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RESEARCH TRENDS IN PLANT SCIENCE

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PREFACE

Plants have always held a profound significance for humanity, not only as a vital source of sustenance but also as intricate living organisms that play a crucial role in maintaining the balance of our ecosystems. As we navigate the challenges of a rapidly changing world, understanding the complexities of plant life becomes increasingly crucial for our sustainable existence and the well-being of our planet.

The journey of plant science has been an incredible voyage of exploration, spanning centuries of curious minds delving into the mysteries of plants and their intricate mechanisms. From the pioneering studies of Gregor Mendel, the father of modern genetics, to the groundbreaking work on photosynthesis by Melvin Calvin, plant scientists have continuously unveiled the marvels hidden within the green world.

Today, we find ourselves in the midst of an unparalleled era of scientific discovery and technological advancement. With the advent of genomics, proteomics, metabolomics, and other high-throughput technologies, we have gained unprecedented insights into the inner workings of plants at the molecular level. The integration of computational approaches, artificial intelligence, and machine learning has further revolutionized the way we analyze and interpret complex plant data, unlocking new avenues for exploration and innovation.

In this book, we have endeavored to bring together a diverse collection of chapters that showcase the multidisciplinary nature of plant science research. From fundamental aspects of plant physiology and molecular biology to the fascinating world of plant-microbe interactions, from the challenges of climate change and agriculture to the potential of plant biotechnology, each chapter offers a glimpse into the forefront of scientific inquiry.

We are immensely grateful to the eminent researchers, scholars, and experts who have contributed their insights to this volume, sharing their passion and knowledge to inspire the next generation of plant scientists. Their collective efforts have resulted in a treasure trove of information that will undoubtedly spark curiosity, provoke thought, and encourage dialogue within the scientific community and beyond.

As we delve into the pages of "Research Trends in Plant Science," we hope that readers will be captivated by the captivating stories of scientific exploration, the compelling discoveries, and the transformative potential of plant research. We envision this book as a valuable resource for students, educators, researchers, policymakers, and all those who share a fascination for the wonders of the plant kingdom.

Editors

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PLANT Β-1,3-GLUCANASES: BIOLOGICAL ROLE AND TRANSGENIC EXPRESSION AGAINST PHYTO-PATHOGENIC FUNGI TO ENHANCE DISEASE RESISTANCE

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Abstract:

Numerous defence mechanisms have evolved in crop plants to combat biotic and abiotic stresses. Plants express a large number of pathogenesis-related (PR) proteins in response to pathogen invasion. Plants contain many 1,3-glucanases, which have been characterised from a variety of species. They are essential for cell division, material transport through plasmodesmata, resistance to abiotic stresses, and flower formation through seed maturation. The ability to isolate and characterise the genes encoding such proteins, including chitinases, has been made possible by developments in recombinant DNA technology and our understanding of plant-microbe interactions at the molecular level. Additionally, crop plants have successfully overexpressed genes for 1,3-glucanase derived from plants to fight off fungus pathogens. It has been successful to engineer crops like tomato, potato, maize, groundnut, mustard, finger millet, cotton, lychee, banana, grape, wheat, and rice to be resistant to fungi, either using -1,3-glucanase alone or in conjunction with other PR proteins. They belong to the family of pathogenesis-related (PR) proteins known as PR-2. A feasible method to build long-lasting resistance in crop plants against fungal pathogens is to use 1,3-glucanase genes as transgenes in conjunction with other antifungal genes. These genes, which came from alfalfa, barley, soybean, tobacco, and wheat, have been co-expressed in a variety of crop plants with promising results. These results include chitinases, peroxidases, thaumatin-like proteins, and -1-purothionin, in various crop plants that are discussed in this review.

Introduction:

In order to detect microbial infections and respond appropriately by triggering the necessary defence responses, plants have developed a number of mechanisms. Pathogens are initially repelled by formed primary barriers, such as constitutively expressed waxes, cell walls reinforced with callose, lignin, and suberin, antimicrobial peptides, proteins, and non proteinaceous secondary metabolites. From the observation that plants are vulnerable to a lack of secondary metabolite production or to pathogens' ability to degrade these metabolites, the significance of such primary barriers has been understood (Osbourn 2001). Once these defences are breached, pathogen elicitors trigger a series of protective reactions in the plant cell, including cell wall rigidification and increased callose, lignin, and suberin deposition, as well as cell death that is accelerated (hypersensitive reaction), production of reactive oxygen species (ROS), phytoalexins, phenolic compounds, pathogenesis-related (PR) proteins and many other defence related proteins (Hematy *et al.,* 2009). These protective reactions are collectively known as ''defence responses'' of higher plants. These inducible or constitutive defence mechanisms of higher plants are fairly conserved among various plant species during evolution. Therefore, irrespective of their morphological differences most plants synthesize or build up structurally and functionally similar protective proteins under certain situations (de Jonge *et al.,* 2011).

Rigidification is one of the plant cell's defence responses. The host plant produces several classes of novel proteins called PR-proteins in response to pathogens like bacteria, fungi, viruses, and viroids. Additionally, the use of chemicals like plant hormones like ethylene (ET), jasmonic acid (JA), and salicylic acid (SA) that mimic the effects of pathogen infection or cause comparable stresses results in the induction of these PR-proteins (Sels *et al.,* 2008). The first PR-proteins were discovered in tobacco leaf extracts that were hypersensitive to the tobacco mosaic virus (TMV) (van Loon and van Kammen, 1970). Numerous species of monocotyledonous and dicotyledonous plants have proteins that resemble tobacco PR-proteins in terms of immunology and amino acid sequence. based on serologic or immunologic relationships, similar sequences, and enzymatic properties, all the recognized PR-proteins from plants are classified into 17 families (Leubner-Metzger and Meins, 1999). This review describes the recent advances in understanding b-1,3-glucanase enzymes, members of the PR-2 family, with respect to their functions in growth and development of plants, antimicrobial potential and their utility in genetic engineering for enhancing disease resistance in crop plants. plays a role in the development, growth, and reproduction of plants Overwintering In the skin of red table grapes, the function of a class I b-1,3-glucanase gene (Vcgns1) as a cryoprotectant was investigated (*Vitis vinifera cv. Cardinal*). After three days of storage at 0° C without treatment, Vcgns1 mRNA levels in the skin of untreated grapes increased, pointing to their potential role in cryoprotection. Reiterating their role in preventing low temperature conditions in red table grapes, purified VcGNS1 protein from recombinant expression displayed high stability at 0C and in vitro assay demonstrated a cryoprotective activity for the freeze labile l-lactate dehydrogenase (LDH) enzyme (Romero *et al.,* 2008).

In winter rye (*Secale cereale* L.), b-1,3-glucanases along with b-(1,3)(1,4)-glucanases are expressed at freezing temperatures and limit the growth in ice crystals in the apoplast by binding to the surface of ice crystals. Their limited hydrolytic activity at freezing temperature also gives plants protection against psychrophilic pathogens. In addition to this, they also modify carbohydrates resulting in osmoprotection and osmotic inhibition of ice crystal formation in the apoplast. Two cDNAs, ScGLU-2, ScGLU-3 coding for b-1,3-glucanase were prepared from mRNA recovered from cold-acclimated winter rye. These cDNAs were cloned in pGEX-KG vector and overexpressed in *E. coli* BL21(DE3). In vitro assays with purified recombinant glucanases showed antifreeze activities and partial hydrolytic activity at subzero temperatures (4^{o}C) highlighting their role in cold tolerance (Yaish *et al.,* 2006). By interacting with the cis acting element DRE/CRT, the dehydrationresponsive element-binding protein 1 s (DREB1 s)/Crepeat-binding factors (CBFs) of *Arabidopsis* regulate the expression of numerous stress-related genes (Sakuma *et al.*, 2002). The rice OsDREB1A and OsDREB1B genes that are induced by cold stress are discovered to be DREB1 orthologs. B-1,3-glucanases and a variety of other proteins, including dehydrins, a-amylase, ACC oxidase, cytochrome P450, receptor-like protein kinase, ATPase family, glycine-rich protein, protease inhibitor, and an unidentified protein, were expressed more abundantly in transgenic rice (*Oryza sativa cv. Kita-ake*) (Ito *et al.*, 2006). Pollination and male gametophyte development Osg1, a gene that encodes a b-1,3-glucanase, was expressed throughout the entire rice plant (*O. sativa L.*), with higher levels in the florets, leaf sheaths, and leaf blades. The late meiosis, early microspore, and middle microspore stages of the florets were when Osg1 was expressed most prominently, according to real-time PCR, immunocytochemical analysis, and reporter gene (GUS) expression driven by the Osg1 promoter. Due to the disruption of callose degradation in the anther locules at the early microspore stage, male sterility was caused when the Osg1 gene was silenced by RNA interference in transgenic rice (Wan *et al.*, 2010).

Different expression groups for the Arabidopsis b-1,3-glucanase genes have been identified using microarray expression and phylogenetic analyses. Group H and K b-1,3-glucanase genes have flower/reproductive-specific functions. Two "other specific genes", At4g14080 and At3g23770, as well as three additional weakly expressed genes make up the expression group K. These additional distinct genes are in charge of breaking down the callose covering the tetrad, releasing the individual microspores into an additional locule. AtPTEN, the tyrosine phosphatase gene, was discovered to coexpress with two pollen/stamen-specific genes (At5g20390 and At5g64790), which are essential for pollen development and maturation (Doxey *et al.*, 2007). Both plants' germination was inhibited when pollen grains of *Solanum chacoense* and *Lilium orientalis* were exposed to high concentrations of lyticase (which specifically hydrolyzes callose), with the former exhibiting bursting. Additional research on the pollen cell wall components of *L. orientalis* revealed that moderate amounts of the b-1,3-glucanase enzyme stimulated pollen grain germination while high concentrations (3 mg/ml) caused bursting. When cellulase and lyticase were added separately, this outcome was not seen, indicating that both callose and cellulose at the pollen aperture prevent the bursting of pollen grains and must be hydrolyzed for germination (Parre and Geitmann, 2005).

From the cell walls of *L. longiflorum* pollen tubes, two full-length exo-b-glucanases, LP-ExoI and LP-ExoII, were isolated as cDNAs. According to molecular analysis, the DNA and amino acid compositions of LP-ExoI and LP-ExoII are about 80% similar. All tissues examined contained LP-ExoII, but LP-ExoI was only in high concentrations in pollen grains and tubes. Both enzymes were able to act on different substrates, including b-1,4-glucans, b-1,3:1,4-glucans, and b-1,6-glucans, in addition to b-1,3-glucans. Both of these were created and kept in developed pollen grains. These two genes' expression increased during pollen elongation, and their mRNA transcript abundance never decreased after pollen germination. The growth of pollen tubes was significantly inhibited by the glucoside inhibitors D-gluconolactone and nojirimycin, suggesting that glucanases' hydrolysis of glucans is necessary for lily pollen tube growth. The pollen tube tip's primary wall degradation (which facilitates tip expansion), callose degradation (which prevents tip clogging), and opening the callosic wall for pollen tube-style communication are the mechanisms proposed for the action of these exo-b-glucanases (Li *et al.*, 2001; Takeda *et al.*, 2004). In pET28A, a cDNA, Tag1 corresponding to a tobacco b-1,3-, glucanase was cloned and overexpressed in *E. coli BL21*(DE3. The activity of b-1,3- glucanase in pollen development was confirmed by antibodies raised against the Tag1 recombinant protein, which showed a 33 kDa protein accumulating only in anthers at the tetrad and free microspore stages (Bucciaglia *et al.*, 2003). development and germination of seeds ZmGlucA, a class A b-1,3-glucanase gene, was discovered in maize seeds by Branco *et al.*, (2011). ZmGlucA was exclusively expressed in seeds and its regulation during seed development, according to mRNA and protein analysis. The strong expression of ZmGlucA in the seeds of mutant dek 827Kpro1, defective in embryo and endosperm development, supports the idea that b-1,3-glucanase is required for seed development. Arabidopsis b-1,3-glucanase genes with flower-specific functions, such as At2g39640, At3g57240, At5g18220, At5g20390, and At5g64790, were studied for expression and microarray results ripening (Doxey *et al.*, 2007). Numerous solanaceous plants, such as tobacco (*Nicotiana tabacum L.*), have coat-imposed seed dormancy due to the obstacles the testa and endosperm present. In the air-dry, minimally hydrated tobacco seeds, b-1,3-glucanase I (bGlu I) was found to express itself briefly and at a low level. RT-PCR, immunoblot analyses, reporter gene assays, and enzyme activity assays all revealed that bGlu I is expressed in the inner testa during after-ripening. The expression of this glucanase during after-ripening promotes testicular rupture in transgenic tobacco seeds modified with sense and antisense forms of bGlu I, assisting in the release of coat-imposed seed dormancy (LeubnerMetzger, 2005). Class I b-1,3-glucanase was stimulated in tobacco (*Nicotiana tabacum* L.) during germination, more specifically in the endosperm cap tissue just before radicle emergence. This b-1,3-glucanase weakens the endosperm by hydrolyzing the cell wall and encourages the radicle to protrude to complete germination (Leubner-Metzger, 2003). Strong expression of the b-1,3-glucanase cDNA, PsGNS2, was found in the seed coat and all four whorls of the pea (*Pisum sativumcv. Finale*) flower. When the embryo reached the late heart stage, it had an elevated expression in the seed coat that persisted until the mid-seed-filling stage. PsGNS2 was only detected in a strip of the inner parenchyma tissue of the seed coat that is specialised in starch metabolism, according to in situ hybridization. The seed coat and endosperm of the medicago truncatula plant that was transgenically expressed a reporter gene under the control of the 50-genomic region PsGNS2 also displayed the highest activity (Buchner *et al.*, 2002).

Fruit physiology

Goni *et al.*, (2010) reported in ripe cherimoya fruits, a constitutive chitinase (27 kDa) is expressed more frequently, and new acidic chitinases (26 kDa) and acidic b-1,3-glucanases (51 kDa) are induced (*Annona cherimola Mill*.). Similar to overripe fruit, a basic constitutive 76 kDa b-1,3-glucanase and a new basic 33 kDa chitinase were both induced. *Botrytis cinerea*, a necrotrophic fungal pathogen of grapes, was inhibited in vitro by extracts of these basic and acidic proteins. The Rasthali (AAB), Kanthali (AB), and Monthan (ABB) cultivars of banana were used in the analysis of mRNA and protein levels of b-1,3-glucanases during ripening. These three cultivars varied in the enzymatic activity of glucanase and the rate at which the fruit pulp softened. Banana pulp harvested at the peak of ripeness contained a full-length cDNA for b-1,3-gluc, which was cloned into the expression vector pQE30:UA. In E. coli M15, it was transformed and expressed (pREP-4). The purified glucanase's in vitro activity against Laminarin suggests that it plays a role in the ripening of banana fruit (Choudhury *et al.,* 2009). Numerous soluble skin proteins are expressed during ripening and exhibit distinct distributions at various stages, according to analysis of proteome maps obtained from the skin tissue of grapes. Defense proteins predominate at the berry's harvest stage, especially various chitinase and b-1,3-glucanase isoforms that aid in fruit ripening and pathogen defence (Wang *et al.,* 2009). Sanchez-Ballesta *et al.,* (2008) studied changes in the postharvest physiological problems of citrus (*Citrus sinensis L*.) fruits' CrglcQ mRNA accumulation, which encodes an acidic class III b-1,3-glucanase. The expression of CrglcQ mRNA is linked to chilling-induced damage that occurs during storage of fruits at a chilling temperature, according to the expression pattern in varieties susceptible to chilling injury and non-chilling peel pitting (2oC). Additionally, mechanical wounding and exogenous ethylene application at low temperatures up-regulated the CrGlcQ transcript, reducing the chilling injury.

Anti-pathogenic potential of b-1,3-glucanases

By hydrolyzing the cell walls of fungal pathogens, plant b-1,3-glucanases directly contribute to defence. They do this most frequently in conjunction with chitinase isozymes. B-1,3-glucanases directly attack fungal pathogens by degrading b-1,3/1,6-glucans, and chitinases attack the bond between the C1 and C4 of two consecutive N-acetylglucosamines of chitins in fungal cell walls, according to in vitro research. The pathogenic fungi's hyphal cell wall deterioration not only makes it vulnerable to cell lysis, but also to the effects of other elements of fungal defence responses (Mohammadi and Karr, 2002). Both of these PR-proteins can also trigger plant defence mechanisms by releasing b-1,3-glucan and chitin oligosaccharides, which act as elicitors. These elicitors are identified by plant surveillance systems, and they are then transmitted via signalling cascades, which results in the initiation of a variety of localised and systemic defensive responses (Somssich and Hahlbrock, 1998). When the black spot pathogen Alternaria brassicicola was introduced to the rocket salad plant (Eruca sativa Mill.), the resistant cultivar (RTM-2002) significantly outperformed the susceptible cultivar in terms of 33 kDa b-1,3-glucanase and 32 kDa chitinase (T-27). Although both cultivars showed an increase in these PR-proteins when compared to the control, RTM-2002 showed a faster rate of protein accumulation than T-27 (Gupta *et al.,* 2012). Salim *et al.,* (2011) investigated how the tomato (*Solanum lycopersicum* L.) landraces LE996, LE150, and LE1165, as well as its wild relative *S. hirsutum Vahl*. genotypes *SeijimaJeisei* and I979, responded defensively to infection by the early blight pathogen *Alternaria solani. Seijimajeisei*, I979, and LE150 are susceptible genotypes of these LE996, while LE1165 and LE996 are resistant genotypes. The early blight pathogen was challenged in vivo, and the results showed that the resistant genotypes produced three distinct antifungal proteins 6 h earlier than susceptible genotypes. In comparison to LE150, LE996 displayed higher levels of b-1,3-glucanase (71%) and chitinases (63%) expression. Other antifungal proteins, such as peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase, also displayed increased expression in LE996 (PAL). *Bipolarissorokiniana*, a spot-blotch pathogen, was introduced to susceptible and resistant wheat varieties (*Triticum aestivum L.*), and the expression of PR-protein genes was analysed using semi-quantitative RT-PCR. Within 24 hours of inoculation, the resistant genotype Chiriya-3 (PDI = 1.3) displayed higher levels of b-1,3-glucanase expression than the susceptible genotype Agra Local (PDI = 40.1). Also reported to be seven times as much co-expression of the actin gene 24 hours after inoculation (Aggarwal *et al.*, 2011). When the stripe rust fungus *Puccinia striiformis f. sp. tritici* infected wheat (*T. aestivum cv. Suwon 11*), TaGluse (PPO) and phenylalanine ammonia-lyase were induced (PAL). When compared to incompatible reactions, compatible reactions significantly increased the expression level of the basic b-1,3-glucanase that TaGlu codes for, which has 334 amino acids. TaGlu was primarily found over the extrahaustorial matrix and in the host cell wall (Liu *et al.*, 2009). Numerous additional reports describe how B-1,3-glucanases, chitinases, and many other PR-proteins are all induced during pathogen infection.

Transgenic expression of *b*-1,3-glucanases

To combat plant pathogens in this era of environmentally friendly and sustainable agriculture, one tactic is to create transgenic varieties that are pathogen-resistant. Although this "way" to deal with plant pathogens isn't yet trustworthy, it is appealing and might be fruitful. Additionally, genetic engineering provides advantages over conventional breeding in that it allows for the sourcing of resistant genes from heterologous sources across the spectrum of living organisms (Desai *et al.*, 2010). Individual transgenic pea (*P. sativum* L.) plants were created by introducing the family 19 chitinase gene (Chit30) from *Streptomyces olivaceoviridis*, barley (*Hordeum vulgare* L.), and the gluc gene from barley. Transgenes were introgressed by crossing transformants to create transgenic hybrids. Numerous molecular level analyses supported the transgenes' stable integration, expression, and inheritance. Crude extract from the transgenic hybrid had inhibitory effects on the spore germination of *Trichoderma harzianum* and *Colletotrichum acutatum* as well as the hyphal growth of *B. cinerea* and *Ascochyta pisi*, according to in vitro tests (Amian *et al.*, 2011). *Arachis hypogaea* L., a transgenic plant, was created that overexpressed tobacco b-1,3-glucanases. When the transgenics were challenged with the leaf spot pathogen *Cercospora arachidicola*, they not only displayed a decrease in the number of spots but also a delay in the onset of the disease. Using *Aspergillus flavus* as a challenge resulted in slower hyphal spread and less aflatoxin buildup in seeds (Sundaresha *et al.*, 2010). By introducing transgenes for wheat a-1-purothionin, barley thaumatin-like protein 1, and barley b-1,3-glucanase, Mackintosh *et al.*, (2007) transformed the wheat cultivar *Bobwhite*. All of the transgenic plants displayed enhanced resistance to FHB when *Fusarium graminearum* was used to challenge these transgenic wheat plants in a greenhouse and in the field. In comparison to Bobwhite, a transgenic line carrying the b-1,3-glucanase gene displayed less severe FHB, lower levels of the mycotoxin deoxynivalenol (DON), and fewer visually scabby kernels (VSK). Many other transgenic crops have demonstrated resistance to phytopathogenic fungi by co-expressing plant b-1,3-glucanases and other antifungal proteins.

Conclusions:

Numerous scientific studies on plants have been published. We now know more about their structure, how their expression is regulated, and the numerous roles they play both directly and indirectly in plants thanks to b-1,3-glucanases. In different plant developmental and reproductive stages, such as microsporogenesis, pollen germination, pollen tube growth through the style, fruit development and ripening, seed development and germination, and somatic embryogenesis, b-1,3-glucanases are localised in various organs. Plants also express this protein group to withstand abiotic stresses. Regarding their expression during pathogen infection and their broad-spectrum antimicrobial activity, b-1,3- Glucanases are the subject of the most research. They also prevent the spread of viruses from cell to cell in plants by controlling the callose turnover at plasmodesmata. The management of destructive plant diseases might be aided by greater understanding of the molecular mechanisms underlying plant b-1,3-glucanases and improved recombinant DNA technologies. As defence transgenes, b-1,3-glucanase genes have the potential to help humanity practise agriculture in a sustainable and environmentally friendly manner.

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HORTICULTURE FARMING AND ISSUES

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Abstract:

Present paper provides a comprehensive review on the horticulture farming and issues for horticultural development in India. There's a critical need to strengthen exploration on horticultural crops to develop demand- driven technology through bettered cultivar diversity, pest controlled in both fields. The development of horticulture requires a minimum of introductory product factors, optimal crop operation structure, post-harvest structure, entrepreneurial operation and horticultural know- style, logistical structure and supporting fiscal structure. The development of the horticultural sector should go hand in hand with the growth of the agro-processing application.

Keywords: Horticulture, Crop management, entrepreneurial operation

Introduction:

The horticultural sector is an essential part of food and nutrition sanctuary in the country. Horticulture is the main section, while the colorful sub-segments include fruits, vegetables, sweet and herbal shops, flowers, spices and colony crops. All of this is considered an essential demand for profitable security. It is the art and wisdom of growing fruits, vegetables, flowers, and other shops for mortal consumption, on-food purposes, and social causes. India is home to multitudinous horticultural crops of marketable significance. Horticulture accounts for 30 of India's GDP and generates about37.1 of total agrarian goods exports. In 2017- 2018, the product of about306.8 million tons was estimated and participated over planting material, each- fed horticulture, lack of request openings, post-harvest operation, lack of processing installations and rightly professed labor force, etc. Agrarian product cast using space, The Government of India Agro meteorology and Land- Grounded compliances (FASAL) program aims to give multiple product vaticinators and pre-harvest crops at public/ state/ quarter position The different agro climatic conditions of India blessing the civilization of a wide variety of horticultural crops, including root and tuber crops, mushrooms, cosmetic shops and colony crops similar as coconut, areca nut, cashew and cocoa. The Government of India has accepted horticulture as a means of environmentally friendly diversification of husbandry through resourceful land use and ideal use of natural coffers. Horticulture attempts to produce multitudinous occupation chances, particularly for jobless youth and women. India has maintained its commanding position in the product of numerous inventories similar as mango, banana, sour lime, coconut, areca nut, cashew, gusto, turmeric and black pepper. It's presently the second largest fruit and vegetable patron in the world. India is alternate only to China in terms of area and product of vegetables, and ranks first in the world for the product of cauliflower, alternate for onions and third for cabbage. India has also made conspicuous progress in flower product. In addition, it's the largest patron, consumer and exporter of spices. In India there's a wide variety of spices similar as black pepper, cardamom (small and large), gusto, garlic, turmeric, chili and a wide variety of tree and seed spices. Nearly every state in the country grows one or further spices.

Horticulture farming in India

The development of horticulture wasn't a precedence in India until a many times ago. In the period from 1948 to 1980, the countries focus was on grain. No major planned trouble had been made for the development of horticulture piecemeal from some specialized support and development sweats for specific goods similar as spices, coconuts and potatoes. The times 1980-92 saw a connection of institutional support and a planned process of developing horticulture. In thepost-1993 period, particular attention was paid to the development of horticulture through an enhancement in plan allocation and knowledge- grounded technology. Although this decade is called the golden revolution in horticultural product, the productivity of horticultural crops has increased. Also, in 2005 – 2006, the National Horticulture Mission was established by the Government of India with the accreditation to promote integrated development in horticulture and to help coordinate, promote and maintain the product and processing of fruits and vegetables and make a solid structure in product, processing and marketing with a focus on post-harvest operation to reduce losses. This huge increase in horticultural yield has made India the second largest fruit and vegetable patron in the world after China. The periodic growth in area and product of fruit and vegetables in India was 2.6 percent and 3.6 percent over the period 1991-2005. This growth is relatively significant compared to the decline in grain realty and grain product. Indian horticultural sector faces severe constraints like low crop productivity, limited irrigation installations and underdeveloped structure support like cold stores, requests, roads, transportation installations etc. Significant post-harvest and processing losses do, performing in low productivity per unit area and high product costs. On the other hand, India's long growing season and different soil and climate conditions in several agro ecological regions give ample occasion for growing a variety of horticulture crops. Hence, efforts are required to capitalize on our strengths and remove obstacles to achieve the goal of impressive growth of horticulture in India. The 2004-2011 foreign trade policy emphasized the need to boost agricultural exports, growth and promotion of horticultural product exports.

After the Green Revolution in the mid-sixties, it came clear that horticulture, for which the Indian geomorphology and agrarian climate are well suited, was the stylish option. India has surfaced as the world's largest patron of mangoes, bananas and cashews and the second largest patron of fruits and vegetables. The most significant development of the last decade is that horticulture has shifted from pastoral areas to marketable product and this changing script has led to private sector investment in the operation of product systems. In the once decade, technological inventions similar as micro-irrigation, perfection husbandry, hothouse civilization and bettered post-harvest operation have impacted development, but colorful problems have surfaced along the way. India has a wide variety of climates and soils where a large number of horticultural crops similar as fruits, vegetables, potatoes, tropical tubers, mushrooms, cosmetic, medicinal and sweet shops, colony crops, spices, cashews, and cocoa and betel vine are grown.

After independence in 1947, the Indian government placed great emphasis on food tone-adequacy, especially grain. Still, after the Green Revolution of the 1960s, it came clear that horticulture, for which the Indian geomorphology and agrarian climate are well suited, was an ideal system of achieving small- scale sustainability. Still, it wasn't until themid-1980s that the Indian government honored the need for diversification to make husbandry more profitable through effective land use; Creation of good jobs for the pastoral population and for women and optimization of the use of natural coffers (soil, water and terrain). Sweats so far have proved satisfying in terms of increased product and productivity of horticultural crops. India has come the world's largest patron of coconut, areca, cashews and tea, and the second largest patron of fruits and vegetables. The changing script encourages private investment. As a result, horticulture has shifted from pastoral areas to marketable gambles that attract youthful people as it has proven intellectually satisfying and economically satisfying.

Issues of horticulture farming

Cultivation pattern in India

Agricultural diversification is an important tool for economic growth. Diversification largely depends on farmers' opportunities and responsiveness to technological breakthroughs, consumer demand, government policies, trade agreements, and the development of irrigation, roads and other infrastructure (Kumar and Mittal, 2003). Changes in patterns respond to these factors. The country's aggregate cropping patterns are represented by the distribution of gross acreage among different crops and commodity groups. India has experienced a significant level of crop diversification since the Green Revolution in terms of changes in the acreage of different crops, largely targeting food grains to achieve the country's goal of self-sufficiency and food security. Over the past decade, changes in production patterns have been more geared towards the horticultural sector and commercial crops such as cotton.

Management of pest and diseases

Rising pests and pathogens as well as adding fungicide resistance continue to pose major challenges for horticulture. Superficial damage to fresh vegetable and fruit products is inferior to consumers, but goes hand in hand with the desire not to have fungicide remainders in food. The adding global trade in factory material and fresh yield has increased the threat of introducing and establishing alien pests and pathogens in new surroundings. At the same time, growing regulations mean smaller conventional fungicides are available to farmers to control pests and conditions. Innovative styles are needed to control new and being pests and conditions. Responsible use of fungicides, for illustration through further targeted use, is essential to guarding the terrain while producing high- quality fresh vegetables and fruit. We need to develop new effective pesticide composites that are less patient in the terrain and have lower toxin to non-target organisms. New innovative semi chemical control styles are being developed for subsistence husbandry, similar as drive- pull strategies for pest control. The coming challenge is to extend analogous approaches to mechanized husbandry systems. There's growing interest in the use of salutary microbes and/ or the manipulation of the phytobiome for crop protection with a view to controlling specific pathogens or perfecting overall factory resistance/ forbearance. A number of challenges remain in the development.

Production of new varieties with the help of precious techniques

Utmost crop product and operation practices are acclimatized to specific genotypes. Furnishing new cultivars through rapid-fire parentage isn't only necessary to reduce crop operation sweats, but also to address challenges in meeting demand in the force chain (e.g. product under completely controlled environmental conditions) as well as changing consumer preferences in to manage with in terms of product quality. For numerous imperishable horticultural crops, the traditional parentage cycle is generally 815 times; for similar crops, specialized tools are urgently demanded to dock the length of breeding cycles (i.e., rapid-fire parentage). Ways for precise gene manipulation, similar as e.g. Targeted gene editing, can significantly increase breeding effectiveness, especially for traits that are genetically controlled by a many important genes similar resistance to pathogens. Still, nonsupervisory hurdles may still need to be overcome, as well as consumer resistance to accepting the use of similar gene-editing tools in horticultural crop parentage. Experimenters need to work with controllers and work with the public to demonstrate the safety of these technology tools.

Conclusions:

The development of husbandry in India requires some pivotal operation inputs, especially force chain operation – collaboration between different stakeholders as well as effective perpendicular and vertical integration. The horticultural sector in particular must give precedence to the development of exploration in genetics, biotechnology, integrated and sustainable product systems, post-harvest treatment, storehouse, and marketing and consumer education. Diversification offers a seductive option and an important source of driving growth in the agrarian sector. While technological advances and associated institutional changes are linked as precedence areas for unborn development of the horticultural sector, exports are seen as most important for the growth of the sector. India can look forward to getting a major patron of horticultural products, thereby securing decent request access for its agrarian exports, which depend largely on competitive technologies that will help boost import eventuality. This development will also contribute to the overall growth of the frugality by generating fresh foreign exchange, creating employment openings and also encouraging small and smallholder growers, which will easily have a positive impact on income and employment. Government should produce a positive terrain that ensures a mutually salutary relationship between growers and the organized sector. The diversification of horticultural crops should be promoted through the mixed civilization of horticultural and non-horticultural crops. This will affect in further food, further income, and better soil health.

In order to increase the product and productivity of fruits and vegetables, introducing vegetables into crop gyration and espousing recommended practices is veritably important. The use of vegetables in catch crops also helps to increase growers' income during the period when the estate isn't economically feasible. The horticultural sector diversification plan needs to identify implicit crops by area, and the realty of low- yield vegetables and fruits should be shifted to more productive and profitable areas.

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IMPORTANT MEDICINAL MUSHROOMS AND THEIR MEDICINAL USES

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Introduction:

Plants have traditionally been used as a source of most medical systems and as such herbal medicines constitute an important part of traditional and evidence based medicine worldwide. In contrast though, the broad medicinal use of mushrooms was for a very long time restricted to Asian countries. Nowadays, the medicinal use of mushrooms, so called, medicinal mushrooms', is increasing also in Western and other countries.

Medicinal mushrooms can be defined as macroscopic fungi, mostly higher Basidiomycetes, which are used in the form of extracts or powder for prevention, alleviation, or healing of diseases and/or for nutritional reasons. The former desirable property of mushrooms derives out of their rich contents of vitamins, trace elements, and minerals and appetite-enhancing flavours, but the therapeutic values result from its antioxidant and immunomodulatory activities, from its ability to activate very specific neuronal receptors, and from its capacity to rejuvenate cellular metabolism. Mushrooms contain many biologically dynamic compounds, some of which are neuroactive substances, free radical scavengers, anti-apoptotic factors, and nerve growth factor stimulators that exert positive effects on brain cells, all of which essentially qualify them as good neuro-nutraceuticals that help in the protection of different neural cells (Remya *et al.*, 2019)

Medicinal mushrooms include both non edible, poisonous mushrooms and edible mushrooms. Interventional strategies using edible mushrooms as functional foods and as preventive agents for age-related diseases, including neurodegenerative diseases, are gaining rapt recognition. Edible medicinal mushrooms have a wide variety of bioactive and nutritional components that could scavenge reactive oxygen species (ROS) and up regulate cellular antioxidant defence mechanisms. It is mainly by virtue of their strong antioxidant activity; mushrooms exert neuroprotective effects and promote neuritogenesis and neuroregeneration. It is shown that specific polysaccharides contained in mushrooms play an important medicinal role.

Mushrooms are well-known for its micronutrients, excessive vitamins, free radical scavengers, and potent antioxidants (Abdullah *et al.*, 2012; Aruoma *et al.*, 1999). Administration of either fresh, dried under shade, freeze-dried, or oven dried, fermented, or processed mushrooms contains a variety of phenolic compounds, free radical scavengers, ferric reducing substances, anti-carotene bleaching compounds, and a variety of polysaccharides (Wong *et al.*, 2009; Han *et al.*, 2013; Zhang *et al.*, 2012).

Important medicinal mushroom

The important medicinal mushroom species are *Ganoderma lucidum*, *Lentinula edodes*, *Cordyceps sinensis*, *Hericium erinaceus* and some others.

Lion's mane mushroom (*Hericium erinaceus*)

Hericium erinaceus is a well-known culinary and medicinal mushroom in Japan and China. *H. erinaceus*, also known as "Yamabushitake" in Japan, "Houtou" in China, or "Lion's mane" in Western countries, is a mushroom that typically grows on old or dead broadleaf trees (Mori *et al.*, 2008; Wang *et al.*, 2004). *H. erinaceus* is a mushroom that is commonly consumed for culinary purposes in countries like Japan and China, but it is also consumed as a supplementary medicine. It is widely studied for its beneficial effects on neuronal health. It possesses a range of therapeutic properties such as antioxidant, hypolipidemic, hemagglutinating, antimicrobial, anti-tumorigenic, and endoplasmic reticulum (ER) stress modulatory activities (Li *et al.*, 2014).

H. erinaceus is being investigated for its therapeutic potential as an inducer of neuronal differentiation and for its neuroprotective properties (Mori *et al.*, 2009). The extracts of *H. erinaceus* have also been used to study the mechanisms through which they contribute to the induction of nerve differentiation and promote neuronal survival. *H. erinaceus* has been found to significantly increase lipoxin A4, a protein known to have anti-inflammatory properties in most areas of the brain (Salinaro *et al.,* 2018). *Hericium erinaceus* is indicated in several CNS (anxiety, depression, multiple sclerosis, Parkinson's disease) and PNS (such as gastric ulcers, diabetes mellitus, cancer, and hyper lipidemia) disorders.

Interestingly, *H. erinaceus* has also been used to reduce obesity. In a controlled prospective clinical study, depression and anxiety were reduced, and the quality of sleep was found to be better in volunteers on a month's diet with *H. erinaceus* containing snacks (Nagano *et al.,* 2010). Erinacine A is the active constituent from mycelium of *Hericium erinaceus*. This information supports the notion that *H. erinaceus* benefits patients with neurodegenerative diseases as a functional food by reducing ER stress. *H. erinaceus* mycelium inhibits reactive oxygen species, oxidized and nitrated proteins, and lipid peroxidation.

Cordyceps (*Cordyceps sinensis*)

Cordyceps sinensis has been described as annual Ascomycetes fungus closely related to the mushroom occur in old Chinese medical books and Tibetan medicine. The name cordyceps comes from Latin words meaning club and head. The people of North Sikkim call the fungus/mushroom/herb *yarsa gumba*; its Tibetan name winter (yarsa) and summer (gumba). In the literature, "gunba" or "gonba" have also been used instead of "gumba." It is called *Keera Jhari* (insect herb) by the local Nepalese. It is also known as the Himalayan Viagra. It grows only in high-altitude regions of about 3800 m above sea level, in cold, grassy, alpine meadows of the Himalayan Mountains. The fungus is parasitic in nature. The base of the

mushroom first originates from an insect larval host (*Hepialis armoricanus* family Hepialidaceae) and ends at the club-like cap, including the *stipe* and *stroma*. The fruit body is yellow to pink, and the root of organism, the larval body pervaded by the mycelium, is yellowish to brown color (Kinjo and Zang 2001).The immature larvae (host) on which *cordyceps* grows usually lies about 6 inches below the surface of the ground. As the fungus approaches maturity, it consumes more than 90% of the infected insect effectively mummifying its host. The average weight of cordyceps is about 300–500 mg. It costs approximately 6.77 U.S. dollar per piece in the international market.

Initially local herders observed that yak, goat, sheep, etc. consuming *C. sinensis* during their grazing in the forest became very strong and stout. This observation paved the way for the discovery of its medicinal value. Thereafter, local people and herders used the fungus powder with jaggery to increase milk production, and improve reproductive capacity and vitality of their cattle. Then its relevant medicinal properties were explored, collecting only the aerial part (fruiting body/stroma), which they dried in sunlight as primary processing. Then they themselves consumed it and became convinced of its medicinal effects in enhancing vigor and vitality.

Traditional healers in Sikkim recommend the fungus/mushroom *Cordyceps sinensis* for "all illnesses" as a tonic; because they claim that it improves energy, appetite, stamina, libido, endurance, and sleeping patterns. *C. sinensis* effective against hepatoma, lung, breast, and stomach cancers (Youn *et al.,* 2009). The major chemical constituent is cordycepic acid with other amino acids, vitamins and minerals. It also use for the treatment of 21 ailments, including cancer, bronchial asthma, bronchitis, TB, diabetes, cough and cold, erectile dysfunction, BHP, jaundice, alcoholic hepatitis, etc., were noted. Various pharmacological and biological studies establishing the curative effect of corydeps involving various experimental models (*in vitro* and *in vivo*) and some clinical trials in volunteer athletes. *C. Sinensis* exhibits very broad biological and pharmacological actions in hepatic, renal, and cardiovascular diseases. It has effects on immunological disorders including cancer. Pharmacological actions of cordyceps are primarily due to bioactive polysaccharides, modified nucleosides, and cyclosporine like metabolites (Zhu *et al.,* 1998).

Ganoderma (Ganoderma lucidum)

It is a large, dark mushroom with a glossy exterior and a woody texture. The Latin word *lucidus* means "shiny" or "brilliant" and refers to the varnished appearance of the surface of the mushroom. In Chinese, the name lingzhi represents a combination of spiritual potency and essence of immortality, and is regarded as the "herb of spiritual potency," symbolizing success, well-being, divine power, and longevity whereas in Japan the name for the Ganodermataceae family is reishi or mannentake. Ganoderma is a genus of polypore fungi in the family Ganodermataceae that includes about 80 species, many from tropical regions. They have a high genetic diversity and are used in traditional Asian medicines. Ganoderma can be differentiated

from other polypores because they have a double-walled basidiospore. *Ganoderma* are wood-decaying fungi with a cosmopolitan distribution. They can grow on both coniferous and hardwood species. They are white-rot fungi with enzymes that allow them to break down wood components, such as lignin and cellulose.

But it is an oriental medicinal mushroom that has a long history of promoting health and longevity in countries like China, Japan, and other Asian countries (Wachtel-Galor *et al.*, 2011). A variety of commercial *G. lucidum* products are available in various forms, such as powders, dietary supplements, and tea. These are produced from different parts of the mushroom, including mycelia, spores, and fruit body. As a widely accepted medicinal mushroom, there are several research studies on the potential benefits *G. lucidum* for various cancers (breast and prostate) and neurodegenerative disorders, along with the molecular mechanisms of mentioned effects.

So far, *G. lucidum* has been noted for its neuroprotective properties. In addition, *Ganoderma lucidum* can serve as a complimentary adjunct medicine for the treatment of chronic fatigue syndrome and liver damage. Most studies used extracts of *G. lucidum* to investigate the molecular mechanism of their neuroprotective effects. In addition to exhibiting the beneficial effects of *G. lucidum*, some specific chemicals have been isolated from the mushroom to be studied for their potential use as a drug. Elemental analysis of log-cultivated fruit bodies of *G. lucidum* revealed phosphorus, silica, sulfur, potassium, calcium, and magnesium to be their main mineral components. Iron, sodium, zinc, copper, manganese, and strontium were also detected in lower amounts, as were the heavy metals lead, cadmium, and mercury (Chen *et al.*, 1998).

Lentinula edodes

The *Lentinula edodes* (shiitake) is an edible mushroom native to East Asia, which is now cultivated and consumed around the globe. It is considered a medicinal mushroom in some forms of traditional medicine. The fungus was first described scientifically as *Agaricus edodes* by Miles Joseph Berkeley in 1877. It was placed in the genus *Lentinula* by David Pegler in 1976. Shiitake grow in groups on the decaying wood of deciduous trees, particularly shii and other chinquapins, chestnut, oak, maple, beech, sweetgum, poplar, hornbea m, ironwood, and mulberry.

In a 100gram raw shiitake mushrooms provide 34 kilocalories of food energy, 6.8 grams carbohydrates, 2.2 grams protein, 0.5 gram fat, 2.5 grams fibres, 4 milligrams niacin, 0.2 milligrams vitamin B6, 0.2 milligrams riboflavin, 18 I.U. vitamin D, 0.2 milligrams manganese, 112 milligrams phosphorus, 5.7 micrograms selenium, 0.1 milligrams copper, 1 milligram zinc, 304 milligrams potassium, 20 milligrams magnesium, 0.4 milligrams iron and also contain moderate levels of some dietary minerals. Like all mushrooms, shiitakes produce vitamin D2 upon exposure of their internal ergo sterol to ultraviolet B (UVB) rays from sunlight or broadband UVB fluorescent tubes. The fungus has been used extensively in traditional Asian medicine and has a number of biologically active chemicals, such as the polysaccharide lentinan, that some studies sugget m ay prove beneficial against cancer & other diseases.

Shiitake mushrooms contain eritadenine, a compound known to reduce cholesterol levels in the blood. They also contain beta-glucans that reduce inflammation and help prevent the intestines from absorbing cholesterol. It is rich in polysaccharides like lentinans and other beta-glucans. These compounds protect against cell damage, help in immune system, and boost white blood cell production for fighting off microbes. Polysaccharides also have anti-inflammatory properties. The researchers concluded by suggesting that shiitake mushrooms can help prevent body weight gain, fat deposition and plasma triacylglycerols when added to a high-fat diet. Certain components of the shiitake mushroom have hypolipidaemic (fat-reducing) effects, such as eritadenine and b-glucan, a soluble dietary fiber that's also found in barley, rye and oats. Studies have reported that b-glucan can increase satiety, reduce food intake, delay nutrition absorption and reduce plasma lipid (fat) levels.

It is great source of B vitamins, which help support adrenal function and turn nutrients from food into useable energy. They have proven to help balance hormones naturally and break through brain fog to maintain focus all day long even improving cognitive performance. It contains 5.7 milligrams of selenium in hundred grams of shiitake mushrooms so act as a natural acne treatment because when selenium is taken with vitamins A and E, it can help reduce the severity of acne and the scarring that can occur afterward. Many studies indicate that the fibers found in shiitake mushrooms may support digestive and gut health. They are known to inhibit inflammation in the gut, which is critical for maintaining a healthy digestive system.

Some others medicinal mushrooms

Pleurotus giganteus

Pleurotus giganteus, or "Seri Pagi" in Malaysia, and "Zhudugu" in China, is a culinary mushroom that is gaining popularity for its organoleptic properties *P. giganteus* can reduce blood glucose, cholesterol, and blood pressure levels in hyperglycaemic patients. In addition to alleviating symptoms of diabetes mellitus, *P. giganteus* also has dietary and neurological benefits, for it has a high carbohydrate, fiber, potassium, and phenolic compounds and triterpenoids, making it a prime candidate to be developed as a nutraceutical for neurodegenerative diseases.

Dictyophora indusiata

Dictyophora indusiata, also known as veiled lady mushroom or bamboo mushroom, is both a culinary and medicinal mushroom found in Asian countries (Journal *et al.*, 2015). These mushrooms contain neuroprotective compounds, dictyoquinazols A, B, and C, that can reduce excitatory neurotoxin-induced cell death.

Inonotus obliquus

Inonotus obliquus, also known as "Chaga," is a medicinal mushroom known for its antitumor and diuretic properties that has also been studied for its benefits to neuronal health. Its antioxidant properties found that the pepsin extract from this mushroom exhibited a protective effect on H_2O_2 induced DNA damage and a reduction of ROS generation (Kim *et al.*, 2011).

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MUTATIONAL BREEDING IN NIGER CROP (*GUIZOTIA ABYSSINICA*): AN OILSEED CROP

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Abstract:

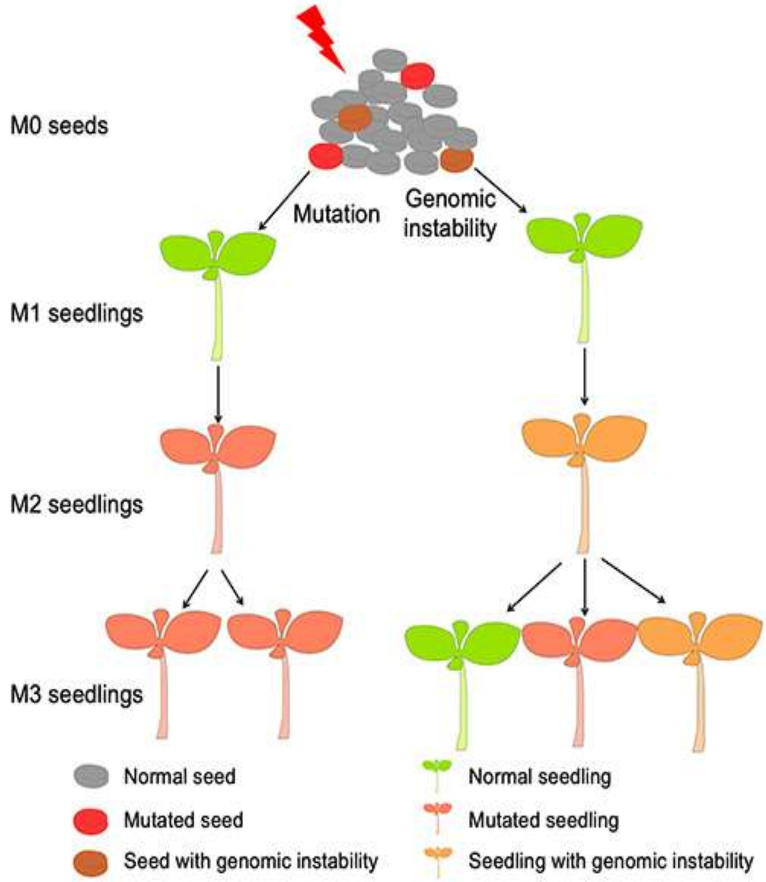
India grows a little amount of oilseed in Niger. It is grown in India and Ethiopia. The crop is grown on barren, marginal, and mountainous terrain. Farmers are growing regional variants of it since it has been neglected. It is a crop that is very self-incompatible and cross-pollinated. The capitulate are extremely tiny and difficult to hybridize. Therefore, the alternative to develop variants is mutation breeding. Mutations are produced by chemical and physical mutagens. A review of the mutant breeding in Niger was attempted. A relatively small amount of research was done on mutant breeding in the Niger. Mutants for high oleic acid and high oil output, enhanced oil content and early blooming, and an increase in seed weight per plant were all discovered. There have also been reports of further mutagenic effects of physical and chemical mutagens in Niger. Studying the meiotic characteristics of C1 plants was also done. Using mutation breeding, desirable mutant characteristics may be isolated. To increase seed output and oil content, this crop requires extra care.

Introduction:

Niger is one of the significant minor oilseed crops grown in Ethiopia and India (*Guizotia abyssinica* Cass). According to [1], it accounts for around 50% of Ethiopian and 3% of Indian oilseed output. It is grown in Ethiopia on flooded soils, where most crops and all other oilseeds are unable to thrive, and it makes a significant contribution to soil preservation and land rehabilitation. It is grown in India on marginal soils, in mountainous terrain, and by tribal people. However, this crop does best on deep, loamy soils with good texture and drainage. There are other names for it, including Ramtil or Kalatil in India and Noog in Ethiopia. The world's major Niger- growing nations include India, Ethiopia, East Africa, the West Indies, and Zimbabwe. Due to its growth on marginal areas with little inputs and the lack of available cultivars for a variety of agro-climatic situations, the output is extremely low and variable. Crop Niger has chromosomal number 2n = 30 and is cross pollinated. The family Compositae, tribe Heliantheae, and subtribe Coreopsidinae all contain the genus *Guizotia*. According to [2], the oil in the seed has a fatty acid composition of 75–80% linoleic, 7-8% palmitic and stearic, and 5-8% oleic acids. Since Niger has been deemed one of the "neglected and underutilized" crops [1], the scientific community has paid it little attention so far. Despite having enormous potential, it is not being improved by current breeding efforts. There are no causes of male sterility, and the crop is incompatible with itself. This results in real-world issues with crop improvement. Some of the main goals of niger breeding are producing male sterility, finding self-compatible lines, increasing seed output, oil content, and diversifying oil quality. Due to the difficulty of hybridization in Niger, it is necessary to breed differences through mutations in order to get beyond the practical issues outlined above.

About mutation breeding

Genetic variation is a prerequisite for selection and crop improvement. The variations that are found in nature do not represent the original spectra of spontaneous mutations. Rather, they are the result of genotypes recombining within populations and their continuous interaction with environmental factors [3]. Mutations are the primary source of all genetic variations existing in any organism, including plant [4]. The ensuing variance fuels evolution and serves as the starting point for natural selection. It is more challenging to exploit spontaneous mutations in plant breeding programs due to their rarity and unpredictable timing [5]. The original approach of plant breeding relied on the occurrence of spontaneous mutations and involved the straightforward selection of suitable offspring's.



Both the hybridization process and intentional mutations produce variations. If the gathered germplasm does not include any naturally occurring variants for the specific characteristic, then generating the variation is the next course of action. Mutation breeding refers to the trait-specific generation of mutant types via purposefully causing the mutations. Therefore, mutation breeding entails creating and using genetic heterogeneity by chemical and physical mutagenesis in order to create new types. Along with recombinant breeding and transgenic breeding, it is currently a cornerstone of contemporary plant breeding [6].

According to Roychowdhury and Tah [7], mutagenesis is the process by which an organism's genetic information undergoes abrupt heritable changes that are not brought on by genetic segregation or genetic recombination but rather are brought on by chemical, physical, or biological factors. The process of inducing mutations by exposure to chemical mutagens or radiation (such as gamma rays, X-rays, or ion beams). Hugo de Vries, who also created the term "mutation," initially discovered mutations as a process for producing variety in the late nineteenth century. Following the discovery of the mutagenic activity of X-rays proven in maize, barley, and wheat by Stadler [8], the area of radiation-induced mutations as a method for producing unique genetic variety in plants advanced.

Mutagens used for mutational breeding

Mutagens are substances that cause artificial mutations in living things. Chemical and physical mutagens are the two main categories into which they are often divided [9]. Planting materials are subjected to both physical and chemical mutagenic ants in order to cause mutations in crops. All types of planting materials can be exposed to mutagens, including complete plants, typically seedlings, various plant propagules such bulbs, tubers, corms, and rhizomes in vegetatively propagated plants, as well as in vitro cultivated cells. Planting materials are subjected to both physical and chemical mutagenic ants in order to cause mutations in crops All types of planting materials can be exposed to mutagens, including complete plants, typically seedlings, various plant propagules such bulbs, tubers, corms, and rhizomes in vegetatively propagated plants, as well as in vitro cultivated cells. Radiations such as alpha, beta, gamma, UV, X, neutrons, protons, and ion beams are physical mutagens. Ethyl methane sulphonate (EMS), methyl methane sulphonate, alkylating agents, base analogues, etc. are among the compounds that are considered chemical mutagens. Inducing polyploidy in plants with the use of the chemical mutagen colchicine also results in variances (mutations) in the plants. By interfering with the orientation and structure of the mitotic fibers and spindle fiber’s, this chemical is known to prevent mitosis in a variety of plant and animal cells. Since microtubules are responsible for chromosomal segregation, colchicine is used to prevent mitosis in order to cause polyploidy and mutations in plant cells. The effectiveness of mutagenesis is influenced by the mutagen concentration, the duration of the treatment, and the experiment's temperature. Therefore, the primary benefit of mutational breeding is the ability to enhance one or two quantitative features without altering the genotypes of the other characters.

Mutation breeding in Niger crop

An effort was made to review the mutation breeding in Niger. A very few efforts were made in Niger and explained as below. Mutants were isolated for high oleic acid and high oil yield [10] and improved oil content and early flowering [11] in Niger. Improvement in other characters was also reported in Niger. Mutagenic effects of chemical mutagens (Ethyl Methane

Sulfonate (EMS) and Methyl Methane Sulfonate (MMS) were studied by Maloo and Agrawal [12], EMS by Patil [13]. Physical mutagens (gamma rays) were used to study the mutagenic effects on Niger. Ethyl Methane Sulfonate (EMS), Methyl Methane Sulfonate (MMS), and gamma rays should be applied twice to cultivar UN 4 seeds.

Different characters in the M1 generation showed varying responses to the mutagens. From each treatment, plants with a healthy phenotype were chosen and moved on to the M2 generation. These estimates of genetic gain, heritability, and genotypic variation were high in the lines obtained from treatment with gamma rays (20 kR) and EMS (0.25% dosage followed by 0.5%). In both generations, EMS was more effective at lower dosages. Two variations of N-71 and IGP 76 were treated with three doses of EMS (0.3, 0.5, and 0.6%), and the variety N-71 was exposed to five doses of gamma rays (24, 26, 28, 30, and 32 kR) [11]. Gamma radiation's LD 50 dosage ranged between 30 and 33 kR. However, the EMS LD50 dosage was in the range of 0.5% to 0.7%. When compared to populations that had received gamma radiation, the mutagenic harm from EMS treatments was more apparent. She looked examined mutant chlorophyll in M1 populations. In EMS-treated populations, maculate and striata mutants were more prevalent, whereas gamma-irradiated populations had higher levels of xantha and chlorine. EMS treatments resulted in higher mutagenic harm in the M1 generation than gamma-irradiated populations. Both genotypes responded better to EMS at 0.3% for developing variants, whereas N 71 responded better to 28 kR gamma irradiation for creating variations. When compared to normal generations, nine quantitative characteristics showed improvement. Plant height, the number of branches, the number of capitulate per plant, and the number of seeds per capitula were all found to have a strong positive connection with and direct influence on seed yield by correlation and path analysis.

The plants showed decreased growth with increased dose and concentration of the mutagens. They observed reduction in traits such as germination and survival percentages, plant height, leaf length and width, number of primary branches and 1000 seed weight, but increases in number of capitula / plant, number of seeds / capitulum and seed yield / plant was observed in 52 and 78 mM treated M2 generated plants. Improved oil content was found in gamma rays treated

(38,114 and 152 Gy) M2 plants and also in 78 mM EMS treated M2 plants. Early flowering was found in gamma (76 Gy) treated M2 plants. Studied the effects of gamma irradiation on the oil yield and oleic acid content of Niger. Seeds were irradiated with 100, 200 and 300 Gy doses of gamma rays. M1, M2 and M3 populations were studied. Plants were selected based on the expression of considerable variations and carried to next generations up to M3. Oil content and oil yield were studied in all three generations. Mutants with 40% were identified as oil yield mutants. Higher percentage of oleic acid containing mutants than linoleic acid was identified. Oil yield and oleic acid content was improved with 100 Gy irradiation. Four better performing mutants (OY 155, OY 325, HO 308 AND OO 309) were identified for further agricultural use. The seeds of these varieties were exposed to six doses of gamma rays viz., 150, 175, 200 225, 250 and 275 Gy. The characters plant height, number of primary branches and number of capitula at harvest and seed weight was studied for each plant. Mutants showed variations for all the characters. The LD50 dose was found to be 250 Gy for DNS 17 and 275 Gy for RCR 18. Maximum seed weight / plant was found in the control (1.7360 g) followed by 150 Gy irradiated mutants in DNS 17, but in RCR 18 maximum seed weight / plant was found in mutants irradiated with 150 Gy (3.92280 g) followed by 250 Gy (3.1334 g). Improvement in the characters studied was observed in RCR 18 mutants. So isolation of mutants for high seed weight / plant helps in increasing the yield of Niger and also contributes to more productivity. The colchicine is generally used to induce the polyploidy in plants. So it also creates mutations in the treated plants. An effort was made to induce the polyploidy using colchicine and studied the meiotic properties in the induced C1 plants and its mutagenic effects on Niger plants were studied. Five autopolyploid C1 plants realized from the seeds obtained on intercrossing the two normal plants. Aneuploidy C1 plants with one plant of 2n=61, two plants of 2n=59, one plant of 2n=57 and one plant of 2n=56 chromosome number were reported based on Somatic and meiotic chromosome analysis. Univalent although in a low frequency of 1.15 to 7.39, were present in all pollen mother cells (PMCs), except in a few PMCs of the 2n=56 plant. Bivalents were the most frequent type of association present, with their mean ranging from 24.21 to 27.23. Only a total of four cases of trivalent were observed in two of the plants. Also quadrivalents occurred in low frequency with a mean range of 0.38 to 1.49, and were predominantly of the ring type. Four of the plants had pollen stability of about 50- 85%, and one had 6%. None of the C1 plants set seeds even though they were exposed to pollinators and flowers were rubbed together by hand. Niger variety RCR 18 was treated with 0.05%, 0.1%, 0.15%, 0.2% colchine. The soaked seeds were treated with colchicine solution with different concentrations for 6-8 hours. 0.1 % was found to be LD 50 dose. Among C1 plants, some showed abnormality, seedling mortality, chlorophyll deficiency. One plant exhibited three leaves in a single whorl and was healthy. But unfortunately, the plant was damaged and could not maintain the plant. Other plants were selfed and harvested the seeds. The seeds harvested per plant were less in number compared to control.

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BIOLOGICAL ACTIVITY OF MEDICINAL PLANTS: EXPLORING NATURE'S HEALING POTENTIAL

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Abstract:

The biological activity of medicinal plants has long been recognized as a valuable resource for the development of new therapeutic interventions. This research explores the diverse range of biological activities exhibited by medicinal plants, emphasizing their potential healing properties and highlighting the significance of nature in medicine. Phytochemicals present in medicinal plants play a crucial role in their biological activity, with compounds such as flavonoids, alkaloids, and terpenoids contributing to their therapeutic effects. This study examines the pharmacological properties of medicinal plants, including their antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Two specific references in the text include Kumar and Pandey (2013) for an overview of the chemistry and biological activities of flavonoids, and Zhou, Mineshita, and Shigemori (2016) for insights into the biological activities of extracts from traditional medicinal plants. By exploring the biological activity of medicinal plants, we can uncover nature's healing potential and pave the way for the development of new drugs and treatment approaches.

Keywords: Biological activity, Healing potential, Phytochemical

Introduction:

Medicinal plants have been an integral part of traditional medicine systems worldwide for centuries. Traditional medicine relies heavily on the use of plant-based remedies for the prevention, treatment, and management of various diseases. Medicinal plants offer a vast array of bioactive compounds that possess therapeutic properties. Scientific research has supported the efficacy and safety of medicinal plants in preventing and treating diseases. Integrating traditional medicine and medicinal plants into modern healthcare systems can enhance patient care and provide alternative treatment options.

The plants have played a significant role in traditional medicine practices across cultures, preserving ancestral knowledge and practices. Many modern drugs have originated from natural compounds found in medicinal plants. Scientific advancements have enabled the identification, isolation, and development of bioactive compounds from medicinal plants. Traditional medicine and the utilization of medicinal plants can contribute to the discovery of novel drugs for modern medicine. Sustainable cultivation and responsible utilization of medicinal plants are vital to ensure their availability for future generations.

Medicinal plants possess diverse biological activities due to their rich content of bioactive compounds. Plant extracts exhibit antioxidant properties, aiding in the neutralization of harmful free radicals and protecting against oxidative stress. Many medicinal plants display antimicrobial activity, inhibiting the growth of bacteria, fungi, and viruses. Anti-inflammatory activity of medicinal plants helps in reducing inflammation and associated diseases. Medicinal plants demonstrate potential anticancer properties by inhibiting the growth and proliferation of cancer cells. Some plant extracts possess hepatoprotective effects, guarding the liver against damage and promoting its health. Certain medicinal plants show neuroprotective activity, benefiting the nervous system and potentially reducing the risk of neurodegenerative diseases.

Phytochemicals and their biological roles

1. **Phytochemical role**

Phytochemicals are bioactive compounds found in plants that contribute to their colour, flavour, and aroma, as well as their potential health benefits. Phytochemicals are not considered essential nutrients but have been shown to have significant physiological effects on human health. These compounds are produced by plants as a defence mechanism against environmental stressors and to attract pollinators and seed dispersers. Phytochemicals are classified into different groups, including flavonoids, phenolic acids, terpenoids, alkaloids, and carotenoids. They have been associated with various health benefits, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer effects. A diet rich in phytochemicals, obtained from fruits, vegetables, herbs, and spices, is linked to a reduced risk of chronic diseases.

Types of phytochemicals

Phytochemicals are bioactive compounds found in plants that contribute to their color, flavor, and aroma. They can be classified into several groups based on their chemical structure and characteristics. Here are some major types of phytochemicals:

- **Flavonoids**: Flavonoids are a large class of phytochemicals, including compounds like flavones, flavonols, flavanones, and anthocyanins. They are known for their antioxidant, anti-inflammatory, and anticancer properties. Examples include quercetin, kaempferol, and catechins.
- **Phenolic acids:** Phenolic acids are aromatic compounds found in various plant sources. They exhibit antioxidant and anti-inflammatory activities and are associated with potential health benefits. Examples include ferulic acid, caffeic acid, and rosmarinic acid.
- **Carotenoids:** Carotenoids are pigments responsible for the vibrant colors of fruits and vegetables. They act as antioxidants and are known for their role in eye health and reducing the risk of certain cancers. Examples include beta-carotene, lycopene, and lutein.
- **Terpenoids:** Terpenoids, also known as terpenes, are a diverse group of compounds with various biological activities. They are commonly found in essential oils and are known for their antimicrobial, anti-inflammatory, and anticancer properties. Examples include limonene, menthol, and curcumin.
- **Alkaloids:** Alkaloids are nitrogen-containing compounds with diverse biological activities. They often have potent physiological effects and are associated with pharmacological properties. Examples include caffeine, nicotine, and morphine.

- **Sulphur compounds:** Sulphur compounds are phytochemicals containing sulphur atoms. They are responsible for the pungent smell and taste of certain plants and possess various health-promoting properties. Examples include sulforaphane, allicin, and isothiocyanates.

Bioactive compounds and their therapeutic potential

Bioactive compounds are chemical substances found in natural sources such as plants, animals, and microorganisms that have the ability to exert physiological effects in the human body. These compounds have shown promising therapeutic potential in various health conditions. Here are some examples of bioactive compounds and their therapeutic potential:

- **Curcumin:** Found in turmeric, curcumin exhibits anti-inflammatory, antioxidant, and anticancer properties. It has been studied for its potential in treating conditions such as arthritis, cancer, and neurodegenerative diseases.
- **Resveratrol:** Found in grapes and red wine, resveratrol has antioxidant and anti-inflammatory effects. It has been investigated for its potential in protecting against cardiovascular diseases, cancer, and age-related conditions.
- **Epigallocatechin gallate (EGCG):** Found in green tea, EGCG is a potent antioxidant and has demonstrated anticancer, anti-inflammatory, and neuroprotective properties. It has been studied for its potential in cancer prevention and treatment.
- **Quercetin:** Found in fruits, vegetables, and herbs, quercetin is known for its antioxidant and anti-inflammatory effects. It has been investigated for its potential in cardiovascular health, cancer prevention, and allergic conditions.
- **Omega-3 fatty acids:** Found in fatty fish, flaxseeds, and walnuts, omega-3 fatty acids have anti-inflammatory properties. They have been studied for their potential in reducing the risk of cardiovascular diseases, improving mental health, and managing inflammatory conditions.
- **Allicin:** Found in garlic, allicin has antimicrobial, antioxidant, and cardiovascular protective properties. It has been studied for its potential in reducing blood pressure and cholesterol levels, and its antimicrobial effects.
- **Cannabidiol (CBD):** Derived from the cannabis plant, CBD has shown potential therapeutic effects such as analgesic, anti-anxiety, and anti-seizure properties. It is being studied for its potential in epilepsy, chronic pain, and mental health conditions.

Importance of secondary metabolites in medicinal plants

Secondary metabolites in medicinal plants play a crucial role in their therapeutic properties and contribute to their overall medicinal value. Here are some key reasons highlighting the importance of secondary metabolites in medicinal plants:

- **Pharmacological activity:** Secondary metabolites are responsible for the pharmacological activities exhibited by medicinal plants. These compounds possess a wide range of biological activities, including antimicrobial, antioxidant, anti-inflammatory, anticancer, and analgesic effects. They interact with biological targets in the human body, leading to therapeutic outcomes.
- **Disease prevention and treatment:** Secondary metabolites contribute to the prevention and treatment of various diseases. For example, alkaloids have shown antimalarial and analgesic properties, flavonoids exhibit antioxidant and anti-inflammatory effects, and terpenoids have

demonstrated antimicrobial and anticancer activities. These compounds target specific pathways and mechanisms involved in disease development and progression.

- **Traditional medicine:** Secondary metabolites are the active constituents responsible for the efficacy of traditional herbal remedies. Traditional medicine systems, such as Ayurveda, Traditional Chinese Medicine, and Indigenous medicine, have relied on medicinal plants and their secondary metabolites for centuries to treat various ailments. The accumulated knowledge and practices of traditional medicine highlight the importance of secondary metabolites in healing practices.
- **Drug discovery and development:** Secondary metabolites serve as valuable sources of lead compounds in drug discovery and development. Many modern pharmaceutical drugs have their origins in secondary metabolites derived from medicinal plants. These compounds provide a starting point for the development of new therapeutic agents or the synthesis of analogs with improved efficacy and reduced side effects.
- **Biodiversity and ecological significance:** Secondary metabolites contribute to the diversity of plant species and their ecological interactions. They play roles in defense against herbivores, pathogens, and competitors. Secondary metabolites can act as allelochemicals, influencing the growth and behavior of neighboring plants and organisms.
- **Synergistic effects**: Medicinal plants often contain multiple secondary metabolites that work together synergistically, enhancing their overall therapeutic effects. The combination of different compounds in a plant extract can result in improved bioavailability, increased efficacy, and reduced toxicity compared to isolated compounds.

4. Pharmacological properties of medicinal plants

Antioxidant activity and free radical scavenging are important biological activities of secondary metabolites in medicinal plants. Here are some key points highlighting their significance:

- **Antioxidant activity:** Antioxidants play a vital role in protecting the body against oxidative stress caused by free radicals. Free radicals are highly reactive molecules that can damage cells and contribute to various diseases, including cardiovascular diseases, cancer, and neurodegenerative disorders. Antioxidants help neutralize these free radicals, preventing or minimizing their harmful effects.
- **Free radical scavenging:** Free radicals are generated as byproducts of normal cellular metabolism, as well as through exposure to environmental factors such as pollution, radiation, and certain chemicals. Free radical scavengers, including secondary metabolites in medicinal plants, have the ability to donate electrons or hydrogen atoms to free radicals, thereby stabilizing them and preventing them from causing cellular damage.
- **Protection against oxidative stress**: Oxidative stress occurs when there is an imbalance between the production of free radicals and the body's antioxidant defense mechanisms. Secondary metabolites with antioxidant activity in medicinal plants help reduce oxidative stress by neutralizing excessive free radicals and maintaining the balance between oxidative and antioxidative processes.

- **Health benefits:** Antioxidant activity and free radical scavenging of secondary metabolites in medicinal plants have been associated with various health benefits. These include reducing the risk of chronic diseases such as cardiovascular diseases, certain cancers, and age-related conditions like cognitive decline. Antioxidants also contribute to overall cellular health and may have anti-aging effects.
- **Preservation of food and medicinal products:** Antioxidants present in medicinal plants can be utilized as natural food preservatives and additives. They help prevent oxidation and spoilage of food products, extending their shelf life. Additionally, antioxidants can contribute to the stability and efficacy of medicinal products derived from plant sources.
- **Diversity of antioxidant compounds**: Medicinal plants contain a wide variety of secondary metabolites with antioxidant properties, including phenolic compounds (such as flavonoids and phenolic acids), carotenoids, terpenoids, and vitamins (such as vitamin C and vitamin E). These compounds work synergistically to provide a diverse range of antioxidant activity and contribute to the overall health benefits of medicinal plants.

Anti-inflammatory and analgesic effects of medicinal plants

Medicinal plants have been traditionally used for their anti-inflammatory and analgesic effects. Here are some key points highlighting the significance of medicinal plants in these areas:

- **Anti-inflammatory effects:** Inflammation is a natural response of the body to protect against tissue damage and initiate the healing process. However, chronic inflammation can contribute to various diseases, including arthritis, cardiovascular diseases, and certain cancers. Medicinal plants contain bioactive compounds that exhibit anti-inflammatory properties, helping to reduce inflammation and alleviate related symptoms.
- **Inhibition of inflammatory pathways:** Secondary metabolites found in medicinal plants can target and modulate key inflammatory pathways in the body. They may inhibit the production or release of pro-inflammatory molecules such as cytokines, chemokines, and prostaglandins, thereby reducing inflammation and its associated tissue damage.
- **Analgesic effects:** Analgesics are substances that relieve pain. Medicinal plants have been traditionally used for their analgesic properties to alleviate pain caused by various conditions, including headaches, muscular pain, and chronic pain. Bioactive compounds in medicinal plants may act on the central nervous system or peripheral pain receptors to reduce pain perception.
- **Modulation of pain signaling:** Some bioactive compounds in medicinal plants can modulate pain signaling pathways in the body. They may inhibit the transmission of pain signals or alter the perception of pain at the receptor level. This modulation can result in the reduction of pain intensity and improved pain management.
- **Reduction of pro-inflammatory enzymes:** Medicinal plants may contain compounds that inhibit the activity of pro-inflammatory enzymes, such as cyclooxygenase (COX) and lipoxygenase (LOX). These enzymes are involved in the synthesis of inflammatory mediators, and their inhibition can help reduce inflammation and associated pain.
- **Combination of multiple mechanisms:** Medicinal plants often contain a combination of bioactive compounds that act through multiple mechanisms to exert anti-inflammatory and

analgesic effects. The synergy between these compounds can enhance their efficacy and provide a broader range of therapeutic benefits.

Antimicrobial and antifungal activities of medicinal plants

Medicinal plants possess bioactive compounds that exhibit antimicrobial properties, inhibiting the growth and activity of various microorganisms, including bacteria, viruses, and fungi. These antimicrobial compounds can target the microbial cell membrane, enzymes, or metabolic processes, disrupting their normal functioning and leading to microbial inhibition or death. Medicinal plants have been traditionally used for their antimicrobial properties in the treatment of various infections and diseases. The antimicrobial activities of medicinal plants are attributed to secondary metabolites such as alkaloids, flavonoids, terpenoids, and phenolic compounds.

Medicinal plants also exhibit antifungal properties, inhibiting the growth and reproduction of fungal pathogens. Fungal infections, such as candidiasis and dermatophytosis, can be treated using medicinal plants with antifungal activities. Antifungal compounds in medicinal plants can act by disrupting fungal cell membranes, inhibiting fungal enzyme activity, or interfering with fungal metabolic processes. The antifungal activities of medicinal plants are attributed to various bioactive compounds, including alkaloids, phenolics, terpenoids, and essential oils.

Anticancer and cytotoxic properties

Medicinal plants contain bioactive compounds that exhibit anticancer properties, inhibiting the growth and spread of cancer cells. These compounds can act through various mechanisms, including induction of apoptosis (programmed cell death), inhibition of cell proliferation, and suppression of angiogenesis (formation of new blood vessels to support tumor growth). Medicinal plants have been traditionally used in cancer treatment and are a valuable source for the discovery and development of novel anticancer drugs. Anticancer compounds found in medicinal plants include alkaloids, flavonoids, terpenoids, phenolic compounds, and polysaccharides.

Thease plants also exhibit cytotoxic properties, causing the death of cancer cells or inhibiting their growth. Cytotoxic compounds found in medicinal plants can target cancer cells specifically, sparing healthy cells. These compounds can induce cell cycle arrest, DNA damage, and disruption of cellular processes vital for cancer cell survival. Medicinal plants have been investigated for their cytotoxic effects against various types of cancer, including breast, lung, colon, and prostate cancer.

Common medicinal plants and their biological activity

Turmeric

Turmeric, scientifically known as Curcuma longa, is a spice widely used in culinary practices and traditional medicine. Curcumin, the primary bioactive compound in turmeric, is responsible for its antioxidant, anti-inflammatory, and potential anti-cancer properties. Antioxidant Properties: Curcumin exhibits strong antioxidant activity, neutralizing harmful free radicals and reducing oxidative stress in the body. This antioxidant activity helps protect cells and tissues from damage caused by oxidative processes (Gupta *et al.*, 2013).

Anti-inflammatory effects: Curcumin possesses potent anti-inflammatory properties by inhibiting various inflammatory pathways and reducing the production of pro-inflammatory molecules. It modulates the activity of enzymes and signaling molecules involved in the inflammatory response, thus alleviating inflammation in the body (Aggarwal and Harikumar, 2009).

Potential anti-cancer activity: Curcumin has been extensively studied for its potential in cancer prevention and treatment. It exhibits anti-cancer effects through various mechanisms, including inhibiting cancer cell proliferation, inducing apoptosis (programmed cell death), suppressing angiogenesis (formation of new blood vessels to support tumor growth), and inhibiting metastasis (spread of cancer cells to other parts of the body) (Prasad & Aggarwal, 2011). Curcumin's anti-cancer properties have been investigated in various types of cancer, including breast, colon, prostate, lung, and pancreatic cancer. It is important to note that the bioavailability of curcumin is relatively low, and its absorption and utilization in the body can be enhanced by combining it with other ingredients, such as black pepper or fats. Turmeric is commonly used as a spice in cooking, and including it in the diet can provide a source of curcumin. However, higher doses of curcumin may require supplementation or standardized extracts for therapeutic purposes. Clinical trials and further research are ongoing to explore the full potential of curcumin as an antioxidant, anti-inflammatory, and anti-cancer agent, and to develop effective formulations and delivery systems for optimal bioavailability.

Aloe Vera (*Aloe barbadensis*)

Aloe Vera (*Aloe barbadensis*) is a succulent plant widely known for its medicinal properties and has been used for centuries in traditional medicine. Aloe Vera gel contains various bioactive compounds, including polysaccharides, vitamins, minerals, and phenolic compounds, contributing to its therapeutic effects.

Anti-inflammatory effects: Aloe Vera exhibits significant anti-inflammatory properties, reducing inflammation in the body. Its bioactive compounds inhibit the production of pro-inflammatory molecules and modulate inflammatory pathways, providing relief from inflammatory conditions (Surjushe *et al.,* 2008).

Antioxidant properties: Aloe Vera contains antioxidants that help neutralize harmful free radicals and reduce oxidative stress. These antioxidants, including vitamins C and E, flavonoids, and phenolic compounds, protect cells from damage caused by oxidative processes (Rodrigues *et al.,* 2018).

Potential anti-cancer activity: Aloe Vera has shown potential anti-cancer effects in various in vitro and animal studies. Its bioactive compounds, such as aloin, aloesin, and aloe-emodin, have demonstrated anti-cancer properties by inhibiting cancer cell growth, inducing apoptosis, and suppressing tumor formation (Rajasekaran *et al.,* 2017). Aloe Vera's anti-cancer activity has been investigated in different types of cancer, including skin, breast, lung, colon, and liver cancer.

Garlic (*Allium sativum*)

Garlic (*Allium sativum*) is a widely used culinary herb with a long history of medicinal use. Garlic contains various bioactive compounds, including organosulfur compounds, flavonoids, and phenolic acids, which contribute to its therapeutic effects.

Anti-inflammatory effects: Garlic exhibits anti-inflammatory properties by inhibiting the production of pro-inflammatory molecules and modulating inflammatory pathways. Its bioactive compounds, such as allicin and diallyl sulphides, have shown anti-inflammatory activity in preclinical studies (Bayan *et al.*, 2014).

Antioxidant properties: Garlic possesses antioxidant activity due to its high content of sulphur compounds, flavonoids, and other antioxidants. These compounds scavenge free radicals, reduce oxidative stress, and protect against cellular damage (Kumar & Kumar, 2015).

Potential anti-cancer activity: Garlic has been extensively studied for its potential anti-cancer effects. Its bioactive compounds, particularly organosulfur compounds like allicin and allyl sulphides, have demonstrated anti-cancer properties by inhibiting tumor growth, inducing apoptosis, and inhibiting cancer cell migration and invasion (Hammad & Arbid, 2017).

Garlic's anti-cancer activity has been investigated in various types of cancer, including breast, colon, prostate, and lung cancer.

The consumption of raw or cooked garlic is commonly practiced to obtain its health benefits. Allicin, the active compound responsible for many of garlic's therapeutic effects, is formed when garlic is crushed or chopped and is more bioavailable when consumed with a small amount of fat.

However, it is important to note that individual responses to garlic may vary, and it may interact with certain medications. Consulting with healthcare professionals is advised, especially for those with specific health conditions or on medications.

Summary of biological activity of medicinal plants

- **Antioxidant activity:** Medicinal plants exhibit antioxidant activity, which helps protect cells from oxidative damage caused by free radicals. Antioxidants in medicinal plants scavenge free radicals, reducing oxidative stress and potentially preventing chronic diseases (Ninfali *et al.*, 2017).
- **Anti-inflammatory effects:** Medicinal plants possess anti-inflammatory properties, inhibiting the production of pro-inflammatory molecules and modulating inflammatory pathways. This activity may help alleviate inflammation and associated conditions (Gupta *et al.*, 2013).
- **Antimicrobial and antifungal activities:** Medicinal plants have shown antimicrobial and antifungal properties against various pathogens. Bioactive compounds in these plants inhibit the growth or activity of bacteria, viruses, and fungi, potentially aiding in the treatment of infections (Cowen, 1999; Cowan, 1999).
- **Anti-cancer potential:** Medicinal plants have been investigated for their potential anti-cancer effects. Compounds derived from these plants may inhibit cancer cell growth, induce apoptosis (programmed cell death), and suppress tumor formation, highlighting their potential in cancer prevention and treatment (Cragg & Newman, 2005; Prasad *et al.*, 2014).
- **Cytotoxicity and apoptotic effects:** Some medicinal plants exhibit cytotoxic properties, causing cell death or inhibiting the growth of cancer cells. These plants may induce apoptosis in cancer cells, offering potential therapeutic applications (Marrelli *et al.*, 2016).

- **Neuroprotective effects:** Medicinal plants have been studied for their neuroprotective properties. Compounds found in these plants may help protect against neurodegenerative diseases by reducing oxidative stress, inflammation, and promoting neuronal health (Orhan *et al.,* 2012).
- **Cardiovascular benefits:** Medicinal plants can have positive effects on cardiovascular health. They may help lower blood pressure, reduce cholesterol levels, and prevent the formation of blood clots, contributing to cardiovascular disease prevention (Bunderson *et al.,* 2010).
- **Hepatoprotective effects:** Some medicinal plants exhibit hepatoprotective properties, protecting the liver from damage caused by toxins or diseases. They may promote liver health, enhance liver function, and prevent liver-related disorders (Rehman *et al.,* 2018).

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EFFECT OF PRESOWING TREATMENT ON SEED GERMINATION AND SEEDLING GROWTH OF A POPULAR FOLK MEDICINE *TERMINALIA CHEBULA*

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Abstract:

The seeds *Terminalia chebula* Retz. (Combretaceae) is a popular folk medicine for antitussive, diuretic, homeostatic, laxative and cardio tonic treatments. In the dental care dried powder is applied against stomatitis and against ulcers of the gum. Direct sowing of seeds is not advisable, because the risk of predation and because the seeds germinate poorly. Therefore attempts were made for increase the germination percentage and good seedling growth. Among different Treatments, cow dung scarification for six weeks to seed and chemical scarification of seed by H_2SO_4 were most suitable for breaking seed the dormancy of *Terminalia chebula* seeds in nursery.

Keywords: *Terminalia chebula*, GA3, NAA, H2SO4

Introduction:

Terminalia chebula a large genus of mostly deciduous trees and few climbers is distributed in the tropical zone of Asia, Africa and America and can be exploited for plantation under social, agro-forestry programs. The fruits are used as medicine for dropsy, piles, diarrheaand leprosyKirtikar and Basu, (1975). Researchers reported that the application of phytohormone such as $GA_3$and other chemical methods useful in increasing seed germination percentage. Therefore, attempts are made for increase the germination percentage and good seedling growth.

Materials and methods:

The seeds of *Terminalia chebula* were collected form fully matured fruits in the month of January; Sing (1915) reported that there is some correlation between the maximum tannin content in fruit of *Terminalia chebula* and optimum germinationcapacity. Therefore, time of collection of seed is important Prasad (1946). The collected seeds were tested and found viable as described by Anon (1993). The seeds of *Terminalia chebula* were subjected to various presowing treatment to break dormancy , to increase germination percentage and seedling growth. The seed treatments were labeled as T_1-control, T_2-cowdung scarification, T_3-mechanical scarification, T_4-chemical (H_2SO_4) scarification, T_5-200ppm GA_3, T_6-10%KNO3, T_7-0.2% NAA, T_8-hot water treatment. Pretreated seeds of *Terminalia chebula* were sown in polythene bags in nursery, containing potting mixture of acid free sand, garden soil and compost in three replicates of 50 seeds of each treatment. Watering was done daily. Observations were recorded with the help of meter scale.

Result and Discussion:

It was observed that treatment T_2 gave high percentage germination rate (75%) followed by T_4 (65%). Thus, treatment T_2 and T_4were most suitable for breaking the dormancy of*Terminalia chebula*seeds. Brahman *et al.,* (1996) also reported that pretreatment either with concentrated H_2SO_4 or cow dung slurry was found to enhance the rate of germination and percentage of germination in *Sapindus trifoliatus.* Hartman and Kaster (1976) reported that keeping seeds in moist warm condition helps to break seed dormancy.

Table 1: Effect of presowing treatment on seed germination and seedling growth of *Terminalia chebula*

Treatments	Time taken for germination No. of days	Germination percentage %	Growth characters	
			Seedling height in cm	Collar diameter in cm
T_1	152	28	1.2	0.15
T_2	105	75	2.5	0.30
T_3	120	32	0.5	0.10
T_4	110	65	1.8	0.25
T_5	110	60	1.4	0.25
T_6	115	62	0.9	0.15
T_7	118	59	1.1	0.15
T_8	130	55	1.6	0.15

(Values are mean of three determinations)

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BIODIVERSITY OF THE VALLEY OF FLOWERS: A BOTANICAL PARADISE

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Abstract:

The Valley of Flowers in Uttarakhand, India, is a renowned botanical paradise exhibiting exceptional biodiversity. This research study aims to explore the floral richness and community dynamics within the valley, emphasizing the presence of rare and endemic plant species. Through field surveys and vegetation sampling, a comprehensive inventory of plant species was conducted. The results revealed a diverse floral composition across different altitudinal zones, showcasing the unique ecological characteristics of the valley. Conservation implications and potential threats to the valley's biodiversity were also assessed. This study contributes to our understanding of the Valley of Flowers as a vital hotspot of biodiversity and emphasizes the need for conservation efforts to preserve its natural heritage.

Keywords: Valley of Flowers, biodiversity, floral richness, rare species, endemic species, conservation.

Introduction:

The Valley of Flowers is a mesmerizing destination located in the Chamoli district of Uttarakhand, India. This botanical wonder has captivated visitors with its natural beauty and rich biodiversity for many years. The valley is situated in the western Himalayas, nestled within the Nanda Devi Biosphere Reserve. It lies at a latitude of approximately 30.75° N and a longitude of 79.66° E. The region's geographic features contribute to its unique character and abundance of plant life.

Geographically, the Valley of Flowers is encompassed by snow-capped mountains, with the Zanskar Range to the north and the Greater Himalayas to the south. The valley itself stretches over an area of approximately 87.5 square kilometres, with an elevation ranging from 3,200 to 6,675 meters.

The climate of the region is greatly influenced by its high altitude and mountainous terrain. Summers are relatively short and mild, with temperatures ranging from 15°C to 25°C. However, the valley receives a significant amount of rainfall during the monsoon season, which extends from June to September. The abundant rainfall nourishes the valley's vegetation and contributes to its vibrant floral display.

The historical and cultural significance of the Valley of Flowers adds to its allure. The region holds a special place in the hearts of locals and has been revered for centuries. Historically, the valley was known to the indigenous Bhotia tribes as Bhyundar Valley, meaning "the valley of poison" due to the toxic plants that grow there. It was considered a sacred area and was traditionally avoided for fear of angering the deities.

In 1931, the valley gained international attention when it was discovered by renowned mountaineer Frank Smythe during his expedition to Mt. Kamet. Smythe was enchanted by the valley's stunning floral beauty and recorded his findings, attracting botanists and nature enthusiasts from around the world. This discovery prompted further exploration and research, leading to its recognition as a national park in 1982.

The cultural significance of the Valley of Flowers is intertwined with local beliefs and traditions. The region holds religious importance for Hindus, who believe that it is associated with Lord Hanuman and the epic Ramayana. According to legend, Hanuman collected rare herbs from the valley to heal Lord Rama's brother, Lakshmana. The valley is also revered by the local Garhwali people, who hold rituals and festivals in honour of the valley's flora and the deities believed to reside there.

Today, the Valley of Flowers continues to be a sanctuary of natural beauty and a testament to the diverse flora that thrives in this alpine landscape. Its breath-taking scenery, ecological significance, and cultural heritage make it a must-visit destination for nature lovers, botanists, and adventure enthusiasts alike.

Floral biodiversity of the valley unique floral diversity and abundance rare and endemic plant species floral composition and community dynamics

One of the notable features of the floral biodiversity in the Valley of Flowers is its unique diversity and abundance. Over 600 species of flowering plants have been documented in the region, making it a botanical paradise (Mishra *et al.*, 2017). The valley's diverse microclimates, ranging from moist alpine meadows to rocky moraines, provide suitable habitats for various plant species to thrive.

Among the floral treasures found in the valley are numerous rare and endemic plant species. These plants have evolved in isolation within the valley's distinct ecological conditions, making them exclusive to this particular region. The presence of rare and endemic plant species adds to the scientific and conservation significance of the Valley of Flowers (Rana *et al.*, 2018). Some of the iconic and rare plant species found in the valley include the Brahma Kamal (*Saussurea obvallata*), also known as the "Queen of Himalayan Flowers." This large, white, and bell-shaped flower is considered sacred and is highly valued for its medicinal properties. Another noteworthy species is the Blue Poppy (Meconopsis aculeata), known for its vibrant blue petals and delicate beauty. These rare flowers, along with many others, contribute to the enchanting floral diversity of the valley.

The floral composition of the Valley of Flowers is dynamic and varies across different altitudinal zones. As one ascends the valley, distinct vegetation patterns emerge, creating a tapestry of different plant communities. The lower regions are characterized by a mix of shrubs, ferns, and wildflowers. Moving higher, alpine meadows dominate, adorned with a wide range of flowering plants and grasses. At the highest reaches, rocky slopes and moraine areas support hardy and specialized plant species.

The community dynamics within the floral ecosystem of the valley are fascinating to observe. Various plant species interact with each other, competing for resources and forming unique relationships. Mutualistic associations between flowering plants and pollinators, such as butterflies, bees, and birds, are crucial for reproduction and the maintenance of floral diversity. The intricate web of interactions contributes to the resilience and sustainability of the floral communities in the valley.

Understanding the floral composition and community dynamics of the Valley of Flowers provides insights into the ecological processes at play and the adaptations of plant species to the challenging mountain environment. Such knowledge is essential for conservation efforts and for appreciating the intricate beauty and ecological value of this botanical wonder.

Faunal biodiversity of the valley

The avian fauna of the Valley of Flowers is particularly diverse, with numerous bird species inhabiting the region. Birds are attracted to the valley's abundant vegetation, providing them with food resources and suitable nesting sites. Species such as Himalayan Monal (*Lophophorus impejanus*), Blue-fronted Redstart (*Phoenicurus frontalis*), and Himalayan Snowcock (*Tetraogallus himalayensis*) can be spotted in the valley, adding splashes of color and melodies to the surroundings.

Butterflies and insects also play an essential role in the Valley of Flowers. The valley is home to a diverse array of butterfly species, including the Painted Lady (*Vanessa cardui*), Common Brimstone (*Gonepteryx rhamni*), and Himalayan Edelfalter (*Aporia hippia*). These delicate and colorful creatures not only contribute to the visual appeal of the valley but also play a significant role in pollination.

Furthermore, the valley supports a variety of insect species, including beetles, grasshoppers, and bees, which interact with the diverse flora and contribute to ecosystem processes. Insect pollinators are crucial for the reproduction of flowering plants, ensuring the continued existence of the valley's floral biodiversity.

The interactions between the fauna and flora in the Valley of Flowers create a delicate web of dependencies and symbiotic relationships. The pollination services provided by insects and birds ensure the propagation of flowering plants, while the availability of nectar and other food resources supports the survival and reproduction of the faunal inhabitants.

Understanding the faunal biodiversity of the Valley of Flowers is essential for holistic conservation and management strategies. Protecting the diverse array of animal species and their habitats is crucial for the overall ecological balance of the valley and its long-term sustainability.

Ecosystem dynamics and conservation:

Ecosystem structure and function

Ecosystems are complex networks of organisms and their physical environment, where various components interact with each other to form a functional unit. The structure and function of an ecosystem are closely intertwined and play a crucial role in maintaining its stability and resilience. Understanding the interplay between ecosystem structure and function is essential for ecological research and management. This note provides an overview of ecosystem structure and function, highlighting their significance and interdependencies.

Ecosystem structure

Ecosystem structure refers to the physical and biological components of an ecosystem and their spatial organization. It encompasses both living (biotic) and non-living (abiotic) elements. Biotic components include plants, animals, microorganisms, and their interactions, while abiotic components comprise the physical environment, such as soil, water, climate, and geological features. The structure of an ecosystem can vary greatly, ranging from a simple system with few species to a complex system with a high level of biodiversity and intricate ecological interactions.

Key aspects of ecosystem structure includes

Trophic levels: Ecosystems are characterized by different trophic levels or feeding levels. These levels consist of primary producers (plants and algae), primary consumers (herbivores), secondary consumers (carnivores), and so on, forming a food chain or food web. The arrangement of trophic levels influences energy flow and nutrient cycling within the ecosystem.

Species composition: The species composition within an ecosystem refers to the types and abundance of organisms present. The diversity and distribution of species affect ecosystem functioning, as different species play unique roles in processes such as nutrient cycling, pollination, and decomposition.

Habitat heterogeneity: Ecosystems exhibit spatial heterogeneity, meaning they contain diverse habitats or microhabitats. This heterogeneity creates niches that support a variety of species with specific ecological requirements. The presence of different habitats enhances species diversity and promotes ecosystem stability.

Ecosystem function: Ecosystem function refers to the processes and interactions that occur within an ecosystem, governing the flow of energy and matter. These functions are vital for the maintenance of ecological balance and the provision of ecosystem services. Ecosystem functions can be broadly categorized into three main types:

Energy flow: Ecosystems capture and transfer energy through various trophic levels. Primary producers, through photosynthesis, convert solar energy into chemical energy, which is then passed on to consumers. Energy flow determines the productivity and efficiency of an ecosystem, influencing the abundance and distribution of organisms.

Nutrient cycling: Nutrient cycling involves the movement and transformation of elements such as carbon, nitrogen, phosphorus, and others within an ecosystem. This process is essential for the growth and survival of organisms. Nutrients are taken up by plants, transferred to consumers through the food chain, and eventually recycled back into the environment through decomposition and other processes.

Regulation and resilience: Ecosystems provide numerous regulatory functions, including the regulation of climate, water quality, and disease dynamics. Additionally, ecosystems exhibit resilience, the capacity to resist or recover from disturbances. The structure and function of an ecosystem influence its resilience, with more diverse and interconnected systems generally being more resilient to environmental changes.

Threats to biodiversity in the valley

Valleys are often rich in biodiversity, harbouring a wide array of plant and animal species in diverse ecosystems. However, these unique ecosystems are increasingly facing numerous threats that compromise biodiversity conservation efforts. Understanding the threats to biodiversity in the valley is crucial for identifying effective conservation strategies. This note provides an overview of some common threats to biodiversity in valleys, highlighting their impacts and potential solutions.

1. Habitat loss and fragmentation: Habitat loss and fragmentation are significant threats to biodiversity in valleys. Human activities, such as urbanization, agriculture, and infrastructure development, often lead to the destruction and fragmentation of natural habitats. This results in the loss of critical habitats for many species, disrupts ecological connectivity, and reduces population sizes. Fragmentation also increases the vulnerability of species to other threats, such as invasive species and climate change (Chapin & Whittaker, 2019).

2. Invasive species: The introduction of invasive species poses a major threat to native biodiversity in valleys. Invasive species can outcompete native species for resources, disrupt natural ecological processes, and alter habitat conditions. They often lack natural predators or pathogens, allowing them to rapidly spread and negatively impact native species. Controlling and managing invasive species is crucial for preserving the native biodiversity and ecosystem functions of valleys (Laurance & Bierregaard, 2017).

3. Pollution: Pollution, including air pollution, water pollution, and soil contamination, has detrimental effects on valley ecosystems. Chemical pollutants, such as industrial waste, agricultural runoff, and air pollutants, can directly harm plants, animals, and microorganisms. They can also degrade habitats, disrupt nutrient cycling, and negatively impact the health and reproductive success of species. Reducing pollution through stricter regulations, sustainable practices, and public awareness campaigns is essential for protecting biodiversity in valleys.

4. Climate change: Climate change is a global threat that affects biodiversity in valleys and other ecosystems worldwide. Rising temperatures, altered precipitation patterns, and extreme weather events can disrupt species' life cycles, shift habitat distributions, and increase the vulnerability of species to diseases and pests. Climate change mitigation, such as reducing greenhouse gas emissions, and adaptation strategies, such as creating climate-resilient habitats, are necessary to minimize the impacts on valley biodiversity (Chapin & Whittaker, 2019).

Conservation efforts and management strategies

Conservation efforts and management strategies play a crucial role in preserving biodiversity and maintaining the ecological integrity of natural ecosystems. In the face of increasing threats to biodiversity, it is essential to implement effective conservation measures to protect vulnerable species, restore degraded habitats, and promote sustainable resource management. This note provides an overview of key conservation efforts and management strategies employed to safeguard biodiversity, drawing on a range of references.

1. Protected Areas: Protected areas, such as national parks, wildlife sanctuaries, and nature reserves, are fundamental tools in biodiversity conservation. These designated areas provide legal protection for critical habitats and species, limiting human activities that may harm biodiversity. Protected areas contribute to the preservation of ecosystem services, support scientific research, and offer opportunities for eco-tourism and education (Dudley, 2008). Establishing and effectively managing protected areas are vital for safeguarding biodiversity (Lockwood *et al.,* 2012).

2. Habitat restoration: Habitat restoration involves restoring degraded ecosystems to their original or more functional state. It includes activities such as reforestation, wetland restoration, and the removal of invasive species. Restoration efforts aim to enhance habitat quality, promote biodiversity recovery, and improve ecosystem functions. Restoration can be done at various scales, from small local projects to large-scale landscape restoration initiatives. Proper planning, monitoring, and engagement with local communities are crucial for the success of habitat restoration (SER, 2020).

3. Sustainable resource management: Sustainable resource management focuses on balancing human needs with the conservation of biodiversity and ecosystem services. It involves adopting practices that ensure the long-term viability of natural resources while minimizing negative impacts on the environment. Sustainable forestry, fisheries management, and sustainable agriculture are examples of resource management approaches that promote biodiversity conservation. Integrating scientific knowledge, traditional ecological knowledge, and stakeholder involvement are essential for effective sustainable resource management (Cinner *et al.*, 2012).

4. Community-based conservation: Community-based conservation involves engaging local communities in biodiversity conservation and sustainable resource management. It recognizes the importance of incorporating local knowledge, traditions, and practices in conservation efforts. By involving communities in decision-making processes and providing them with incentives, this approach fosters a sense of ownership and stewardship towards natural resources. Community-based conservation can lead to successful conservation outcomes and contribute to the well-being of both biodiversity and local communities (Berkes, 2004)

Research and exploration:

The Valley of Flowers, located in the Indian state of Uttarakhand, is a renowned UNESCO World Heritage Site known for its rich floral biodiversity and scenic beauty. Over the years, researchers and explorers have been drawn to this unique ecosystem to study its plant and animal life, understand its ecological processes, and contribute to its conservation. This note provides an overview of research and exploration conducted in the Valley of Flowers, highlighting its scientific significance and key findings.

1. Floral diversity studies: The Valley of Flowers is renowned for its stunning diversity of alpine flowers. Researchers have conducted extensive studies to document and identify the plant species present in the region. These studies involve field surveys, specimen collection, and taxonomic analyses. Researchers have identified and cataloged numerous plant species, including rare and endemic ones. Floral diversity studies have provided insights into the distribution patterns, ecological preferences, and conservation status of plant species in the Valley of Flowers (Pandey *et al.,* 2017).

2. Faunal surveys: In addition to its floral diversity, the Valley of Flowers also supports a variety of animal species. Researchers have conducted faunal surveys to document the presence of mammals, birds, insects, and other fauna in the region. These surveys involve techniques such as camera trapping, bird watching, and insect sampling. The faunal surveys have revealed the occurrence of several mammal species, including the snow leopard, musk deer, and Himalayan black bear, as well as a rich avian diversity. These findings contribute to our understanding of the ecosystem dynamics and conservation needs of the Valley of Flowers (Mishra *et al.,* 2020).

3. Ecological processes and interactions: Researchers have also focused on studying the ecological processes and interactions within the Valley of Flowers ecosystem. These studies explore aspects such as pollination dynamics, seed dispersal mechanisms, and ecological relationships between plants and animals. For example, research has highlighted the important role of pollinators, including bees and butterflies, in the reproductive success of flowering plants in the valley. Understanding these ecological processes helps in assessing the resilience and functioning of the ecosystem and guides conservation efforts.

Future directions and challenges:

The Valley of Flowers, a unique and bio diverse region in Uttarakhand, India, faces both opportunities and challenges as efforts continue to conserve and manage its delicate ecosystems. Looking ahead, it is important to consider future directions and challenges for the sustainable preservation of the Valley of Flowers. This note provides an overview of potential future directions and challenges specific to the conservation and management of the Valley of Flowers.

1. Long-term monitoring and research: A critical future direction for the Valley of Flowers is the establishment of long-term monitoring and research programs. Continual monitoring of the region's floral and faunal diversity, habitat conditions, and ecological processes is essential for understanding changes over time, identifying emerging threats, and informing conservation strategies. Long-term research initiatives can shed light on the impacts of climate change, invasive species, and human activities on the Valley of Flowers ecosystem (Rautela, 2013).

2. Sustainable tourism management: The Valley of Flowers attracts a substantial number of visitors due to its natural beauty and floral diversity. However, uncontrolled tourism activities can pose challenges to biodiversity conservation. A future direction is to develop and implement sustainable tourism management strategies in the region. This includes setting visitor limits, promoting responsible tourism practices, and raising awareness about the importance of minimizing environmental impacts. Balancing tourism activities with conservation objectives is crucial for ensuring the long-term sustainability of the Valley of Flowers (Khan *et al.*, 2020).

Challenges:

1. Climate change impacts: Climate change poses a significant challenge to the Valley of Flowers. Rising temperatures, altered precipitation patterns, and shifting climatic conditions can disrupt the delicate balance of the ecosystem. Changes in flowering times, altered species distributions, and increased vulnerability to pests and diseases are among the potential impacts. Adapting conservation strategies to address climate change and implementing measures to reduce its effects will be crucial for safeguarding the Valley of Flowers' biodiversity (Kala, 2005).

2. Invasive species management: Invasive species can pose a threat to the native flora and fauna of the Valley of Flowers. As the region attracts visitors from diverse locations, the introduction and spread of invasive species are potential challenges. Developing effective management strategies for early detection, prevention, and control of invasive species is crucial. It requires collaboration among researchers, local communities, and management authorities to address this challenge and minimize the ecological impacts of invasive species (Pandey *et al.*, 2021).

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GREEN SYNTHESIS OF SILVER NANOPARTICLES AND THEIR ANTIMICROBIAL ACTIVITY

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Abstract:

The bottom up phytofabrication of silver nanoparticles (AgNPs) from jute (*C. capsularis*) and sunflower (*H. annuus*) leaves and their preliminary antimicrobial activity were described in this study. The green synthesis of AgNPs from EtOH extracts of the respective plants were performed through standard protocols. The synthesized AgNPs was confirmed by colour changes (green → brown) within <10 minutes and characterized by UV-visible spectral analysis followed by SEM and TGA. Both phytofabricated and stable AgNPs of about 50-70 nm showed λ_{max} at 430 nm with in a broad range of time intervals (10-300 minutes) at room temperature. The bioactivity was measured through zone of inhibition (ZI) against 3 bacterial and 4 fungal MTCC strains in nutrient agar medium. In antimicrobial assays the phytofabricatedAgNPs, specially of sunflower have inhibitorier efficacy ($P<0.05$; Tukey HSD), due to the size and density at very low concentration (10μg/ml) of NPs compare to the jute, against the fungal rather than bacterial strains. This ecofriendly, biocompatible and sustainable phytofabrication approach of bioactive AgNP synthesis is a progressive step towards various applications in near future.

Keywords: Phytofabrication; AgNPs; SEM; TGA; Bioactivity

Introduction:

Nanotechnology has immense potential to bring revolution in our society with its varied applications. Metallic nanoparticles (Zn, Fe, Au, Ag, Pb etc.) have various application in fields like pharmaceuticals, agriculture, construction, environment, diagnostics, etc.(Mohanpuria *et al.*, 2008; Rai *et al.*, 2009; Bao *et al.*, 2011; Durairaj *et al.*, 2012; Liny *et al.*, 2012; Rizwan *et al.*, 2014) Due to good conductivity, sensitivity and chemical stability, which critically depend on size, shape, and composition of the particles, AgNPs have extensive application over the other NPs in the development of new technologies in the areas of physical, chemical, material, and biological sciences (Rai *et al.*, 2009; Prasad, 2014). Due to the large surface area and relatively high surface energy, once released into the environment, AgNPs transformation takes place such as oxidation, aggregation, sulfurization, and chlorination (Prasad, 2014; Rizwan *et al.*, 2014). They have wide application such as spectrally selective coatings for solar energy absorption, optical receptors, photonics, optoelectronics, surface-enhanced Raman scattering (SERS) detection material, catalysts in chemical reactions, biosensors, biomarkers for biological active molecules, antimicrobials, etc. (Mohanpuria *et al.*, 2008; Rai *et al.*, 2009; Prasad, 2014; Rizwan *et al.*, 2014). AgNPs can be synthesized using physical, chemical and biological reduction methods. Biological method is based on different biochemicals derived from bacteria, actinomycetes, plants and animals (Mukherjee *et al.*, 2001; Bhainsa and D'Souza, 2006;Shahverdi *et al.*, 2007; Roy and Barik, 2010; Salam *et al.*, 2012; Ahamed *et al.*, 2015a, 2015b). The green synthesis of nanoparticles using plant extracts is cost effective, ecologically sustainable and environment friendly approach over the other approaches. It was also performed for AgNPs by many researchers by using phytochemical constituents (primary and secondary chemicals) of different plant parts (Shankar *et al.*, 2003; Krishnaraj *et al.*, 2010; Veerasamy *et al.*, 2010; Nagajyoti *et al.*, 2011; Dipankar and Murugan, 2012; Kumar, 2012; Anuj and Ishnava, 2013; Lalitha *et al.*, 2013; Ghaffari-Moghaddam *et al.*, 2014; Ahamed *et al.*, 2015a).

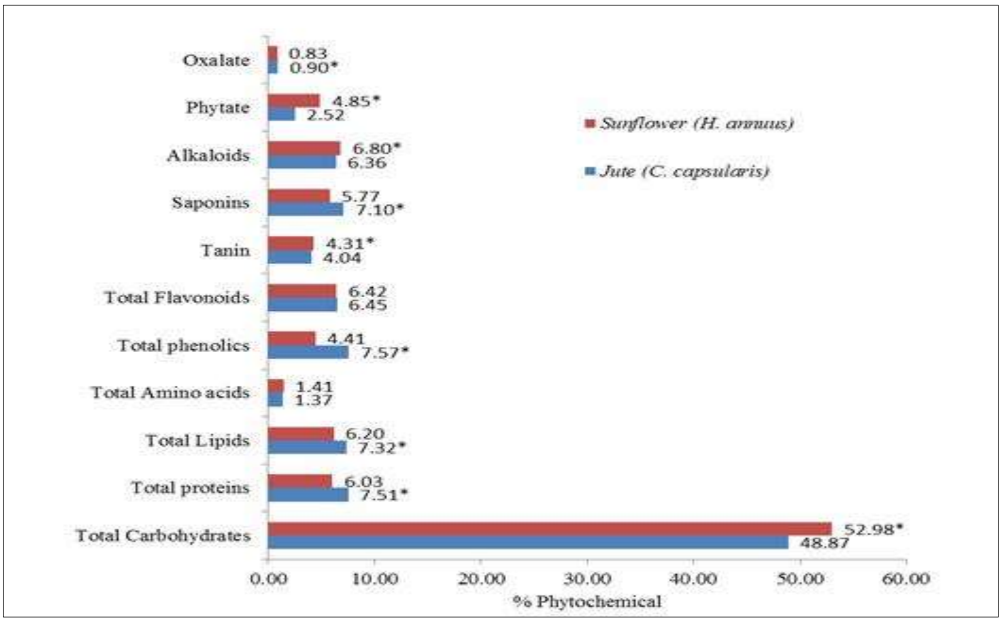


Figure 1: Phytochemical variations of the Jute (*C. capsularis*) and sunflower (*H. annuus*) leaves
Note: Asteroid (*) over the bars indicate that the mean % of 3 observations are significantly ($P < 0.05$) higher than the other, while comparing one type of plant leaf with the other within the column

Phytochemical mediated reduction is the most common bottom-up approach for syntheses of AgNPs (Sivaraman *et al.*, 2009; Song and Kim, 2009; Mohammad, 2010; Krishnaraj *et al.*, 2010; Roy and Barik, 2010; Kumar, 2012; Lalitha *et al.*, 2013). There are still several scopes for improvement in green pathway for a single step rapid synthesis of AgNPs at room temperature by different modifications. Jute (*C. capsularis*) and sunflower (*H. annuus*) both are the best economic crops having many uses throughout the world including India (Roy and Barik, 2012a, 2012b Roy, 2014, 2015). They are well known for producing a variety of secondary chemicals instead of primary one and some of which possess a wide variety of biological, pharmaceutical as well as reducing properties (Leela and Vivekanandan, 2008; Mohammad, 2010; Mukherjee *et al.*, 2012; Roy and Barik, 2012b; Cao *et al.*, 2013; Roy, 2014). Thus, sunflower leaf extract and sunflower oil was already found to be promising in the development of AgNPs (Leela and Vivekanand, 2008; Mohammad, 2010; Thakore *et al.*, 2014). Also microwave assisted in situ growth of AgNPs on TEMPO-oxidized jute fibers was reported by Cao *et al.*, 2013. However, the synthesis of AgNPs using such plant constituents has not yet been fully studied along with their antimicrobial activity. So keeping in view the advantages, by using the reducing properties of jute and sunflower leaf constituents, we have synthesized AgNPs by single step green bottom-up approach through $AgNO_3$ at room temperature within few minutes and check its antibacterial as well as antifungal activity.

Materials and Methods:

1. Collection and preparation of the plant extract

The freshly harvested mature leaves of *C. capsularis and H. annuus*were collected randomly growing in fields near Chinsurah Rice Research Center (22°53' N, 88°23' E), Hooghly, West Bengal, India. Leaves were initially rinsed with distilled water and dried by paper toweling for extraction and analysis of phytochemicals. Each kind of leaves (10g) were cut into fine pieces and extracted through 100 ml 30% ethanol (EtOH) for 24h at room temperature (30°C). The crude extracts were filtered through Whatman`s No.41 filter paper and stored at 4°C for the synthesis of AgNPs.

2. Phtochemical analysis of the plants

The leaves were dipped in different solvents for extraction of different primary and secondary chemicals. The chemicals were estimated by various biochemical analysis, such as total carbohydrates (DuBois *et al.*, 1956), total proteins (Lowry *et al.*, 1951), total lipids (Folch *et al.*, 1957), total amino acids (More and Stein 1948), total phenolics (Bray and Thorpe, 1953), total flavonoids (Zhishen*et al.,* 1999), tanin (Trease and Evans, 1983), saponins (Trease and Evans, 1983) and alkaloids (Harborne, 1973), phytate (Reddy and Love, 1999) and oxalate (Day and Undrwood, 1986). Determination of each biochemical analysis was repeated for three times and expressed in percent dry weight basis.

3. Synthesis of AgNPs

The aqueous solution of 1 mM silver nitrate ($AgNO_3$) [analytical grade (AR), purchased from E. Mark (India)] was prepared and used for the synthesis of AgNPs. Each kind of plant extract (5 ml) was added into 50 ml of aqueous solution of 1 mM $AgNO_3$ for reduction of Ag+ to Ag^0. The reaction mixture was incubated (10 minutes) at room temperature till the turn up of green to brown colour. The particles were isolated by centrifugation (6000 rpm up to 15 minutes), repeated washing and drying at 70°C for further characterization.

4. Characterization of synthesized AgNPs

The reduction of Ag^+ to Ag^0 was monitored by measuring the UV-Vis spectrum of each reaction mixture at different time intervals (10, 20, 30, 40, 50, 60, 120, 180, 240, 300 minutes) within the range of 370-500 nm in the UV-Vis spectrophotometer (Shimadzu UV-VIS Spectrophotometer) because the absorption spectrum of aqueous $AgNO_3$ and green synthesized AgNPs solution exhibited λ_{max} at about 220 nm and 430 nm, respectively. The EtOH used as a blank reference. The isolated dried and powdered AgNPs were used for SEM and TGA study. A thin film of each sample was prepared separately on a small glass cover slip (3x3 mm), and set on a copper stab for electron microscopy using Hitachi made Scanning Electron Microscope (SEM) (Model: S530 with IB2 ion cotter, Japan). A carefully weighted quantity of the AgNPs of each was subjected to TGA on a Parkin-Elmer Diamond TG/DTA instrument at heating rate of 10˚C/min under nitrogen atmosphere.

5. Antimicrobial assay

The green synthesized AgNPs from Jute (*C. capsularis*) and sunflower (*H. annuus*) leaf extracts were tested for their antimicrobial activity against three bacterial strains [*Enterobacter aerogenes* (MTCC-111), *Staphylococcus aureus* (MTCC-9542) and *Escherichia coli* (MTCC-8933)] and four Fungal strains [*Colletotrichum gloeosporioides* (MTCC-3414), *C. lindemuthianum* (MTCC-2015), *Fusarium moniliformis (MTCC-1254)* and *Alternaria alternata (MTCC-8459)*], by well diffusion method as described by Thombre*et al.*, 2012. In these bioassay (antibacterial and antifungal) experiments, six treatments [aqueous $AgNO_3$ (A), EtOH (B), jute leaf extract (J), sunflower leaf extract (S), jute AgNPs (J2) and sunflower AgNPs (S2)] were considered to compare their relative antimicrobial efficacy in terms of their zone of inhibition (ZI). Among the six treatments, aqueous $AgNO_3$ (A) and EtOH (B) were used as negative control. The plant extract (J, S) and green synthesized AgNPs (J2, S2) were added in the well at primary concentration of 10µg/ml whereas $AgNO_3$ (A) and EtOH (B) were used in 1 mM and 30% concentration, respectively. Each experiment was done in triplicate.

5.1 Antibacterial assay

In a Bio safety chamber, under aseptic condition, 30 ml of nutrient agar (13g nutrient broth powder in 1000 ml double distilled water) was poured into pre-sterilized Petri plates (9 cm diameter) and allowed to solidify (5-6 mm thickness). Once the media solidifies it was then inoculated /spreaded with 10 µl of broth culture of *E. aerogenes*, *S. aureus* and *E. coli*, seperately. With the help of sterile cork borer(s) 3 wells (5 mm diameter) were formed separately in each agar plates and 10 µl of each treatment were added aseptically. All plates were then incubated at 37^0C for 24 hrs and the different levels of ZI around the well as indicated by clear area devoid of microbial growth were measured (mm) and expressed as average of three readings.

5.2 Antifungal assay

The antifungal activity of Ag-NPs against *C. gloeosporioides*, *C. lindemuthianum*, *F. moniliformis* and *A. alternata*as models for fungi was investigated. The nutrient agar medium (NA) under aseptic condition was prepared in Petri plates (9 cm diameter) and dried overnight in an incubator at 37^0C. Next day the Malt Extract Soft Agar (MESA) was prepared in test tubes and respective fungal spore (stock) suspensions were added in each pre-marked test tubes. Then MESA with fungal spore suspensions were overlay in respective pre-marked plates and dried 1 hour. After dried the plates, four cups (5 mm diameter) were cut in each plate separately with the help of sterile cork borer(s). Lastly, 10µl of each treatment (J, S, J2 and S2) was poured into the pre-marked respective cups aseptically to find the ZI. The bioassay was also conducted for A and B (negative control) side by side in separate Petri plates but due to their null effect we discard them. All plate were then incubated at 37^0C for 24 hrs and the different levels of zone of inhibition were measured similarly as mentioned in antibacterial assay.

6. Data analysis

All the data of phytochemical regime and antimicrobial activity were analyzed using one way ANOVA, Tukey HSD and Pearson correlation (Zar, 1999). All the statistical analysis was performed using the statistical program SPSS v. 13.0 (SPSS, 2004).

Results:

1. Phytochemical regime

The biochemical analyses of jute and sunflower leaves represents variation in both primary (carbohydrates, proteins, lipids and amino acids) and secondary (Phenols, flavonoids, tanin, saponins, alkaloids, phytate and oxalate) chemicals which is very much similar with the previous report of Roy and Barik, 2012b and Roy, 2015. The phytochemical regimes of the two plants are presented in figure 1. In the primary chemicals, only carbohydrates (52.98%) was significantly ($P < 0.05$) higher in sunflower whereas proteins (7.51%) and lipids (7.32%) was significantly ($P < 0.05$) higher in jute (figure 1). But in secondary chemicals, Tanin (4.31%), alkaloids (6.80%) and phytate (4.85%) was significantly ($P < 0.05$) higher in sunflower whereas phenols (7.57%), saponins (7.10%) and oxalate (0.90%) was significantly ($P < 0.05$) higher in jute (figure 1). The relative concentration of primary to secondary chemicals in jute and sunflower was 1.86 and 2.00, respectively.

2. Chacterstics of phytofabricated AgNPs

During green synthesis of AgNPs through the plant extracts changes in colour from green to brown was observed spectral analysis in frequency range of 270 to 500 nm at room temperature and which was represented the peak at around 420-430 nm for long time interval (10-300 minutes) specific for the synthesis of AgNPs with longer stability (figure 3).

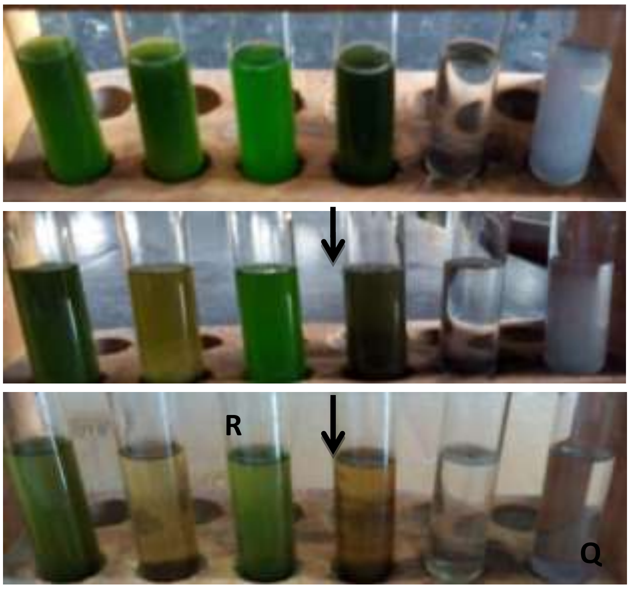


Figure 2: Picture show the colour change during the reaction of Ag^+ into AgNPs of Jute (*Chorchoruscapsularis*) and sunflower (*Helianthus annuus* L.) leaves (before reaction [P], reaction after <10 minutes [Q] and reaction after >240 minutes [R] only in J2 and S2) . Note: From left to right side the test tubes are representing EtOH extract of Jute leaf [J], jute AgNPs [J2], EtOH extract of sunflower leaf [S], sunflower AgNPs [S2], EtOH [B] and Aqueous $AgNO_3$ [A], respective

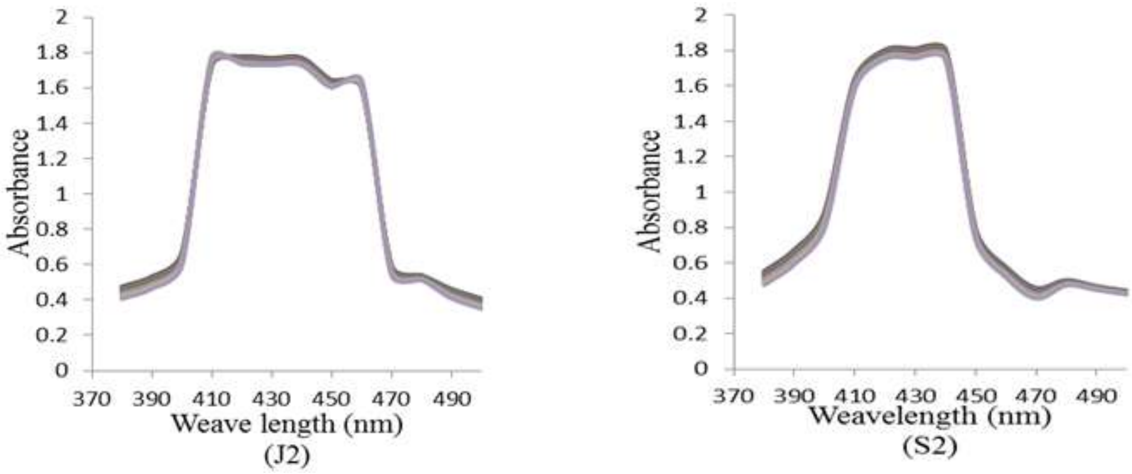
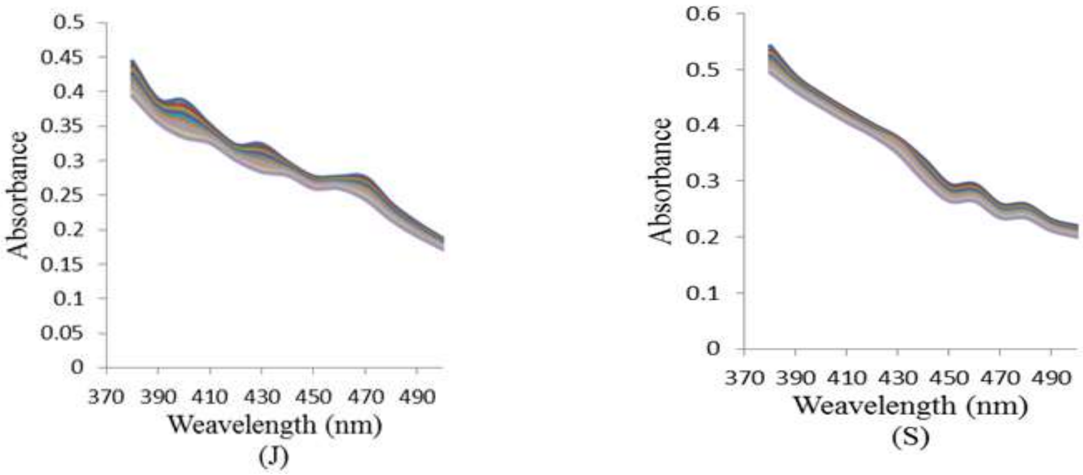
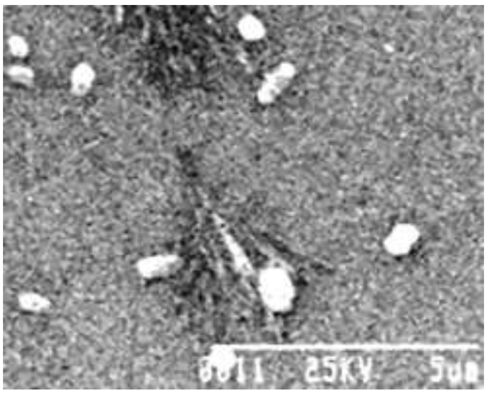
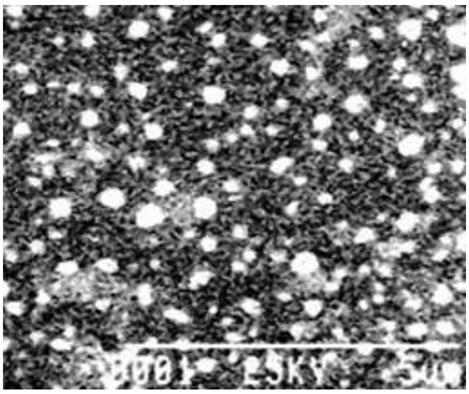


Figure 3: UV-Vis absorption spectra recorded at different time intervals (10, 20, 30, 40, 50, 60, 120, 180, 240, 300 minutes) of AgNPs synthesized from Jute (*C. capsularis*) leaves (J2) and sunflower (*H. annuus* L.) leaves (S2) like previous researchers (figure 2). The brown colour due to the reduction of Ag^+ confirms the formation of AgNPs and was characterized by UV-Vis Spectroscopy, SEM (scanning electron microscope) and TGA (Thermal gravimetric analysis) as in Roy and Barik, 2010. The reduction of pure silver ions (Ag^+) was estimated by UV-Vis





Jute AgNPs



Sunflower AgNPs

Figure 4: UV-Vis absorption spectra recorded at different time intervals (10, 20, 30, 40, 50, 60, 120, 180, 240, 300 minutes) of EtOH extract of Jute (*C. capsularis*) (J) and sunflower (*H. annuus*) leaves (S)

Figure 5 The SEM images of AgNPs synthesized from Jute Note: (*C. capsularis*) and sunflower (*H. annuus* L.) leaves at 25.0 kV × 1 k

The band at 420-430 nm can be attributed to the property surface plasmon resonance (SPR) due to oscillation of electrons (Mie scattering) for strong interaction of light with the AgNPs. Whereas the EtOH extract of jute (J) and sunflower (S) showed almost straight gradually downward spectrum with minute undulation (figure 4). In both cases EtOH act as blank. The λ_{max} of AgNPs (1.741-1.729 and 1.811-1.754 for J2 and S2, respectively) was observed at around 430 nm whereas in EtOH extract it was 0.444-0.394 and 0.542-0.495 at around 380 nm, respectively within the time span of 10-300 minutes. The SEM image provided roughly spherical
Figure 6 Plates showing antibacterial activity of the six treatments (Aqueous $AgNO_3$ [A], *EtOH [B], EtOH extract of Jute leaf [J], EtOH extract of sunflower leaf [S], jute AgNPs [J2] and sunflower AgNPs [S2]) against three bacterial strains (*E. aerogenes* [MTCC-111], *S. aureus* [MTCC-9542] and *E. coli* [MTCC-8933]).*Treatment(s) without any activity topography of AgNPs was about 65±5 (J2) and 55±5 (S2) nm in size (figure 5). The density of the synthesized AgNPs was higher in case of sunflower mediated phytofabrication than the jute leaves (figure 5). TGA data of the synthesized AgNPs showed steady weight loss (14.23 and 16.54% in J2 and S2, respectively) due to desorption of its bioorganic compounds with increasing temperature range of 160˚–550˚C as in Roy and Barik, 2010.

Table 1: One way ANOVA result between groups (treatments {Aqueous $AgNO_3$ [A], *EtOH [B], EtOH extract of Jute leaf [J], EtOH extract of sunflower leaf [S], jute AgNPs [J2] and sunflower AgNPs [S2]}) against the three bacterial and four fungal strains

Microbs	*Sum of Squares*	*Mean Square*	$F_{5,17}$	*p*
Bacterial Strain				
E. aerogenes	985.167	197.033	545.631	0.0001
S. aureus	1303.000	260.600	625.440	0.0001
E. coli	1680.278	336.056	1099.818	0.0001
Fungal strain				
C. gloeosporioides	381.944	76.389	1417.526	0.0001
C. lindemuthianum	365.629	73.126	1196.605	0.0001
F. moniliformis	1902.389	380.478	2337.407	0.0001
A. alternata	331.612	66.322	1388.142	0.0001

Note: Treatment without any activity

Table 2 Correlations (Pearson) between/ within treatments and microbial strains

Microbs	Treatments	EA	SA	EC	CG	CL	FM
Bacterial strains							
E. aerogenes	0.375						
S. aureus	0.029	0.129					
E. coli	0.293	0.731**	0.525*				
Fungal strains							
C. gloeosporioides	0.793**	0.536*	0.319	0.733**			
C. lindemuthianum	0.785**	0.541*	0.324	0.735**	0.997**		
F. moniliformis	0.834**	0.497*	0.280	0.712**	0.979**	0.973**	
A. alternata	0.803**	0.530*	0.312	0.732**	0.999**	0.996**	0.986**

Note: orrelation is significant at 0.05 (*) and 0.01 (**) level [2-tailed]; Here, EA- *E. aerogenes;* SA- *S. aureus* EC-*E. coli;* CG- *C. gloeosporioides; CL- C. lindemuthianum; FM- F. moniliformis*

3. Antimicrobial activity

During antimicrobial (antibacterial and antifungal) activity the ZI was measured for all the treatments except EtOH (B) because of its higher evaporation and diffusion created null effect against the microbes. In antibacterial bioassay aquous $AgNO_3$ (1mM) have insignificant ($P>0.05$; Tukey HSD) ZI of 15.17±0.60, 17.17±0.44 and 18.50±0.29 mm on the three strains (*E. aerogenes*, *S. aureus* and *E. coli*) respectively after 24 hr of incubation (figure 6, 7). Significant ZI was observed in *S. aureus* (21.17±0.60 mm) and *E. aerogenes* (14.83±0.44 mm) for EtOH extract of the leaves (J and S) respectively (figure 6, 7). The inhibitory (ZI) effacasy of jute AgNPs (J2) [12.50±0.29, 11.50±0.29 and 17.00±0.58 mm] was significantly ($P<0.05$; Tukey HSD) lesser than sunflower AgNPs (S2) [18.50±0.29, 18.17±0.44 and 21.83±0.44 mm] in respective bacterial strains. The toxic response of both AgNPs (J2 and S2) was higher in *E. coli* ($F_{5,17}=1099.818$ at $P<0.0001$) relative to the other bacterial strains (table 1). Positive but insignificant ($P>0.05$) correlations with treatments were also observed (0.375, 0.028, and 0.293) with the three (*E. aerogenes*, *S. aureus* and *E. coli*) respective strains (table 2).

In antifungal response, only the phytofabricatedAgNPs showed ZI on the selected 4 fungal (*C. gloeosporioides*, *C. lindemuthianum*, *F. moniliformis*and*A. alternate*) strains (figure 8,9). The ZI for the sunflower AgNPs (S2) was significantly ($P<0.05$) higher (10.83±0.23, 10.77±0.23, 20.93±0.49 and 9.87±0.23 mm) than jute mediated AgNPs (J2) (8.50±0.23, 8.07±0.26, 22.63±0.29 and 8.23±0.20 mm) against the 4 strains (*C. gloeosporioides*, *C. lindemuthianum*, *F. moniliformis*and *A. alternate*), respectively (figure 8,9). The treatments and respective strains (*C. gloeosporioides*, *C. lindemuthianum*, *F. moniliformis*and *A. alternate*) were showed positively significant correlation [0.793, 0.785, 0.834 and 0.803 ($P<0.01$)] with each other (table 2). The toxic response of both AgNPs was higher in *F. moniliformis* ($F_{5,17}=2337.407$ at $P<0.0001$) relative to the other fungal strains (table 1).

The bioactive AgNPs have showed highly significant ($P<0.0001$; df=5,17) value when compared between treatments against the selected MTCC strains of bacteria (*E. aerogenes*

[F=545.631], *S. aureus* [F=625.440] and *E. coli* [F=1099.818]) and fungai (*C. gloeosporioides*[F=1417.526], *C. lindemuthianum*[F=1196.605], *F. moniliformis*[F=2337.407] and *A. alternate* [F=1388.142]) (table 1). In both antimicrobial assays the phytofabricatedAgNPs, specially of sunflower have inhibitorier efficacy, due to the size and density of NPs compare to the other, against the fungal rather than bacterial strains (table 1).

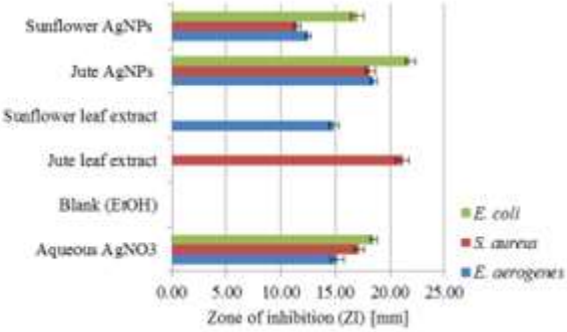


Figure 7: Antibacterial activity of aqueous AgNO3, plant extracts and synthesized AgNPs along with blank against three bacterial strains (E. aerogenes [MTCC-111], S. aureus [MTCC-9542] and E. coli [MTCC-8933)]

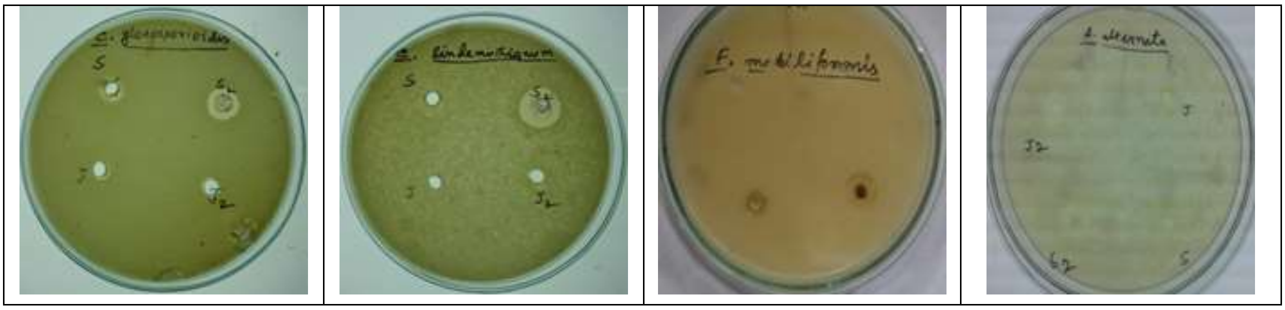


Figure 8: Plates showing antifungal activity of the six treatments (*Aqueous $AgNO_3$ [A], *EtOH [B], EtOH extract of Jute leaf [J], EtOH extract of sunflower leaf [S], jute AgNPs [J2] and sunflower AgNPs [S2]) against four fungal strains (*C. gloeosporioides*[MTCC-3414], *C. lindemuthianum* [MTCC-2015], *F. moniliformis*[MTCC-1254]and *A. alternata*[MTCC-8459]). *Treatment(s) without any activity

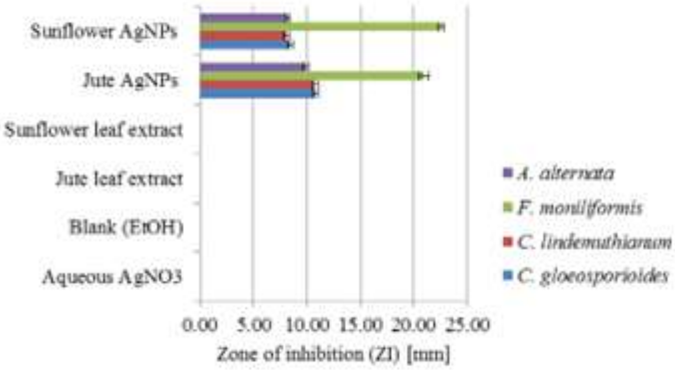


Figure 9: Antifungal activity of aqueous $AgNO_3$, plant extracts and synthesized AgNPs along with blank against four fungal strains (*C. gloeosporioides*[MTCC-3414], *C. lindemuthianum* [MTCC-2015], *F. moniliformis*[MTCC-1254]and *A. alternata*[MTCC-8459])

Discussion:

Biological synthesis of AgNPs have proven to be better methods over the other due to slower kinetics and better manipulation on control over synthesis and their stabilization (Ahamed *et al.*, 2015a, 2015b; Ghaffari-Moghaddam*et al.*, 2014; Salam *et al.*, 2012; Prasad, 2014). In the present scenario, plants are better synthesizers due to theabundance of the plant resources when compared to other forms of biological resources (Dipankar and Murugan 2012; Ghaffari-Moghaddam and Hadi-Dabanlou, 2014; Krishnaraj*et al.*, 2010; Kumar, 2012; Lalitha *et al.*, 2013). Also, plants provide a better platform for nanoparticles synthesis as they possess a broad variability of secondary metabolites for particle formation and stabilization through natural capping agents (Nagajyoti *et al.*, 2011; Shankar *et al.*, 2003; Song and Kim, 2009; Veerasamy *et al.*, 2010; Roy and Barik, 2010; Rout *et al.*, 2012). It was reported that plants extracts contain biomolecules including carbohydrates, proteins, polyphenols, flavonoids, sterols, triterpenes, alkaloids, alcoholic compounds, polysaccharides, saponins and reducing enzymes which could be used as reductant and stabilizar to the formation of AgNPs (Vigneshwaran *et al.*, 2006; Cao *et al.*, 2013; Thakore *et al.*, 2014; Prasad, 2014).

Several researchers including India have employed a single-step green approach to synthesize AgNPs from plants as its biomolecules induce the reduction of Ag^+ from $AgNO_3$ to AgNPs (Ahmed *et al.*, 2015a, 2015b; Anuj and Ishnava, 2013; Prashar *et al.*, 2009; Roy and Barik, 2010; Salam *et al.*, 2012; Saxena *et al.*, 2010; Song and Kim, 2009; Veerasamy *et al.*, 2010; Rout *et al.*, 2012; Ghaffari-Moghaddam and Hadi-Dabanlou, 2014; Krishnaraj*et al.*, 2010; Kumar, 2012; Lalitha *et al.*, 2013). The process of reduction is extra cellular, fast, ecofriendly and cost effective leading to the development of in vitro green AgNPs (Sivaraman *et al.*, 2009; Salam *et al.*, 2012; Song and Kim, 2009). Though, plants during glycolysis produce a large amount of H+ ions along with NAD which acts as a strong reducing agent and responsible for in vivo formation of AgNPs (Salam *et al.*, 2012; Prasad, 2014). A number of plants are being currently investigated for their role in the synthesis of AgNPs (Ahmed *et al.*, 2015a; Anuj and Ishnava, 2013; Salam *et al.*, 2012; Prasad, 2014). The polyol components and the water-soluble heterocyclic components are largely accountable for the reduction of Ag^+ and the stabilization of the nanoparticles, respectively (Salam *et al.*, 2012; Prasad, 2014). There are also reports on reductases and polysaccharides as factors involved in biosynthesis and stabilization of the AgNPs, respectively (Salam *et al.*, 2012; Prasad, 2014; Vigneshwaran *et al.*, 2006). In *Capsicum annuum*and *Azadiractaindica*, proteins (amine groups), terpenoids and reducing sugars have played a reducing and controlling role during the formation of AgNPs (Li *et al.*, 2007; Namratha and Monica, 2013). Sunflower oil has capability to reduce and stabilize the hexagonal growth of AgNPs due to its extended phenolic system and they have cytotoxicity against human lung carcinoma (Thakore *et al.*, 2014).

Today, many antimicrobial agents are available but limited in clinical applications, because of their many complications after using these drugs. So requiring new drugs with less complication like AgNPs having antimicrobial activities and which from different sources were successfully investigated throughout the world. PhytofabricatedAgNPs with antimicrobial properties have also been investigated against different microbes which actually depend on size, shape, environmental conditions (pH, ionic strength) and capping agent (Baker *et al.*, 2005; Durairaj *et al.,* 2012; Gordon *et al.,* 2010; Jo *et al.,* 2009; Kim *et al.,* 2012; Kora and Arunachalam, 2011). Recently, efficient antimicrobial activity of green AgNPs was observed against multi drug resistant (MDR) and highly pathogenic bacteria (*P. aeruginosa*, *S. aureus*, *S. typhi*, *S. epidermidis* and *E. coli*) (Singh *et al.*, 2014; Rai *et al.,* 2009; Rai *et al.,* 2012) and fungai (*C. gloeosporioides*) (Chowdappa *et al.*, 2014). Historically recognized inhibitory action of Ag^{+} and AgNPs on bacterial cells is due to the interaction with thiol groups in their key respiratory enzymes (Gordon *et al.,* 2010; Kora and Arunachalam, 2011). Whereas, antifungal drugs or AgNPs inhibit the normal budding process through the destruction of ergosterol mediated membrane integrity by the formation of ‘‘pits’’ on their surfaces to form pores and then cell death (Jo *et al.,* 2009; Kim *et al.,* 2012).Due to their antimicrobial properties, AgNPs have been used most widely in the health industry (biomedical and nanomedical fields), food storage, textile coatings, agriculture, constraction and a number of environmental applications (Bao *et al.*, 2011; Gordon *et al.,* 2010; Mohanpuria *et al.,* 2008; Sukritha *et al.,* 2012; Namratha and Monica, 2013; Rai *et al.*, 2009, 2012; Rout *et al.*, 2012; Singh *et al.*, 2014; Prasad, 2014; Rizwan *et al.*, 2014).

In our study, the bacterial strains (*E. aerogenes*, *S. aureus* and *E. coli*) were nonpathogenic but opportunistic pathogenicity found due to direct infection or due to the production of toxins (Samuel, 1996).In other instances,the fungal strains (*C. gloeosporioides*, *C. lindemuthianum*, *F. moniliformis*and *A. alternate*) were also nonpathogenic but they become pathogenic in different conditions. *Colletotrichum* is one of the major plant pathogen causing anthracnose or black spot disease on variety of hosts [*C. gloeosporioides* on mango (Gautam, 2014), *C. lindemuthianum* common bean (Pinto *et al.*, 2012)] whereas *F. moniliformis*is primarily a pathogen of maizeand alsocause disease in other crop species (Jurgenson *et al.*, 2002). Even *Alternaria alternata* produce different host-specific toxins to cause diseases like leaf spot in strawberry and other diseases on several host plants (Tsuge *et al.*, 2013).

The growth inhibition effect (ZI) of AgNPs synthesized from jute and sunflower leaves relative to the plant phytochemical constituents were investigated against the selected microbial strains along with controls similar to the previous reports of many researchers. Apparently, the ZI against bacterial strains was significantly larger than the fungal strains (figure 7, 9). But the green synthesized AgNPs exhibited a significant as well as potent antifungal activity compare to antibacterial activity (table 1) at very low concentration (10µg/ml), due to more reactivity for their large surface area, over the phyto-constituents of the selected plants. Even the sunflower mediated AgNPs have more potency due to their size and abundance against the fungal strains rather than the bacterial strains (table 1). Therefore, it can be concluded that synthesized green AgNPs from sunflower (S2) can be used in aquatic suspension or mist against the selected fungal pathogens of different plant species including economic crops to fruit plants but deserving further investigation for broad scale agricultural applications. The use of AgNPs can specially opens a new scope in agriculture for the preparation of resistant (disease and drought) varieties as well as biologically degradable target specific synthetic pesticides for the economic pathogens and pests in near future. They can also provide major application in medicine, disinfectant, environment, building materials, water quality, textiles, cosmetics, paints, soil fertility, pest control, agriculture and aquaculture, etc. The phytofabricated AgNPs will also eliminate the problem of chemical agents as well as factor regulation and thus it become simple, biocompatible, benign to environment, economically sustainable and eco-friendly to us.

Conclusion:

The bottom up phytofabrication of silver nanoparticles (AgNPs) from jute (*C. capsularis*) and sunflower (*H. annuus*) leaves and their preliminary antimicrobial activity were described in this study. The green synthesis of AgNPs from EtOH extracts of the respective plants were performed through standard protocols. The synthesized AgNPs was confirmed by colour changes (green → brown) within <10 minutes and characterized by UV-visible spectral analysis followed by SEM and TGA. Both phytofabricated and stable AgNPs of about 50-70 nm showed λ_{max} at 430 nm with in a broad range of time intervals (10-300 minutes) at room temperature. The bioactivity was measured through zone of inhibition (ZI) against 3 bacterial and 4 fungal MTCC strains in nutrient agar medium. In antimicrobial assays the phytofabricatedAgNPs, specially of sunflower have inhibitorier efficacy ($P<0.05$; Tukey HSD), due to the size and density at very low concentration (10µg/ml) of NPs compare to the jute, against the fungal rather than bacterial strains.

Author's contributions

SKC designed the whole study including sample collection, chemical analysis, synthesis of AgNPs, data analysis and prepares the manuscript and conducted the antibacterial assay at microbiology laboratory of SKC conducted the antifungal assay at plant physiology and Biochemistry laboratory, GourBanga University.

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REVIEW ON *ASPARAGUS RACEMOSUS* (SHATAWARI) AND ITS PHARMACOLOGICAL ACTIONS

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Abstract:

Asparagus racemosus has a good history of usage as food and herbal remedy. For the treatment of stomach ulcers, liver problems, inflammation, immunological disorders brought on by stress, dyspepsia, and other conditions, all portions of this plant are therapeutically significant. It also acts as a galactogogue and lowers apoptosis. Its water and alcohol extracts have potent anti-inflammatory, immune-stimulating, and antitussive properties. It controls the amounts of cholesterol and blood lipids. Its bioactive components, including as phytochemicals (flavonoids and saponins), are employed in a variety of pharmacological processes. *Asparagus racemosus* is in risk of extinction because of its numerous applications and increasing demand. In order to meet the demand, suitable agricultural techniques and other technology are needed. Shatawari is now offered in a variety of commercial formulations. This brief study sheds insight on the *Asparagus racemosus*'s significance for treating various illnesses.

Keywords: *Asparagus racemosus*, extracts, bioactive moieties, medicinally important.

Introduction:

Perennial *Asparagus racemosus* has a horizontal root stock, substantial roots, and long young shoots that are consumed as vegetables. The Indian herb Shatavari is another name for it. The meaning of the name Shatavari is "the one who has a hundred husbands or is likeable to many." It is crucial for the female reproductive system and is also regarded as a tonic for problems with the reproductive system [1]. In India and the Himalayas, this plant is primarily grown in tropical and subtropical climates. Additionally, this plant is grown in Indonesia, Australia, Sri Lanka, and tropical Africa [2]. The Asparagaceae family includes asparagus [3]. The plant *Asparagus racemosus* has a woody stalk, needle-like leaves, and a tiny white flower [4]. The Greek words "Stalk" and "Shoot" are the origin of the name asparagus. In the realm of asparagus, there are about 200 species. It is typically grown in India. Natural medicine uses some of its species, including Asparagus gonaclades and Asparagus odsendens. *Asparagus racemosus* root has a bitter-sweet flavor and functions as a tonic, aphrodisiac, stomachic, cooling, binding, galactagogue, diuretic, rejuvenating, carminative, and antibacterial. *A. racemosus* root has many positive effects in the treatment of numerous illnesses, including nervous breakdown, diarrhea, inflammation, liver problems, cough, bronchitis, and many other infectious disorders [3]. They also produce highly useful shoots. Aldehyde, ketones, vanillin, and asparagine are found in them. Thiazole and its methyl and ethyl ester are utilized to provide flavor. On a dry basis, their ripe fruit and blossom contain 2.5% rutin and quercetin. In leaves, you can find diosgenin and quercetin 3-glucuronide. *Asparagus racemosus* bark had antifungal and antibacterial effects.

Powdered roots include about 3% protein, 5.4% saponins, 52.8% carbs, 18% crude fiber, 4.1% inorganic matter, and 5% oil. Due to their diuretic properties, asparagus was employed by the ancient Greeks and Romans. It aids in renal cleansing and inhibits kidney stone development. Additionally, it greatly enhances kidney cellular activity and urine production [5]. Herbalism, often known as ecological healing because it operates within the framework of humanity's shared ecological and evolutionary legacy with the plant kingdom [6], is a marvelously simple yet astonishingly sophisticated method of healing in sync with nature. Herbs have been used for a very long time in cosmetics, food, teas, and most crucially in treatments known as phytomedicine or phytotherapy [7]. The World Health Organization (WHO) estimates that 80 percent of the people in various Asian and African nations utilize herbal medicines, with a focus on specific primary health care issues. There are undoubtedly thousands of different herbs that may be found all over the world, but each one has a unique medicinal use that makes it an Ayurvedic rasayana. It aids in slowing down the ageing process, boosts toughness, improves nervous system health, guards against tumors, neuropathy, dyspepsia, inflammation, and hepatopathy [8,9]. *A. racemosus* is a member of the Liliaceae family and is also known as Satamuli, Satawar, and Satavari. It grows in low elevations all over India. The most bioactive components of asparagus are a category of steroidal saponins, which are found in the plant species *Asparagus racemosus*, which is rich in phytoestrogens. Along with minerals like Mg, Ca, P, Fe, and folic acid, this plant also contains vitamins A, B1, B2, C, and E. Essential oils, asparagine, flavonoids, arginine, tyrosine (kaempferol, quercetin, and rutin), tannin, and resin are some of the other main chemical components of asparagus [10, 11].

Figure 1: Pictures of *A. racemosus* showing flowers, leaves, fruits, tuberous roots

Plant characteristics

Kingdom: Plantae

Clade: Angiosperms

Clade: Monocots

Order: Asparagales
Family: Asparagaceae
Sub family: Asparagoideae
Genus: *Asparagus*
Species: *Asparagus racemosus* L

Medicinal significance

The primary healthy plant of tropical and semitropical India may be *Asparagus racemosus*. Its beneficial use has been acknowledged in Indian and British formularies, and it is frequently recognised for its phytoestrogenic properties. *Asparagus racemosus* has been classified as a rasayana herb in writing and has occasionally been used as an adaptogen to increase an organism's general resistance to a variety of stresses. In addition to its use in the treatment of symptoms and infectious diseases, the plant also has inhibitory, immunostimulant, antidyspepsia, and medicinal effects. In Ayurvedic medicine, the roots are utilized after a procedure and drying regimen. It is frequently used as a female internal reproductive organ tonic, as an agalactogogue (to stimulate breast milk production), in cases of acidity, and as a general health tonic. *A. racemosus* is an old galactogogue that has been used to treat duodenal ulcers in humans as well as in animals. This plant's antioxitocin activity has long been known, and its immunomodulatory qualities are thought to be its underlying cause. It is well known that higher plants contain antifungal and antimicrobial compounds. These compounds have served as a source of inspiration for the development of novel drug compounds, and plant-derived medicines, such as those used in the Unani and Ayurvedic medical systems, have significantly contributed to the treatment of human diseases [12].

Pharmacological activities:

1. Antimicrobial activity

The most common cause of life-threatening illnesses in humans is bacteria, both Gramme positive and Gramme negative, such as various types of Bacillus, Staphylococcus, Salmonella, and Pseudomonas. Because of their numerous natural niches, these species have the capacity to endure extreme conditions [13]. The majority of fatal infectious bacterial illnesses occurred in both developed and developing nations [14]. *A. racemosus* has anti-bacterial and anti-fungal properties in both its inorganic and organic solvent extracts [15]. Using the traditional cylinder technique, the antibacterial properties of *A. racemosus* root extracts were examined. *Staphylococcus aureus, Staphylococcus werneri, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Kleibsella pneumonia*, and *Pseudomonas putida* were among the microorganisms employed. The extract was effective against both gram-positive and gram-negative bacteria. Staphylococcus aureus, a gram-positive bacterium, was the most impacted by the ethanol extracts, which had antibacterial activity equivalent to that of the reference standard medication Gentamycin (25g) [16]. According to experimental results, methanol extracts were highly anticandidal to several Candida species. The MFC (Minimum fungicidal concentration) values varied from 5 to 0.625 mg/ml, whereas the MIC (Minimum inhibitory concentration) values ranged from 2.5 to 0.312 mg/ml [17].

Phytoestrogenic activity

Plant-derived oestrogens are in demand now since hormone replacement treatment is risky and ineffective [18]. Phytoestrogens are chemical substances found in any plant that resemble ovarian, placental, and their active metabolites in terms of structure or activity [19]. The root of *A. racemosus* has a good lactogenic action [20]. In the Ayurvedic medical system, root extract from *A. racemosus* is highly helpful in promoting milk supply during lactation [21]. In vivo uterine contractions caused by oxytocin were discovered to be inhibited by the saponin-rich portion of the plant [22]. Due to the effect of released corticoids or prolactin, an aqueous extract of *A. racemosus* roots increases the weight of mammary glands in post-partum and oestrogen-primed rats as well as the weight of the uterus in the oestrogen-primed group [23]. While the alcoholic extract of *A. racemosus* has an oestrogenic impact on both the genital organs and the female mammary glands. Women with inadequate milk supply can benefit from *Asparagus racemosus* formulations like Ricalex® tablets (Aphali Pharmaceuticals; 40 mg concentrated root extract per tablet) [24]. The Himalaya Drug Co., Bangalore created the herbal remedy "U-3107" or EveCare®, which contains 32 mg of *Asparagus racemosus* extract per 5ml of syrup and is used to treat a variety of menstrual diseases and threatening abortions [26]. Additionally, "EveCare" pills have been shown to be successful in treating dysfunctional uterine bleeding (DUB). The regularisation of the menstrual cycle can also be related to the endometrium's localised endovascular thrombosis, which was brought on by large dosages of phytoestrogens [27]. Premenstrual syndrome (PMS) in female humans has been successfully treated using a proprietary medication made from *Asparagus racemosus* (approximately 85 parts) [28]. Patients with dysmenorrhea were also shown to benefit with "EveCare" [29]. Another polyherbal formulation that increases uterine weight and uterine glycogen is Menosan®, which contains 110 mg of *Asparagus racemosus* extract per pill [30]. Without increasing the amount of endogenous oestrogen, phytoestrogen functions by interacting directly with the oestrogen receptor. Additionally, "Menosan" has been investigated as a potential postmenopausal symptom therapy [31]. The effect of Satavari on the male reproductive system is likewise well recognised. Rats given *A. racemosus* were shown to have noticeably large testes [32].

Effect in neurodegenerative disorders

The psychotropic medication "Mentat" is very effective in treating alcohol withdrawal symptoms like tremors, convulsions, hallucinations, and anxiety in ethanol-administered rats [33]. Various formulations of *A. racemosus* have effective neurodegenerative potential. While "EuMil" therapy restored normalcy to the disturbed levels of nor-adrenalin, dopamine, and 5-hydroxytryptamine in animal brains [34]. The duration of immobility time was significantly reduced in the groups treated with methanolic extract of *A. racemosus* (100, 200, and 400 mg/kg), an antidepressant drug, and the forced swimming test (FST), a rodent model of depression used to evaluate the potential antidepressant activity. Methanolic plant extract (100, 200, and 400 mg/kg) pretreatment for 7 days prior to the learned helplessness test (LH) significantly boosted avoidance responses and decreased escape failures compared to control rats [35]. *A. racemosus* methanolic extract activates the adrenergic system and improves serotonergic-mediated behaviour, indicating that the serotonergic pathway is involved in the antidepressant effect.

Anti-oxidant activity

An investigational study on the ability of methanolic extract from *Asparagus racemosus* roots to protect mice's hippocampus and striatal neurons from damage caused by kainic acid (KA). Mice under anaesthesia were given intrahippocampal and intrastriatal injections of KA, which resulted in excitotoxic lesions in the brain. GSH acts as a nucleophilic scavenger of toxic compounds and as a substrate in the GPx-mediated destruction of hydroperoxides to stop the accumulation of toxic levels in brain tissues, so GSH is considered to have good antioxidant property. After KA injection, decreased glutathione peroxidase (GPx) activity and reduced glutathione (GSH) content were observed. The GPx activity and GSH content were improved in the *Asparagus racemosus* extract-treated mice, whereas membranal lipid peroxidation and protein carbonyl levels were decreased. The study's findings led researchers to the conclusion that plant extracts work as antioxidants by reducing the oxidative damage caused by free radicals [36].

Anti-diarrhoeal activity

One of the most significant health issues is diarrhoea. In one year, diarrhoea is projected to cause the deaths of 2.2 million people worldwide, the majority of whom being newborns and young children under the age of five [37]. According to Ayurvedic scriptures including the Sushruta Samhita and Sharangdhar Samhita, satavari is useful in treating Atisar (diarrhoea), Pravahika (dysentery), and Pittajshool (gastritis) [38]. Based on the ability of castor oil to release ricinoleic acid, which causes irritation and inflammation of the intestinal mucosa and, in turn, causes the release of prostaglandins, which stimulate motility and secretion and cause diarrhoea in rats, evaluation of the ethanol and aqueous extracts of *A. racemosus* roots was conducted. The action of this extract can be ascribed to the reduction of prostaglandin manufacture, which in turn reduces gastrointestinal motility and secretion. Very considerable anti-diarrheal activity was discovered, and results shown were similar to loperamide, an anti-diarrheal medicine.

Anti-inflammatory activity

NO is a key player in the immune system and is detected in high concentrations during the development of tumours, autoimmune processes, infectious and inflammatory disorders, and other immunopathological conditions. Increased levels of tumour necrosis factor (TNF) synthesis have been linked to the onset of diabetes, septic shock, tumorigenesis, rheumatoid arthritis, psoriatic arthritis, and inflammatory bowel disease. The methanolic extracts significantly inhibited production of TNF- and IL-1 in mouse macrophage cells. generation is linked to both acute and long-term inflammation. Methanolic and aqueous extracts at 100 g/mL inhibited NO generation in a statistically significant way. The methanol and aqueous extracts of the three root extracts were shown to have considerable inhibitory effects on NO generation in this investigation. Inflammation is mostly controlled by macrophages [39].

Anti-dyspepsia activity

A. racemosus has furthermore been utilised in Ayurveda to cure dyspepsia. The herb was discovered to exhibit effects equivalent to those of the allopathic medicine metoclopramide, a dopamine antagonist used to speed up stomach emptying in the treatment of dyspepsia. In this investigation, the stomach emptying half-time was measured after administration of 2g of powdered *A. racemosus* root vs a conventional dose of metoclopramide (10 mg pill). The effects of *A. racemosus* and metoclopramide did not differ statistically significantly. According to this study's hypothesis, Satavari may work as a moderate dopamine agonist [40].

Adaptogenic activity

A class of plant medicines known as "Rasayana" not only strengthens the body's defence mechanisms but also fosters physical and mental health, as well as strength and a long life. The goals of 'rasayanas' include rogapaharanasamartha (raising resistance to diseases), medhabalakaram (boosting intelligence and physical strength), vayasthapana (delaying ageing), ayukaram (expanding life span), and ayukaram (retarding ageing) [41]. These are comparable to "adaptogens," which are substances that boost an organism's non-specific resistance to a range of stimuli. *A. racemosus* is being researched as a possible treatment for cisplatin adverse effects such normalised intestinal hypermotility and stomach emptying. *A. racemosus* normalised cisplatin-induced intestinal hypermotility and reversed the effects of cisplatin on gastric emptying [42]. The major component of "Satavari mandur" (SM), an ayurvedic herbo-mineral preparation, is the root extract of *A. racemosus*, a potent antiulcerogenic drug. The herbal supplement "Siotone" has considerable adaptogenic properties and can reverse the effects of chronic stress on the body's biochemical, physiological, and behavioural systems [43]. Like "Siotone," "EuMil" demonstrated notable adaptogenic and antistress action. Studies on the acute and subacute toxicity of "Siotone" and "EuMil" revealed that neither substance had any harmful effects. *Asparagus racemosus*' effects on Amlapitta (hyperacidity), Grahani (ulcerative colitis), Parinam shool (septic ulcer), and Vataj shool (spastic colon) were also studied, and it was found that the symptoms improved. Fresh *Asparagus racemosus* roots extracted with methanol significantly reduced the risk of developing acute stomach ulcers brought on by cold constraint stress, acetic acid, pylorus ligation, aspirin combined with pylorus ligation, and cysteamine-induced duodenal ulcers [44].

Anticancer activity

Asparagus racemosus roots aqueous extract may function as a potent formulation to stop hepatocarcinogenesis brought on by DEN therapy. In Wistar rats pretreated with the aqueous extract of *Asparagus racemosus* roots, immunohistochemical staining of the hepatic tissues revealed the absence of mutated p53 foci while clusters of cells expressing the mutated p53 protein were present. As a result, hepatocarcinogenesis was avoided in Wistar rats that had previously received an aqueous extract of *Asparagus racemosus*' roots [45]. The rat mammary carcinogenesis caused by DMBA is inhibited by *Asparagus racemosus*. Rats fed a diet containing 2% *Asparagus racemosus* displayed a decrease in cancer incidence and the mean number of tumours per animal bearing tumours [46].

Teratogenic effects

By administering methanolic extract of *A. racemosus*, teratogenic effects such as swelling of the legs, slow growth of the foetal body and placental part, and an increase in foetus resorption were observed in post-natal studies, resulting in a smaller litter of pups with higher mortality and delayed development [47].

Cardio protective activity

Due to the production of reactive oxygen species and an increase in serum lipid levels like cholesterol, coronary artery disease and atherosclerosis are caused. A Herbo-mineral compound called Abana' was discovered to have a considerable hypocholesterolaemic impact in rats, indicating the possibility of usage as a cardio-protective drug [48]. Supplements containing *A. racemosus* root powder reduced lipid peroxidation and lowered lipid profiles in a dose-dependent manner. In addition to plasma LDL (low-density lipoprotein) and VLDL (very low-density lipoprotein)-cholesterol decreasing by more than 30%, total lipids, total cholesterol, and triglycerides also fell in the liver [49].

Anti-bacterial activity

The methanol extract of *A. racemosus* roots has also demonstrated significant antibacterial efficacy in vitro against Escherichia coli, Shigella dysenteriae, Shigella sonnei, Shigella flexneri, Vibrio cholerae, Salmonella typhi, Salmonella typhimurium, Pseudomonas putida, Bacillus subtilis, and Staphylococcus aureus [50].

Immunoadjuvant effects

As the animals fed daily with *A. racemosus* aqueous root extract had a considerable rise in antibody titres to Bordetella pertussis as compared to the untreated animals, *A. racemosus* was examined in experimental animals as an immunoadjuvant. Animals with high immunoadjuvant potential had lower death rates and overall better health, which was evidence that a robust immune system had developed. Leucopenia, anaemia, and other adverse effects are brought on by the immunopathological illnesses that cyclophosphamide (CP) is used to treat. In (CP)-treated mice, *A. racemosus* lessens these negative effects while maintaining haemolytic antibody titres. Therefore, *A. racemosus* extracts and formulations help to raise white cell counts, absolute neutrophil counts, and haemagglutinating. As macrophages contribute to the development of intraperitoneal adhesions, *A. racemosus*' increase in macrophages aids in the prevention and control of postoperative adhesions. In addition to Withania somnifera, Tinospora cordifolia, and Picrorhiza kurroa, it has been shown that *A. racemosus*, an immunomodulator and immunostimulant, also dramatically reduces macrophage chemotactic activity and the generation of interleukin-1 and TNF-. According to Ayurveda, the sickness of diminished 'ojas' (essential energy of the body) is known as AIDS. In the ancient medical system, satavari is referred to as the creation of "ojas." As a result, the use of *A. racemosus* as an immunoadjuvant in adjuvant therapy for the treatment of HIV can be carefully considered [51].

Antitussive activity

When mice were given sulphur dioxide to produce coughing, the methanol extract of *A. racemosus* roots had considerable antitussive effect, with the cough inhibition being comparable to that of 10–20 mg/kg of codeine phosphate [52].

Anticandatial activity

The in vitro anticandidal activity of *Asparagus racemosus* roots and tubers extract was examined against Candida parapsilosis, Candida stellatoida, Candida albicans, Candida tropicalis, Candida krusei, Candida guillermondii, and Candida krusei, which are isolated from patients with vaginal thrush. The *Asparagus racemosus* extract displayed a high level of effectiveness against every Candida strain. The extract's inhibitory effect on all of the tested Candida was found to be comparable to that of commonly used antibiotics [53].

Conclusion:

Asparagus racemosus has enormous traditional significance and is utilised in numerous medications. *Asparagus racemosus* is also utilised in herbal treatments including Ayurveda, Siddha, and Unani. Traditional practises have been supported by numerous investigations and scientific studies. The medical and commercial uses for this plant are quite promising. Although significant research has been done on the biological activity and medicinal uses of plants, there are still many opportunities for pharmacological use that need to be investigated. These plants offer a wide range of therapeutic qualities, including antibacterial, hepatoprotective, anti-HIV, antioxidant, and cardiac. There is numerous research that use plant extraction as their reporting method. *Asparagus racemosus* should only be used during pregnancy with caution and in therapeutic doses. Stability can be attained by applying biotechnological techniques like micropropagation and callus culture. Additionally, the development of suitable agro methods and the improvement of environmental conditions will improve the quality of total production, guaranteeing good quality and having phytochemicals in optimal yields. *A. racemosus* commercial production would be encouraged by farmers in order to prevent the exploitation of this plant in the wild and to support the conservation effort. A species with enormous potential is *Asparagus racemosus*. Through contemporary testing and evaluation (pre-clinical and clinical trials) in various medical states, the therapeutic efficacy of *A. racemosus*, which is widely employed in Indian System of Medicine, has been established. These investigations position this homegrown medication as a cutting-edge contender for bioprospecting and drug development for conditions like cancer, ulcer, diabetes, heart disease, male and female infertility, and postmenopausal syndrome. Numerous areas of this plant's function, including its medical uses, still provide endless opportunities for research. Therefore, these plants' phytochemicals and minerals will make it possible to utilise them for therapeutic purposes.

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IN VITRO SHOOT INDUCTION FROM NODAL EXPLANTS OF *CUCUMIS SATIVUS* (L)

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Abstract:

The effect of BAP and NAA + Kn combination on *In vitro* shoot regeneration and elongation was analysed in nodal explants. The percentage of response was increased upto 2.0mgl/L to 3.0 mgl/L Kn and later gradually decreased at high concentrations. Plantlet regeneration through Cotyledon and shoottip explants and streptomycin resistant plantlet using in vitro mutagenesis was also developed in *S. Surattence* (Muukherjee *et al.*, 2011; Swamy *et al.*, 2005; Nasar *et al.*, 1997) plant regeneration callus induction. *In vitro* regeneration in the *Cucumis sativus* (L) study of multiple shoots MS Containing media with different and combinations of growth regulators. The effect of plant growth regulators on rooting was investigated 4-6 weeks after culture on MS medium (Subashri *et al.*, 2014; Devendra *et al.*, 2011; Verma, 1996; Sunderraj *et al.*, 1989). Due to over exploitation for high medicinal values and destruction of the habit this (Sunderraj *et al.*, 1989; Nasir *et al.*, 1997) meristem shoottip cultures hence there is need for *ex situ* conservation through tissue culture methods. The stem node explants were inoculated on MS medium supplemented with various cytokinins i.e., BAP and NAA. Addition of BAP at 3.0 mgl/L concentration or NAA at 3.0 mgl/L to the MS basal medium, induced regeneration from the stem node explants. Micro propagation involves multiplication of genetically identical individual by asexual reproduction within a short span of time with tremendous potential for the production of high quality plant based medicines (Murch *et al.*, 2000). Multiple shoot induction was achieved in one of the important medicinal plant of *Cucumis sativus* (L) MS medium supplemented with 1.0 mg/l BAP + 2.0 mg/l NAA and 3.0 mg/l L-Glutamicacid was found to be optimum to induce shoots. The present study established reliable and reproducible protocol for rapid multiple shoot induction from stem node explants of *Cucumis sativus* using different concentrations and combinations of Cytokinins.

Keywords: Stem node, NAA BAP, *Cucumis sativus*, Shoot induction

Introduction:

Stem node explants of *Cucumis sativus* (L) on MS medium fortified with plant growth regulators along with coconut milk and Amino acids. The plants of Cucurbitaceae suffer from several diseases including the water melon mosoic virus (Greber, 1978), Cucumber green mottle mosoic virus (Nijsden, 1984) and *Cucumis sativus* (L) also suffers from downey and powdery mildews which seriously limits the crop production. Auxiliary buds from pumpkin were reported by Jelaska (1972). *In Vitro* Regeneration of plants via Somatic embryo genesis has much potential for plant propagation and gene transfer In Soybean somatic embryo have been obtained from cultured immature cotyledons Soybean from embryogenic callus line derived from the shoot tip explants (Parvathi, Venkateshwarlu M-2018) *In Vitro* regeneration Via somatic embryo genesis has drawn more attention than other methods because it can produce a large number of plants in a relatively short time (Walker *et. al* 2001) The number of protoplasts showed increase during shorter treatment time and reaches a peak at 4-5 hours of after this many reports of soyabean somatic embryogeneis were published (Pathak. *et.al* 2014) The development of protoplast systems has increased the plants for use in both Biochemical and genetic research. Ugandhar *et. al* (2011) has been also reported that high amount of Cytokinin and lower amount of Auxins is the best combination for Somatic embryogenesis which is in accordance of our study.

Materials and Methods:

The presence of Cytokinin unducing shoot organogenesis well developed nodal explants of *Cucumis sativus* experimental data. The capacity of shoot bud differentiation and shoot proliferation from nodal explants of Coconut milk (CM) depended on hormonal variation. The percentage of response was increased gradually from 2.0 to 3.0 mgl/L BAP, the shoots raised *in vitro* 2-3 cm long were cultured on MS medium supplemented with various concentrations multiple shoot formation from shoot apics was obtained on MS medium supplemented with 1.0mgl/L NAA (Deca *et al.,* 1999 and Anju *et al.,* 2005) nodalexplants showed a positive response on MS medium containing BAP (1.0 mgl/L-2.0mgl/L) combination with NAA (1.0mgl/L to 2.0 mgl/l) That clearly implies the additive effect of IAA (1.0mgl/l to 2.0 mgl/L) combination of auxin and Cytokinin favored shoot bud differentiation in many plants. BAP was superior to Kn in inducing high frequency shoot regeneration in many numbers of plants. (Srilatha, venkateshwarlu, 2019) planlet regeneration from Cytoledon culture Soybean All media were adjusted to PH 5-8 before addition of 0.8% agar-agar and auto claved at 121°C and 103K Pg for 20Min Cultures 25x 150nm cultures tubes. MS Basal Medium was supplemented with various plant growth regulators and 3.0% Sucrose. The PH of the media was adjusted to 5.8, solidified with 0.8% Difco-bacto Agar and Autocloved at 103.4 KPa or 121°C for 15-20min. A single explants was placed in each culture tube and incubated (at25± 1°C with a 16h photoperiod under fluorescent light (40-50m^2 s^{-1}). Explants with In Vitro multiple shoots proliferated on TD2-containing media were transferred to MS Medium containing different concentrations (BAP2.0-3.0mgl+) Multiple shoot intonation from shoot tip explants was observed within 20-25days after inoculation. The effect of different five types of growth regulators on direct plantlef regeneration of tamato from shoot tip explants (Ugandar & Venkateshwarlu, 2018).

The Result from this study has shown that BAP induced the activation of Totipotency at the stem node explants, which resulted in the formation of multiple shoots. The stem node segments of 2.0 – 3.0 cm long were cultured and surface sterilized with 0.1% $HgCl_2$ for 5-7 minutes and rinsed with sterile distilled water. They were cultured on MS medium containing 2.5% sucrose and 0.8% Agar-Agar and different concentrations of BAP, NAA and L-Glutamic acid. The pH of the medium was adjusted to 5.8 and later was autoclaved at 120^0C for 17 minutes. Cultures were incubated under 16 hrs, illumination (250 lux) at 25±2^0C temperature. Raising the level of BAP (0.5 to 2.0 mg/l) resulted in the increase in the number of shoots from hypocotyls and cotyledon explants of Niger (Nikam and Shitole, 1993). Cotyledonary explants (3-4 weeks old) of different sizes (0.5-1.0mm) were cultured with the abaxial surface in contact with induction Ms Medium consisting (1.0-2.0 mgl/L BAP) and (1.0 to 3.0 mgl/L) NAA for maturation and plant regeneration.

Results and Discussion:

The hormonal supplement was selected because it was optimum for callus formation stem node explants of *Cucumis sativus* developed (60%) with increase in concentration of NAA in MS Medium. The regenerated plants will be useful for constant supply of uniform raw materials for commercial secondary metabolic extraction according to their observation. BAP, Kn were superior for multiple shoot formation has been reported as it was observed in the present investigations. Then the plantlets were transferred to polypots containing pre chamber set at 28°C and 60-70% relative humidity. After 2-4 weeks they were transplanted to polybags containing mixture of soil+sand+manure in 1:1:1 ratio and kept under shade house for a period of 6 days to 4 weeks. Multiple shoot buds were initiated on the callus cultured in MS Medium supplemented with different combinations (BAP, NAA & Kn). The regenerated elongated shoots were transferred to Indole Byutyric acid (IBA) (2.0mgl/L-4.0mgl/L) for root induction. Sub cutting of callus into fresh medium containing the same concentrations of growth regulates resulted in the emergence of callus. The Stem node cuttings were inoculated on MS basal medium fortified with various cytokinins i.e., BAP and NAA. Coconut water also had a role in triggering the formation of multiple shoots. Raising the level of BAP (3 mg/l to 4 mg/l) resulted in an increase in the percentage of shoots developed from Stem node cuttings. There was no significant increase in the number of shoots on NAA at low and high concentration. Low concentration of L-glutamic acid (0.5 – 1.0 mg/l, along with BAP (2.0 mg/l, has produced significant mean number of multiple shoots that ranged from 2-3 to 5-6 in both the explants. The mean number of shoots developed on the explants ranged from 1-4 to 2-3 by the addition of different concentrations of BAP and NAA. The number of shoots developed on the explants ranged from 1-4 to 2-3 by the addition of BAP at a concentration of 2.0 mg/l or NAA at 2.5 mg/l. (Plate – I, Table – I). MS medium fortified with 1.0 mg/l BAP or 2.0 mg/l L-Glutamic acid also induced shoot buds on Stem node explants. Addition of NAA failed to produce many shoots but enlarged the stem node segments. Lower levels of coconut milk (6, 12%) induced callus formation. The results from study have shown the initiation of shoot buds and formation of multiple shoots from different explants i.e. Stem node cuttings of *Cucumis sativus* (L). Among all explants used Stem node segments were the best for multiple shoot induction. With an increase in the level of BAP 2.0 – 3.0 mg/l the percentage of explants producing shoots also increased.

Table 1: *In Vitro* Shoot induction from nodal explants of *Cucumis sativus* (L)

Growth Regulators	Nodal explants	
	% frequency of Shoots	Mean no. of Shoots
MS + 0.5 mg/l BAP + 1.0 L-Glutamic acid +IBA	30	Green Callus
MS + 1.0 mg/l BAP + 2.0 L-Glutamic acid+IBA	40	Green Callus
MS + 2.0 mg/l BAP + 3.0 L-Glutamic acid+IBA	35	Callus + shoots (1-3)
MS + 3.0 mg/l BAP + 4.0 L-Glutamic acid+IBA	30	Callus + shoots (4-6)
MS + 1.5 mg/l NAA + 0.5mg/l	25	Green Callus
MS + 1.0 mg/l NAA + 2.0mg/l	22	Green Callus
MS + 2.5 mg/l NAA + 2.5mg/l	20	Callus + shoots (2-4)
MS + 3.0 Mg/l NAA + 3.0mg/l	15	Callus + shoots (1-3)
MS + 3.5 mg/l NAA + 3.0mg/l	10	Callus + shoots (3-6)

Explant **Callus** **Regenaration**

Plate I: *In Vitro* shoot Induction from nodal explants of *Cucumis sativus* (L).

Conclusion:

Maximum shoot bud differentiation from callus culture was achieved on MS Medium supplemented with BAP, NAA and KN, therefore their MS Medium was designated as multiple shoots. The callus showed maximum number of shoot bus (2-4) The method of repeated transfer of explant is considered to be useful for large scale production of plants, as it avoids isolation and culture of new explants. This is considered as one of the methods to increase the response in explants has suggested that repeated transfer of explants on multiplication media containing cytokinins succeeds in activating the plant materials. The purpose of this work was to study the effect of different concentrations of growth regulators on direct plantlet regeneration of *Cucumis sativus* from stem node explants. Rooted plantlets were successfully hardened under culture conditions and established in the field conditions.

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A PRELIMINARY REPORT ON THE USE OF LEAFY VEGETABLES BY THE NATIVE OF BALANGIR DISTRICT, WESTERN ODISHA, INDIA

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Abstract:

The present study was conducted for the documentation of the leafy vegetables used by the native of Balangir district, Western Odisha. These green leafy vegetables were very nutritive and rich in minerals. These are good sources of natural antioxidants which are responsible for maintaining good health. Data about 49 plant species from 28 families and 38 genera were collected in this survey and investigation. The dominant family is Brassicaceae contributing six species. It is followed by the Amaranthaceae and Fabaceae family; each contributed five species. Four species belong to the Cucurbitaceae family. The families Apiaceae, Solanaceae, Chenopodiaceae, Convolvulaceae, Portulaceae and Tiliaceae contribute two species individually. Remaining 19 families contributed one species each. *Brassica* is the dominant genus containing five species. The leafy vegetables include 34 numbers of herbs (69.4%), six numbers of shrubs (12.2%), four numbers of climbers (8.2%) and five numbers of trees (10.2%). Most extensively used species were *Amaranthus oleraceous* L., *Amaranthus viridis* L., *Brassica oleracea* var. *capitata* L., *Brassica oleracea* var. *botrytis* L., *Corchorus capsularis* L., *Coriandrum sativum* L. and *Cinnamom umtamala* Nees. Documentation of leafy green vegetables improves both the economic condition of native peoples and also helps towards the conservation of biodiversity.

Keywords: Leafy vegetables, Native, Balangir district, Western Odisha.

Introduction:

Green leafy vegetables, also known as leafy greens, are the edible plant leaves. Some leafy greens can be eating raw as salad e.g. broccoli, some white onion, spinach, cabbage etc.; while others leafy vegetables may require cooking (Sahu and Sahu, 2022). Green leafy vegetables are consumed since the remote time as a resource of food as they are very nutritious and rich in minerals which are useful in maintaining human fitness. The physical strength (health) and nutrition of growing world populations are the most important forthcoming challenge mainly in developing countries. Plant foods are the source of energy, micronutrients and nutrients crucial to health, in addition to phytochemicals with further health benefits together with glycemic control, immune-stimulation or antioxidant activity. The leafy greens grip a vital place in a healthy and balanced diet and are the cheapest of all the vegetables, being richest in their dietetic value. Being deficient in information/knowledge particularly on the dietary value of these leafy greens among the community in most cases are the major negative aspect in their lesser utilization. Leafy greens, either locally collected or cultivated, are diversified sources of nutrition and phytonutrients. These are identified as excellent resources of naturally occurring antioxidants for example tocophenols, vitamin c and polyphenols which are responsible for fitness and defend against cardiovascular disease and tumour (Kumar *et al.,* 2013).

Leafy vegetables have a significant value in the nutritional necessity of the tribal and local population. In this district ethno-medicinal leafy plants and their products are used during different types of diseases among the tribal groups. Wild edible leafy vegetables are important resources of nutrients mostly in rural parts. These are known as admirable sources of natural antioxidants. Many people are not aware of the nutritional values of wild leafy vegetables (Sandey and Sharma, 2019).

In several developing nations, the humanitarian diet is influenced by a common principal food. Besides that, it has negligible quantities of various foodstuffs, leading to a high threat of insufficient ingestion of both macro and micronutrients. The chief diets, in India, are rice/wheat based. Parboiled-polished rice is the principal food. Again, other food items, for example- pulses, vegetables, fish, animal products and fruits, constitute a very negligible portion of Indian diets. Vegetables, mainly green leafy ones, are an excellent resource to get nutrition, as they are rich not only in proteins but also give carbohydrates and vegetable oils. They contain ascorbic acid, fibers, folic acid, various vitamins, β-carotene and minerals like Calcium, Iron, Potassium, Phosphorus, Sodium and Znic (Sahu and Ekka, 2021, Sahu and Sahu, 2022).

Leafy vegetables are a very erratic as well as consumable category of leafy crop type plants that is available naturally and can be developed commercially with modern features and can be ingested (Mohanty and Routaray, 2020). Leafy greens mainly contain antioxidants, nutritional fibres, minerals, α-linoleic acid and vitamins. It possesses different health benefits such as anti-diabetic properties, prevents cardiovascular disease, anti-hypertensive, anti-canceric, anti-anemic properties and helps to improve gut health. They also contain Ferous, Magnecium, Kalium, phosphorus, calcium like minerals. Furthermore, it contains a very low amount of carbohydrates, lipids, sodium and cholesterols. The leafy vegetables provide a considerable quantity of a B-vitamin i.e. folate that promotes heart health and helps to prevent certain birth defects. To improve your daily diet, eat about 2cups of dark leafy vegetables daily as it is equivalent to a cup of vegetables. Mostly 2.5cup is suggested to take regularly to get a diet of 2000 calories (Aslam *et al.,* 2020).

Green leafy vegetables help to fight against belly bloat, makes skin glow, relieve anxiety, improves inflammatory response, support healthy aging, control the toxins, boost digestive enzymes and also maintain immunity. Mostly these leafy vegetables are short lived. As leafy vegetables perform photosynthesis, their vitamin K level is high. The most common form of this vitamin i.e. Phylloquinone is directly involved in photosynthesis. We can add these leafy vegetables to our diet in many ways including salads, soups, juice, sauces, curry etc.

The use of leafy vegetables by the native of Balangir district is not reported yet. Keeping these in mind the present the objective of the present study is to documentations of leafy vegetables used by the native of Balangir district, Odisha, India.

Materials and Methods:

Study area

Balangir district lies between 20º11′40″ to 21º05′08″north latitude and 82º41′15″ to 83º40′22″ east longitude (Figure 1). The district comprises an area of 6575 sq. Kilometers among which 1543.85 sq. Km (23.48%) covered by forest. The total population of this district is 16,48,997 including 83,00,97 males and 8,18,900 females. Balangir district is divided into three subdivisions, 14 blocks, two municipalities, three NACs, and 1783 villages. Minimum temperature measured in this district is 16.6ºC and maximum is 48.7ºC.

Data collection

For documentation of the utilization of native green leafy vegetable plants, the area of survey was regularly visited and close communication was made mainly with the tribal people related to phytomedicines. Plant specimens were collected and identified from the books (Saxena and Bramhan 1994-96, Haines 2008). Further the local names were cross checked by using different studies carried out by various authors in Odisha (Sahu *et al.*, 2010; Sahu *et al.*, 2013; Sahu *et al.,* 2016; Sahu *et al.*, 2018; Rana *et al.*, 2020; Sahu *et al.*, 2020a, b, c, d; Behera *et al.*, 2021; Sahu *et al.*, 2021a, b; Sahu and Mishra 2022; Sahu and Ekka 2020, 2021; Mishra *et al.*, 2022; Behera and Sahu, 2023; Behera *et al.*, 2023; Rout and Sahu, 2023; Sahu and Sahu, 2017, 2019, 2020, 2022, 2023). Some of the people with knowledge of such medicines did not want to disclose the specifics of some plants as it is believed that revealing the knowledge might make the medicinal properties ineffective. As tradition has it, the information is undisclosed and carefully passed down from time immemorial. Therefore, the survey was designed with alternative approaches to collect the information. The collected leafy vegetable plants were written in a table having a botanical name, followed by its local name, family, habit, plant parts used and mode of use.

Results:

In this present study a total of about 49 plant species from 38 genus and 28 families were documented on the survey (Table-1), that were used by the native of Balangir district, Odisha, India. The data were documented as botanical name followed by local name, family, habit, plant parts used and mode of use. Among the 38 genus the Brassica genus contains highest numbers of species contributing five species i.e. *Brassica olerocea* var. *capitata* L., *Brassica olerocea* var. *botrytis* L., *Brassica napus* L. var. *glauca* (Roxb.) Schulz, *Brassica oleracea* L. var. *gongylodes* L. and *Brassica oleracea* var. *italica*. It followed by Amaranthus genus contributed four numbers of species i.e. *Amaranthus tricolor* L., *Amaranthus soinosus* L., *Amaranthus viridis* L. and *Amaranthus oleraceous* L. The genera Cucurbita, Momordica, Portulaca and Ipomea contributed two species each, while the remaining 32 genera contributed one species each. Among 28 families, the dominant family is Brassicaceae contributing six species. It followed by the families Amaranthaceae and Fabaceae contributed five species each respectively. The Cucurbitaceae family contributed four species.

Table 1: List of plant species used as of leafy vegetable species used by the native of Balangir district, Western Odisha, India

Botanical Name	Local Name	Family	Habit	Parts Used	Mode of use
Spinacia oleraceae L.	Palangsaag	Chenopodiaceae	H	Leaves and tender shoots	Used as food and also for making medicines. It is used to treat obesity, memory and thinking skills, fatigue, cancer and many other conditions
Cucurbita maxima Duchesne	Makhan	Cucurbitaceae	C	Leaves, Flower, Fruit, Seed	Used as food and also have meditational values. It is used to treat gastrointestinal diseases and intestinal parasites.
Amaranthus tricolor L.	Lal khada	Amaranthaceae	H	Leaves and young stem	Used as food by cooking the leaves and young stem
Brassica olerocea var. *Capitata* L	Bandhakobi	Brassicaceae	H	Leaves	Used as food either by cooking it or can be eaten raw. It also has medicinal values like anti-diabetic, anticancer, anti-hypertensive, anti-cholesterolmic, anti-oxidant properties.
Brassica olerocea var. *Botrytis* L.	Phulakobi	Brassicaceae	H	Leaves	Used as food by cooking it and have medicinal values also. It has glucosinolates which helps to prevent cancer and have anti-inflammatory, antiviral and antibacterial effects.
Allium cepa L	Ueil	Amaryllidaceae	H	Leaves/modified leaves,	Used as food by cooking it or may use as raw and it helps to make medicines and also used for skin diseases, and to prevent cancer and heart disease.
Moringa oleifera Lam.	Munga	Moraginaceae	T	Leaves, seeds, bark, roots, sap and flowers.	Used as food by cooking and have some medicinal values like anti-asthmatic, anti-diabetic, hepatoprotective, anti-inflammatory, anticancer, antimicrobial, antioxidant, antiulcer, cardiovascular properties.
Mentha spicata L. *Emend.*Nathh	Podina	Lamiaceae	H	Leaves, Flowers, Stem, Bark and seeds	Used as food either by cooking or raw and also used to treat gastrointestinal, respiratory, bad breath, carminative diseases and possesses antispasmodic, diuretic and sedative agents.

Amaranthus spinosus L.	kantaLeutia	Amaranthaceae	H	Leaves	Used as food by cooing it and also used to treat asthma, arthritis, diarrhea, internal hemorrhage, neurological disorders and skin diseases.
Colocasia esculenta (L) schott	Saru	Araceae	H	Corms, Leaves, Stems	Used as food and to treat asthma, arthritis, diarrhea, internal hemorrhage, neurological disorders and skin disorders.
Amaranthus viridis L.	Khada	Amaranthaceae	H	Leaves, stem	Used as food and have anti-inflammatory, diuretic, anti-rheumatoid, antiulcer, analgesic, anti-anemic, laxative properties and also used to treat asthma.
Bryophyllum pinnatum	Patargaja	Crassulaceae	H	Leaves	Have some medicinal values like astringent, analgesic, and useful in diarrhea and vomiting.
Corchorus capsularis L.	Nalta	Tiliaceae	H	Leaves, young fruits	Used for edible purpose and rich in antioxidants.
Bauhinia purpura L.	Kuler	Casaloiniaceae	T	Leaves, Flowers, Flower buds, Young pods	Used for edible purpose and have anti-bacterial, anti- diabetic analgesic, anti-inflammatory, anti-diarrheal, anti- cancerous, and thyroid hormone regulating properties.
Coriandrum sativum L.	Dhania	Apiaceae	H	Seeds, Leaves	Used for edible purpose by cooking it or it may be eaten raw and also used for its fragrances. It is used to make medicines.
Momordica charantia L.	Karla	Cucurbitaceae	C	Leaves, fruits	Used as food by cooking it and also used to treat jaundice and other liver diseases and to cure ulcers and burns.
Trachyspermum ammi L.	Juani	Apiaceae	H	Leaves, flowers, seeds	It has medicinal values as it possesses stimulant, antispasmodic, carminative properties.
Murray akoenigii L. Spreng	Mersinga	Rutaceae	S	Leaves, Roots	Used as aromatic food and also used for treatment of night blindness, Dysentery, diarrhea, vomiting, bites of poisonous animals' bruises eruption.

Basella alba L.	Poi	Bassillaceae	H	Leaves, shoots	Used as food by cooing and also used to treat dysentery, diarrhea, anemia, cancer.
Axlypha indica L.	Kaphgajri	Euphorbiaceae	H	Leaves	It has medicinal values as it possesses anti-helmintic, anti-diabetes, anti-hyperlipidemic, anti-obesity, ant venom, hypoxia properties.
Cinnamomum tamala Nees	Tejpatar	Lauraceae	T	Leaves, Bark	Used as spices and used to treat dental diseases, cough asthma
Portulaca oleracea L.	Chantisaag	Portulaceae	H	Leaves	Used as food by cooking and have antibacterial, anti-inflammatory, antioxidant and wound healing properties.
Raphanus sativus L.	Mula	Brassicaceae	S	Leaves, Roots	Used as food by cooking it or it can be raw by making salad. It is used to treat constipation, chronic tracheitis and hypertension.
Azadirachta indica A. Juss	Neem	Meliaceae	T	Stem, Root bark, Fruit, Leaves	Used for edible purpose and also to treat skin disorder. It has inhibitory effect on microbial growth.
Bacopa monnieri (L.) pennell	Bramhi	Scrophulariaceae	H	Leaves	Used as food and for making medicines for promoting neuron communications.
Marsilea minuta L.	Sunsuniasaag	Marsileaceae	S	Leaves, Shoots	Used as food and to treat cough and respiratory troubles.
*Cordia oblique*Willd	Bahal	Boraginaceae	T	Leaves, Flowers	Used as food and have medicinal values. It used to treat cough. It has astringent, analgesic, anti-inflammatory properties.
Ipomoea aquatic L.	Kalama saag	Convolvulaceae	H	Leaves, Tender shoot tips	Used as food by cooking it.
Cicer arientinum L.	Chana saag	Fabaceae	H	Leaves, Fruits	Used for edible purpose and have medicinal properties like aiding weight management, improving digestion and rescue the risk of many diseases.

Boerhavia diffusa L.	Gadhapurnisa ag	Nyctaginaceae	H	Leaves	Used as food by cooking and also used to treat accumulation of fluids in the body.
Portulaca quadrifid L.	Nunisaag	Portulacaceae	H	Leaves	Used as food by cooking it and juice of leaves is applied to abscesses and decoction is given in dysentery
Commelina benghalensis L.	Kenasaag	Commelinaceae	H	Leaves	Used as food by cooking it and also used to treat leprosy, sore throat, opthalmia, burns, pain and inflammation
Glinus oppositifolius (L.) A, DC.	Pita saag	Molluyinaceae	H	Leaves	Used as food and to treat joint pains, inflammation, diarrhea, intestinal parasites, fevers, furuncles. Skin disorders.
Alternanthera sessilis L. R. Br.	Madrangasaag	Amaranthaceae	H	Leaves, Flowers	Used as food and also used to treat jaundice.
Momordica dioica Roxb.	Kankdo	Cucurbitaceae	C	Leaves, Fruits, Tuber roots.	Used as food and have ant diabetic activity.
Lablab purpureus (L.) Sweet	Simba	Fabaceae	H	Leaves, Fruits, Pods	Used as food and used to prevent pregnancy and diarrhea.
*Brassica napus*L. Var. *glauca* (Roxb.) Schul	Sursoo	Brassicaceae	H	Leaves, Flowers, Seeds	Used as food and leaves used as salads and other dishes and from seeds oil can be extracted and used as medicines. Used to treat the common cold, joint pain, arthritis, lungs illnesses and other conditions.
Trigonella foenum graecum L.	Methi	Fabaceae	H	Seeds, Leaves	Used as food and to make traditional medicines as an antibacterial, a gastric stimulant, an anti-diabetic
Sesbania grandiflora L.	Agasti	Fabaceae	S	Leaves, Flowers	Used as vegetables and added to curries or salads. It can also use to treat diarrhea, dysentery, microbial infections and inflammatory diseases.
Brassica oleracea L. Var. *gongylod*es L.	Gaintkobi	Brassicaceae	S	Leaves	Used as food and also used to prevent oxidative stress, induce detoxification enzymes, stimulate immune system, decrease risk of cancer.

Ipomea aquatica Forssk	Kanda	Convolvulaceae	H	Leaves, Tender shoot tips	Used as food
Chenopodium album L.	Batha	Chenopodiaceae	H	Leaves	Used as food and for treatment of rheumatism, bug bites, sunstroke, and skin problems.
Cuculligo orchiodes Gaertn	Kanjher	Hypoxidaceae	H	Leaves	Used as food and as tonic demulcent, diuretic and anticancer, anti-diabetic.
Solanum lycopersicum L.	Patalghanta	Solanaceae	H	Leaves, fruits	Used as food and to make sauce. It also used as medicine as rich source of vitamins, minerals and fibres.
Cucurbita maxima	Kakharu	Cucurbitaceae	C	Leaves, Fruits	Used as food and also used to treat gastrointestinal disorders.
Capsicum annuum	Mircha	Solanaceae	H	Leaves, Fruits	Used as food and as medicine for poor circulation, upset stomach, toothache, fever, heart disease prevention.
Indigo feracassioides Rottler ex.	Girdi	Fabaceae	S	Leaves, Flowers, roots	Used as food and also root is dried, and its powder form applied externally in the treatment of pains in chest.
Amaranthus oleraceous L.	Bhaji	Amaranthaceae	H	Leaves, Young stem	Used as food and as medicine for ulcer, diarrhea, swollen mouth and Throat.
Brassica oleracea var. *italica*	Broccoli	Brassicaceae	H	Leaves	Used as food by cooking it or by making salads.

The families Apiaceae, Chenopodiaceae, Convolvulaceae, Portulaceae, and Solanaceae contributed two species each. Rest of 19 families i.e. Amaryllidaceae, Araceae, Bassilbaceae, Boraginaceae, Crassulaceae, Casalpiniaceae, Commelinaceae, Euphorbiaceae, Hypoxidaceae, Lamiaceae, Lauriaceae, Meliaceae, Moraginaceae, Marsileaceae, Molluyinaceae, Nyctaginaceae, Rutaceae Scrophulariaceae and Tiliaceae contributed one species each (Figure 2). The leafy vegetables include 34 numbers of herbs (69.4%), six numbers of shrubs (12.2%), four numbers of climbers (8.2%) and five numbers of trees (10.2%). (Table 1, Figure 3). Most extensively used species are *Amaranthus oleraceous* L., *Brassica olerocea* var. *capitata* L., *Brassica olerocea* var. Botrytis L., *Amaranthus viridis* L., *Corchorus capsularis* L., *Coriandrum sativum* L. and *Cinnamom umtamala* Nees.

The native peoples mostly depend on naturally occurring leafy vegetables as compared to the cultivated leafy greens. Several of these were also used for therapeutic purposes. Plants or the leafy greens are generally collected in the young stage of the leaves. The leafy vegetables were also collected by local sellers for vending these leafy vegetables. They sold various types of green leafy vegetables like *Amaranthus viridis* L., *Chenopodium album* L., *Cucurbita maxima* Duchesne., *Coriandrum sativum* L., *Ipomoea aquatica* Forssk., *Moringa oleifera* Lam., *Spinacia oleraceae* L., *Raphanus sativus* L. Etc. The natural leafy vegetables were highly nutritive and rich in minerals also.

Table 2: Family wise distribution of leafy vegetable species used by the native of Balangir district, Western Odisha, India

Family	Number of Species	% age of contribution
Amaranthaceae	5	10.2
Amaryllidaceae	1	2.0
Araceae	1	2.0
Apiaceae	2	4.1
Brassicaceae	6	12.2
Bassilbaceae	1	2.0
Boranginaceae	1	2.0
Chenopodiaceae	2	4.1
Cucurbitaceae	4	8.2
Crassulaceae	1	2.0
Caesalpiniaceae	1	2.0
Convolvulaceae	2	4.1
Commelinaceae	1	2.0
Euphorbiaceae	1	2.0
Fabaceae	5	10.2
Hypoxidaceae	1	2.0
Lamiaceae	1	2.0
Lauriaceae	1	2.0
Meliaceae	1	2.0
Moraginaceae	1	2.0
Marsileaceae	1	2.0
Molluyinaceae	1	2.0
Nyctaginaceae	1	2.0
Portulariaceae	2	4.1
Rutaceae	1	2.0
Solanaceae	2	4.1
Scrophulariaceae	1	2.0
Tiliaceae	1	2.0

Table 3: Habit wise plant distribution of study sides.

Habit	No. of species	% age of Contribution
Climber	4	8.2
Herb	34	67.4
Shrub	6	12.2
Tree	5	10.2

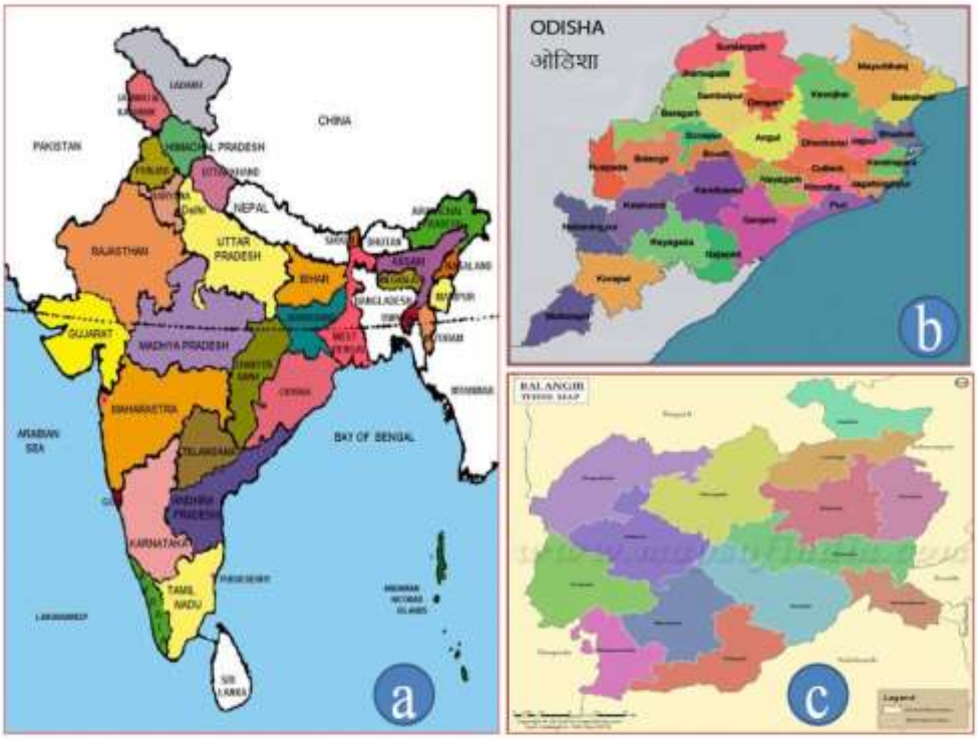

Figure 1: **Map showing the location of Balangir in the state of Odisha**

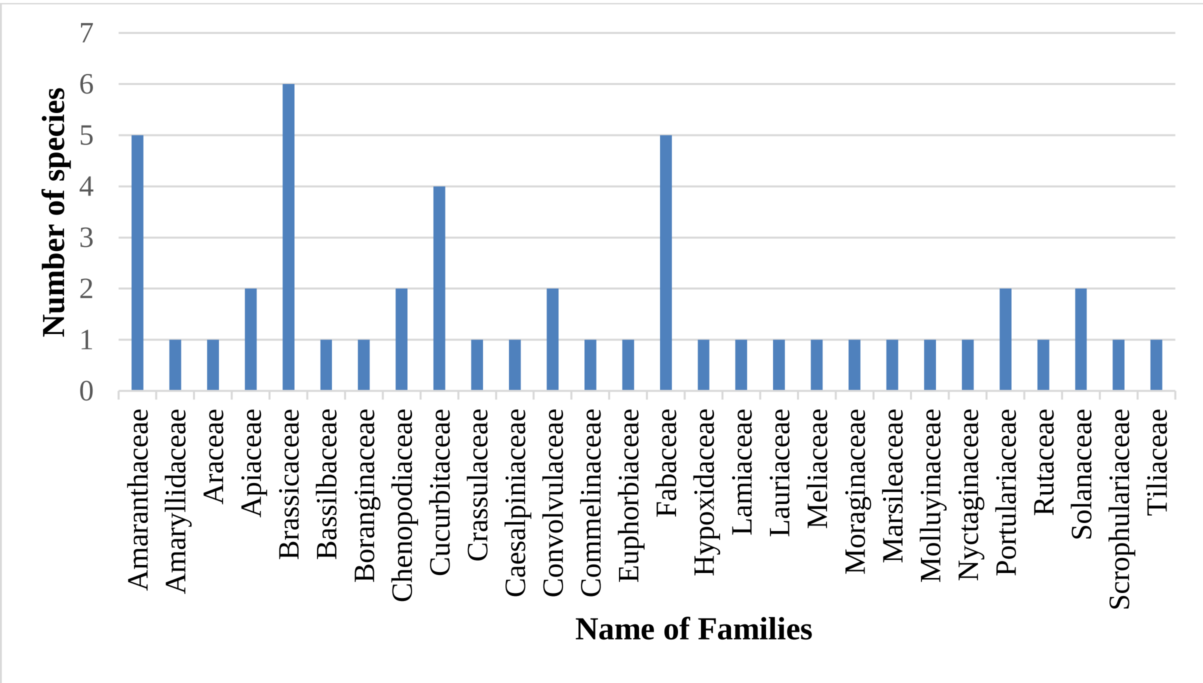

Figure 2: Family wise distribution of leafy vegetables used by the native of Balangir district, Western Odisha, India

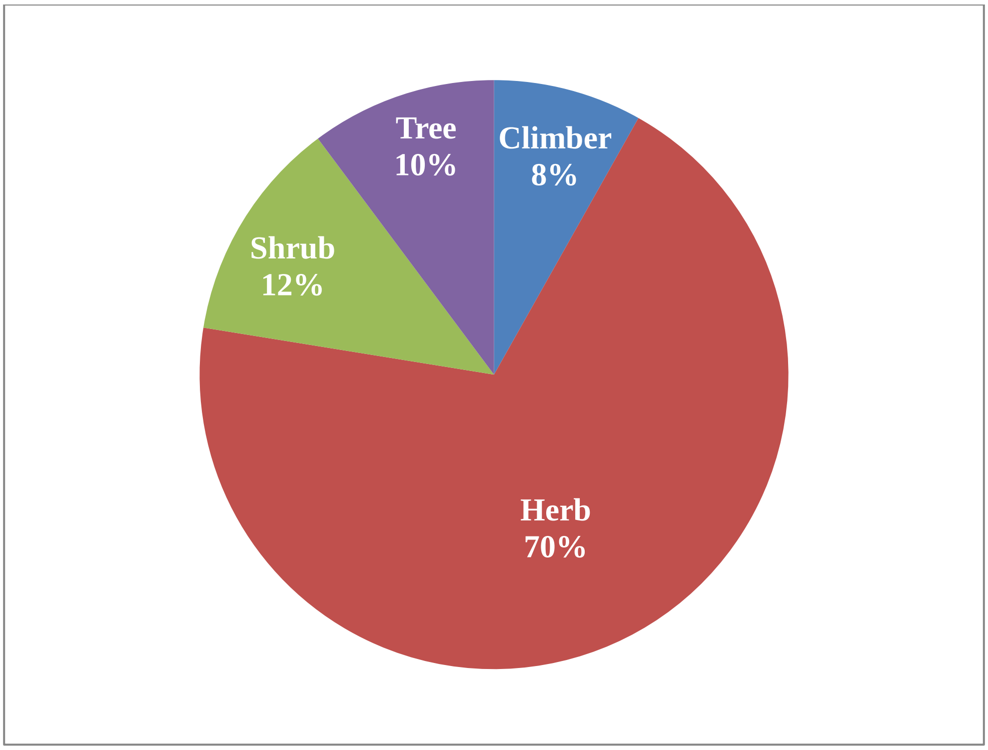

Figure 3: Pie-chart showing diversity of plant species by habit of the study sites

Discussion:

Leafy vegetables may be herb, shrub, climber or tree where leaf is suitable for eating. It is observed that information of green leafy vegetables may be vanished in near future, if we do not alert younger generation about their therapeutic values, government policies should be need to change to advance the leafy vegetables importance, whose potential source of nutrition is under values at present. Due to high nutritional value, the leafy greens or leafy vegetables occupy a significant place in the human diet. Previously many authors also studied the leafy vegetables of different areas. In this current survey I had documented a total 49 numbers of plant species from 38 genus and 28 families. Among the 38 genera, *Brassica* genus consists of highest number of five numbers of species (i.e. *Brassica olerocea* var. *capitata* L., *Brassica olerocea* var. *botrytis* L., *Brassica napus* L. var. *glauca* (Roxb.) Schulz, *Brassica oleracea* L. var. *gongylodes* L. and *Brassica oleracea* var. italica). It followed by Amaranthus genus contributing four species (i.e. *Amaranthus tricolor* L., *Amaranthus soinosus* L., *Amaranthus viridis* L. and *Amaranthus oleraceous* L.). Five genera named as *Cucurbita*, *Momordica*, *Portulaca* and *Ipomea* contributed two species each. The Remaining 32 genera contributed one species each. Among the 28 families Brassicaceae is the dominant family contributing six species and is followed by Amaranthaceae and Fabaceae families contributing five species each. The Cucurbitaceae family contributed four species and the families Apiaceae, Chenopodiaceae, Convolvulaceae, Portulaceae, and Solanaceae contributed two species each. Rest of the 19 families contributed one species each. Similar kinds of results were also reported by various authors that were described as below.

Mohanty and Routray (2020) reported the leafy vegetables used near the Gandhamardan hills, Nrusinghnath, Bargarh district. They recorded 43 green leafy vegetables, among which about 25 species were similar to my survey report. In their report they did not motion any specific medicinal use of a specific plant which was motioned in this document. Sandey and Sharma (2019) studied leafy vegetables used by tribal populations of various areas of Kondagaon district of Chhattisgarh 36 species were motioned which were the source of leafy vegetables whereas 64 were motioned as ethno-medicinal leafy vegetables. Parida and Mahalik (2020) they studied seven tribal communities to assess the diversity of plant species and documented 48 species belonging to 26 families; among those Amaranthaceae was the dominated species containing eight plants. In this present manuscript we had reported the Brassicaceae family as a dominant family because a total of six species (*Brassica olerocea* var. *capitata* L, *Brassica olerocea* var. *botrytis* L., *Raphanus sativus* L., *Brassica napus* L. var. *glauca* (Roxb.) Schul, *Brassica oleracea* L. var. *gongylod*es L., and *Brassica oleracea* var. *italica*) were belonging to this family. Sahu and Ekka (2021) studied the leafy vegetables used by the residents or locals of Bargarh district and recorded 39 species. About 21 species of my record were similar to their work. But the medicinal values of the plants were not motioned in their report. Sahu and Sahu (2022) reported a total of 44 plant species, belonging to 35 genera and 27 families as green leafy vegetables by the tribal peoples of Jharigaon Block of Nabarangpur district, Odisha, India. They also reported that the Amaranthaceae and Brassicaceae families were jointly dominant over other families and contributed about five species each.

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NUTRITIVE ANALYSIS OF AN UNDERUTILIZED EDIBLE FRUIT OF THE MYRTACEAE FAMILY; *SYZYGIUM OCCIDENTALE* (BOURD.) GANDHI

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Abstract:

Syzygium occidentale is a tree species endemic to the southern Western Ghats. This species is distributed along the margins of shallow streams, prone to seasonal floods and is enlisted as vulnerable as per IUCN red list. The present investigation addresses the nutritive analysis of this underutilized edible fruit. The investigations as per standard protocols revealed that the fruits of this plant hold appreciable amount of nutrients.

Keywords: *S. occidentale*, Fruit, Nutrition.

Introduction:

The etymology of 'Myrtaceae' is from a shrub 'Myrtus' which is occurring in the Mediterranean in North Africa and in South America and is an ecologically important angiosperm family comprising both shrubs and trees (Mitra *et al.*, 2012). Govaerts *et al.*, (2008) reports that about 140 genera and 3800 - 5650 species are included in this family. The Myrtaceae family as well as the major genus *Syzygium*, on this family includes many plants bearing edible fruits and some of them are integral part of income generation among the rural people of Indian subcontinent.

Syzygium occidentale is a beautiful small riparian tree species endemic to the southern Western Ghats. The common tree species occurring along with *S. occidentale* in includes *Ochlandra travancorica* (Bedd.) Gamble, *Hopea parviflora* Bedd., *Lophopetalum wightianum* Arn., *Madhuca longifolia* (J. Koenig ex L.) J.F. Macbr., *Calophyllum apetalum* Willd., *Holigarna arnottiana* Hook.f., *Lagerstroemia speciosa* (L.) Pers . *Memecylon talboltianum* Brandis, *Xanthophyllum arnottianum* Wight, *Humboldtia vahliana* Wight, *Mangifera indica* L. etc.

Materials and Methods:

The present study was carried out on two populations of *S. occidentale* located in Chittar river bank (Thiruvananthapuram, Kerala) and one population occurring in Athirappilly river bank of Thrissur District (Kerala). The study aims on the nutritive analysis of fruits of *S. occidentale* on selected study sites. The habitat comprises mostly of riparian evergreen forest. The study was carried out during 2017-2018, 2018-2019 and 2019-2020 (three fruiting seasons).

Nutritive analysis of the *S. occidentale* fruits were carried out following slandered protocols. The total carbohydrate present in the fruits of *S. occidentale* was estimated as per Hedge *et al.*, (1962). Reducing sugar content in fruits was estimated as per the protocol of Miller

(1972). The total protein content in *S. occidentale* fruit was estimated as per Bradford's method (1976). The Ninhydrin reagent test suggested by Moore and Stein (1948) was followed to calculate the total amino acid content. The method suggested by Bligh and Dyer (1959) was followed to estimate lipid content in the sample fruits. Vitamin A (Carotene) content of the sample was estimated according to (Bayfield and Cole, 1980). The Ascorbic acid or Vitamin C was estimated according to Sadasivam and Balasubramanian (1987).

Results and Discussion:

The fruits of *S. occidentale* are initially green which turn pink in colour on ripening and they are bitter in taste. The fruit nutritive analysis of *S. occidentale* was done as per the standard protocols revealed that the fruit is rich in protein, reducing sugars and vitamins. The fruits of *S. occidentale* hold a total carbohydrate content of 2.80±0.04mg/g, reducing sugar content of 0.30±.07 mg/g and 1.32±0.11 mg/g of protein. The fruits of *S. occidentale* also contain Lipid (0.80±.04 mg/g) and amino acid (0.76±0.09mg/g). The fruit possess Vitamin A and Vitamin C (0.57±0.06 mg/g and 0.32±0.02 mg/g) respectively which makes this lesser-known underutilized edible fruit as a good nutritional supplement.

Table 1: Nutritional analysis of fruits of *S. occidentale*

Sl. No.	Nutrient	Quantity present (mg/g)
1	Total carbohydrates	2.80 ± 0.04
2	Reducing Sugars	0.30 ± 0.07
3	Protein	1.32 ± 0.11
4	Amino acid	0.76 ± 0.09
5	Lipids	0.80 ± 0.04
6	Vitamin A	0.57 ± 0.06
7	Vitamin C	0.32± 0.02

Debela *et al.* (2011) states that wild edible fruits contribute a major share in food diet that supply the body with vital nutrients and protein. In developing nations including India, the rural people traditionally harvest wide range of wild edible fruits owing to its taste, aroma, in connection with various cultural events, ethnomedicinal practices and food shortage (FAO, 2008). The estimated energy value of wild edible fruits like *Phoenix loreirii* (265.26±0.002), *Syzygium cumini* (247.91±1.6), *Carissa spinarum* (237.3±0.002) etc. are comparatively high (Anand and Deborah 2016). These results explained that these plants hold good source of nutrients such as carbohydrates and proteins. Earlier works on species such as *S. zeylanicum and S. caryophyllatum* proves that they are rich in protein contents. The Protein content of these fruits was 3.68% and 3.37% respectively (Shilpa and Krishnakumar, 2015). These species are also rich in the crude fibre and percentage of fiber in S. *caryophyllatum* was 4.12. The fruit of *S. zeylanicum* contained slight higher percentage of crude fibre (12.67). *Syzygium caryophyllatum* possess 50 mg/100 g of vitamin C. Even though it is a lesser amount when compared to guava

(212 mg/100 g), it is appreciably more in comparison to the vitamin C content in apple Nutritive analysis of fruits of *Syzygium* species is not yet addressed well.

The fruits of *S. occidentale* are rich in protein, lipids, reducing sugars and vitamins. The fruits possess a total carbohydrate content of 2.80±0.04 mg/g, reducing sugar content of 0.30±0.07 mg/g and 1.32± 0.11 mg/g of protein. This observation is very similar to other species of *Syzygium* like *S. zylanicum* and *S. caryophyllatum* which contain more quantity of protein than other nutrients as per Shilpa and Krishnakumar (2105). *S. occidentale* fruits accounts a high content of Lipid (0.80 ± 0.04 mg/g) and amino acid (0.76± 0.09mg/g) and can be recommended as a good dietary supplement. It also contains promising amounts of Vitamin A and Vitamin C (0.57± 0.06 mg/g and 0.32± 0.02mg/g) respectively which makes this lesser-known underutilized edible fruit as a good nutritional supplement.

Conclusion

Syzygium occidentale, belongs to the Myrtaceae family is a small tree occurring in southern parts of Western Ghats along the river margins and often on rock crevices. The plant is enlisted as vulnerable as per IUCN red list. The present investigation on the nutritive analysis of fruits of *S. occidentale* revealed that it holds appreciable quantities of nutrition. This underutilized edible fruit can be promoted as a dietary supplement that in turn generate an income to the rural people and adds to the conservation, cultivation and popularization of this endemic species.

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PROXIMATE EVALUATION AND ESTIMATION OF TANNINS HAVING ANTI-MICROBIAL POTENTIAL FROM *MUCUNA PRURIENS* AND *MORINGA OLEIFERA* LEAF EXTRACTS

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Abstract:

Mucuna pruriens commonly known as velvet beans is a tropical legume belonging to the fabaceae family which is native to tropical. Fabaceae or Leguminosae family is widely known for its nutritional factors and different constituents supporting the therapeutic importance. *Moringa oleifera* is a plant that is often called the drumstick tree; Moringa has been used for centuries due to its medicinal properties and health benefits. It also has antifungal, antiviral, antidepressant, and anti-inflammatory properties. *Moringa oleifera* is a plant that has been praised for its health benefits for thousands of years. It is very rich in healthy antioxidants and bioactive plant compounds. So far, scientists have only investigated a fraction of the many reputed health benefits from both the plants. Proximate analysis in plants gives valuable information and helps to assess the quality of the sample. It provides information on moisture content, ash content, volatile matter content, ash, fixed carbon etc. which can be a cause of a pharmacological effect. Extraction of phytoconstituents and standardization of solvents for getting maximum yield is of paramount importance in the world of Natural Therapeutics. Present study aims at the overall impact of different solvents on tannin content and its antimicrobial potential. Tannins are a group of polyphenols that are astringent in nature having anti-inflammatory, antioxidant, anti-microbial and other activities. In the current chapter, we are focusing upon few parameters of proximate evaluation including estimation of tannin content by colorimetry and anti-microbial potential by disc diffusion assay and MIC.

Keywords: Proximate evaluation, tannins, extraction, Disc diffusion method, MIC

Some abbreviations used in the chapter: MIC- Minimum Inhibitory Concentration

Introduction:

India has been on forefront for the use of drugs of herbal origin as per the knowledge of traditional systems of medicines such as Unani and Ayurveda from ancient times. The drugs are derived from different organs of plant or are secondary metabolites or are waste products of the plants such as gum, resins and latex. Herbal medicines are popular in developing countries due to their easy access and cost effectiveness. Side effects of modern medicine include dry mouth, gastrointestinal side effects, hepatotoxicity, seizure, and inconsistent weight loss.[13]Plants synthesize and preserve a variety of biochemical products, many of which are extractables and are used as chemical feed stocks or as raw material for various scientific investigations.[1] Many secondary metabolites of plants are commercially important and are used in/as number of pharmaceutical compounds.[1] Proximate evaluation of the two plants reveal the information about ash content and extractable matter. Ash value indicates the quality and purity of the drug which is in the powder form. Ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salts). A high ash value is an indication of contamination, substitution, adulteration in the crude drug.[11] The ash remaining followed by ignition of herbal drugs is determined for various parameters like total ash, acid-insoluble ash and water-soluble ash.

Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. Water soluble ash is the difference in weight between the total ash and the residues after treatment of the total ash with water. This is used as a means of evaluation of the crude drugs for the presence of active constituents in the herbal material. An excess of water in herbal crude drug encourages microbial growth, fungal growth, and deterioration. Moisture is an evitable component of crude drugs, which needs to be eliminated to aid their preservation. The test for loss on drying of crude drug determines the presence of both water and volatile matter for the materials that absorb moisture easily or deteriorate in presence of water.

In some cases, the crude extract of medicinal plants may be used as medicaments. Here we are trying to estimate tannins belonging to the class of polyphenols.

Tannins are naturally occurring plant polyphenols which bind and precipitate proteins. There are different classes of tannins.

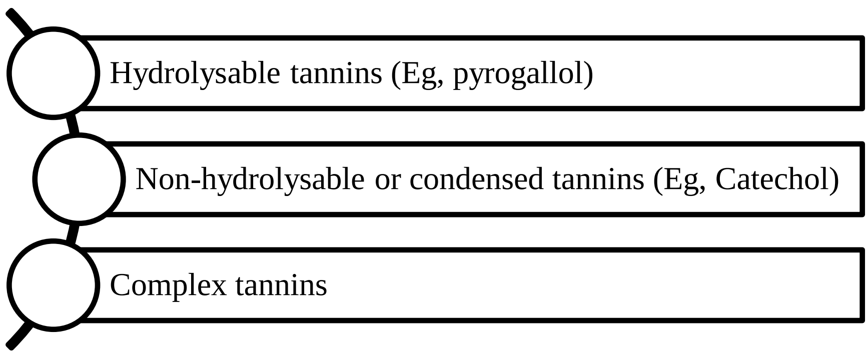


Figure 1: Types of tannins with example

Standardization is the process of implementing and developing technical standards based on the consensus of the standard organization. It helps to maximize compatibility, safety, repeatability, and quality. Solvents are used to dissolve or dilute other substances. Standardization is important as it will provide the information on the solvent which is most compatible with analyte of interest. While standardizing the solvents, properties like compatibility with target analyte, cost-effectiveness, availability, etc. are considered.

Depending upon the properties of solvent and analyte recovery we can decide upon which solvent can be used further for the maximum extraction of phytoconstituents. [2,3]

Folin-Dennis method is colorimetric quantification method for tannins. The phenolic group present in tannins is an excellent hydrogen donor that forms strong hydrogen bonds with proteins carboxy group. Folin-Denis reagent is a mixture of phosphomolybdic and phosphotungstic acid and are reduced to molybdenum and tungsten oxides by -OH groups in phenols. Tannins will reduce phosphotungstomolybdic acid in the presence of alkaline conditions due to sodium bicarbonate to produce a highly colored blue solution. Intensity of the colour is proportional to concentration of tannins.[4]

Many plants have gained commercial importance because of their antimicrobial traits, which are due to phytochemicals synthesized in the secondary metabolism of the plant. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found in vitro to have antimicrobial properties.[5] Antimicrobial agents play a key role in controlling and curing infectious disease. Antimicrobial agents use different mechanisms against bacteria to prevent their pathogenesis and they can be classified as bactericidal. Antibiotics are one of the antimicrobial agents which have several classes, each with different targets.[14]

Tannic acid has been reported to present the activity against Influeneza A virus, Papilloma viruses, noroviruses, Herpes simplex virus type 1 and 2, and human immunodeficiency virus (HIV) as well as activity against both Gram-positive and Gram-negative bacteria as *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Listeria innocua*.[15]

The methods for assessing antimicrobial resistance (AMR) of plants by some molecular methods like PCR, DNA microarray, whole-genome sequencing and metagenomics, and matrix-assisted laser desorption ionization-time of flight mass spectrometry.[16] These methods are used for identification of AMR genes. Some non-conventional methods include whole genome sequencing (WGS), matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) spectrometry, Fourier transform infrared (FTIR) spectroscopy, and microfluidics technology.[17] In the current study simple, conventional and manual methods are used for assessment of anti-microbial potential of the plant extracts i.e, MIC and Disc diffusion methods. These methods are easy to perform, simple and rapid. Results are obtained and are observed without a specialized instrumentation.

Inefficiency of medical therapies used to cure patients with bacterial infections requires not only to actively look for new therapeutic strategies but also to carefully select antibiotics based on variety of parameters. Minimum Inhibitory Concentration (MIC) defines in-vitro levels of resistance or susceptibility of specific bacterial strains to applied antibiotics. The increasing resistance of bacteria to antibiotics calls for searching ways to reduce it and improve the effectiveness of the infection therapies. MIC is the lowest Concentration of an antibacterial agent expressed in ug/mL or mg/L which is assessed under strictly controlled in-vitro strain of an organism. MIC values describe the susceptibility of a specific bacterium to a specific antimicrobial. In general, however the larger the MIC value the lower the susceptibility of the organism.

Agar disc diffusion is one of the methods which is used to evaluate antimicrobial activity of plant extract. Disc diffusion method helps to estimate the activity of the extract against the bacterial culture. This method is based on the principle of antibiotic-impregnated disc, placed on agar previously inoculated with micro-organism, and the disc previously dipped in herbal extracts diffuse radially outward through the agar medium producing an antimicrobial activity. A clear zone or ring is formed around a disc after incubation if the agent inhibits the growth of micro-organisms.[6]

Procedure:

1. Fresh plants were collected and authenticated for further analysis.
2. **Proximate Evaluation:** Plants were sun dried and ground in course form. Plant monographs are unavailable hence proximate evaluation was carried out. Plant powders were analyzed for total ash content, acid insoluble ash, water soluble ash, moisture content (loss on drying) and extractive value. Protocols for these tests are followed as per the Ayurvedic Pharmacopoeia of India.
3. **Quantitative estimation of tannins:** Purified Gallic acid equivalent was used as a standard and quantification was performed by calibration curve method. Standards ranging from 2ug/mL to 10ug/mL from 1mg/mL working stock were prepared and 1mL of 20% sodium carbonate was added in all the tubes along with 0.5mL of Folin Dennis reagent. 0.5gm of *Mucuna pruriens* and *Moringa oleifera* leaf powder was macerated respectively in 20mL and 10mL of the three solvents. 0.5mL of the extract was taken in the tubes and processed the tubes in the same manner as the standards.

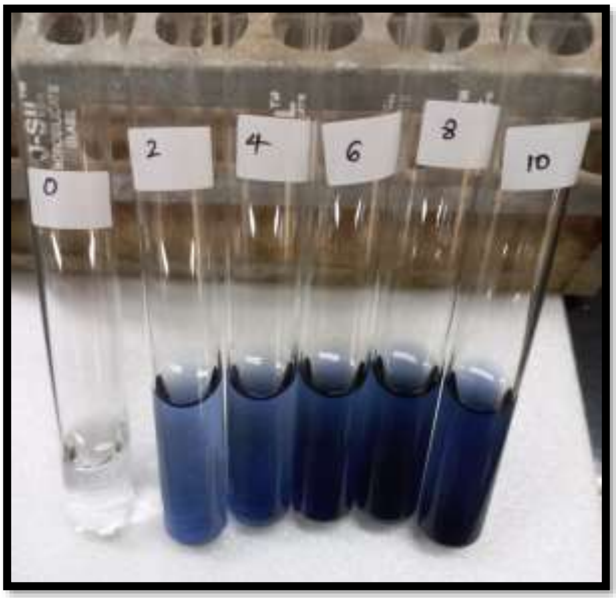


Figure 2: Standard series of Gallic acid ranging from 2ug/mL to 10ug/mL

4. **Test for Assessment of MIC:**
   a. Minimum Inhibitory Concentration was carried out adding equal volumes of all the above three extracts and culture suspensions of *K. pneumoniae* and *E.coli* in 9ml of Sterile Nutrient broth.
   b. The extracts were further serially diluted up to 10 dilutions each from original concentration of 2,50,00,000mg/L to 2.4 * 10^{-6}mg/L.

c. All the tubes were incubated at 37^0 Celsius for 24hours and evaluated based on the turbidity observed.

5. **Disc Diffusion test:** The sterile nutrient agar plate is swabbed with a *K. pneumoniae* and *E. coli* suspension respectively and sterile paper discs dipped in all the three extracts are placed in four quadrants along with a control. The results were observed after the incubation at 37^0C for 24hours.

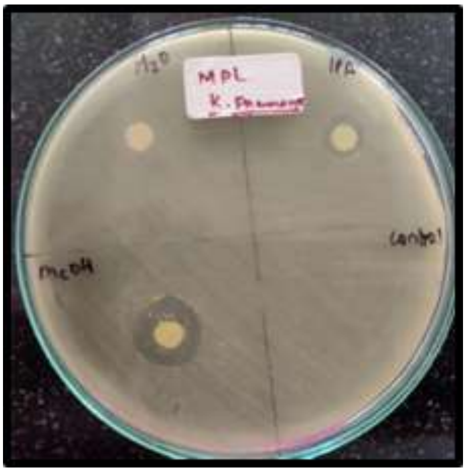


Figure 3: Disc diffusion assay of solvent extract against *K. pneumoniae*

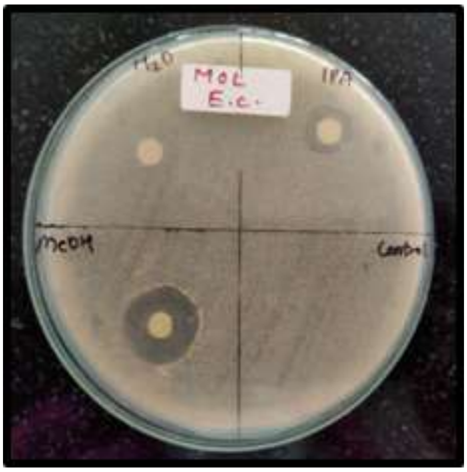


Figure 4: Disc diffusion assay of solvent extract against *E. coli*

Result:

Proximate evaluation

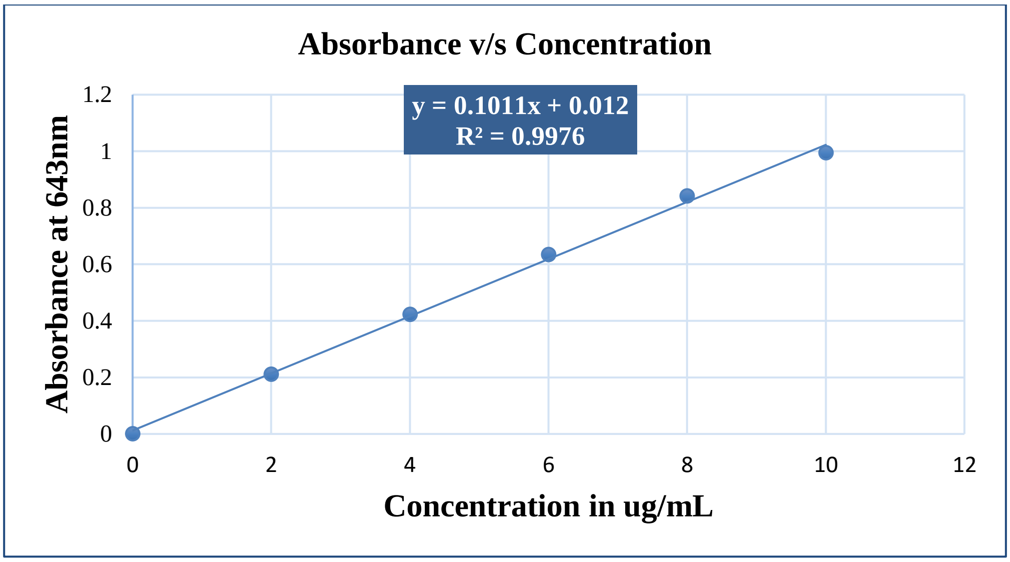
Table 1: Percentage ash contents in proximate evaluation

Plant name and part	Percentage Total ash	Percentage acid insoluble ash	Percentage water soluble ash	Percentage loss on drying
Mucuna pruriens leaves	10.75	2.6	0.55	10.08
Moringa oleifera leaves	9.6	1.55	1.15	11

Table 2: Extractive values

Name of solvent	Plant name and part	% extractable matter
Methanol	*Mucuna pruriens* leaves	14.100
Water		4.800
Isopropanol		5.100
Methanol	*Moringa oleifera* leaves	17.100
Water		7.200
Isopropanol		8.200

Quantification of tannins



Graph 1: Standard Calibration curve of tannins

Table 3: Concentration of tannins from three different solvents

Name of the solvents used	Plant name and part used	Tannin concentration (ug/mL)	Tannin content (%)
Water	*Mucuna pruriens* leaves	4.38	0.35
Methanol		5.08	0.41
IPA		1.29	0.1
Water	*Moringa oleifera* leaves	7.68	0.77
Methanol		9.56	0.96
IPA		2.39	0.24

Anti-microbial activity of the extract

Table 4: Solvent extracts and Zone of inhibition

Name of the solvent	**Average of zones of inhibition for Mucuna pruriens leaves extract against *K. pneumoniae* (mm)**	**Average of zones of inhibition for *Moringa oleifera* leaves extract against *E.coli* (mm)**
Water extract	0	0
Methanol extract	14	19
IPA extract	8	11

Table 5: MIC of extracts

Name of the solvents	MIC for Mucuna pruriens leaves extract against *K. pneumoniae* mg/L	MIC for *Moringa oleifera* leaves extract against *E. coli* mg/L
Water extract	1250000	2500000
Methanol extract	156.25	0.78125
IPA extract	62500	125000

Conclusion:

As shown in the summarized result table of proximate ash content evaluation, considerable variation was observed. This is due to the physiological and biochemical composition of the plant. This will reflect upon the biological or pharmacological action of the plant. Higher extractive values in polar solvents like methanol for both the plant extracts show presence of high amount of polar components in leaves. Extractive value of crude drugs gives us prima facia information about chemical nature of the components present. It further helps in standardization of solvent for quantitative analysis. Standardization of solvents for evaluating the extraction efficiency of polyphenols i.e, tannins from both the plants shows concentration of tannins highest in methanol extract followed by water followed by IPA. Least concentration of tannins was found in IPA extract. Though methanol being good organic solvent giving the maximum tannin content, water can be preferred considering the environmental toxicity of methanol. As tannins are water soluble and they contain hydroxyl groups along with non-polar aromatic phenol, they can be extracted in the mixture of polar (i.e., water) and mid-polar (i.e., methanol) solvents. Water is a universal solvent but is not stable for longer periods of storage of extract. Water extracts are easy targets for contaminants and fungus. Also, water extracts might not be compatible for further instrumental analysis.

After comparing the zones of inhibition with the control it was found that methanol extract shows highest antimicrobial activity against the organism followed by IPA and water extract. The extracts are assessed for the activity against a selected organism because of the primary knowledge of activity against possible infections caused by these bacteria. Zone of inhibition is highest for methanol extract. Methanol is an organic solvent which also contributes to antimicrobial potential. Also considering MIC, methanol extract has shown the most promising effect followed by IPA and water.

From the results, it is evident that methanol is the best suited solvent for extraction as it showed potential promising action against the organismsas compared to IPA and water. Considering the ideal properties of solvents; methanol can be used for further analysis of plant leaf extract. Also *Mucuna pruriens* leaves extract and *Moringa oleifera* leaf extract has shown the inhibition of *K. pneumoniae* and *E. coli* organisms respectively proving its activity against the infection. Animal studies can be performed for further verification of action against Pneumonia.

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CONTEMPORARY TRENDS IN PLANT RESEARCH FROM GLOBAL PERSPECTIVE

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Introduction:

Plant research, also known as botanical research or plant science, is a field of study that focuses on the study of plants, their biology, ecology, evolution, and interactions with the environment. It encompasses a wide range of topics and disciplines, including botany, plant genetics, physiology, ecology, molecular biology, and biotechnology.

Plant research aims to understand the fundamental aspects of plant life, as well as their importance and impact on the planet and human society. Some of the key areas of study in plant research that will be discussed in this chapter are as follows:

Functional genomics

Advances in DNA sequencing technologies have revolutionized the field of plant genomics. Researchers are using high-throughput sequencing to study the functional elements of plant genomes, including gene expression patterns, epigenetics, and gene regulatory networks.

Functional genomics is a field of study within molecular biology that aims to understand the functions and interactions of genes at a genome-wide level. It combines various experimental and computational approaches to analyze the functions of genes and their products, such as RNA and proteins, within the context of cellular processes and organismal development.

The main objectives of functional genomics include:

i. Gene function elucidation: Functional genomics aims to determine the roles and functions of individual genes. This involves identifying the specific biological processes or pathways in which these genes are involved.
ii. Gene regulation: Understanding how genes are regulated is a critical aspect of functional genomics. Researchers investigate the mechanisms that control when and where genes are expressed, and how they are turned on or off in response to various internal and external signals.
iii. Genetic interactions: Functional genomics examines how genes interact with each other within the genome and how these interactions influence cellular functions and phenotypes.
iv. Comparative genomics: By comparing the genomes of different species, researchers can gain insights into the evolutionary relationships between genes and understand how similar or different gene functions are across organisms.

To achieve these objectives, functional genomics employs various experimental techniques, such as:

i. Transcriptomics: This involves studying the expression levels and patterns of all genes (transcripts) in a cell or tissue at a given time. Techniques like microarrays and RNA sequencing (RNA-seq) are commonly used for transcriptomic studies.
ii. Proteomics: Analyzing the full set of proteins expressed in a cell or organism, proteomics helps researchers understand how genes translate into functional proteins and their roles in cellular processes.
iii. Epigenomics: This field studies heritable changes in gene expression that do not involve alterations in the underlying DNA sequence. Epigenetic modifications play a crucial role in gene regulation.
iv. Functional RNA analysis: Studying non-coding RNAs, such as microRNAs and long non-coding RNAs, can provide insights into their regulatory functions.
v. Genetic screens: These are used to identify genes associated with specific functions or phenotypes by systematically inactivating or overexpressing individual genes and observing the resulting effects.
vi. CRISPR-Cas9 technology: The revolutionary gene editing tool CRISPR-Cas9 is widely used in functional genomics to selectively modify specific genes and study their functions.

The data generated from these experiments are often complex and require advanced bioinformatics and computational approaches for analysis and interpretation.

Functional genomics is fundamental to understanding the molecular basis of various diseases, identifying potential drug targets, and unraveling the complexities of cellular processes and organismal development.

Plant epigenetics

Epigenetics involves the study of heritable changes in gene expression that do not involve alterations in the DNA sequence. Researchers are investigating the role of epigenetic modifications, such as DNA methylation and histone modifications, in regulating various aspects of plant growth, development, and responses to environmental cues.

Plant epigenetics is a fascinating field of study that explores the heritable changes in gene expression and function that occur without any alteration in the DNA sequence itself. Epigenetics refers to the modifications or marks on the DNA and associated proteins that can influence how genes are regulated and expressed, ultimately impacting various aspects of an organism's development, adaptation, and response to the environment.

Epigenetic modifications in plants can be reversible and can occur in response to internal signals (such as developmental cues) or external factors (like environmental stresses). Some of the key epigenetic mechanisms in plants include DNA methylation, histone modifications, and small RNA-mediated gene regulation.

i. DNA Methylation: DNA methylation is a common epigenetic modification in plants. It involves the addition of a methyl group to specific cytosine bases in the DNA sequence, leading to changes in gene expression. Methylation patterns can be passed on from one cell generation to another and even from one generation of plants to the next.

ii. Histone Modifications: Histones are proteins around which DNA is wrapped, forming a complex called chromatin. Chemical modifications to histone proteins, such as acetylation, methylation, phosphorylation, etc., can affect how tightly the DNA is wrapped and, in turn, influence gene accessibility for transcription.
iii. Small RNA-mediated gene regulation: Small RNAs, such as microRNAs and small interfering RNAs (siRNAs), can interact with the messenger RNAs (mRNAs) of specific genes, leading to their degradation or translational repression. This process can fine-tune gene expression levels and response to different environmental stimuli.

Plant epigenetics plays a critical role in various aspects of plant biology, including:

i. Developmental processes: Epigenetic modifications are essential for controlling cell differentiation and tissue-specific gene expression during plant growth and development.
ii. Stress response: Plants can use epigenetic mechanisms to respond to environmental changes, such as drought, temperature fluctuations, or pathogen attacks.
iii. Reproduction: Epigenetic changes can be inherited and play a role in regulating gene expression during seed development and germination.
iv. Adaptation and evolution: Epigenetic modifications can provide a mechanism for plants to adapt to changing environmental conditions without alterations in their DNA sequence. Additionally, they may contribute to the evolution of new traits over generations.

Studying plant epigenetics can provide valuable insights into how plants adapt to their surroundings and how we might be able to manipulate epigenetic processes to improve crop productivity, environmental resilience, and other desirable traits in plants. However, it's essential to continue research in this area to fully understand the complexity and potential applications of plant epigenetics.

Plant-pathogen interactions

Understanding how plants defend themselves against pathogens and pests is a crucial area of research. Scientists are studying the molecular mechanisms underlying plant immunity and pathogen virulence to develop novel strategies for disease control in crops.

Plant-pathogen interactions refer to the relationships between plants and various microorganisms, such as pathogens, that can cause diseases in plants. These interactions can be intricate and dynamic, involving a constant battle between the plants' defense mechanisms and the pathogens' strategies to invade and colonize their hosts. Understanding these interactions is crucial for agricultural practices, as they can significantly impact crop health, yield, and overall food security.

There are three main types of plant-pathogen interactions:

i. Compatible Interactions: In compatible interactions, the pathogen successfully infects the plant, leading to disease development. The pathogen has evolved mechanisms to overcome the plant's defense responses and exploit the host's resources for its own growth and reproduction. These interactions often result in visible symptoms, such as wilting, leaf spots, or necrosis.

ii. Incompatible Interactions: In incompatible interactions, the plant is resistant to the pathogen's invasion, preventing the development of disease. Plants possess a range of defense mechanisms, such as physical barriers, chemical toxins, and immune responses, which can impede the pathogen's growth and spread. This resistance can be qualitative (controlled by a single gene) or quantitative (controlled by multiple genes).

iii. Non-host Interactions: Non-host interactions occur when a pathogen cannot infect a particular plant species. Non-host resistance is generally stronger and more broad-spectrum than host resistance. It is governed by multiple layers of defense, making the plant immune to a wide range of pathogens that would otherwise infect other plant species.

The interaction between plants and pathogens involves a series of events:

i. Recognition: Plants have receptors that can detect specific molecules or structures present in the pathogen, known as pathogen-associated molecular patterns (PAMPs). The recognition of PAMPs triggers an initial immune response called PAMP-triggered immunity (PTI).

ii. Effector-triggered immunity (ETI): Some pathogens secrete effector molecules into the plant cells to suppress PTI. In response, plants have resistance (R) proteins that can detect these effectors and trigger a stronger immune response called effector-triggered immunity (ETI).

iii. Systemic Acquired Resistance (SAR): After an initial infection, plants can develop a more extensive and long-lasting resistance to various pathogens throughout the entire plant system, known as systemic acquired resistance.

iv. Pathogen Adaptation: Over time, pathogens can evolve to overcome the plant's defense mechanisms through mutations that change their effectors or other virulence factors. This continuous arms race between plants and pathogens leads to an ongoing evolution of resistance and virulence strategies.

Researchers and plant breeders work to better understand these interactions to develop disease-resistant crops through traditional breeding methods or genetic engineering. By enhancing plant defenses or incorporating resistance genes, they aim to improve crop resilience and reduce the dependence on chemical pesticides.

Plant-microbe interactions:

The study of plant interactions with beneficial microbes, such as rhizobia and mycorrhizal fungi, has gained significant attention. Researchers are exploring the mutualistic relationships between plants and microbes, including nutrient exchange, disease resistance, and stress tolerance.

Plant-microbe interactions refer to the relationships between plants and various microorganisms, including bacteria, fungi, viruses, and other microorganisms, that can positively or negatively impact the plants' growth, health, and overall well-being.

These interactions can be highly complex and have significant implications for agriculture, ecology, and environmental sustainability. Let's explore some of the key aspects of plant-microbe interactions:

i. Symbiotic relationships: Some microorganisms form mutually beneficial relationships with plants, known as symbiosis. There are two main types of symbiosis:
   a. Mutualism: In mutualistic interactions, both the plant and microbe benefit. An example is the association between mycorrhizal fungi and plant roots. The fungi help the plant absorb nutrients, such as phosphorus and nitrogen, from the soil, while the plant provides the fungi with sugars produced through photosynthesis.
   b. Commensalism: In commensalistic interactions, the microbe benefits from the association, but the plant is neither significantly helped nor harmed. One example is the presence of epiphytic bacteria on leaf surfaces, which may not actively harm the plant but can impact the plant's surface and microbial community.
ii. Pathogenic interactions: Some microorganisms can cause diseases in plants, leading to reduced growth, yield, or even plant death. These pathogenic interactions can have severe economic and ecological consequences. Plant pathogens include bacteria, fungi, viruses, and nematodes, among others.
   a. Bacterial and fungal diseases: Bacterial diseases like bacterial blight and fungal diseases like powdery mildew are examples of plant-pathogen interactions.
   b. Viral diseases: Viruses can infect plants and disrupt their normal functions, leading to various symptoms such as mosaic patterns on leaves and stunted growth.
iii. Induced systemic resistance (ISR): Some beneficial microorganisms can activate the plant's defense responses against pathogens without causing disease themselves. This phenomenon is known as induced systemic resistance (ISR) and can help plants defend against future pathogen attacks.
iv. Biocontrol: Certain beneficial microorganisms act as biocontrol agents, helping to suppress plant pathogens and protect plants from diseases. This strategy is environmentally friendly and reduces the reliance on chemical pesticides.
v. Nitrogen fixation: Some bacteria, known as nitrogen-fixing bacteria, can convert atmospheric nitrogen into a usable form for plants. This process enriches the soil with nitrogen, benefiting plant growth and ecosystem productivity.
vi. Plant growth promotion: Some microorganisms can promote plant growth by producing growth-promoting substances like phytohormones, solubilizing nutrients, and enhancing nutrient uptake.

Understanding plant-microbe interactions is crucial for sustainable agriculture, as it allows us to harness beneficial interactions while mitigating the harmful effects of pathogenic ones. Researchers continue to study these interactions to develop innovative approaches for disease control, crop enhancement, and ecological restoration.

Synthetic biology and genetic engineering

Advances in genetic engineering techniques are enabling scientists to modify plant genomes more precisely. Researchers are using these tools to engineer plants with improved traits, such as enhanced nutritional content, increased crop yield, and tolerance to abiotic and biotic stresses.

Synthetic biology and genetic engineering are two closely related fields that involve manipulating biological organisms and systems to create new functionalities, products, or solutions. While they share some common principles and techniques, they have distinct approaches and applications.

A. Genetic Engineering:

Genetic engineering is the process of directly manipulating an organism's DNA using various molecular biology techniques. This manipulation can involve inserting, deleting, or modifying specific genes to alter the organism's characteristics or produce desired traits. Genetic engineering has been extensively used in biotechnology, agriculture, medicine, and research.

Key aspects of genetic engineering include:

i. Recombinant DNA Technology: Genetic engineers use restriction enzymes and other molecular tools to cut and paste DNA from one organism into another, creating recombinant DNA molecules.
ii. Gene Editing: Techniques like CRISPR-Cas9 have revolutionized genetic engineering by enabling precise modifications in an organism's genome, leading to targeted gene editing and gene knockout (deactivation).
iii. Transgenic Organisms: Genetic engineering can produce transgenic organisms by introducing genes from different species into the host organism, resulting in organisms expressing foreign genes.
iv. Applications: Genetic engineering has applications in agriculture to create genetically modified crops with improved traits, in medicine for gene therapy to treat genetic diseases, and in research to study gene function and regulation.

B. Synthetic Biology:

Synthetic biology is a multidisciplinary field that combines principles from biology, engineering, computer science, and other disciplines to design and construct new biological parts, devices, and systems that do not exist naturally. It aims to engineer living systems to perform specific functions or produce valuable products.

Key aspects of synthetic biology include:

i. Standardized Biological Parts: Synthetic biologists use standardized genetic components (such as promoters, coding sequences, and terminators) to create genetic circuits that can be easily assembled and modified.
ii. Modular Design: The field adopts a modular approach to build complex biological systems, similar to how engineers use standardized parts to build diverse machines.
iii. Bottom-Up Approach: Synthetic biology starts with basic biological components and assembles them into larger systems to create novel organisms or functionalities.
iv. Applications: Synthetic biology has applications in biofuel production, pharmaceuticals, bioremediation, and the creation of biosensors and biomaterials.

While both synthetic biology and genetic engineering involve modifying biological systems, synthetic biology emphasizes the design and construction of new biological parts and

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systems, while genetic engineering often focuses on specific gene manipulations within existing organisms.

Both fields hold great potential for addressing various challenges in medicine, agriculture, industry, and environmental sustainability. However, they also raise ethical considerations, and responsible research and application are essential in their development.

Plant metabolomics:

Metabolomics involves the comprehensive analysis of small molecules (metabolites) in plants. Scientists are using advanced analytical techniques to identify and quantify metabolites, providing insights into the metabolic pathways involved in plant growth, development, and responses to environmental cues.

Plant metabolomics is a subfield of metabolomics that focuses on the comprehensive analysis of small molecules, known as metabolites, in plant tissues and cells. Metabolomics is a powerful analytical approach that aims to identify and quantify the complete set of metabolites present in a biological system. These metabolites include primary metabolites (e.g., sugars, amino acids, organic acids) and secondary metabolites (e.g., alkaloids, flavonoids, terpenoids).

The primary goal of plant metabolomics is to understand the metabolic processes that occur within plants and how these processes respond to different environmental factors, genetic variations, and other perturbations. By analyzing the metabolic profile of plants, researchers can gain insights into the physiological and biochemical status of the plant, assess its health, and understand how it interacts with its surroundings.

Plant metabolomics typically involves several key steps:

i. Sample preparation: Plant tissues or cells are collected and subjected to extraction procedures to isolate metabolites for analysis.
ii. Analytical techniques: Various analytical techniques are used to identify and quantify the metabolites in the samples. These techniques include mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and others.
iii. Data analysis: The obtained data is processed and analyzed using sophisticated bioinformatics tools and statistical methods. This step involves identifying the metabolites, comparing different samples, and finding patterns and correlations in the data.
iv. Interpretation and functional analysis: Researchers interpret the results to understand the metabolic pathways and networks involved in specific biological processes or responses. This can help uncover how plants adapt to environmental changes, respond to stressors, or interact with other organisms.

Applications of plant metabolomics are wide-ranging and include:

i. Studying plant responses to abiotic stresses (e.g., drought, heat, cold) and biotic stresses (e.g., pathogen attacks, insect herbivory).
ii. Identifying and characterizing bioactive compounds with potential medicinal, pharmaceutical, or agricultural applications.
iii. Assessing the nutritional quality and safety of food crops.

iv. Understanding plant-pathogen interactions and identifying potential targets for disease control.
v. Studying plant metabolic engineering for improving desirable traits in crops.

Overall, plant metabolomics plays a crucial role in advancing our knowledge of plant biology and holds great promise for applications in agriculture, medicine, and other fields.

Various significance of plant researches all across the world

1. Food Security: Plants are the primary source of food for humans and animals. Understanding plant genetics, physiology, and ecology helps improve crop yield, nutritional content, and resistance to pests and diseases, ensuring food security for a growing global population.
2. Sustainable Agriculture: Plant research leads to the development of sustainable farming practices that reduce environmental impacts, conserve resources, and promote long-term agricultural productivity.
3. Climate Change Mitigation: Plants play a critical role in carbon sequestration and climate regulation. Research on plant responses to climate change helps us understand their ability to mitigate greenhouse gas emissions and adapt to shifting environmental conditions.
4. Biodiversity Conservation: Studying plant species and their ecosystems aids in biodiversity conservation efforts, which are essential for maintaining ecological balance and preserving endangered species.
5. Medicine and Health: Many pharmaceuticals are derived from plants or plant compounds. Plant research can lead to the discovery of new drugs and treatments for various diseases and medical conditions.
6. Renewable Energy: Certain plant species are used to produce biofuels, providing a renewable and cleaner alternative to fossil fuels.
7. Environmental Cleanup: Plants can be used for phytoremediation, a process that helps clean up contaminated environments by absorbing and detoxifying pollutants.
8. Horticulture and Landscaping: Plant research contributes to the development of attractive and adaptable ornamental plants, enhancing the aesthetics of landscapes and gardens.
9. Ecosystem Restoration: Understanding plant interactions within ecosystems aids in ecosystem restoration efforts, including reforestation and habitat rehabilitation.
10. Genetic Engineering and Biotechnology: Plant research enables the development of genetically modified crops with improved traits, such as increased yield, drought resistance, and pest tolerance, addressing agricultural challenges.
11. Economic Impact: Agriculture, horticulture, forestry, and other plant-related industries contribute significantly to the global economy. Plant research helps boost productivity and economic growth in these sectors.
12. Education and Knowledge: Plant research adds to our understanding of biology, ecology, and the natural world, enriching scientific knowledge and inspiring future generations of researchers.

13. Societal Benefits: Plants provide numerous resources for human societies, such as timber, fiber, and medicinal products. Plant research helps optimize the sustainable use of these resources.
14. Innovation and Technology: Plant research fosters innovation in various fields, from agriculture and biotechnology to food science and environmental engineering.

Plant research plays a crucial role in addressing global challenges such as food security, climate change, environmental degradation, and the sustainable use of natural resources. It also contributes to the development of new medicines, agricultural practices, and biotechnological advancements that benefit society as a whole. By understanding plants better, researchers can make informed decisions and develop innovative solutions to enhance our relationship with the natural world.

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PLANT QUARANTINE AND INSPECTION

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Abstract:

Plant quarantine in India is a crucial phytosanitary measure aimed at safeguarding the nation's agriculture and environment from the introduction and spread of pests, diseases, and invasive plant species. As a major agricultural country and a key player in international trade, India faces significant risks of unintentional pest and disease transmission. Administered by the Department of Agriculture, Cooperation & Farmers Welfare, the plant quarantine program operates under relevant legislation and follows International Plant Protection Convention (IPPC) guidelines to harmonize with global standards. Through risk assessment and management, imported plants and plant products undergo thorough inspection and, if necessary, phytosanitary treatments to prevent the establishment of exotic pests and diseases. India continually adapts its plant quarantine system to address emerging challenges and collaborates with international organizations and neighboring countries to strengthen regional pest surveillance and management. The success of India's plant quarantine program contributes to agricultural biodiversity, sustainable practices, and overall economic and environmental well-being.

Keywords: Plant Quarantine, NBPGR, Diseases, Phytosanitary measures

Introduction:

Plant quarantine is a critical phytosanitary measure implemented by governments and agricultural authorities worldwide to prevent the introduction and spread of pests, diseases, and invasive plant species. It is an essential component of biosecurity and plays a crucial role in safeguarding agricultural and natural ecosystems, as well as protecting the economy and public health. The movement of plants and plant products across international borders has increased significantly due to globalization and international trade. While this has facilitated economic growth and cultural exchange, it has also increased the risk of unintentional introduction and establishment of harmful pests and diseases in new regions. These pests and diseases can cause severe damage to crops, forests, gardens, and natural habitats, leading to devastating consequences for agricultural productivity and biodiversity. The primary goal of plant quarantine is to regulate and control the import, export, and domestic movement of plants, plant products, and related materials to prevent the introduction and spread of harmful organisms. Quarantine measures aim to detect and intercept potential threats at ports of entry, border checkpoints, and within a country's territory. If a potentially harmful organism is identified, appropriate measures are taken to manage or eradicate the threat effectively. Plant quarantine operates on the principle of risk assessment and management, wherein the likelihood of introducing harmful organisms is evaluated based on factors such as the origin of the plants or plant products, known pests in the region of origin, and the intended use or destination of the consignment. High-risk commodities and pathways are subject to more stringent quarantine measures, while low-risk items may be granted easier access. International cooperation and adherence to phytosanitary standards are essential for effective plant quarantine. Countries often collaborate through organizations like the International Plant Protection Convention (IPPC), which establishes guidelines for phytosanitary measures and facilitates the harmonization of quarantine procedures globally. In conclusion, plant quarantine is a crucial tool for preventing the spread of pests, diseases, and invasive species that could have devastating impacts on agriculture, ecosystems, and economies. By implementing rigorous quarantine measures and promoting international cooperation, countries can mitigate the risks associated with the movement of plants and protect their agricultural and environmental assets.

Why is plant quarantine necessary?

1. Rapid arrival and establishment or development of destructive new pests.
2. Epidemics of plant pests can result in agricultural loss.
3. This can affect food production or export trade.
4. Therefore, the main aim is to prevent from exotic pests or diseases by applying laws.

The objectives of plant quarantine order

1. To prohibit the import of plants/plant material.
2. To prohibit the import of germplasm/GMOS/ transgenic plant material. No consignment of germplasm/transgenic/Genetically Modified Organisms (GMOs) shall be imported into India for research/experimental purpose without valid permit issued by the Director, National Bureau of Plant Genetic Resources, New Delhi -110012.
3. To prohibit the import of deleterious weed species.
4. To regulate the import of insects/fungi &other microbial cultures/bio-agents**.**
5. To regulate import of timber & bulk shipment of food grain) No consignment of timber shall be permitted import unless the following conditions and requirements are met with, namely:
   a) The timber shall be stripped off its bark, either be squared or rounded and accompanied by an official statement that the wood has been appropriately fumigated/treated and such treatment shall be endorsed in the phytosanitary certificate issued at the country of origin or re-export, as the case may be;
   b) The timber shall be marked 'kiln-dried' or with any internationally recognized mark.
6. To regulate packaging material of plant origin in imports.
7. To regulate imports of soil/peat or sphagnum-moss etc**.**
8. To regulate the import of transit consignments. (Kumar *et al*., 2020).

Agencies involved in plant quarantine

- Directorate of Plant Protection, Quarantine and Storage, Faridabad.
- National Bureau of Plant Genetic Resources (NBPGR) New Delhi.
- Crop specific Research Institutes of Indian council of Agricultural Research (ICAR).
- Head of Plant Pathology Division of State Agricultural University (SAU).
- State Agricultural and Horticultural Departments. (Kumar *et al*., 2020).

Plant quarantine acts in India (Laxmi *et al*., 2014)

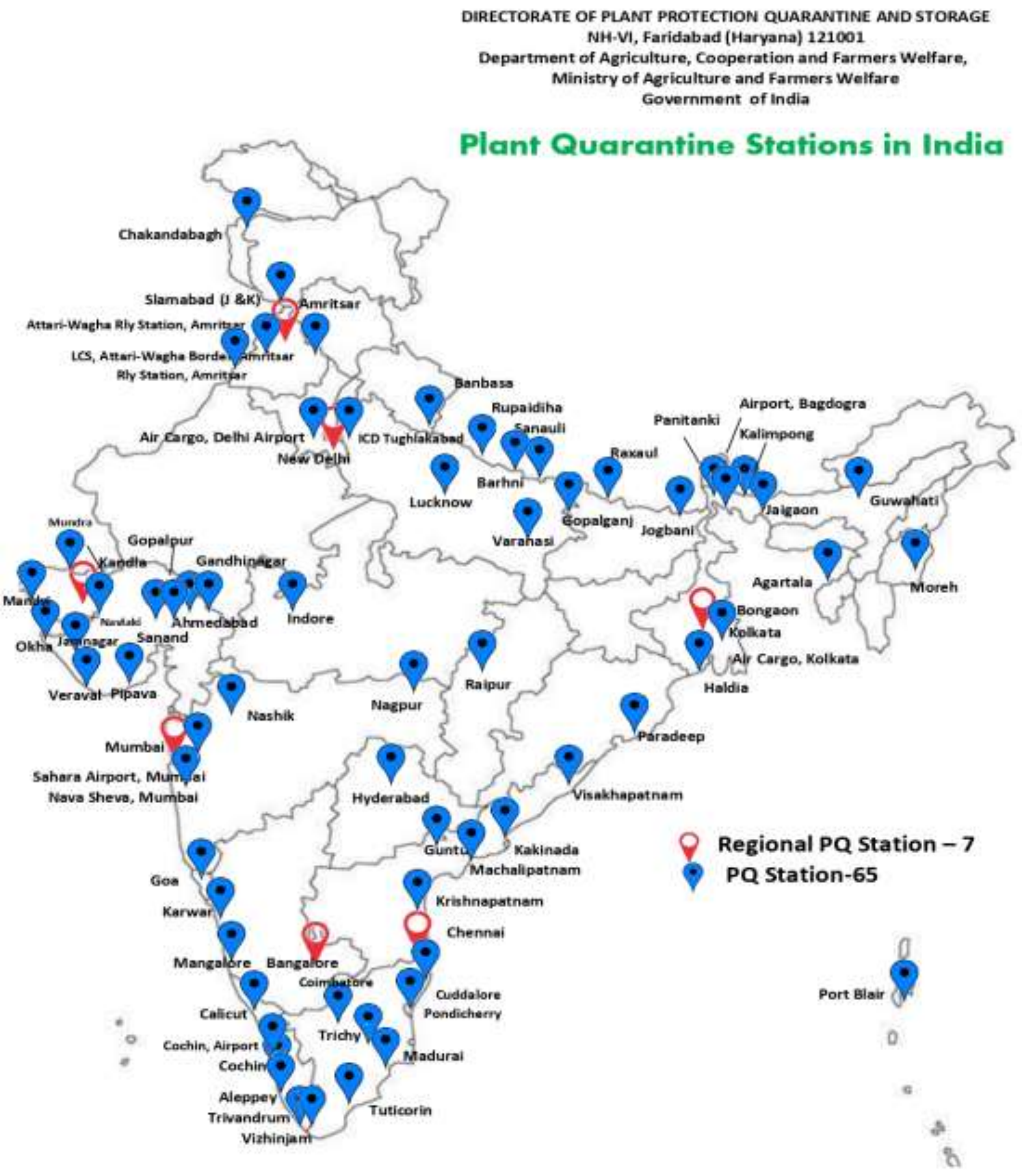
1914	Destructive Insects and Pests Act.
1946	Plant Quarantine processing of exotic germplasm initiated at the then Imperial Agricultural Research Institute (now Indian Agricultural Research Institute) for Plant Introduction Scheme.
1956	Plant Quarantine Units in the Division of Entomology and Plant Pathology established at IARI, New Delhi.
1961	Plant Introduction Division established in IARI; germplasm exchange and plant quarantine activities increased manifold.
1967	Division of Nematology established at IARI, which housed Nematology unit of plant quarantine
1976	NBPGR established
1981	Post-entry quarantine of imported (chemically treated) wheat, barley and triticale started in isolation nursery.
1983	Post-entry quarantine for detection of seed transmitted viruses in exotic legumes started.
1984	Plants, Fruits and Seeds (PFS) (Regulation of Import into India) Order issued under DIP Act
1988	New Policy on Seed Development (NPSD) announced, resulting in increased import of seed material
1989	PFS Order (1984) revised to meet the requirements of NPSD and increasing imports.
2003	Plant Quarantine (Regulation of Import into India) Order.

Alien plant pathogen in India (Singh *et al*., 2016)

Sr. No.	Diseases	Native Place	Year of Introduction
1	Coffee rust (*Hemileia vastatrix*)	Sri Lanka	1879
2	Late blight of potato (*Phytophthora infestans)*	England	1883
3	Flag smut of wheat (*Urocystis tritici*)	Australia	1906
4	Downy mildew of grapes (*Plasmapara viticola)*	Europe	1910
5	Cucurbit powdery mildew *(Erysiphae chicoracearum)*	Sri Lanka	1910
6	Maize downy mildew (*Sclerospora phillipinensis)*	Java	1912
7	Blast of paddy (*Pyricularia oryzae*)	South East Asia	1918
8	Crucifer black rot (*Xanthomonas campestris pv. campestris)*	Java	1929
9	Rubber powdery mildew (*Oidium heavea)*	Malaya	1938
10	Tobacco black shank (*phytophthora nicotianae)*	Holland	1938
11	Banana bunchy top	Sri Lanka	1940
12	Hairy root of apple	England	1940
13	Potato wart (*Synchytrium endobioticum*)	Netherlands	1953
14	Onion smut (*Urocystis cepulae)*	Europe	1958

Plant quarantine stations in India

Presently, there are 73 Plant quarantine Stations at different international airports, seaports and land frontiers implementing the plant quarantine stations regulations with its Headquarters at Faridabad. Presently, there are 73 Plant quarantine Stations at different International Airports, Seaports and Land Frontiers implementing the plant quarantine stations regulations with its headquarters at faridabad. the list of existing plant quarantine stations are following:



Plant quarantine methods

There are number of plant quarantine methods which are used separately or collectively to prevent or retard the introduction and establishment of exotic pests and pathogens. The components of plant quarantine activities are:

1. Complete embargoes: It involves absolute prohibition or exclusion of specified plants and plant products from a country infected or infested with highly destructive pests or diseases that could be transmitted by the plant or plant products under consideration and against which no effective plant quarantine treatment can be applied or is not available for application.

2. Partial embargoes: Partial embargoes, applying when a pest or disease of quarantine importance to an importing country is known to occur only in well defined area of the exporting country and an effectively operating internal plant quarantine service exists that is able to contain the pest or disease within this area.

3. Inspection and treatment at point of origin: It involves the inspection and treatment of a given commodity when it originates from a country where pest/disease of quarantine importance to importing country is known to occur.

4**. Inspection and certification at point of origin:** It involves pre-shipment inspection by the importing country in cooperation with exporting country and certification in accordance with quarantine requirements of importing country.

5. Inspection at the point of entry: It involves inspection of plant material immediately upon arrival at the prescribed port of entry and if necessary subject to treatment before the same related.

6. Utilization of post entry plant quarantine facilities: It involves growing of introduced plant propagating material under isolated or confined conditions (Shukla *et al*., 2007).

Forms of quarantine

Grouped into five classes-

1. International quarantine: Legislation to prevent the introduction of new pests and weeds from foreign countries.

2. Domestic quarantine: Legislation to prevent the spread of already established pests, diseases, and weeds from one part of the country to another. Most of the states in India have plant quarantine laws to avoid entry of plant pests and diseases

- **A: Bunchy top of banana:** The export and the transport from the States of Assam, Kerala, Orissa, West Bengal, Tamil Nadu to any other State of Banana plant or any other plant of the genus Musa, including sucker, stem, leaf, flower, and any other part thereof which may be used for propagation, or the materials of banana plant or any other plant of the genus Musa, which are used for packing and wrapping, excluding the banana fruit is prohibited.
- **B: Banana mosaic:** The export and transport from the States of Maharashtra and Gujarat of any plant of Banana or any other plant of genus Musa including the sucker, stem, flower and any other part thereof, but excluding leaf and fruit thereof is prohibited; vide Government of India notification No.F. 6-10-PPS dated the 11thApril, 1961.
- **C: Potato wart:** The export to potato tubers from the State of West Bengal to any other State or territory of India is prohibited.
- **D: Apple scab:** The Directorate of Horticulture, Himachal Pradesh worked out a detailed scheme for the eradication of scab, and also issued a notification No.NIC.20/76 dated 28 December 1978, prohibiting the export of planting material of apple outside the State.

3. Farmer's quarantine: Legislation to enforce farmers to apply effective control measures to prevent damage by already established pests.

4. Food quarantine: Legislation to prevent the adulteration and misbranding of insecticides and determine their permissible residue tolerance levels in food stuffs.

5. Quarantine for pest control operations: Legislation to regulate the activities of people engage.

Pest/ pathogen detection technique

A wide variety of pests and pathogens (insects, mites, nematodes, fungi, bacteria, viruses, viroid/MLOs, spiroplasma, etc.) and weeds are the objects for quarantine consideration. Similarly, planting material also may be introduced in a variety of forms, i.e., true seed, corms, bulbs, rhizomes, suckers, runners, bud wood, scions, cuttings and rooted plants. Therefore, detection techniques would vary depending on the type of material, the host species and the type of pests/pathogens involved. Many a times, more than one technique would have to be used. Detection techniques may broadly be classified into two groups: (a) generalized tests which would reveal a wide range of pests/pathogens; and (b) specialized or specific tests which are used to detect specific pests/pathogens.

Generalized tests

A very widely used method is the inspection of dry seed with the naked eye or under the low power of microscope. This method would reveal a wide range of free moving insects, their eggs and larval stages, mites on or with the seed, weeds, soil, infected/infested plant debris, fungal fructifications like sclerotia, smut and bunt balls, nematode galls, discolored or deformed seeds mixed with seed; oospore or bacterial crusts, acervuli, pycnidia, sclerotia and even free spores of rusts, smuts and many other fungi on the seed surface. Examination of dry seed under UV or NUV light may reveal infections of certain fungi and bacteria through emission of fluorescence of different colours. Examination of seed washings may reveal surface contamination by rusts, smuts, downy mildews and a large number of other fungi. Most commonly used incubation methods for the detection of fungi are the common moist blotter and agar tests wherein seeds are incubated on these media for a specific length of time (generally about a week) at a suitable temperature under alternating light and dark cycles. These two media reveal a wide range of internally seed-borne fungal and some bacterial pathogens in a wide variety of crops. Seedling symptom test and the growout test are quite versatile and reveal the symptoms produced by any category of plant pathogens including fungi, bacteria and viruses. Growout test is the simplest of the tests extensively used for the detection of viruses. However, some viruses may be carried symptomlessly in the plant and, therefore, it should be used in combination with other tests like indexing on indicator test plants and serology.

Specialized tests

Insects

X-ray radiography has been used very successfully all over the world for the detection of hidden infestation (with no apparent sign of infestation on the seed surface) of insects, particularly seed infesting chalcids and bruchids. Seed transparency test (boiling the seeds in lacto phenol to make them transparent) may also be used for the detection of hidden infestation and extraction of the insects for identification. X-ray radiography is also very effective in salvaging infested seed lots.

Nematodes

For the detection of seed-borne nematodes, seeds are soaked in water for about 24 hours. This makes the nematodes active, which then come out of the seed into the water, or the seeds may be teased out with the help of forceps and a needle and examined for detection of nematodes under a stereo microscope. In rooted plants, the accompanying soil and plant debris may similarly be soaked in water and nematodes may be extracted for identification using nematological sieves or tissue paper.

Fungi, bacteria and viruses

Serological tests are very effective for the detection and identification of viruses and bacterial pathogens and are being used in various plant quarantine stations with great success. Phage-plague technique is still more sensitive for bacterial pathogens as even strains of bacteria can be identified. Indicator test plants are also very helpful as they may reveal pathogenic races within a species of a fungus, bacterium and specific strains within a virus. Modifications of the generalized incubation tests (agar and blotter tests) have also been used for the detection of specific plant pathogens. Deep-freezing blotter test and 2,4-D blotter test are very efficient for detection of black-leg pathogen (*Phoma lingam*) in crucifer crops. Potato-dextrose-oxgall agar is useful for the detection of *Septoria nodorum* in wheat while PCNB agar is a selective medium for detection of Fusarium species in cereals. In the case of vegetative propagules, laboratory methods may suffice for the detection of insects and mites, nematodes, majority of fungi and certain bacteria. However, for the detection of systemic fungal pathogens, bacteria, viruses, viroid and MLOs, isolation growing for a season or a year or more in quarantine glass-houses/net-houses is required (Borkar *et al*., 2021).

Plant quarantine treatment

Once a pest, pathogen or a weed has been detected in the introduced planting material, quarantine officials must make all efforts to disinfect/decontaminate the material and make it available for further exploitation in the country without undue delay. Such an effort on the part of quarantine officials would help to restore a positive image to plant quarantine. However, it may be kept in mind that treatments, which only reduce the inoculum, may be acceptable for general agricultural practices, but they are not acceptable in plant quarantine. For quarantine purposes, tolerances are zero and, therefore, no residual inoculum of exotic pests/pathogens must remain. Fool-proof eradicative treatments are required to be employed before release of the planting material from quarantine.

Fumigation

Fumigation of the material under atmospheric or under reduced pressure has been found acceptable as a quarantine treatment against insects and mites. Fumigants like methyl bromide, HCN, phosphine and EDCT (ethylene dichloride + carbon tetrachloride mixture) are commonly used.

Heat treatment

Hot water treatment or hot air treatment are also used in quarantine for eradication of insects, mites, nematodes, fungi, bacteria and viruses. The basic principle involved is that treatment temperature should be sufficiently high to kill the associated pest/pathogen but not the host. However, in most cases, margin of safety is very narrow and, therefore, the temperature should be very accurately controlled. Some recommended hot water treatments (Kahn, 1977) are:

1. Against nematodes: Flower bulbs, 44° C for 240 min; chrysanthemum, 48° C for 25 min; potato tubers, 45° C for 5 min;
2. Against insects and mites: Narcissus bulbs, 44° C for 180 min; strawberry runners, 46° C for 10 min;
3. Against viruses: Grape vine, 45° C for 120-180 min; sugarcane setts, 50° C for 120 min.; potato tubers, 50° C for 17 min;
4. Against fungi: Celery seed, 50° C for 25 min;
5. wheat seed, 52-54° C for 10 min;

Lambat *et al.* (1974) reported eradication of *Phoma betae* in sugar beet seed by hot water treatment at 50° C for 30 min. Hot water seed treatment has also been reported to eradicate certain bacterial pathogens like black-rot pathogen *(Xanthomonas campestris pv. campestris*) in crucifer seeds at 50° C for 30 min; bacterial blight of cluster bean (*X. campestris pv. cyamopsidis*) at 56° C for 10 min; and bacterial blight of sesame (*X. campestris pv. sesami*) at 52° C for 10 min.

Chemical treatments

Chemicals may be applied as dust, slurry, spray or as dip. It should be ensured that dosage of chemical should be enough to eradicate the inoculum but should not kill the host and the chemical should not be hazardous to personnel handling the treated seed. Treatment should be given on arrival and only after ascertaining the health status of the material. The choice of the chemical and dosage to be used should be made depending upon the pest/pathogen involved. Seeds treated at origin are not only difficult to examine but are hazardous to inspect also. Heavily treated seed, which makes inspection difficult, should be denied entry.

Tissue culture

Tissue culture as a safeguard in quarantine has been advocated by Kahn (1979). This method reduces the pest/pathogen introduction risk in two ways: (i) the size of the consignment is very much reduced since the introductions are represented by meristem tips, excised buds or embryos, and (ii) the aseptic plantlet system has built-in pest/pathogen detection capability. All insects, mites, nematodes and most fungi can be eliminated. Symptoms on young seedlings, and growth of the organisms on the agar medium, if any, may be visible through the transparent culture tubes, and these could be discarded. Tissue culture in combination with thermotherapy and chemotherapy is an excellent safeguard from quarantine angle. However, certain systemically infecting pathogens like rusts, downy mildews, bacteria, viruses, viroids and MLOs, may still get transported. Therefore, as an additional safeguard, the tissue culture material could be passed through post-entry quarantine isolation growing and indexed/tested for the suspected pathogens. Indeed, tissue culture technology provides an exciting prospect for large scale exchange of genetic stocks with very little pest/pathogen introduction risk (Borkar *et al*., 2021)

Case studies

First report of Puccinia horiana causing white rust of chrysanthemum in India

A quarantine disease of United States and Canada *Puccinia horiana* causal organism of White Rust of Chrysanthemum first reported in 2013 and 2014, n in fields in and around Bengaluru, Karnataka state, India. Conversations with chrysanthemum growers revealed that such occurrences had also been noticed in Udagamandalam district of Tamil Nadu state since 2012, with a renewed occurrence there in 2014. Chrysanthemum (*Dendranthema grandiflora*) is an important export-oriented cut-flower crop in the state of Tamil Nadu.

A survey was conducted for the occurrence of fungal diseases in chrysanthemum in Kothagiri Hills of Nilgiris District and Yercaud Hills of Salem District in 2013. A variety, Saffin Pink, of chrysanthemum exhibited pale-green to sunken yellow spots on the upper surface of the leaf accompanied with raised, buff or pinkish, waxy pustules on the corresponding lower surface. The disease affected mainly the leaves, but under severe infestation, spread to stems, bracts, or even the flowers. Under severe infection, the pustules coalesced leading to complete drying and death of the plant. The disease incidence ranged from 70 to 80% across all the areas surveyed and was observed in three crops cultivated throughout the year. Observation under stereo zoom microscope revealed the presence of dull white, raised, waxy telia covered with pubescence of the leaf. Teliospores were bicelled, pedicellate, oblong to clavate, smooth, and yellowish to gray with pale-yellow cell wall. The mean diameter of the teliospore cluster varied from 58 to 92 µm at 400× magnification. Size of individual teliospores ranged from 10 to 13 µm width to 50 to 85 µm length at 400× magnification. On the basis of morphological features, the rust pathogen was identified as Puccinia horiana (Pedley 2009) and the disease was recognized as Chrysanthemum white rust (CWR). Recently it was seen in Seobagh, Kullu district of Himachal Pradesh and Ranghat – II Block, Nadia district, (Sriram *et al*., 2015).

Conclusion:

- The purpose of the health authorities was to establish adequate detention period. The term ‘Quarantine’ came to be only used for the detention and the practices connected with it. Plant Quarantine regulations are promulgated by the national and the state governments to prevent the introduction and spread of harmful pests and pathogens.

- Phytosanitary or health certificate is a certificate which should accompany a plant or plant material or seed which is to be moved from one place to another place
- NBPGR plays important role for issuance of Import Permit and to undertake quarantine processing of all imported PGR and trial material for results.
- Quarantine and Fumigation stations under DPPQS handle bulk imports for commerce and for planting.
- Government need to concern about Domestic Plant quarantine at state level, inert state border and check of seed/stack certificates or nursery infection.

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BIOSYNTHESIS OF PLANT SECONDARY METABOLITES IN HETEROLOGOUS MICROORGANISMS THROUGH METABOLIC ENGINEERING

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Abstract:

In recent era plant natural products have gained worldwide attraction and importance in global market for their diverse application in many fields like pharmaceuticals, nutraceuticals, seasonings, food, and drug industries having huge commercial values. More than 200,000 plant natural products reported till date have shown pharmacological activities and biotechnological importance. The successful extraction of natural products through plant have limited by several environmental factors. Likewise, the chemical synthesis of natural products is also reported to be inefficient due to the complexity of the chemical structures involving enantioselectivity and regioselectivity. Currently researchers are urged to search for an alternative means for overproducing valuable natural products using microorganisms. Using the inexpensive renewable resources like reconstructing the biosynthetic pathway in heterologous microorganisms have provided a significant result for producing enough of a desired natural product. Since earlier days the people are looking for the resource-conserving and environment-friendly synthesis pathway for natural plant products using microbial cell factories. Recently various metabolic engineering strategies have been developed to produce natural products using microorganisms. Microbial expression systems to produce various valuable compounds includes polyculture and co-culture consortiums for carrying out robust biosynthesis. The two most important heterologous hosts widely used as a cell factory to produce natural products are Escherichia coli and Saccharomyces cerevisiae. They are most advantageous than other organisms because of their easy availability, high cell density culture, high growth rate and many more so they act as a well-established tools and strategies for metabolic engineering. After the complete study and elucidation of biosynthetic pathways and regulatory factors, it has helped to metabolically engineer new capabilities in planta as well as successful engineer the whole pathways into microbial systems. The present chapter focusses mainly on the plant secondary metabolites and its application for generating valuable compounds in microbial expression platforms and in plant metabolic engineering.

Keywords: Metabolic engineering, heterologous microorganisms, Plant natural products, application.

Introduction:

Since the advent of recombinant DNA technology, genetic engineering of cells, particularly microorganisms, has been successfully practiced for the development of strains capable of overproducing recombinant proteins and small molecule chemicals. For the later, strategies beyond simple genetic engineering are often required as they are synthesized through multiple intracellular reactions, which are further complicated by various factors including cofactor balance and regulatory circuits. Metabolic engineering can be defined as purposeful modification of cellular metabolism using recombinant DNA and other molecular biological techniques (Baiza *et al.*, 1999).

Secondary metabolites are broadly defined as natural products synthesized by an organism that are not essential to support growth and life. The plant kingdom manufactures over 200,000 distinct chemical compounds, most of which arise from specialized metabolism. While these compounds play important roles in interspecies competition and defense, many plants natural products have been exploited for use as medicines, fragrances, flavors, nutrients, repellants, and colorants. Despite this vast chemical diversity, many secondary metabolites are present at very low concentrations in planta, eliminating crop-based manufacturing as a means of attaining these important products. The structural and stereochemical complexity of specialized metabolites hinders most attempts to access these compounds using chemical synthesis. Although native plants can be engineered to accumulate target pathway metabolites (Zhou *et al.*, 2009; Glenn *et al.*, 2013; Lange and Ahkami, 2013; Wilson and Roberts, 2014; Tatsis and O'Connor, 2016), metabolic engineering is technically morechallenging in plants than in microbes. Advancements in synthetic biology have stimulated the synthesis of valuable natural products in tractable laboratory microbes by interfacing plant secondary pathways with core host metabolism. Microbial synthesis overcomes many of the obstacles hindering traditional chemical synthesis and plant metabolic engineering, thus providing an alternative avenue for exploring plant specialized pathways (Becker *et al.*, 2003). This Update provides a brief overview of engineering plant secondary metabolism in microbial systems. We briefly outline biosynthetic pathways mediating formation of the major classes of natural products with an emphasis on high-value terpenoids, alkaloids, phenylpropanoids, and polyketides. We also highlight common themes, strategies, and challenges underlying efforts to reconstruct and engineer these pathways in microbial hosts (Watts *et al.*, 2006). We focus chiefly on de novo biosynthetic approaches in which plant specialized metabolites are synthesized directly from sugar feedstocks rather than supplemented precursors or intermediates. Readers are directed to a selection of pioneering supplementation studies within the context of microbially-sourced plant natural products (Kaneko *et al.*, 2003; Yan *et al.*, 2005; Leonard *et al.*, 2007; Hawkins and Smolke, 2008; Leonard *et al.*, 2008; Fossati *et al.*, 2014; Fossati *et al.*, 2015).

Metabolic engineering of terpenoids

Engineering precursor availability the availability of precursors is an important issue in metabolic engineering. Whether the concentration of a certain isoprenoid precursor is limiting to produce terpenoids probably depends on the plant species, the tissue and the physiological state of the plant. Theinitial step toward the biosynthesis of IDP (isopentenyl diphosphate) through the MEP pathway is catalyzed by 1-deoxy-D-xylulose-5-phosphate (DXP) synthase (DXS). Over-expression and down-regulation of DXS in Arabidopsis affected the levels of various isoprenoids including chlorophylls, tocopherols, carotenoids, ABA, and GAs (Estevez *et al.,* 2001). Up-regulation of DXP reductoisomerase (DXR), which converts DXP to methylerythritol phosphate, in transgenic peppermint (Mentha piperita) plants resulted in a 50% increase in essential oil yield (Mahmoud & Croteau, 2001). As well as DXS and DXR, hydroxymethylbutenyl diphosphate reductase (HDR), the enzyme conducting the last step in the pathway generating IDP and DMADP, has also been suggested to be a rate-limiting step in the MEP pathway in both tomato (Lycopersicon esculentum) and Arabidopsis (Botella-Pavia *et al.,* 2004). When plants expressing taxadiene synthase (TXS) (Engineering diterpenoids and triterpenoids) under the cauliflower mosaic virus (CaMV) 35S promoter (Besumbes *et al.,* 2004) were crossed with plants over-expressing DXS or HDR, increases of 6.5 times and 13 times, respectively, were detected in the accumulation of taxadiene compared with plants over-expressing only TXS (Botella-Pavia *et al.,* 2004). The results of many studies reported to date suggest that, in general, the direct precursor for monoterpene biosynthesis (i.e., GDP) is largely available to introduced monoterpene synthases and, in some cases, does not seem to be limiting. For example, Lucker *et al.* (2004) reported that, in the flowers of transgenic tobacco (*Nicotiana tabacum*) plants expressing three different monoterpene synthases, the levels of products corresponding to the three enzymes were high but did not affect the level of the endogenous linalool production. Enhancing the precursor supply for the biosynthesis of isoprenoids in the mevalonate pathway has been attempted mainly by altering expression levels of the gene encoding 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), which is considered to be a rate-limiting step in the pathway (Chappell, Wolf, Proulx, & Saunders, 1995; Harker, Hellyer, John, Lanot, & Safford, 2003; Schaller *et al.,* 1995). Constitutive over-expression of HMGR in tobacco resulted in a three- to tenfold increase in total-sterol levels (Chappell *et al.,* 1995). Levels of other isoprenoids, including sesquiterpenes, were not altered, possibly because of compartmentation, channelling or the presence of other rate-limiting steps. In a study, overproduction of FDP synthase in Artemisia annua resulted in 2-3 fold increases in levels of the artemisinin (sesquiterpenes) in transgenic plants compared with those in wild-type plants (Chen *et al.,* 2000).

Engineering alkaloid biosynthesis

Alkaloids: a large family of plant pharmaceutical compounds and is a diverse group of small, heterocyclic, nitrogen-containing molecules that are thought to be involved in defending plants against herbivores and pathogens. Several alkaloids are exploited for their pharmaceutical properties. For example, morphine and codeine are analgesics, vinblastine, vincristine and taxol are anti-cancer agents, scopolamine widely used in medicine for its anticholinergic activity, colchicine is a gout suppressant, tubocurarine is a muscle relaxant and sanguinarine is an antibiotic. Most of these alkaloids are extracted from plants or are derived either chemically or via biotransformation from natural precursors. There is an increasing demand from the pharmaceutical industry for anti-tumor agents like taxol and vinblastine, and for nicotine for the fabrication of gums and patches designed to help with tobacco weaning. Alkaloid biosynthesis pathways are often more complex than the flavonoid pathway and, at the moment, only a few structural genes from the tropane and benzylisoquinoline alkaloid pathways have been isolated (Facchini, 2001; Memerlink. Valenzano *et al.,* 2006). To date, the best-known alkaloid biosynthetic pathway at the gene level leads to the formation of terpenoid indole alkaloids (TIAs) in periwinkle (*Catharanthus roseus*) (Gantet & Memelink, 2002; Fig. 1).

Table1: Reports of metabolic engineering of terpenoids, alkaloids, polyketids, and flavonoids

Compounds class	Engineered species	Target	Subcellular location	Regulation	Altered profilea	References
Terpenoids	*A. annua*	hmgrsqs	Cytosol	Constitutive	Artemisinin □ Artemisinin □	Aquil *et al.* (2009)
	A. annua	FDP synthase ipt	CytosolCyto	ConstitutiveC	Artemisinin □ Artemisinin □	Zhang *et al.* (2009)
	A. annua	cyp71av1 cyp71av1	solCytosolC	onstitutiveCon	Artemisininc acid □	Han *et al.* (2006) Sa *et*
	A. annua	Limonene synthase	ytosolCytos	stitutiveConsti	Dihydroartemisinic acid□	*al.* (2001)
	Chicory Yeast	Menthofuran	ol Plastid	tutiveConstitut	Several productsb□ □	de Kraker *et al.* (2003)
	Mint Mint	synthase Limonene	ER	iveConstitutiv	Menthofuran□, pulegone	Chang *et al.* (2007)
		hydroxylase		eConstitutive	□, menthol □	Diemer *et al.* (2001)
	Mint		ER		Limonene □, menthofuran	Mahmoud & Croteau,
		Limonene synthase		Constitutive	□, isomenthone□, menthol	(2001)
		Limonene synthase			□	Mahmoud *et al.*
		Trichodiene			Limonene □	(2004)
	Tobacco	synthase Amorpha-	Cytosol	Constitutive	Limonene □	Lucker *et al.* (2004)
	TobaccoToba	4,11–	Plastid	ConstitutiveC	Trichodiene□, oxygenated	Ohara *et al.* (2003)
	cco	diene synthase	Cytosol	onstitutive	trichodienec□	Hohn *et al.* (1991)
	Tobacco	Cembratrien-ol	Cytosol	Constitutive	Amorpha-4,11–diene □	Wallaart *et al.* (2001)
		hydroxylase			Cembratriene-diol □	
	Tobacco	Limonene	ER	Constitutive		Wang *et al.* (2001)
	Tobacco	hydroxylase Paclitaxel synthase	ER	Constitutive	Isopiperitenol and derivatives□	Lucker *et al.* (2004)
	E. coli		Cytosol	Constitutive	Paclitaxel □	Han *et al.* (2006)

HMGR, hydroxymethylglutaryl CoA reductase; FDP, farnesyl diphosphate; ipt, isopentenyl 5-phosphate transferase; SQS, squalene synthase; cyp71av1, cytochrome P450 monooxygenase; H6H, hyoscyamine-6-hydroxylase; PMT, putrescine N- methyltransferase; TRP I, tropinone reductase I; TRP II, tropinone II; str; strictosidine synthase; tdc, tyrosine decarboxylase; DXS, 1-deoxy-D-xuylulose 5-phosphate synthase; G10H, geraniol-10-hydroxylase; PKS, polyketid synthase; vhb, Vitreoscilla hemoglobin; DEBS, deoxyerythronolide B synthase ; PAL, phenylalanine ammonia lyase; 4CL, 4-coumarate: coenzyme A ligase; CHS, chalcone synthase; C4H, cinnamate-4-hydroxylase; TAL, tyrosine ammonia lyase; MatB, manolyl-CoA synthetase.

a. Compounds that have been reduced (-) or increased (+) in amount.

b. Overexpression sometimes resulted in cosuppression. Therefore, in different plants, levels of certain compounds were up- or downregulated.

c. Formation of oxygenated trichodiene was detected after induction by an elicitor.

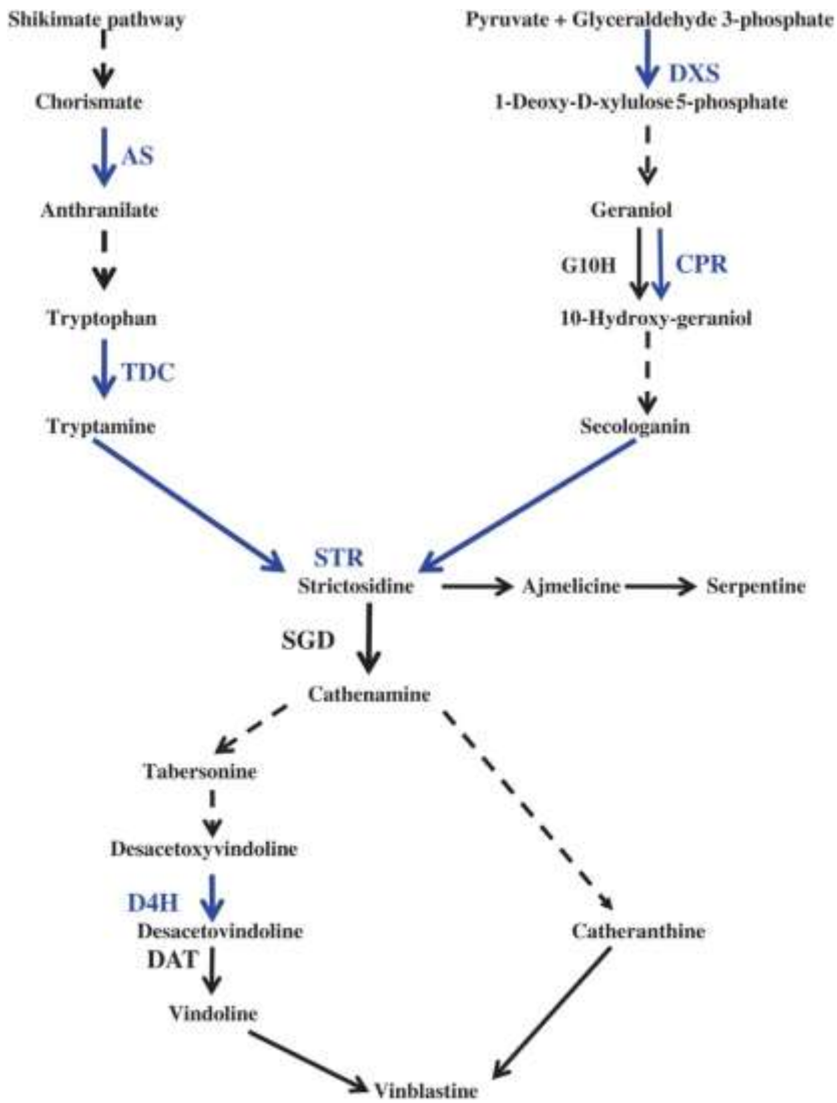

Figure 1: Regulation of terpenoid indole alkaloid biosynthesis in Catharanthus roseus cells by ORCA3. Solid arrows indicate a single enzymatic step and dashed arrows indicate multiple enzymatic steps. Blue arrows indicate that the gene encoding the enzyme is regulated by ORCA3. Abbreviations: AS, anthranilate synthase; CPR, cytochrome P450-reductase; DAT, deacetylvindoline acetyltransferase, D4H, desacetoxyvindoline 4-hydroxylase, DXS, D-1-deoxyxylulose 5-phosphate synthase; G10H, geraniol-10-hy-droxylase; ORCA3, octadecanoidresponsive Catharanthus AP2-domain protein 3; SGD, strictosidine□-D-glucosidase; STR, strictosidine synthase; TDC, tryptophan decarboxyl-ase. (Gantet&Memelink, 2002)

This plant species produces the monomeric alkaloids serpentine and aj- malicine, which are used as a tranquilizer and to reduce hypertension, re- spectively. Dimeric alkaloids from periwinkle, vincristine and vinblastine, and their hemisynthetic derivatives, including vinorelbine and vinflunine, are used extensively in the treatment of many cancers. Dimeric alkaloids are present at very low levels in the plant and are restricted to specific leaf cell types (St-Pierre *et al.*, 1999). For this reason, the more abundant monomeric precursors, catharanthine and vindoline, are isolated and coupled chemically to form dimeric vinblastine. But even monomeric al- kaloid levels are low in plants. Palazon, Ocana, Vazquez, & Mirjalili (2008) found that the scopolamine content of *A. belladonna* was relatively high in the seedling and vegetative stages but progressively decreased toward the flowering stage and in the same time, the total alkaloid content had considerably increased. They reported that by over-expressing the genes (PMT, tropinone reductase and h6h) of scopolamine biosynthesis, up to four-fold enhancement in scopolamine production was recorded (Palazon *et al.*, 2008; Fig. 2).

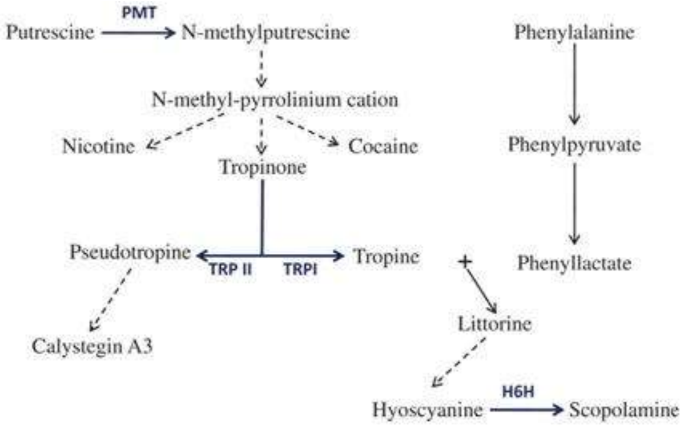

Figure 2: Overview of the most important steps in the scopolamine biosynthetic path- way. The enzymes over-expressed in scopolamine-producing hairy root cultures are in blue. Broken arrows indicate multiple steps. PMT, Putrescine N-methyltransferase; TRP I, Tropinone reductase I; TRP II, Tropinone reductase II, H6H, hyoscyamine -6ß-hydroxy- lase. (Palazon *et al.*, 2008)

Metabolic engineering of plants for medicinal applications

Numerous pharmacological compounds that are produced by plants and have applications for human health have undergone extensive reviews. Medicines made from natural products have often been taken orally as unprocessed extracts. However, given that crude extracts sometimes contain undesirable or harmful intermediates or have insufficient concentrations of the desired bioactive compound(s), this delivery raises questions about safety. To improve plant synthesis of important metabolites of medical significance, there has been a rise in investment in genetic and metabolic engineering. Model organisms like *Saccharomyces cerevisiae* and *Escherichia coli* are used as microbial hosts for the generation of biosynthetic molecules as part of this effort's ongoing expansion into the microbial realm. Since biopharming has the potential to be more cost-effective, facilitates compound purification, has fewer contamination issues, and is easier to regulate, it is a good option for pharmaceutical development because it allows for greater control over the emergence of potentially harmful side products. But as efforts to boost the production of a single metabolite or end-product have advanced, it has become evident that this is typically not possible through direct genetic overexpression or feeding of particular substrates.

Conclusion:

A full understanding of not only the fundamental pathways but also of flux, metabolic bottlenecks, genes and transcription factors, as well as the effects of metabolic channeling and metabolon formation on the entire system, is necessary for the dynamic and challenging process of biosynthetic engineering. There are a number of extra challenges that arise when these pathways are artificially generated in microbial hosts, such as how the route will interact with natural metabolism and the possibility that this might produce unanticipated intermediates and end products. But as technology has continued to level the playing field, more complex and ambitious pharmaceutical natural product engineering projects have become possible. The following analyses certain natural product biosynthesis routes with an emphasis on biotechnology-based modifications that affect particular chemicals of therapeutic relevance.

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