIVE ASPECTS OF MUSHROOM CULTIVATION

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ABSTRACT
Mushrooms possess many medical and pharmacological properties. It is not only satisfy food requirement but also provide health benefits with different varieties having different medicinal properties. Mushroom has huge potential to be used as bioprospecting agents. Bioprospecting is the process of extraction and commercialization of new products based on biological resources. The role of mushrooms as anti-oxidant, anti-cancer, its anti- genotoxicity and anti-mutagenic agent, open up the new gates for pharmaceutical industries. In this chapter, bioprospecting aspects of mushroom cultivation have been discussed. In the near-term, mushrooms will be used for developing medicines for curing cancer like diseases and neurological disorders.

Keywords: mushroom, pharmacological properties, anti-genotoxic properties, bioprospective agent.

1. INTRODUCTION
The number of mushrooms on Earth is estimated at 140,000, yet only 10% (approximately 14,000 named species) are known. Mushrooms comprise of a vast and yet largely untapped source of pharmaceutical products. For millennia, mushrooms have been valued by humankind as an edible and medical resource. A number of bioactive molecules, including antitumor substances have been identified in many mushroom species. Polysaccharides are the best known and most potent mushroom derived substances with antitumor and immunomodulating properties (Mizuno 1996, 1999a, b, 2002; Lorenzen and Anke 1998; Borchers et al. 1999; Ooi and Liu 1999; Wasser and Weis 1999; Tzianabos 2000; Reshetnikov et al. 2001). In the Far East where knowledge and practice of mushroom use primarily originated, hot-water-soluble fractions (decoctions and essences) from medicinal mushroomshave been in use as medicine for a long time. (Hobbs 1995, 2000). Mushrooms such as *Ganoderma lucidum* (Reishi), *Lentinus edodes* (Shiitake), *Inonotus obliquus* (Chaga) and many others have been collected and used for hundreds of years in Korea, China, Japan, and eastern Russia. Those practices still form the basis of modern scientific studies of fungal medical activities, especially in the - field of stomach, prostate, and lung cancers (Wasser et al., 2002).

2. WORLDWIDE PRODUCTION OF MUSHROOM
- Globally, the production and per capita consumption of mushroom has increased at a rapid rate for last 5 decades.
- According to the United Nations Food and Agriculture Organization statistics, the average annual growth rate of edible fungi is 5.6% worldwide.
- During 1997-2012, annual per capita consumption of mushrooms increased from about 1 kg to over 4 kg. The main producer and consumer of mushroom is China (Royse 2014).
- In India, more than 40,600 tons of mushrooms are produced annually (Pandey et al. 2014).
- Mushroom cultivation is one of the eco-friendly ways to recycle agricultural and agro-industrial wastes.
- In 2016, market value of cultivated edible mushroom species was about 30-34 billion dollars and medicinal mushroom species was 10-12 billion dollars (Mushroom fact sheet, 2016).
- Therefore, it is a billion dollar agribusiness which provide great opportunities to the farmers and their families as well as others who are interested in cultivatingmushroom in spare time and get an additional source of income.
- In India, the total production of mushroom is about 1,29,782 metric tonnes (Sharma *et al*., 2017), of which major share is contributed (73%) by button mushroom production by Agro Dutch, Lalru (Patiyala), followed by oyster mushroom which contributes about 16% in Punjab leading to a production of around 18,000 metric tonnes/annum (Sharma *et al*., 2017).

3. MUSHROOM CULTIVATION
Mushrooms can be cultivated in two ways: Mushroom fruit bodies and mycelial biomass. Solid state fermentation produces mushroom fruit bodies and liquid culture produces mycelial biomass. Both of them have their own nutritional and medicinal properties. Depending upon the requirement, either one of them could be cultivated. Composition of the substrate is an important factor which affects the nutritional content, production and yield of mushroom.

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4. METHODS OF CULTIVATION

In solid state cultivation method, solid waste of agro-industries can be used for cultivation. The substrate is soaked in bavistin or formaldehyde containing solution overnight and then spread on a clean surface for removing excessive amount of water to maintain the moisture content upto 75%. The moist substrate is then sterilized by autoclaving at 121°C for 30 min orby free flowing steam at 100 °C for 2hrs. This is followed by filling the substrate in plastic bags and inoculating it with 2% spawn. The inoculated bags are incubated at appropriated temperature and humidity content which varies according to the mushroom species.

Solid state cultivation process also involves the tray cultivation methods. In tray cultivation method, trays are filled with sterilized substrate, inoculated with the spawn and incubated at appropriate temperature and conditions. After spawn run, the whole substrate is covered with mushroom mycelium. This is followed by the addition of casing layer with the caution that it must not penetrate the substrate layer.

Another method of solid state cultivation process is log cultivation method which is mainly used for shiitake mushroom. In this method, wooden logs are used for cultivation. Holes are drilled in the logs for the inoculation of mushroom spawn. The inoculated logs are incubated in appropriate condition for the production of mushroom fruit bodies.

All these methods provide mushroom fruit bodies for the consumption purpose. However, mushroom biomass can be produced by liquid culture method. This is performed in fermenter. The mushroom mycelium is cultivated as seed culture in the fermenter and this starter culture is used in the fermenter for the production of mycelial biomass in appropriate conditions. These conditions vary according to the mushroom species used.

5. NUTRITIONAL CONTENT OF MUSHROOMS

Mushrooms are rich source of protein which can be consumed to combat protein deficiency diseases like marasmus and kwashiorkor. They also possess crude fiber content and minerals such as calcium, iron, zinc, magnesium, manganese, selenium and arsenic (Alam et al., 2008). Mushrooms in diet can help to combat mineral deficiency related problems. It is good source of vitamin D which is used in calcium absorption (Feeney et al. 2014) and required for strengthening the bones. Low amount of fat and carbohydrate content in mushroom makes it suitable for consumption by diabetic patients.

6. ANTI-GENOTOXIC PROPERTIES OF MUSHROOM

The bacterial reversed mutation assay is used to evaluate the mutagenic properties. The test uses amino acid-dependent strains of *S. typhimurium.* In the absence of an external histidine source, the cells cannot form colonies. Colony growth is resumed if a reversion of the mutation occurs, allowing the production of histidine to be resumed. The independent experimental results by (Prabakaran et al., 2011;Taherkhani, 2014; Devi et al., 2015)showed that *Phellinus spp*. extracts had antimutagenic activity when tested with S. *typhimurium* strains TA98 and TA100, especially the ethanol extract of *P. rimosus*. In the case of TA98, the ethanol extract of *P. rimosus* had strong activity at 1mg/ plate, whereas ethanol extract of *P. rimosus* showed strong antimutagenic activity at the concentration of 2 mg/plate for TA100. The ethanol fraction had the most active antimutagenic activity.Based on these findings, it is suggested that the antimutagenicity activity of ethanolic extract from *P. rimosus*is due to the presence of phenolic compounds in this mushroom. The mechanism by which the extract shows antimutagenicity is not clear. However, the antimutagenic activity of the extract against direct acting mutagens is probably due to the direct inactivation of the mutagens by complex formation with the ingredients present in the extract. The antimutagenic effect may also be a resultof protecting the bacterial genome from the directly acting mutagens. This protection can be rendered by the rapid elimination of mutagens from bacteria before their interaction with the DNA, which may be mediated by facilitating or stimulating the transmembrane export system in bacteria(Ajith and Janardhanan, 2011; Laovachirasuwan et al., 2016). The anti-genotoxic potential of mushrooms is shown in table 1.

Table-1: Anti-genotoxic properties of mushroom.

Agaricus blazei	*Agaricus blazei* is shown to decrease H_2O_2 induced DNA damage within 15 mins of its application, maximum potency being reached at 30 mins.	Živković et al., 2017
89 different mushroom species	Five extracts (*Cortinarius evernius, Rozites caperatus, Lactarius vellereus, Russula integra* and *Pleurotus cornucopiae*) inhibit UV induced mutations	Filipic et al., 2002
Ramaria largentii (orange coral mushroom	*Ramaria largentii* extract has a remarkable DNA protective activity against H_2O_2-induced damage	Aprotosoaie et al., 2017

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Agaricus brasiliensis	Co-treatment with quercetin (100 and 500 µmol/L) significantly reduced DNA damage caused by Thymol (200 µg/mL)	Radakovic et al., 2015

7. MUSHROOMS AS IMMUNITY BOOSTERS

Mushrooms are also known for their ability to boost up the immune response. Immunomodulatory properties of mushroom are shown in table 2. The main component for inducing immunity in the humans is mushroom glycans (also known as β -glucans. In mushroom glycans, the structural backbone is composed of glucose which has been reported to activate both the innate and adaptive components of immune system.These carbohydrate moieties act upon innate immune cells such as natural killer cells (NK), macrophages, neutrophils and dendritic cells to activate them to release important cytokines or reactive species in order to kill infectious microbes or tumor cells. The cytokines released in turn also activate the adaptive immune cells which along with the innate immune cells act upon foreign antigens by augmenting both the cellular and humoral immunities. An immunomodulatory heteroglycan isolated from the fruit bodies of *Astraeus hygrometricus* has been reported to induce immune stimulation in Dalton's Lymphoma tumor bearing mice which reduced tumor progression and increased the survival rate in the animal. In another study, heteroglycan isolated from the mycelia of *Pleurotus ostreatus* has also shown to inhibit tumor progression (in both Dalton's Lymphoma and solid Sarcoma 180 model) by augmenting the immune system. Therefore, mushroom especially, glycans can be an important source for immunomodulatory pharmaceutical products (Devi and Maiti, 2016).

Table-2: Immunomodulatory properties of mushroom

Mushroom	Model	Cellular Response	Dose	Reference
Grifola frondosa	*In vivo*, mouse colon cancer	Increase in Tumor-specific CD8+ and CD4+ T cells, NK cells	20 or 80 mg/kg PO for 20 d; Maitake D-fraction	Masuda et al, 2013
Ganoderma lucidum	*In vivo*, mouse cancer cell line CT26	Increase in Phagocytosis via TLR4	50, 100, 200 mg/kg IP; standardized PSG-1 polysaccharide	Zhang et al, 2013
Agaricus bisporus	*In vivo*, mouse leukemia	Increase in CD3, CD19, CD11b, Liver weight, Spleen weight, NK activity	3 or 6 mg/kg PO × 3 wk; hot water extract	Lin et al, 2012
Ganoderma lucidum	*In vitro*, human inflammatory breast cancer line	Decrease in MMP-9	0.5, 1.0 mg/mL every 48 h of 96 h; extract of fruiting body and cracked spores	Martinez-Monte mayor et al, 2011
Cordyceps	*In vitro*, human bladder cancer cell lines 5637 and T-24	Decrease in MMP-9, NF-κB	50, 100, 200 µg/mL; cordycepin	Lee, Kim, and Moon, 2010
Turkey Tail	*In vivo*, human breast cancer, phase I clinical trial	Increase in Lymphocyte count, NK activity,CD8+ T cells,CD19+ B cells	6 or 9 g PO daily for 6 wk	Torkelson et al, 2012

8. ROLE AS ANTIOXIDANT

A number of secondary metabolites of mushroom including terpenes, polyketides, steroids and phenolic compounds are the source of antioxidant activity (Table 3). Among the all compounds, polyphenols have highest antioxidant activity. Polyphenols are the group of compounds which bear an aromatic ring and one or more hydroxyl groups. The mostly known polyphenols are flavonoids, flavones and anthocyanin. Polyphenols have free radical scavenging , metal chelation, enzyme modulation activities. Besides, these polyphenols can inhibit low-density lipoprotein (LDL) oxidation.

Glutathione (GSH) plays a critical role as the master antioxidant in mammalian cells and tissues. Recent studies have also identified ergothioneine as a potentially important antioxidant/nutrient with preventive properties. It has been reported that mushrooms (particularly the yellow oyster and porcini) are a rich dietary source of these critical antioxidants. Both antioxidants have been reported to be more concentrated in pileus than stipe tissues in selected mushrooms species. Kalaras et al., (2017) have reported that *Agaricus bisporus* harvested during the third cropping flush contained higher levels of ergothioneine and glutathione compared to the first flush, possibly as a response to increased oxidative stress. They may have important translational implications,

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suggesting that mushroom consumption may be associated with reductions in oxidative stress related diseases and disorders (Kalaras et al., 2017).

Table-3: Antioxidant properties of mushroom

S. No.	mushroom	Antioxidant properties	mechanism	reference
1.	*Armillaria mellea, Cantharellus cibarius, Lactarius deliciosus, Leccinum aurantiacum, Suillus luteus,* and *Boletus badius*	*B. badius* and *S. luteus* have antioxidant potential due to presence of phenolics.	dried using both freeze drying and convection drying at 50°C. water and ethanolic extracts were prepared. Hot-air drying shows more frequently negative effects as compare to positive effects.	Radzki et al. (2014)
2	*Terfezia boudieri Chatin, Boletus edulis,* and *Lactarius volemus*	High contents of phenolic and flavonoid compounds. *B. edulis* showed the higher TAC and TPC; highest inhibitory effect on DPPH and on reactive oxygen species (ROS).	A microwave-assisted extraction (MAE) process for polyphenols was used to prepare methanol concentration of 80%, extraction temperature of 80 °C, and extraction time of 5 min. Different antioxidant assays (i.e., total antioxidant capacity (TAC) and total phenolic content (TPC)) were utilized to evaluate the antioxidant capacity of the methanolic extracts of mushrooms.	Özyürek et al., 2014
3	*Geranium nepalense*	abundant contents of total polyphenol and flavonoid contents	*G.nepalense* scavenged DPPH and ABTS radicals in a dose-dependent manner and strongly suppressed cellular reactive oxygen species, thereby protecting H_2O_2-induced cytotoxicity. It may be useful in preventing oxidative stress-induced diseases including Alzheimer's disease, respiratory inflammatory disease, and chronic kidney diseases.	Sim et al., 2017
4	*Fomitopsis betulina*	Phenolic acids (syringic, gallic, p-hydroxybenzoic, 3,4-dihydrophenylacetic), indole compounds (L-tryptophan, 5-hydroxy-L-tryptophan, 5-methyltryptamine), sterols (ergosterol, ergosterol peroxide, hexestrol, cholecalciferol), and triterpenes (betulinic acid, betulin)	Phenolics have antioxidant ability. The mycelium extract exhibited significant cytotoxic activity against prostate cancer cells, while the fruiting body extract indicated a moderate effect on the viability of melanoma and prostate cancer.	Sułkowska-Ziaja et al., 2018
5	*Pleurotus djamor*	Phenolic, flavonoid contents of methanol and aqueous extracts of mushroom	Radical scavenging activities of methanol and aqueous extracts was assessed using 1,1-diphenyl-2-picryl-hydrazyl (DPPH), N,N-dimethyl-p-phenylenediamine (DMPD), total Fe^{3+} reducing power, phosphomolybdenum, metal chelating activity, and lipid peroxidation inhibition assays. Both extract types showed efficient radical scavenging activities against DPPH and DMPD radicals, ferric (Fe^{3+}) and cupric (Cu^{2+}) ion reducing powers, metal chelating activities, and lipid peroxidation inhibition.	Sudha et al., 2016
6	Lactarius salmonicolor	p-hydroxybenzoic acid,	antioxidant capacity was assessed	Athanasakis et

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		total phenolic content	by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method and total phenolics and antioxidant activity wasreported to be significantly higher in the fractions of increased polarity.	al., 2013
7	*Boletus chrysenteron, B. edulis, Leccinum scabrum, L. aurantiacum,* and *Macrolepiota procera*	Scavenging ability was the highest for B. edulis and B. chrysenteron. Boletus chrysenteron and B. edulis were characterized by high scores of polyphenol contents and antioxidant activity in the FRAP, TEAC, and DPPH assays.	Antioxidant activity was measured with the FRAP, TEAC, DPPH scavenging ability and ferrous ions chelating ability assays which show the variation in the antioxidant properties of mushroom.	Witkowska et al., 2011
8	*Pleurotus ostreatus, Morchella esculenta, P. ostreatus (Black), P. ostreatus (Yellow)* and *Pleurotus sajor-caju*		The DPPH radical scavenging activity was highest in *Morchella esculenta.*	Ahmad et al., 2014
9	*Lycoperdon perlatum, Cantharellus cibarius, Clavaria vermiculris, Ramaria formosa, Marasmius oreades, Pleurotus pulmonarius*	bioactive components revealed that total phenols are the major bioactive component found in extracts of isolates.	Antioxidant efficiency by inhibitory concentration on 1,1-Diphenly-2-picrylhydrazyl (DPPH) was found significant when compared to standard antioxidant like Buthylated hydroxyanisol (BHA). The concentration (IC(50)) ranged from 0.94 ± 0.27 mg/mL to 7.57 ± 0.21 mg/mL.	Ramesh and Pattar, 2010

9. ROLE AS ANTI-CANCER AGENT

Cancer is characterized by the aberrant behavior of a single cell type, which is difficult to treat by chemotherapy due to the lack of compounds specifically targeting only malignant cells. Even the most successful anticancer drugs were typically chosen because of their ability to preferentially inhibit rapidly dividing cells. Though, these drugs are aimed to eliminate neoplastic cells, while causing minimal damage to normal cells, their undesirable effects are inevitable. There is an increasing demand for novel diagnostic and medical therapies in cancer treatment. Lectins can play an important role in targeted drug delivery by increasing the efficacy of the treatment by enhancing drug exposure to the targeted site and limiting the side effects of the drug on normal and healthy tissues.

Mushrooms are widely known for their anti-cancer properties.The anti-cancer activity in glycans of mushroom varies widely from the simple homopolymers to the complex heteropolymers. Mushroom polysaccharides that are bound to proteins or peptides have been noted to exhibit a higher anti -tumor potential as compared to the polysaccharides without any protein or peptide complexation. Mushroom glycans mediate their antitumor function mostly by augmenting the immune response of the host. It has been reported that the aqueous extract derived from *Cordyceps militaris* induces apoptosis by increasing Bax expression in A549 cells. In another case, protein bound glycan derived from *Phellinus linteus* was shown to inhibit SW480 colon cancer cells by inducing G2/M cell cycle arrest. Polysaccharides or glycans can also induce necrosis or programmed cell death as another mechanism for inhibiting tumor proliferation.

Macrophages secrete a repertoire of cytokines like TNF-α and NO that shows cytotoxicity to tumor cells. NO exerts its toxic effect by inactivating enzymes that contain iron-sulfur clusterandthereby inhibiting DNA-binding proteins by releasing zinc finger transcription factors and by disbalancing the mitochondrial membrane potential. Acidic glycans extracted from *Phellinus linteus* produced both NO and TNF-α which contributed to anti-tumoricidal functions. In addition, polysaccharopeptides from *Ganoderma lucidum* have shown a different mechanism of tumor inhibitory functions on vascular cell proliferation(anti-angiogenesis) as well as by imparting inhibition on VEGF secretion(Devi and Maiti, 2016).

Tumor lectinology has identified cytochemical and histochemical differences between normal and tumor tissues, between different types of tumors, and within a single class of tumors. Alteration in protein glycosylation and increased sialylation are hallmark features of cancer cell surfaces serving as targets for lectin-

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based markers in histochemical studies. Therefore, lectins can be used to reveal the stages of carcinogenesis and can be very useful tools for histochemical and other methods for the identification of cancer and the degree of metastasis. There has been a tendency to shift the application of lectins from detection to combat cancer. Recent research shows cytotoxic, apoptotic, and necrosis inducing effects of certain lectins. Comparative studies using several lectins as antitumor or cytotoxic agents have shown a variety of effects depending on lectin source, cell line or cancer type. Some lectins inhibit cell proliferation in malignant cells by cross-linking cell surface glycoconjugates without any need for internalization. Other lectins like plant toxin ricin bind to glycoproteins on the cell surface from where it is endocytosed to the cytosol and enzymatically inhibits protein synthesis.

Initial studies on the role of lectins for human cancer treatment have focused on cytotoxic properties of plant lectin ricin (RCA) and abrin. Mistletoe lectin from *Viscum album* has also been widely studied against cancer, whereas phytohemagglutinin (PHA) and wheat germ agglutinin (WGA) are well known for their toxic effects on intestinal epithelial cells. Various other plant lectins with antiproliferative effects have also been reported, namely GSA (*Griffonia simplicifolia* agglutinin), Con-A (Concanavalin A), PNA (*Arachis hypogea*), SBA (*Soyabean agglutinin*), and VFA (*Vicia foba* agglutinin). The antiproliferative lectins from higher fungi are less documented, although some mushroom lectins have profound inhibitory effects on cell proliferation of malignant cells. They have been reported to be cytostatic and non-cytotoxic and their antiproliferative action is reversible. These properties of mushroom lectins combined with their unique carbohydrate specificity make them very attractive molecules for investigating their antiproliferative potential and possible role in cancer therapeutics (Singh et al., 2016).

In a study by Wang et al. (2014) water -soluble polysaccharide -BEP, has been reported to be successfully isolated and purified from the fruiting bodies of B. *edulis*, with an average molecular weight of 113,432 Da. BEP contains no protein and uronic acidbut has a high carbohydrate content of 93.4%. Monosaccharide component analysis indicated that BEP was composed of glucose, galactose, rhamnose and arabinose with molar ratios of 2.9:3.2:1.3:1.6. The data obtained from GC/MS, IR and NMR (1H and 13C) analysis revealed that BEP has a backbone consisting of (1→6)-linked--d-glucopyranosyl, (1→2,6)-linked-- d-galactopyranosyl, (1→6)-linked--d-galactopyranosyl, (1→3)- linked--d-rhamnopyranosyl residues that terminated with a single terminal (1→)-linked--l-arabinofuranosyl residue at the O- 2 position of (1→2,6)-linked--d-galactopyranosyl residue along the main chain in the ratio of 2.9:1.6:1.6:1.4:1.5.

BEP was shown to significantly inhibit the growth of Renca transplanted in mice during in vivo antitumor experiment and had no cytotoxicity to hematological system and liver/kidney function of tumor-bearing mice at test doses. Acute toxicity was also investigated by oral administration of serial BEP dilution (from 500 mg/kg to 2000 mg/kg body weight) in 200 L PBS to the normal mice. Acute toxicity results proved that there was no toxicity up to a concentration of 2000 mg/kg body weight in BALB/c mice as evidenced by absence of significant behavioral changes and animal death (data now shown). Furthermore, BEP was reported to significantly stimulate splenocytes proliferation, increase the spleen and thymus indices, elevate the activities of NK cell and CTL in spleen, and promote the secretion of the cytokines IL-2 and TNF- in Renca tumor-bearing mice. The above results suggested that the BEP had indirect anti-tumor activity achieved by improving immune response (Wang et al., 2014).

10. COMMERCIALLY AVAILABLE CHEMOTHERAPEUTIC FORMULATIONS

Mushrooms havemedicinal applications and commercial products have been developed. A few of them have been mention in the table 4. Lu et al. (2011b)reported the extraction of polysaccharide Krestin (PSK), a non-toxic immunomodulator, from medicinal mushroom*Coriolus versicolor*. When it is used for standard neoadjuvant therapyand administered withpaclitaxel and trastuzumab augments, it provide anti-tumor immunity and results in improved pathological complete response rate and overall survival in mouse models of HER2+/ER- and triple negative and locally advanced breast cancer. Paclitaxel/Docetaxel and PSK may work together to auto immunize the patients of their own tumors, resulting in tumor-destructive immunity.

Another mushroom, *Ganoderma lucidum* also known as Reishi enhances the function of immunosuppressing anti-tumor agent Cyclophosphamide and Cisplatin as reported by Zhu et al. (2007) and Masuda et al. (2009). Hence, it becomes very important to categorically analyze and select mushrooms of medicinal use and put them into application. Mushrooms are commercially also available in cosmetics as well supplements and food products for consumption.

Table-4: Chemotherapeutic agents isolated from mushrooms.

Chemotherapeutic Agent	Indicated Mushroom	Reference
Trastudzumab	PSK (turkey tail *i.e. Coriolus versicolor*)	Lu et al, 2011b

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Cyclophosphamide	Reishi	Zhu et al, 2007
Cisplatin	Maitake (*Grifola frondosa*)*Cordyceps*, Reishi (*Ganoderma* spp.)	Masuda et al, 2009, Yao et al, 2012
Docetaxel	PSK (turkey tail *i.e. Coriolus versicolor*)	Kinoshita et al, 2009, Wenner et al, 2012
Doxorubicin	*Agaricus*	Lee and Hong, 2011

11. CONCLUSION

Mushrooms are being increasingly researched and used for their important health benefits with different varieties having different medicinal properties. The role of mushrooms as anti-oxidant, anti-cancer, its anti-genotoxicity and anti-mutagenic agent, open up wide areas in which mushrooms could be used. It has now become essential to harness the untapped potential of mushrooms in various fields and clinically and commercially make it available to everyone. More translational research is needed to understand different types of mushrooms and whether in extract form or other forms it can have a better effect. Further, a detailed research in this direction, can lead us to use these as strong medicine in future.

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APPROACHES AND APPLICATIONS OF BIOPROSPECTING OF GENES FOR CROP IMPROVEMENT

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ABSTRACT
Bioprospecting is the exploration of biodiversity for new resources which could be used for social and commercial value. It is systematic search for whole organism, genes and natural compounds for useful purpose. The microbial diversity thriving in extreme heats in deserts, extreme cold even in volcanic eruption could be great source of genes. These genes can helps crop plants to overcome several abiotic stresses. Even the animals such as camels and goats possess unique adaptive mechanism for abiotic stress tolerance and several fishes has unique adaptive tolerance for cold and salinity and these genes can be explored for crop improvement. For crop improvement there are several approaches which could be applied for bioprospecting of genes, it could be done by using heterologous probes or primers, using purified proteins, transcriptome profiling, insertional mutagenesis, map based cloning and integrated approaches. Among them transcriptome profiling is very efficient method for bioprospecting and it could be achieved by DDRT-PCR, cDNA AFLP, microarray, SAGE and massively parallel sequencing. Emphasis should be given to master regulators such as transcription factors and signaling components which play major roles. Ultimate aim of bioprospecting is to find new resources and products from nature that can be used by humans for social and economic value increment. Improving human health, through both medicine and better nutrition are main thrust area in agriculture. Bioprospecting plays a dominant role in discovering leads for drug development, since existing/known compounds for developing drugs for human use are limited. Nature can provide original novelty and complexity that can be modified in the laboratory and then could be utilized for crop improvement.

INTRODUCTION
The search for and utilization of biological resources is as old as human kind and has been key to the survival, adaptation, and evolution of the human species. In recent times, agronomists and professionals from related sciences have done considerable agricultural prospecting looking for landraces having agronomic advantages, particularly higher yields, pest and disease resistance, and adaptation to specific microhabitats. Improved crop varieties and hybrids has been considered for gene prospecting by indigenous communities. Even plant breeding no longer depends only on genes from wild types and ancestors of modern crops. Now a day's Genetic engineeringhas been used to improved new varieties. For example, with designer genes that confer resistance to pests and frog skin inspired genetic engineers to design resistance genesfor the bactericidal and fungicidal peptides (Nader and Hill, 1999).

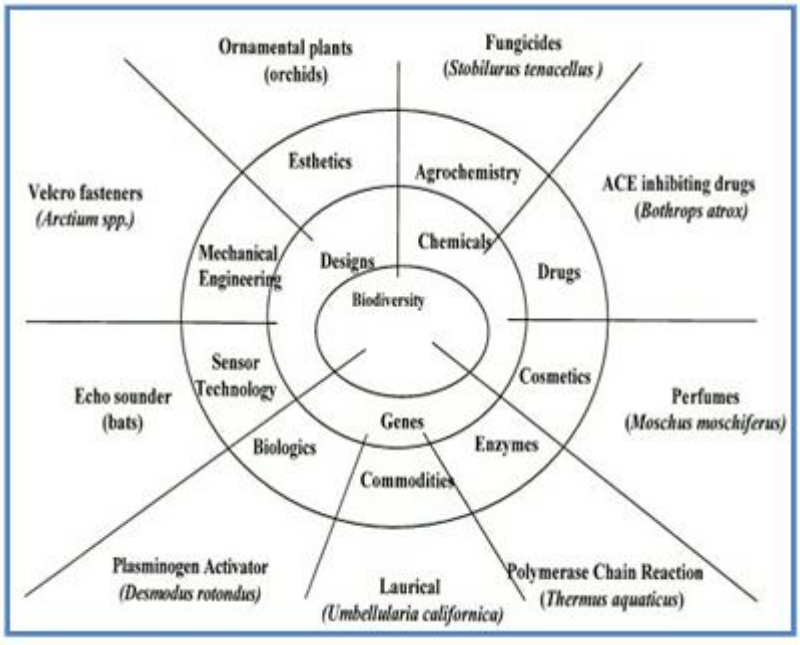

Figure-1: The three principal sources of inspirations from biodiversity and their applications. Products examples are described in the (with variations derived from Nader and Hill, 1999.

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MODERN BIO PROSPECTING

Modern science and technology have provided substitutes for biological products like natural colorants and plant-derived natural pesticides like rotenone, nicotine, and ryanodine. Yet human ingenuity still depends in great part on inspirations from wild life-forms to create these substitutes. On this basis of development of novel products, able to discover and expose the principle of a biological function. Nature provides solutions to most of life's technical problems. Therefore, natural selection has imposed on living organisms for minimum of material and energy accomplishes a maximum of efficiency and stability. This provide resources and increasing environmental problems (Hill,1997).

CHEMICAL PROSPECTING

The major defence mechanisms of plants against herbivores rely on chemicals: ''The world is not colored green to the herbivore's eyes, but rather is painted morphine, L-DOPA, calciumoxalate ,cannabinol, caffeine, mustard oil, strychnine, rotenone, etc.'' (Janzen, 1975). The co- evolution of herbivores and their feeding plants has created diversity on both the species and the molecular levels (Ehrlich and Raven, 1964). Communication, competition, sexual attraction or rejection, and pollination are also based to a great degree on chemistry and have contributed to the development of diversity. To help conserve this chemical wealth for future generations, Thomas Eisner developed the concept of chemical prospecting, a collaborative effort among conservationists, scientists, the pharmaceutical industry, and biodiversity-rich countries to develop products from bio- diversity and to generate income for its conservation (Reid et al., 1993).

Although human ingenuity has created a huge variety of chemical compounds with exceptional pharmaceutical activity without inspiration from nature—like the anxiolytic benzodiazepines valium and librium— natural compounds and their derivatives are still of central importance in drug discovery.

GENE PROSPECTING

Numerous products derived from animals are already entering into the market (Tamayo et al., 1997). A powerful protein to treat acute heart attack was discovered biorationally in the saliva of the common vampire bat, *Desmodusrotundus*. While feeding on the blood of their victims, these bats release analgesics and coagulation inhibitors with their saliva, including the Desmodus Plasminogen Activator (DPA), which dissolves thrombolytic blood clots and allows clot-free drinking. Recombinant human tissue Plasminogen Activator (tPA) has been approved as a therapeutic agent against heart attack in the United States and Europe, although researchers at Schering AG found that DPA is more efficient and safer for therapeutic application. Consequently other blood-sucking organisms like ticks, mosquitoes and hookworms are currently under investigation in attempts to discover novel anticlotting, antiplatelet, and vessel-constricting principles.

One example of the value of traditional knowledge is the development of the recombinant pharmaceutical protein hirudin, a potent anticoagulant derived from the saliva of leeches (*Hirudomedicinalis*). Leech therapy has been used in traditional medicine in Europe and Asia for centuries as the only efficient treatment of thrombosis, thrombophlebitis, and hypertension.

ECONOMIC VALUE AND BENEFIT SHARING

Biodiversity-Related Markets: Markets related to products derived from biodiversity are significant, as shown in Table 1. The estimated growth of the world drug market is about 6% per year, from 1997 sales of U.S. $295 billion to $378 billion in the year 2001 (Thayer, 1998). Recently developed natural products have been more effective than already available pharmaceutical product. Bioprospecting needs to provide large numbers of natural compounds at high speed, to compete with synthetic and combinatorial chemistry efforts

Table-I: Markets of Various Products and Services

Market.	U.S. $ (billions)
Drug market (1997)	295
Natural product-based drugs (1993)	50
Phytomedicines (1993)	12.4
Pesticides	30
Seeds (estimate for 2000)	50
Horticulture (U.K., 1991)	1.6
Enzymes (1996)	2.5
Cosmetics (U.S.A., 1994)	20
Natural cosmetics	0.5
Tourism (1995)	3400
Nature tourism (1988)	12

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BIOPROSPECTING AND CONSERVATION

The initial meaning of prospecting was the search for precious metals and oil in the soil and subsoil. Bioprospecting has been used to indicate the search for new sources of chemical compounds, genes, proteins, microorganisms, and other products that have economic potential and that can be found in the world's diversity of plants, animals, and microorganisms. It was highlighted earlier that the present and potential value of bioprospectible designs, genes, and chemicals is enormous and justifies the use of intelligent conservation strategies. To be effective as an element of conservation, bioprospecting must contribute to the generation of wealth through R&D and must also alleviate poverty in biodiversity rich regions. In this context, bioprospecting programs at the national level can help to develop national capacity and provide economic returns to conservation projects if investments are made in science, technology, and market research. However, this may not be sufficient to achieve sustainable development: communities inside or bordering protected areas must participate in conservation and must benefit from bioprospecting activities in their own regions. The utilization of biodiversity linked to added value at the local level appears to be a promising avenue to generate economic growth at the community level and to create incentives for conservation. Economic benefits at the community level could include: growing, grading, and packing of medicinal plants; cultivation of new ornamental plants; domestication, cultivation, and primary production of biopesticides; cultivation and processing of aromatic teas; and exploration and evaluation of local land races with resistant genes of interest. In all cases, a critical prerequisite for engaging in added-value biodiversity development at the community level is market research.

BIOPIRACY

Our world would be a totally different place today if crops and domestic animals had not been moved from their centers of origins and domestication to other parts of the world. Coffee to the Americas, potatoes to Europe, corn to Africa, and wheat to China are just a few examples of how germplasm has become the "patrimony of humanity".The ratification of the Convention on Biological Diversity (CBD) and the development and implementation of property rights regimes have changed completely the prior existing scenarios on the spread and use of germplasm. Today, in many cases genetic and biochemical resources are jealously guarded and are the cause of conflict (when appropriate and legally sound negotiations are absent) in many parts of the world. The term biopiracy has been coined to reflect the illegal appropriation or exploitation of genetic and biochemical resources. Even national researchers, when dealing with poorly defined or cumbersome legal procedures, tend to take shortcuts in their quest for useful genetic and biochemical resources.

CONCLUSION

The recently born concept of modern bioprospecting is already being challenged by new developments in science and technology. In an endeavour to reduce risks and to increase the probability of success, companies are trying to substitute natural (incalculable) products with artificial (calculable) products wherever possible. The success rate in drug discovery is estimated to be about one product out of 10,000 screened chemicals or plant species for one field of diseases. It is too early to judge whether combinatorial chemistry will be as successful as natural sources in the generation of new drug candidates. Many scientists doubt that novel lead structures will result from these combinatorial efforts, which can compete with those evolved in nature during an evolutionary process of millions of years. However, the technology can be a powerful tool to complement natural sources by generating millions of derivatives from natural leads. It should be noted that many highly successful drugs, which have been introduced to the market only recently, are derivatives of natural leads that were discovered many decades ago.

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ANALYSIS OF GENETIC VARIATION AMONG THE ACCESSIONS OF *BACOPA MONNIERI* COLLECTED FROM REGIONS OF MADHYA PRADESH USING RAPD

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ABSTRACT
Bacopa monnieri L. Commomnly known as Brahmi, one of the important medicinal plants, used in Ayurvedic medicine for over 3000 years and classified as a "Medhya Rasayana" a drug used to improve memory and intellect. Bacopa has been used as a brain tonic to enhance memory concentration and it also provides positive benefits for sufferers of epilepsy and anxiety.

Several DNA based marker systems are used to study the genetic diversity of medicinal plants. These methods require detailed genetic information about the genome. Although less information is available about the genome of Bacopa. In the present study genetic variability between 12 accessions of Bacopa collected from various regions of Madhya Pradesh were analysed by RAPD method using 31 primers of 10 mer obtained from "Operon Inc" USA.

Amplified DNA fragments with each primers for all the 12 accessions were separated by agarose gel electrophoresis along with a 250 bp ladder and reproducible bands from each experiment were recorded. Clear and reproducible bands from each experiment were recorded. A total of 175 amplicons were obtained from 31 RAPD primers. Total 149 non-polymorphic bands and 26 unique bands were found. Using 31 primers between the Bacopa accessions of Madhya Pradesh regions 14.86% average polymorphism was observed. The dendogram developed based on Similarity index (SI) showed low level genetic variation among the accessions of Madhya Pradesh region.

Keywords: RAPD, Bacopa monnieri, plant tissue culture.

INTRODUCTION
Bacopa monnieri (2n = 64) commonly known Brahmi a small creeping herb with light purple color flowers, member of family Scrophulariaceae. It grows prolifically in wet soil, shallow water and marshes in India and the tropics (Malhotra *et al.,* 1959). It has been used as a brain tonic to enhance learning, memory and concentration (Joshi *et al.,* 2006) as well as providing positive benefits for sufferers of epilepsy and anxiety (Singh *et al.,* 1980). The standardized methanol extract of *Bacopa monnieri* also possesses potential antidepressant activity in rodent models of depression (Sairam *et al.,* 2002). Bacoside is known to stimulate GABA which has anticonvulsant, pain relieving, anti stress and sedative effects (Singh *et al.,* 1996). Bacosides appear to have antioxidant activity in the hippocampus, frontal, cortex and striatum regions of brain (Bhattacharya *et al.,* 2000). Animal research has shown *Bacopa* extracts modulate the expression of enzymes in generation and scavenging of reactive oxygen species in the brain (Chowdhari *et al.,* 2002). The significant molecule responsible for pharmacological benefits of Bacopa is reported as Bacoside, a Tetra-cyclic triterpenoid Saponin (Chatterji *et al.,* 1965).

Madhya Pradesh is one of the richest /sources of medicinal plants in India. *Bacopa monnieri,* however, has been declared as one of the threatened medicinal plants due to human destruction and also environmental changes (FRLHT, 2006, Red Data List of endangered species of Madhya Pradesh, IUCN, 2010). Present study, there fore, was carried out germplasm existing in the *in situ* conditions at different regions of Madhya Pradesh and to assess the genetic diversity in the collected accessions using RAPD analysis.

MATERIAL AND METHODS
Bacopa monnieri L. accessions growing *in situ* conditions were collected from different agro-climatic regions of Madhya Pradesh and were allocated with a code name (BME1:Jabalpur), (BME:2 Chitrakoot). (BME3:Khandwa), (BME4:Bhopal), (BME5:Alirajpur), (BME6:Shivpuri), (BME7: Muraina), (BME8:Amarkantak), (BM9:Reeva), (BM10:Gwalior), (BME11:Swarnayanti park Bhopal), (BME12:Mandla).

Genomic DNA of all the accessions was isolated by modified Saghai - Maroof Method (Saghai-Maroof *et.al.,* 1984). The genomic DNA concentration and purity was measuring spectrometrically and about 50-60µg DNA was used in each RAPD reaction mixture. The Master-Mix used for the RAPD analysis was consisted of 10X buffer (2.0 µl), $MgCl_2$ (2.8 µl), 10mM d NTP mix (1.5 µl), 3U/ µl Taq DNA Pol (0.2 µl), Primer (2.0 µl), nuclease free Water (9.5 µl) and Genomic DNA (2.0 µl).

The PCR program used was as follows:

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STEP 1:	Initial Denaturation	94 °C	5 min
STEP 2:	Denaturation	94 °C	1 min
STEP 3:	Annealing	37 °C	1 min
STEP 4:	Extension	72 °C	2 min
STEP 5:	Final extension	72°C	10 min
STEP 6:	Hold	4 °C	α min

PCR was performed with above condition for each primer with 12 accessions at a time in identical environment in **'T-gradient thermocycler'** of **Biorad Company**. After completion of 40 cycles, the samples with amplified fragments (amplicons) (for each primer for 12 samples) were visualized on 1.5 % agarose gel, along with 250ng DNA adder.

The experiment was repeated and the gel pictures were used to assess the total number of amplified fragments, non polymeric, polymeric bands for each accession with single primer. Genetic diversity of the accession was assessed using dendogram , prepared prepared using PAST software: (http://palaeo-electronica.org/2001_1/past/issue1_01.htm). Percent polymorphism was evaluated for each of the primer using data on number of Polymorphic and Non-polymorphic bands.

RESULTS AND DISCUSSION

Inspite of extensive reports available on biochemical, pharmacological studies on this very important medicinal plant, limited work have been carried out on its genetic and phylogenetic aspects. *Bacopa monnieri* is reported to be an octaploid with basic chromosome number Eight. However any evidence for the nature of its ploidy (auto-allo) is not available (Khanuja *et al.,* 1997). Darokar *et al.,* 2001 reported low levels of genetic diversity by RAPD analysis of 40 RAPD primers in 23 accessions of Bacopa.

In the present study, amplified DNA fragments with each primer for all the 12 accessions were separated by agarose gel electrophoresis along with a 250bp ladder. Clear and reproducible bands from each experiment were recorded. Data for total number of bands, number of polymorphic and non-polymorphic bands generated by each primer were recorded. The number of amplicons produced by the primers varied from 1to11. Primer OPB-02 resulted highest number of bands i.e. 11. While primer OPB-07 and OPB-23 showed the least number *i.e*. single (amplicon) of bands.

A total of 175 amplicon were obtained from 31 RAPD primers. Out of which 149 bands were non-polymorphic bands while 26 bands were found to be unique. The primers OPB-01, OPB-05, OPB-06, OPB-12, OPB-15, OPB-16, OPB-17, OPB-18, OPB-19, OPB-21, OPB-22, OPB-24, OPB-28, OPB-29, and OPB-31 regenerated specific polymorphic bands. Rest of the primers generated non-polymorphic bands. The number of polymorphic bands generated by each primer varied from one to as high as five. OPB-19 produced highest polymorphic bands followed by OPB -17 and OPB 15, 21, 22and 23.

The percentage polymorphism and the molecular weight of the polymorphic bands generated by each primer were recorded. A total percentage of (average) polymorphism observed was 14.86% using 31 primers. The 15 primers showing polymorphism between the 12 accessions were OPB-01 (14.28%), OPB-05 (25.0%), OPB-06 (11.1%), OPB-12 (20.0%), OPB-15 (33.3%), OPB-16 (16.6%), OPB-17 (57.14 %), OPB-18 (16.6%), OPB-19 (71.43%), OPB-21 (40.0%), OPB-22 (25.0%), OPB-24 (25.0%), OPB-28 (20.0%), OPB-29 (14.28%) and OPB-31 (22.22%). The primer showing least polymorphism is OPB-06, while primer OPB-19 has shown maximum polymorphism. Therefore, the range of polymorphism is 71.43% to 11.11%.

The dendrogram, given in Figure 1, was developed based on Similarity Index (SI) analyzed through RAPD primer to determine the extent of genetic variability and phylogenetic relationship in between the 12 accessions collected from 12 different locations of Madhya Pradesh using PAST software. The dendrogram produced by *Bacopa* accessions (Figure 4.24) showed two main clusters i.e. "Cluster A" and "Cluster B". Cluster A comprises of accessions collected from (BMG-1) (1-Jabalpur, BMG-2 (2- Chtrakoot), BMG-3 (3- katni) and BMG-4 (4- Bhopal). The second major Cluster B consisted of accessions collected from BMG-5 (5- Alirajpur), BMG-6 (6- Shivpuri), BMG-7 (7- Morena), BMG-8 (8- Amarkantak), BMG-9 (9- Rewa), BMG-10 (10-Gwalior), BMG-11. (11- Bhopal sp) and BMG-12 (12- Mandla). Cluster B is further subdivided into two different sub-clusters classified as cluster I and II respectively. Cluster I consisted of accessions BMG-5, BMG-6, BMG-7, and BMG-8 while cluster II consisted of the accession from region BMG-9, BMG-10, BMG-11., and BMG-12. Genetic distance levels of all 12 collected accessions *Bacopa monnieri* ranged from 1.46 to 7.4 (Figure-1). The accessions from BMG-5 and BMG-6 are linked at a genetic distance of 2.4, while BMG-7 and BMG-8 are linked at 3.2 of genetic distance level. The accessions from BMG-10 and BMG-11 are linked at

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1.46 genetic distance level. The accessions from BMG-1 and BMG-2 are linked at a genetic distance of 4.11, while BMG-3 and BMG-4 are linked at 4.00 of genetic distance level.

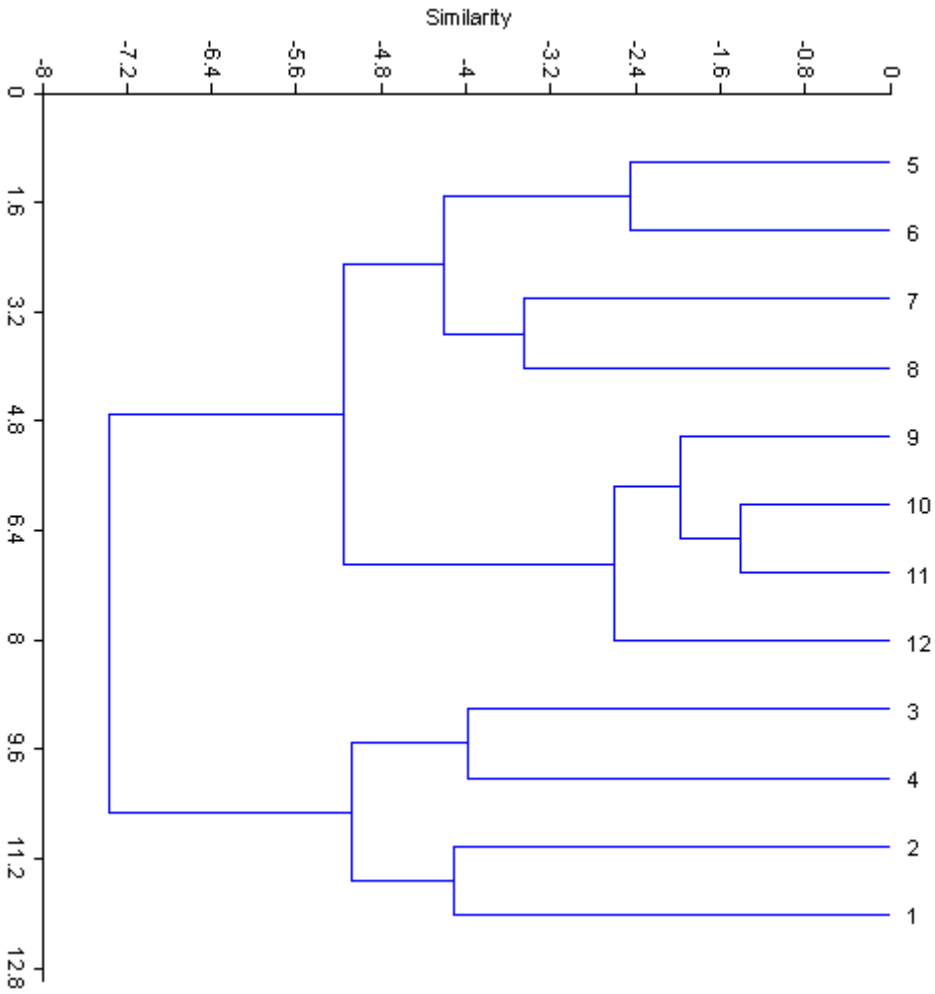

Figure-1: Dendrogram representing relation among the 12 germplasm of Madhya Pradesh region.

RAPD (Random Amplified Polymorphic DNA) analysis has been demonstrated as good tool for the purpose of assessing genetic diversity among the various accessions of a plant (Virk et al., 1995). The *Bacopa monnieri* germplasm collection has been assessed for diversity at DNA level, to define its core diversity and identify of individual accessions. 40 random primers tested, 29 primers generated one or more polymorphic bands. The number of polymorphic bands generated varieed from 2 to maximum of 8. Results demonstrated existence of low level of genetic variability in the species in Bacopa accessions from different regions of Pune. (Darokar et al., 2001). RAPD markers were used in vivo and in vitro plants of bacopa (Ashutosh et al., 2013; Muthaiah et al.2013) and also to assess the genetic diversity among the accessions collected from south india (Kumar et al. 2013) as well as in central India (Neraj et al. 2012).

Little is known about the genetic structure of population and phylogeny of *Bacopa monnieri*. It is believed that *Bacopa* is self compatible and has an insect mediated pollination and shows both sexual and vegetative mode of reproduction. *Bacopa monnieri* is an octaploid with chromosome number 8 (n=8). Any evidences about nature of its ploidy (auto/allo) are not available (Darokar *et al*., 2007). Present studies conclude that analysis of genetic variability among the 12 accessions of one single state i.e. Madhya Pradesh using RAPD technique have shown low genetic variability, like the earlier reports and emphasizes the need to application of other molecular marker for further analysis of genetic diversity .

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CURRENT ADVANCEMENT AND FUTURE APPRAISAL OF MUTATION BREEDING FOR GENETIC IMPROVEMENT OF CROPS

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ABSTRACT
Currently there are over 3220 officially released mutant cultivars in over 210 plant species. In breeding mutant traits are used to improve breeding goals to increase yield, quality, and disease pest resistance, tolerance to abiotic stresses, post-harvest degradation and innovative use. Mutation breeding in plants with reference to physical mutagenesis is effectively being utilized in breeding programmes. It serves as a base material for plant mutation breeding which involves some basic and advance techniques with other methods for the Genetic improvement in crop plant. Various mutagens (physical, chemical and biological) are used in inducing mutation. Future prospects of mutation breeding in crops are mainly focused to yield increment with high nutrients value and smart crop varieties and global food security can be achieved by this approach. Bioprospecting and allele mining of genes for biotic and abiotic stresses can be achieved with help of marker assisted mutation breeding. The method is being applied in several crops including rice, wheat, soybean, groundnut etc and identifying novel genotypes to be used in further breeding programme.

INTRODUCTION
Crop improvement programmes through mutations were originated about many years ago. Mutation breeding for genetic improvement of crops has been further introduced into modern plant breeding in the early 1940's.The released official plant mutant varieties of 3222 out of 170 different plant species across 60 countries worldwide. The developed varieties increasebiodiversity and provide breeding material for conventionalplant breeding thus directly contributing to the conservationand use of plant genetic resource [1]and still these works are continued by number of scientists.

Mutation breeding has been successfully utilised for the improvement of crops as well as to supplementthe effortsmade usingtraditional methods of plant breeding [2].Alteration in genes have been easily possible through induced mutation other than plant breeding procedures [3]. According to experience available, the efficiency of mutant genes can be depending on the genotypic background.

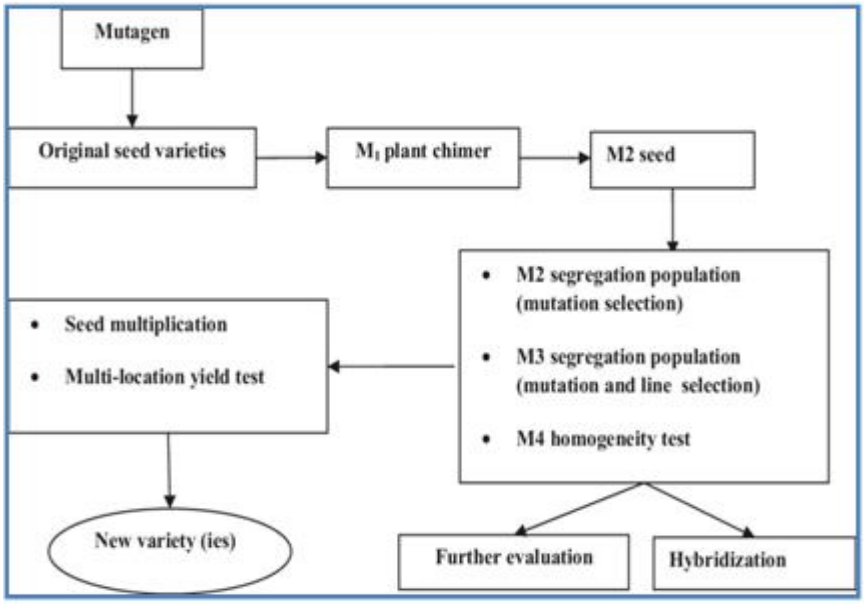

Fig-1: Mutation chain

The interactions between mutant genes and genotypic background cannot be justified. Therefore, mutants with valuable or desirable traits should be crossed with many varieties and strains in order to generate positive and negative interactions. Therefore selection of geneotypes, representing mutant gene is easy without showing negative effects. Mutation breeding is predominantly used in annual diploid and allopolyploid self-fertilizing crops, while it causes much more difficulties in cross-pollinating species.to induce genetic variability in various

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pulse crops such as Cicerarietinum [4] Viciafaba [5],Vignamungo [6],Lens culinaris [7],Hordeumvulgare [8] Vignaunguiculata [9],Vignaradiata [10],Glycine max [11].

As early as 1942, the first disease resistant mutant was reported in barley [12]. In cereals 1468 and legumes 370 varieties and majority of cultivars came from rice (434), barley (269) and wheat (197) [13]. The induction of mutation has already been recognised as a potential technique for crop improvement since the discovery of mutation effects of X-rays [14].

Current status of mutation breeding

For the purpose of high yielding crops, induced mutations have been used for new and valuable alteration in plant characterssignificantly in 80 years ago.The primary motive of the mutation breeding is to enlarge the frequency and spectrum of mutations [15] and also to increase the incidence of viable mutations. The aim of mutationhas developed varieties by altering characters like maturity, seedsize and disease resistance for increasing yield and yield related characters. Rice more than 800 varieties have been developed worldwide, from induced mutations or as a result of crossing such mutants with other breeding lines [16]. Through mutation breeding, abiotic and biotic stresses, duration of maturity and flowering and other yield contributingcharacters have been improvedThe role of mutation breeding in increasing food production and provide sustainable nutrition is well established [17].

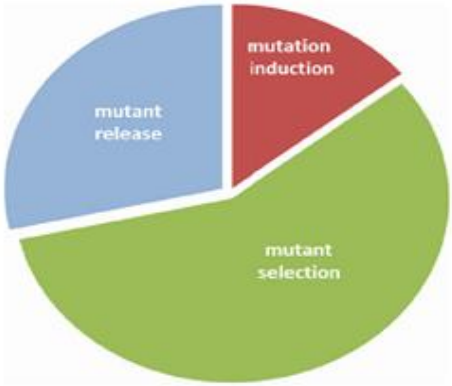

Fig-2: Three step Mutation Breeding

This possibility has been exploited in both cereals and legumes, as is evident from the list of mutant cultivars developed in legumes and cereals. Now a days RFLP, microsatellite-based DNA fingerprintinghas been used for mapping plant genes and transgenesis.In China(1957)the first rice varieties KT 20-74 and SH 30-21, developed through induced mutation were released and the first variety Yenhsing-1, developed by a cross-breeding programme with a mutant. Soon afterwards, Japan released the semi dwarf mutant Reimei which have significantly increased yield because of their lodging resistance. In Pakistan, a new variety 'Kashmir Basmati' was derived from induced mutation in Basmati 370which matures early and has cold tolerance and retains the aroma and cooking quality of the parent. The induction of thermo sensitive genic male-sterile (TGMS) mutant in Japonica rice mutant PL-12, which is controlled by a single recessive gene has an immense contribution in designing the strategies for the production of hybrid rice varieties. In China '26 Zhaizao' was developed by gamma ray irradiation of indica rice, as seen in fig.3

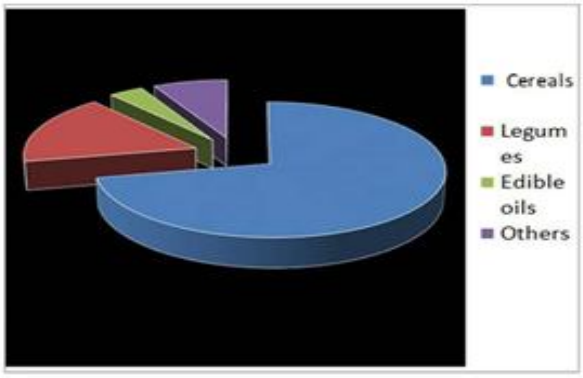

Fig. 3. Number of released varieties developed through mutation breeding in cereals and legumes
Data source: Officially released mutant varieties The FAO-IAEA Database, 2000

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Future appraisal of mutation breeding

In recent years our interest has been rationalised in mutation research because the induced mutagenesis is gaining importance and preference in plant molecular biology as a useful tool to identify and isolate genes to study their characteristics. These studies will definitely have a major impact on the future crop improvement programmes and improving the crops and developing the global food security [18]. In vitro mutagenesis technique has enriched the crop yield andimprovedresistance traits day by day. These studies will definitely have a major impact on the future crop improvement programmes [19]. A small amount of tissues and calli can be exposed tomutagenesis for the improvement of crop species. Currently, the use of in-vitro mutagenesis is low, very little number of plants such as banana and sugarcane have been regenerated through this technique.Recently, heavy ion beam irradiation has emerged as an effective and efficient way of inducing mutation in many plant varieties because of its broad spectrum and high frequency [20].

CONCLUSION

Induced mutagenesis are one of the most important approaches for broadening the genetic diversity in crops to circumvent the bottleneck conditions.Mutagenesis,decades old technique demonstrably can contribute to unleashing the potentials of plant genetic resources and thereby avail plant breeders the raw materials required to generate the envisaged smart crop varieties. Crop varieties generated through mutation breeding highly useful and using world widely and regulate the global food security.

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PRELIMINARY STUDIES ON LOW-DENSITY POLYETHYLENE (LDPE) DEGRADING BACTERIA ISOLATED FROM VARIOUS MUNICIPAL WASTE DUMPING SITES AT GWALIOR M.P.

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ABSTRACT
Plastic materials have broad spectrum in every aspect in this modern era. Materials are demolished and generated all the time, but if there is anything that goes against this rule, it can create disaster to the entire ecosystem. Due to its recalcitrant nature, it persists in the environment for a long period of time. Hence one of the biggest challenges in front of modern society is the proper handling and management of solid waste being created indiscriminately. In this study, biodegradation of polyethylene was carried out in Liquid culture medium under laboratory conditions. Two Gram positive and one Gram negative polyethylene degrading bacteria were isolated and identified as Staphylococcus spp., Bacillus spp. and Pseudomonas spp., respectively. Among them, Pseudomonas spp. was found to be the most significant polyethylene degrading bacteria. Percent weight loss of polyethylene was determined with each isolates separately and it was found to be 30% in case of Pseudomonas spp. followed by 26% in Bacillus spp., and 22% in Staphylococcus spp. as compared to control (0%) in liquid culture medium over two months of incubation period at 37°C. Further study shall be carried out to identify more bacterial spp. having strong capability for the degradation of plastics, such activity shall be helpful in removal of plastic from contaminated sites as bioremediating agents which lead to the implementation of sustainable and plastics free environment.

Keywords: Biodegradation, Polyethylene, Plastics, Pollution, and Environment

INTRODUCTION
Plastic has become the one of the main cause for environmental pollution. Its production and consumption increases gradually. The worldwide consumption of polyethylene is extending at a rate of 12% per annum and approximately 140 million tons of synthetic polymers are produced every year at international level (Shimao, 2001). Plastics have found large spectrum in every aspect in this modern era; along its uses it also causes harmful effects on micro and macro organisms and surrounding ecosystem as well. Due its recalcitrant property to biodegradation it persists in the environment in its natural form for longer duration. Plastics are man-made synthetic polymer. Varieties of plastics products are available are as follows.-Low-Density Polyethylene(LDPE), High-Density Polyethylene (HDPE),Polyvinyl Chloride(PVC), Polyethylene Terephthalate(PET), Polypropylene(PP), Polystyrene(PS) and others. Naturally degradation of plastics takes places slowly.

Biodegradation is a slow, natural and environmental friendly process because it cannot cause any kind of harmful impact to ecosystem. Biodegradation of plastic product by microbes occurs by the action of certain enzymes that cause cleavage of the polymer chains into oligomers and monomer, then after they further metabolized by the microbial cells. The end product formed as a result of aerobic and anaerobic degradation is carbon dioxide and water and methane respectively.

Many Bacterial species were previously reported those significantly involved in the biodegradation process included as *Bacillus, Pseudomonas, Klebsiella, Actinomycetes, Nocardia, Streptomyces, Thermoactinomycetes, Micromonospora, Mycobacterium, Rhodococcus, Flavobacterium, Comamonas, Escherichia, Azotobacter* and *Alcaligenes* (some of them can accumulate polymer up to 90% of their dry mass (Sangale *et al.,* 2012). There are some important fungal species, that actively participating in the biodegradation process are *Sporotrichum, Talaromyces, Phanerochaete, Ganoderma, Thermoascus, Thielavia, Paecilomyces, Thermomyces, Geotrichum, Cladosporium, Phlebia, Trametes, Candida, Penicillium, Chaetomium,* and *Aerobasidium.*

The purpose of this study was to isolate bacteria from polluted waste dumped soil area that have potential to degrade plastic and screening of the polyethylene degrading bacteria and identifying the high potential bacteria that degrade the plastics.

MATERIALS AND METHODS
Polyethylene sample: -Polyethylene films of 30 micron were obtained from Gwalior plastics industry.

Sample collection: - Soil samples (waste disposable site dumped with **p**olyethylene bag and plastic cup) were collected from different municipal waste sites of Gwalior. The soil samples were collected at a depth of 3-5cm,

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in a sterile zip lock polyethylene bags and then air dried at room temperature. The soil samples were stored at 4^0C for upcoming use to carry out different studies.

Isolation of Polyethylene Degrading Microorganisms
One gram of soil sample after get air dried was transferred in to a conical flask containing 10 ml of sterile distilled water. This content was shaken and serially diluted. To isolate microorganisms associated with materials (polyethylene bags and plastics cups) by pour plate method using nutrient agar medium. For each dilution, three replica plates were made. The plates were then incubated at 35-37 ^{0}C for 2-7 days. The developed colonies were isolates and sub cultured repeatedly to get pure colonies and then preserved at 4^0C in slants.

Screening of Polyethylene Degrading Microorganisms by Clear Zone Method
Polyethylene powder was added in mineral salt medium theat a fine concentration and the mixture was kept in shaker for 1 month. The isolated organisms were inoculated on polymer containing agar plates and then incubated at 37°C. The organisms, producing zone of clearance around their colonies were selected for further analysis.

Identification of Polyethylene Degrading Microorganism
The identification of bacteria was performed on the basis of colonies morphology on plate , Gram's staining , microscopic and macroscopic examination and biochemical test according to Bergey;s manual of determinative bacteriology.

Microbial Degradation of Polyethylene and Plastics under laboratory conditions
Liquid culture method: - The Pre-weighed discs of 2cm diameter prepared from polyethylene film were aseptically transferred to the conical flask containing 250 ml of liquid culture medium, inoculated with different bacterial species. Control was maintained with plastic discs in the microbe-free medium. Different flasks were maintained for each treatment and left in a shaker. After two month of shaking, the plastic discs were collected, washed thoroughly using distilled water, shade-dried and then weighed for final weight. From the data collected, weight loss of the polyethylene was calculated.

RESULTS
Isolation and identification of plastic degrading bacteria associated with soil sample
Soil sample was collected from municipal waste disposal sites polluted with plastic materials, used as a source for isolating bacteria having the ability to degrade the polythene. These bacterial colonies were obtained from soil having ability to adhere to the polyethylene the dominant strains isolated after serial dilution.

The bacterial strains were identified macroscopically by examining colony morphology, surface pigment, shape and size of nutrient plate. Microscopic examination included Gram's staining to study the staining behavior, shape and cell arrangement. (Fig no. 1& Fig no. 2)

Further characterization was done by performing the following biochemical test. (Table no. 1)

Screening of Polyethylene degrading studies by Clear Zone Method
The polyethylene containing mineral salt agar plates were inoculated with the isolated bacterial strains. All isolates were screened for their degradative activity. Clear zone was observed after 10 days of incubation period at 37^0C around the colony (Fig no.3) .On this screening the no. of bacterial strains that showed degradative activity were as follows:

The three bacterial isolates that were identified were *Pseudomonas* spp., *Bacillus spp, and Staphylococcus spp.*

Microbial Degradation of Polyethylene in laboratory conditions
Selected bacterial isolates were further tested in the laboratory conditions to check the capability of degrading plastic .The isolates were allowed to degrade the plastic for period of two month .After the period of incubation, the plastic /polyethylene were collected, washed thoroughly using distilled water, shade-dried and then weighed to check final weight shows analysis of polyethylene weight losses with different bacterial strains under laboratory conditions. From the data collected, weight loss of the polythene was calculated (table no.1).This study was done to analyze the degradative ability of the isolates we recovered from the soil. The isolates were inoculated on polymer containing Mineral salt medium agar plates and their zones of clearance around their growth were observed. Here also, *Pseudomonas* spp. *and Staphylococcus spp*. showed the clear zones better than the other bacterial isolates.

In this study the biodegradation of plastics was done by the liquid culture method. It is clear that most recalcitrant polymers can be degraded to some extent in the appropriate environment at the right concentration. The most convenient method to determine the degradation is to measure the weight loss. During the degradation

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time, the systems were maintained as undisturbed with no addition and removal of medium which indicates that the microorganism used the polythene film as carbon source.

Microbial degradation of a solid polymer like polythene requires the formation of a biofilm on the polymer surface to enable the microbes to efficiently utilize the non-soluble substrates by enzymatic degradation activities. Developments of multicellular microbial communities known as biofilm, attached to the surface of synthetic wastes have been found to be powerful degrading agents in nature.

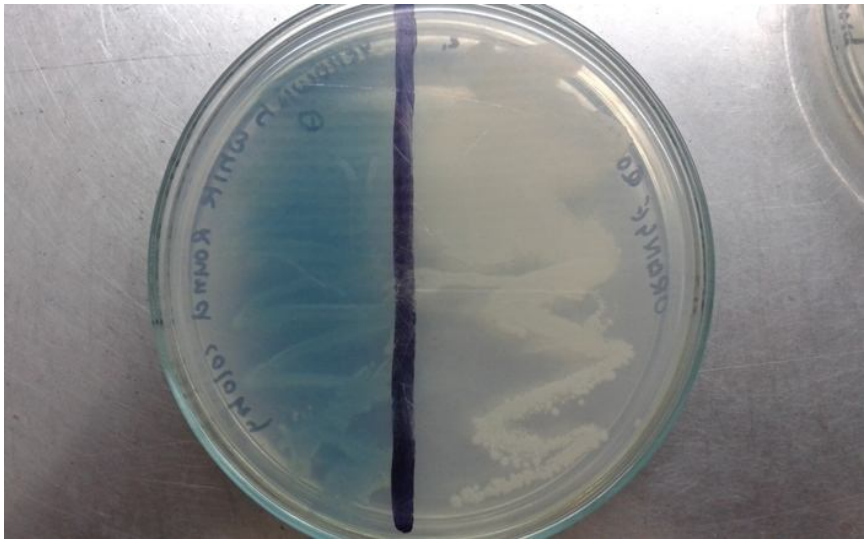

Figure no-1: Isolates on NAM plate

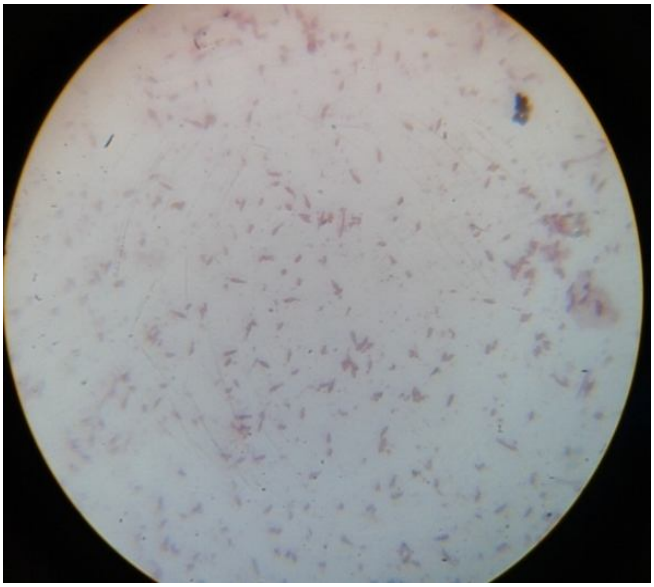

Fig no-2: Microscopic view of isolate

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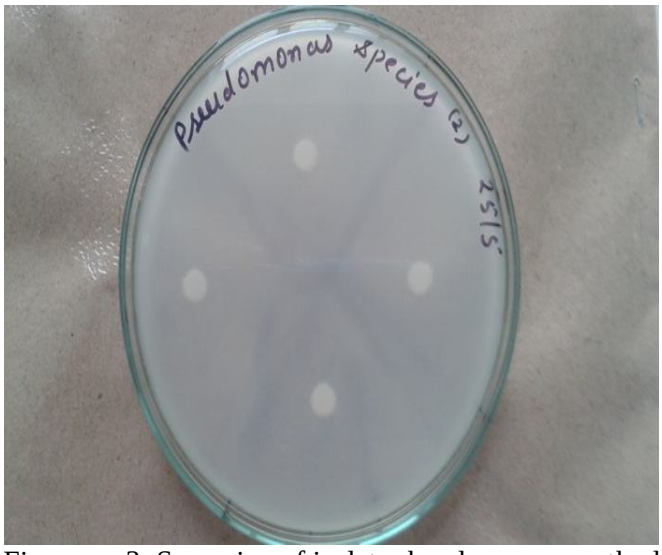

Figure no-3: Screening of isolates by clear zone method

Table No-1: Identification of polyethylene degrading bacterial isolates

Characteristics	**Strain 1**	**Strain 2**	**Strain 3**
Colony Characteristics			
Shape	Round	Round	Round
Size	Large	Small	Large
Colour	Green	White	White
Surface	Convex	Dull, granular	Shiny
Margin	Undulate	Entire	Undulate
Morphology			
Straight rod	+	+	-
Cocci	-	-	+
Gram stain	-	+	+
Cell arrangement	Short chain, single	Short rod, single	Irregular clusters
Spore	C	C	-
Motility	+	+	-
Enzyme production			
Lipase	+	+	+
Gelatinase	+	+	+
Carbohydrate Fermentation			
Glucose	+	A/-	A/-
Fructose	-/-	A/-	-/-
Sucrose	-/-	A/-	A/-
Urease	-	-	-
TSI	-	Y/Y	+
SIM	-	-	-
Oxidase	+	+	-
Catalase	+	-	+
Indole production	-	-	-
Methyl Red	-	-	+
Voges Proskauer	-	+	+
Citrate Utilization	+	-	-
Identified Microorganisms	*Pseudomona-sas sp.*	*Bacillus sp.*	*Staphyloco-ccus sp.*

Table No-2: Result of Degradation of Polyethylene Sample by Bacterial Isolates after an Incubation Period of Two Month

Strain no.	**Initial wt. (mg)**	**Final wt.(mg)**	**Difference**	**Weight loss after two months in %**
Pseudomonas spp.	50	35	15	30
Bacillus spp.	50	37	13	26

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Staphylococcus spp.	50	39	11	22
Control	50	50	0	0

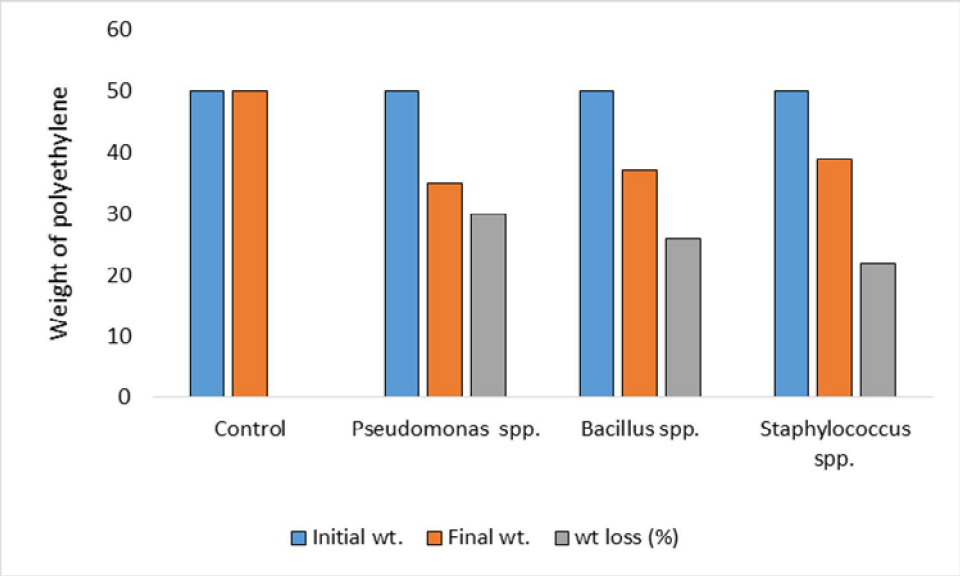

Figure No-4: Graphical representation of biodegradation by isolates

DISSCUSSION

Nowadays the major discussion has covered the valuable concerns about the natural and synthetic polymers, their types, uses and degradability. Also it has looked at the disposal methods and the standards used in assessing polymer degradation. Plastic polyethylene wastes accumulating in the environment are posing an ever increasing ecological threat. Many previous reports highlight that microorganisms can degrade plastic and describe their association with polythenes and plastics. Over 90 genera, from bacteria and fungi, among them *Bacillus* megaterium, *Pseudomonas* sp., *Azotobacter*, *Ralstonia* eutropha, *Halomonas* sp., etc are the best examples (Chee *et al*. 2010). Plastic degradation by microbes due to the activity of certain enzymes that cause cleavage of the polymer chains into monomers and oligomers. Plastic that has been enzymatically broken down further absorbed by the microbial cells to be metabolized. Aerobic metabolism produces carbon dioxide and water. Instead of anaerobic metabolism produces carbon dioxide, water, and methane as end products (Usha *et al*. 2011).

A study was performed by Ch Vijaya et al conclude that on the basis of test perfomed in the lab the weight in loss was 2.9-4.5% for HDPE and 10.5 – 11.6%for LDPE films by using Bacillus species. This study indicates that the biodegradation of polythene films at very slow rate in natural environment.(Vijaya *et.al.* 2008).

Pseudomonas sp. isolated from household garbage dump(P2) gave the lowest biodegradability of 31.4% and 16.3% for natural and synthetic polyethylene, respectively.However, *Pseudomonas* sp. isolated from textile effluents drainage site gave an intermediate biodegradability of 39.7%and 19.6% for natural and synthetic polyethylene, respectively.(Nanda *et.al*. 2010). In the previous study microorganisms - *Bacillus, Pseudomonas, Aspergillus, Penicillum* able to degrade polyethylene were isolated from compost soil and characterized. Physicochemical analysis of PE was done by Scanning electron Microscopy (SEM) & Fourier Infrared Spectroscopy (FTIR). The degraded products were analyzed by Gas Chromatography-Mass-Spectrometer (GC-MS) (Mahalakshmi *et .al.*, 2012). Recently M. Ariba Begum reported that *Pseudomonas alcaligenes* exhibited significant polythene degradation ability and in the near future, *Pseudomonas alcaligenes* degrade 10.5% ,14.7 % and 16.2% of polythene bags at 10, 20, and 30 days of incubation and this bacterial species can be used to reduce the quantity of plastic waste, which is rapidly accumulating in the natural environment.(Ariba *et.al*.,2015)

Gauri *et.al* 2016recently using -*Bacillus*, *Staphylococcus*, and *Pseudomonas* as bacterial consortiumfor degradation of polythene of 10 micron and 40 micron and individually these species also examine for degradation . According to this it showed that bacterial species degrade plastic efficiently individually as compared to bacterial consortium.Bacillus sp. showed 42.5% as compared to Staphylococcus sp. 20% Pseudomonas sp. 7.5 % and consortium 5% on the basis of this result we can conclude that Bacillus sp. may act as solution for the problem caused by polythene in nature

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CONCLUSION

The status of polyethylene pollution should be updated area wise. Some wakefulness programs regarding to reduce the use of polythene should be promoted among the public. Microorganisms isolated from the soil samples, sample was collected from waste disposal municipal sites contaminated with polyethylene, and they show the capability to degrade polyethylene in synthetic condition. These microbes used polyethylene as a carbon source and degrade the polyethylene and converted into toxic form, which may lead be less pollutant to the environment.

The bacteria were identified to be *Pseudomonas spp., Bacillus spp., and Staphylococcus spp. Pseudomonas* spp. (strain 1) degrades polyethylene/plastic more than that of other bacterial species. *Bacillus spp.* (strain 2) has less capacity to degrade polyethylene as compared to *Pseudomonas spp.Staphylococcus* spp. (strain 3) has less capacity to degrade polyethylene as compared to other isolated bacteria.(Fig No. 4)

The isolated microbes were native to the place of plastic waste dumping and shown some degradability in natural conditions, yet they also exhibited biodegradation in laboratory conditions on minimal synthetic media. This gives some knowledge that these microbes can used in both natural and artificial conditions for the purpose of degradation of polyethylene.This knowledge can be used further as a valuable application in commercial degradation of plastics and used for the preparation of bio plastics/biopolythenes or ecofriendly polyethylene's to solve the plastic waste problems using a microbial tool. Bio plastics can control environmental pollution and easily degradable.

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PHYTOREMEDIAL TREATMENT OF DOMESTIC WASTEWATER AT GWALIOR (M.P.) BY *CHRYSOPOGON ZIZANIOIDES* (VETIVER GRASS)

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ABSTRACT
Phytoremediation technique is most prominently used in modern world to remediate pollutants from the contaminated sites. As the water percentage on this planet is 75%, still fresh water resources are sinking day by day. The study was conducted to assess the removal efficiency of pollutants from domestic wastewater by vetiver grass (Chrysopogon zizanioides). The plant had possessed the capacity to filtrate, absorb and accumulate various pollutants from domestic wastewater. Results had demonstrated that there was a significant reduction of various physiochemical parameters like pH, Conductivity, TDS, Total Hardness, permanent hardness, Chlorides, Nitrates and Phosphates from the wastewater and there was significant increment in DO concentration after treatment. The rate of reduction was 40% to 60% and reduction rate was increased after 30th day of treatment. This technique is considered as valuable technique for removing contaminants from domestic wastewater. This study had revealed that phytoremediation is cost-effective and ecofriendly technique employed for the removal of contaminants from various types of wastewaters.

Keyword: Phytoremediation, Vetiver grass, Physiochemical parameters, Domestic Wastewater

INTRODUCTION

Water is essential for existence of life and all other human activities like agriculture, industrial and domestic purposes. The availability of water is important determinants of the human environment and their surroundings (Patz, *et.al,* 2005). The natural water resources like rivers, lakes and streams restrain enough factors for growth of various organisms in the aquatic body (Dhasarathan *et.al,* 2006). The unwanted and unnecessary substances which change the physical, chemical and biological characteristics of water and interferes with its use for legitimate purpose is called water pollution (Kneese, 2015). The water pollution is continuously growing and a lot of measures have been taken to overcome that problem. There is threat to the survivability of human life by increased pollution in major lakes, rivers, reservoirs of the world (Meybeck *et al.,* 1996). The surface area of earth swathes almost 3/4th liquid water approximately, while only 3% as fresh water. Out of which 69% fresh water is locked up in glaciers and ice caps, groundwater 30%, and 0.3% surface water (USGS 2004). The various parameters which indicates the quality of water involves gross changes in dissolved oxygen, biological oxygen demand, pH, electrical conductivity, total solids, Total dissolved solids, color turbidity taste etc. These changes may be short and long term consequences on aquatic ecosystem (Kumar 2013).

Currently, low-tech system like phytoremediation has a substantial interest in its use for wastewater treatment and resource recovery at low cost and minimal maintenance. The phytoremedial treatment technology with plants has possessed a certain degree of purification of various types of polluted water (Cajuste & Laird, 2000). The plants with strong absorption and adsorption of pollutants and good tolerance to toxic substance could be planted in the polluted water which accordingly fixes and removes water pollutants for water purification. The removing of pollutants by the application of plants had gained importance as they were proved to be efficient in wastewater management. Vetiver grass (*Chrysopogon. zizanioides* (L.) Nash) is a perennial and fast growing grass is not a hydrophyte but it prefers wet and waterlogged habitat. The plant posses deep root system and high biomass production. Due to its unique morphological characteristics and its tolerance of adverse environmental conditions, it has been used effectively for wastewater treatment (Troung 2003). On seeing the growth ability and potential of *C. zizanioides* to survive at various climatic as well as different ecosystems, the present study was carried out to assess the impact of said plant for the treatment of domestic wastewater. The objective of this study was to reveal the treatment efficiency of vetiver in treating domestic wastewater by growing hydroponically and determination of nutrient accumulation by said plant.

MATERIAL & METHODS

The experiment i.e. phytoremediation of pollutants from wastewater by *Chrysopogon zizanioides* was carried out during 2017-2018 at School of Studies in Botany Jiwaji University, Gwalior. The water sample were collected from open drainage system of Mahalgaon area and test plant were taken from CIMAP Lucknow U.P. Gwalior is the northern-most city of Madhya Pradesh is located at 26.22^0 North latitudes and 78.18^0 East longitudes with average elevation about 197 metres above msl.

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The experiment was carried out in 20 litre camphor. First of all the plant was thoroughly washed with tap water, followed by distilled water and then wrapped by filter paper to remove excess water. Then plant samples were planted into camphor in 3 replicates. Following physico-chemical parameters i.e. pH, Electric Conductance, Total Dissolved Solids, Chlorides, Total Hardness, Permanent hardness, Total Alkalinity, Nitrates, Phosphate & Potassium were analysed in waste water in comparison with tap water by the standard analytical methods of APHA 2005. The analysis of the said parameters was carried out at initial (0 days) and after (15 days) interval of experiment for approximately two months. Results were computed on the basis of average of all three consecutive reading of each experimental setup (Table 1).

RESULTS AND DISCUSSION

Table-1: Physico-chemical analysis of different concentration of experimental setup with *C. zizanioides*

Parameters	Samples	Time period				
		0 days	15 days	30 days	45 days	60 days
pH	sample 1	7.36	7.26	7.18	7.15	7.11
	sample 2	8.36	8.20	8.00	7.75	7.63
EC (µS/m)	sample 1	679.33	593.67	552.33	482.33	448.00
	sample 2	1597.33	1307.33	1117.33	1069.67	944.33
TDS (mg/l)	sample 1	624.33	587.67	554.67	527.00	508.67
	sample 2	1754.00	1684.33	1573.00	1336.33	1213.00
Total Hardness (mg/l)	sample 1	657.44	584.00	524.67	491.00	473.00
	sample 2	2010.33	1704.00	1616.67	1412.33	1337.67
permanent hardness (mg/l)	sample 1	310.00	285.67	267.00	254.33	243.67
	sample 2	1010.67	982.33	863.00	760.33	730.33
Nitrates (mg/l)	sample 1	3.53	3.07	2.53	1.98	1.59
	sample 2	10.44	8.22	7.53	6.17	5.82
Chlorides (mg/l)	sample 1	16.55	14.23	13.65	13.14	12.76
	sample 2	65.82	55.93	53.14	51.34	48.81
DO (mg/l)	sample 1	8.07	7.97	7.63	7.77	7.23
	sample 2	1.17	2.43	3.53	4.03	4.43
Phosphate (mg/l)	sample 1	4.16	4.02	3.72	3.37	3.21
	sample 2	8.65	7.48	6.94	5.50	4.27
Potassium (ppm)	sample 1	21.87	20.40	19.23	17.87	16.90
	Sample 2	39.47	37.87	36.83	35.07	34.67

Sample 1 = tap water, Sample 2 = Domestic wastewaster, TDS, Total Dissolved solids, DO = Dissolved Oxygen

The wastewater contains a considerable amount of nutrients which are considered vital for maintaining the fertility of soil and also enhances the growth and productivity of plants. The maximum change in pH was observed from 30 to 45 days and the rate of change of pH within the treatment period was observed as 8.73 % (Graph I). The toxicity affects the solubility of many substances and water becomes more toxic with slight increase of pH (Shu *et al*., 2005). It was found that pH values of effluent samples collected from dyes and textile industries showed extremely high content of total dissolved solid (Dhingra *et al.*, 2015). Electrical conductivity was reduced maximum from day 15 to 30th day with a decrement of 18.1% and the overall decrement of EC within the whole period was 40.88% (Graph2). After 45 days of treatment there was huge reduction in total solids, Total hardness and permanent hardness with a rate of reduction from 40% to 50% and maximum reduction had been shown from day 30th to 60 (Graph 3). The treatment showed maximum rate of reduction of nitrates from 45 to 60 days i.e. 21.88% (Graph 6) and the overall rate of reduction of nitrates was observed as 53.83%. Maximum reduction of phosphates and nitrates revealed that the current test plants play a great role in removing organic waste from domestic wastewater. The plant also shows high recovery rate for N upto 70% from industrial wastes (Smeal, et. al. 2003). Similarly there was maximum reduction of chlorides from the wastewater and the concentration of chlorides within 60 days was reduced from 65.82 mg/l to 48.81mg/l with a %age reduction of 25.84 (Graph 5).

One of the important parameters in drinking water is DO, but in wastewater, the concentration of DO was very low and there was the increment of DO from 1.17 to 4.43 mg/l (Graph 4) within 60 days of treatment in domestic wastewater. DO in the aeration tank must be maintained at 1-3 mg/L for effective growth of microbial biomass, which is timely and expensive to reestablish wasteful use of resources which increases the treatment efficiency

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(Randall, et.al. 1998). After 45 days of treatment the results for phosphates was evaluated and highest reduction was observed from 30 to 45 days with 20.7% and overall reduction of the said parameter was observed 50.6% (Graph 6) Potassium is considered the third most important macronutrient required by the plant but it is not in too much concentration. Reduction of potassium was observed maximum from 15 to 60 days i.e. 2.74% and the overall reduction were observed as 13.84% (Graph 7). The study also revealed that there is not too much reduction of K from the said treatment technique.

CONCLUSION

The value before and after treatment were evaluated and were found that plant showed the good capacity to remove pollutants from the aqueous solutions. Other than Dissolved oxygen all the parameters showed decrease /reduction. The rate of reduction had increased with time and it had been observed that the reduction rate was uplifted from 30^{th} days and then there occurs a huge reduction of pollutants. The results of this experiment depicts that this plant is very efficient for the treatment of wastewater especially domestic wastewater. This treatment technique showed that the experiment is very efficient, eco-friendly, cost effective and best alternative methods for domestic waste water treatment which converts complex organic matter into simpler forms.

The principal objective of our study was to check the best possible method for treatment of domestic waste water. The measures and efforts are made in such a way to bring the pollutants present in waste water within the limits and to improve the environmental quality and maintain as for as possible the "Ecosystem Balance'' by low cost, ecofriendly approaches.

The present study still need further research to investigate the role of plants in detail with special reference to any effect on other living forms and its potential to remove toxic chemicals present in our environment. According to Uggetti (2009) it is an effective process using plant life to help in the necessary process of treating some of the products of human communities.

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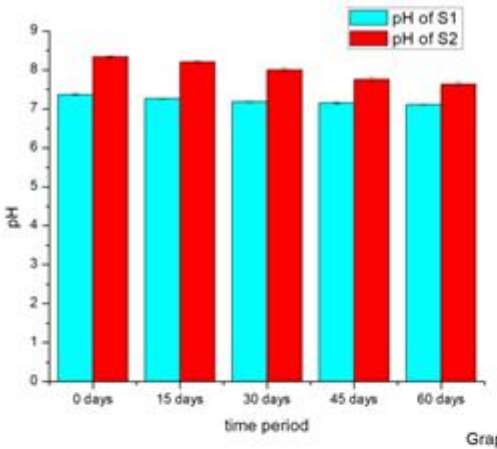
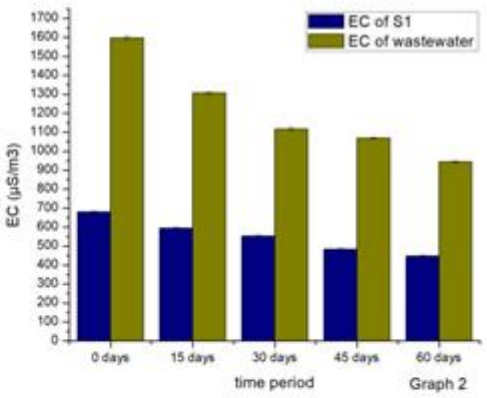
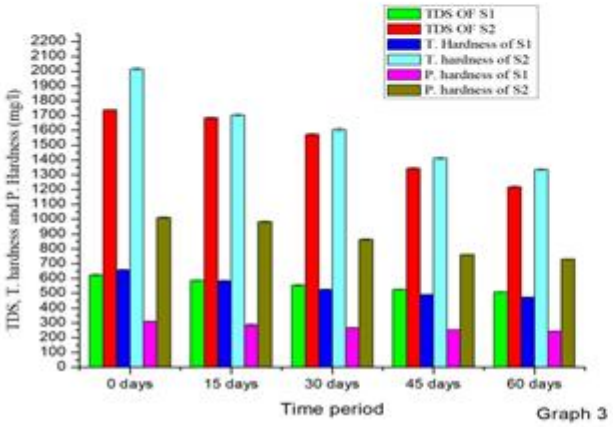


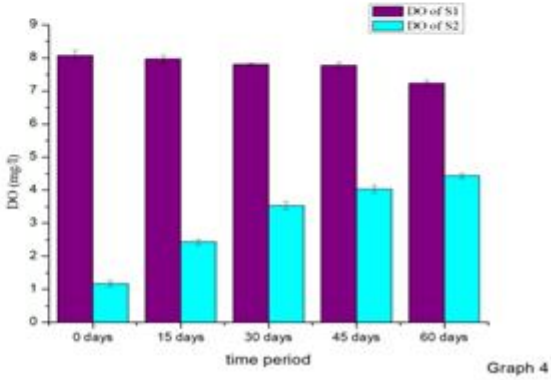
Graph 1



Graph 2



Graph 3



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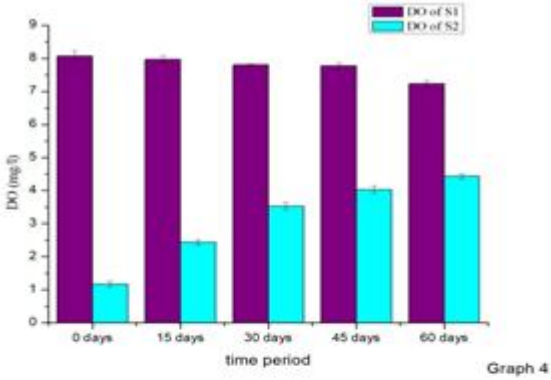
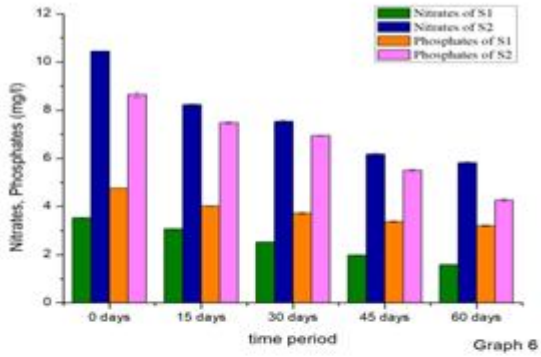
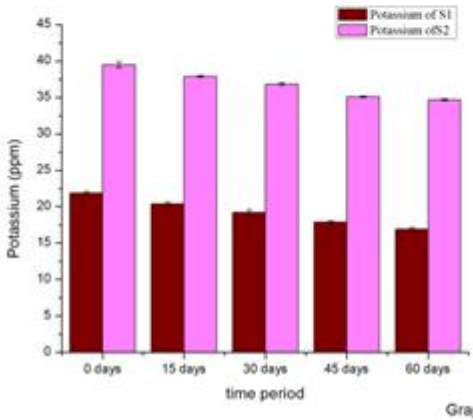


Graph 4



Graph 6



Graph 7

S1 = tap water, S2 = Domestic wastewater

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PHYTOREMEDIATION OF HEAVY METALS FROM INDUSTRIAL WASTEWATER BY CONSTRUCTED WETLAND TREATMENT TECHNOLOGY. A REVIEW

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ABSTRACT
Phytoremediation is a cost effective and affordable technical solution for extraction and stabilization of inactive metals and metal pollutants from polluted soil and water in the present era. Phytoremediation can be potentially used to remediate heavy metals in the contaminated sites. Heavy metals are the most important contaminant in the environment to be sorted out. This study revealed the impact of heavy metals on the plants growing in the industrial sites and capacity of metal tolerant plants growing within these sites to filtrate, absorb and accumulate the metals like copper, zinc, iron, magnesium, lead, chromium from the sediments and then their accumulation within different plant parts. The heavy metal accumulated by the plants growing in the wetland at the industrial waste effluents samples are either to be extracted or stabilized. The constructed wetland treatment technology provides various opportunities to the plants to show the better option for remediation of pollutants. The remediation potential of the plants would be analyzed at various vegetative phases of the plant. It is to be expected that the accumulation of heavy metals would be higher in the roots as compared to the shoots and the concentration would increases towards the end of growing season. This technology is becoming ecofriendly and potentially most effective. This paper aims to pile up information about various heavy metals, their sources, effects and treatment. In addition the study also reveals the factors which affect the uptake mechanisms of plants and the advantages and limitations of phytoremedial technology. Some of the recommended plants which are frequently used for reduction of contaminants are also reported.

Keywords: Phytoremediation, Heavy Metals, Industrial sites, constructed wetlands

INTRODUCTION
Bioremediation is an innovative and promising technology available for removal of heavy metals from contaminated industrial sites as it is environmental friendly technology for decontaminating those sites which are contaminated with wide range of pollutants. (Kulshreshtha *et al,* 2014). Heavy metals are elements with metallic properties and an atomic mass of > 20. Heavy metal pollution is a global challenge that requires joint efforts of governments, scientists, and communities. Accumulation of heavy metals often results in soil and water degradation and ecosystem malfunction (He *et al,* 2015). Moreover, heavy metals enter food chains from polluted soil, water and air, and consequently cause food contamination, thus posing a threat to human and animal health (Satpathy *et al,* 2014) The anthropogenic activities like mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping, and melting operations makes a significant contribution to environment (Welch 1995; Samarghandhi *et al,* 2007). Heavy metal contamination of soil, water and air has posed various ill effects on humans and other life forms of the ecosystem (Baker *et al,* 2008). There are several techniques to remove these heavy metals, including chemical precipitation, oxidation or reduction, filtration, ion-exchange, reverse osmosis, membrane technology, evaporation and electrochemical treatment (USEPA 2008). But most of these techniques become ineffective when the concentrations of heavy metals are less than 100 mg/l (Gunatilake 2015).

Phytoremediation technology has been receiving attention as the name given to a set of technologies that use different plants as a containment destruction, or an extraction technique which indicate a cost effective technique compared to conventional treatments (Saber *et al,* 2016). Phytoremediation is an area of active current research and mostly new efficient metal hyperaccumulators are to be explored for applications in phytoremediation and phytomining (EPA. 2000).

PHYTOREMEDIATION – THE CONCEPT
During the 1980s the US government initiated a program for the development of environmental cleanup technologies which has accelerated the growth of new productive research field worldwide (Kramer, 2005). The U.S. Environmental Protection Agency (EPA 2002) seeks to protect human health and environment from the risks associated with hazardous waste sites while encouraging development of innovative technologies (Chaney et al, 2005; Huang et al, 2004).

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HOW DOES PHYTOREMEDIATION WORK?

Nature has blessed us with a variety of plants. The degradation and detoxification as well as hyper accumulation of heavy metals ranging from 100mg/kg dry mass for Cd to 1000 mg/kg for Cu, Co, Cr. (Ahemad,2014 & Baker, et al.,1994). The mechanisms are:-

PHYTOEXTRATION

The plants used for phytoextraction are known as hyperaccumulators that sequester extremely large amounts of heavy metals in their tissues. This technique is generally used for metal contaminates like nickel, zinc, copper, lead, chromium, and cadmium (Cluis. 2004). Accumulation factor for plants was calculated as:

$$AF = \frac{\text{Metal plant Conc } (\mu g\ g-1)\ \ (\text{roots} + \text{stems} + \text{Leaves})}{\text{Mean Soil available } (\mu g\ g-1) \text{ Concentration}}$$

PHYTOVOLATIZATION

This process involves the uptake of contaminants from soil and waste water, transforming them into volatilized compound and then transpiring into the atmosphere (Pivertz, 2001), particularly used for the removal of some volatile heavy metals like Hg and Se from polluted soils (Karami, & Shamsuddin, 2010) by using special matters secreted by root (Bizily et al, 1999).

PHYTOSTIMULATION

Phytostimulation is referred as enhanced rhizosphere, biodegradation and plant assisted bioremediation/ degradation. Plants release natural substances through their roots, thereby supplying nutrients to microorganisms, and these microorganisms increase the biological degradation of toxic contaminants from soil (Miller, 1996; Lasat, 2000). The organic substances and oxygen released by plants leads to increase microbial activity, which in turn results in the stimulation of natural degradation of organic contaminants (Meers and Tack, 2004).

PHYTOSTABILIZATION

Phytostabilization is a simple, cost-effective, and less environmental invasive approach to stabilize and reduce the bioavailability of contaminants by plants. (Raskin and Ensley, 2000) established a vegetation cover where sequestration (binding and sorption) of immobilize metals within the plant rhizosphere occurs and reduces metal bioavailability to livestock, wildlife, and human exposure (Kumpiene et al. 2007).

RHIZOFILTRATION

The technique is designed to colonize plants endophytically which can't be reduced to a few genetic traits and different bacteria have evolved differently in their adaptation to the plant environment (Birgit *et al.,* 2013). Terrestrial plants are more suitable for rhizofiltration as they produce longer and more substantial root system with large surface areas for metal sorption (Young, 2010).

HEAVY METALS AND THEIR TOXICITY

Heavy metal pollution has caused increasing concerns all over the world because heavy metals are easily bioaccumulated through food chains even at low contents in the environment (Pejman *et al,* 2015) and then pose a serious threat to human health, other living organisms and natural ecosystems (Zhang et al., 2009; Deforest et al., 2007; Maanan *et al,* 2015). Heavy metals in the soil have been considered to be powerful tracers for monitoring the impacts of human activities because heavy metals pollution in the environment is derived from anthropogenic sources (Guo et al., 2012). The heavy metals which are most commonly associated with possible toxicity in dietary supplements are arsenic, cadmium, chromium, lead, and mercury (WHO, 1998) and/or specified in the NSF International (NSF) and American National Standards Institute (ANSI) Standard 173 for dietary supplements (NSF International, 2008). Metals in ionic form, such as Pb(II), Cu(II), Cd(II), As(III), Co(II), Ni(II), Zn(II), Cr(VI), Hg(II), are toxic and found in different types of wastewater. Toxic metals such as cadmium (Cd), lead (Pb), arsenic (As), chromium (Cr), and thallium (Tl) do not have beneficial effects in humans. Long-term exposures to these metals may cause severe disruptions in the normal functioning of the organs and organ systems where the metals are accumulated. Pb is linked with a broad range of negative pregnancy outcomes, including cardiovascular diseases, early membrane rupture and spontaneous abortion, and erectile dysfunction (EPA, 2005). Cadmium is very toxic and is linked to several cases of poisoning through food. Small quantities of cadmium cause adverse changes in the arteries of human kidney causing kidney damage. Moreover, cadmium replaces zinc biochemically and causes high blood pressures (Rajappa et al. 2010). Metals such as Cd, Cr, V, As, Mn, Ti, Co, Cu, Fe, Pb, Ni, Zn, and their compounds have been suggested to be initiators or promoters of carcinogenic activity in animals .Furthermore, Be, Sb, Al, Hg, Ni, Cd and Co can cause undesirable reproductive/fertility problems (Nriagu. , 1998). Zinc toxicity can leads to diarrhea (Osibanjo et al. 2012), manganese may slow down the intellectual development of the child (Buschnann et al.

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2008). Iron has been associated with repeated blood transfusions, genetic and metabolic diseases (Fraga & Oteiza. 2002) and copper toxicity is related to several health concerns, including kidney disease, stomach cramps, nausea, vomiting, diarrhoea, cancer, and liver damage (EPA 2002).

CONSTRUCTED WETLAND

Constructed wetland allows wastewater to flow through specially prepared impervious bed in which specific wetland plants are grown (Chavan and Dhulap, 2012). The large contact area between water and the bed particles allow eliminating nitrates and phosphates by processes, absorption and chemical precipitation. The aerobic and anaerobic bacteria carry out active role in the reduction of COD and BOD of wastewater (Vymazal, 2009). The Plant plays a significant role in CWs by their root zone and substrate in order to absorb ionic heavy metal. Mengzhi *et al,* (2009) investigated the effectiveness of constructed wetlands (CWs) for removal of three heavy metals (Zn, Cu and Pb). The results revealed that CW with coke and gravel showed the first dynamic rate constants of Zn in CWs as 0.2326 h^{-1} and 0.1222 h^{-1}, respectively.

IMPORTANCE OF PHYTOREMEDIATION PROCESSES FOR INDUSTRIAL EFFLUENTS

Constructed wetlands planted with commonly available aquatic macrophytes (*Erianthus arundinaceus* and *Typha angustifolia and Phragmites* australis) has been found to be efficient for the removal of heavy metals from pulp and paper industrial area effluents. The average removal efficiencies for the heavy metals varied between 70 and 74% and was in the order of Cu > Fe > Cd > Ni > Zn > Mn (Arivoli et al, 2015). The land irrigated with loamy drain water were analyzed and different metals showed different enrichment factor for loamy drain water irrigated soil i.e., Cd 30% (max), Pb 26%, Zn 18%, Cr 5%, Cu 5%, Ni 2% (min). For plant samples collected at polluted sites are Ni 46% spinach (whole plant) (max), Zn 42% spinach (whole plant), Cr 39% spinach (whole plant), Cu 33% spinach (whole plant), Pb 20% potato tuber, Cd 20% potato tuber (min).The levels of Zn 145, Cu 5.25, and Ni 39.25 µg/ g in spinach, Pb 29.25, Cr 38.25 and Cd 3.2 µg/g in potato tuber grown on polluted soil irrigated with contaminated drain water were found more than the reference value (Sahu *et al,* 2007). The DTPA, extratable concentrations of Agricultural soils 0f Ada district of Central Ethiopia were as copper 40.5mg/kg, iron 35.5 mg/kg, manganese 134mg/kg, nickel 2.1mg/kg, lead 2.6mg/kg and zinc 53.5mg/kg. The low soil organic carbon, high CEC and high phosphorus fertilization could lead to poor heavy metal availability to plants. The available micronutrients seem to be adequate for plant growth, and their toxicity value seems to be lower than above critical value (Minase, 2016). Variations in heavy metal content in the soil were attributed due to differences in land use types and disparity in organic sources amongst the plant and animal composites. For better crop productivity, more organic matter should be added to the soils. The traditional nutrient cycling practices, such as manure application, allow crop residue to decay on fields, fallowing and rotational cropping has to be followed to rehabilitate the soil and buildup both macro and micronutrients to their natural level (Nigatu *et al,* 2016). Histidine and citrate prevent the Ni uptake in the root exudate (Raskin, 2000). As a guideline, FAO/WHO defined the permissible limits (ppm) of the various heavy metals in the consumed medicinal herbs for different countries as: From the comparison of the results with the defined permissible limits, it was concluded that the levels of heavy metals present in the herbs fall in the permissible range for consumed medicinal herbs as defined for different countries.chromium (2), manganese (44.6 to 339), iron (261 to 1239), cobalt (0.14 to 0.48), nickel (1.63), copper (20 to 150), zinc (27.4), cadmium (0.3), mercury (0.1) and lead (10). (Moses et al., 2013). Contaminated water samples were collected from Bellandur, Varthur and Hebbal lakes of Banglore and were subjected to biosorption by the modified leaf and bark powders according to the parameters optimized for seed powders. The reduction in heavy metal content was observed by Thin Layer Chromatography (TLC) and Atomic Absorption Spectroscopy (AAS) methods. Other physico chemical parameters like turbidity BOD, COD, DO, Nitrate and Phosphate content were examined to emphasize the bioremedial property of chemically modified *Moringa olifera* leaf and bark extracts.

SIGNIFICANCE OF PHYTOREMEDIATION TECHNOLOGY IN ENVIRONMENTAL CLEAN UP?

Species grown in substrata with elevated metals contained significantly higher metals. Metals accumulated by plants were mostly distributed in root tissues suggesting that an exclusion strategy for metal tolerance (Nouri et al, 2009). Plant species differ widely in their ability to accumulate heavy metals. The root tissues accumulate higher concentrations of metals than shoots, which indicates greater plant availability of the substrate metals, as well as interior limited mobility of the plant (Fitzgerald *et al,* 2003). The effect of irrigation with industrial effluents in combination with vesicular arbuscular mycorrhiza (VAM) on growth, uptake of nutrients and yield was evaluated on growth uptake of nutrients and yield of wheat (*Triticum aestivum* Giza 164) and Faba beans (*Vicia faba* Giza 461). VAM was used as biological indicator to overcome the harmful effects of pollution with Heavy metals. Irrigation of plants with Industrial effluents led to marked changes in the growth criteria depending on plant and stage of growth as it has been observed that the total carbohydrate and nitrogen contents in both shoots and roots changed (Wafaa, M., 2001).

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PLANT STRESS RESPONSE AND MECHANISM FOR THE ACCUMULATION OF HEAVY METAL

The factors affecting metal accumulation during phytoremediation include metal concentrations, pH, electrical conductivity, and nutrient status in substrata. Mostly the concentrations of zinc and copper in above ground and underground tissues of the plants were significantly, positively related to their total substrata, while iron, zinc, and copper were negatively correlated to soil phosphorous (Nouri et al. 2009). Water hyancinth (Eichhornia crassipes Mart. Solms) posseses the translocation ability for the metals like Cu, BP, Cd, Ni, and Zn. The ratio for translocated metals were in the order of Cu>BP>Cod>Ni>Zn. The concentrations in the roots were 3 to 15 times higher than those in the shoots. The absoption capacity for water hyacinth was estimated at 0.24 kg/ha for Cod, 5.42 kg/ha for BP, 21.62 kg/ha for copper, 26.17kg/ha for Zn and 13.46 kg/ha for Ni (Suchismita Das, et al. 2015). The plant line of pea (*Pisum sativum*) SGE and its mutant SGECdt, having increased tolerance to and accumulation of Cd, but decreased tolerance to and accumulation of Hg (Andrew et al. 2016). The industrial wastewater effluent of Unnao district (U.P), india with concentration of metals were found in order Cr>Ni>Zn>Cu. *Eichhornia crassipes* Mart. were exposed to various concentrations of above evaluated industrial waste water (0, 25, 50, 75 and 100%) for 10 days. The accumulation of heavy metals was observed more in root than the leaves (Cr>Ni>Zn>Cu). These metals also inhibited biochemical constituents (pigments, protein and sugar contents and amylase activity) including antioxidants (catalase, peroxidase activity and proline content) in *E. crassipes* (Sahu k et al. 2007). Increasing urbanisation, industrialisation and over population is one of the leading causes of environmental degredation. Heavy metal pollution is mostly associated with industrialization. Heavy metals even at low concentration are very toxic to living organism. Beside several physical and chemical processes for remediation of heavy metals, phytoremediation is an important counterbalance to industrialisation pollution. Phytoremediation is an emerging technology for metal accumulation as the plants uses the mechanism to degrade, extract, accumulate, stabilize or even filtrate from soil and water.

CONCLUSION

Contamination of soil and water by Heavy metal in changing environment poses a serious threat to public and food safety. Heavy metals pollution is now an emerging and major health hazard to humans and plants. Besides several physical and chemical processes for remediation of heavy metals, phytoremediation is an important counterbalance to industrialisation pollution. There is a need to improve the possibilities of accumulation of heavy metals in plants using the genetic engineering. Transgenic species have to over expressing phytochelatins and metallothioneins that can form complexes with heavy metals and translocate them into vacuoles to maximize phytoaccumulation. This technology can be applied insitu to remediate pollutants from soil ground water and surface water bodies.

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EFFECT OF PHYTOHORMONES ON INVITRO REGENERATION OF *COSTUS SPECIOSUS*

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INTRODUCTION

Costus speciosus (Koen) Sm medicinal important plant belong to family Zingiberaceae. All plant parts of Costus speciosus have different uses so demand of this plant increased day by day .So now a days Costus speciosus become endangered species. Tissue culture technique play an important role to conserve the endangered species.Different concentration of various phytohormones auxin,cytokinin,gibberellins directly effect on invitro regeneration of Costus speciosus.The ratio of auxin and cytokinin hormone in media are totally responsible for root and shoot initiation. Auxin are useful for the formation of roots. Cytokinin promote cell division and responsible for shoot regeneration. Different auxins like IAA,NAA and cytokinins like BAP,Kinetin etc. This type of conservation method saving the plant from extinction . The plant are useful as an ornamental purpose and medicinal point of view.The rhizome are the main source of diosgonin . It is also used for diabetes and jaundice. The root extract is useful in fever,cough ,skin disease and snake bites. The important morphological applications of plant tissue culture in micropropagation ,small amounts of tissue can be used to raised plantlets due to the presence of totipotency capacity.

MATERIALS AND METHODS

Costus speciosus plant collected from Vindhya herbal , Bhopal. Rhizomes were washed thoroughly in Tween - 20 for 15 min.,and then washed under running tap water for 2 -3 hrs. Whole rhizome were surface decontaminated with 70% ethanol and 0.1% $Hgcl_2$. Rhizomes cut into small pieces and inoculate in MS Media containing with growth hormones. MS medium was used as basal medium and media was supplemented with sucrose and agar . Different phytohormones at different concentration were added to the medium. The ph of the media was adjusted to 5.8 before being autoclaved at 121 ^{0}C for 20 min. and 15 lbs sq inch pressure. Explant is the starting material for micropropagation. Specific medium selection is necessary with special reference to concentration of particular growth hormones. Plant growth hormone added as per requirement and dissolved properly. Different concentration of BAP and NAA were used for shoot induction and study the effect of different hormones on regeneration.All the cultures were incubated at 25 ◦C under white fluorescent tube. The initiated shoots can be subcultured . The normal period between subcultures is 3 weeks and during this time the number of shoots available for subculture.

SHOOT INDUCTION EXPERIMENT

The effect of season,age of explants and the effect of various hormones on initiation of shoots were studied simultaneously. For these studies the sterile rhizomes were inoculated on Murshige and Skoog (1962) basal medium supplemented with cytokinin like BAP and Auxin NAA in different concentration . This medium containing with sucrose and agar also.

MEDIUM USED IN SHOOT INITIATION

Table-1

MS Medium	Hormone conc.
Medium 1	MS +0.3 ml BAP +0.05 NAA
Medium 2	MS + 0.625BAP+0.125 NAA
Medium 3	MS +1.2 BAP +0.25 NAA
Medium 4	MS +2.4 BAP +0.5 NAA
Medium 5	MS +4.8 BAP+1 NAA

Number of experiment carried out for initiation of shoot from rhizome explants. Number of shoot based on combination and concentration of hormones.

RESULTS AND OBSERVATION

In preliminary studies conducted it was found that the response of the explants in culture was dependent on the age of the explants and season of inoculation. Rhizome used as a explants shows better results during the month of November to January were more vigorous and responded more.The time period of the treatment of surface disinfectant (0.1% $Hgcl_2$) was standardized from 2-3 minutes for explants.

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The inoculated culture were maintained at 25^0 C and 16 hour photoperiod.After 25 days small shoot initiate from the rhizome .The best response was observed in the medium supplemented with BAP combination with NAA. In these media the explants induced healthy and vigorous shoots which was elongated to 4-5 cm in 30-50 days

The present work has shown that cutting the rhizome into segments and cultured them on to suitable medium supplemented with phytohormones can initiate the explants. The success was not achieved when the rhizome segment were cultured on MS media. Initiation was observed when MS media supplemented with NAA and BAP.

Table-2

S. No.	Medium + Growth hormones mg/l	No.of shoots per culture	Average shoot legth in cm.	Callussing
1	MS +0.3 ml BAP +0.05 NAA	1	1-3 cm.	-
2	MS + 0.625BAP+0.125 NAA	1	1-3 cm.	-
3	MS +1.2 BAP +0.25 NAA	2	2 cm.	-
4	MS +2.4 BAP +0.5 NAA	2	2cm.	-
5	MS +4.8 BAP+1 NAA	3	3 cm.	-

CONCLUSION

In India several important and necessary steps have to be taken up for its propagation. *In vitro* studies on *Costus speciosus* resulted in fast regeneration of this plant and thus to fulfill the growing demand of *Costus speciosus* diosgonin. Due to the medicinal importance of rhizome, *Costus* speciosus is rapidly disappearing from its natural habitat. Therefore it's important to develop methods for the propagation and and conservation of this endangered species.

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CHARACTERIZATION OF CHROMIUM (Cr (VI) REDUCING BACTERIA FROM SOIL AND WASTE WATER

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ABSTRACT
Hexavalent chromium is one of the most toxic heavy metals in the environment. It gets accumulated in soil and waste water, causing serious health hazards to environment and mankind. Among the different forms of chromium, Cr (III) and Cr (VI) are the most toxic forms of chromium due to their high permeability to biological cell membranes. Industrial workers are at a high risk due to the nature of their work environment. The present study deals with the isolation and characterization of chromium reducing bacteria from different industrial and sewage samples collected from different industrial sites of Gwalior city and also to investigate the ability of bacteria for reduction of chromium at different optimized conditions. In this study, a total of 9 bacterial isolates were obtained after enrichment of the samples up to 100mg/l concentration of chromium. Two isolates- S1A and S2C, showed maximum tolerance level up to 175mg/L, were selected for optimization and Cr (VI) reduction study. S1A showed a 32% of Cr (VI) reduction, while S2C had shown an increased reduction with 40% at 25mg/L Cr (VI) concentration, 200 uL inoculum size, pH 8.0, 37°C temperature at 48 h and on the basis of molecular characterization was identified as- Bacillus altitudins JUGS2C. Therefore, the isolate can be used for the bioremediation and as a bio-indicator of sites contaminated with different forms of chromium.

Keywords: Bio-indicator, Bioremidation, Chromium (VI), contamination, hazardous.

INTRODUCTION
As a result of modern industrialization a huge amount of toxic heavy metals are introduced in environment. There is a great need to reduce mobility, toxicity and bioavailability of heavy metals to overcome this problem (Sharma and Gupta, 2017). Chromium is the seventh most abundant element present on the earth which exists in several oxidation states. The two prevalent forms of chromium are present in the environment i.e., hexavalent and trivalent (Chung *et al.,* 2006). Chromium is a one of the most toxic heavy metal causing health hazards to the plants, animals and also increasing pressure on flora and fauna. It is released into the environment by a large number of industrial operations, including chrome plating, petroleum refining, leather tanning, wood preservation, textile manufacturing, and pulp processing (Ezaka and Anyanwu. 2011). If Cr(VI) concentration into the environment exceeds >0.05 mg/L, then it may affect the human physiology and if enter in the food chain, it may cause severe health hazards such as skin irritation, nasal irritation, ulceration, eardrum perforation, and lung carcinoma etc. (WHO, 2011; Srinath *et al*., 2002). Moreover, it targets the eyes, kidneys, respiratory system, skin and liver (Morshed, 2016). The primary risk factor is the exposure to the environment for chromium toxicity; due to the nature of their work environment, the industrial welders are at a high risk to this exposure. Compound containing hexavalent chromium when comes to skin contact, it causes rashes and ulcers on the skin.

So it is very necessary to use bioremediation technique for the removal of toxic heavy metal from the environment. Biosorption can be defined as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake (Fourest and Roux, 1992). The present study was focused to isolate chromium resistant bacteria from contaminated environment, to investigate the ability of these bacteria for reduction of chromium at different optimized conditions and also molecular characterization of selected isolates through 16S rRNA sequencing. In this study, *Bacillus altitudins JUG S2C* had shown excellent ability to reduce hexavalent chromium under optimized condition.

MATERIALS AND METHODS
Sample collection
Three Soil (S1, S2, S3) and one waste water (S4) samples were collected in a pre-sterilized polybags and bottles from two industrial sites of Gwalior city. The selected samples were then immediately transferred to the lab and processed.

Enrichment of samples
1 gm soil and 1 ml water samples were enriched in 50 ml of nutrient broth medium containing Cr (VI) concentration of 25 mg/L and incubated at 37°C and 120 rpm for 96 h in a shaker incubator (REMI CLS- 24

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plus), serving as the initial enrichment culture. Subsequent enrichment at increased concentrations was done by transferring 1 ml of broth from previous concentration as inoculum in 50 ml of nutrient broth containing 50 mg/L and 100 mg/L chromium concentration. After incubation, broth was withdrawn at regular time intervals up to 96 h for growth observation. Broth was centrifuged at 10000 rpm and 4ºC for 10 min (REMI C-24 PLUS) and supernatant was used for determination of residual chromium concentration. The chromium concentration was analysed by Diphenylcarbazide assay (APHA, 2012).

Isolation and determination of Maximum tolerance level (MTL) of chromium resistant bacterial isolates
Isolation of the bacterial isolates was done using a serial dilution method and 100 µl of diluted broth was spread on nutrient agar plates. The inoculated plates were incubated at 37 ºC for 24 h. After incubation isolated bacterial colonies were picked and pure cultures were obtained for further study.

Maximum tolerance level of bacterial isolates was determined by standard solid agar plate method given by Malik and Jaiswal (2010). Bacterial isolates were streaked on nutrient agar plates containing different Cr (VI) concentrations- 25 mg/L, 50 mg/L, 75 mg/L, 100 mg/L, 125 mg/L, 150 mg/L, 175 mg/L and incubated at 37°C for 24-48 h. Bacterial growth was then compared on visual basis and the highest concentration supporting bacterial growth was selected as maximum tolerance level for relevant bacteria for further study.

Morphological, Biochemical and Molecular characterisation of selected bacterial isolates
For morphological characterization, Gram's staining, endospore staining and motility test were performed. For biochemical characterization following tests were performed i.e., catalase, oxidase, gelatinase, amylase, nitrate reduction, urease, Indole test, Methyl red test, Voger Proskauer test and Citrate utilization test, according to the Bergey's manual of systematic bacteriology (Holt *et al.*, 2000). S2-C showed highest chromium reduction at optimized parameters and was sent to the CMBR lab (Bhopal) for 16S rRNA sequencing for molecular identification.

Determination of optimum growth conditions for chromium reduction
Optimization of growth conditions for maximum Cr (VI) reduction output was done i.e. pH, temperature, inoculum size and initial chromium concentration. For optimization of pH, temperature and inoculum concentration, Nutrient broth containing 25 mg/L chromium concentration was used and concentrations were prepared by using potassium dichromate stock solution.

For optimum pH determination, Nutrient Broth was adjusted at pH 4, 6, 8, 10, and 6.8. Then for temperature optimization, Nutrient Broth after inoculation was incubated at different temperatures i.e. 30ºC, 40ºC, 50ºC and 37ºC. After that inoculum optimization was performed at different inoculum size i.e., 100 µl, 200 µl, 300 µl and 400 µl of fresh bacterial culture were inoculated. In the last optimization of initial chromium concentration was done, Nutrient Broth with different chromium concentrations i.e., 25 mg/L, 50 mg/L, 75 mg/L and 100 mg/L was inoculated and all were incubated at 37ºC, 120 rpm for 72 h. For determination of bacterial growth and chromium concentration broth was withdrawn at regular time intervals of 24, 48 and 72 h. Bacterial growth was determined as optical density measured at 600 nm and analysis of remaining chromium concentration in the supernatant was done by Diphenyl carbazide method at 540 nm, that was obtained after centrifugation of broth at 10,000 rpm and 4ºC by UV spectrophotometer (Schimadzu, UV-1800).

Chromium reduction study at optimized conditions
Chromium reduction study was performed at optimum pH, temperature, inoculum size and initial chromium concentration. 25 ml of Nutrient broth were adjusted at pH 8, 200 µl fresh inoculum was inoculated having 25 mg/L of chromium concentration and incubated at 30°C, at 120 rpm for 72 h. Broth was withdrawn at regular time intervals of 24, 48 and 72 h for determination of bacterial growth and remaining chromium concentration.

RESULT AND DISCUSSION

Enrichment of samples
A total of four samples were enriched at three Cr (VI) concentrations i.e. 25, 50 and 100 mg/L. During enrichment, percentage of chromium reduction at 25 mg/L was observed highest in S4 (48%) and S2 (40%) as compared to S1 (32%) and S3 (28%) and at 50 mg/l chromium concentration, maximum percentage of chromium reduction was observed in sample S2 (72%) while minimum reduction was found in S1 (16%). Percentage of chromium reduction was very low at 100 mg/L of chromium concentration but maximum in S2 (29%) and minimum in S1 (7%) and S4 (7%) (Fig.1).

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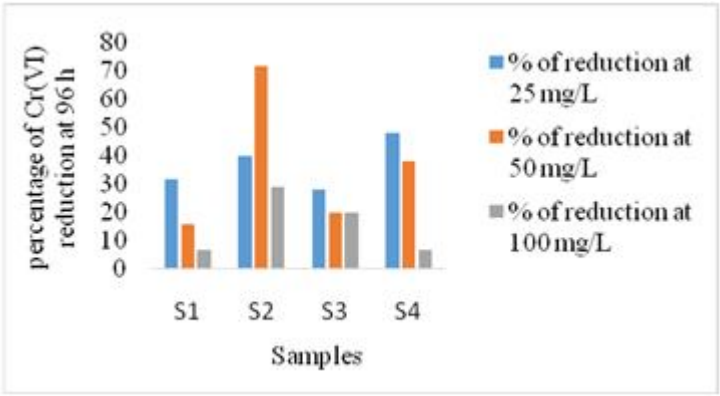

Fig-1: Percentage of chromium reduction by different soil and water samples during enrichment process after 96 h.

Isolation and determination of maximum tolerance level (MTL) of chromium resistant bacterial isolates

A total no. of 9 chromium resistant bacterial isolates was obtained from all four samples. Screening for MTL was carried out at different chromium concentrations from 25 mg/L up to 175 mg/L. S1-A and S2-C had shown maximum tolerance at 175 mg/L (Table 1.) and were selected for further Cr (VI) reduction study.

Table-1: Screening of bacterial isolates for maximum tolerance level at different Cr (VI) concentrations.

Name of isolates	25mg/L	50mg/l	75mg/l	100mg/l	125mg/l	150mg/l	175mg/l
S1-A	R++	R++	R++	R+	R+	R	R
S1-B	R++	R++	R++	R+	R+	R	-
S1-C	R++	R+	R++	R++	R+	R	-
S2-A	R++	R+	R++	R+	R+	-	-
S2-B	R++	R+	R++	R+	R	-	-
S2-C	R++	R+	R++	R+	R+	R	R
S4-A	R++	R+	R++	R+	R	R	-
S4-B	R++	R+	R+	R	-	-	-
S4-C	R++	R	-	-	-	-	-

(R)- Normal growth, (R+) - Moderate growth, (R++) - Maximum growth (-) - No growth

Morphological and Biochemical characterization of selected bacterial isolates

Selected isolates were examined for their morphology and biochemical characterization. S1A and S2C were found gram positive with endospore, motile in nature. Details of shape and arrangement of cells and biochemical tests are mentioned in Table 2. On the basis of biochemical characterization, both of bacterial isolate were identified as *Bacillus sp.*

Table-2: Morphological and Biochemical characteristics of selected bacterial isolates.

Bacterial isolates	S1-A	S2-C
Morphological characteristics		
Colony morphology	Creamy, small, circular, opaque, Entire, Flat	Creamy, small, circular, opaque, Entire, Flat
Gram staining	Gram +ve	Gram +ve
Shape and arrangement	Rod shape, single cell	Rod shape streptococcus arrangement
Endospore staining	Central endospore	Central endospore
Motility	Motile	Motile
Biochemical characterization		
Catalase, Oxidase	+ve	+ve
Gelatinase	-ve	-ve
Amylase production	+ve	-ve
Nitrate, Urease	+ve	+ve
Sugar fermentation test		

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Sucrose, Glucose	+ve	+ve
Maltose, Manitol	-ve	+ve
Xylose	-ve	+ve
Inositol, Lactose	-ve	-ve
IMViC		
Indole	-ve	-ve
MR VP test	+ve	+ve
Citrate utilization	-ve	-ve
Tentative identification	*Bacillus sp.*	*Bacillus sp.*

The phylogenetic affiliation of the strain S2C showing maximum Cr (VI) reduction was performed by 16SrRNA gene sequencing by dideoxy sanger method. On the basis of 16SrRNA gene sequence comparison with already submitted sequences in nucleotide database using BLAST N search showed its 99% similarity with *Bacillus altitudinis* strain MGB4026. Phylogenetic analysis was done by neighbor joining method (Saitou and Nei, 1987) and it showed that *Bacillus altitudinis strain JUG S2C* was closely related with above strain MGB4026 that is submitted from Mangrove soil, China. The genbank accession number of *Bacillus altitudinis* strain JUG S2C is MK106103. Phylogenetic tree was constructed and evolutionary distances were computed using the Neighbour joining method (Tamura and Kumar, 2004) by Mega7 software (Kumar *et al.*, 2015).

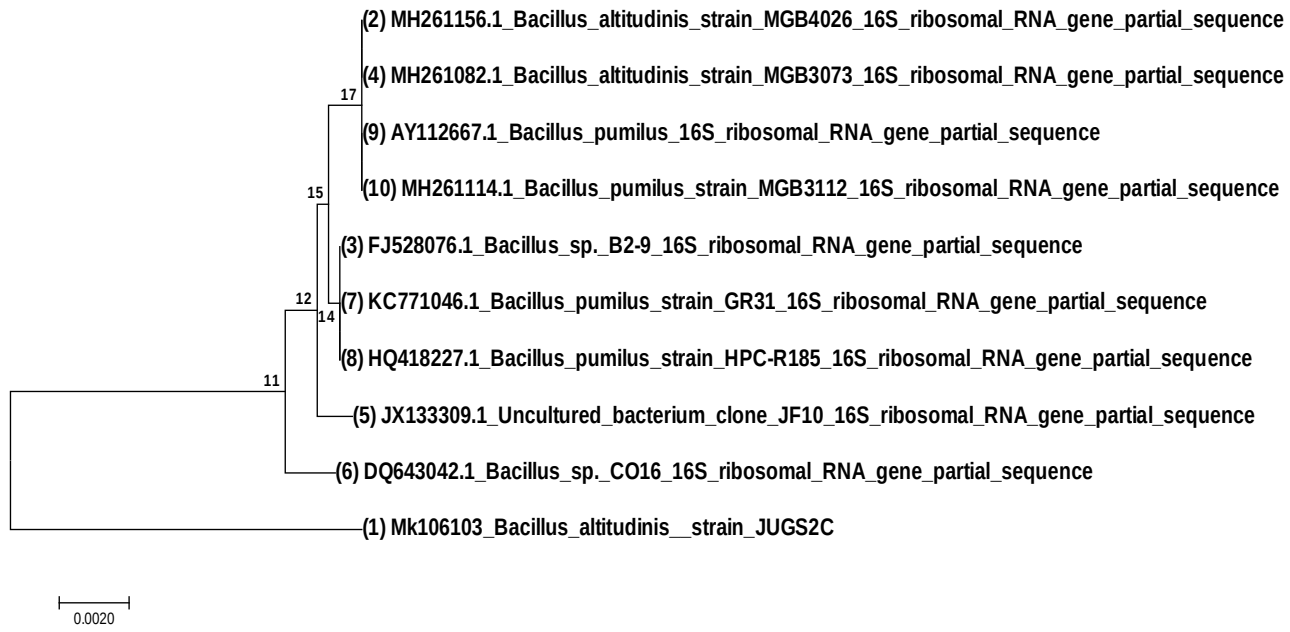

Fig-2: Molecular phylogenetic analysis of MK 106103 -*Bacillus altitudinis* JUGS2C by Neighbor Joining Method (Saitou N. and Nei M., 1987)

Determination of optimum growth conditions for chromium reduction

On the basis of MTL, S1A and S2C were selected for further study and on optimization of pH, S1A had shown maximum reduction at pH 10 (36%) and S2C had shown at both pH 8 and 10 (70%) (Fig.3). Similar results had also been reported by Singh and Gupta (2019) that during Cr (VI) reduction study optimum pH values for maximum reduction for three bacterial isolates- *Enterococcus faecalis, Bacillus megaterium* and *Bacillus cereus* were- pH 6.8, pH 10 and pH 8 respectively. Kafilzadeh and Saberifard (2016) had also reported that the optimum growth conditions for Cr (VI) reduction was within the temperature range of 30 and 40°C and pH range of 7.0 to 8.0.

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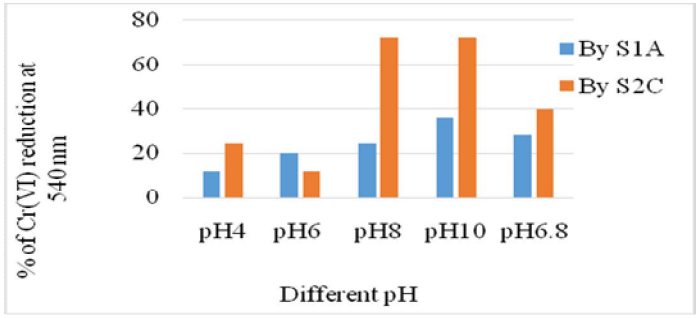

Fig-3: Percentage of chromium reduction by S1A and S2C at different pH values, 37°C, 120 rpm at 48 h.

For the optimization of incubation temperature Nutrient broth were incubated at different temperature range (30-50°C) (Fig. 4). Growth of bacterial isolates was found best at 30°C and percentage of chromium reduction was also maximum for S1-A 56% and S2-C 70% respectively. Zakaria *et al.*, (2006) have already reported the optimal growth of *Acinetobacter haemolyticus* was at 30°C for chromium reduction study. Sarangi and Krishnan (2007) had also found that the growth of *Bacillus* KCH3 isolate was also stimulated at temperature of 30°C and inhibited at temperature below 15°C and over 40°C.

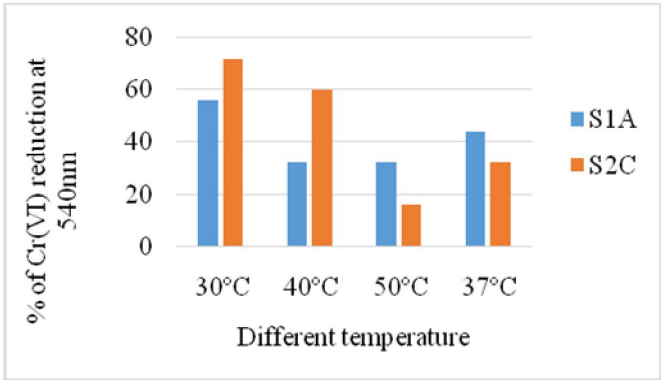

Fig-4: Percentage of chromium reduction at different temperatures, 25mg/L Cr (VI) concentration, 120 rpm for 48h.

Inoculum size was optimized by inoculating Nutrient broth with different inoculum amount i.e. 100, 200, 300 and 400 µl and incubated at 37°C for 48 hrs at 120 rpm. Growth of S2-C was found best at 200 µl size of inoculum. In addition chromium reduction was found best of isolate S1-A and S2-C (100%) at 200 µl size of inoculum. In another study, Biswas (2015) *et al.*, had reported that *Alcaligenes faecalis* strain P-2 showed the optimum growth when medium contained 5% of inoculum.

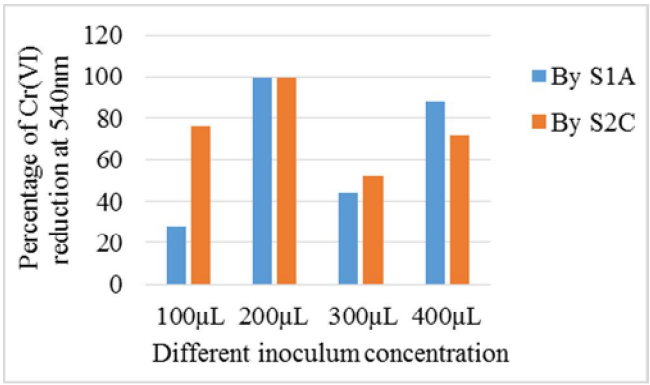

Fig-5: Percentage of chromium reduction at different inoculum size, 25mg/L Cr (VI) concentration, 37°C, 120 rpm for 48 h

In initial chromium concentration optimization maximum growth of both was found at 75 mg/L. S2C showed maximum reduction of 60% at 25 mg/L and was 54% at 50mg/L, while S1A had shown 48% of reduction at 25mg/L and 53% at 50mg/L. In a similar study Essahale *et al.*, had also reported that the maximum reduction was at 25 mg/L concentration but decreased continuously with increasing chromium concentration.

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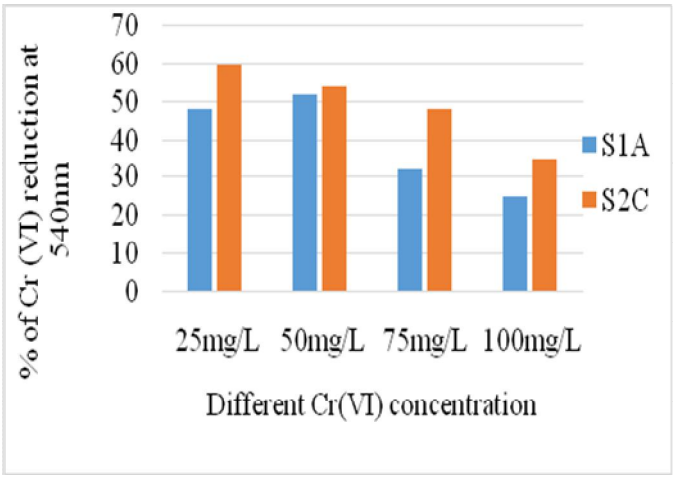

Fig-6: Percentage of chromium reduction at different Cr (VI) concentrations, 37°C, 120 rpm, for 48 h.

Chromium reduction study at optimized conditions

Finally the Cr (VI) reduction study was performed at optimized growth conditions and the maximum amount of reduction was obtained when the isolates S1-AB and S2-C were grown at 30°C, pH 8.0, with 200 µL of inoculum size and initial Cr (VI) concentration of 25 mg/L in Nutrient Broth. Thus chromium reduction was determined by DPC method. S2C had shown maximum chromium reduction of 40% while, S1-A had shown maximum reduction of 32%. Similar work is reported by Basu *et al.*, (2014) that *Bacillus subtilis* had shown 97% removal of chromium with an initial concentration of 2.5 mg/L.

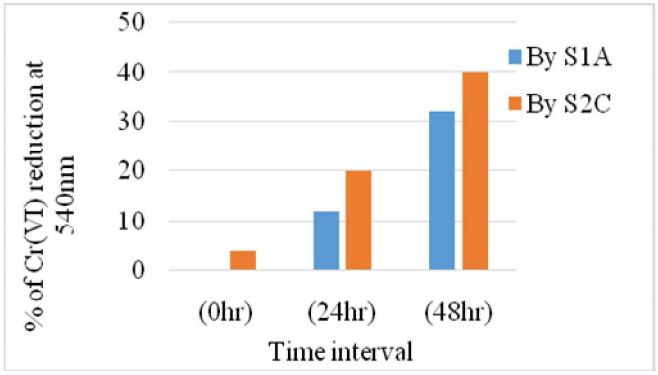

Fig-7: Percentage of chromium reduction at 25mg/L Cr (VI) concentration, 200 µL inoculum size, 30°C for 48 h.

CONCLUSION

The present study concludes that *Bacillus* species play a prominent role in bioremediation of heavy metals in the environment and showing their ability to degrade the highly toxic Cr (VI) pollutant in soil and water. Out of total 9 isolates only two isolates were selected on the basis of maximum tolerance level. Further optimization of growth conditions was done and reduction study was carried out in nutrient broth at following optimized values i.e., pH 8.0, with inoculum size of 200µl, with 25mg/L initial Cr (VI) concentration at 30°C. Isolate S2-C showed highest Cr (VI) reduction of 40% than by S1A- 38% at optimized reduction conditions. On molecular characterization S2C was identified as *Bacillus altitudinis JUG S2C* (MK 106103). So the study infers that the isolate could be helpful in bioremediation of contaminated sites after certain specific research.

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IN VITRO MULTIPLICATION OF *CHLOROPHYTUM BORIVILIUM* L. FOR COMMERCIAL CULTIVATION - A REVIEW

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ABSTRACT
Chlorophytum borivilianum, sixth most important medicinal herb in India, belonging to the family Liliaceae and commonly known as 'safed musli', "Indian Viagra", "roots of gold" ,"herbal Viagra", "the wonder crop". C. borivilianum used in many ayurvedic vital tonics and aphrodisiac formulations. The economically and pharmacologically important part, roots, contains steroidal saponins, fructans, fructooligosaccharide (FOS), acetylated mannans, phenolic compound and proteins. The steroidal saponins viz. neotigogenin, steagmasterol, tokoregenin confer aphrodisiac properties.

Safed musli is propagated either vegetatively through stem discs and tubers or by seeds. The growth rate in vegetative method is very low and produces limited number of plants. Seeds are having long dormancy period (about 10 months) with poor seed germination percentage (11-24%).

Increasing demand at national and international market, rapid declaration in the natural habitats, slow growth (vegetatively), poor seed viability has led to the application of biotechnological methods Viz. clonal and in vitro micropropagation of this crop for commercial purposes. This review gives work done so far and the success of application of this technique in commercial cultivation.

INTRODUCTION
Chlorophytum borivilianum is an important medicinal herbaceous plant belongs to monocotyledons, family Liliaceae.

Botanical Classification
Kingdom: Plantae

Clade: Angiosperm

Clade: Monocots

Order: Asparagales

Family: Asparagaceae

Subfamily: Agavoideae

Genus: *Chlorophytum*

Species: *borivilianum*

It is a perennial rhizomatous herb, commonly known as "Safed musli" in India and also famous world wide as "Indian Viagra", "roots of gold" ,"herbal Viagra", "the wonder crop**"**(Jakkulwar and wadhai,2012). The roots of *C. borivilianum* are a constituent of 'Chyawanprash' an outstanding rejuvenator. *Chlorophytum* grows naturally in various parts of India viz. Assam, Eastern Ghats, Himalayas, Bihar, Madhya Pradesh, Gujarat and Andra Pradesh (Desai et al, 2018).

About 300 species are reported in the Genus *Chlorophytum,* distributed throughout the tropical and sub-tropical parts of the world and seventeen among them are found in India (Kumar and Subramanium ,1986) . In Madhya Pradesh, three main species are reported and all the three species are under cultivation and the most common and commercial being *Chlorophytum borivilianum* which is diploid with basic chromosome number 2n=16 (Kumar and Subramanium ,1986) .

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Safed musli holds a unique place in Ayurveda, Unani and Allopathic medicine due the presence of various components of pharmacological importance. Roots are rich in carbohydrates (35-45%), proteins (5-10%), fibers (25-35%), minerals, vitamins and they are also rich in phytochemical like steroidal saponins, alkaloids. Steroidal saponins presence has conferred the medicinal properties of the *Chlorophytum borivilianum* (Archana et al, 2009).

Roots are the economic part and having aphrodisiac properties due to presence of steroidal saponins viz. neotigogenin, steagmasterol, tokoregenin (Acharya et al., 2009).These saponins are the major secondary metabolites responsible for the medicinal properties viz. aphrodisiac, antioxidants, anticancer and immune booster (Gayathri et al., 2012). *Chlorophytum* is also used to cure physical illness and for general weakness as a revitalizers, for diabetes, arthritis, as curative for diarrhea, dysentery, gonorrhea, leucorrhea etc. (Thakur et al., 2009).

The dried fasciculated roots are in great demand in national and international market (Sautrik and Timir, 2014) and increasing demand for dried roots has led to over exploitation of the species from the natural habitants. International union for conservation of nature and natural resources (IUCN), has declared *C.borivilianum* status as endangered species. The Medicinal Plant Board of India has recognized *C.borivilianum* as the sixth most important herb to be protected and promoted. The government of India has also been extending subsidies to the farmers for the cultivation of this herb through 'National Horticulture Board' (Kaur et al., 2009).

Safed musli is propagated vegetatively through stem disc as well as tubers and sexually by seeds. The growth rate in vegetative method is very low and produces limited number of plants. Seeds are difficult to germinate with low seed viability, long dormancy period (about 10 months), and with only 11-24% germination ((Desai et al, 2018).).

Increasing demand at national and international market, rapid declaration in the natural habitants, slow growth (vegetatively), poor seed viability has led to the application of biotechnological methods Viz. clonal and *in vitro* micropropagation of this crop for commercial purposes.

Plant tissue culture techniques play an important role in the breeding, production, and improvement of crops and medicinal plants. *In vitro* micro propagation of plants holds a tremendous potential for rapid production of high quality, disease free uniform planting material irrespective of the season and weather. However, standardization of all the physicochemical parameters *in vitro* conditions has to be done commercial production of the plants.

Tissue source, the physiological and genetic condition of the parental plant age of the organ and size of the explant, physical conditions, nutrients and growth regulators applied at different stages of in vitro culture and during acclimatization and hardening of the regenerated plants.

The present paper reviews the work done so far in the multiplication of *Chlorophytum borivilianum* using various *in vitro* techniques.

PLANT MATERIAL FOR MICROPROPAGATION

Selection of an explant and establishing aseptic cultures are the initial and most important step in the in vitro micropropagation of plants. In general, meristems, shoot tips, auxillary buds, hypocotyls, epicotyls, seeds, nodes, Stem, leaf, root (Sharma et al, 2017), floral stem buds (Kumar and Prabha , 2016) tuber are commonly used as initial experimental material for tissue culture studies.

Young shoot apices obtained from the tuberous roots of the field grown plants or multiple shoots raised from cultured shoot apices, of about 1-1.5 cm with shoot apex, were commonly used for plant regeneration in *Chlorophytum* (Rizvi et al, 2006; Ashraf et al, 2014).

Basu and Jha (2014) used 0.5 to 1.3 cm long basal portion of *in vitro* grown mature leaves, while Sikha et al (2018) collected 1.5-2.0 cm long crowned shoot buds with roots for micropropagation studies. Where as, Jakkulwar and Wadhai in 2012 reported use of stem disc and shoot base**.**

Leaf tips, leaf sheath, rhizome from the *in vitro* raised plants were taken for callus induction and saponins production by Bathoju and Giri (2012).

In vitro culture techniques were also applied for the analysis of factors influencing callus induction, tuberization biochemical analysis of cultures. Joshi et al 1999; Gaikwad et al 2003; Archana and Harsh 2010 reported callus induction from stem disc, shoot bud, root disc and seeds for callus induction. Inflorescence and even the flower buds, pedicles for noticed to be good source as explants for callus induction, organogenesis and plant regeneration (Archana and harsh 2009; Goyal et al 2009; Rani et al, 2017) in *C.borivilianum.*

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Use of nodal segments from inflorescence avoids the destruction of mother plants, and the conservation threatened and endangered species and also minimize the fungal and bacterial contaimination arising from the use of explant, Sharma and Mohan (2006) used actively growing immature floral buds attached to the inflorescence axis for clonal propagation and cultivation of *Chlorophytum borivilianum.*

Immature inflorescence has been recognized as an important source of totipotent cultures. Samantaray et al (2009) has taken young inflorescence of ~8.0 cm in length (after cutting the peduncle) with intact floral buds and used the apex segment of 0.0-2.5 cm (type-1), the middle segment of 2.5-5.0 cm (type-2) and the basal segment of 5.0-8.0 cm (type-3) as initial explant for tissue culture studies in safed musli.

Plant material for micro propagation

Selection of an explant and establishing aseptic cultures are the initial and most important step in the *in vitro* micropropagation of plants. Meristems, shoot tips, auxillary buds, seeds, nodes, stem, leaf, root (Sharma et al, 2017), inflorescence (Kumar and Prabha 2016), tubers were commonly used as initial experimental material by many scientists for tissue culture studies.

The basic nutrient medium is another critical parameter that controls the success. Murashige and Skoog's (MS) medium is the most commonly and widely used basic composition of nutrients for *Chlorophytum* for callus induction, organogenesis and also for tuberization experiments. Purohit et al., in 1994 initially reported *in vitro* using MS medium.

Application of nutrient media such as Gamborg's (B5) medium, Whites medium was also reported (Basu and Jha, 2014).

Plant growth regulators, Auxins and Cytokinins) play an important role in the differentiation in cultures. They are used either alone or in combinations, according to the aim, plant and explant. Cytokinins play major role in shoot induction, multiplication and the promotion of cell division and expansion (Mehndi et al., 2014). Widely used cytokinine in Chlorophytum tissue culture studies are- 6- benzyl amino purine (BAP) and Kinetin (Kn), Benzyl adenine (BA), Zeatin (ZN) etc. While Auxins are used to induce cell division, cell elongation, root differentiation. NAA(α-naphthalene acetic acid), IAA (Indole -3-acetic acid), IBA (Indole-3- buteric acid), 2,4-D (2,4-dichlorophenoxy acetic acid) are the commonly used auxins for callusing and rooting. The ratio of cytokinine and auxins is a crucial determinant of the type of differentiation in the plant tissue cultures (Skoog and Miller, 1957).

Callus is an undifferentiated mass of cells. Indirect organogenesis and *in vitro* plantlet regeneration, somaclonal variants development and production of valuable metabolite are few important applications. Studies on callus induction and plant regeneration are limited in this crop and are used basically for the analysis of metabolites production.

Young shoot apices obtained from the tuberous roots of the field grown plants, multiple shoots raised from cultured shoot apices (of about 1-1.5 cm) were used for plant regeneration in *Chlorophytum* by Rizvi et al, (2006) and Ashraf et al, (2014).

Joshi et al, (1999); Gaikwad et al, (2003); Archana and Harsh (2010) reported callus induction from stem disc, shoot bud, root disc and seeds of *Chlorophytum*. While, leaf tips, leaf sheath, rhizome from the *in vitro* rose plants were taken for studying saponins production in callus cultures by Bathoju and Giri (2012). Inflorescence, flower buds, pedicles were reported to be good source as explants for callus production (Archana and Harsh 2009; Goyal et al 2009; Rani et al, 2017).

Auxins are most commonly supplemented in basic nutrient medium for *in vitro* callus initiation from explants. However, addition of cytokinins in small quantities gives better response in monocots. Combinations of 6-benzylaminopurine and α-naphthalene acetic acid (NAA) were most commonly applied in callus initiation and maintenance from different explants in *Chlorophytum (*Jakkulwar and Wadhai, 2012; *)*. While, Archana and Harsh (2011) recorded kinetin + NAA as suitable combination for raising callus from stem disc, shoot buds, root disc and seeds. Nakhte et al., (2014) used MS medium supplemented with BAP + NAA (0.25mg/l+0.50mg/l) for callusing from auxillary bud of 11 species of *Chlorophytum borivilianum.*

In vitro plantlet regeneration and micropropagation of plants is achieved either by embryogenesis- a single step process, where shoot and root primordial are present in the embryo or by organogenesis, where first shoots are induced and then rooting is induced in 2nd step.

Multiple shoot induction, adventitious shoot formation in *Chlorophytum* from plant parts such as shoot apices, shoot tips, auxillary buds, hypocotyls, epicotyls, seeds, nodes, Stem, leaf, root, floral stem buds, tubers has been

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reported (Kumar and Prabha , 2016; Sharma et al., 2017). Basu and Jha (2014) used 0.5 to 1.3 cm long basal portion of *in vitro* grown mature leaves, While Sikha et al (2018) collected 1.5-2.0 cm long crowned shoot buds with roots for micropropagation studies. Whereas, Jakkulwar and Wadhai in 2012 reported use of stem disc and shoot base. Inflorescence, flower buds, pedicles were reported to be good source as explants for callus production induction, organogenesis and plant regeneration (Archana and Harsh 2009; Goyal et al 2009; Rani et al, 2017) in *C.borivilianum.* Use of nodal segments from inflorescence avoids the destruction of mother plants and also minimizes the fungal and bacterial contaimination arising from the use of explants. Sharma and Mohan (2006) used actively growing immature floral buds attached to the inflorescence axis for clonal propagation and cultivation of *Chlorophytum borivilianum*. Samantaray et al ,(2009) has taken young inflorescence of ~8.0 cm in length (after cutting the peduncle) with intact floral buds and used the apex segment of 0.0-2.5 cm (type-1), the middle segment of 2.5-5.0 cm (type-2) and the basal segment of 5.0-8.0 cm (type-3) as initial explants.

Four basic nutrient media (MS, B5, Whites and Schenk and Hildebrandt) were screened by Prasad et al.,(2007) for *in vitro* shoot initiation in safed musli and found that MS medium was best for stem disc cultures. Comparative analysis of shoot initiation efficiency of BAP and Kn were also carried out by various scientists and their work confirmed that BAP was more effective than the Kn (Kemat et al., 2010; Sharma and Mohan, 2006; Desai et al., 2018). According to Mehndi et al.,(2014), BAP alone (8.88-26.6µM) was significantly effective on shoot multiplication, while Kn alone (8.88-26.6µM) for effective shoot elongation. Sharma and Mohan (2006), however, observed that use of 0.5-2mg/l Kn produced healthy shoots when compared to BAP. Whereas Desai et al.,(2018) used MS + 1mg/l BAP + 1mg/l Kn and MS + 2mg/l BAP + 3mg/l Kn shoot multiplication.

Basu and Jha, (2014) tested MS and Gamborg's basal media supplemented with varying levels of 6-benzyladenine (1.11-3.90µM) and thidiazuron (1.14-9.08µM) for multiple shoot induction. They reported that MS + 4.54 µM thidiazuron was ideal for shoot induction and has given rise to numerous shoot primordia within three weeks without any callus phase from leaf explant of two varieties of safed musli.

Nakhte et al.,(2014) used MS medium supplemented with BAP (0.5mg/l) for shoot induction, and IAA(0.25mg/l) root induction respectively from auxillary bud explants of 11 species of *Chlorophytum borivilianum.* While Rizvi et al., (2007), tested a wide range of BAP concentrations levels (2.2 µM - 40.0µM) and found that BAP at 22.2µM level was best for *in vitro* shoot multiplication from the shoot apices obtained from the tuberous roots of field grown plants. According to Sharma and Mohan (2006) report, MS + 2mg/l kinetin + 0.1mg/l 2, 4-D proved to be the best for multiple shoot induction and MS + 2mg/l BAP for maximum number of shoots (35) production. Where as MS medium supplemented with vitamins, sucrose 3%, 0.8% agar and 5mg'l BAP induced good number of multiple shoots in cultures of stem discs possessing shoot buds (Garima and Shruti, 2012; Thakur et al.,2013)

Samantaray et al.,(2009) reported that direct shoot regeneration could be achieved from immature inflorescence of *Chlorophytum arundinaceum* and *C. borivilianum* on half strength MS medium supplemented with 3.0 mg/l BA, 150 mg/l adenine sulphate, 0.1mg/l NAA and 3% (w/v) sucrose under a 16 –h photoperiod and the proliferation frequency increased on subsequent sub culturing on to the same medium.

Rhizogenesis is the 2nd and an important step for plantlet regeneration and in vitro micropropagation work. In Chlorophytum, shoots raised from different explants have been reported to show differential rooting response on media supplemented with different auxins such as IAA, NAA, and IBA and also to basic salt concentration.

Full, half and ¾ strength MS media were applied for rhizogenesis (Jha et al., 2014). In safed musli, all the auxins- IAA, NAA, IBA have resulted in root initiation. However, the combination of basic nutrients with these auxins, source of explant has shown great influence the time for initiation, number of roots and also on the length of the roots (Jakkulwar and Wadhai, 2012; Ranjan, 1998).

Full strength MS salt concentration medium has been demonstrated to induce highest number of roots per shoot in *Chlorophytum*, while decrease in the salt concentration reduced the number and increased the length of roots (Jakkulwar and Wadhai, 2012)

As early as in 1994, Purohit et al., reported successful root induction on MS medium supplemented with 2mg/l IBA, 3% sucrose and 0.9% agar.

Kemat et al., (2006) observed 100% rooting of shoots on all auxins (IAA, NAA, IBA) containing media as well as on basal medium. However they reported that 1mg/l IBA in full strength MS medium was best for successful rooting from young shoot buds for rapid multiplication of musli.

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Urvashi and Mohan (2006) published rooting of shoots (86.7%) with maximum fasciculated roots (5nos) on Knop medium containing iron and vitamins stocks of MS medium + 2mg/l IBA + 0.1% activated charcoal.

According to Sharma et al (2017), ½ MS+1mg/l IBA +10%CM medium promoted root initiation in leaf base cultures after 39 days of inoculation and sub culturing on to higher concentration of hormones (2mg/l IBA +15% CM) has enhanced the number of roots. Where as, Desai et al., (2018) observed *in vitro* rooting on half strength MS medium supplemented with 2 mg/l IBA. Maximum number of shoots (12.75-1.32) obtained from crown shoot bud as an explant. While Basu and Jha (2014); Sanghamitra et al.,(2009) recorded rooting from healthy shoots on half strength MS supplemented with varying concentration of IAA (2.28-11.42µm), IBA (1.97-9.80µm) and NAA (2.7-10.8µM) from leaf explant cultures.

Arora et al. in 2006 reported Somatic embyogenesis from callus cultures of seedling and leaf explants. The shoots were developed *in vitro* from somatic embryos. Rooting and complete plantlets were raised on B5 medium supplemented with 0.57µM IAA. Arora et al., also observed variation in regeneration capacity in long term cultures and variation in the ploidy of the plant.

Acclimatization, Hardening and transfer to field

Acclimatization and Hardening of *In vitro* raised plants is the most crucial step in micropropagation. The ability of crop variety to become adapted to natural climatic conditions is known as acclimatization.

In vitro regenerated plants are grown in aseptic and high humid conditions and they will be soft and delicate.

To acclimatize the micropropagated plants, different workers have employed different approach toward successful establishment of *in vitro* raised plants under *ex vitro* condition.

The *in vitro* regenerated plants are very soft and delicate to face ambient environmental conditions during acclimatization (Bhojwani and Razdan, 1992). During this period, the leaves of the plants develop cuticle and notice starting of photosynthetic system functioning gradually under green house conditions. The overall success of tissue culture raised plants depends on successful hardening and transplantation into the field. Bhojwani and Razdan (1983) have emphasized that high humidity conditions are best for initial few days after transplantation. Temperature of the environment plays crucial role in the survival as well as the growth of transplanted plants.

Samantaray et al.,(2009) reported survival of about 90% *in vitro* raised plants up on transfer from green house to the field conditions.

Sharma and Mohan (2006) observed that during the acclimatization period, the fasciculation of roots play important in the better survival of the plantlets. They also noticed that activated charcoal presence in the medium has induced fasciculation of roots irrespective of the nutrient media employed and auxins applied in the medium.

The rooted plantlets will be carefully taken from culture flask and agar is removed using sterile distilled water and then transferred to pots/ root trainers or containers containing different mixture of natural or artificial soil like vermiculite.

Thakur et al., (2013) observed small and thick root hairs in three weeks old *Chlorophytum* plantlets on sub culturing on to filter paper wick containing ½ MS liquid medium without growth regulators and these plants on successive sub culturing on to same medium with three weeks time intervals developed tubers.

In 2014, Basu and Jha published that 85% of tissue culture raised plants have developed new leaves and showed expansion after three weeks of acclimatization. These plants under green house conditions have also shown normal growth. Nearly 100 plants that were transferred to *ex vitro* conditions at the onset of monsoon (June-July) and out of them more than 80% survived the monsoon rains with normal fasciculated roots.

Differential response in average survival of *in vitro* plants during primary and secondary hardening was observed by Desai et al., in 2018 among nine different genotypes of *Chlorophytum*. The average survival rate during the primary and secondary hardening of all the genotypes was reported to be 65% and the highest survival rate (74%) was recorded in Vireshwar and the lowest (54%) in Pipalakhunt variety.

Tuberization

Establishment of *in vitro* tuberization of *Chlorophytum borivilianum* using solid and liquid culture systems. A high *in vitro* tuberization rate on solid and stationary liquid Murashige and Skoog media observed by Mehndi et al.,(2013).

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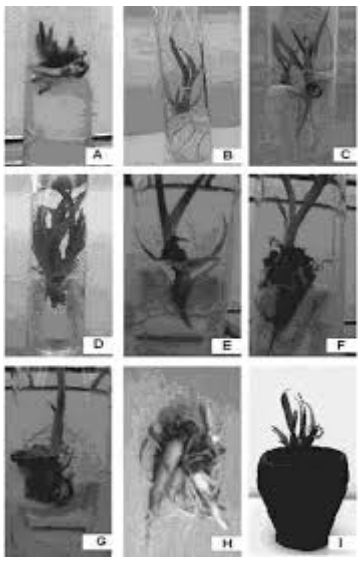
Garima and Shruti, (2012) studied tuberization of roots in tissue culture raised plants, both in the open field as well as in nursery shade conditions. They reported that more than 90% sprouted both in the open field and in polybags. The plants rose from the tuberous roots developed from tissue culture and used as secondary propagules produced on average 14 tuberous roots.



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According to Prasad et al., (2007) *in vitro* plantlets were best hardened on verminicompost: sand (1:1) mixture with 70% survival and prolonged incubation in the dark for 75 days resulted in *in vitro* tuberization.

While Mehndi et al., (2012) noticed that most suitable combination for *in vitro* tuberization of *C.borivilianum* was 950µM CCC with 60g/l sucrose.

In *Chlorophytum*, sterilized vermiculite, sand and organic matters, or sterilized soil was most commonly used for the transplantation. Among all the composition tested, the highest percentage (83%) of plantlets survival was observed in vermiculite: organic matters (1:1) (Agretious et al., 1996; Anand et al., 1997).

Desai et al, (2018) treated the rooted shoots with 200 ppm carbendazim-50%, 200 ppm rhidomil and 200 ppm blytox (each for 2 minutes) followed by rinsing with distilled water before transferring in to pots. They used plastic cups containing moist and autoclaved substrates (pot mix-sand: soil: cocopeat,1:1:1) initial hardening. The plants were covered with polythene and watered daily to maintain high relative humidity (95%). The plants were gradually weaned to natural conditions and transferred to polyhouse for secondary hardening.

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REVIEW ON ANTIMICROBIAL ACTIVITY OF POMEGRANATE: *PUNICA GRANATUM*

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ABSTRACT
Pomegranate [*Punica granatum*] is known for hundred of years for its multiple health benefits in addition to is nutritional value it has antimicrobial activity. It has strong antibacterial activity against the pathogenic bacteria such as *Salmonella typhi, vibrio cholera, Shigella species, Escherichia coli, Listeria monocytogenes.*

This review aims in exploiting the medicinal value of pomegranate in the treatment of various diseases and the antioxidant potential of active ingredient of pomegranate extracts.

An extensive and systemic review of literature was carried out and the data reveals that pomegranate and there extracts may serve as a natural alternative against various bacterial, viral and fungal pathogens. Nearly every part of pomegranate plant has been tested for its antimicrobial activity including bark, leaves, juice, and peel and many study reveals success with peel extract as it has high amount of antioxidants. The various phytochemicals such as hydrolyzable tannin and anthroganin isolated from pomegranate shows antioxidant property.

The objective of the present study is to evaluate the antibacterial activity of different part of pomegranate. Several studies has also been conducted which shows medicinal value of pomegranate part and the current studies do support to the potential benefit of its . A more integrated approach is needed to verify the medicinal use of pomegranate on different bacteria and to understand the effect of extracts on different human diseases , also to see its bactericidal activity on humans

Keyword: *Punicagranatum*, antibacterial activity, antimicrobial activity, antioxidants.

Content

INTRODUCTION
Punica granatum commonly vocalized as pomegranate, mostly cultivated in Iran, North India and in Mediterranean region. Pomegranate has high antioxidants which helps in reducing free radicals form the body. Pomegranate is traditionally being used for Diarrhea scince ancient times[1].

High antibacterial activity of pomegranate is also been absorbed against the different bacteria like *Escherchiacoli, Salmonella thypi, Vibrio cholera, Klebsiella pneumonia, Staphylococcus aureus, etc* from its peel extracts *(POPx) [2-3]. A*lso research is beendone on antifungal activity of pomegranate peel extract (hydroalcholic extract) against the Dermatphtes (*epidermophyton, trichophyton and microsporum)[4]*. going with therapeutic activity of pomegranate its is been found that, Pomegranate rind methanolic extract is found to inhibit the inflammation and allergies [5].

Also there are evidence for pomegranate to control the viruses affecting the human body. The viruses are food borne viruses include human Noroviruses, Hepatitis A virus, Hepatitis E virus, Rotaviruses, Aichi virus and other human enteroviruses and small round structured virus[6]. And among of all these viruses human Norovirus are the main cause of viral gastroenteritis [7-9].

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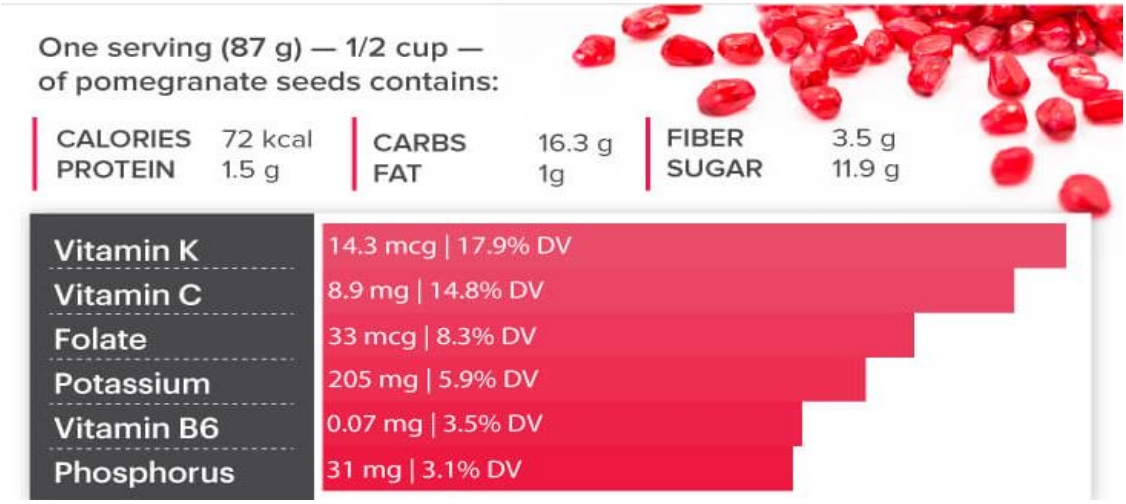
MEDICINAL USE

Traditionally pomegranate is used as medicine for diarrhea, helminthiasis, dysentery and respiratory pathologies[10-13]. The antimicrobial activity of pomegranate has been found with peel extracts and also the therapeutic potential has been widely recognized. The high antioxidant potential of pomegranate peel extracts shows a strong medicinal use of it[14]. Pomegranate is also rich in vitamin C and vitamin K. Here vitamin C helps in development and repair of all body tissues and vitamin K helps to treat and prevent lowering levels of certain substances (blood clotting factors) that your body naturally produces. These are the substances which help in thickining blood and stop bleeding naturally/noramlly.



ANTIBACTERIAL ACTIVITY OF POMEGRANATE

It is been recognized that the pomegranate shows the bactericidal activity as in Thailand a study was conducted on three different strain of *E coli* [15]which shows that the pomegranate ethonolic extract shows strong inhibition on the *E coli O157;h7* (both bacteriostatic and bactericidal) which is a evidence that pomegranate extract is effective against the infection caused by the *E coli O157;h7*[16].

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Pomegranate also shows the antibacterial activity against waterborne pathogenic bacteria includes *Salmonella typhi, Vibrio cholerae , Yersinia entrocolitica, Shigella spp. , and Listeria monocytogenes[17].* Typhoid fever is one of the deadly enteric infection caused by bacteria and it can easily be in body with contaminated food or water. Extract of pomegranate fruit pericarp is been tested with it and it inhibits the growth of bacteria[18]. Another study shows that methonolic extract of pomegranate exhibits highly bactericidal activity against *V. cholerae[19].* Pomegranate peel extract which is infused with tea were effective against *Shigella spp.(*imp cause of diarrhea)[20]. It is been observed that the effects of different concentrations of a methanolic peel extract[PoPx] .at 12 mg/mL, 8 mg/mL, and 4 mg/mL on growth of dental bacteria were compared using the disc diffusion method . Every concentrations of the pomegranate extract had bacterialcidal activity against Staphylococcus aureus and Staphylococcus epidermidis. Extract concentrations of 12 mg/mL and 8 mg/mL were effective against *L. acidophilus, S. mutans, and S. salivarius* and the extract is unable to inhibit *Actinomyces viscosus*[21].

ANTIVIRAL ACTIVITY OF POMEGRANATE

There are less number of research conducted on the antiviral activity of pomegranate, but the research done on the virus us reported against the Influenza virus, herpes virus, pox virus, and human immunodeficiency virus (HIV-1) [22-24]. In one study, among four flavonoid compounds associated with pomegranates, only punicalagin was shown to have inhibitory effects on influenza virus [25].Researcher have found that polyphenol from pomegranate are antiviral against inluenza A virus, here they supressed the virus replication in host and agglutination of chicken red blood cell is inhibited which is caused by the virus using the real polymerase chain reaction(PCR), a plaque assay and a median culture of tisue infective dose 50% hemagglutination assay[22]. They also showed that among four polyphenols, punicalagin was found to be the most effective anti-influenza component, blocking replication of influenza virus RNA and inhibitingagglutination of chicken red blood cells by the virus. In order to understand the mechanism of action against human norovirus , the host cell monolayers for the respective viruses were treated with pomegranate juice and polyphenols prior to or after infection, where reduced infectivity of FCV-F9 and MNV-1 【murine norovirus (MNV-1) and (B) feline calicivirus (FCV-F9)】 obtained [26]. Greater effects in titer reduction were observed when the treatment was performed prior to infection, than after infection (corresponding to replication stage), suggesting that pomegranate juice and its polyphenol may have role in stopping virus binding to the host cell receptors by covering/blocking cell surface where receptors are present or the virus surface ligands it is been theorised that with the help of transmitting electron microscopy (TEM) we may determine if these polyphenol cause the structural or functional damage to the virus or to virus capsid.

When the vaccines are not discovered for HIV, antiretroviral chemotherapeutic have been used to decreas the symptoms of HIV-1 mainly in doveloped countries. Scientists [23] showed that HIV-1 entry inhibitors from pomegranate juice are adsorbed onto corn starch, block HIV-1 binding to CD4 and CXCR4/CCR5 host cell receptors, and constrains the infection by primary virus clades group A to G and group O. These researchers founded the potential of producing anti-HIV-1 from naturally safe food sources.

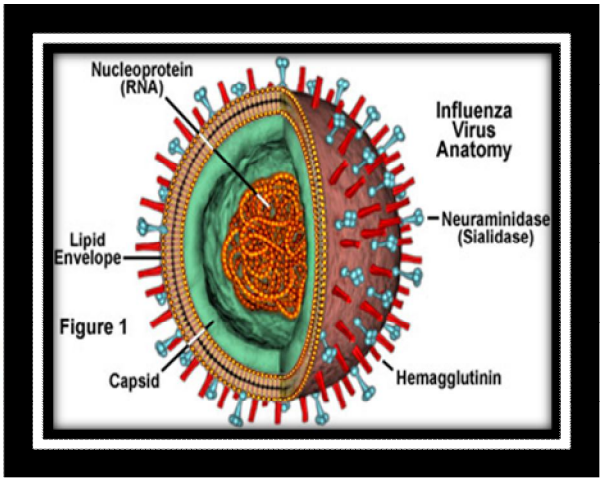

Figure 1

ANTIFUNGAL ACTIVITY OF POMEGRANATE

An investigation by some researchers have also been found that there is an antifungal activity of pomegranate against *candida* species and per of pomegranate is most effective against the *Candida albicans* [27-28]. Researcher explained that pomegranate husk extract had a strong inhibition on *Candida truncatum, Candida coccodes, and Rhizoctonia solani,* but it fails to inhibit the *Alternaria alternata and Fusarium solani [29].*

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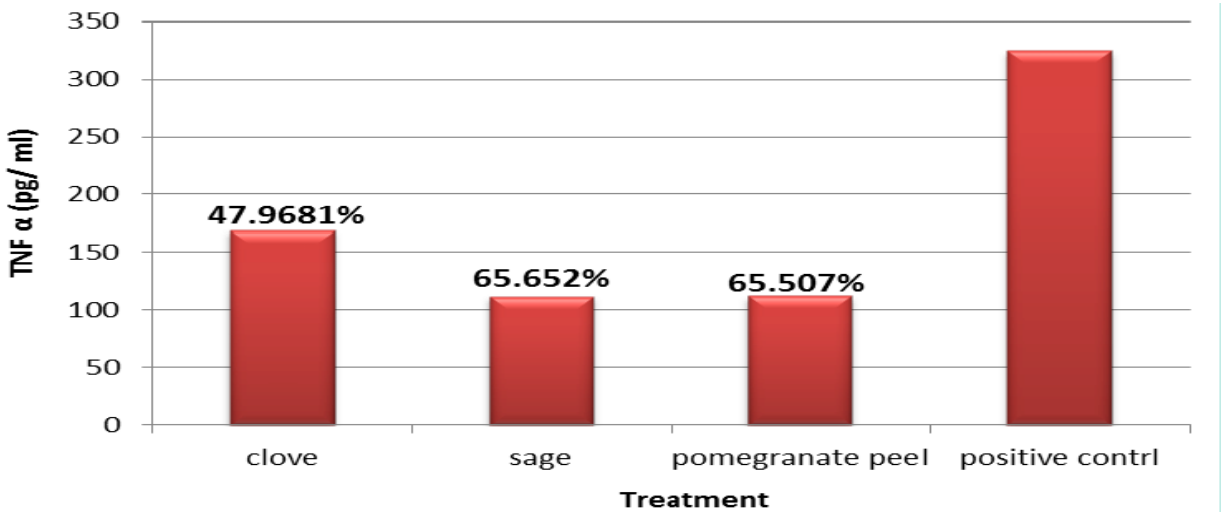
Dermatophytes species (*epidermophyton, trichophyton and microsporum)* are the species that infect epidermis and appendages with serious health consequences. The hydroalcholic pomegranate peel extract (PoPx) had inhibition effect against the dermatophyte fungi *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum canis* and *microsporum gypseum.* The pomegranate crude peel extract shows the inhibitory activity against the *Dermatophyte Trichophyton mentagrophytes*, *Trichophytum rubrum*, *Microsporum canis* and *microsporum gypseum.* The crude extract and punicalagin showed antifungal activity against the conidial and hyphal stages of the fungi. In contrast with mammalian cell the cytoxicity assay is selectively for fungal cells. Therefore it indicate that the crude extract and punicalagin had a greater antifungal activity against *Trichophytonrubrum*, Therefore it is a possible to obtain a antifungal medicine for Dermatophytes [30].

ANTI – INFLAMMATORY AND ANTI-ALLERGIC EFFECT OF POMEGRANATE

Scientist had found that the therapeutic benefit of pomegranate is in high value and it's fraction has built a scientific consensus that pomegranate rind methanolic extract gas the ability to inhibit the Inflammation and Allergies[8]. The Anti Inflammatory component of pomegranate peel i.e. punicalagin, punicalin, strictinin A and granatum B has tendency to reduce the nitric oxide by inhibiting the expansion of pro-Inflammatory protien[31-32]. Evidently, inflammatory cells including neutrophils, macrophages and monocytes may inflict damage to nearby tissues, an event thought to be of pathogenic significance in a large number of diseases such as emphysema, acute respiratory distress syndrome, athero-sclerosis, reperfusion injury, malignancy and rheumatoid arthritis[33].

Anti inflammatory assay graph for tumor necrosis factor alpha (TNF-a)[34].



CONCLUSION

There are number of studies done on pomegranate fruit and it's anti microbial and anti fungal activity of pomegranate and it is found that pomegranate, pomegranate peel, pomegranate rind, pomegranate husk and pomegranate seed has high value of antioxidants and vitamins. From this point of view the pomegranate has wife acceptance in the medicinal use against the different bacterial disease, viral disease, fungal disease, allergies, inflammation and digestive disorders.

The high medicinal use of this fruit shows it's importance to prevent human and animals from different diseases. Therefore pomegranate is good target to study and to obtain a new medicine from the pomegranate and from its different parts.

Hence it can be very useful in the future for the cure of various disease.

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SYNTHESIS, CHARACTERIZATION AND ANALYSIS OF PHOTOCALYTIC EFFICACY OF GREEN COPPER NANOPARTICLES USING *TAMARINDUS INDICA* L. LEAF EXTRACT

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ABSTRACT
Nanomaterials have unique properties that distinguish them from the corresponding bulk materials. The shape and size of metal nanoparticles influence their optical, catalytic and conductive properties. Copper oxide in nanoscale dimensions (CuO NPs) is a multifunctional material with promising applications in the catalysis, gas sensing, batteries, solar energy, etc. In the present work, we report biogenic synthesis of copper nanospheres by utilizing the tamarind leaf extracts. The method employed is totally eco-friendly, facile and economic. Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS), UV-Vis spectroscopic analysis have been used for the characterization of biosynthesized CuO NPs. The SEM results show the formation of uniformly distributed nanospheres, 50-100 nm in diameter. EDS results have confirmed that the comprising elements (Cu and O) are in stoichiometry. Optical results have revealed the formation of CuO NPs. The photocatalytic activity of synthesized samples was observed by monitoring the degradation of methylene blue dye under visible light using a UV-Visible spectrophotometer. It was found that the CuO nanospheres degrade about 60% of the dye within 2 hrs of light exposure. The present work confirms high efficiency of the nanospheres as a photocatalyst and has opened a promising route for the removal of hazardous dyes from industrial effluents contributing directly to environmental cleanup process.

INTRODUCTION
The growing population and rapid industrialization are majorly contributing for increasing pollution of water bodies. Textile, leather, paper and other industrial effluents are the major contributors for effluents such as methylene blue, Congo red, Tatrazine etc. (De alba et al., 2002; Ramsay et al., 2007; Kobylewski and Jacobsen, 2012). Traditional methods of polluted water treatment were found to be not much productive. Photocatalysis, an oxidation process, has been realized as an efficient process to degrade these organic pollutants (Roman and Nely, 2013).

Photocatalysts are materials which produce strong oxidizing agents such as- superoxide and hydroxyl radicals, by consuming the electron hole pairs generated in the presence of water and UV or visible light (Sabeen et al., 2017), which in turn help in the removal of organic pollutants from the water (Liu, et al., 2003). Materials that can use visible light of solar light would be economical in degradation of organic pollutants in the effluent water.

Recently, focus of research has been shifted to the application of metal oxide nanomaterials as photacatalytic materials, as these materials with small size and large surface area have been proved to be strong photocatalysts. Further, the biogenic green metal oxide nanoparticles are economically cheap to prepare, simple to synthesize and are reported to be better photocatalysts which can absorb visible region of sunlight and degrade organic pollutants in waste water (Vanaja et al., 2014).

Copper oxide nanostructures are being synthesized through various methods including chemical and green methods as well (Oral et al., 2004; Izaki et al., 2011; Ghidan et al., 2016; Kei et al., 2017). Use of chemical methods has many disadvantages including difficulty in separation and purification of the nanoparticles, energy consumption and use of hazardous chemicals. The biogenic synthesis of the copper nanoparticles is a recent trend (Ghidan et al., 2016; Kei et al., 2017). Biogenic synthesis of CuO nanostructures and their utilization in the remediation of water pollutants is in totality environmental friendly. In the present work we have synthesized the CuO nanostructures from young leaf extract (aqueous) of *Tamarindus indica* L. and were utilized to analyze their photolytic activity in the degradation of methylene blue (MB) dye.

MATERIAL AND METHOD
Young leaves of *Tamarindus indica*, 1-3 cm in size and red to light green in color, were used in the present study as reducing agent for CuO nanoparticles synthesis. These leaves were collected from Hyderabad, Telangana state vegetable markets during July-August of 2018. The leaves were washed and dried in shade on fresh news papers. Dried leaves were crushed into fine power and about 2.0 g of leaf powder was suspended in 50ml of distilled water in a 500 ml Borosil beaker and was boiled in a microwave oven for ten minutes. The mixture was thoroughly mixed with short intervals, during the boiling. Finally, the extract was filtered through

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Wattman filter paper No.1 and the filtrate volume was made to 100 ml. The leaf extract thus obtained was stored in a refrigerator for further use.

PREPARATION OF COPPER NANOPARTICLES

Solution of 0.1M $CuCl_2.2H_2O$ was prepared in triple distilled water. 15ml of leaf aqueous extract mixed in 150 ml of 0.1M Copper Chloride solution was left in dark for overnight. The precipitate was collected by the centrifugation of the resulting mixture and was washed with distilled water while the centrifugation process and finally dried in an oven at 30 °C. Synthesized powder was collected and studied for its size, shape, composition, optical and photocatalytic properties.

CHARACTERIZATION OF CU OXIDE NANOPARTICLES

The formation of CuONPs was observed visually during the change in the color of the mixture. Further confirmation was done by optical density analysis using UV-visible spectrophotometer 9400-800 nm) as described by Tejovathi et al. in 2018. The size and characterization of CuONPs was done by SEM, and Energy Dispersive Spectroscopy (EDS) analysis.

PHOTOCATALYTIC ACTIVITY ANALYSIS

The photocatalytic behavior of the synthesized Cu nanoparticles was observed by the degradation analyses of methylene blue dye, as per the procedure given by Vanaja et al., 2014. The nanoparticles (40 mg) were dispersed into the aqueous solution (100 ml) of methylene blue (4 ppm). Concentration change in methylene blue dye, with and without catalyst (CuNPs), in the presence of visible light was observed up to 2 hours with 20 minutes interval by recording the absorbance of the solution at 660 nm wavelength.

RESULTS AND DISCUSSIONS

A clear and rapid change in the color of the solution from blue to pale green was observed as soon as both the solutions were mixed. The SEM micrograph of the synthesized biogenic CuO nanostructures, given in the Figure 1, shows spherical structure of nanoparticles having dimensions in the range 50-100 nm.

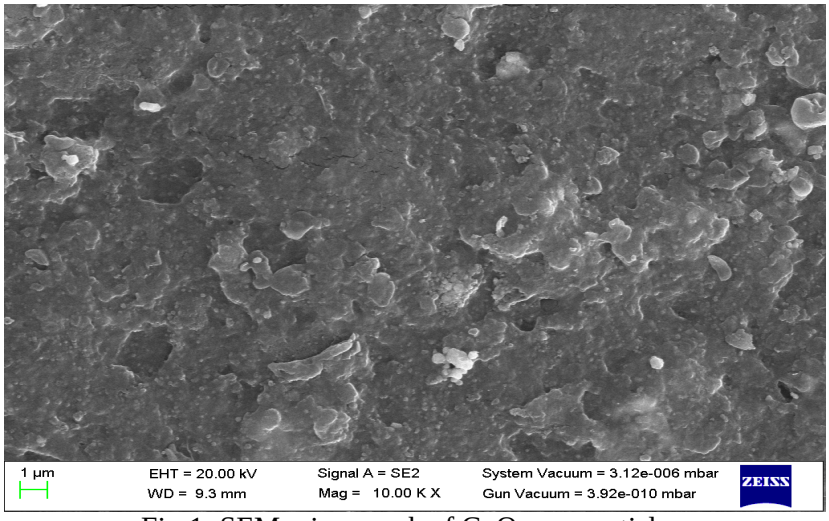

Fig-1: SEM micrograph of CuO nanoparticles.

Identification of the comprising elements in the nanomaterials has been analyzed by Energy Dispersive Spectroscopy (EDS) and the results are shown in the Figure 2 Spectrum of nanoparticles confirms the presence of copper and oxygen elements. The total atomic percentage of copper and oxygen are in stoichiometric ratio (Figure2). In addition there are other peaks that may be attributed to the compounds present in the leaf extract used for capping, reducing and synthesis of CuONPs.

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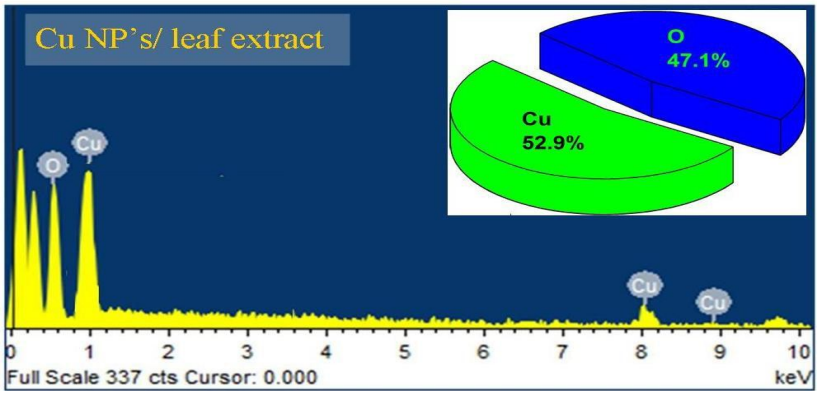

Fig-2: EDS spectrum of CuO nanoparticles with inset pi-chart showing atomic percentage of copper and oxygen.

Optical properties of the synthesized CuO nanoparticles were observed using a double beam UV-Vis spectrophotometer. The absorption spectrum that was recorded between 300-800 nm wavelengths, shown in figure. 3(a) depicts that the nanoparticles have good absorbance in the visible region whereas the leaf extract has negligible absorbance in comparison.

The visible light absorbing nature of the nanoparticles make them to be good visible light driven photocatalysts. The band gap, calculated from the linear portion of the Tauc plot, shown in Figure 3(b), was around 2.75 eV and indicates good photocatalytic efficiency and the value matches well with the earlier reported value for CuONPs [Oral et al., 2004].

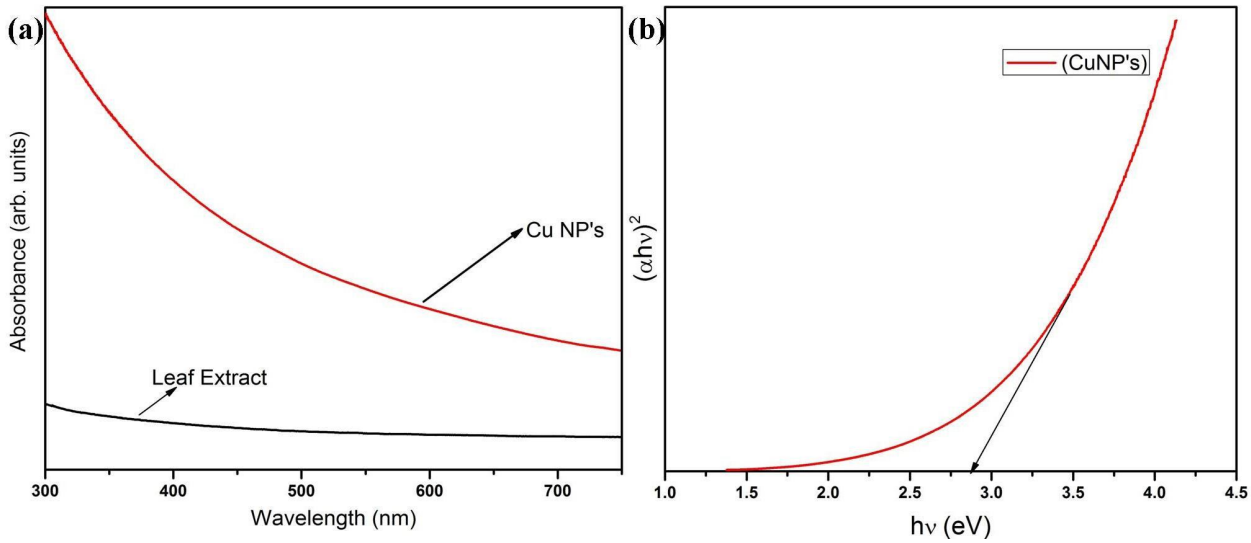

Fig-3: (a) Absorption spectra and (b) band gap plot of CuO nanoparticles.

The photocatalytic property of CuO nanoparticles (as catalyst) was analyzed against methylene blue dye. The solution without nanoparticles was used as control. The absorbance of the solution with or without catalyst in the visible light was recorded at 660nm wavelength in 20 minutes intervals against control, given in Figure 4, shows decrease in the absorbance with increase in the exposure time period. This decrease in the absorbance confirms the degradation of the dye by the nanoparticles. While control solution, without catalyst, has not shown any considerable decay in the concentration of methylene blue dye. Present results clearly show that CuONPs synthesized degrade 60 % of the dye in just 2 hrs of visible light illumination, thus, CuO nanoparticles acted as good visible light driven photocatalysts.

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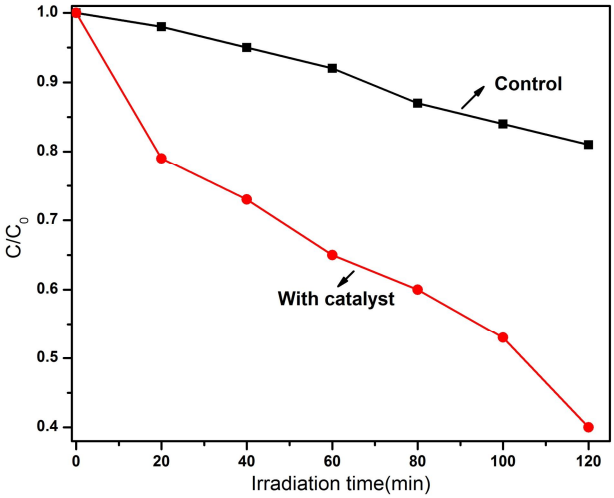

Fig-4: Photocatalytic degradation curve with and without CuO nanoparticles.

CONCLUSIONS

Green synthesis of copper oxide nanoparticles (CuO NPs) is a facile, economical and ecofriendly method compared with chemical and physical methods. Green biogenic method that was employed for the synthesis of CuO nanoparticles utilizing the young leaf extract of *Tamarindus indica* resulted spherical nanoparticles, almost uniformly distributed having diameters in the range of 50-100nm. Photocatalytic activity analysis of the synthesized CuO nanoparticles investigated against methylene blue has shown that the nanoparticles degraded 60% of the dye in just 2 h of exposure to visible light. The present study, thus, concludes that NPs developed with young leaf extract of *Tamarindus* hold good photocatalytic property against methylene blue dye and further investigation of this property against other organic effluents in the water needs to be analyzed.

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GILOE (TINOSPORA CORDIFOLIA WILLD.): MULTI-EFFICACIOUS PLANT OF MEDICINAL VALUE

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ABSTRACT
Medicinal plants have been used as natural medicines and in India enormous biodiversity of medicinal plants is bestowed. Among them, Tinospora cordifolia (Willd.) Miers, (Guduchi) is one of the important plants belongs to the family Menispermaceae. In Hindi, the plant is commonly known as Giloe, is a plant prescribed in Ayurveda, the Indian traditional system of medicine as a Rasayana. The biological significance of this plant is mainly because of leaves, barks and roots contain bioactive compounds such as alkaloids, glycosides, lactones, steroids and aliphatic compounds having various medicinal importance viz., immunomodulatory, anti-tumour, antioxidant, anti-malaria, hepatoprotective, anti-allergic, anti-cancerous. In conclusion, Tinospora cordifolia suggests, a huge biological potential of this plant. Various reports on its multiple medicinal use attracted attention for commercial exploitation of the plant to meet the requirements of the growing pharmaceutical industry. It is an excellent drug, which could be a good remedy for various ailments of animal as well as human beings. This plant species has huge therapeutic potential, it has been over exploited by several human activities. Conservation of this plant species can be done by plant tissue culture for rapid propagation of the plant and for the enhancement of secondary products. Further studies are required in the field of tissue culture to increases the success rate, molecular analysis of any genetic changes if comes through DNA fingerprinting and exploration of a variety of fungal endophytes to reveal the exact pathway of natural product synthesis in the plant cells.

Keywords: Anti-cancer activity ; Immuno-modulatory activity; Hepatoprotective activity; Antidiabetic activity; Tinosporides; Berberine; Tinospora cordifolia .

1. INTRODUCTION
Tinospora cordifolia (Willd.) Miers is an extensively spreading, glabrous, succulent, climbing shrub belonging to the family Menispermaceae [CSIR, Nadkarni 1976]. It is distributed throughout the tropical region of Nepal, India, Sri Lanka and China, ascending to an altitude of 1,200 m asl. It thrives in the tropical region in forests and other habitat. Stems, roots, leaves and starch obtained from the roots and stems are used for medicinal purpose, especially in Ayurveda. The root is a powerful emetic and is used for visceral obstructions; its water extract is used in leprosy. The root also exhibit antidiabetic effect[Gupta et al. 1967]. The extracts of stem, leaves, barks and roots show strong antioxidant activities [Stanley et al.1999]. The bitter principle present in the stem is used in the treatment of debility, dyspepsia, fever and urinary disease and the decoction of the leaves is used for the treatment of gout. The pharmaceutical significance of this plant is mainly due to the presence of various bioactive compounds such as glucosides, and alkaloides including berberine.

Various reports on its multiple medicinal use attracted attention for commercial exploitation of the plant to meet the requirements of the growing pharmaceutical industry. T. Cordifolia natural stands are now fast disappearing and are threatened due to indiscriminate collection and over-exploitation. Conventional vegetative propagation of this plant has limited potential for large scale cultivation .Due to the presence of immense medicinal properties, this plant has been overexploited by pharmaceutical companies and folk people for traditional remedies have led to the acute scarcity of this plant to meet the present-day demand. Due to its high demand, T. Cordifolia has been listed amongst 32 highly prioritized medicinal plants of agro climatic zone 8 (Rajasthan, U.P. and M.P.) of India as identified by National Medicinal Plant Board, New Delhi, Government of India.

T. cordifolia can be propagated by seeds and vegetative cuttings. However, both the ways are not suitable for large scale production and having problems in traditional methods of propagation. Viability of seeds is very less, poor seed set and germination of seeds are the main problems associated with its clonal propagation. Vegetative cuttings are also not suitable due to less productivity and also dependent upon weather conditions for its further growth. Keeping in view the vegetative propagation constraints, plant tissue culture techniques may be suitable methods for its large scale production in a lesser time and space . Today , world over there is an increasing awareness of traditional medicine mainly so because we have come to realize that it holds tremendous potential of untapped resources. What it lacks is hard-core evidence and documentation on proper scientific methods. In 20th century a new mode for comprehensive study of medicinal plants was initiated . India is bestowed with vast biodiversity including those of medicinal plant species. Among them Tinospora

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cordifolia belongs to family Menispermaceae and is well known as Gulancha tinospora in English . In common Indian literature it is called Giloe or Gurcha . Six species of genus Tinospora are reported world wide, which however have not been analysed extensively for their genetics and molecular biology.

T. cordifolia is widely distributed through out tropical India ascending to an altitude of 300m from Kumanon to Kanyakumari .While T. malabarica and T. crispa are found in the forests of Assam and Chennai . Among all the species Tinospora cordifolia attained a wide research importance because of the presence of numerous important chemical compounds.

2. BIOCHEMICAL PROPERTIES

A variety of constituents have been isolated from Tinospora cordifolia belonging to different classes such as alkaloids, diterpenoid lactones, glycosides, steroids and phenolics.

3. BIOLOGICAL ACTIVITIES

The major biological activities of Tinospora cordifolia are as follows :

Plant part System of medicine	Active components	Compound	Biological activity (In human being)	References
Stem, Ayurvedic Root	Alkaloids	Berberine, Choline, Tembetarine, Magnoflorine, Tinosporin , Palmetine, Isocolumbin, Aporphine alkaloids, Jatrorrhizine, Tetrahydropalmatine,	Anti-viral infections, Anticancer, anti-diabetes, inflammation, Neurological, immunomodulatory, psychiatric conditions.	UpadhayaAK(2010); Rout GR(2006); Patel SS(2009); Gupta R(2011); Jagetia GC (2002); Patel MB(2011)
Whole plant Ayurvedic	Diterpenoid Lactones	Furanolactone, Clerodane derivatives [(5R,10R)-4R-8Rdihydroxy-2S-3R:15,16-diepoxy-cleroda-13 (16), 14-dieno-17,12S:18,1Sdilactone], Tinosporon, Tinosporides, Jateorine, Columbin	Vasorelaxant: relaxes norepinephrine induced contractions, inhibits Ca++influx, anti-inflammatory, anti-microbial, antihypertensive, anti-viral. Induce apoptosis in leukemia by activating caspase-3and bax, inhibits bcl-2.	Sriramaneni RN(2010); Yang S,Devid K *et al.*(2010); Zhao F(2008); Kohno H(2002); Dhanasekaran M(2009)
Stem Ayurvedic	Glycosides	18-norclerodane glucoside, Furanoid diterpene glucoside, Tinocordiside, Tinocordifolioside, Cordioside, Cordifolioside Syringin, Syringin-apiosylglycoside, Pregnane glycoside, Palmatosides,Cordifoliosi de A, B, C, D and E	Treats neurological disorders like ALS, Parkinsons, Dementia,motor and cognitive deficits and neuron loss in spine and hypothalamus, Immunomodulation, Inhibits NF-kBand act as nitric oxide scavenger to show anticancer activities.	Ly PT(2007); Karpova EA(1991); Kapil A and Sharma S(1997); Chen S(2001); Badwin AS (2001); Yang JH, Caoh *et al.*(2010); Kim SK(2008)
Shoot Ayurvedic	Steroids	β–sitosterol, δ-sitosterol, 20 β-hydroxyecdysone, Ecdysterone, Makisterone A, Giloinsterol	IgA neuropathy, glucocorticoid induced osteoporosis in early inflammatory arthritis, induce cell cycle arrest in G2/M phase and apoptosis through c-Myc suppression. Inhibits TNF- α, IL-1 β, IL-6 and COX-2.	Lv J, Woodward *et al.*(2012); McKeown E, Hitchon CA *et al.*(2012); Sundarraj S, Achiraman S *et al.*(2012)
Stem Ayurvedic	Sesquiterpe noid	Tinocordifolin	Antiseptic	Maurya R (1998)
Whole Ayurvedic plant	Aliphatic compound	Octacosanol, Heptacosanol Nonacosan-15-one	Anti-nociceptive and anti-inflammatory. Protection against 6-	De-Oliveria AM(2012); Wang T(2010); Thippeswamy G(2008)

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Root Ayurvedic whole plant	Others	Dichloromethane 3,(a,4-di hydroxy-3-methoxy-benzyl)-4-(4-compounds hydroxy-3-methoxy-benzyl)-tetrahydrofuran, Jatrorrhizine, Tinosporidine, Cordifol, Cordifelone, Giloinin, Giloin, N-transferuloyltyramine as diacetate, Tinosporic acid.	hydroxydopamine induced parkinsonisms in rats. Down regulate VEGF and inhibits TFN-α from binding to the DNA . Protease inhibitors for HIV and drug resistant HIV.	Ghosh AK(2008); Mukherjee R(2010)

3.1 Anti-Diabetic Activities

Tinospora cordifolia is widely used in Ayurvedic medicine for treatment of diabetes mellitus. The stem of Tinospora cordifolia is used to cure diabetes by regulating level of blood sugar [Sangeetha MK, 2011].

It act as anti-diabetic drug through explanatory oxidative stress, promoting insulin secretion by inhibiting gluconeogenesis and glycogenolysis. The root extract of plant decreases the blood sugar level [Umamaheshwari S, 2007].

3.2 Immunomodulatory Activities

T. cordifolia is used to improve the immune system. A variety of active compounds 11-hydroxymuskatone, N-methyle-2-pyrrolidone, N formyla-nnonain, cordifolioside A, magnoflorine, tinocordioside and syringing have potential immunomodulatory and cytotoxic effects [Sharma P, 2012]. These compounds improve the phagocytic activity of macrophages, enhancement in nitric acid production by stimulation of splenocyte, [Upadhyay PR, 2011] and production of reactive oxygen species (ROS) in human neutrophil cells [More P, 2010]

3.3 Anti-Microbial Activities

Methanolic extract of T. cordifolia has been used against microbial infection [55]. Antibacterial activity of T. cordifolia extract has been bio assayed against Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Salmonella typhi, Shigella flexneri, Salmonella paratyphi, Salmonella typhimurium, Pseudomonas aeruginosa, Enterobacter aeruginosa, Enterobacter aerogene [Narayanan AS, 2011; Jayachandran R, 2003; Ambedkar DH, 2009]. T. cordifolia extract bacterial growth and improved phagocytic and intracellular bacterial capacities of neutrophils in mice [Sengupta S, 2009].

3.4 Anti-HIV Activities

Root extract of Tinospora cordifolia decrease in the regular resistance against HIV [Kalikae MV, 2008]. This anti HIV effect was exposed by reduction in eosinophil count, stimulation of B lymphocytes, macrophages, level of haemoglobin and polymorphonuclear leucocytes [Kalikae MV, 2008; Akhtar S, 2010].

3.5 Anti-Cancer Activities

The extract of T. cordifolia was found to increase humoral immune response, as seen from the increase in plaque forming cells in spleen and circulating antibody titre and to produce an enhancement in macrophage activation. Root extract of T. cordifolia has been shown radio protective role. Dichloromethane extracts of TC shows cytotoxic effects owing to lipid peroxidation and release of LDH and decline in GST [Jagetia GC, 2006]. In pre-irradiating mice, root extract has widely affected radiation, induced rise in lipid peroxidation and resulted in the decline of GSH in testes [Upadhyay PR, 2011]. Most of the synthetic chemotherapeutic agents laid toxic side effects on the living organisms [Diwanay S, 2004]. The effect of Giloy has been reported better than doxorubicin treatment [Narayanan AS, 2011].

3.6 Anti-stress Activity

Ethanol extract of T. cordifolia at the dose of 100 mg/kg exhibited significant anti-stress activity compared with diazepam at the dose of 2.5 mg/kg [Sarma et al., 1996].

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3.7 Digestive Activity
T. cordifolia fight against Entamoeba histolytica.The antiamoebic drug containing Tinospora cordifolia .According to Sohni et al. (1995) reported varying degrees in inhibition of of the enzymes, viz., DNase, RNase, acid phosphatase, alkaline phosphatase and protease activities of crude extracts of axenically cultured amoebae.

3.8 Anti-inflammatory Activity
Water extract of T. cordifolia has been used in oedema arthritis and on human arthritis. The dried stem of T. cordifolia have significant anti-inflammatory effect in both acute and sub-acute models of inflammation. T. cordifolia was more effective than acetylsalicylic acid in acute inflammation [Jana et al., 1999].

3.9 Antioxidant Activity
T. cordifolia exhibited excellent antioxidant activity in ethanol, methanol and water extracts. Stem of guduchi have high potential as a source of natural antioxidants to reduce oxidative stress with consequent health benefit [Bhawya D 2010]. The decreased concentration of glutathione (GSH), activity of superoxide dismutase (SOD) and catalase in liver and kidney of diabetic rats [Prince et al., 2004]. Methew and Kuttan (1997) reported the antioxidant activity and amelioration of cyclophosphamide-induced toxicity. The direct and indirect antioxidant actions of T. cordifolia probably act in corroboration to manifest the overall radio-protective effect [Goel et al., 2002].

3.10 Cognition (Learning and Memory) Activity/ Mental disorders
Failing memory and other intellectual functions with little disturbance in consciousness [Ropper et al., 2009]. Both aqueous and alcoholic extracts of T. cordifolia help to enhance cognition (learning and memory) in normal and cyclosporine induced memory deficit rats and also used as mental disorders [Kulkami and Verma, 1993].

3.11 Hepatoprotective Activity
Effect of T. cordifolia extract on modulation of hepatic functions . It help to prevent fibrosis and stimulates regeneration of hepatic tissue. It helps in fatty liver. Tinospora cordifolia extracts treatment in CCl4 intoxicated rat was found that liver was protected [Bishayi et al., 2002]. The chloroform extract of T. cordifolia failed to reduce the liver toxicity in test. [Reddy et al.1993]. It also exhibited in vitro inactivating property against Hepatitis B and E surface antigen in 48-72 hrs [Mehrotra et al., 2000].

3.12 Antiulcer activity
Ethanolic root extracts of T.cordifolia use to protect from ulcers, it induces a marked protective activity against an 8h' restrain stress induced ulcerization,which is equal to that of diazepam. [D.N.K.Sarma et al. 1995]

3.13 Mental disorder
The whole plant and the juice of the leaves are used in various mental disorders.This is one of the best psychotropic drugs in India.[allayurveda.com]

3.14 Against dengue
The capsule developed from Guduchi is useful in the treatment of Dengue [Planet ayurveda].

4. INTERACTIONS WITH OTHER DRUGS

- Medications for diabetes (Antidiabetes drugs) interact with Tinospora cordifolia Tinospora cordifolia might decrease blood sugar. Taking Tinospora cordifolia along with diabetes medications might cause blood sugar too low. Monitor your blood sugar levels. The doses of your diabetes medications might need to be changed.
- Medications that decrease the immune system (Immunosuppresants) interacts with Tinospora cordifolia Tinospora cordifolia might cause the immune system more active and this could increase the symptoms of autoimmune diseases.Taking it along with some medications that decrease the immune system might decrease the effect of these medications.

5. TOXICITY

Tinospora cordifolia seems to be safe when used short term. The safety of long-term use, more than 8 weeks ,is not known.

6. DOSAGE

For allergic rhinitis: 300mg of a specific Tinospora cordifolia aqueous stem extract (Tinofend, Verdure Sciences) three times daily for 8 weeks.

7. CONCLUSION

Tinospora cordifolia is also known as Guduchi . The plant is used in ayurvedic ,"Rasayanas" to improve the immune system and the body resistant infections. Tinospora cordifolia is used in many diseases .There are many

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herbal plants in the world, Among them Guduchi is considered to be having greater medicinal value. The pharmaceutical significance of this plant is mainly due to the presence of various bioactive compounds such as glucosides, and alkaloides including berberine.

Various reports on its multiple medicinal use attracted attention for commercial exploitation of the plant to meet the requirements of the growing pharmaceutical industry. T. Cordifolia natural stands are now fast disappearing and are threatened due to indiscriminate collection and over-exploitation. Conventional vegetative propagation of this plant has limited potential for large scale cultivation. T. cordifolia can be propagated by seeds and vegetative cuttings. However, both the ways are not suitable for large scale production and having problems in traditional methods of propagation.Viability of seeds is very less, poor seed set and germination of seeds are the main problems associated with its clonal propagation. Vegetative cuttings are also not suitable due to less productivity and also dependent upon weather conditions for its further growth. Keeping in view the vegetative propagation constraints, plant tissue culture techniques may be suitable methods for its large scale production in a lesser time and space .

Plants produces diversity of secondary metabolites which not only plays an important role in adaption according to the environment but also represent an important source of active pharmaceuticals. Wide array of biological activities including anti-inflammatory, anti-carcinogenic antioxidant, anti mutagenic etc have been attributed to many secondary compounds isolated from plant sources.

Biotechnology offers a great opportunity to exploit these secondary metabolites via different methods. Plant cell and tissue culture is one of the emerging fields of biotechnology to investigate and enhance the production of various secondary metabolites. Undifferentiated cell cultures like callus and cell suspension cultures have been studied widely for secondary production. Careful survey of literature reveals that in vitro approaches have been applied in Tinospora cordifolia for micropropagation , molecular DNA fingerprinting and production of phenolics and secondary metabolites. However still several gaps existing in available knowledge. With regard to production of biological active secondary metabolites in vitro in callus and cell suspension cultures which extend posers to fulfill the gaps and broaden knowledge base regarding production of secondary metabolites ie. tinosporoside etc.

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EFFECTS OF *RHIZOBACTERIUM* ON SALT STRESSED COWPEA SEEDLINGS

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ABSTRACT
Salinity is one of the most severe abiotic problems in arid and semi-arid regions of the world. This experiment was carried out to evaluate the influence of bradyrhizobacterium (BR) application on the early stage of plant growth i.e seed germination stage of cowpea, in saline water. Cowpea variety – Pusa Sukomal and BR2 and BR3 strains of bradyrhizobacterium for seed inoculation were used in the experiment. The NaCl at 0mM (control), 25, 50 and 75 mM concentrations were used and the influence of salinity was observed at 7 and 15th day of germination. Percentage germination, shoot and root length, fresh weight and dry weight and photosynthetic pigments - chlorophyll a, b and carotenoids content were used as growth parameters. The results, in general, have shown reduction in all parameters analysed at both 7 and 15 days of germination. The reduction was high with NaCl salinity level increased. Further, this was commonly noticed in without and with application of BR2 or BR3 symbiotic microorganisms. However, the application of BR2 and BR3 strains has improved the performance of the seedlings as compared to seeds germinated without application. This study, thus, reports that Bradyrhizobacterium (BR2 and BR3) mitigated the harmful effects of salinity stress at seedling stage.

Keywords: Salinity, Cowpea, Bradyrhizobacterium, seedling BR2 and BR3

INTRODUCTION
Salinity is one of the major limiting abiotic stress factors that influence crops' productivity. About 20% of the total cultivated land over worldwide is estimated to be affected by salt stress and this is increasing every day (Gupta and Huang, 2014). Salinity inhibits plant growth because it affects important metabolic processes like photosynthesis, transpiration, cell growth and seed germination. Seed germination is the most perceptible phase in salinity conditions, because salinity affects absorption of water (Meloni et. al., 2007). Germination andseedling growth parameters are the most basic, important and viable criteria used for screening and selecting salt tolerant crop plants (Mordi et. al., 2013).

The legumes are both ecologically and agriculturally very important crops because they have the ability to fix nitrogen in the root nodules in a symbiotic interaction with soil rhizobia however; they are highly sensitive to salinity and drought status. The physiological consequences of environmental stress in legumes have been reported to be dependent on the specific characteristics of these plants. A better understanding of the mechanisms involved in the response to abiotic stress may be the possible method to improve crop productivity (Crespi and Galvez 2000)

Cowpea,*Vigna unguiculata* (L.) Walps, is an important food legume crop with high protein content (20-25%) (Diouf and Hilu, 2005; Kareem and Taiwo, 2007; Sharmar et al., 2013).

Plant growth promoting Rhizobacteria (PGPR) - Rhizobia present in the rhizosphere are known to enhance plant growth under saline stress (Ahmad *et al.,* 2011). Rhizobacteria are also known to improve productivity in salinity conditions (Shukla *et al.,* 2012).

Salinity is the most widespread problem in symbiotic nitrogen fixation and it restricts the extension of legume cultivation (Faghire *et al.,* 2011; Farissi *et al.,* 2011, 013). The initiation, development, and function of nodules are reported to be effected more than the symbiotic nitrogen fixation (Saadallah *et al.,* 2001; Rao *et al.,* 2002; Faghire *et al.,* 2011). Thus, the process of infection appears to be the most sensitive to salt (Payakapong *et al.,* 2006). Selection of host genotypes that are tolerant to high-salt conditions, Rhizobacteria tolerance and the selection of salt-tolerant plant-rhizobia combinations might improve plant productivity under symbiosis (Kenenil *et al.,* 2010).

MATERIAL AND METHODS-
Collection of plant material and Rhizobacterium
In this experiment, the role of *Rhizobacterium* application on Cowpea seed germination in saline conditions was studied using Cowpea variety- Pusa Sukomal obtained from Indian Agricultural Research Institute, New Delhi and two strains of Bradyrhizobacterial (BR) cultures- BR2 and BR3, specific for cowpea, obtained from IARI, Delhi were used.

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NaCl salinity was applied at 0, 25, 50 and 75mM concentrations and the effect was studied at 7 and 15 days of germination by measuring the growth parameters like shoot and root length (in cm), Biomass (Fresh and dry weight, in g) and Photosynthetic pigments –Chlorophyll a (*Chl a*), Chlorophyll b (*Chi b*) and Carotenioids (*Car*) Chlorophyll content.

Experiments were performed in 3 replicates. In each set, two groups- one without BR application (control) and With BR application, containing 50 seeds per NaCl concentration were used.

On 7th and 15th day of germination, randomly 5 seedlings were selected per replicate and used for the Chlorophyll a, b, and carotenoides estimation and the rest of the seedlings were used for recording growth parameters. Method given by Sibley et al., 2014 was adopted for the estimation of chlorophyll content in the samples. The data from all the replicates were pooled, mean and standard error was calculated.

RESULTS AND DISCUSSION

The effect of salinity on the growth of Cowpea was studied at seedling stage using 0, 25, 50 and 75mM NaCl saline water. The influence of Rhizobacterium was observed by applying BR2 and BR3 strains of bradyrhizobacterium on seeds of variety Pusa Sukomal before exposing to salinity.

SEED GERMINATION

One of the most worldwide spread methods for determination of plant tolerance to salts is the germination percentage in salt solutions. Germination percentage of variety Pusa Sukomal without and with BR inoculation was recorded on 7th day is given in Figure 1. The percentage germination of seeds in control was 100% and it has reduced to 96.67%, 93.33% and 89.33% respectively on exposed to 25mM, 50mM and 75mM NaCl and in the absence of BR strains. The seeds inoculated with BR2 before exposing NaCl solution have shown 100% germination in control (0mM) as well as in 25mM NaCl water. The percentage was reduced to 96.67% and 90.67% when the NaCl concentration was increased to 50mM and 75mM respectively (Figure 1). Seeds with BR3 also ecorded 100% germination in control as well as in 25mM NaCl and 98.67% and 94.67% on 50 and 75mM the NaCl concentration (Figure 1).

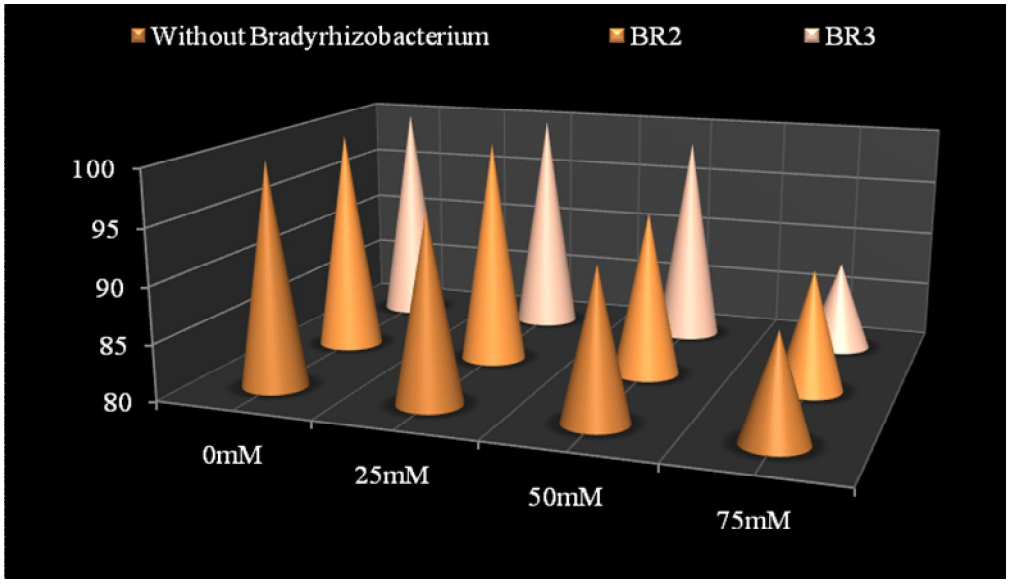

Figure-1: Effect of salinity on percentage germination of Pusa Sukomal seeds without and with Bradyrhizobacterium (BR2 & BR3) application

The control 7 days old seedlings have recorded 22.64cms shoot length under 0mM salinity. While on exposure to NaCl, the shoot length was recorded as 21.12±1.07 (25mM), 15.52±1.18 (50 mM) and 12.40±1.28cms (75mM). On application of BR2 strain, seedlings have recorded 27.48±1.33cms in 0mM, 23.52±0.71cms in 25mM, 18.16±1.39cms in 50mM and 12.44±1.03cms in 75mM NaCl salinity. Similarly on BR3 strain inoculation, have registered 29.76±1.37cms, 25.28±0.94cms, 21.50±0.65cms and 12.30±0.54cms shoot length in 0 (control), 25, 50 and 75mM NaCl concentrations respectively (Table 1).

The 15 day old seedlings have recorded 28.20±1.23, 24.10±1.15, 22.50±1.48 and 18.90±1.13cms shoot length in the absence of BR strains on four NaCl- 0, 25, 50, 75mM concentrations. On application of strain BR2, length of shoots measured 31.20±1.65, 27.50±1.55, 28.30±1.89, and 23.50±1.35cms and on BR3 strain inoculation 33.70±1.18, 30.30±1.41, 28.50±1.44 and 24.80±0.63cms respectively under 0, 25, 50 and 75mM NaCl salinity (Table 1).

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The root length of 7 day old seedlings, grown under 0, 25, 50 and 75mM NaCl concentrations and without BR strain application, was 11.36±0.53, 9.70 ± 0.49, 7.04 ± 0.43 and 5.24±0.44cms respectively (Table 2). In the BR2 presence, the roots of the seedlings have measured 12.20±0.40, 10.44±0.74, 9.12±0.42 and 8.72±0.85cms and with BR3 presence, root length of the seedlings was noticed to be 13.08±0.40, 12.70±0.30, 10.92±0.65 and 9.68 ± 0.80cms under 0, 25, 50 and 75mM NaCl saline conditions (Figure 2).

Similarly, roots in 15 day old control seedlings have recorded 14.80cms length at 0mM NaCl and on exposure to salinity (25mM-75mM) the length was decreased to 12.80, 12.30 and 11.80cms. On application of BR strains, roots have grown 15.10, 13.20, 13.10 and 11.20cms in length with BR2 strain and 16.40, 14.60, 13.30 and 11.20cms with BR3 at 0, 25, 50 and 75mM NaCl concentrations respectively (Table 2).

Fresh and Dry weight

The fresh and dry weight data obtained from 7 and 15 day old seedlings after exposure to NaCl, with or without BR2 and BR3 inoculation is presented in the Figure. Control seedlings gained 7.58g and 11.56g of fresh weight and 0.92±0.07 and 1.37±0.09 dry weight in 7 and 15 days. When the seeds were exposed to NaCl salinity, the fresh weight was 6.50g (25mM), 4.96g (50mM), 2.54g (75mM) on 7th day and 9.39g (25mM), 8.11g (50mM) and 5.92g (75mM) on 15th day (Table 3).

Application of BR2 stain on seeds before exposure to salinity, the seedling have shown 10.14g, 8.12g, 5.59g and 4.09g fresh weight on 7th day of germination and 13.59g, 12.15g, 10.17g, and 9.57g fresh weight on 15th day of germination at 0 (control), 25, 50 and 75mM NaCl concentrations respectively (Table 3). These seedlings on drying recorded 1.28±0.09g, 1.06±0.12g, 0.99±0.03g and 0.81±0.07g dry weight in 7th day seedlings and 2.01±0.11g, 1.49±0.08g, 1.33±0.05g and 1.18±0.09g dry weight in 15 days of exposure to 0, 25, 50 and 75mM salinity (Table) .

Similarly when BR3 strain was inoculated on seeds, 7 day old seedlings under 0, 25, 50 and 75mM concentration have recorded 11.05g, 9.18g, 6.25g and 4.51g fresh weight and 1.46±0.12g, 1.25±0.07g, 1.09±0.08g, 0.86±0.08g dry weight respectively at different salinity levels (Table 3 & 4). While 15 day old seedling attained 14.33g (0mM), 13.17g (25mM), 11.39g (50mM) and 9.36g (75mM) fresh weight and 2.08±0.07g, 1.55±0.12g, 1.43±0.12g and 1.16±0.07g dry weight (Table 3 and Figure 3).

Seedling establishment is a crucial process in a plant life, especially in the presence of adverse environment factors (Bohnert, *et al.,* 1995). Many researchers have reported that salinity causes adverse effects on plant growth and seed germination percentage (Lobato *et al.,* 2009; Rajpur *et.al.,* 2002, Guljar *et.al,*. 2003; Kaymakanova, 2009), fresh and dry weight (Raptan *et al.,* 2001; Yupnis *et al.,* 2001; Ghoulam *et al.,* 2002) in various plants including in chick pea, Cowpea, lettuce (Murrillo-Amador and Troyo-Die Guez, 2000; Ashraf and Waheed, 1992; Barassi *et al.,* 2006). Rhizobacteria are known to ameliorate the effect of salt stress on germination (Sheikh Hasna Habib *et al.* 2016). Present studies with Cowpea var. Pusa Sukomal also have shown remarkable reduction in germination percentage with increase in salt concentration, which in accordance to earlier reportes in cowpea (Murrillo-Amador and Troyo-Die Guez, 2000) in chickpea (Ashraf and Waheed, 1992).

Barassi *et al.,* in 2006 observed that rhizobacteria has improved the seed germination in lettuce in saline conditions. The application of BR strains BR2 and BR3 on seeds, in the present study, also have definitely improved the seed germination and shoot, root lengths, fresh and dry weight in 7 as well as 15 day old seedlings.

NaCl concentrations	Shoot length (in Cms)					
	7th day			15th day		
	Without BR strain (Control)	With BR2	With BR3	Without BR strain (Control)	With BR2	With BR3
0mM	22.64±0.8	27.48±1.33	29.76±1.37	28.20±1.23	31.20±1.65	33.70±1.18
25mM	21.12±1.07	23.52±0.71	25.28±0.94	24.10±1.15	27.50±1.55	30.30±1.41
50mM	15.52±1.18	18.16±1.39	21.50±0.65	22.50±1.48	28.30±1.89	28.50±1.44
75mM	12.40±1.28	12.44±1.03	12.30±0.54	18.90±1.13	23.50±1.35	24.80±0.63

Table-1: Effect of NaCl salinity on the shoot length of 7thand 15th day old seedlings in the presence and absence of inoculation of Rhizobacterium BR2 & BR3 of Cowpea var. Pusa Sukomal.

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NaCl Concentrations	Root length (in cms)					
	7th day			15th day		
	Without BR strain (Control)	With BR2	With BR3	Without BR strain (Control)	With BR2	With BR3
0mM	11.36±0.53	12.20±0.40	13.08±0.40	14.80±0.68	15.10±0.66	16.40±0.67
25mM	9.70±0.49	10.44±0.74	12.70±0.30	12.80±0.81	13.20±0.66	14.60±0.78
50mM	7.04±0.43	9.12±0.74	10.92±0.65	12.30±0.67	13.10±0.84	13.30.±0.68
75mM	5.24±0.44	8.72±0.85	9.68±0.80	11.80±0.59	11.20±0.73	11.20±0.65

Table-2: Effect of NaCl salinity on the root length of Pusa Sukomal seedlings with or without Rhizobacterium BR2 &BR3 inoculation.

NaCl concentrations	Fresh weight (in gm)					
	7th day			15th day		
	Without BR strain (Control)	With BR2	With BR3	Without BR strain (Control)	With BR2	With BR3
0mM	07.58±0.42	10.14±0.57	11.05±.0.53	11.56±0.33	13.59±0.41	14.33±0.6
25mM	06.50±0.57	08.12±0.52	09.18±0.37	09.39±0.39	12.15±0.57	13.17±0.57
50mM	04.96±0.74	05.59±0.29	06.25±0.36	08.11±0.21	10.17±0.23	11.39±0.59
75mM	02.54±0.33	04.09±0.51	04.51±0.35	05.92±0.85	09.57±0.71	09.36±0.37

Table-3: Effect of NaCl salinity on the fresh weight of Pusa Sukomal seedlings with or without Rhizobacterium BR2 &BR3 inoculation

NaCl Concentrations	Dry weight (in gm)					
	7th day			15th day		
	Without BR strain (Control)	With BR2	With BR3	Without BR strain (Control)	With BR2	With BR3
0mM	0.92±0.07	1.28±0.09	1.46±0.12	1.37±0.09	2.01±0.11	2.08±0.07
25mM	0.82±0.02	1.06±0.12	1.25±0.07	1.25±0.08	1.49±0.08	1.55±0.12
50mM	0.72±0.03	0.99±0.03	1.09±0.08	1.07±0.03	1.33±0.05	1.43±0.12
75mM	0.39±0.02	0.81±0.07	0.86±0.08	0.86±0.05	1.18±0.09	1.16±0.07

Table-4: Effect of NaCl salinity on the dry weight of Pusa Sukomal seedlings with or without Rhizobacterium

Photosynthetic pigments

The Photosynthetic pigments- Chlorophyll a (*Chl a*), Chlorophyll b (*Chl b*) and carotenoids (*caro*) content (µg/gfw) in 7 and 15 days old seedlings was analyzed in the seedlings exposed to salinity both in the presence and absence of bradyrhizobacterium (BR2 and BR3) strains and the data obtained has been presented in the Table 5.

According to the data, the control seedlings of age 7 days have recorded 192.33µg/gfw of *Chl a*, 89.27µg/gfw of *Chl b* and 56.06µg/g fw of *Caro*. While the seedlings of age 15 days have estimated to contain 313.09 µg/gfw *Chl a*, 281.01µg/gfw *Chl b and* 64.64µg/gfw *Caro* (Table 5). On exposure to salinity, the amount of *Chl a* was decreased from 155.03µg/gfw to 80.60µg/gfw, the *Chl b* level was decreased to 68.45µg/gfw to 55.65µg/gfw, and the *Car* level from 51.33µg/gfw to 24.72µg/gfw in 7 day old seedling raised in 25 to 75mM NaCl saline waters (Table 5). Similarly the 15 days seedlings have shown change in the levels from 313.10±0.31 µg/gfw to199.92±0.76 µg/gfw in *Chl a*, 281.02±10.54 µg/gfw to 127.67±8.42 µg/gfw in *Chl b* and 64.64±1.94 µg/gfw to 37.65 ±1.30 µg/gfw in *Car* concentration as the NaCl level raised from 25 to 75mM (Table).

The application of BR2 has shown increase in the levels of chlorophyll pigments- *Chl a* (241.43 / 354.85µg/gfw); *Chl b* (116.42 / 250.98µg/gfw) and *Car* (72.33/ 79.72µg/gfw) in 7 and 15 days' old seedlings respectively under non saline conditions. On exposure to 25mM salinity stress, the *Chl a* contents was found to be between 178.312µg/gfw and 297.96µg/gfw; *Chl b* content was between 93.46µg/gfw and 147.96µg/gfw and the *Caro* level was between 50.10µg/gfw and 68.40µg/gfw in 7 and 15 days old seedlings respectively (Table 5).

The 50mM NaCl salinity has resulted further reduction to 131.90µg/gfw (*Chl a*), 89.37µg/gfw (*Chl b*) and 35.39µg/gfw (*Caro*) in 7 days and 275.69µg/g (*Chla*), 190.02µg/gfw (*Chl b*) and 153.61µg/gfw (*Caro*) in 15 days old seedlings. Similarly, at 75mM NaCl these seedlings have exhibited 129.84µg/gfw and 252.49µg/gfw of *Chl a*, 95.67µg/gfw and 130.96µg/gfw of *Chl b* and 33.23µg/gfw and 38.66 µg/gfw of *Caro* in 7 days and 15 days old seedlings respectively (Table 5).

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The photosynthetic pigments- *Chl a, Chl b and Caro* levels estimated in the seedlings germinated in the presence of strain BR3 have recorded 237.28µg/gfw, 229.17µg/gfw, 216.79µg/gfw and 213.14µg/gfw in 7 days and 421.99µg/gfw, 392.70µg/gfw, 348.330µg/gfw and 324.54µg/gfw of *Chl a* in 15 days old seedlings under 0, 25, 50, 75mM NaCl salinity stress. Similarly, they have recorded *Chl b* content as 122.90µg/gfw, 114.38µg/gfw, 102.70µg/gfw and 92.46µg/gfw in 7 days old seedlings and 359.51µg/gfw, 312.45µg/gfw, 252.99µg/gfw and 154.18µg/gfw in 15 days old seedlings that were germinated under 0, 25, 50 and 75mM NaCl (Table 5). While the amount of carotenoids was 89.52µg/gfw and 92.70µg/gfw (0mM), 70.04µg/gfw and 69.66µg/gfw (25mM), 61.97µg/gfw and 67.62µg/gfw (50mM) and 54.77µg/gfw and 76.59µg/gfw (75mM) in 7 and 15 day seedlings respectively (Table 5).

Reduction in plant growth under salt stress and salt toxicity conditions is associated with the reduction in photosynthesis (Gama et al., 2007). The photosynthetic pigment content in plants has been considered as one of the parameter for salt tolerance in crop plants (Hernandez et al 1995, Mitsuya et al 2002, Hasan et al 2005).

Reduction in chlorophyll content at higher concentrations of salt was commonly observed in *Brassica juncea* (Iqbal *et al.,* 2006), Mulberry (Jamil *et al.,* 2012) *Aegiceros cornicuatum* (Parida and Das, 2005) also in *Vigna ungiculata* (Oyetuni and Imade, 2014), soybean (Han and Lee, 2005). However, the presence of rhizobacteria has shown increase in chlorophyll and proline contents in faba bean plants when subjected to salt stress (Metwali *et al.,* 2015). In the present study, the overall the photosynthetic pigments Chlorophyll a, b and carotenoids content in 7 and 15 days seedlings showed significantly progressive decline with increasing concentration of NaCl. Rhizobacterium strains BR2 and BR3 coculture with seed has shown clear increase in *Chl a, b total chl* and carotenoids in 7 days and in 15 days seedlings. Overall BR3 strain showed highest amount of chlorophyll content in comparison with BR2 strain of rhizobacteria and also without rhizobacterial inoculation under different concentrations of NaCl salinity stress (Table 5).

In conclusion, the application of Bradyrhizobacterial (BR) strains on Cowpea seeds-Pusa Sukomal has enhanced the saline toxicity tolerance. The BR3 strain has more effectively reduced the harmful effects of salt stress, in comparison with control and BR2. The Pusa Sukomal with BR3 combination was found to be better than other combinations used. Selection of plant variety and Rhizobacterium strains (Salt tolerant) and cultivation of Cowpea with prior inoculation of Rhizobacterial culture on seeds in saline conditions may improve seed growth in saline conditions.

Photosynthetic pigments content(in µg/gfw)						
NaCl concention	**Chl a**		**Chl b**		**Carotinoides**	
Control	7^{th}	15^{th}	7^{th}	15^{th}	7^{th}	7^{th}
0mM	192.330±0.530	313.095±0.308	89.272±0.280	281.018±10.536	56.062±0.373	56.062±0.373
25mM	155.030±0.843	275.937±0.750	68.459±0.126	219.770±10.499	51.330±0.640	51.330±0.640
50mM	143.602±0.502	248.344±0.277	52.355±0.308	198.567±8.847	35.544±0.300	35.544±0.300
75mM	80.603±0.668	199.926±0.757	55.655±0.322	127.670±8.422	24.723±0.529	24.723±0.529
With Rhizobacteria BR2						
0mM	241.438±0.328	354.859±0.413	116.422±1.849	250.984±15.306	72.335±0.276	79.722±2.691
25mM	178.312±0.721	297.960±0.728	93.468±0.489	147.968±14.944	50.108±0.032	68.404±3.369
50mM	131.898±1.822	275.695±0.531	89.371±0.733	190.018±12.578	35.393±0.047	53.608±2.371
75mM	129.840±0.976	252.495±00.282	95.674±0.375	130.959±13.044	33.231±0.154	38.657±2.444
With Rhizobacteria BR3						
0mM	237.289±3.694	421.994±0.422	122.908±2.706	359.519±13.811	89.521±0.494	92.701±2.525
25mM	229.176±0.151	392.701±0.322	114.388±1.679	312.454±14.801	70.044±0.556	69.661±2.495
50mM	216.793±0.415	348.330±1.039	102.708±0.992	252.992±15.044	61.979±0.521	67.627±2.549
75mM	213.141±0.089	324.543±0.444	92.464±1.546	154.183±16.911	54.773±0.276	76.595±3.232

Table-5: Effect of NaCl salinity on the chlorophyll (*Chla, Chlb, tChl* and *Caro*) content of Pusa Sukomal seedlings with or without Rhizobacterium BR2 &BR3 inoculation in 7 and 15 days.

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ANALYSIS OF PHYTOCHEMICAL AND *IN VITRO* ANTIMICROBIAL ACTIVITY OF *BUTEA MONOSPERMA* AQUEOUS AND ALCOHOLIC LEAF EXTRACTS AGAINST FIVE BACTERIAL AND TWO FUNGAL STRAINS

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ABSTRACT
Medicinal plants have enormous ability to synthesize wide variety of secondary metabolites with antimicrobial activity. Butea monosperma Lam., also known as palash, is a tree belongs to the family fabaceae. Qualitative analysis of alcoholic and aqueous extract of B. monosperma leaves for phytochemicals has revealed that the ethanol extract possessed alkaloids, flavonoids, tannins, saponins, carbohydrates, glycosides and anthroquinones. While the methanol extract had flavonoides, tannins, saponins, carbohydrates, phytosteroles, glycosides and protein.. Phytochemicals alkaloids, flavonoides, tannins, saponins, carbohydrates and anthroquinones were present in the aqueous extract.

In vitro studies of antimicrobial property of B. monosperma leaf ethanol, methanol and aqueous extract against gram +ve bacterial strains L. acidophilus, B. subtilis and S. aureus and gram –ve bacterial strains S. typhi and E. coli and two fungal strains C. albicans and A. niger were carried out. The antimicrobial property was analyzed at three concentrations i.e. 50, 100 and 200µg. The leaf methanol extract has alone shown antimicrobial activity against all gram +ve bacteria and fungal strains. S. typhi was sensitive for methanolic and aqueous extracts. The E. coli strain was found to be highly insensitive to the leaf extracts and B. subtilis was highly sensitive to Butea leaf extract.

Keywords: B. monosperma, antimicrobial activity, phytochemicals, leaf extract

INTRODUCTION
B. monosperma, commonly known as palash, has been extensively used in Ayurveda, Unani, Homeopathic medicine and in folk medicines as astringent, tonic, diuretics leprosy, gout and skin diseases (India Meteria Medica 2002). It is reported to possess pharmacological properties like antimicrobial (Lohitha *et al.*, 2010), antifertility, antihemintic, antidiarrhoeal, wound healing, antitumor (Khan *et al.*, 2009), antidiabetic (Akhtar *et al.*, 2010), anti-inflammatory (Shahavi *et al*., 2008), hepatoprotective (Wagner *et al*., 1986), antiulser and antistress activity in their different parts extracts.

Presence of natural compounds in plant makes medicinal plants very effectible against general disease as they show antimicrobial properties. That's why medicinal plants play important role in therapeutic treatment (Iwu *et al.*, 1999). Plant, thus, are source of new chemical compounds, phytochemicals, including vitamins, carotenoids, flavonoids, Polyphenols, peptides, minerals, tannins, saponins, pigments, carbohydrates (Madhuri and Pandey, 2009). Chemical constituents that present in the leaf of *Butea* extracts have been reported (Kasture *et al.*, 2000). The leaves are useful in diabetes and also used to treat leucorrhea (Somani *et al.*, 2006; Rmanjaneyulu *et al.*, 2011). Limited reports are available (Lin *et al.*, 1999 and Shailendra, 2008) on the antimicrobial property of *B. monosperma* leaf extracts. In the present study we are reporting the qualitative phytochemical analysis and *in vitro* antimicrobial activity of *B. monosperma* leaf alcoholic and aqueous extracts.

MATERIAL AND METHODS
Leaf material of *B. monosperma was* collected in bulk from different regions of Gwalior between years 2012 - 2014 and processed according to the procedure earlier given by us (Pratibha *et al.*, 2018). The leaf alcoholic and aqueous extracts thus obtained were utilized for the phytochemical screening. The qualitative chemical composition of these extracts were done using commonly employed precipitation and coloration procedure given by Trease and Evans (1989); Harbone (1998); Sofowora (1993). The *in vitro* antimicrobial property of the extracts was studied at 50, 100 and 200 µg concentration by *in vitro* sensitivity test using Agar well diffusion method. The zone of inhibition was recorded to the nearest size in mm.

The antimicrobial property of leaf extracts was analyzed and compared with the standard antibiotics (Hexa g+7 comb- Amphicillin, Cephalothin, Clindamycin, Erythromycin, Oxacillin and Vancomycin) and antifungal drugs (Fluconazole, Itraconazole, Metroconazole, Ketoconazole including streptomycin.

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Five bacterial strains : three gram +ve - *L. acidophilus* (MTCC 10307), *B. subtilis* (MTCC 441) and *S. aureus* (MTCC 3160) ; two gram –ve - *S. typhi* (MTCC 3224) and *E. coli* (1610) and two fungal strains *C. albicans* (MTCC 3017) and *A. niger* (MTCC 478) were used for the present work. All the bacterial cultures were maintained on Nutrient Agar medium and fungal cultures were maintained on Potato Dextrose Agar medium.

RESULT AND DISCUSSION

Qualitative phytochemical analysis of ethanol, methanol and aqueous extract of *B. monosperma* leaves, data is present in Table 1, revealed that the ethanol extract possessed alkaloids, flavonoids, tannins, saponins, carbohydrates, glycosides and anthroquinones. While phytosterols, protein and reducing sugars were absent. While methanol extract recorded presence of flavonoids, tannins, saponins, carbohydrates, phytosterols, glycosides and protein and gave negative results for alkaloids, reducing sugars and anthroquinones testes indicating their absence. Phytochemical tests for alkaloids, flavonoids, tannins, saponins, carbohydrates and anthroquinones have given positive results indicating their presence in aqueous extract. Whereas protein, phytosterols, glycosides and reducing sugars tests were negative, indicating their absence in the aqueous extract

(Table 1)

S. No.	Phytochemical Name	Leaf		
		Ethanol Extract	Methanol Extract	Aqueous Extract
1	Alkaloids	+	-	+
2	Flavonoids	+	+	+
3	Tannins	+	+	+
4	Saponins	+	+	+
5	Carbohydrates	+	+	+
6	Phytosterols	-	+	-
7	Glycosides	+	+	-
8	Protein	-	+	-
9	Reducing sugars	-	-	-
10	Anthroquinones	+	-	+

(+) present; (-)absent

Table 1: Qualitative profile of phytochemicals present in *B. monosperma* leaf ethanol, methanol and aqueous extracts.

Antibiotics	Concentration	Inhibition zone size (Diameter in mm ± SD)									
		L. acidophilus		*B. subtilis*		*S. aureus*		*S. typhi*		*E. coli*	
Amp	10mcg	30.4±1.21	++++	0±0.0	-	0±0.0		11±3.08	+++	0±0.0	-
Cep	30mcg	24.6±1.86	++++	26±3.11	++++	9.2±0.37	+++	14.4±0.68	++++	14±0.55	++++
CD	2mcg	21.4±0.93	++++	16.8±1.39	++++	0±0.0	-	7±0.32	+	10.4±0.51	+++
E	15mcg	23.6±1.12	++++	24.4±0.51	++++	0±0.0	-	12.4±0.51	++++	11.4±0.24	++++
OX	1mcg	21.8±1.71	++++	13±0.84	++++	0±0.0	-	0±0.0	-	0±0.0	-
Va	30mcg	17±1.87	++++	17±1.67	++++	0±0.0	-	0±0.0	-	5.4±0.40	+

Sensitivity levels- : +=5.0 to 7.0 mm; ++ = 7.1 to 9.0; +++ =9.1 to 11.0, ++++=11.1 above.

Table-2: *In vitro* Sensitivity (inhibition zone size in mm) of five bacterial cultures to standard antibiotics present in Hexa g+7 (Hi media) antibiotic comb.

The inhibition zone (IZ) size obtained from *in vitro* sensitivity response of all bacterial strains to standard antibiotics present in Himedia multi comb hexa g+7, is given in the Table 2. The results clearly have shown that *L. acidophilus* is very highly (++++) sensitive to all the antibiotics tested. The diameter of inhibition zone varied from 17.0±1.87 to 30.4±1.21mm. Similarly *B.subtilis* also has recorded very high sensitivity (++++) against all the antibiotics used except the ampicillin (Table 2). The least inhibition zone was recorded as 13.0±0.84mm against OX and maximum inhibition zone of 26±3.11mm recorded against Cep. *S. aureus* has shown sensitivity to Cep alone with 9.2±0.37mm zone of inhibition. Both *S. typhi* and *E. coli* have recorded sensitivity against four out of the six antibiotics tested. *S. typhi* recorded very high (++++) sensitivity to Amp

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(11±3.08mm), Cep (14.4±0.68mm) and E (12.4±0.51mm) and *E. coli* for Cep (14±0.55mm) and E(11.4±0.24mm) (Table 2).

Similarly the *in vitro* sensitivity of two fungal strains to five antifungal drugs was evaluated and the data on zone of inhibition (in mm) is given in Table 3 fungal strains *C. albicans* and *A. niger* were highly sensitive to Fluconazole with 18.2±0.58mm and 16.2±0.37mm IZ and metroconazole (6.4±0.51mm and 5.4±0.24mm IZ) . *C. albicans* alone has further shown sensitivity to Streptomycin (Table 3).

Concentration	Antifungal drugs	Inhibition zone size (Diameter in mm ± SD)			
		C. albicans		*A. niger*	
100µg	Streptomycin	10.2±3.80	+++	0±0.0	-
100µg	Fluconazole	18.2±0.58	++++	16.2±0.37	++++
100µg	Itraconazole	0±0.0	-	0±0.0	-
100µg	Ketoconazole	0±0.0	-	0±0.0	-
100µg	Metroconazole	6.4±0.51	+	5.4±0.24	+

Sensitivity levels- : +=5.0 to 7.0 mm; ++ = 7.1 to 9.0; +++ =9.1 to 11.0, ++++=11.1 above.

Table 3: *In vitro* Sensitivity of two fungal strains against five selected antifungal drugs

Strains name	Inhibition zone (IZ) size (in mm+-SD)								
	Ethanol extract con. (µg)			Methanol extract con. ((µg)			Aqueous extract con. ((µg)		
	50	100	200	50	100	200	50	100	200
L. acidophilus	0±0.0	0±0.0	0±0.0	6.4±0.89	9.4±0.89	11.8±0.45	0±0.0	0±0.0	0±0.0
	-	-	-	+	+++	++++	-	-	-
B. subtilis	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	13±0.71	0±0.0	0±0.0	0±0.0
	-	-	-	-	-	++++	-	-	-
S.aureus	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	10.4±1.52	0±0.0	0±0.0	0±0.0
	-	-	-	-	-	+++	-	-	-
S. typhi	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	9.6±2.19	0±0.0	0±0.0	10.2±0.84
	-	-	-	-	-	+++	-	-	+++
E. coli	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
	-	-	-	-	-	-	-	-	-
C. albicans	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	6.0±0.71	0±0.0	0±0.0	0±0.0
	-	-	-	-	-	+	-	-	-
A. niger	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	8.4±0.89	0±0.0	0±0.0	0±0.0
	-	-	-	-	-	-	-	-	-

Sensitivity levels- : + = 5.0 to 7.0 mm; ++ = 7.1 to 9.0; +++ = 9.1 to 11.0; ++++ = 11.1 above.

Table 4: *In vitro* antimicrobial activity of ethanol, methanol and aqueous extracts of *B. monosperma* against bacterial and fungal strains

Results obtained from antimicrobial activity studies against five bacterial and two fungal strains are presented in Table 4, clearly have shown that only methanolic extract of leaf has restricted the growth of six out of the seven strains tested at 200µg concentration level (Table 4).

Methanolic extracts, leaf developed zone of inhibition against *L. acidophilus, B. subtilis, S. aureus, S. typhi, C. albicans* and *A. niger*. Leaf extract at 50, 100 and 200µg concentrations developed 6.4±0.89, 9.4±0.89 and

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11.8±0.45mm size IZ *in vitro* cultures of against *L. acidophilus*. *B. subtilis* recorded 13±0.71mm IZ while *S. aureus* showed 10.4±1.52mm size of IZ. Culture of *S. typhi* was inhibited up to 9.6±2.19mm diameter. The fungal cultures of *C. albicans* and *A. niger* demonstrated clear zone of 6.0±0.71mm and 8.4±0.89 mm size (Table 4).

Ethanolic extract has completely failed to inhibit any of the microbial growth. While the aqueous extract was effective only against *S. typhi* with 10.2±0.84mm at 200µg concentration. Further, *E. coli* has demonstrated insensitivity to all the three extracts at all the three concentrations tested (Table4)

Comparison of *in vitro* antimicrobial activity of leaf extracts of *B. monosperma* with standard antibiotics and antifungal drugs reveals that Most of the microbial cultures were highly sensitive to standard antibiotics than the leaf extracts. *E. coli* was highly resistant to leaf extracts.

CONCLUSION

The alcoholic and aqueous extracts of *B. monosperma* plant leaf was used for phytochemical analysis and antimicrobial activity against bacterial and fungal strains. The methanolic extract of *Butea* leaf has has shown high antimicrobial activity and while aqueous extract as limited effect. *B. subtilis* is highly sensitive to extract followed by *L. acidophilus, S. aureus, S. typhi* and fungal strains *A. niger* and *C.albicans*. *E. coli* was highly resistant. Further work, however, needs to be careered out on effect of concentrations and role of each phytochemical and its concentration on its antimicrobial property.

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- **Text Book:**

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