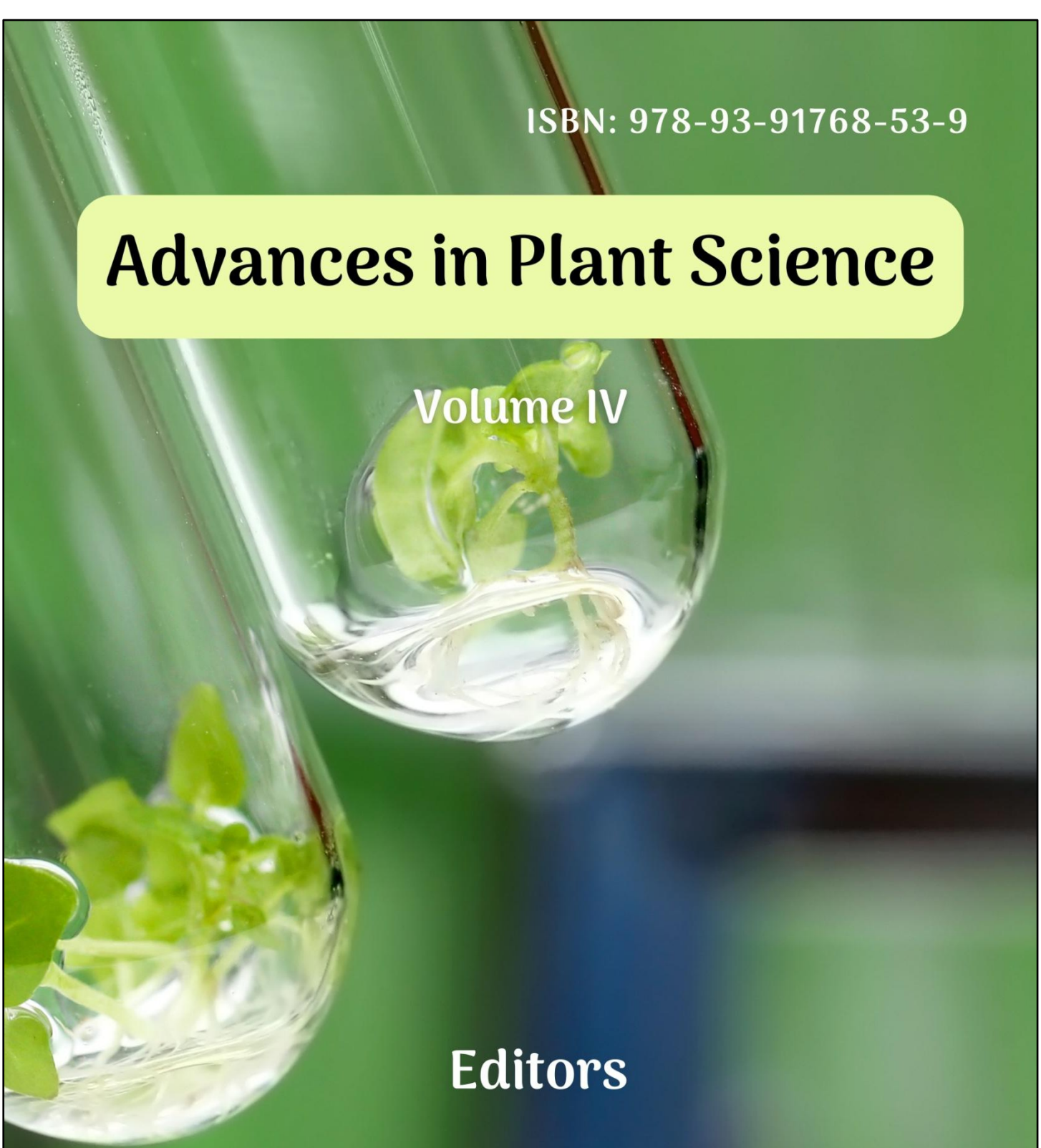
ISBN: 978-93-91768-53-9

Advances in Plant Science

Volume IV



Editors

Dr. Bhagwan D. Bulchandani

Dr. Kavita Chahal

Dr. Parashurama T. R

Dr. Minal S. Patil

Bhumi Publishing

Advances in Plant Science

Volume IV

(ISBN: 978-93-91768-53-9)

Editors

Dr. Bhagwan Das Bulchandani
Department of Botany,
M. D. College,
Merta City, Nagaur, Rajasthan

Dr. Kavita Chahal
Department of Botany,
Government College Bichhua,
Chhindwara, M. P.

Dr. Parashurama T. R
Department of Botany,
Kumadvathi First Grade College,
Shikaripura, Karnataka

Dr. Minal Shriram Patil
Department of Pharmacognosy,
DCS's Annasaheb Ramesh Ajmera
College of Pharmacy,
Nagaon, Dhule, M. S.

Bhumi Publishing

ISBN: **978-93-91768-53-9**

Published by:

Bhumi Publishing,

Nigave Khalasa, Kolhapur 416207, Maharashtra, India

Website: www.bhumipublishing.com

E-mail: bhumipublishing@gmail.com

Book Available online at:

https://www.bhumipublishing.com/books/

PREFACE

We are delighted to publish our book entitled ***"Advances in Plant Science Volume lV".*** *This book is the compilation of esteemed articles of acknowledged experts in the fields of plant science providing a sufficient depth of the subject to satisfy the need of a level which will be comprehensive and interesting. lt is an assemblage of variety of information about advances and developments in plant science. With its application oriented and interdisciplinary approach, we hope that the students, teachers, researchers, scientists and policy makers will find this book much more useful.*

The articles in the book have been contributed by eminent scientists, academicians. Our special thanks and appreciation goes to experts and research workers whose contributions have enriched this book. We thank our publisher Bhumi Publishing, lndia for compilation of such nice data in the form of this book.

Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

- Editors

Advances in Plant Science Volume lV

CONTENTS

Sr. No.	Book Chapter and Author(s)	Page no.
1.	**TRICHOMES AND ESSENTIAL OILS OF *PELARGONIUM GRAVEOLENS*** Zohra Mohammedi	1 – 16
2.	**EFFECT OF DIFFERENT CONCENTRATION OF *EUCALYPTUS GLOBULUS* LABILL LEAF LEACHATES ON GERMINATION AND GROWTH OF *ELEUSINE CORACANA* (L). C.V. DAPOLI – 1** Vikram P. Masal	17 – 20
3.	**ANTIDIABETIC EFFECTS OF BIOFLAVONOID MORIN** Shali K Sivan and Mini S	21 – 33
4.	**POTASH SOLUBILIZING BACTERIA: A GREEN ALTERNATIVE TO CHEMICAL FERTILIZER** Varsha D. Jayaswal, Harsha D. Pardeshi, Leena P. Shirsath and Sandip P. Patil	34 – 44
5.	**ANATOMICAL STUDIES ON *FICUS BENGHALENSIS* L. VAR. *KRISHNAE* (C. DC.) CORNER (MORACEAE)** E. Munuswamy	45 – 50
6.	**INTEGRATED PEST MANAGEMENT STRATEGIES** Amrit Mohapatra and Dibyajyoti Swain	51 – 63
7.	**ECOSYSTEM SERVICES: CONCEPT AND VALUATION** Kuldeep Joshi and Lalit Upadhyay	64 – 70
8.	**PRELIMINARY STUDY OF PROXIMAL COMPOSITIONS IN WEED PLANT *INDIGOFERA*** Smita P. Gudadhe	71 – 81

9.	**EFFECT OF SOIL CARBON SEQUESTRATION ON CLIMATE CHANGE MITIGATION** Sumana Balo	82 – 93
10.	**PLANT TISSUE CULTURE: AN OVERVIEW** Kanchan Awasthi and Ankita Yadav	94 – 101
11.	**STRATEGIES FOR UPSCALING PLANT SECONDARY METABOLITE PRODUCTION UNDER *IN VITRO* CULTURE SYSTEMS** Rajesh . S and Radhamani. T	102 – 115

TRICHOMES AND ESSENTIAL OILS OF *PELARGONIUM GRAVEOLENS*

Zohra Mohammedi

Department of Biology, Faculty of Natural Sciences and Life,

University of Mustapha Stambouli, BP 305, Mamounia, Mascara 29000, Algeria

Corresponding author E-mail: mdi3zhr@gmail.com

Introduction to the genus *Pelargonium*

Geranium is a large family that includes about 800 species. The principal genera are: *Erodium*, *Geranium* and *pelargonium* (Boukhris *et al.*, 2013). The species of the genus *Geranium* L., includes approximatively 300 species, distributed throughout the world, especially in the temperate belt and the montane areas of the tropics (Kremer *et al.*, 2013). Some of these species have been described for Europe and Croatia. The genus *Geranium* comprises about 430 species, divided into three subgenera: *Geranium*, *Eurodioïdea*, *Robertium*. The subgenera is the largest with over 370 species (Esfandani-Bozchaloy *et al.*, 2018).

The genus *Erodium* is distributed across all continents. A major center of diversity is observed in the Mediterranean region (63 species), whereas other regions harbor only few native species: North America, South America, Australia, and Asia. The potential for introduction by man makes it difficult to differentiate between natural and introduced status of some *Erodium* species in numerous floras (Fiz *et al.*, 2006).

Pelargonium genus contains about 280 species among which at least 30 species are odorants (Blerot *et al.*, 2018). The species of this genus largely fall into fairly easily recognizable groups, although the borders between the groups can sometimes be obscured, and the issue may be clouded by species of uncertain affinity. Traditionally, the genus has been divided into sections (Maggs *et al.*, 1995). The name "*Pelargonium*" is derived from "*Pelargos*" which means "*Stork*" in Greek. The fruit shape is reminiscent of the beak of the stork. *Pelargonium* species are widely distributed in South Africa. It has been introduced into Europe, Asia and North Africa (Boukhris *et al.*, 2013).

Aroma produced by aromatic *Pellargonium* species are remarkably diverse such as rose, mint, lemon, mutmeg, ginger and many others scents. *Pelargonium* species exhibiting a wild range of variation in leaf and floral morphology. Phylogenetic analyses led to a structuration of the *Pelargonium* genus into five main clades comprising 16 sections, most of the odorant *Pelargonium* belonging to clades A. The rose-scented *Pelargonium*, also named *Pelargonium*

rosat, are the most famous hybrids for their produced of essential oil (EO) (Blerot *et al.*, 2018), with biological activities.

Pelargonium graveolens

Pelargonium graveolens (*rose geranium*, *rose-scented geranium*, *sweet scented geranium*, *old fashion rose geranium*) is one of various species within the *Pelargonium* genus and that are native to the southern parts of Africa, firstly described by Muller (1963) and vincent (1995). It is known in several countries including Algeria, Tunisia, Morocco, Egypt, Spain and France (Hamidpour *et al.*, 2017; Boukhris *et al.*, 2013).

Among the *scented Pelargonium* hybrids, the *rose scented P. x hybridum* cultivars, also named *Pelargonium rosat*, are the most emblematic cultivars and are often used to replace the expensive *Rosa damascena* essential oil. These hybrids descend from several crossing between *Pelargonium graveolens* or *Pelargonium radens* in one hand and *Pelargonium capitatum* in the other hand. Cultivars used in EO production are somatically multiplied from cuttings, but the knowledge of their exact botanical origin has been lost over time (Blerot *et al.*, 2018).

Pelargonium graveolens is an erect, multi-branched shrub, that grows up to 1.5 m and has a spread of 1 m. The leaves are deeply incised, velvety and soft to the touch (due to glandular hairs). The flowers vary from pale pink to almost white and the plant flowers from August to January. The leaves may be strongly *rose-scented*, although the leaf shape and scent vary. Some plants are very strongly scented and others have little or no scent. Some leaves are deeply incised and others less so, being slightly lobed (Kochhar, 2016).

Morphology and structure of trichomes

1. Glandular and non glandular trichomes

Glandular trichomes (figure 1), frequently called glands, are epidermal appendices formed by the head of a single cell or pluricellular secretory cells and a non-glandular stalk. They secrete essential oils and vary in the number of secretory cells, number and length of the stalk cell(s), the amount of oil secreted, density, and arrangement in the epidermis (Gonçalves *et al.*, 2010; Maffei *et al.*, 1989). Non-glandular trichomes (figure 1) are a type of plant trichomes without glandular heads. They have a thin apex. Non-glandular trichomes can be unicellular or multicellular. In addition, they can be branched or not. Most non-glandular trichomes are simple, essential branched and star-shaped. On the physiological scale, the non-glandular trichomes were known by their role in reducing the water loss and improving the resistance of the xerophytes plants to arid environments (Boukhris *et al.*, 2013).

Glandular trichome is anatomically divided into three regions: basal, stalk and head; which may be unicellular or multicellular. In addition, glandular trichomes can be divided into

two types: peltate and capitate. Peltate trichomes are considered to be involved predominantly in essential oil production, and have a large sub-cuticular space where the essential oil is stored . Capitate trichomes have been reported to secrete small amounts of essential oils and flavonoids, in addition to a complex mixture of polysaccharides, proteins and mucilages. However, capitate trichomes contain a limited storage capacity and the secreted materials accumulate in the cell lumen. Whatever the exact nature of the capitate trichome secretory products, it is clear that the bulk of the essential oil is produced by and stored in peltate trichomes (Martínez-Natarén *et al.*, 2011).

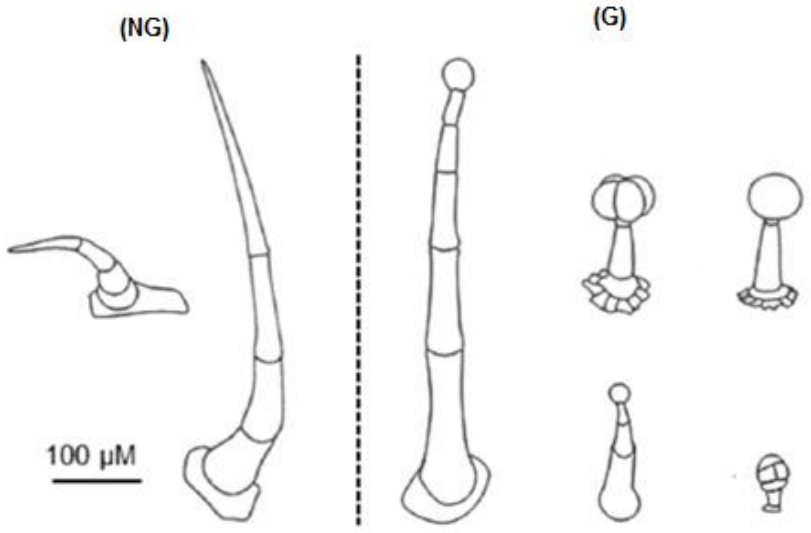


Figure 1: Structure of some trichomes, demonstrates difference between glandular (G) and non glangular (NG) trichomes (According to Tissier, 2012)

2. Morphology and structure of *Pelargonium graveolens glandular trichomes*

As regards the variety of trichomes in Geraniaceae, four different types of trichomes are noted, all on both surfaces of leaves: long tector, short curved, short glandular, and totally glandular, of which the latter is most frequent (Romitelli and Martins, 2013). The surfaces of *Pelargonium graveolens* vegetative and reproductive organs contain non-glandular and glandular trichomes (figure 2). The non-glandular trichomes are present on all aerial parts. In fact, they are more numerous on the abaxial than on adaxial surface of the leaf lamina. They are also situated above the veins on the leaf margins. These trichomes are often unicellular and uniseriate that is always attached to the epidermis. Some bicellular and multicellular structures were observed (Boukhris *et al.*, 2013).

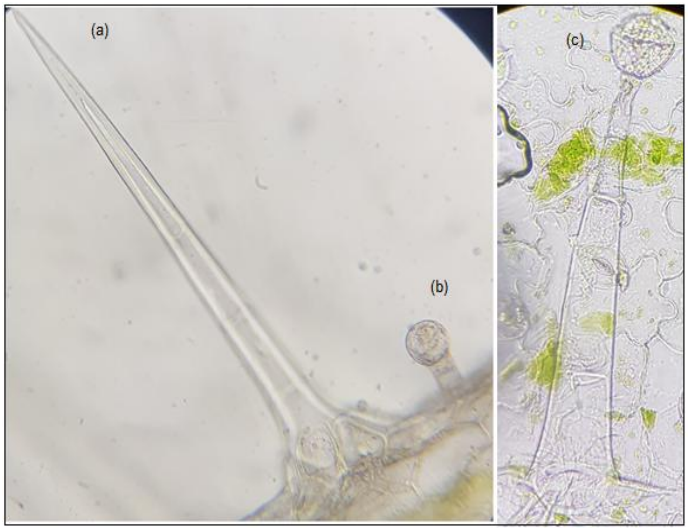


Figure 2: Non-glandular (a) and glandular (b, c) trichomes in *Pelargonium graveolens*

The glandular trichomes have a great variety in their morphologies, because of different development processes. Boukhris *et al.* (2013) have observed two types of glandular trichomes: capitates and peltates. The capitates trichomes, corresponding to three types, type I, type II and type III in respect to the shape and structure of stalk and head cell. Type I shows an elongated large head (one cell) and a short cylindrical stalk (three cells), type II has a spherical, small head (one cell) and a long conical stalk (five cells). The peltate glandular trichomes are present especially in stems and sepals. They have a base including epidermis cells, a thick stalk and a large flattered head. The number of cells forming the disk in peltate trichomes depends on the development stage (figure 3). From Romitelli and Martins (2013), *Pelargonium graveolens* has a glandular trichome composed of a basal cell, two short stalks and an elliptical apical cell (figure 4).

Two groups of trichomes were observed on the *Pelargonium graveolens* leaves by Khetsha et al. (2021). One group consisted of one type of non-glandular trichome, which is regarded as an attenuated kind (figure 5A). The attenuated trichome is characterised by the long and gradual taper. The second group consisted of three types of glandular trichomes, of which one type belonged to the peltate kind. The peltate kind was characterised by a short neck with bigger round tips and regarded as the brevicollate trichome (figure 5B). The other two types belong to the capitate kind. One capitate kind consisted of a smaller trichome type, characterised by a short segmented capitate with a columnar hatchet-shaped tip that has a slightly bent apical cell pointing at the leaf apex, known as the asciiform trichome type (figure 5C). The second capitate kind consisted of elongated segment flask-shaped bodies that incorporated a round head, having similar characteristics as the asciiform and was regarded as the elongated-capitate type (figure 5D).

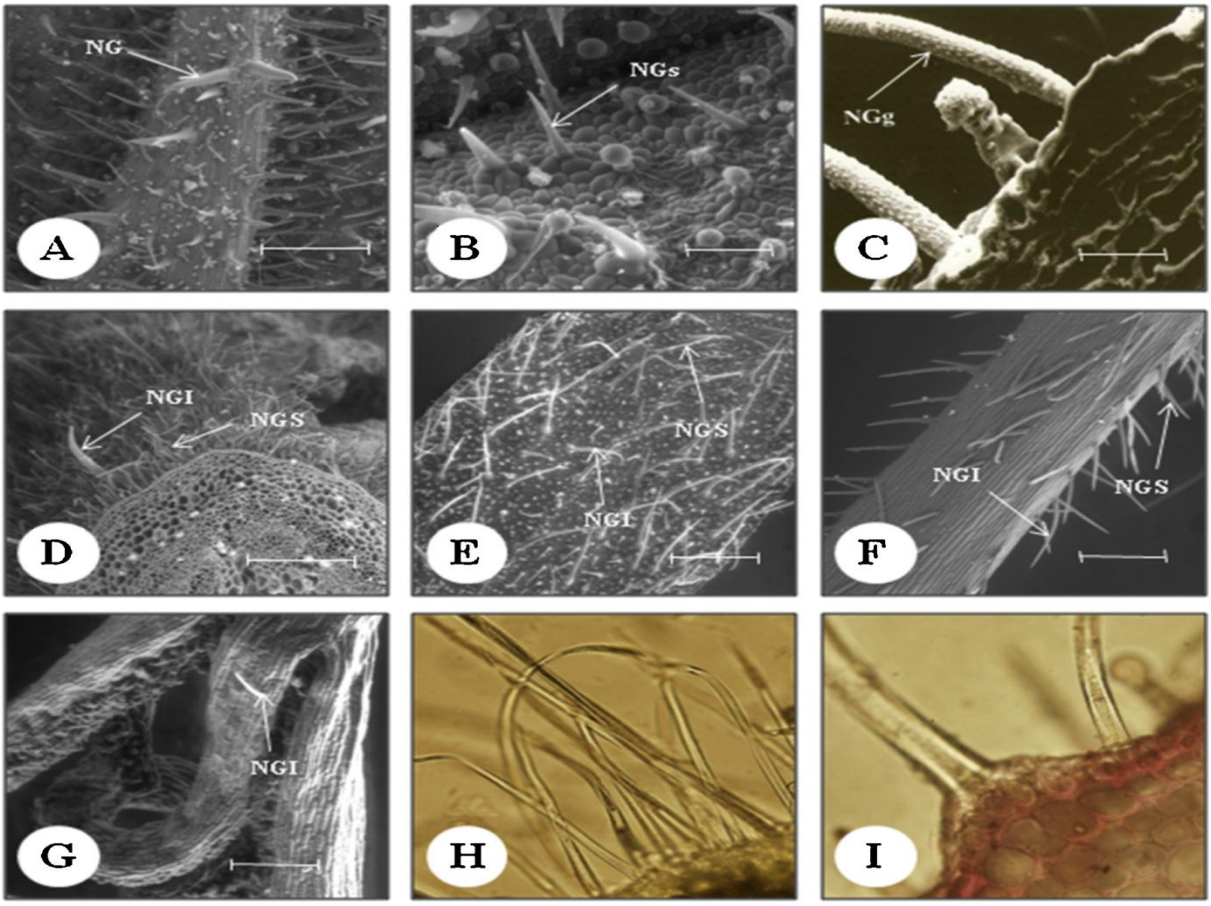


Figure 3: Non-glandular trichomes in the aerial parts of *Pelargonium graveolens*; (A–G) SEM, (H–I) LM. (A: SEM view of the abaxial side of a portion of a leaf lamina with dense mass of non-glandular trichomes. B: The adaxial side of a portion of a leaf lamina with scattered non-glandular trichomes with smooth surface (NGs). C: The abaxial side of a portion of a leaf lamina showing a basal part of two non-glandular trichomes with granulate surface (NGg) (fixed material). D: Cross section of a portion of a stem covered with a large number of straight non-glandular trichomes (NGs) and incurved non-glandular trichomes (NGI). E: The abaxial side of a portion of a sepal covered with dense mass of straight non glandular trichomes (NGs) and incurved non-glandular trichomes (NGI). F: A style surface showing straight non glandular trichomes (NGs) mixed with incurved non-glandular trichomes (NGI). G: A stigmata surface showing one short incurved non-glandular trichomes (NGI). H: Numerous non-glandular trichomes tall straight (NGS) or incurved non-glandular trichomes (NGI) obvious on upper epidermis of pedicel. I: Transverse section in leaf lamina showing a basal part of a uniseriate non-glandular trichomes) (*According to Boukhris et al., 2013*).

Figure 4: Capitate glandular trichome in *Pelargonium graveolens* (According to Romitelli and Martins, 2013)

Variation in trichome types may play a different role in plant physiology and ecology with variable morphological, mechanical and phytochemical characteristics. Thus, all trichome group types were densely spaced on the abaxial leaf surface and scattered on the adaxial leaf surface. However, the attenuated trichome group tended to occur more densely near the midrib on the abaxial leaf surface. The hemispherical peltate trichome group tended to occur evenly spaced on the abaxial leaf blade surface but was mostly absent from the epidermis above the midrib vein. Dense populations of trichomes on the abaxial leaf surface could be associated with the promotion of photosynthetic competence and establishment of phytochemical defence of leaves when mature. The density of trichomes may vary with variations in the environmental conditions, indicating the trade-offs between trichome traits, mainly to increase the resistance and the cost of trichome production (Khetsha *et al.,* 2021).

Electron-microscopic observations showed that density of small glandular trichomes decreased with an increase in irrigation frequency. The increase in glandular trichome density in the stressed treatments could have resulted from a decrease in epidermal cell size suggested that unlike leaf area, trichome number per leaf is less sensitive to environmental stresses, implying that the apparent increase in trichome density observed in water-stressed conditions mainly arose from a reduction in leaf size (Eiasu *et al.,* 2012). In another study, Gupta et al. (2016) evaluate synergistic effect of microbes *Trichoderma harzianum ThU*, *Glomus intraradices* and *Bacillus subtilis CIM* on glandular trichome number in *Pelargonium graveolens*. A positive and direct correlation was observed between essential oil content and number of glandular trichomes in all treatments.

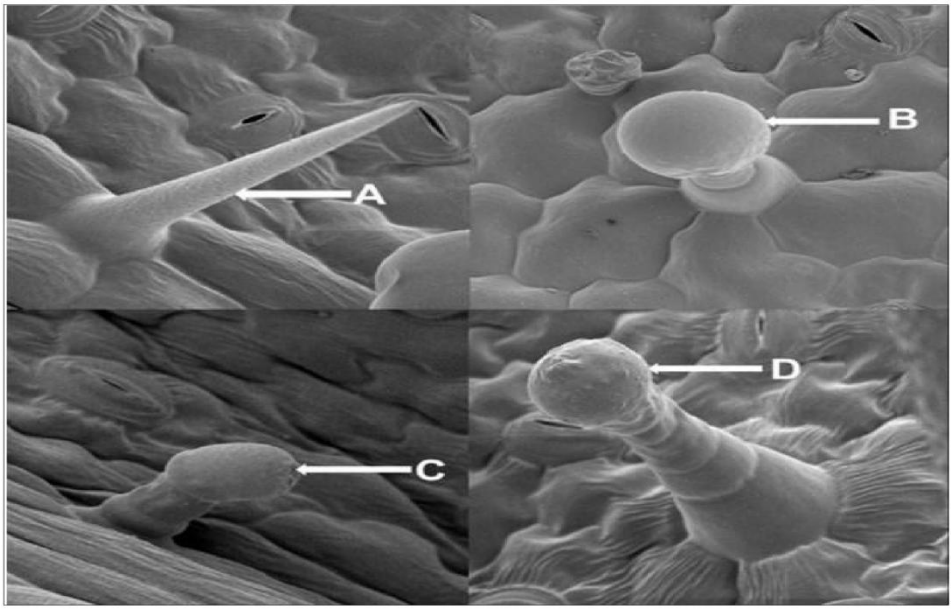


Figure 5. The four different types of trichomes found on the leaves of *Pelargonium graveolens* as observed under a scanning electron microscope (400X magnifications) (Thitapoho farm, Tweespruit). (A: non-glandular trichome; B: Brevicollate trichome; C: Asciiform trichome; D: Elongated-capitate trichome) (According to Khetsha *et al.*, 2021)

Essential oils produced by glandular trichomes

1. Composition

Pelargonium EO consists primarily of oxygenated C10 monoterpenoid volatiles with variable amounts of C15 sesquiterpenes. While the monoterpenoid fraction of Pelargonium EO varies according to the cultivar and season (Verma *et al.*, 2013), it consists primarily of acyclic monoterpene alcohols geraniol and (-)-citronellol (and their aldehyde, acid, and ester derivatives, referred to collectively here as "citronelloid monoterpenes"), oxygenated p-menthanes such as (+)-menthone and (-)-isomenthone, and lesser amounts of olefinic hydrocarbons such as limonene and β-phellandrene. These volatile compounds are thought to accumulate in type I and II capitate glandular trichomes on the leaf surface. Phytochemical analysis has suggested the total volatile complement of *Pelargonium* consists of more than 100 compounds, the bulk of which are terpenoids (Bergman *et al.*, 2020).

The EO yield of *Pelargonium graveolens* depends on the organ type. It varied from 0.038 to 1.0% (Gomes *et al.*, 2004; Motsa, 2006; Dhifi *et al.*, 2011; Ghannadi *et al.*, 2012; Boukhris *et al.*, 2013). *Pelargonium* EO consists primarily of oxygenated C_{10} monoterpenoid volatiles with variable amounts of C_{15} sesquiterpenes (figure 6) (Bergman *et al.*, 2020).

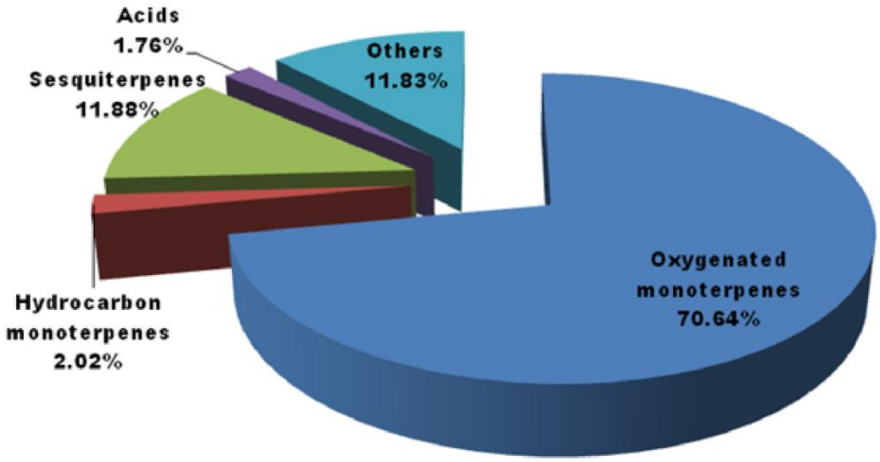


Figure 6: Percentage variation in the chemical composition of *Pelargonium graveolens* leaves essential oil (According to Dhifi *et al.*, 2011)

A total of 45 compounds of the *Pelargonium graveolens* L. EO growing in Algeria were identified. The main constituents were citronellol (30.2%), citronellyl formate (9.3%) and geraniol (7.6%) (Boukhatem *et al.,* 2013). The chemical profile of the volatile oil from Morocco was investigated by Chraibi et al. (2016) and Sadiki et al. (2019). The analyses carried out by the first authors revealed 61 different compounds. The major compounds were citronellol (26.98%), geraniol (14.12%), isomenthone (8.80%), linalool (4.97%), citronellylformate (3.1%), followed by geranylformate (4.07%) and guai-6,9-diene (4.24%), whereas the second authors identified 54 components whose the major components are citronellol (25.24 %), geraniol (23.36 %), citronellyl formate (8.35 %), linalool (7.11 %), β-eudesmol (6.13 %), geranyl formate (4.26 %) and *iso*-menthone (3.37 %). In another side, Fayed (2009) have identified 32 compounds in Egyptian *Pelargonium graveolens*. The major components were citronellol (29.90 %), trans-geraniol (18.03 %), 10-epi-γ-eudesmol (8.27 %), isomenthone (5.44 %), linalool (5.13 %), geranyl acetate (4.52 %), γ-cadinene (2.89 %), geranyl butyrate (2.53 %), geranyl tiglate (2.50 %) and gemacrene D (2.05 %). The Tunisian Rose-scented geranium, *Pelargonium graveolens* EO, contains high levels of the following compounds: β-citronellol, geraniol, citronel-lyl formate, geranyl formate and δ-selinene. The EO leaves and flowers of *Pelargonium graveolens* organs exhibit a β-citronellol and geraniol chemotype, but EO stems shows δ-selinene and β-citronellol chemotype. The oxygenated monotepenes represented the major compounds (70.3%, 62.92% and 43.3%) of the total of the EOs in flowers, leaves and stems (Boukhris *et al.*, 2013). These authors have identified 64 compounds in the EO of *Pelargonium graveolens* with 43 in flowers, 45 in leaves and only 35 in stems, while 36 compounds only were detected by Dhifi et al. (2011) in *Pelargonium* graveolens EO from Tunissia and is characterized by the

predominance of two compounds: citronellol and geraniol with respective amounts of 27.53 and 25.85 %. In another study of the chemical composition of the EO of *Pelargonium graveolens* from Tunisia, GC–MS analyses led to the identification of 42 different compounds. The oil contained a complex mixture, of which the prominent components were linalool (6.54%), citronellol (27.53%), geraniol (25.85%), 6-octen-1-ol, 3,7-dimethyl-, formate (8.75) and **Δ**-selinene (8.15%). These different results explain the variability in the content and composition of *Pelargonium graveolens* essential oil not only in the Mediterranean basin, but also in the same geographical region. For *Pelargonium graveolens* growing in other regions of the world, the variations are noticeable, but the predominance of monoterpene compounds, and their esters were observed, followed by sesquiterpenes and their esters. For examples, 30 compounds of Indian *Pelargonium graveolens* EO were identified. The main components were citronellol (33.6 %), geraniol (26.8 %), linalool (10.5 %), citronellyl formate (9.7 %) and p-menthone (6.0 %) (Rana *et al.*, 2002). In volatile oil from Iranian *Pelargonium graveolens*, 24 compounds were identified. The main constituents were *β*-citronellol (36.4%), citronellyl formate (12.1%) and geraniol (10.7%) (Ghannadi *et al.*, 2012), while 61 components were detected, of which 43 were identified in Romanian *Pelargonium graveolens* EO. Citronellol (39.97-43.67 %) was found to be the main component, followed by geraniol (2.57–9.66 %), linalool (1.41-4.77 %) and their esters: citronellyl formate (11.23-13.55 %), geranyl formate (1.15-2.21 %), citronellyl butyrate (1.01-1.54 %) and geranyl tiglate (1.22-1.75 %) (Gâlea and Hancu, 2014).

2. Biosynthesis

The essential oil, it is produced by glandular trichomes which are generally accepted made as the functionally specialized tissues. EO are stored or volatilized at the plant surface (Boukhris *et al.*, 2013). In *pelargonium*, biosynthesis of terpenes takes place in leaves in specialised structures known as glandular trichomes, or oil glands, that are composed of a secretory cell producing EO in a subcuticular storage cavity (Blerot *et al.*, 2018).

Bergman *et al.* (2020) determined two distinct monoterpene biosynthetic pathways contribute to their volatile profiles, namely, cyclic *p*-menthanes such as (-)-isomenthone and acyclic monoterpene alcohols such as geraniol and (-)-citronellol and their derivatives citronelloid monoterpenes. Untargeted volatile profiling of 22 seed-grown *Pelargonium graveolens* lines demonstrated distinct chemotypes that preferentially accumulate (-)-isomenthone, geraniol, or (-)-citronellol along with approximately 85 minor volatile products. Whole plant $^{13}CO_2$ isotopic labeling performed under physiological conditions permitted to measure the *in vivo* rates of monoterpenoid accumulation in these lines and quantify differences

in metabolic modes between chemotypes. These authors determined that *p*-menthane monoterpenoids in *Pelargonium* are likely synthesized from (+)-limonene via (+)-piperitone rather than (+)-pulegone.

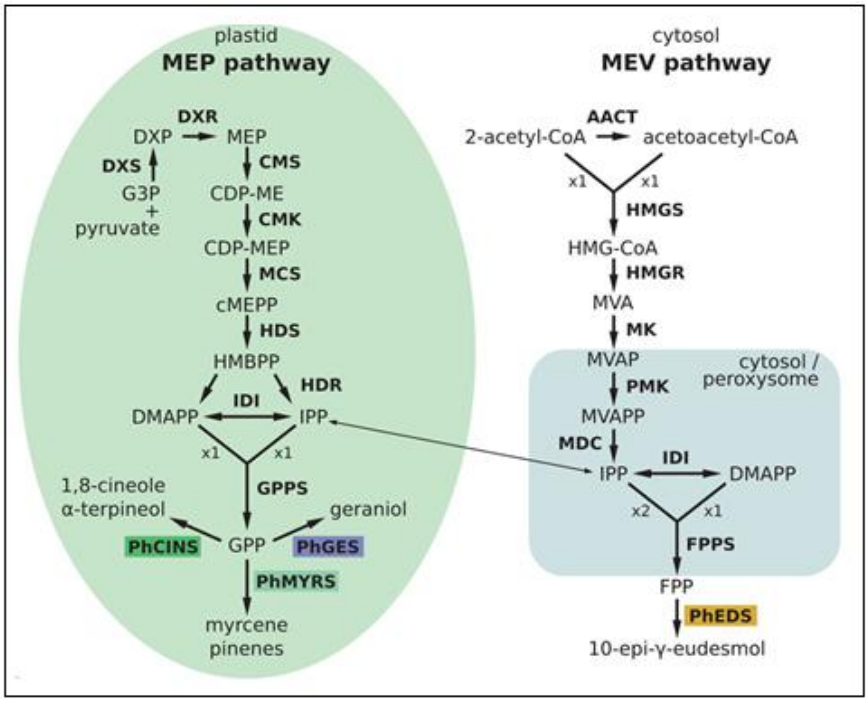


Figure 7: Enzymatic steps in the biosynthetic pathways of mono and sesquiterpenes.(AACT, acetoacetyl-coenzyme A thiolyase; CDP-ME, 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol; CDP-MEP, CDP-ME-2-phosphate; cMEPP, 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate; PhCINS, cineole synthase; CMK, 4-(cytidine 5′-diphospho) 2-*C*-methyl-D-erythritol kinase; CMS, 2-*C*-methyl-D-erythritol 4-phosphate transferase; DMAPP, dimethylallyl diphosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; DXR, DXP reductoisomerase; DXS, DXP synthase; PhEDS, eudesmol synthase, FPP, farnesyl diphosphate; FPPS, FPP synthase; PhGES, geraniol synthase; G3P, glyceraldehyde 3-phosphate; GPP, geranyl diphosphate; GPPS, GPP synthase; HDS, 1-hydroxy-2-methyl-2(*E*)-butenyl 4-diphosphate synthase; HDR, 1-hydroxy-2-methyl-2(*E*)-butenyl 4-diphosphate reductase; HMBPP, 1-hydroxy-2-methyl-2(*E*)-butenyl 4-diphosphate; HMGCoA, 3-hydroxy-3-methylglutaryl-CoA; HMGR, hydroxymethylglutaryl-CoA reductase; HMGS, hydroxymethylglutaryl-CoA synthase; IDI, isopentenyl diphosphate isomerase; IPP, isopentenyl diphosphate; MDC, mevalonate pyrophosphate decarboxylase; MCS, 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate synthase; MEP, 2-*C*-methyl-D-erythritol 4 phosphate; MK, mevalonate kinase; MVA, mevalonate; MVAP, MVA-5-phosphate; MVAPP, MVA-5-diphosphate; PhMYRS, myrcene synthase; PMK, 5-phosphomevalonate kinase). (According to Blerot *et al.*, 2018)

It is generally accepted that IPP/DMAPP provided by the plastidial methyl erythritol phosphate (MEP) and cytosolic mevalonate (MVA) pathways are used, respectively, for the biosynthesis of monoterpenes and sesquiterpenes, but several works have pointed out cross-talks between these two metabolic routes (figure 7). Balance between primary terpenes and their secondary derivatives largely influences the fragrance produced by aromatic plants. Regulation of terpene synthases (TPS) and other enzymes involved in terpene biosynthesis is both controlled transcriptionally and by IPP/DMAPP supply. In pelargonium, a gene encoding for 1-deoxy-D-xylulose-5-phosphate synthase (DXS), the first step providing IPP/DMAPP by the MEP pathway, has been characterised. Over expression of the *DXS* gene led to a slight increase in EO content (Blerot *et al.*, 2018).

Physiological role of trichomes

Trichomes differ between species and genotypes and have an important role in adaptation of the plant to its environment (Dehimeche, 2021). The non-glandular trichomes can have several functions: protection against mechanical damage, protection against lightning. The trichomes produce shadows on the epidermis. This occurs for example in the leaves of olive trees, maintenance of a proper microclimate since they retain moisture on the surface of the epidermis.

The non-glandular trichomes serve mainly as mechanical barriers on the leaf surface. They may be involved in protection against insect herbivores and fungi by preventing or disrupting their movements on the leaves. They may also have a protective role against UV by covering the epidermal layer and could also affect the transpiration of the plant by limiting the diffusion of water.Glandular trichomes produce and store various secondary metabolites which can be volatile, semi-volatile or non-volatile; the most stored substances are mainly terpenoids but also phenylpropenoids, flavonoids, methylketones, acyl sugars and defensive proteins. Glandular trichomes potentially protect leaves against various stresses via the secretion and storage of defense compounds, such as terpenoids. They can accumulate large amounts of essential oils and have been associated with resistance against insect pests. They are also involved in reducing water loss and filtering UV radiation. Some plants can tolerate high levels of metals, thanks to cysteine-rich defensive proteins secreted in the glandular trichomes. They can also secrete saline ions, leading to calcium balance in the plant (Dehimeche, 2021).

Physiological role of essential oils produced by *Pelargonium graveolens*

The EO produced by the glandular trichomes of *Plargonium graveolens* play an important physiological role in the interactions of the plant with its external environment and in the protection of the plant from various threats and aggressions like pathogens.

The insecticidal/antifeedant property of geranium oil was well known for the past many years against various types of insects. L-quisqualic acid ($C_5H_7N_3O_5$), an excitatory amino acid, isolated from the petals of *Pelargoniu,* displayed a paralytic effect on Japanese beetles, also essential oil exhibits insecticidal properties against the insect *Rhyzopertha dominica*. The EO from *Pelargonium graveolens* exhibited remarkable antifungal properties against *Rhizoctonia solani,* a plant pathogenic fungus. The effect of the individual component of EO was estimated, and results suggested that citronellol exhibits the most effective fungicidal property, followed by geraniol, isomenthone, geranyl formate and citronellyl formate (Bouzenna and Krichen, 2013). Essential oils have also activity against others fungi such as *Alternaria alternata*, *Fusarium graminearum*, *Fusarium oxysporum*, *Fusarium culmorum* (Ben Hsouna and Hamdi, 2012), *Fusarium tricinctum*, *Phomopsis helianthi*, *Phoma macdonaldii* (Džamić *et al.,* 2014), and toxic effect against some species of fungi, which are responsible for wood decay, such as *Coniophora puteana*, *Coriolus versicolor*, *Poria* placenta and *Gloeophyllum trabeum* (Moutaouafiq *et al.,* 2019).

Biological activities of *Pelargonium graveolens* and interest for human

Geranium species are known as decorative and aromatic, with considerable quantities of essential oils. Some species are used in traditional medicine due to their antidiabetic, hemostatic, antihemorrhoidal, and antidiarrheal activities, as well as for the treatment of pain, fevers and gastrointestinal ailments. It is used in traditional medicines as an astringent, an antidiarrheal, for treatment of eyes infections, for kidney and gall stones, and for male fertility improvement. In folk medicine, it is used in the form of an infusion and decoction for stomach ailments and some Geranium species have considerable antimicrobial, acaricidal, and antioxidant activity (Kremer *et al.,* 2013; Asgarpanah and Ramezanloo, 2015). *Plargonium graveolens* EO is largely utilized in the perfumery, cosmetic and aromatherapy industries all over the world. It has since become indispensable aromatherapy oil (Asgarpanah and Ramizanloo, 2015).

Plargonium graveolens EO showed antibacterial activity against human pathogenic bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia Coli*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Salmonella enterica* (Dhifi *et al.,* 2011; Ghannadi *et al.,* 2012; Chraibi *et al.,* 2016; Sadiki *et al.,* 2019; Ben Hsouna and Hamdi, 2012), and antifungal activity against moulds and yeasts like *Candida albicans* (Dhifi *et al.,* 2011), *Candida tropicalis*, *Aspergillus niger*, *Penicillium expansum* , *Aspergillus flavus*, *Trichophyton menthagrophytes* (Chraibi *et al.,* 2016; Ben Hsouna and Hamdi, 2012; Džamić *et al.*, 2014). *Plargonium graveolens* in the food industry is being used for its antimicrobial activity. The EO has shown through multiple studies to be effective in fighting bacteria and fungi like *Rhizopus nigricans,*

Fusarium graminearum, Fusarium oxysporum, Fusarium culmorum, Alternaria alternata. This antimicrobial action has led to testing of the oil against food spoilage pathogens and has shown promising results. Enough so that it has been highly considered in the food industry as a preservative agent (Hamidpour *et al.,* 2017; Ben Hsouna and Hamdi, 2012).

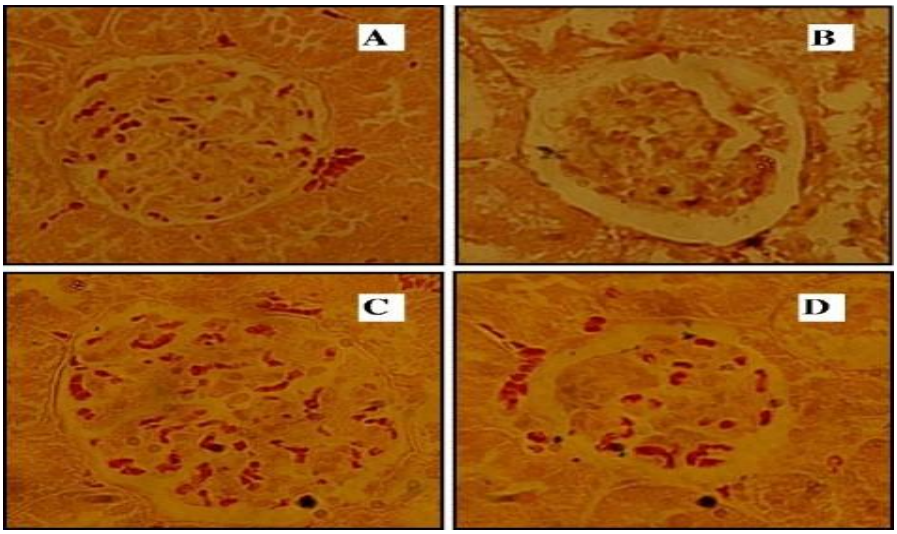


Figure 8: (A) Normal rat kidney. (B) Diabetic treated rat kidney: tubular epithelial damage messangial capillary proliferation and fatty infiltration. (C) Diabetic rat treated with Glibeclemide and (D) Diabetic rat treated with essential oil (150 mg/kg): a positive effect was observed (H&E 100×) (According to *Boukhris et al.*, 2012)

Plargonium graveolens EO exhibits antioxidant, anticancer and hypoglycemic activities. The EO reduced the concentration of DPPH free radical with EC50 value of 66.45µg/ml (Fayed, 2009). Author suggests that the antiradical scavenging activity of oils might be attributed to the replacement of hydroxyl groups in the aromatic ring systems of the phenolic compounds. The EO of *Plargonium graveolens* was able to release free groups with a high capacity to oxidize free radicals of DPPH molecule. Also, the latter authors have understood the effect of EO on human promyelocytic leukemia cells, experiments were conducted using cultured HL-60 and NB4 cell lines. Results of the viability were measured using trypan blue assay. The results found that the incubation of HL-60 cells with *Plargonium graveolens* EO at all concentrations (25 – 200 µg/ml) for 24 hour reduced the viability of these cells. The dead cells were increased by increasing the concentration of EO. A highest HL-60 dead cell (%) was recorded by EO (79.27%) for concentration of 200 µg/ml. The hypoglycemic effect of *Plargonium* graveolens at the dose of 150 mg/kg b.w. was significantly more effective than that of glibenclamide. It is through the histological findings in hepatic and renal tissues of diabetic rats (figure 8) that these beneficial effects of EO were confirmed (Boukhris and al., 2012).

References:

Asgarpanah J, Ramezanloo F (2015): An overview on phytopharmacology of Pelargonium graveolens L., Indian Journal of Traditional Knowledge, 14(4): 558-563.

Ben Hsouna A, Hamdi N (2012): Phytochemical composition and antimicrobial activities of the essential oils and organic extracts from *pelargonium graveolens* growing in Tunisia, Lipids in Health and Disease, 11(167): 1-7.

Bergman ME, Chávez Á, Ferrer A, Phillips MA (2020): Distinct metabolic pathways drive monoterpenoid biosynthesis in a natural population of *Pelargonium graveolens*. J Exp Bot, 71(1): 258-271.

Blerot B, Martinelli L, Prunier C, Saint-Marcoux D, Legrand S, Bony A, Sarrabère L, Gros F, Boyer N, Caissard JC, Baudino S, Jullien F (2018): Functional Analysis of Four Terpene Synthases in Rose-Scented Pelargonium Cultivars (*Pelargonium* x *hybridum*) and Evolution of Scent in the Pelargonium Genus, Frontiers in Plant Science, 9(1435): 1-18.

Boukhatem MN, Kameli A, Saidi F (2013): Essential oil of Algerian rose-scented geranium (*Pelargonium graveolens*): Chemical composition and antimicrobial activity against food spoilage pathogens, Food Control, 34(1): 208-213.

Boukhris M, Bouaziz M, Feki I, Jemai H, El Feki A, Sayadi S (2012): Hypoglycemic and antioxidant effects of leaf essential oil of *Pelargonium graveolens* L'Hér. in alloxan induced diabetic rats, Lipids Health Dis, 11(81): 1-10.

Boukhris M, Ben Nasri-Ayachi M, Mezghania I, Bouaziz Mohamed, Boukhris M, Sayadi S. (2013): Trichomes morphology, structure and essential oils of Pelargoniumgraveolens L'Hér. (Geraniaceae), Industrial Crops and Products, 50: 604-610.

Bouzenna H, Krichen L (2013): *Pelargonium graveolens* L'Her. and *Artemisia arborescens* L. essential oils: chemical composition, antifungal activity against *Rhizoctonia solani* and insecticidal activity against *Rhysopertha dominica*, Nat Prod Res, 27(9):841-846.

Chraibi M, Farah A, Balouiri M, Barkai H, Sadiki M, Fikri Benbrahim K (2016): Chemical characterization and antimicrobial activity of Moroccan *Pelargonium asperum* essential oil, Journal of Applied Pharmaceutical Science, 6 (12): 42-46.

Dehimeche N (2021): Rôle des trichomes et des composés organiques volatils dans le dépôt d'ozone et la réponse des plantes de tomate au stress, Thèse Doctorat, Écologie Fonctionnelle et Sciences Agronomique, Université de Montpellier, 189p.

Dhifi W, Mnif W, Jelali N, Baaziz H, Hadded A, Hamdi N (2011): Characterization of Leaves Essential oil of *Pelargonium graveolens* Originating from Tunisia: Chemical Composition, Antioxidant and Biological Activities, Journal of Essential Oil Bearing Plants, 14(6): 761-769.

Džamić AM, Soković MD, Ristić MS , Grujić SM , Mileski KS, Marin P D (2014): Chemical composition, antifungal and antioxidant activity of *Pelargonium graveolens* essential oil, Journal of Applied Pharmaceutical Science, 4(03): 1-5.

Eiasu BK, Steyn JM, Soundy P (2012): Physiomorphological response of rose-scented geranium (Pelargonium spp.) to irrigation frequency, South African Journal of Botany, 78: 96-103.

Esfandani-Bozchaloyi S, Sheidai M, Keshavarzi M, Noormohammadi Z (2018): Species identification and population structure analysis in Geranium subg. Geranium (Geraniaceae), Hacquetia, 17(2): 235-246.

Fayed SA (2009): Antioxidant and Anticancer Activities of *Citrus reticulate* (Petitgrain Mandarin) and *Pelargonium graveolens* (Geranium) Essential Oils, Res J Agric Biol Sci, 5(5): 740-747.

Fiz O, Vargas P, Alarcón ML, Aldasoro JJ (2006): Phylogenetic Relationships and Evolution in Erodium (Geraniaceae) based on trnL-trnF Sequences, Systematic Botany, 31(4): 739-763.

Gâlea C, Hancu G (2014): Antimicrobial and Antifungal Activity of *Pelargonium roseum* Essential Oils, Adv Pharm Bull, 4(Suppl 2): 511-514.

Ghannadi A, Bagherinejad MR, Abedi D, Jalali M, Absalan B, Sadeghi N (2012): Antibacterial activity and composition of essential oils from *Pelargonium graveolens* L'Her and *Vitex agnus-castus* L, Iran J Microbiol, 4(4): 171-176.

Gomes PB, Mata Vera G, Rodrigues AE (2004): Characterization of Portuguese grown geranium oil (Pelargonium sp.), Journal of Essential Oil Research, 16: 490-495.

Gonçalves LA, Azevedo AA, Otoni WC (2010): Characterization and ontogeny of the glandular trichomes of Ocimum selloi Benth. (Lamiaceae), Acta bot bras, 24(4): 909-915.

Gupta R, Singh A, Pandey R (2016): Microbe-based technology ameliorates glandular trichomes, secondary metabolites and antioxidants in Pelargonium graveolens L'Hér, J Sci Agric, 96(12): 4151-4159.

Hamidpour R, Hamidpour S, Hamidpour M, Marshall V, Hamidpour R (2017): Pelargonium graveolens (Rose Geranium) – A Novel Therapeutic Agent for Antibacterial, Antioxidant, Antifungal and Diabetics, Archives in Cancer Research, 5(1):1-6.

Khetsha ZP, Sedibe MM, Pretorius RJ, Watt E (2021): Cytokinin, gibberellic acid and defoliation on density and morphology of trichome of *Pelargonium graveolens* L'Hér for essential oil biosynthesis, Agrociencia, 55: 331-346.

Kochhar SL (2016): Economic Botany-A Comprehensive Study, Cambridge University Press, 5th edition, 680p.

Kremer D, Čepo DV, Dunkić V, Müller ID, Kosalec I, Bezić N, Stabentheiner E (2013): Phytochemical and Micromorphological Traits of *Geranium dalmaticum* and *G. macrorrhizum* (Geraniaceae), Natural Product Communications, 8(5): 645-650.

Maffei M, Chialva F, SaccoT (1989):Glandular trichomes and essential oils in developing peppermint leaves- I. Variation of peltate trichome number and terpene distribution within leaves, New- Phytol, 111: 707-716.

Maggs GL, Vorster P , Walt JJA (1995): Taxonomy of the genus *Pelargonium* (Geraniaceae): the section *Polyactium.*1. Circumscription and intrasectional classification, S Afr J Bol, 61(2): 53-59.

Martínez-Natarén DA, Parra-Tabla V, Dzib G, Calvo-Irabién LM (2011): Morphology and density of glandular trichomes in populations of Mexican oregano (*Lippia graveolens* H.B.K., Verbenaceae), and the relationship between trichome density and climate, Journal of the Torrey Botanical Society ,138(2):134-144.

Motsa NM (2006): Essential oil yield and composition of rose-scented geranium (*Pelargonium* sp) as influenced by harvesting frequency and plant shoot age, Ph. D Thesis, Agronomy, University of Pretoria, 112p.

Moutaouafiq S, Farah A, Ez zoubi Y, Ghanmi M, Satrani B, Bousta D (2019): Antifungal Activity of *Pelargonium graveolens* Essential Oil and its Fractions Against Wood Decay Fungi, Journal of Essential Oil Bearing Plants, 22(4): 1104-1114.

Rana VS, Juyal JP, Amparo Blazquez M (2002): Chemical constituents of essential oil of *Pelargonium graveolens* leaves, Int J Aromatherapy, 12: 216-218.

Romitelli I, Martins MBG (2013): Comparison of leaf morphology and anatomy among *Malva sylvestris* ("gerânioaromático"), *Pelargonium graveolens* ("falsa-malva") and *Pelargonium odoratissimum* ("gerânio-de-cheiro"), Rev Bras Pl Med, Botucatu, 15(1): 91-97.

Sadiki FZ, El Idrissi M, Sbiti M, Rahou A. (2019): Antibacterial properties of the essential oil of *Pelargonium Graveolens* L'Hér. RHAZES: Green and Applied Chemistry, 4(4): 17-23.

Tissier A (2012): Trichome Specific Expression: Promoters and Their Applications, IntehOpen, Chapter 17, 27p.

Verma RK, Chauhan A, Verma RS, Rahman LU, Bisht A. (2013): Improving production potential and resources use efficiency of peppermint (*Mentha piperita* L.) intercropped with geranium (*Pelargonium graveolens* L. Herit ex Ait) under different plant density. Ind Crop Prod, 44: 577-582.

EFFECT OF DIFFERENT CONCENTRATION OF *EUCALYPTUS GLOBULUS* (LABILL) LEAF LEACHATES ON GERMINATION AND GROWTH OF *ELEUSINE CORACANA* (L). C.V. DAPOLI – 1

Vikram P. Masal

PG Research Lab, Dapoli Urban Bank Senior Science College,

Dapoli, Dist. Ratnagiri (M.S.) 415 712

Corresponding author E-mail: masalvikram@gmail.com

Abstract:

Allelopathy generally referees to the inhibitory or stimulatory effect of one plant species on the germination, growth or development of another plant species (Molisch, 1937). The effects depend on the concentration of the release of compounds in the environment. The allelochemicals shows positive or negative effects on seed germination, growth and development of other plants. In the present investigation deals with the study of significant allelopathic effects of *Eucalyptus globulus* (Labill) leaf leachates on the germination and growth of *Eleusine coracana* (L). During the experimental period the temperature of Konkan region was ranged from 12.02^{0}C to 34.87^{0}C and humidity 62% to 93.9%.

Keywords: Allelopathy, *Eucalyptus globulus* Labill**,** *Eleusine coracana* (L)., Konkan.

Introduction:

Muller (1969) shown that proper use of Allelopathy may reduce the dependence on pesticides herbicides, fungicides, nematicides and insecticides. In many instance, the chemicals leached from the plants have an Allelopathy influence on the germination and growth of subsequent crops. Allelopathy influence of certain plants on crops has been reported by Bhatt *et al*. (1993), Hollis *et al.* (1982). Although toxic metabolites are distributed in all plant tissues but the bark and leaves are the major sources (Hollis and *et al.*, 1982).

Material and Methods:

The Laboratory Experiment was conducted in Department of Botany, Dapoli Urban Bank Senior Science College Dapoli. The tobacco yellowish leaves were collected from Sangli District of Maharashtra state during excursion tours. The plant materials were oven dried at 80 ^{0c} for 42

hrs and then ground to a fine powder. The extract was prepared by soaking 50 grams of dry ground tobacco leaves powder in 200 ml Distilled water for 24 hours.

The leaf leachates were filtered and the filtrate made up 200 ml volume by using distilled water. Which were considered as 100% and then diluted with distilled water and prepare solution of 20%, 40%, 60%, 80%, and 100%. The treatment was replicated four times by using R. B. D. design.

Eleusine coracana (L) seeds were treated with 0.1% mercuric chloride and washed thrice with distilled water and dried on sterile absorbent paper to avoid fungal attack. Twenty five seeds of *Eleusine coracana* (L) were tested for germination in 20 cm diameter petridishes containing germinating paper saturated with above concentration of leaf leachates. The moistened petridishes was maintained by adding 2.5 ml leaf leachates solutions.

The percentage of germination, root and shoot length and biomass production of the seedling was recorded after 3 DAS, 5DAS and 7 Days after sowing.

Result and Discussion:

The water extract of *Eucalyptus globulus* (Labill) leaves decreased the germination and seedling growth of *Eleusine coracana* (L) is depicted in Table 1. The 100% leaf extract of *Eucalyptus globulus* (Labill) inhibit the germination percentage (i.e. 28.33%) followed by 80%,60%,40% and 20% leaf extract respectively.

Bhatia *et al.* (2005) observed the germination percentage of wheat decreased with the increase in rice straw leachates concentration as compared to control. Rai and Tripathi (1982) reported the leaf leachates from *Eupatorium riparium* Regel.significantly inhibited the radical and plumule length of *E. adenophorum and Trifolium repens.* Rao *et al*. (1977) reported that aqueous extract of dry leaves of *Parthenium hysterophrus* L.inhibit the dry weight of plumule and radicals of *Triticum vulgare* L.

From above similar line of our observations it was concluded that 100% leaf leachates decreases root and shoot length i.e. 0.93 and 0.86 cm followed by 80%,60%,40% and 20% leaf extract respectively as shown in the above Table No.1. It was also predicted that the Leaf leachates of *Eucalyptus globulus* Labill. will be used as herbicide in different agronomic crops as well as different vegetable crops.

Table 1: Effect of different concentration of *Eucalyptus globules (Labill*) leaf leachates on germination and growth of *Eleusine coracana (L).*

Treatment	Germination % Days after soaking					Length of Radical cm Days after soaking					Length of plumule cm Days after soaking				
	3	4	5	6	7	3	4	5	6	7	3	4	5	6	7
T_0 control	88.0	96.00	96.00	96.00	96.00	0.90	1.13	2.0	2.66	3.33	0.63	0.66	1.73	0.9	2.1
T_1 (20%)	80.00	88.00	88.00	88.00	88.00	0.60	0.80	2.0	2.66	3.28	0.36	0.46	1.70	1.06	1.83
T_2 (40%)	58.66	72.00	72.00	72.00	72.00	0.53	0.70	1.50	2.22	3.26	0.26	0.36	1.66	1.13	2.10
T_3 (60%)	40.00	69.33	69.33	69.33	69.66	0.36	0.71	1.43	2.00	3.13	0.20	0.26	1.26	0.66	1.66
T_4 (80%)	24.00	30.66	30.66	30.66	30.66	0.34	0.46	1.20	1.80	2.20	0.16	0.23	0.96	0.43	1.33
T_5 (100%)	12.00	25.33	25.33	25.33	28.33	0.33	0.36	0.53	0.80	0.93	0.10	0.16	0.50	0.23	0.86
SE $\pm$ =	0.544	1.088	1.093	1.211	1.988	0.046	0.0250	0.0172	0.0544	0.1420	0.0389	0.0136	0.0554	0.062	0.080
CD at 5%	1.713	3.427	3.443	3.815	6.262	0.144	0.078	0.0541	0.1713	0.447	0.122	0.428	0.174	0.196	0.252

REFERENCES:

Bhatt, B.P., Chauhan D.S.and Todaria N.P. (1993): Phototoxic effect of tree crop on germination and radical extension of some food crop Trop.sci. 33:69-73

Bhatia, R.K., Sukhpreet, Sindhu, M.S., Mehara, S.P., Singh Gurupratap. (2005): Allelopathic effect of rice crop residue of Wheat and Mustard.Journal of research .Volume, 42 :pp 412.

Gaynar, D.G.and B.B.Jadhave (1992): Allelopathic effects on Terminalia tomentosa Roth. On germination of rice and cowpea. Indian journal of Plant physiology 35 (3):288-291.

Hollis, C.A., Smith J.E.and Fisher R.F. (1982): Allelopathic effects of common under storey species on germination and growth of southern pines.For.sci.28:509-515.

Kendrick, R.E.and Frankland, B. (1969): Photo control of germination in Amaranths caudatus. Planate 85:326-329.

Molisch H. (1937): Der Enflusslinear Pflanze auf die andere Allelopathic Germany:Gustav Fischer, Jena.

Muller C.H. (1969): Allelopathy as a factor in ecological process.Vegetation 18:348-357.

Panse, N.G.and Suckhatme P.V. (1985): Statistical methods for Agricultural workers. I.C.A.R., New Delhi, Forth Edition.

Rao, J.V.S., K.Ram Mohan Rao and S.S.Murthy (1979): Allelopathic effect of some weed of vegetable crop on the germination and early seedling growth of bajara. Trop. Eco. 20 (1):5-8

Rao A.N., D.C. Dager and P.S.Dubey (1977): Phyto allelopathic potentials of Parthenium hystrophorus Linn.Ind.j.weed science. 9 (1):24-32.

Rice E.K. (1974): Allelopathy.Academic press, New York.

Rai, JPN and Tripathi, R.S. (1982): Allelopathy as a factor contributing to dominance of Eupatorium riparium Regal. Indiand .J.Ecol. 9 (01):14 – 20.

ANTIDIABETIC EFFECTS OF BIOFLAVONOID MORIN

Shali K Sivan and Mini S*

Department of Biochemistry,

University of Kerala, Trivandrum, Kerala, India-695581

*Corresponding author email: minis@keralauniversity.ac.in

Abstract:

Diabetes mellitus is one of the most prevalent metabolic diseases worldwide and is associated with severe clinical complications including diabetic retinopathy, nephropathy, cardiomyopathy and nephropathy. Currently available treatment methods for diabetes suchas oral hypoglycemic drugs or insulin therapy have some side effects and also exerts economic burden on society. So, there is an urgent need for cost-effective antidiabetic agents from natural plants with lesser side effects. Recent studies suggest that flavonoids from plant have beneficial effects in the management of diabetes. Morin is a bioflavonoid, abundantly present in Moraceae family plants, exhibits several pharmacological properties such as anticancer, anti-inflammatory, antidiabetic, antioxidant, cardioprotective, neuroprotective and nephroprotective activities. Studies using various *in vitro* and *in vivo* models proved that morin is effective in maintaining glycemic levels and reducing the complications of diabetes. In this review, we evaluated the effect of dietary flavonoid morin on various complications of diabetes and the molecular mechanism behind it.

Abbreviations:

DM, Diabetes mellitus; DR, Diabetic retinopathy; DN, Diabetic nephropathy; PTP1B, Protein tyrosine phosphatase 1B; AIF, Apoptosis inducing factor; ROS, Reactive oxygen species; RNS, Reactive nitrogen species; MMS, Methyl methanesulfonate; Nrf2, Nuclear factor erythroid 2-related factor 2; SOD, Super oxide dismutase; GSH, Reduced glutathione; STZ, Streptozotocin; IL-6, Interleukin 6; IL-1β, Interleukin 1 β; TNF-α, Tumor necrosis factor -α; VEGF, Vascular endothelial growth factor; IRS, Insulin receptor substrate; IR, Insulin resistance

Introduction:

Diabetes mellitus (DM) is a serious metabolic disorder with a high incidence rate and a challenging public health problem worldwide (Al-Ishaq *et al.,* 2019). In 2021, the number of people living with diabetes (among adults ages 20–79) is estimated to be 536. 6 million and this

will increase up to 783. 2 million by 2045 (Sun *et al.,* 2021). Diabetes is characterized by chronic hyperglycemia resulting from impaired insulin secretion, insulin action or both. It leads to altered carbohydrate, protein and lipid metabolism. Diabetes is associated with numerous microvascular and macrovascular problems including ketoacidosis, dyslipidemia, hypertension, nephropathy, retinopathy, neuropathy, cardiovascular and atherosclerotic complications (Proença *et al.,* 2020). Studies suggested that 30%–40% of diabetic patients will have at least one complication within ten years after the onset of diabetes with a higher probability of amputation. The remarkable feature is that we cannot reverse the complications of diabetes through drug treatment (Huang *et al.,* 2020).

Currently available treatment methods for diabetes include the use of different classes of oral hypoglycemic drugs such as biguanides, sulfonylureas, thiazolidinediones, α-glucosidase inhibitors, and non-sulfonylureas secretagogues and insulin therapy. The use of these antidiabetic drugs results in some adverse effects such as drug resistance and toxicity, so the demand for new antidiabetic agents from natural sources is growing (Salehi *et al.,* 2019).

Several studies proved that flavonoids have beneficial effects in the management of diabetes. Morin (3,5,7,2′ ,4′ -pentahydroxyflavone) is a bioflavonoid isolated from a variety of plants, particularly those belonging to the Moraceae family. Morin possesses several pharmacological properties such as antioxidant, anti-inflammatory, anti-cancer, antidiabetic, neuroprotective, nephroprotective and cardioprotective activities (Rajput *et al.,* 2021). Morin is easily available and comparatively cost-effective. A number of *in vitro* and *in vivo* models proved that morin is effective in maintaining glycemic levels and reducing the major complications of diabetes. Morin administration has not been associated with any side effects (Choudhury *et al.,* 2017). The present review discussed the therapeutic effect of morin on diabetes complications and the molecular mechanisms underlying this beneficial effect.

Background information on morin

Morin is abundantly present in almond (*Prunus dulcis*), fruit and leaves of guava plant (*Psidium guajava* L.), osage orange (*Maclura pomifera*), onion (*Allium cepa*), white mulberry (*Morus alba* L.), apple (*Malus pumila*), tea (*Camellia sinensis*), fig (*Ficus carica* L.), sweet chestnut (*Castanea sativa*), jack fruit (*Artocarpus heterophyllus*), old fustic (*Maclura tinctoria*), red wine, coffee and cereal grains (Fig. 1) (Choudhury *et al.,* 2017; Rajput *et al.,* 2021).

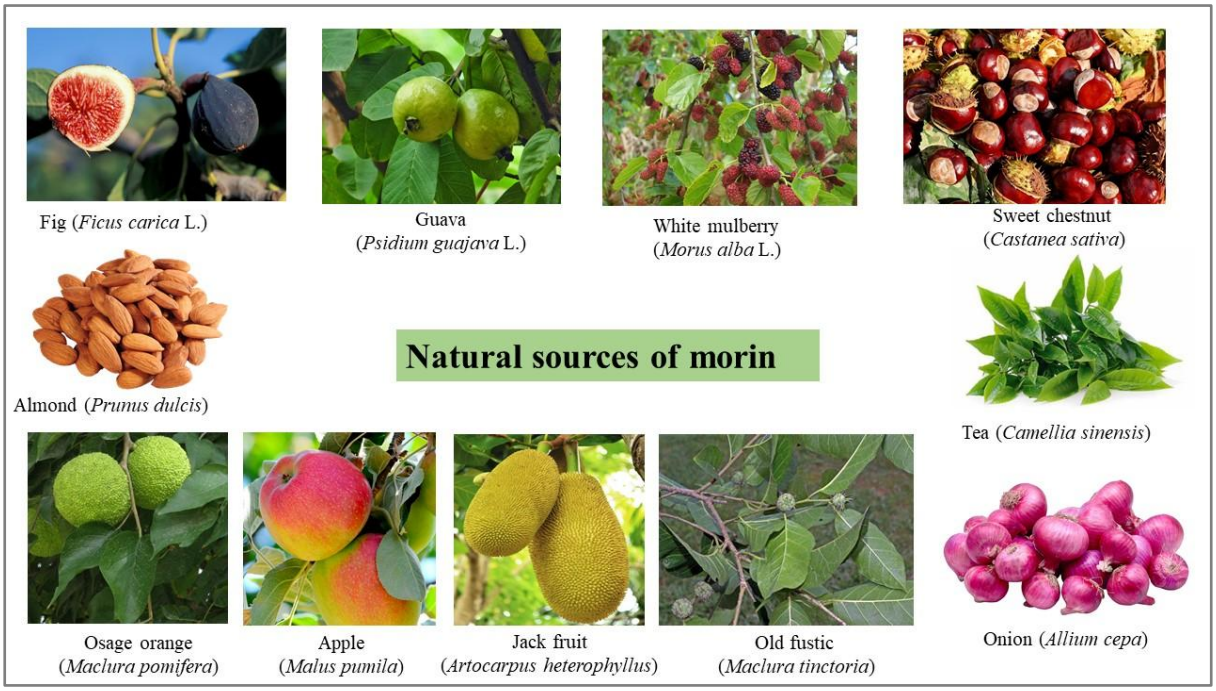


Figure 1: Natural sources of morin

Morin is a yellow colour pigment with a molecular weight of 302. 2357 g/mol. The structure of morin hydrate shows that it is an isomeric form of quercetin with a carbonyl group in position 4, an OH in position 3, a resorcinol part and it differ only in the hydroxylation pattern on B-ring. Hydroxylation is at meta-position in morin and ortho position in quercetin (Fig. 2). Morin is soluble in methanol (50 mg /ml), water (0. 25 mg/ml at 20 °C, 0. 94 mg/ml at 100 °C) and slightly soluble in ether and acetic acid (Sinha *et al.*, 2016).

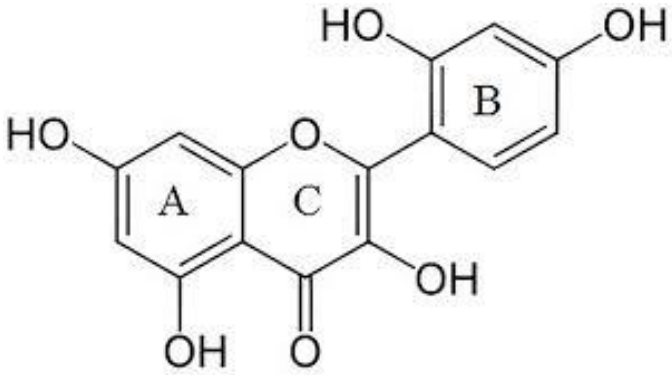


Figure 2: Chemical structure of morin

Morin is found in plant sources as a free phytochemical or its glycosylated derivatives. During dietary intake, morin and its derivatives are converted into their respective aglycon form in the gut, which helps the easy uptake of derivatives from the gut. However, unabsorbed portions of morin were converted into aglycon in the large intestine by the bacteria (Choudhury

et al., 2017). Morin and its metabolites were detected in the serum for up to 120 minutes after consumption in the diet (Rajput *et al.*, 2021).

Beneficial effects of morin on diabetes complications

Several *in vivo* (Table 1) and *in vitro* (Table 2) studies proved the efficacy of morin in reducing the complications of diabetes.

1. Morin in diabetic retinopathy

Diabetic retinopathy (DR) is one of the common microvascular complications of diabetes and is the leading cause of blindness among diabetic patients in the working-age population (Rodríguez *et al.,* 2019). Characteristics of DR include microaneurysms, pericyte and neuronal cell loss, acellular-occluded capillaries formation and vascular basement membrane thickening (Guzman *et al.,* 2017). Recent data suggested that morin supplementation reduced the progression of DR in STZ induced type 1 diabetic rats by improving the endogenous antioxidant enzyme activity, decreasing the levels of lipid peroxidation products, interleukin 1β (IL-1β), tumor necrosis factor -α (TNF-α), and vascular endothelial growth factor (VEGF) in retina. Morin treatment also improved the thickness of the retina and the number of ganglion cells in diabetic rats (Jiang *et al.,* 2020).

2. Morin in diabetic osteopenia

Alternations in bone and mineral metabolism during diabetes lead to the development of diabetic osteopenia. Osteopenia results in the increased occurrence of bone fractures and delayed healing of bone fractures (Liang *et al.,* 2011). Elevated oxidative stress is the major reason for the development of osteopenia in diabetic condition (Hamada *et al.,* 2009). In STZ induced diabetic rats, morin treatment prevents bone loss by decreasing the bone turnover parameters like osteocalcin (OC), deoxypyridinoline cross-links (DPD), bone-specific alkaline phosphatase (BALP), and telopeptides of collagen type I (CTX). Bone micro-CT analysis of morin treated diabetic rats showed a reduction in both trabecular bone mass loss and its microarchitecture deterioration. Moreover, morin treatment significantly reduced the level of lipid peroxidation products, inflammatory markers and improved the activity of antioxidant enzymes in rat models of diabetic osteopenia (Abuohashish *et al.,* 2013).

3. Morin in diabetes-induced liver damage

Liver injury is a common complication of diabetes and elevated levels of oxidative stress and inflammatory response are the reasons behind the hepatocyte damage (Mohamed *et al.,* 2016). In human HepG2 cells, high glucose treatment produces an upregulated expression of MicroRNA29a (miR-29a) and thereby increases the expressions of its target genes (PI3K and IRS2) and decrease gluconeogenesis by downregulating the expression of PEPCK. While morin

positively control the alternations in the expressions of miR-29a and related genes (Razavi *et al.,* 2019). In STZ induced diabetic rats, morin supplementation improved the activities of glycolytic enzyme (hexokinase), reduced the activities of gluconeogenic enzymes (glucose-6-phosphatase and fructose-1,6-bisphosphatase) and increased the glycogen content in the liver (Vanitha *et al.,* 2014). Moreover, morin act as a non-competitive inhibitor of protein tyrosine phosphatase 1B (PTP1B) and thus increases the phosphorylation of insulin receptor and Akt. Morin enhances glycogen synthesis and inhibits gluconeogenesis in HepG2 cells (Paoli *et al.,* 2013). Studies using rat primary hepatocytes proved that high glucose stress increased the reactive oxygen species (ROS) generation and thus reduced the viability of the cell. Additionally, high glucose treatment lost the mitochondrial membrane integrity, elevated translocation of apoptotic proteins including cytochrome-c, endonuclease-G and AIF (Apoptosis inducing factor), along with changes in the expression patterns of Bax and Bcl-2 in primary rat hepatocytes. Whereas treatment with morin reduced the alternations induced by high glucose stress (Kapoor and Kakkar, 2012).

4. Morin in diabetes-induced brain damage

Diabetes is associated with mild to moderate reduction in cognitive function with changes in brain function (Moheet *et al.,* 2015). Morin supplementation effectively reduced the diabetes-induced brain damage by improving antioxidant enzymes and neurotrophic factors level, reducing the concentration of lipid peroxidation products and inflammatory markers in STZ induced diabetic rats (Ola *et al.,* 2014). Recent evidences suggest that morin treatment significantly improved the motor and sensory nerve conduction velocities and nerve blood flow in the brain of STZ induced diabetic rats. Studies using mouse neuroblastoma cell line, Neuro 2A (N2A), showed that morin reduced the mitochondrial ROS generation under high glucose conditions. Morin suppresses NF-κB mediated inflammatory pathway and upregulates Nrf2 mediated pathway in high glucose treated N2A cells (Bachewal *et al.,* 2021).

5. Diabetic nephropathy

Diabetic nephropathy (DN) is another major complication of diabetes and the mechanism behind the DN is ROS, hyperglycemia and impaired podocyte autophagy, affecting 30% of patients with diabetes (Hu *et al.,* 2021). Aleisa *et al.* (2013) reported that morin supplementation prevents diabetes-induced kidney damage by decreasing the oxidative stress in STZ induced diabetic rats. Studies in rat glomerular mesangial cells (MCs) showed that morin prevents cell proliferation and fibronectin accumulation in high glucose treated condition via modulating p38 MAPK and JNK pathways (Ke *et al.,* 2016).

Molecular mechanism of morin in diabetes complications

The central events in the pathophysiology of diabetes are β cell dysfunction and peripheral insulin resistance (Mahler and Adler, 1999). Mechanisms by which the development chronic hyperglycemia mediated diabetic complications include; increased activation of PKC pathway, polyol pathway, hexosamine pathway, AGE formation and action, and alterations of signal transduction pathways. These pathways ultimately result in elevated oxidative stress, varying gene expressions and protein function resulting in vascular tissue damage (Kitada *et al.,* 2010). Morin reduce the complications of diabetes through decreasing the oxidative stress, inflammation, insulin resistance and improving the beta cell function (Fig. 3).

1. Antioxidant effect of morin in diabetes complications

ROS and reactive nitrogen species (RNS) are formed in our body under physiological conditions and these are removed by the action of antioxidant enzymes and compounds. However, excess production of these free radicals or any defects in the action of the antioxidant defence system leads to the development of oxidative stress and causes damages to lipid, proteins and DNA (Réus *et al.,* 2019). Vanitha *et al.* (2017) proved that morin reduces oxidative stress induced by three genotoxic agents - H_2O_2, STZ and methyl methanesulfonate (MMS) in INS-1E rat insulinoma β-cell line through increasing the activity of antioxidant enzymes and activating nuclear factor erythroid 2-related factor 2 (Nrf2) pathway. Studies in high glucose treated primary rat hepatocytes showed that morin treatment regulates the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase, reduced glutathione (GSH) and glutathione peroxidase and expressions of the antioxidant gene (Kapoor and Kakkar, 2012). Additionally, morin treatment improved the activities of antioxidant enzymes in retinal tissue in diabetic retinopathy (Jiang *et al.,* 2020) and in brain tissue in diabetic encephalopathy (Ola *et al.,* 2014).

2. Anti-inflammatory effect of morin in diabetes complications

Inflammatory biomarkers have been linked to the development of diabetes and the main inflammatory markers involved are white blood cell count, adipokines, chemotactic proteins, cytokines and acute phase proteins. Whereas, adipose tissue, immune cells, pancreas and liver are major organs in chronic inflammation related to diabetes (Lontchi-Yimagou *et al.,* 2013). Abuohashish *et al.* (2013) reported that morin treatment reduced the level of inflammatory markers such as IL-6, IL-1β and TNF-α in rats with experimentally induced diabetic osteopenia. In the diabetic brain, morin reduced the levels of inflammatory markers (TNFα, IL1β, and IL-6) considerably (Ola *et al.,* 2014). Jiang *et al.* (2020) proved that morin treatment decreased the level of inflammatory markers (TNF-α, IL-1β) in the retina of STZ induced diabetic animals.

Table 1: Effect of morin in diabetic complications- *in vivo* studies

Study models and method	Dose/ period	Diabetic complication	Major findings/mechanisms	References
Single intraperitoneal injection of STZ 60mg/kg, Wistar albino rats	Morin is given at a dose of 25 and 50 mg/kg for 60 days	Diabetic retinopathy	Morin prevents progression of diabetic retinopathy through improving the antioxidant enzyme activity and lowering the levels of TNF-α, IL-1β, and VEGF.	Jiang *et al.*, 2020
Single intraperitoneal injection of STZ 55mg/kg,Wistar albino rats	Morin is given orally at a dose of 15 and 30mg/kg for 5 consecutive weeks	Diabetic osteopenia	Morin treatment prevents the bone loss, microarchitecture deterioration through its antioxidant and anti-inflammatory properties	Abuohashish *et al.*, 2013
Single intraperitoneal injection of STZ 50mg/kg, Wistar albino rats	Morin is given at a dose of 25 and 50mg/kg for 30 days	Diabetes induced liver and pancreas damage	Morin treatment regulates the activities of enzymes involved in glycolysis and gluconeogenesis in the liver of diabetic rats. Morin improved serum insulin levels and protect the normal histology of pancreas in diabetic rats	Vanitha *et al.*, 2014
Single intraperitoneal injection of STZ 65 mg/kg, Wistar albino rats	Morin is given at a dose of 15 and 30 mg/kg for 5 weeks	Diabetic encephalopathy	Morin treatment decrease oxidative stress, inflammation and improve neurotrophic factors in the brain of diabetic rats.	Ola *et al.*, 2014
Single intraperitoneal injection of STZ 55mg/kg, Sprague Dawley rats	Morin is given at a dose of 50 and 100mg/kg (treatment was started after 6 weeks of diabetes induction) for 2 weeks	Diabetic neuropathy	Morin treatment improved motor and sensory nerve conduction velocities	Bachewal *et al.*, 2021
Single intraperitoneal injection of STZ 65mg/kg, Wistar albino rats	Morin is given at a dose of 15 and 30 mg/kg for five consecutive weeks	Diabetic nephropathy	Morin treatment ameliorate STZ induced diabetic nephropathy via decreasing the oxidative stress	Aleisa *et al.*, 2013

Table 2: Effect of morin in diabetic complications- *in vitro* studies

Cell type	Concentration of morin used	Major findings	References
High glucose treated human HepG2 liver cells	12. 5-100µM	Morin treatment downregulated the miR-29a expression and thereby improves insulin signalling and glucose metabolism.	Razavi *et al.,* 2019
HepG2 cells	20-100 µM	Morin is identified as a small molecular non-competitive inhibitor of PTP1B	Paoli *et al.,* 2013
STZ treated RINm5F cells	10 -50 µM	Morin treatment prevents STZ induced cell damage in RINm5F by promoting the phosphorylation of AMPK and thus activating FOXO3 and catalase.	Wang *et al.,* 2018
MIN6 cells	0. 001 -10µM	Morin treatment promote insulin secretion by activating I-3R	Lin *et al.,* 2017
Neuro 2A (N2A) cells	10 -20 µM	Morin treatment reduce oxidative stress by modulating Nrf2-NFκB pathway	Bachewal *et al.,* 2021
Primary rat hepatocytes	5 µg	Morin treatment prevent primary rat hepatocytes from apoptosis and dysfunction upon high glucose treatment	Kapoor and Kakkar, 2012
Rat glomerular mesangial cells (MCs)	25 and 50µM	Morin treatment suppresses the activation of the p38 MAPK and JNK signalling pathways, thereby decreases high glucose-induced mesangial cell proliferation and ECM expression.	Ke *et al.,* 2016
INS-1E cells	0-25 µM	Morin treatment protects beta cells from oxidative stress-induced DNA damage via activating the Nrf2 mediated signalling pathway.	Vanitha *et al.,* 2017

3. Pancreatic beta cell function and insulin release

A reduced beta cell function is the central event in the development and progression of diabetes (Wysham and Shubrook, 2020). Morin administration improved the serum insulin level in STZ induced diabetic rats and immunohistochemical analysis showed that morin treatment is effective in preserving the normal histological architecture of pancreatic cells along with an increased number of insulin positive cells in diabetic rats (Vanitha *et al.*, 2014). Imidazoline I-3 receptors (I-3Rs) are one subtype of imidazoline receptor (I-R) located in pancreatic beta cells and regulate insulin release. Recent studies using MIN6 cells proved that morin increases insulin secretion by activating I-3R receptors (Lin *et al.*, 2017). Wang *et al.* (2018) showed that morin protects RINm5F pancreatic β cells against STZ induced cell damage by reducing the ROS production and apoptosis of the cell. This is achieved via the activation of the AMPK-FOXO3-catalase pathway by morin in RINm5F cells.

4. Morin on insulin resistance

The main characteristic of insulin resistance (IR) is a decreased insulin response or delayed stimulation of glucose transport. IR results from impaired insulin signalling at one or more intracellular intermediates, such as reduced insulin receptor substrate (IRS) activation or reduced glucose oxidation (Diane *et al.*, 2021). Protein tyrosine phosphatase 1 B (PTP1B) act on phosphorylated insulin receptor and function as a major negative regulator of insulin signalling pathway. Studies using HepG2 cells established that morin can act as a non-competitive inhibitor of PTP1B and thereby promoting the phosphorylation of insulin receptor and Akt (Paoli *et al.*, 2013). In high fat diet-induced obese mouse models, insulin resistance is reduced after morin administration (Naowaboot *et al.*, 2016).

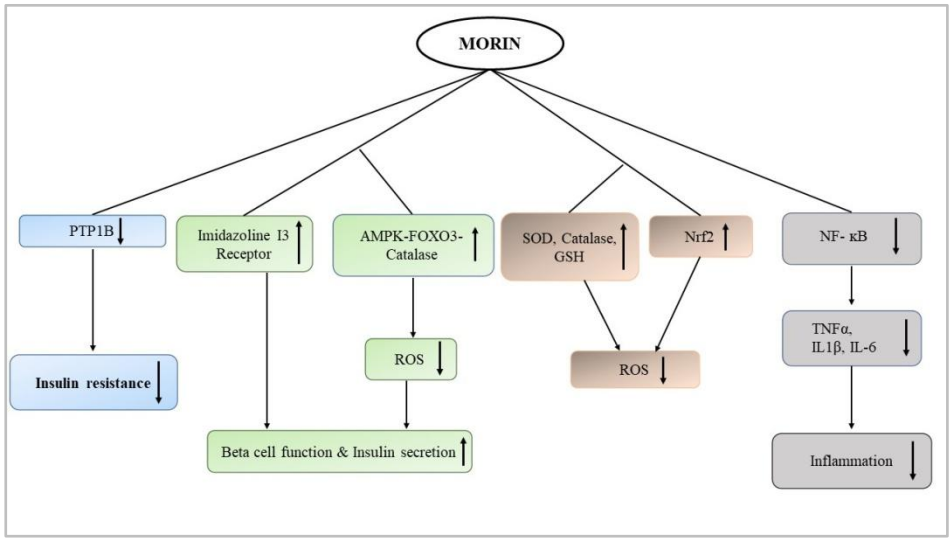


Figure 3: Molecular mechanism of morin in diabetes complications

Conclusion:

Morin has a promising therapeutic potential in the management of diabetes mellitus, according to the current data. The present review outlines the therapeutic effects of morin and its relevant molecular mechanism in diabetes complications.

References:

Abuohashish, H. M., Al-Rejaie, S. S., Al-Hosaini, K. A., Parmar, M. Y., and Ahmed, M. M. (2013). Alleviating effects of morin against experimentally-induced diabetic osteopenia. *Diabetology and metabolic syndrome*, *5*(1), 1-8.

Aleisa, A. M., Al-Rejaie, S. S., Abuohashish, H. M., Ahmed, M. M., and Parmar, M. Y. (2013). Nephro-protective role of morin against experimentally induced diabetic nephropathy. *Digest Journal of Nanomaterials and Biostructures*, *8*(1), 395-401.

Al-Ishaq, R. K., Abotaleb, M., Kubatka, P., Kajo, K., and Büsselberg, D. (2019). Flavonoids and their anti-diabetic effects: cellular mechanisms and effects to improve blood sugar levels. Biomolecules, 9(9), 430.

Asmat, U., Abad, K., and Ismail, K. (2016). *Diabetes mellitus* and oxidative stress—A concise review. *Saudi pharmaceutical journal*, *24*(5), 547-553.

Bachewal, P., Gundu, C., Yerra, V. G., Kalvala, A. K., Areti, A., and Kumar, A. (2018). Morin exerts neuroprotection via attenuation of ROS induced oxidative damage and neuroinflammation in experimental diabetic neuropathy. *Biofactors*, *44*(2), 109-122.

Choudhury, A., Chakraborty, I., Banerjee, T. S., Vana, D. R., and Adapa, D. (2017). Efficacy of morin as a potential therapeutic phytocomponent: Insights into the mechanism of action. International Journal of Medical Research and Health Sciences, 6(11), 175-194.

Diane, A., Abunada, H., Khattab, N., Moin, A. S. M., Butler, A. E., and Dehbi, M. (2021). Role of the DnaJ/Hsp40 family in the pathogenesis of insulin resistance and type 2 diabetes. Ageing Research Reviews, 101313.

Guzman, D. C., Olguín, H. J., García, E. H., Peraza, A. V., de la Cruz, D. Z., and Soto, M. P. (2017). Mechanisms involved in the development of diabetic retinopathy induced by oxidative stress. *Redox Report*, *22*(1), 10-16.

Hamada, Y., Fujii, H., and Fukagawa, M. (2009). Role of oxidative stress in diabetic bone disorder. *Bone*, *45*, S35-S38.

Hu, Q., Qu, C., Xiao, X., Zhang, W., Jiang, Y., Wu, Z., ... and Zhao, Y. (2021). Flavonoids on diabetic nephropathy: advances and therapeutic opportunities. *Chinese medicine*, *16*(1), 1-17.

Huang, D. D., Shi, G., Jiang, Y., Yao, C., and Zhu, C. (2020). A review on the potential of Resveratrol in prevention and therapy of diabetes and diabetic complications. Biomedicine and Pharmacotherapy, 125, 109767.

Jiang, B., Geng, Q., Li, T., Firdous, S. M., and Zhou, X. (2020). Morin attenuates STZ-induced diabetic retinopathy in experimental animals. *Saudi Journal of Biological Sciences*, *27*(8), 2139-2142.

Kapoor, R., and Kakkar, P. (2012). Protective role of morin, a flavonoid, against high glucose induced oxidative stress mediated apoptosis in primary rat hepatocytes.

Ke, Y. Q., Liu, C., Hao, J. B., Lu, L., Lu, N. N., Wu, Z. K., ... and Chen, X. L. (2016). Morin inhibits cell proliferation and fibronectin accumulation in rat glomerular mesangial cells cultured under high glucose condition. *Biomedicine and Pharmacotherapy*, *84*, 622-627.

Kitada, M., Zhang, Z., Mima, A., and King, G. L. (2010). Molecular mechanisms of diabetic vascular complications. *Journal of diabetes investigation*, *1*(3), 77-89.

Liang, W., Luo, Z., Ge, S., Li, M., Du, J., Yang, M., ... and Luo, Z. (2011). Oral administration of quercetin inhibits bone loss in rat model of diabetic osteopenia. *European journal of pharmacology*, *670*(1), 317-324.

Lin, M. H., Hsu, C. C., Lin, J., Cheng, J. T., and Wu, M. C. (2017). Investigation of morin-induced insulin secretion in cultured pancreatic cells. *Clinical and Experimental Pharmacology and Physiology*, *44*(12), 1254-1262.

Lontchi-Yimagou, E., Sobngwi, E., Matsha, T. E., and Kengne, A. P. (2013). *Diabetes mellitus* and inflammation. *Current diabetes reports*, *13*(3), 435-444.

Mahler, R. J., and Adler, M. L. (1999). Type 2 diabetes mellitus: update on diagnosis, pathophysiology, and treatment. *The Journal of Clinical Endocrinology and Metabolism*, *84*(4), 1165-1171.

Mohamed, J., Nafizah, A. N., Zariyantey, A. H., and Budin, S. (2016). Mechanisms of diabetes-induced liver damage: the role of oxidative stress and inflammation. *Sultan Qaboos University Medical Journal*, *16*(2), e132.

Moheet, A., Mangia, S., and Seaquist, E. R. (2015). Impact of diabetes on cognitive function and brain structure. *Annals of the New York Academy of Sciences*, *1353*, 60.

Naowaboot, J., Wannasiri, S., and Pannangpetch, P. (2016). Morin attenuates hepatic insulin resistance in high-fat-diet-induced obese mice. Journal of physiology and biochemistry, 72(2), 269-280.

Ola, M. S., Aleisa, A. M., Al-Rejaie, S. S., Abuohashish, H. M., Parmar, M. Y., Alhomida, A. S., and Ahmed, M. M. (2014). Flavonoid, morin inhibits oxidative stress, inflammation and enhances neurotrophic support in the brain of streptozotocin-induced diabetic rats. *Neurological sciences*, *35*(7), 1003-1008.

Paoli, P., Cirri, P., Caselli, A., Ranaldi, F., Bruschi, G., Santi, A., and Camici, G. (2013). The insulin-mimetic effect of Morin: A promising molecule in diabetes treatment. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1830*(4), 3102-3111.

Proença, C., Ribeiro, D., Freitas, M., and Fernandes, E. (2020). Flavonoids as potential agents in the management of type 2 diabetes through the modulation of α-amylase and α-glucosidase activity: a review. Critical Reviews in Food Science and Nutrition, 1-71.

Rajput, S. A., Wang, X. Q., and Yan, H. C. (2021). Morin hydrate: A comprehensive review on novel natural dietary bioactive compound with versatile biological and pharmacological potential. Biomedicine and Pharmacotherapy, 138, 111511.

Razavi, T., Kouhsari, S. M., and Abnous, K. (2019). Morin exerts anti-diabetic effects in human HepG2 cells via down-regulation of miR-29a. *Experimental and clinical endocrinology and diabetes*, *127*(09), 615-622.

Réus, G. Z., Carlessi, A. S., Silva, R. H., Ceretta, L. B., and Quevedo, J. (2019). Relationship of oxidative stress as a link between *Diabetes mellitus* and major depressive disorder. *Oxidative medicine and cellular longevity*, *2019*.

Rodríguez, M. L., Pérez, S., Mena-Mollá, S., Desco, M. C., and Ortega, Á. L. (2019). Oxidative stress and microvascular alterations in diabetic retinopathy: Future Therapies. *Oxidative medicine and cellular longevity*, *2019*.

Salehi, B., Ata, A., V Anil Kumar, N., Sharopov, F., Ramírez-Alarcón, K., Ruiz-Ortega, A., ... and Sharifi-Rad, J. (2019). Antidiabetic potential of medicinal plants and their active components. Biomolecules, 9(10), 551.

Sinha, K., Ghosh, J., and Sil, P. C. (2016). Morin and its role in chronic diseases. *Anti-inflammatory nutraceuticals and chronic diseases*, 453-471.

Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B., ... and Magliano, D. J. (2021). IDF diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Research and Clinical Practice, 109119.

Vanitha, P., Senthilkumar, S., Dornadula, S., Anandhakumar, S., Rajaguru, P., and Ramkumar, K. M. (2017). Morin activates the Nrf2-ARE pathway and reduces oxidative stress-induced DNA damage in pancreatic beta cells. *European journal of pharmacology*, *801*, 9-18.

Vanitha, P., Uma, C., Suganya, N., Bhakkiyalakshmi, E., Suriyanarayanan, S., Gunasekaran, P., and Ramkumar, K. M. (2014). Modulatory effects of morin on hyperglycemia by attenuating the hepatic key enzymes of carbohydrate metabolism and β-cell function in streptozotocin-induced diabetic rats. *Environmental toxicology and pharmacology*, *37*(1), 326-335.

Wang, N., Zhang, J., Qin, M., Yi, W., Yu, S., Chen, Y., ... and Zhang, R. (2018). Amelioration of streptozotocin-induced pancreatic β cell damage by morin: Involvement of the AMPK-FOXO3-catalase signaling pathway. *International journal of molecular medicine*, *41*(3), 1409-1418.

Wysham, C., and Shubrook, J. (2020). Beta-cell failure in type 2 diabetes: mechanisms, markers, and clinical implications. *Postgraduate Medicine*, *132*(8), 676-686.

POTASH SOLUBILIZING BACTERIA: A GREEN ALTERNATIVE TO CHEMICAL FERTILIZER

Varsha D. Jayaswal, Harsha D. Pardeshi, Leena P. Shirsath and Sandip P. Patil*

Department of Microbiology and Biotechnology,

R. C. Patel Arts, Commerce and Science College, Shirpur-425405, India

*Corresponding author E-mail: patilsandip3@gmail.com

Abstract:

Biofertilizers are the fertilizers that contain living microorganisms, their activities increases soil fertility and produce supplementary substances for plants. Biofertilizers can be produced from vegetable wastes (onion peels, cabbage waste etc.) and fruit wastes (banana peels) as substrates.There is necessity to evaluate efficiency of soil organisms like Potash Solubilizing Bacteria (KSB) in combination with vegetable wastes to formulate it into a biofertilizer and in enhancing the growth of crops. The Potash Mobilizing Bacteria is attributed to potash mobilization in the soil to the plant. Therefore, it is recommended for stimulating growth, productivity and yield components in many crops. The vegetable waste is highly organic and rich in carbohydrates, proteins etc. The Potash mobilizing bacteria utilize complex organic substances present in vegetable waste and converted it into re-usable fertilizer by improving the quality of compost with sufficient amount of organic carbon and nitrogen. The vegetable waste along with efficient soil organisms helps in promoting growth of crops. Present studies focuses on the isolation of potent KSB from soil and its use as the biofertilizer that can serve as the good alternative to chemical fertilizers.

Introduction:

Agricultural microbiology is branch of microbiology which deals with microbial applications for agricultural economy. Nutrients are essential for plant to healthy growth.There are about thirteen mineral nutrients in the soil and they are classified into two categories with are macronutrient and micronutrient (Lack and Evans, 2005).

Nitrogen (N), phosphorus (P) and potassium (K) are primarily macronutrients which are needed in large amounts while copper, iron, boron are the micronutrients that are needed in very small amount or micro quantity. For this reason plants needs in combination of nutrients to live, growth and reproduce. In the soil, mineral nutrients are dissolved in water and absorbed through the plants roots.

Nitrogen

Based on the primary macro nutrients N, P, K requirement, plants required nitrogen in largest amount as compared to phosphorus and potassium. However, unlike carbon dioxide and oxygen, the elemental form of nitrogen is completely unavailable to use directly to majority of green plants (Hillel, 2008). Plants such as legumes gain nitrogen by associated with free living microorganism which can fix the nitrogen from the atmosphere. Besides decaying organic materials, animal excreta and nitrogen fertilizer also contribute as source of nitrogen in soil (Lack and Evans, 2005). Nitrogen is essential for plants since it can promote rapid growth, enhance leaf size and quality, speed up crop maturity and also help in fruit and seed development. Recent studies prove that, nitrogen fixing bacteria can enhance growth and production of various plants significantly (Aseri *et al.*, 2008).

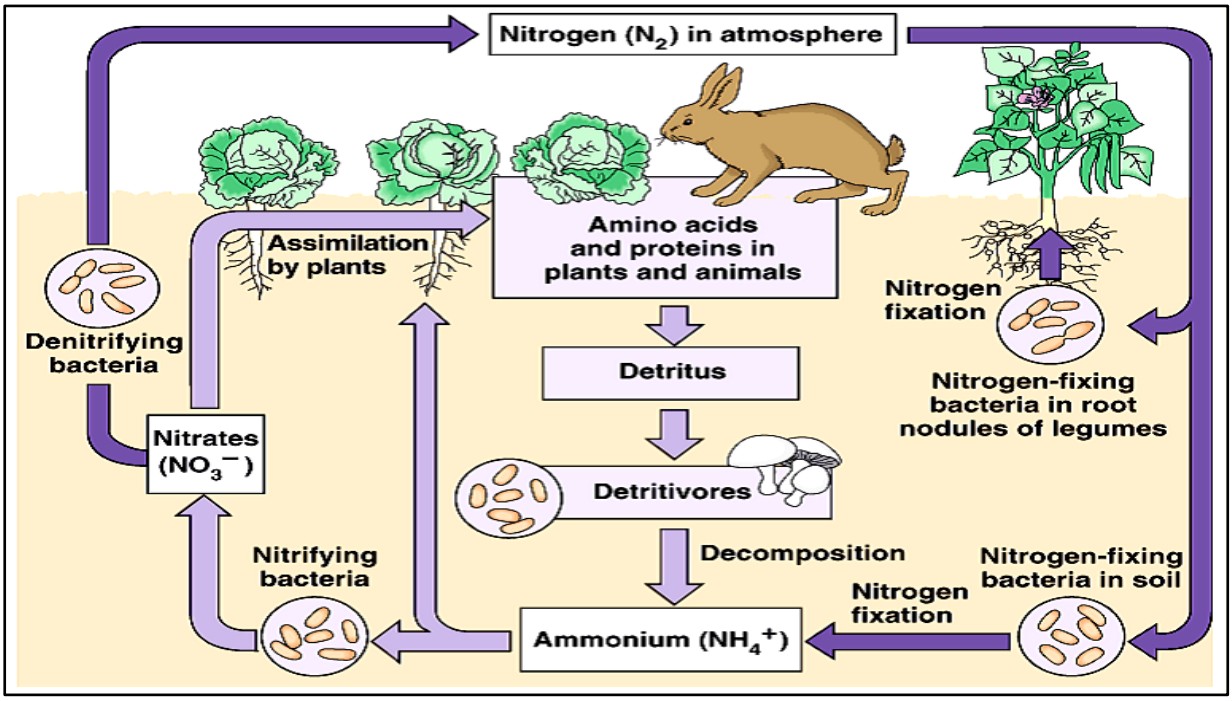


Figure 1:Nitrogen Cycle (Spiro and Stigliani, 2003)

Phosphorus

Various properties of phosphate such as weak mobility, formation of insoluble forms with many cations and fixation in organic soil compounds can lead to its deficiency to the plants (Hirsch, 2006).

Phosphorus deficiency will affect major energy storage and transfer function of plants since ATP is mobile within plants. It also disturbs root development and cause early flowering

and ripening. Then symptoms are characterized by undersized growth, dark green leaves with a leathery texture and reddish purple leaf tips and margin leaf tips and margin.

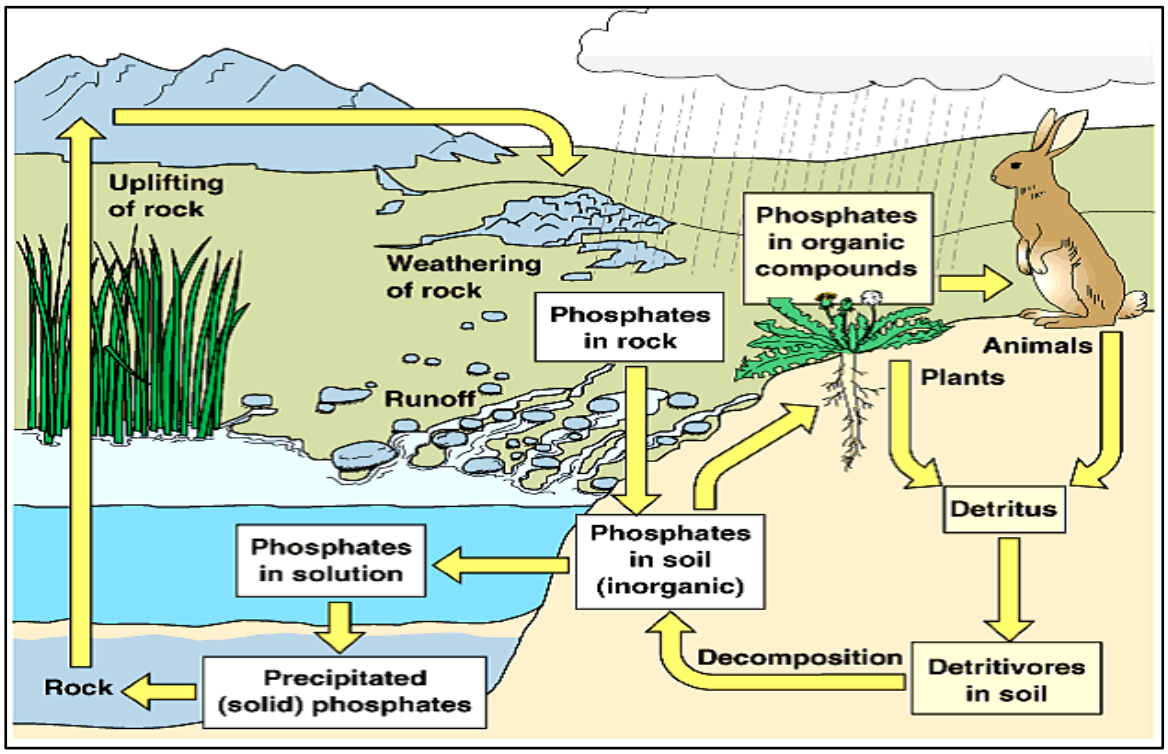


Figure 2: Phosphorus Cycle (Spiro and Stigliani, 2003)

Potassium

Potassium (K) is the seventh most common element in earth's crust. Potassium is the first isolated in 1807 by Sir Humphry Davy who obtained it through the electrolysis of very dry molten caustic potash (KOH). Potassium is the first metal isolated by electrolysis and collected at the cathode (Holmes 2011).

The potassium content of Indian soils varies from less than 0.5 - 3.00% (Mengel, 1987). In Indian soil the soluble (K) potash forms are present in approximately 2% and insoluble are present in range of 98% in form of minerals like biotite, feldspar, mica, muscovite, vermiculite (Goldstein, 1994).

Potash solubilizing bacteria (KSB) in soils enhances the plant growth and provides nutrients in high amount to the plants (Han and Lee, 2005). *F. aurentia* belonging to the family *Pseudomonaceae* obtained from agricultural soil (Banana rhizospheric soil).It solubilized K and it increases crop yield (Ramarthinam and Chandra, 2006). This solubilization effect is generally due to the production of certain organic acids and enzymes by KSB. In addition, they are also known to produce amino acid, vitamin and growth promoting substances like indole 3- acetic acid (IAA) and Gibberelic acid (GA) which help in better growth of the plants (Ponmurugan

and Gopi, 2002). Solubilization of potassium occurs by complex formation between organic acids and metal ions such as Fe^{2+},$Al^{3+}$and Ca^{2+}(Styriakova, 2003).

Fundamentally, K is water soluble and highly mobile element and it is transported in plants via xylem (Lack and Evans, 2005). In plants, potassium act as a regulator since it is constituent of 60 different enzyme systems of drought tolerance and water use efficiency. All crops required potassium especially high carbohydrate plants such as bananas and potatoes (Hillel, 2008). In addition current study has showed that, crops need more potassium for their optimum growth (Simonsson *et al.,* 2007).

Uptake of potassium depends on the rate which it supplied through roots rather than amount of potassium availability in the soil potassium regulates many metabolic processes required for growth, fruit and seed development.

Potassium has many functions in plant growth such as it enhances the progress of cell division and growth, increase disease resistance and drought tolerance, regulate the opening and closing of the stomata required for osmotic regulation. Besides, potassium is essential for photosynthesis process and act as a key to activate enzymes to metabolize carbohydrates for manufacture of amino acid and proteins.

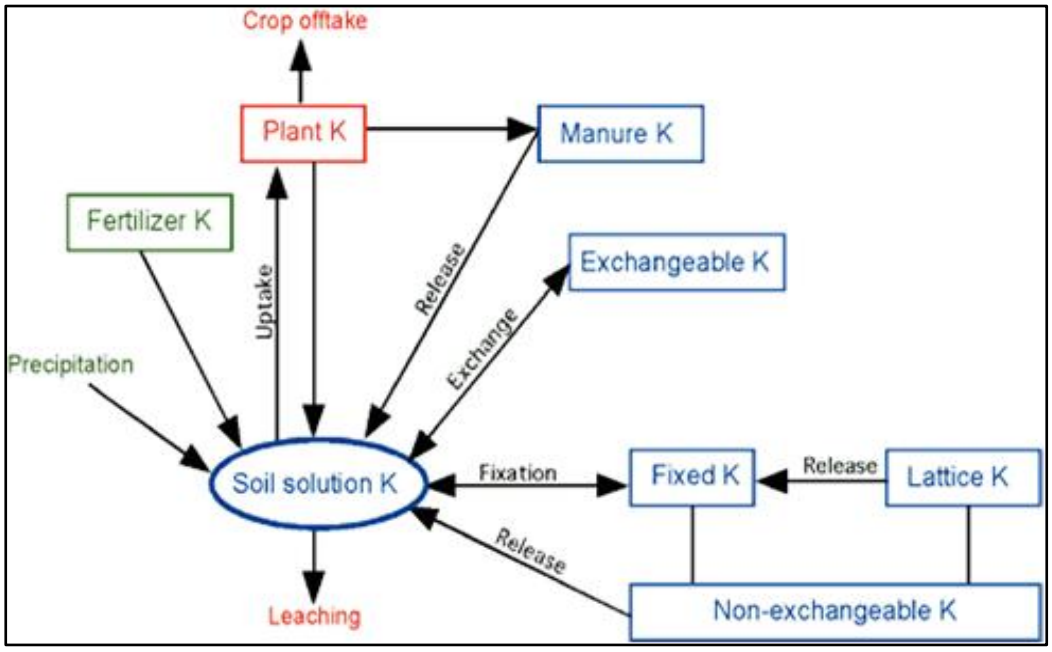


Figure 3: Potassium Cycle in Soil-Plant-Animal System (Syers, 1998)

Potash solubilizer is a beneficial bacterium capable of mobilizing potassium available in soil into the root zone of the plants. The potash mobilizing or solubilizing bacteria can also use in liquid form (as a liquid biofertilizer) which in turn acts as a source of potassium for plant

growth. Potassium is the vital component of plant nutrition package for increased crop yield and quality that performs important biological functions to maintain plant growth. Among the Nitrogen (N), Phosphorus (P), Potassium (K); Potassium is the third important plant nutrient. Potassium is essential macronutrient for plant growth and it plays important role in activation of several metabolic processes including protein synthesis, photosynthesis, enzymes as well as in resistance to diseases and insects etc.

Role of K in plants

a. Potassium helps in formation of amino acids and protein from ammonium ions which absorbed by roots.

b. It increases the hydration of protoplasm.

c. It activates the number of enzymes viz. Alcohol dehydrogenase.

d. It increases the resistance to insect pest and various diseases.

Microorganisms used as potash solubilizing bacteria

Generally, most commonly used potash solubilizing bacterial strains is *Frateuria aurentia*. The representative species of *Pseudomonaceae*, *Bacillus* sp. can also be used as KSB. Other species includes *Bacillus mucilaginosus, Enterobacter hormaechei* as an efficient KSB. A wide range of bacteria namely *Pseudomonas, Burkholderia, Acidothiobacillus ferroxidans, Bacillus edaphicus, Bacillus circulans* and *Paenibacillus* species are also utilized as KSB.

Disadvantages of chemical fertilizers

a. Chemical fertilizers affects on beneficial microorganisms in soil.

b. Overuse of chemical fertilizers responsible for change in pH of soil and makes it acidic.

c. Due to increased use of chemical fertilizers the soil fertility decreases.

d. Chemical fertilizers pollute the groundwater, rivers and lakes.

e. The continuous use of chemical fertilizers has led to degradation of soil health.

f. Overuse of Chemical fertilizers is an increasing concern because it depletes the essential nutrients in the soil.

Materials and Methods:

Sample collection

The sample for the isolation of potash solubilizing bacteria is obtained from rhizospheric soil of banana field.

Isolation of KSB

Isolation of microorganism from above mentioned sample was carried out by using routine microbiological isolation techniques. The dilution of 10^7 of sample was spread on

Glucose Yeast Calcium Agar (GYcaA) aseptically. It was incubated at 37^{o}C for 24 hr. Upon incubation, the isolated colonies from GYcaA medium were transferred into glucose yeast broth for enrichment culture technique. The enriched cultures then used for further experimentation.

Identification and characterization of potential isolates

A. Morphological characteristics

a. Colony characteristic: Colony characters were observed after 24 hours of incubation on Glucose Yeast Agar.

b. Gram's staining: Gram's staining was performed as per protocol to know the Gram character of an organism.

c. Motility: Motility was performed as per regular protocol.

B. Biochemical tests

a. **Sugar fermentation test**: Tubes containing sterile peptone water with Andrade's indicator and 1% respective sugars (mannitol, maltose, etc).

b. **IMViC test**: The suspension of 24 hrs. old culture was inoculated in the test tube containing peptone water for Indol test, glucose phosphate broth for Methyl red and Vogues Proskauer test in respective tubes, Simmons citrate slant for citrate utilization test. All tubes were incubated at 37^{o}C for 24hr. After incubation IMViC reagents were added in respective set of tubes.

c. **Enzyme tests:**

Amylase test: A loopful suspension of 24 hr old culture was spot inoculated on sterile starch agar plate. After incubation Gram's iodine was added.

Catalase test: A loopful suspension of 24 hr old culture is spot inoculated on sterile Nutrient agar plate. After incubation H_2O_2 was added.

Oxidase test: A loopful suspension of 24 hr old culture was inoculated in nutrient broth tubes and added 1% tetramethyl p-phenyl diamine. After addition of this reagent the tube was observed for purple colour formation within next 10 seconds.

Preparation of biofertilizer by using isolated KSB

After the isolation of potent KSB microorganisms from above mentioned samples, it was spread on Glucose Yeast Calcium Agar (GYcaA) medium and incubated at 37^{o}C for 24 hr. Upon incubation, a single colony of KSB from GYcaA plate was picked and inoculated it in glucose yeast calcium broth containing vegetable waste (onion peel powder). Then it was incubated for 48 hr at 37^{o}C for the preparation of KSB biofertilizer.The prepared biofertilizer was applied on

wheat seeds in order to check its efficiency. The rate of germination of seeds and further growth of crop plants was observed and compared with the control plants.

Results and Discussion:

An isolated KSB strain showed its potential of dissolving mineral potassium effectively at 37^{o}C and left a clear zone of clearance around the colony (Fig.4A) (Parmar, 2013). The morphological, cultural and biochemical characteristics of potent strain of KSB are summarized in Table 1. The potent isolate was Gram negative, motile bacillus. The isolate KSB have potential of utilization of various carbohydrates (Sucrose, Lactose, Dextrose, Mannitol, Galactose, Cellobiose, Trehalose) (Table 2) and secretion of various enzymes (Catalase, amylase, oxidase) (Table-4). Isolated bacterial strain showed maximum growth at pH 7.0 (Shanware, 2014). In IMViC test, it does not produced indole and showed negative results in MR-VP tests; but it showed ability to degrade citrate as a sole source of carbon (Table-4). Based on these characteristics, the isolated KSB may be categorized as a species of *Pseudomonas*.

Morphological characteristics

Table 1: Colony characteristics on Glucose yeast calcium agar

Characteristic	Result
Shape	Bacillus
Size	1 mm
Color	White
Opacity	Opaque
Elevation	Convex
Consistency	Smooth
Margin	Entire
Gram character	Gram negative
Motility	Motile

Table 2: Sugar fermentation profile of isolate

Sugar test	Result
Sucrose	+
Lactose	+
Dextrose	+
Mannitol	+
Galactose	+
Arabinose	-
Cellobiose	+
Trehalose	+

Biochemical Test:

a. Sugar fermentation test: After 24 hrs. incubation the isolate showed following results

b. IMViC tests

Table 3: IMViC tests

Test	Result
Indole test	-
Methyl red	-
Vogues prausker	-
Citrate utilization	+

c. Enzyme tests

Table 4: Enzyme secretion tests

Enzyme	Result
Catalase test	+
Amylase test	+
Oxidase test	+

A. **B.** **C.**

Figure 4: A. Isolation of KSB B. Enriched culture broth of KSB C. KSB showed enhanced growth of wheat crop as compared to control

Preparation and effect of Biofertilizer

The isolate KSB have showed ability to solubilize the K reserves from soil and make it available to the plants, which results in promotion of plant growth and minimizing the

application of chemical fertilizers. K solubilization is carried out by a large number of bacteria in natural habitat. Application of KSB as biofertilizer not only enhances plant growth and yield but also decreasing the use of agrochemicals and support eco-friendly crop production.

The prepared biofertilizer when applied on wheat seeds; it showed better rate of seed germination and growth of plants as compared to control (Fig. 4C).

Conclusions:

Isolated Potash solubilizing bacterial strain (KSB) is efficiently solubilizes the potash into the root zone of plant. It increases the water holding capacity of the plant. Isolated potash solubilizing bacteria produces the 3-Indol acetic acid which increases the rate of seed germination. The prepared KSB biofertilizer showed an enhanced growth of wheat crop as compared to control plants. This isolated KSB can serve as a good alternative to the excessive use of chemical fertilizers.

Acknowledgement:

Authors acknowledge the kind help of Dr. D. R. Patil, Principal, R. C. Patel Arts, Commerce and Science College, Shirpur, MS, India for providing suitable infrastructural facilities required to perform this work.

References:

Aseri, G. K., Jain, N., Panwar, J., Rao, A. V. and Meghwal, P. R. (2008): Biofertilizers improve plant growth, fruit yield, nutrition, and metabolism and rhizosphere enzyme activities of pomegranate (Punicagranatum L.) in Indian Thar Desert. Scientiahorticulturae, 117(2), 130-135.

Basak, B. and Biswas, D. (2012): Modification of waste mica for alternative source of potassium: evaluation of potassium release in soil from waste mica treated with potassium solubilizing bacteria (KSB): LAP LAMBERT Academic Publishing.

Cejas, I., Rumlow, A., Turcios, A. E., Engelmann, F., Edel Martinez, M., Yabor, L., and Lorenzo, J. C. (2016): Exposure of common bean seeds to liquid nitrogen modifies mineral composition of young plantlet leaves. American Journal of Plant Sciences 7 (2016), Nr. 12, 7(12), 1612-1617.

Chaffey, N. (2006): Instant notes in plant biology, 2nd edn. Lack A, Evans D. 2005. Oxford: Taylor and Francis, pp 351.

Dash, D., Patro, H., Tiwari, R. C. and Shahid, M. (2010): Effect of organic and inorganic sources of nitrogen on Fe, Mn, Cu and Zn uptake and content of rice grain at harvest and straw at different stages of rice (Oryza sativa) crop growth. Adv. Appl. Sci. Res, 1(3), 36-49.

Dubey R. C. Maheshwari D. K. (2005) practical microbiology. S. Chand and co. New Delhi.

Goldstein, A. H. (1994): Involvement of the quinoprotein glucose dehydrogenase in the solubilization of exogenous phosphates by gram-negative bacteria. Phosphate in microorganisms: cellular and molecular biology. ASM Press, Washington, DC, 197-203.

Han, H. S. and Lee, K. D. (2005): Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity. Res J AgricBiolSci, 1(3), 210-215.

Kalpana, P., SaiBramari, G. and Anitha, L. (2011): Formulation of potential vegetable waste compost in association with microorganisms and Spirulinaplatensis. Asian Journal of Plant Science and Research, 1(3), 49-57.

Kalpana, P., SaiBramari, G. and Anitha, L. (2011): Formulation of potential vegetable waste compost in association with microorganisms and Spirulinaplatensis. Asian Journal of Plant Science and Research, 1(3), 49-57.

Lian, B. F. P. Q., Fu, P. Q., Mo, D. M. and Liu, C. Q. (2002): A comprehensive review of the mechanism of potassium release by silicate bacteria. Acta Mineral Sinica, 22, 179-183.

Magen, H. (2008): Balanced crop nutrition: fertilizing for crop and food quality. Turkish Journal of Agriculture and Forestry, 32(3), 183-193.

Neneng, L. (2020): Formulation of liquid biofertilizer for enhance of soil nutrients in peatland. Budapest International Research in Exact Sciences (BirEx) Journal, 2(3), 314-322.

Parmar, P. and Sindhu, S. S. (2013): Potassium solubilization by rhizosphere bacteria: influence of nutritional and environmental conditions. J Microbiol Res, 3(1), 25-31.7.

Parmar, P. and Sindhu, S. S. (2013): Potassium solubilization by rhizosphere bacteria: influence of nutritional and environmental conditions. J Microbiol Res, 3(1), 25-31.

Ponmurugan, P. and Gopi, C. (2006): In vitro production of growth regulators and phosphatase activity by phosphate solubilizing bacteria. African Journal of biotechnology, 5(4), 348-350.

Prajapati, K. B.and Modi, H. A. (2012): Isolation and characterization of potassium solubilizing bacteria from ceramic industry soil. CIBTech J Microbiol, 1(2-3), 8-14.

Prajapati, K., Sharma, M. C. and Modi, H. A. (2013): Growth promoting effect of potassium solubilizing microorganisms on *Abelmoscus esculantus*. Int J AgricSci, 3(1), 181-188.

Ramamoorthy, B. and Bajaj, J. C. (1969): Soil fertility map of India. Indian Agricultural Research Institute.

Ramarethinam, S. and Chandra, K.(2005): Studies on the effect of potash solubilizing/mobilizing bacteria Frateuriaaurantia on brinjal growth and yield. Pestology, 11, 35-39.

Rapheal, O. and Adebayo, K. S. (2011): Assessment of trace heavy metal contaminations of some selected vegetables irrigated with water from River Benue within Makurdi Metropolis, Benue State Nigeria. Advances in applied science research, 2(5), 590-601.

Seneviratne, G., Jayakody, K., Weerasekara, M. L. M. A. W., Someya, T. and Ryuda, N. (2011): Microbial biofertilizer application versus compost use in agriculture: soil health implications. Soil Microbes and Environmental Health, 4, 81-117.

Shanware, A. S., Kalkar, S. A. and Trivedi, M. M. (2014): Potassium solublisers: occurrence, mechanism and their role as competent biofertilizers. Int J CurrMicrobiol App Sci, 3(9), 622-629.

Simonsson, M. andersson, S. andrist-Rangel, Y., Hillier, S., Mattsson, L. and Öborn, I. (2007): Potassium release and fixation as a function of fertilizer application rate and soil parent material. Geoderma, 140(1-2), 188-198.

Spiro, T. G. and Stigliani, W. M. (2003): Chemistry of the Environment.

Styriakova, I., Styriak, I., Galko, I. G. O. R., Hradil, D. A. V. I. D. and Bezdicka, P. (2003): The release of iron-bearing minerals and dissolution of feldspars by heterotrophic bacteria of Bacillus species. Ceramics Silikaty, 47(1), 20-26.

Zakaria, A. A. B. (2009): Growth optimization of potassium solubilizing bacteria isolated from biofertilizer. Faculty of Chemical and Natural Resources Engineering, University Malaysia Pahang.

ANATOMICAL STUDIES ON *FICUS BENGHALENSIS* L. VAR. *KRISHNAE* (C. DC.) CORNER (MORACEAE)

E. Munuswamy

Department of Plant Biology and Plant Biotechnology,

Loganatha Narayanasamy Government College,

Ponneri, Thirivallur District, 601 204

Corresponding author E-mail: em.sam12@gmail.com

Abstract:

Ficus benghalensis L. Var. *Krishnae* (C. DC.) Corner of the family Moraceae, as per the botanical literature. Some taxon is said to be *Ficus krishnae* the above name is synonym. As per the pharmacological literature *Ficus benghalensis* L. Var *Krishnae* (C. DC) Corner is attributed medicinal properties such as diabetes, diarrhea, diaphoretic, aphrodisiac, spermatorrhea, gonorrhea, dysentery, toothache, bruises, rheumatic joints, diuretic, astringent, leucorrhoea, haemoptysis and fever (Yoganarasimhan, 2000). The regional floras provide the morphological features for identification of the plant. When the plant samples are leathery and woody in condition, it posses difficulty in identification of the herbal. So one has to option for microscopic parameters of various parts of the plant. The present study provides detailed description of anatomical features of leaf, petiole, node, internode, epidermal peeling and leaf venation of *Ficus benghalensis L.Var. Krishnae (C. DC.)* Corner supplemented with the photomicrographs at different magnification studies of the herbal drugs with may help for botanical diagnosis and over come the adulteration and substitutions of the drugs.

Keywords: *Ficus benghalensis* L. Var. *Krishnae (C. DC.)* Corner, Aphrodisiac, spematorrhea, haemoptysis, herbal drugs and adulteration

Introduction:

Ficus benghalensis L.Var. *Krishnae* (C. DC.) Corner of Moraceae is popularly known as per the most of the floras (Gamble, 1921; Mathew, 1983; Henry *et al.*, 1987; Yoganarasimhan, 2000; Madhavachetty *et al.*, 2008). The Tamil equivalent for this plant seems to be controversial. However *Ficus benghalensis* L.Var. *Krishnae* (C. DC.) Corner is credited with many medicinal properties both Indian system of medicine as well as folklore claims. In many taxonomic books,

the external features of provided for identification of this tree is present. But microscopic parameters are not available to supplement the morphological features and to diagnose the botanical identity of samples of the plant. With a review to fill up the tannin and secretory tissues in the botanical studies and throw lighter anatomical diagnostic features of *Ficus benghalensis L.Var. Krishnae (C. DC.) Corner*, the present study was attempted.

Materials and Methods:

The specimen for the present study was collected from Goonipalayam reserve forest located at Thiruvallur District during April 2015. The following organs were studied during the present work.

1. Epidermal peeling
2. Leaf venation
3. Anatomy of leaf, petiole, node and internode.

Epidermal peeling

Fresh leaves of *Ficus benghalensis* L. var. *Krishnae* (C. DC.) Corner was collected and cut into small pieces of laminal materials taken at the mid level and half way between margin and midrib. The leaf bits were immersed Jeffrey's maceration fluid and kept in thermostat till the epidermal layer separates from the mesophyll.

Leaf venation

Fresh leaves of *Ficus benghalensis* L.var. *Krishnae* (C. DC.) Corner was collected and cut into small pieces, kept in test tubes to which 5% sodium hydroxide solution was added. The tubes were kept in thermostat for 2 to 3 days, till the leaves become transparent.

Anatomy

Fresh materials of leaves, petiole, node and internode were collected and fixed as soon as they were collected. The materials were trimmed properly and fixed in FAA (Formalin +Acetic Acid +Alcohol). The materials were dehydrated and infiltrated with paraffin wax by employing ethanol and tertiary butyl alcohol mixtures as prescribed by Saas (1951).

By using Rotary microtome, sections were cut at 10-15 µm thickness. The sections were stained with tannic acid-ferric chloride (Foster, 1934) for primary walls. Alcoholic safranin -Fast green (Johansen, 1940) were used to stain ligninous walls and cytoplasm respectively. The microphotographs were taken by using Nikon Lab-Photo-2.

Observations:

Morphology (Fig. 1)

Figure 1: A small twig with 4 -5 leaves

Tree is common. It has recognized easily by enormous pillar-like aerial roots and profusely spreading branches. Corner (1967) mentioned a variety of *Ficus benghalensis* L. var. *Krishnae* (C. DC) Corner (1902), in which one behalf of the leaf has reflexed and connate to form a very characteristic cup. It often cultivated and called “Krishnae Bor " and " Krishna's cup". Bark grey. Leaf cone shaped structure shining above.

Anatomy

Leaf (Fig. 2 and 3)

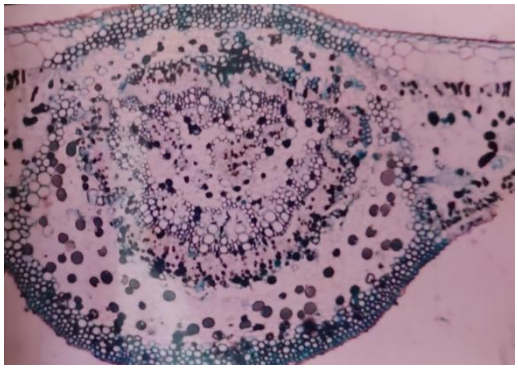


Figure 2: Cross sectional view of leaf midrib (100x)

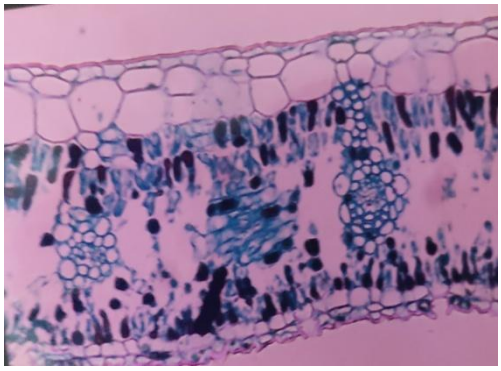


Figure 3: Coss sectional view of leaf laminna

Lamina 1.2 mm thick and of midrib 1.5 mm thick. Epidermis barrel shaped and double layered. Cuticle thin and wavy. Mesophyll has two layers of palisade cells five to six layers of spongy tissue. Diameter of palisade tissue 330 µm and diameter of spongy tissue is 1.0 mm.

Vascular bundle have sclerenchymatous cap on the lamina region. Midrib have three to four layers of collenchyma cells on the both sides.

Petiole base (Fig. 4)

Diameter 1.4 mm. Outline ovate. Epidermis barrel shaped and single layered, compact cells having no intercellular space. Width of the cortex 750 µm. Ground tissue heterogeneous with two to three layers of collenchyma followed by thin walled parenchyma tissue. Vascular strand consist of 6-7 collateral bundles arranged in a discontinuous ring. Tannin and starch grains abundant in the parenchyma cells.

Petiole tip (Fig. 5)

Diameter 1.4 mm. Outline wavy. Epidermis wavy, barrel shaped and single layered compact cells having no intercellular spaces. Cortical thickness 585 µm. Ground tissue have many layers of parenchymatous tissue. Vascular bundles discrete in a discontinuous ring. Tannins and starch grains are abundant.

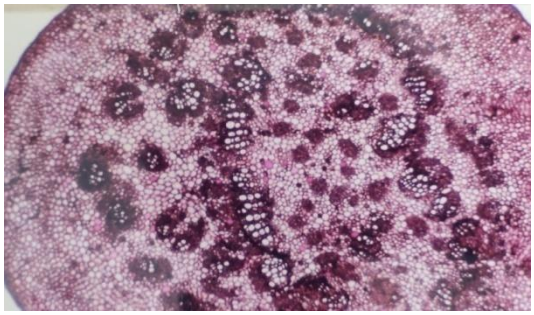


Figure 4: Cross sectional view of petiole base

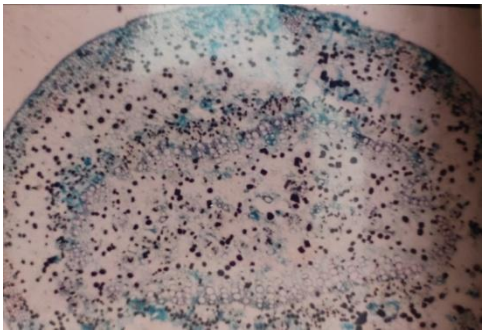


Fig. 5 Cross sectional view of petiole tip (50x)

Node (Fig.6)

Diameter 2.6 mm. Shape circular surface uneven, hairy. But lesser in number. Epidermis cubical and double layered. Thickness of cortex 1.3 mm. Ground tissue have two to three layers of collenchyma, beneath this having seven to eight layers of parenchymatous tissue. Vascular bundles are discrete. Tannin abundant in the cortical region. It has five leaf traces. It is called multilacunar type.

Internode (Fig.7)

Diameter 2.3 mm. Shape elliptic. Surface uneven and hairs present. Epidermis barrel shaped and double or single layered. The width of cortex 855 µm. Cortex consist of three zones, outer one or two sclerenchyma cells, middle zone have four to five layer of chlorenchyma cells

and the inner zone has many layers of parenchyma cells. Vascular strand consist of five to six collateral bundles arranged in the form of ring. Tannin abundant.

Figure 6: Cross sectional view of node (macroscopic)

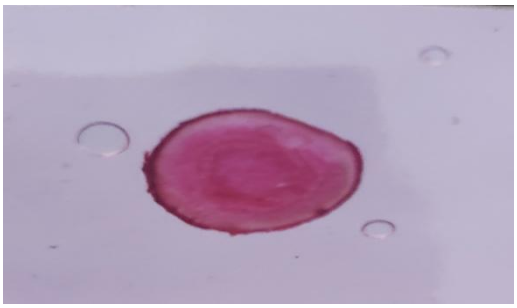


Figure 7: Cross sectional view of internode (macroscopic)

Epidermal peeling (Fig. 8)

Hypostomatic, anomocytic. Stomata small, closely aggregated, stomata sunken. Epidermal hairs absent.

Leaf venation (Fig.9)

Areole cubical and polygonal. Vein ending branched many times and connected to one another.

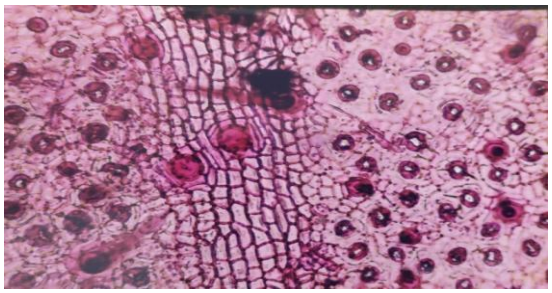


Figure 8: Epidermal peeling of stomata by maceration (250 x)

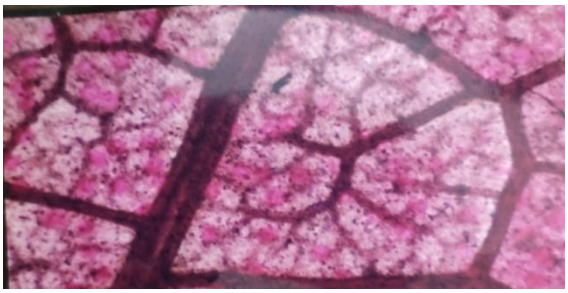


Figure 9: Leaf venation pattern (50 x)

Discussion:

In the present investigation anatomical studies on *Ficus benghalensis* L. var. *Krishnae* (C. DC.) Corner (Moraceae) deals with the anatomy of the node, internode, petiole, leaf, epidermal peeling and leaf venation. In the species studied the internode has wide pith, thin vascular cylinder, heterogeneous cortex and laticifers in all ground tissue. The node exhibits uniformly a multilacunar vascular system with single trace associated with each leaf gap. The petiole of the vascular cylinder consists of thin radial files of continuous cylinder. The leaf has dorsiventral symmetry with adaxial multiple epidermis and cystolith containing chambers. The

midrib projects prominently below the surface of lamina. The vascular system of the midrib contains a ring of collateral, discrete vascular bundles. The stomata are sunken and closely aggregated. The vein islets (areole) are either polygonal in shape with branched, free vein endings.

References:

Corner EJH (1967): *Ficus* in the Solomon Islands and its bearing on the post-jurassic history of Melanesia. Philosophical Transactions of the Royal Society of London, 253(783):23-159.

Foster (1934): The use of tannic acid and iron chloride for staining cell wall of meristematic tissues. Stain technology. 9:91-92.

Gamble, J. S. (1921): The flora of the Presidency of Madras. 2. Vols. Botanical Survey of India, Calcutta, India. p.1075-1079.

Henry, A. N., Kumari, G. R. and Chithra, V. (1987): Flora of TamilNadu, India.2.Vols. Botanical Survey of India, Coimbatore. p.138-147.

Jeffrey EC (1917): The anatomy of woody plants. University of Chicago Press, Chicago, Illinois.

Johansen DA. (1940): Plant microtechnique. New York: McGraw-Hill, 523 p.

Madhavachetty, K Sivaji, K and Tulasi Rao K (2008): Flowering plants of Chittoor District, Andhra Pradesh, India, Student offset printers. Tirupathi, P.490.

Mathew, K. M. (1983): The flora of the TamilNadu. 3.Vols. The Rapinat Herbarium, St. Joseph's college, Tiruchirapalli, TamilNadu, India.p.1182-1186.

Sass JE. (1951): Botanical microtechnique. Ames: Iowa State College Press, 391 p.

Yoganarasimhan, S. N. (2000): Medicinal plants of India. Vol.II. Tamil Nadu Regional Research Institute (A. Y.) Bangalore. India p.715.

INTEGRATED PEST MANAGEMENT STRATEGIES

Amrit Mohapatra*[1] and Dibyajyoti Swain[2]

[1]Department of Entomology,

[2]Department of Plant Pathology,

Faculty of Agricultural Sciences,

Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, Odisha, India

*Corresponding author E-mail: amritmohapatra192@gmail.com

Abstract:

Integrated Pest Management (IPM) is a holistic approach to combat pests (including herbivores, pathogens, and weeds) using a combination of preventive and curative actions, and only applying synthetic pesticides when there is an urgent need. Integrated pest management (IPM) is an environmentally friendly technology. IPM is a multifaceted approach to pest management that seeks to minimize negative impacts on the environment. This technique is an important step towards providing healthy, viable food for a growing global population. Just as the recent recognition that an evolutionary perspective is useful in medicine to understand and predict interactions between hosts, diseases, and medical treatments, we argue that it is crucial to integrate an evolutionary framework in IPM to develop efficient and reliable crop protection strategies that do not lead to resistance development in herbivores, pathogens, and weeds. Such a framework would not only delay resistance evolution in pests, but also optimize each element of the management and increase the synergies between them. The pest control industry is continuously examining novel technologies and products that will improve the way to manage and prevent pests. The general objective is to likewise diminish the effects of various available pesticides on the environment and on non-target creatures, besides the economic influence on bottom lines.

Keywords: Biological control, crop wild relatives, economic injury level, evolutionary application, evolutionary integrated pest management, pesticide resistance, plant resistance, plant tolerance.

Introduction:

Pests are organisms which can damage the crops and compete with them. They cause decrease in the plant density, stunted growth of plant, a lower production capacity, and lessen the

yield or nature of horticultural products. Pathogens, herbivores, and weeds cause ubiquitous problems for crop production, including 11%–59% losses in yields of the major crops in the world (Oerke, 2006). Thus, conventional breeding programs have resulted in crop varieties with very low genetic variation in resistance-related traits and modern agriculture has instead heavily relied on synthetic pesticides (including insecticides, fungicides, and herbicides) to control pests. These synthetic toxins may have adverse effects on humans (Damalas and Eleftherohorinos, 2011; Nicolopoulou-Stamati, Maipas, Kotampasi, Stamatis, and Hens, 2016) and biodiversity (Geiger *et al*., 2010; Rundlöf *et al*., 2015). Moreover, their efficiency has decreased, as numerous pest species have evolved resistance to one or several of the available pesticide compounds (Bass, Denholm, Williamson, and Nauen, 2015; Gould, Brown, and Kuzma, 2018; Ma and Michailides, 2005; Powles and Yu, 2010; Sparks and Nauen, 2015). Thus, pesticides are not always reliable even in cases where they are needed and pesticide resistance has recently been termed a "wicked problem" (Gould *et al*., 2018).

Several techniques for crop protection were developed to prevent and minimize the loss of crops due to pests in the field (pre harvest losses) and during storage (postharvest losses). Crop protection involves products, tools, and practices which can be used by farmers to protect their harvest against insects, disease, and weeds. The food production can be influenced by insects and disease. Farmers around the globe settle on various choices every day with respect to how best to secure their crops by using different practices like biological control, microbial pesticides, pest behavior, genetic manipulation, and plant immunization of pest population. Fortunately, a variety of solutions are available with advances in modern agriculture.

It was recently suggested that an evolutionary framework is also needed in pest management (Hicks *et al*., 2018; Neve, Busi, Renton, and Vila-Aiub, 2014; Thrall *et al*., 2011; Zhan, Thrall, and Burdon, 2014). Such a framework would allow us to test whether an individual control measure is efficient and predict long-term consequences of the method for relevant agro-ecosystems. Here, we develop this concept and argue that an evolutionary perspective is particularly desirable and fruitful for the development of *Integrated Pest Management* (IPM; see also Peterson, Higley, and Pedigo, 2018). IPM is an approach to combat pests and pathogens using a combination of sustainable methods, thereby becoming less dependent on synthetic pesticides. As opposed to pesticides, the goal of IPM is not to eradicate pests, but to manage them at low numbers below economically injurious levels.

History of IPM:

IPM is not a new philosophy. The concept has been around since the 1920's when cotton pest management program was developed. Under this scheme, insect control was "supervised"

by qualified entomologists, and insecticide applications were based on conclusions reached from periodic monitoring of pest and natural-enemy populations. This was viewed as an alternative to calendar-based insecticide programs. Supervised control is based on a sound knowledge of the ecology and analysis of the projected trends in pest and natural enemy populations. In supervised control, (integrated control) the best mix of chemical and biological controls is sought and identified for a given insect pest. The chemical insecticides are used in a manner that is least disruptive to biological control. The chemical controls are applied only after regular monitoring indicates that a pest population had reached an economic threshold level. Thus, such treatment is required to prevent the population from reaching an economic injury level where economic losses would exceed the cost of the artificial control measures. Typically, the main aim of IPM programmes is on agricultural insect pests (IPM Guidelines, 2009). Although, originally developed for agricultural pest management, IPM programmes are now developed to encompass diseases, weeds, and other pests that may interfere with the management objectives of sites such as residential and commercial structures, lawn and turf areas, and home and community gardens. IPM programs use current, comprehensive information on the life cycles of pests and their interaction with the environment. This information, in combination with available pest control methods, is used to manage pest damage by the most economical means, and with the least possible hazard to people, property, and the environment. The IPM approach can be applied to both agricultural and non-agricultural settings, such as the home, garden, and workplace. IPM takes advantage of all appropriate pest management options including, the judicious use of pesticides.

IPM – A philosophy:

1. A pest management strategy

Integrated Pest Management (IPM) is a philosophy that involves the management of a pest instead of controlling or eradicating a pest. It requires a greater knowledge of the pest, crop and the environment. Therefore, its strategy focuses on harnessing inherent strengths within ecosystems and directing the pest populations into acceptable bounds rather than toward eliminating them. This strategy avoids undesirable short term and long term ripple effects and will ensure a sustainable future (Lewis *et al*., 1997).

IPM programs should be operated with “pest management objectives” rather than “pesticide management objectives”. Integrated pest management is a comprehensive long term pest management program based on knowledge of an ecosystem that weighs economic, environmental, and social consequences of interventions (Flint and van den Bosch, 1981). The

foundation for pest management in agricultural systems should be an understanding and shoring up of the full composite of inherent plant defenses, plant mixtures, soil, natural enemies, and other components of the system. These natural ‘‘built in’’ regulators are linked in a web of feedback loops that are renewable and sustainable. The use of pesticides and other ‘‘treat-the-symptoms’’ approaches are unsustainable and should be the last option rather than the first line of defense. A pest management strategy should always start with the question ‘‘Why is the pest a pest?’’. It should also seek to address underlying weaknesses in ecosystems and/or agronomic practice(s) that have allowed organisms to reach pest status (Lewis *et al*., 1997).

2. An integrated process

Integration or compatibility among pest management tactics is central to Integrated Pest Management. Simply mixing different management tactics does not constitute IPM. Mixing the tactics arbitrarily may actually aggravate pest problems or produce other unintended effects. IPM recognizes there is no “cure-all” in pest control (dependence on any one pest management method will have undesirable effects). Reliance on a single tactic will favor pests that are resistant to that practice. In IPM, integrated control seeks to identify the best mix of chemical and biological controls for a given insect pest. The term "integrated" is thus synonymous with "compatibility."

3. Understanding pest biology and ecology

The determination of the correct cause of pest problem (understanding pest biology) and ecology is essential in manipulating the environment to the crop’s advantage and to the detriment of the pest.

4. Acceptable pest levels

IPM recognizes that eradication of a pest is seldom necessary or even desirable, and generally not possible. The primary objective in pest management is not to eliminate a pest organism but to bring it into acceptable bounds (Lawal *et al*., 1997). The emphasis is on control, not eradication. IPM holds that wiping out an entire pest population is often impossible, and the attempt can be expensive and environmentally unsafe. IPM programmes initial task is to establish acceptable pest levels, called action thresholds, and apply controls where the thresholds are crossed. These thresholds are pest and site specific, meaning that it may be acceptable at one site to have for instance a weed such as white clover, but at another site it may not be acceptable. By allowing a pest population to survive at a reasonable threshold, selection pressure is reduced. This stops the pest gaining resistance to chemicals produced by the plant or applied to the crops. If many of the pests are killed, then any that has resistance to the chemical will form the genetic

basis of the future, more resistant, population. By not killing all the pests there are some un-resistant pests left that will dilute any resistant genes that appear (Wikipedia, 2011).

5. IPM a continuum, not an end

Agriculture is a dynamic system that continually changes to changing crop production practices. IPM must continually change to meet pest management challenges. IPM is a continuum that will change with time. Every farmer practices some type of IPM, as long as they make progress to better its management. As new pest control techniques are discovered, the producer and crop advisor must adapt their pest control program to reflect these changes. What is considered a good IPM program today may be considered a chemical intensive program in a few years. Additionally, some good advice to the producer and crop advisor is to try the new changes on a limited scale, while becoming comfortable with the suggested practices before wide-scale changes are made.

IPM process:

IPM is applicable to all types of agriculture and sites such as residential and commercial structures, lawn and turf areas, and home and community gardens. The process includes:

1. Proper identification pest damage and responsible pests

Identification must be the first objective. When the identity of a pest is not known, then, a strategy built to control the pest cannot be transferred from one site to another, primarily, because the pest species or strain (biotype) might behave differently. Thus, a solid foundation must be built on systematic, taxonomy, etiology, and spatial distribution (Irwin, 1999). Cases of mistaken identity may result in ineffective actions. If plant damage is due to over-watering, it could be mistaken for fungal infection, since many fungal and viral infections arise under moist conditions. This could lead to spray costs, but the plant would be no better off.

2. Pest and host life cycles biology

Understanding crop growth and development is an underlying principle of IPM. We cannot just focus on the pest. The interactions between crop and pest (as well as the environment) are very important. To deplore an efficient IPM programme, literature and other data sources about the pest, the pest's life cycle, host range, distribution, movement, and basic biology will have to be researched. At the time you see a pest, it may be too late to do much about it except maybe spray with a pesticide (Metcalf and Luckmann, 1994). Often, there is another stage of the life cycle that is susceptible to preventative actions. For example, weeds

reproducing from last year's seed can be prevented with mulches and pre-emergent herbicide. Also, learning what a pest needs to survive allows you to remove these.

3. Monitor or sample the environment for pest populations

After the pest has been correctly identified, monitoring must begin before it becomes a problem. Sampling and monitoring methodologies must be designed and tested to provide the ability for assessing instantaneous and dynamic aspects of the pest's density, activity, or incidence (Irwin, 1999). Understanding how the environment affects pest and crop development is very important. Understanding interactions with the environment allows crop advisors to react to changing conditions. Environmental influences like drought stress influences pest management recommendations. When a crop is under stress it can be less capable of dealing with the stress caused by insects that extract plant sap (e.g. aphids, leafhoppers) and this stress may slightly lower the economic threshold. Weed populations which would not normally cause an economic loss may do so under drought conditions when they compete with the crop for limited water. The weather is notorious for affecting pest development and survival. Certain weather patterns may affect weed seed germination and explain why certain weeds are more abundant during wet fall or springs.

4. Establish action threshold (economic, health, and aesthetic)

The question here is: how many are too many or how much can be tolerated? In some cases, there are standardized numbers of pests that can be tolerated. Soybeans are quite tolerant of defoliation, so if there are a few caterpillars in the field and their population may not be increasing dramatically; thus, no urgent action may be necessary. Conversely, there is a point at which an action must be taken to control cost. For instance the farmer can control cost at the point when the cost of damage by the pest is more than the cost of control. This is an economic threshold. Tolerance of pests varies according to the health hazard (low tolerance) or merely a cosmetic damage (high tolerance in a non-commercial situation). Different sites may also have varying requirements based on specific areas. For instance, white clover may be perfectly acceptable on the sides of a tee box on a golf course, but unacceptable in the fairway where it could cause confusion in the field of play (Purdue University, 2006).

5. Choose an appropriate combination of management tactics

The word 'integrated' in IPM initially referred to the simultaneous use or integration of any number of tactics in combination, with focus on maintaining a single pest species below its economic injury level. Although, in theory a single strategy results from the simultaneous integration of several tactics, in practice, the integration actually occurs in a step-wise, time delayed fashion. Several of the tactics are compatible, but some are not. Certainly the tactics of

biological control, habitat manipulation, and legal control go alongside. The tactic of host resistance can stand alone or be combined with the other tactics just mentioned. Chemical control is generally compatible with host resistance. Thus, a management strategy integrates one or several compatible tactics into a single package (Irwin, 1999).

6. Evaluate and record results

Evaluation is often one of the most important steps in Integrated Pest Management (Bennett *et al*., 2005). It is the process of reviewing an IPM program and the results it has generated. Asking the following questions is useful: Did the steps one took effectively control the population? Was this method safe enough? Where there any expected side effects? What is the next step? Understanding the effectiveness of the IPM program allows the site manager to make modifications to the IPM plan prior to pests reaching the action threshold and requiring action again.

Pest management tactics:

There are different pest management tactics to suppress pests. They include host resistance, chemical, biological, cultural, mechanical, sanitary and mechanical controls. The primary pest management tactic involves maximization of built-in pest reduction features of an ecosystem. Molecular or genetic mechanisms are potentially manifested in a number of these more specific tactics. Each category, discussed below, employs a different set of mechanisms for suppressing populations.

1. Chemical control

The therapeutic approach of killing pest organisms with toxic chemicals has been the prevailing pest control strategy for over 50 years. Safety problems and ecological disruptions continue to ensue (Wright, 1996), and there are renewed appeals for effective, safe, and economically acceptable alternatives (Benbrook, 1996). Synthetic chemical pesticides are the most widely used method of pest control. The four major problems encountered with conventional pesticides are toxic residues, pest resistance, secondary pests, and pest resurgence (Lewis, 1997). The use of natural pesticides and organophosphates that are more environmentally friendly are encouraged and synthetic pesticides should only be used as a last resort or only used as required and often only at specific times in a pests life cycle.

2. Biological control

This involves the use of other living things that are enemies of a pest in order to control it. Sometimes, the term ‘‘biological control’’ has been used in a broad context to encompass a

full spectrum of biological organisms and biologically based products including pheromones, resistant plant varieties, and autocidal techniques such as sterile insects. IPM is mainly aimed at developing systems based on biological and non-chemical methods as much as possible.

3. Host plant resistance

This involves breeding varieties with desirable economic traits, but less attractive for pests, for egg laying and subsequent development of insect, disease or nematode. It also involves withstanding the infestation/infection or the reduction of pests to level that they are not large numbers during the plant growth period (Sharma, 2007).

4. Cultural measures

This involves practices that suppress pest problems by minimizing the conditions that favour their existence (water, shelter, food). Some of these factors are intrinsic to crop production while making the environment less favourable for survival, growth and reproduction of pest species. If followed in an appropriate manner, the cultural practices can provide significant relief from pests. The selection of appropriate site for the cultivation of field crops and fruit trees can reduce future infestation from insect pests. The culture should be selected in such a manner that it should be suitable for growing in the area and tolerant to important pests diseases of the area.

5. Mechanical control

This is the use of machinery and other tools to control pests. It involves agricultural practices like tillage, slash and burn, and hand weeding. The pruning of infested parts of fruits and forest trees and defoliation in certain crops help reduce the pest population. Chaffing of sorghum/maize stalks and burning of stubbles kills maize borer.

6. Sanitary control

Preventive practices are important part of an IPM programme. These include cleaning field equipment (i.e., tillage equipment, haying equipment, etc.), planting certified seeds and quarantine of infested crops or farmlands. These are methods used to prevent the introduction of a pest into the field.

7. Natural control

Natural control involves the enhancement of naturally occurring pest management methods to combat pests like using beneficial insects and diseases. Here, insecticides will only be used when they are economically feasible and it is apparent that natural enemies will not control the pests.

IPM: A multi-disciplinary approach:

IPM is a management intensive philosophy which stresses a multidisciplinary approach. Pests interact with each other, the crop, and the environment. Similarly, pest and crop management disciplines must work together to develop control recommendations that reflect these interactions. For example, management of the Soybean aphid includes entomologists who study the insect and their damage to soybean, agronomists that identify crop stage which are most vulnerable to soybean aphid damage, plant pathologists who study the viruses transmitted by aphid feeding, and soil scientists who study the aphid's interaction with nutrient deficiencies.

Benefits of an IPM programme:

The benefits of Integrated Pest Management are immense directly to farming and indirectly to society.

- Integrated Pest Management (IPM) protects environment through elimination of unnecessary pesticide applications. In IPM, pesticides are used at the smallest effective dose when other methods of pest control have failed. Also, they are used in bringing a pest organism to acceptable bounds with as little ecological disruption as possible.
- IPM improves profitability. Since IPM programme applies the most economical management pest tactics, profitability is ensured for the grower or farmer.
- It reduces risk of crop loss by a pest. Applying pest management and monitoring tactics will also ensure the reduction of crop loss or damage by pests.
- Long term sociological benefits of IPM would also emerge in areas of employment, public health, and well being of persons associated with agriculture.

Disadvantages of an IPM programme

In spite of the numerous benefits of IPM stated so far, there are also some drawbacks to it:

1. An IPM program requires a higher degree of management

Making the decision not to use pesticides on a routine or regular basis requires advanced planning and therefore, a higher degree of management. This planning includes attention to field histories to anticipate what the pest problems might be, selecting crop varieties which are resistant or tolerant to pest damage, choosing tillage systems that will suppress anticipated pest damage while giving the crop the greatest yield potential.

2. IPM can be more labor intensive

Consistent, timely and accurate field scouting takes time. However, it is this information that is necessary and is the corner stone of IPM programs. Without this information you cannot make intelligent management decision.

3. Success can be weather dependant

Weather can complicate IPM planning. For example you might want to lower herbicide rates and use row cultivation to manage weed pressure. However an extended wet period may reduce (or eliminate) the effectiveness of row cultivation. Therefore, good IPM planners will have a alternate plan for when these problems arise.

Integrated Pest Management (IPM) for dummies:

IPM is a holistic approach to combat herbivores, pathogens, and weeds using several methods, while minimizing applications of chemical pesticides. The concept is often illustrated as a pyramid, where various preventive and curative methods form the foundation and chemical control is used only when the economic injury level (EIL) has been reached.

The science of IPM is the systematic study of the compatibility and optimization of simultaneously implemented methods. Such optimization requires an evolutionary perspective which, to date, is lacking.

Commonly used methods that require evolutionary fine-tuning:

- Chemical control—only to be used as a last option
- Biological control—the use of living organisms to control pests
- Semiochemicals—including insect pheromones and kairomones
- Plant diversity—including intercropping and/or cultivar mixing
- Crop vaccination—including priming and induction of crop defenses
- Plant resistance—including antibiosis and antixenosis
- Plant tolerance—a plant's ability to endure enemy attack without yield loss
- Cultural control—including crop rotation, and watering regime

In addition to these pest controlling measures, IPM programs often include monitoring and forecasting of pest populations, as well as use of decision supporting tools to determine when chemical interventions are necessary. However, evolutionary based support tools that provide robust guidance for combining preventive actions have not yet been developed.

Where the base layers consist of prioritized preventive methods, and the top layer consists of more curative methods (normally chemical control), which is used as the last resort

when other combined actions cannot prevent pests from reaching economic injury levels (EIL; Barzman *et al*., 2015). An important feature of IPM is the *integration* of different methods and exploitation of their combined, rather than individual, effects (Stenberg, 2017). Several strategies may, on their own, retard pests' evolution of resistance to synthetic pesticides (Palumbi, 2001), for example by decreasing population size or rate of reproduction. In addition, the explicit approach of IPM to combine different control measures may generate fluctuating or balancing selection pressures that further retard evolution of resistance (Liu *et al*., 2014; Palumbi, 2001). Thus, IPM may in itself be considered a strategy that is more "evolutionarily smart" than applying a single control method that exerts strong directional selection on pests. However, any single control method may select for resistance in the pests, and there may be preventive and curative methods within IPM that would benefit from knowledge provided by evolutionary research to avoid unwanted evolutionary responses in the pests. Management of resistance to pesticides, and other control methods, should preferably also be explicitly included as a component of IPM. The idea of developing an evolutionary framework around IPM is, however, not just to delay resistance evolution in pests, but also to optimize each element as well as the synergies between the different parts in order to increase their efficiency. To confirm that IPM actually is a more evolution smart strategy, the management consequences must be evaluated in an evolutionary framework. As ecological interactions among species usually have shorter time spans than the genetic changes leading to adaptation, although not always, it is generally easier to observe and study current ecological interactions than their evolutionary outcomes. However, an evolutionary framework is crucial both to understand long term consequences and to manage evolutionary based problems such as resistance development. Here, we thus suggest how an evolutionary perspective could improve both the management and evaluation of control measures, enabling development of IPM as the sustainable and powerful tool required to counter agricultural pest herbivores, pathogens, and weeds.

The concept of Evolutionary Integrated Pest Management as presented in the current paper. Implementation of Evolutionary IPM is dependent on research in several domains to develop new approaches for pest management, integrating these methods and evaluating their pest control efficiency as well as evolutionary consequences (green layer). Implementation is also based on social and economic aspects (peach layer), such as a common understanding across disciplines and research funding for interdisciplinary research. Important when developing and implementing the pest management is to convey the significance of an evolutionary perspective to farmers and decision makers, as well as incorporating the economic aspects for farmers of the

pest management approach. Together, these aspects will facilitate the implementation of Evolutionary IPM (blue layer), which in turn could spur further research as well as an increased understanding in society of the importance of an evolutionary framework (the vertical arrow). Figure inspired by the Sustainable Development Goals "Wedding Cake" made by Azote Images for Stockholm Resilience Centre and presented by Rockström and Sukhdev at Stockholm EAT Food Forum, 2016.

As reported here, we have identified several domains where we believe that an evolutionary framework will play an important role in the development of new IPM strategies and predicting their consequences for pest load and yield. We also discuss the challenges and rewards of interdisciplinary research involving both evolutionary biologists and applied researchers, as well as the importance of transferring evolutionary knowledge to stakeholders and decision makers.

Important domains for evolutionary IPM

IPM technology	Targeted pest	Targeted beneficiaries
Pruning of fruit trees and use of bird net	fruit bat, a pest of economic importance on litchi, longan and mango	Fruit growers and general public
Inoculative releases of predators to control population of Tetranychus urticae	Mite, a pest of economic importance on solaneceous crops, roses and strawberry	Tomato, chilli, eggplant, rose and strawberry growers
Release of parasitoids (Encarsia formosa and Eretmocerus eremicus)	Whitefly, a pest of economic importance in a range of field and greenhouse crops	Growers of food crops and , ornamentals
Field Sanitation using field cages (augmentorium), protein bait and MAT block	Melon fly, Bactrocera cucurbitae, major pest in cucurbits	Around 70 % of food crop growers

References:

Integrated Pest Management (IMP) Principles. United States Environmental Protection Agency. 2012.

Resistance: The Facts - History and overview of resistance (PDF). IRAC. Retrieved 26 February 2020.

Acosta, EW (1995–2006): The History of Integrated Pest Management (IPM). Biocontrol Reference Center.

Benbrook, C. M. (1996): Pest Management at the Crossroads (Consumer's Union, Yonkers, NY).

Bennett, G.W., Owens, J.M., Corrigan, R.M. (2005): Truman's Scientific Guide to Pest Management Operations, 6th Edition, Purdue University, Questex, USA. pp. 10-12,

Charles Perrings; Mark Herbert Williamson; Silvana Dalmazzone (2000): The Economics of Biological Invasions. Edward Elgar Publishing. ISBN 978-1-84064-378-7.

Clercq, P.; Mason, P. G.; Babendreier, D. (2011): Benefits and risks of exotic biological control agents. Biocontrol. 56 (4): 681–698.

Flint, M. L. and van den Bosch, R. (1981): Introduction to Integrated Pest Management (Plenum, New York).

Irwin, M.E. (1999): Agricultural and Forest Meteorology 97: 235–248.

Knipling, EF (1972): Entomology and the Management of Man's Environment. Australian Journal of Entomology. 11: 153–167.

Lewis, W. J., van Lenteren, J. C., Sharad C. Phatak, and Tumlinson, J. H. (1997): Proc. Natl. Acad. Sci. USA 94: 12243–12248.

Paulitz, T. C., and R. R. Bélanger (2001): Biological control in greenhouse systems. Annu. Rev. Phytopathol. 39: 103–133.

Purdue University Turf Pest Management Correspondence Course, Introduction, 2006.

Sharma, B.K. (1997): Environmental Chemistry, Krishna Ltd., Delhi pp. soil-114.

Smith and Adkisson. 1997. The World Food Prize Foundation. Retrieved 15 April 2015.

Smith, R.F.; Smith, G.L. (1949): Supervised control of insects: Utilizes parasites and predators and makes chemical control more efficient (PDF). California Agriculture. 3 (5): 3–12. Archived from the original (PDF) on 2012-04-30.

Stenberg JA (2017): A Conceptual Framework for Integrated Pest Management. Trends in Plant Sci 22:759–769.

Wright, L. (1996): Silent sperm. (A reporter at large.) The New Yorker (Jan. 15): 42, 48, 50- 53, 55.

Wright, M. G.; Hoffmann, M. P.; Kuhar, T. P.; Gardner, J.; Pitcher, S. A. (2005): Evaluating risks of biological control introductions: A probabilistic risk-assessment approach. Biological Control. 35 (3): 338–347.

ECOSYSTEM SERVICES: CONCEPT AND VALUATION

Kuldeep Joshi* and Lalit Upadhyay

Sher e Kashmir University of Agriculture Sciences and Technology, Jammu

*Corresponding author E-mail: joshi.kuldeep2222@gmail.com

Abstract:

Ecosystem services (ES) are the conditions and processes through which natural ecosystems and the species that make them up, sustain and fulfil human life. ES are classified as provisioning services, regulating services, cultural services and supporting services. This includes food production, building materials, medicines, regulation of microclimate, disease prevention, and provision of productive soils and clean water resources, as well as landscape opportunities for recreational and spiritual benefit. ES are often neglected or even ignored by the economy, industry, and local habitants; even though most of them strongly depend on the flow of ES.The Principal techniques for monetary valuation of ES are;Market based production approach comprising of Production function (PF) and Replacement or Restoration cost (RC), Surrogate market with revealed preference such as Travel cost (TC) and Hedonic pricing (HP), Simulated market with stated preference following contingent valuation (CV) method. The challenges in valuating ES are due to their complex, non-linear nature, decisions concerning ecosystem management are socially contentious and fraught with uncertainty. Although judicious application of economic valuation techniques to ES can provide valuable information for conceptualizing decision choices and evaluating management options, there are serious limitations in the economic welfare approach to decision-making.

Introduction:

Ecosystem services are the benefits which humans get through the transformations of environmental resources (including land, water, vegetation and atmosphere) into a flow of essential goods and services including clean air, water, and food (Constanza *et al*., 1997). The term “Ecosystem Service” (ES) accounts for all the goods and services bestowed by nature and its manipulated ecosystems that sustain and support human well-being. Four major categories of ES are provisioning services, regulating services, Cultural services and Supporting services. ES consists of a combination of soil, animals, plants, water, air and other services which contribute to stabilize biodiversity andecology. Depletion of these elements resultsin the inability of an

ecosystem to provide services. ESare of significant value as they support our well-being,however, these services are neglected and undervalued by our society as most of them are intangible and are not marketable, and also their value is not easy to estimate (Daily *et al*., 1997). They are often ignored and not taken into consideration by the economists, industrials, and local habitants; even though most of them strongly depend on the flow of ES. Knowing the economic value of an ecosystem and its services is an important asset, because its major demand is the support of human well being, sustainability, and distributional fairness (Costanza and Farber, 2002). Over the past many decades, we have manipulated theecosystems rapidly and comprehensively due to the high demand for food, water, wood, fibre, and fuel. This conversion of the earth has contributed to significant net gains in economic development andhuman well-being. Economic valuation of ES is still a big challenge; there are several authors and projects which are dealing with classification, quantification, mapping and valuation of ecosystem services in order to integrate the concept into decision making.

Ecosystem functions:

The term "ecosystem functions" (EF) are describesas the internal functioning of an ecosystem (e.g. nutrient cycling and maintaining energy fluxes, nutrient recycling, food–web) De Groot (1992) defined an EF as "the capacity of natural processes and components to provide goods and services that satisfy human needs directly or indirectly". EF are grouped into four major categories, which are as follow.

i Regulation : Regulation of different ecological processes through biogeochemical cycles and other biosphere processes.

ii Production : Goods and services such as food, raw materials, energy resources and genetic material.

iii Habitat : Shelter and breeding habitat to living beings, thereby providing protection to biodiversity and evolutionary processes.

iv Information : Contributes to the maintenance of human health by providing opportunities for reflection, spiritual enrichment, cognitive development, recreation and aesthetic experience.

Valuation:

The idea of valuating ES was first introduce by King (1966) and Helliwell (1969). Diaz *et al*. (2006) defined the ecosystem services as, the benefits provided by ecosystems to humans, who contribute to making human life possible and worth living, It is the widely accepted definition. Earlier the economists were mainly concerned with the direct use values that produce

tangible benefits. However, now they are recognising the growing appreciation for the indirect use, non-use, existence, bequest and option values of an ecosystem and arenow developing techniques to extend monetary valuations to these ecosystem services (Tietenberg, 1992).

Meanings of the word 'value' (adapted from Gilipin, 2000)

Market value	It is the price of a commodity or service in the open market.
Intrinsic value	The value of substance that may have little or no market value, but have use value.
Intrinsic, non-use	The value linked to the environment and life forms for their own purpose.
Existence value	The value related to the knowledge that species, environment and other ecosystem services prevail, even if the individual does not consider ever making active use of them.
Bequest/vicarious values	A disposition to pay to preserve the environment for the benefit of others, intra- and inter generationally.
Present value	The value of a future assettoday, discounted to the present.
Option value	A wish to pay a certain sum today for the future use of that asset.
Quasi-option value	It is the gain in value of preserving options for future use,considering an expectation of increasing knowledge about the working of the natural environment.

Methods of Economic Valuation:

The major techniques for the monetary valuation of environmental goods and services are as follow.

Market	Basis of approach	Main techniques
Market based	Production approach	Production function (PF), Replacement or restoration cost (RC).
Surrogate market	Revealed preference	Travel cost (TC), Hedonic pricing (HP)
Simulated market	Stated preference	Contingent valuation (CV)

Principal techniques for monetary valuation (adapted fromChee, 2004)

i. Production function analysis (PF)
ii. Replacement/restoration cost technique (RC)
iii. Travel cost method (TC)

iv. Hedonic pricing (HP)
v. Contingent valuation (CV)

Production Function Analysis (PF):

The ES those are marketed / marketable such as drinking water, a fish harvest (Maler *et al*., 1994) or a timber production etc. It is based on the cause-effect relationships between the ES being valued and the output level of the marketed commodity. It targets mainly on production or cost data (Ellis and Fisher, 1987). Lack of data creates difficulty in understanding the cause-effect links between the ES and the marketed commodity (Spash, 2000). Ecosystems are dynamic and complex systems whose component variables often relate in nonlinear ways across a range of temporal and spatial scales. This method is dependent on the market demand of a service means that market plays an important role in determining the monetary value of an ES (Sagoff, 1998).

Replacement/Restoration Cost Technique (RC):

It is the cost to replace/restore an ESafter it has been damaged. The objective of replacing/restoring an ES to its pre-damaged state is to reinstate lost consumer surplus and non-use value (Garrod and Willis, 1999). Bockstael *et al*., (2000) briefed that from an economic point of view, the optimal level of restoration must be determined by the value of service benefits to society.RC is valid only if individuals are willing to pay for the natural services which are no longer available.

Travel Cost Method (TCM):

TCM evaluates individual preferences for non-market goods where consumption is commensurate with the costs of travel to acquire it (Garrod and Willis, 1999). TCM is applied to outdoor recreationalactivities such asforest safari. In TCM, information such as travel costs, permit fees, on-site expenses and capital expenditure on safari are calculated. Analysing such costs and predicting safari activity can then be used to derive surrogate demand functions for forest safari at a specific location. More frequent visitors are there for an ecosystemcloser to large human settlements and accountfor a huge aggregate monetary value for the site. Whereas, little or no value for a site having restricted access and far from human settlements. Furthermore, if visitors fail to recognise the importance or existence of a site's characteristic then this will be absent from the valuation.

Hedonic Pricing (HP):

The value an individual place or a service is based on the attributes it possesses (Garrod and Willis, 1999). The economic value of a characteristic of the service is derived from the market price of the service. Price per unit is regressed on the features of the service and the

implicit marginal value of a unit of the characteristic of interest is developed from the parameters of the regression (Sinden, 1994). In real estate markets HP estimates the value of environmental amenities of a site on land for housing.Environmental aspects determine the value of a particular land or house based on characteristics such as land condition, soil fertility, water rights, proximity to clean water, air, urban forests, recreational opportunities, peace and quiet etc. All this is expected to increase the prices of land in certain markets (Geoghegan, 2002).

Contingent Valuation (CV):

This is a 'stated preference' technique credited to Ciracy-Wantrup (1947). It is a hypothetical approach where people are interrogated through questionnaires and/or interviews for their demand function for a certain environmental good/service (Garrod and Willis, 1999). Portney (1994) described that CV is regarded with some reservation because it is not based on actual market behaviour. The CV is based on willingness to pay (WTP) (individual does not own the service) or willingness to accept (WTA) payments (individual owns the service).

This method is difficult with lack of technical conceptual problems. Bingham *et al*., (1995) studied the description and framing of what is to be valued is critical to the reliability of the method. Knowledge about ES, biased opinions and level of understanding in respondents determines the results of CV (Arrow *et al*., 1993). The structure and characteristics of the interrogated mass, their level of income and education determines the magnitude of bids. For example in 1989 in The Exxon Valdez oil spillcase (Gatto and De Leo, 2000) people of the USA were used as a reference group for calculating the damage to ecosystemdue to oil spill using CV methods. The compensations paid to people of Alska were US$5 billion for their losses. High compensations were paid due the high income of the US population. Whereas, if the same accident would have occurred in some under developed nation where salaries are low, then payout would have been veryless (Gatto and De Leo, 2000).

Challenges to Economic Valuation:

Economists lack knowledge regarding the understanding of the ecological production function and related queries in a deeper sense. Whereas, ecologists, on the other hand need to know the essence of trade-off, competing demand on the resources and conflicting choices over temporal and spatial scale.Valuation methods are affected by the individual preferences and theirutility.Traditional fundamentals of economic valuation becomes constrains when sustainability and social equity are included as goals with economic efficiency for ecosystem management (Costanza and Folke, 1997). Measuring and combining ecological knowledge with economics in a time interval is not easy as the ecological cycles are complex ranging from weeks to millennia.

Conclusion:

Ecosystem services should be valued in monetary terms to maintain sustainability in present as well as future and should be given priority during in policy formation by the legislatives. Awareness and programs like Payment for ESs, green credit, carbon credits etc should be made as well strict laws and penalties need be imposed on those involved in degrading ecosystem.

References:

Arrow, K., Solow, R., Portney, P., Leamer, E.E., Radner, R., Schuman, H., (1993): Report of the NOAA Panel on Contingent Valuation. Washington DC, USA.

Bingham, G., Bishop, R., Brody, M., Bromley, D., Clark, E., Cooper, W., Costanza, R., Hale, T., Hayden, G., Kellert, S., Norgaard, R., Norton, B., Payne, J., Russell, C., Suter, G., (1995): Issues in ecosystem valuation: improving information for decision making. Ecological Economics 14, 73–90.

Bockstael, N.E., Freeman, A.M., Kopp, R.J., Portney, P.R., Smith, V.K., (2000): On measuring economic values for nature. Environmental Science and Technology 34, 1384–1389.

Chee, Yuang En, (2004): An ecological perspective on the valuation of ecosystem services. Biological Conservation 120, 549–565.

Ciracy-Wantrup, S.V., (1947): Capital returns from soil conservation practices. Journal of Farm Economics 29, 1188–1190.

Costanza, R., d'Arge, deGroot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O.'Neill, R.V., Paruelo, J., Raskin, R.G., Sutton, P., vander Belt, M., (1997): The value of world's ecosystem services and natural capital. Nature 387, 253–260.

Costanza, R., Farber, S., (2002): Introduction to the special issue on the dynamics and value of ecosystem services: integrating economic and ecological perspectives. Ecol. Econ. 41, 367–373.

Costanza, R., Folke, C., 1997. Valuing ecosystem services with efficiency, fairness and sustainability as goals. In: Daily, G.C.(Ed.), Nature's Services: Societal Dependence on Natural Ecosystems.Island Press, Washington DC, pp. 49–68.

Daily, G.C., (1997): Introduction: what are ecosystem services. In: Daily, G.C. (Ed.), Nature's Services: Societal Dependence on Natural Ecosystems. Island Press, Washington DC, pp. 1–10.

De Groot, R.S., (1992): Functions of Nature: Evaluation of Nature in Environmental Planning, Management and Decision Making. Wolters-Noordhoff, Groningen.

Diaz, S.F.J., Chapin III, F.S., Tilman, D., (2006): Biodiversity loss threatens human wellbeing. PLoS Biol., 4.

Ellis, G.M., Fisher, A.C., (1987): Valuing the environment as input. Journal of Environmental Management 25, 149–156.

Garrod, G., Willis, K.G., (1999): Economic Valuation of the Environment. Edward Elgar Publishing Ltd., Cheltenham, UK.

Gatto, M., De Leo, G., (2000): Pricing biodiversity and ecosystem services: the never-ending story. BioScience 50, 347–355.

Geoghegan, J., (2002): The value of open spaces in residential land use. Land Use Policy 19, 91–98.

Gilpin, A., (2000): Environmental Economics: A Critical Overview. Wiley, Chichester, UK

Helliwell, D.R., (1969): Valuation of wildlife resources. Regional Stud. 3, 41–49.

King, R.T., (1966): Wildlife and man. New York Conservationist 20, 8–11.

MA, M.E.A., (2005): Ecosystems and Human Well-being: Current State and Trends. Island Press, Washington, p. 1.

Maler, K., Gren, I., Folke, C., (1994): Multiple use of environmental resources: a household production function approach to valuing natural capital. In: Jansson, A., Hammer, M., Folke, C., Costanza, R. (Eds.), Investing in Natural Capital. Island Press, Washington DC, pp. 234–249.

Portney, P., (1994): The contingent valuation debate: why should economists care?.Journal of Economic Perspectives 8, 3–17.

Sagoff, M., (1998): Aggregation and deliberation in valuing environmental public goods: a look beyond contingent pricing. Ecological Economics 24, 213–230.

Sinden, J.A., (1994): A review of environmental valuation in Australia. Review of Marketing and Agricultural Economics 62, 337–368.

Spash, C.L., (2000): The Concerted Action on Environmental Valuation in Europe (EVE): an introduction. Environmental Valuation in Europe (EVE), Cambridge Research for the Environment, UK.

Tietenberg, T., (1992): Environmental and Natural Resource Economics. Harper Collins Publishers, New York, USA.

PRELIMINARY STUDY OF PROXIMAL COMPOSITIONS IN WEED PLANT *INDIGOFERA*

Smita P. Gudadhe
Department of Botany,
Arvindbabu Deshmukh Mahavidyalaya, Bharsingi, Tal. Narkhed, Nagpur
Corresponding author E-mail: smitagudadhe@gmail.com

Abstract:

Indigofera is well known for its dye yielding properties. It is also observed that some species of *Indigofera* are medicinal as well as famine food plant. *Indigofera* is generally a wild weed which shows some medicinal properties due to its secondary metabolites mentioned in earlier literature but the present study deals with the mineral content of the two species of *Indigofera* namely *I. linifolia* (Linn.f.)Retz, *I. cordifolia* Heyne ex Roth from bharsingi regioin situated in Narkhed tahsil of Nagpur District. It is found that both species shows the presence of minerals in all parts of plants. It was observed that the percentage of dry matter was high in stems of *I. cordifolia* i.e. 47.7 % while high moisture content was in leaves of the same species. The seeds of both species show the high percentage of nitrogen content which supports the results of Ash content and Nitrogen percentage of earlier reports.

Keywords: *I. linifolia* (Linn.f.)Retz, *I. cordifolia* Heyne ex Roth, Mineral Content.

Introduction:

Plants have primary and secondary metabolites as well as mineral nutrients that play a very vital role in growth and developmental process of plants, they benefit the human life too. Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents which provides a measure of a total amount of minerals within a sample. Analytical techniques for providing information about the total mineral contents are based on the fact that the minerals (the “analyte”) can be distinguished from the all the other components (the “matrix”) within a sample in some measurable way. The most widely used methods are based on the fact that heating does not destroy minerals and that they have a low volatility compared to other sample components. The three main types of analytical procedure used to determine the ash content of samples are based on this principle, dry ashing,

wet ashing and low temperature plasma dry ashing. The method chosen for the particular analysis depends on the results of carrying out the analysis, the type of sample analyzed and the equipment available e.g. muffle furnace etc. Ashing may also be used on the first step in preparing samples for analysis of specific minerals, by some advanced or the various traditional methods. Therefore the "ash content" is a measure of the total amount of minerals present within a food, where as the "mineral content" is a measure of the amount of specific inorganic components present within a food, such as Ca, Na, K and Cl (https://people.umass.edu).

Minerals play a very important role in the plants. High mineral contents are some times and essential for the healthy nutrients, which come from the soil, are dissolved in water and absorbed through a plants roots. There are 13 mineral nutrients and are divisible into two groups i.e. macronutrients and micronutrients on the basis of their importance to the plants. Macronutrients or major nutrients can be broken into two main groups, primary and secondary nutrients. The primary nutrients are Nitrogen (N), Phosphorus (P) and Potassium (K) and secondary nutrients are calcium (Ca), Magnesium (Mn) and Sulphur (S). These mineral nutrients are sometimes spoken of as essential elements because some are needed in relatively large quantities and others in very small amounts, the former are referred to as "minor" or "trace" elements or as micronutrients. (http://agropedia.iitk.ac.in/content/plant-nutrients-and-its-role)

Phosphorus occurs in the form of phosphate compounds. This element, like nitrogen, is closely concerned with the vital growth processes in plants as it is a constituent of nucleic acid, and nuclei in which this occurs are essential parts of living cells. Hence, a deficiency of this element will also be expected to result in greatly restricted growth. Phosphorus is also of importance in seeds and in connection with the metabolism of fats, respiration, root development and the ripening of seeds and fruits (Wallace, 1943).

Calcium plays dominant role in the maintaining the strength of stems and stalks of plants. Calcium functions in plant cell elongation and division, structure and permeability of cell membrane, nitrogen metabolism and carbohydrate translocation. The viability of seeds is directly related to their calcium concentration (Dickinson *et al*., 2000).

Nitrogen is a major constituent of several of the most important substances, which occur in plants. It is of outstanding importance among the essential elements in nitrogen compounds comprise from 40-50% of the dry matter of protoplasm, the living substances of plant cells. For this reason nitrogen is required in relatively large quantities in relation with all growth processes in plants.

Indigofera L. is a dicotyledonous plant and is a member of Leguminoceae-Papillionaeae family of largely herbs, shrubs and trees with a great variety of habitat, which includes

hydrophytes, xerophytes and climbers distributed in temperate and tropical areas (Dallwitz *et al.*, 2000). The vernacular names generally are Nilini or Nil. *Indigofera* is a large genus consisting of over 800 species all over the world (Hutchinson, 1964). It is native to Southern United States through tropical and subtropical South America as well as the Caribbean Islands (Howard, 1988). Recently considering the taxonomic status of leguminales, *Indigofera* is placed in family Fabaceae because Fabaceae is the alternate name of Papillionaceae. According to ICBN, the term Fabales may also be used alternatively for Leguminales (Bhattacharya *et al*., 1998). The species of *Indigofera* are creeping, prostate or erect, they are annual, biannual herb or semi woody under shrub and trees (Dallwitz, 1980). They are distributed throughout the tropical and subtropical regions of the world with a few species reaching the temperate zone in eastern Asia.

Out of these 35 species, 11 Species are present in Amravati district (Dhore, 2002). They are having edible and medicinal value). Along with the primary metabolites and secondary metabolites, the Indian system of medicine also uses several minerals in various formulations (Ramawat, 2004) and the medicinal value of the plants depends on these biochemical constituents and minerals present in them. Now-a-days, Indigo is being cultivated in India, some parts of Bangladesh, Southeast Asia and Africa. Indirubin is a pink colored pigment is synthesized as a by-product of Indigo, which is useful in some therapeutic applications (Aobchey, 2007). The pigment (indigo) is present in the leaves and stems of number of *Indigofera* species (Siddiqui *et al*., 2007).

Various species of *Indigofera* are anthelmintic, anticancer, antiseptic, astringent, and antileukemic and antihypertensive due to this it has got more importance in folk medicines and Ayurveda (Miller *et al*, 1973).

The survey of literature on genus *Indigofera* has proved its valuable importance in medicine and food. It has nutritional, medicinal value and forage is used as crops. Genus *Indigofera* has a long history in trade because of one of its species, *Indigofera tinctoria* L. yield a dye ‘indigo’. In Latin “Indigo” means colour and ‘fera’ means to bear (Dixit, 1977).

Ash values were determined with a purpose to find out the total amount and inorganic solutes present in the plant material (Kadam *et.al.* 2014). Therefore, the genus *Indigofera* with large number of species finds a valuable place in Ayurvedic medicine. There are still hidden treasures to be discovered in most of the species. Very few have been worked out in all aspects. Hence, it is proposed to undertake analysis of mineral constituents only in those species of *Indigofera,* which are not worked out and which will enhance the present knowledge and surely speed up researches in other fields.

Material and Methods:

Collection of Experimental Material

For the mineral analysis two different species of *Indigofera* were selected i.e. *I. linifolia* (Linn.f.) Retz and *I. cordifolia* Heyne ex Roth, were collected from bharsingi regioin situated in Narkhed tahsil of Nagpur District. The plants were collected during September to November and were brought to the laboratory and Herbarium specimens were also prepared.

After the morphological confirmation of both plant species was subjected for Mineral estimation by Ash analysis, quantitative analysis of dry matter and moisture content and estimation of Nitrogen by Kjeldahl method (Oser, 1976; AOAC, 1984; Bazarbarua, 2000; Ahmad, 2005; Sadashivam and Manickam, 2005).

Mineral estimation

Ash analysis

Preparation of sample: Ash analysis was done for stem, leaves, seeds and roots. 5 gm of dried plant parts was taken for preparation of ash and kept in Muffle in Furnace at 400˚C. for 12 hours. Acid soluble ash fraction was analyzed qualitatively for the presence of Ca, P, Mg and S elements. About 0.5 –1gm of ash was dissolved in 10 ml of warm 20 % (v/v) HCL in distilled water and volume was made up to 50 ml. The filtrate was used for following test.

a) Sulphur (S): To the filtrate (about 10 ml) few drops of 5% barium chloride solution was added. Formation of white very fine crystalline precipitate of barium sulphate proves the presence of sulphur.

b) Calcium (Ca): 20 ml of filtrate was taken and made slight alkaline with few drops of dilute ammonium hydroxide (water: ammonium hydroxide, 1**:**1), and filtered few drops of saturated ammonium oxalate solution were added. A white precipitate of calcium oxalate proves the presence of calcium.

c) Magnesium (Mg): Excess of ammonium oxalate solution was added to precipitate calcium of test solution and filtered. Filtrate was then evaporated to a volume of about 5 ml. To the hot filtrate 1 ml of saturated disodium hydrogen phosphate was added, cooled and allowed to stand crystals of ammonium magnesium phosphate $(NH_4)_3MgPO_4$ showed the presence of magnesium. Rubbing the inside of test tube with a glass rod can fasten precipitation.

d) Phosphorus (P): To 10 ml of filtrate ammonium molybdate solution was added. Heated for few minutes on steam bath and cooled. A profuse yellow crystalline precipitate of ammonium phosphomolybdate $(NH_4)_3Po_4\ (MoO_3)_{12}$ shows the presence of phosphorus.

Quantitative analysis

i) Determination of dry matter and moisture content: The weight of the clean and empty dishes (container) was taken firstly and then about 30 gm of sample of leaves and stems was taken in weighing dishes. The dishes with samples were placed in oven at 105^0C overnight for drying and loss of moisture. After that the dishes with dried samples kept for cooling and transfer in desiccators. The weight of the dishes with dried leaves and stems was taken immediately after removing from desiccators.

Calculations

For Dry matter

$$\text{Dry matter (\%)} = \frac{\text{(Wt of dish + Wt of dried sample) - (Wt of dish)}}{\text{Wt of sample before drying}} \times 100$$

$$= \frac{\text{Wt of dry sample} \times 100}{\text{Wt of sample before drying}}$$

For Moisture content

$$\text{Moisture content} = \frac{\text{Wt of fresh sample - Wt of dry sample}}{\text{Wt of Fresh sample}} \times 100$$

ii) Determination of Ash

Procedure: The clean crucible was kept in muffle furnace at 600^0C for 1 hr and after cooling at room temperature kept in desiccators. The weight was taken immediately after removing from the desiccators to prevent from the absorption of moisture. Sam procedure was followed with the ash content of the sample.

Calculations

$$\text{Ash percentage (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \text{ X } 100$$

iii) Estimation of Nitrogen by Kjeldahl method

Procedure: In this procedure first total nitrogen in the plant sample is determined. 300 mg of

sample powder i.e. leaves, stems, seeds are taken in different Kjeldahl's flask with 7.5 ml concentrated H_2SO_4 with a pinch of catalyst. The flasks are kept for 24 hrs at room temperature. After 24 hrs the whole mass is digested on gas burner for about 4 to 5 hrs till the solution is diluted up to 50 ml by adding distilled water. 5 ml of this solution is taken for the estimation of nitrogen in distillation set. In conical flask 5ml of 2% boric acid solution is taken. Ammonia released from the sample reacts with boric to form ammonium borrate. This is titrated against 0.35 N HCL. This titration reading gives % of Nitrogen present in plant sample.

Observations and Result:

Table 1: Qualitative analysis of Minerals

Sr. No.	Minerals	*I. linifolia*				*I. cordifolia*			
		Stem	**Leaves**	**Seeds**	**Roots**	**Stem**	**Leaves**	**Seeds**	**Roots**
1	Sulphur	+	+	+	+	+	+	+	+
2	Calcium	+	+	+	+	+	+	+	+
3	Magnesium	+	+	+	+	+	+	+	+
4	Phosphorus	+	+	+	+	+	+	+	+

Table 2: Determination of Dry matter

Sr. No.	Species	Plant Parts	Fresh weight (gm)	Dry weight (gm)	Dry content (%)
1	*I. linifolia Retz.*	Stem	30	13.2	44
		Leaves	30	11.7	39
2	*I. cordifolia Hyene ex Roth.*	Stem	30	14.3	47.7
		Leaves	30	10.1	33.7

Table 3: Determination of Moisture content

Sr. No.	Species	Plant parts	Weight of dish (gm)	Fresh weight (gm)	Dry weight (gm)	Moisture content (%)
1	*I. linifolia Retz.*	Stem	27.0	30	13.2	56
		Leaves	27.0	30	11.7	61
2	*I. cordifolia Hyene ex Roth.*	Stem	27.0	30	14.3	52.3
		Leaves	27.0	30	10.1	66.3

Table 4: Ash content in *Indigofera* species

Sr. No.	Species	Ash Percentage (%)		
		Stem	**Leaves**	**Seeds**
1	*I .linifolia* Retz.	6.8	5.4	5.8
2	*I. cordifolia* Heyne ex Roth	7.4	4.2	6.6

Table 5: Nitrogen content in *Indigofera* species

Sr. No.	Plant Species	Plant parts	Nitrogen content (%)
1	*I .linifolia* Retz.	Stem	3.8
		Leaves	2.6
		Seeds	5.2
2	*I. cordifolia* Heyne ex Roth	Stem	3.7
		Leaves	4.3
		Seeds	4.9

Qualitative analysis of Minerals

In the qualitative analysis of minerals *Indigofera linifolia* (Linn.f.) Retz and *I. cordifolia* Heyne ex Roth. shows the presence of minerals likes Sulphur (S), Calcium (Ca),Magnesium (Mg) Phosphorus (P) and Nitrogen (N) in the stem, leaves and seed of these species. **(Table:-1)**

Determination of Dry matter

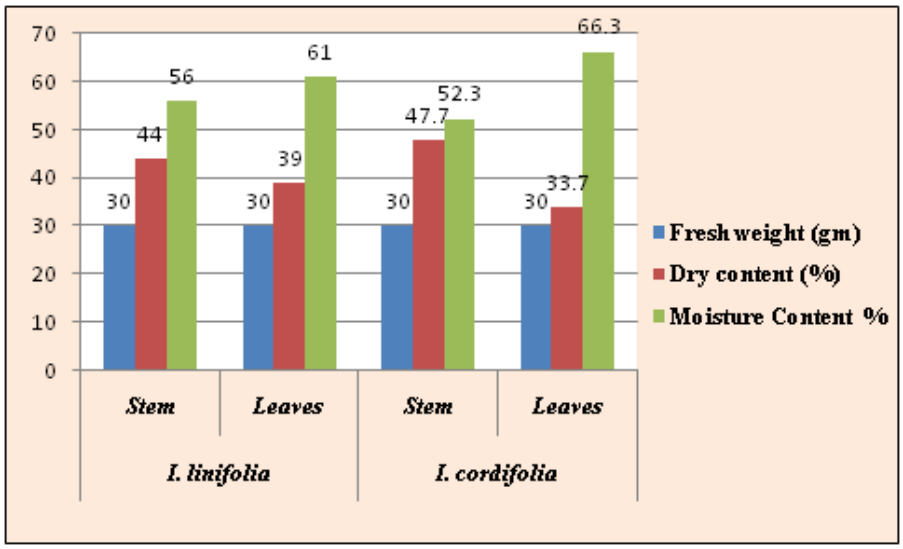
In the quantitative analysis of *Indigofera linifolia* (Linn.f.) Retz it is observed that 30 mg fresh sample of stem shows 13.2 gm dry weight with 44% dry content and leaves with 11.7 mg dry weight with 39 % dry content. In case of *I. cordifolia* Heyne ex Roth. 30 mg fresh sample of stem shows 14.3 mg dry matter with 47.7 % of dry content and leaves shows 10.1 mg dry matter with 33.7% dry content in leaves. (Table:-2; Graph-1)

Determination of Moisture content

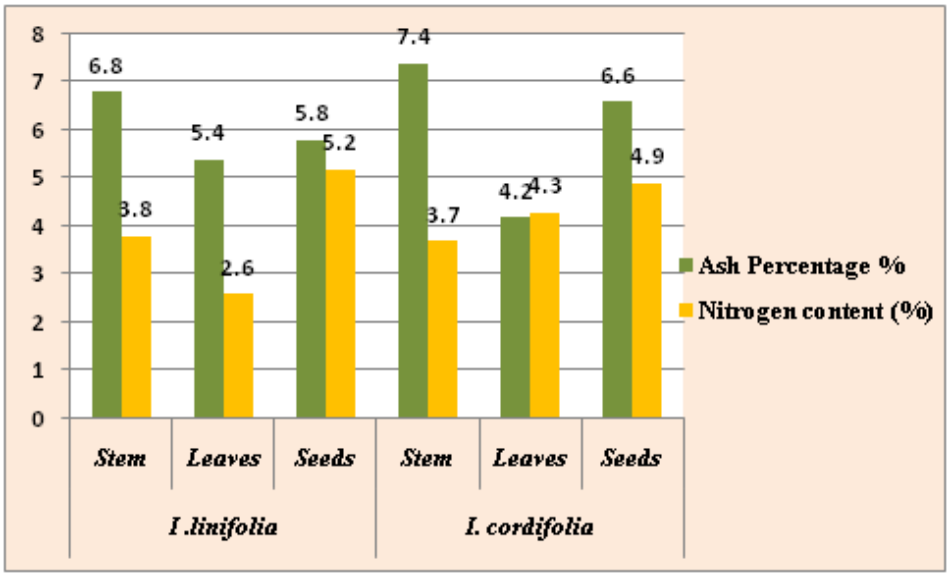
In *Indigofera linifolia* (Linn.f.) Retz. stems shows 56% moisture content where as in leaves it shows 61%. But in case of leaves of *Indigofera cordifolia* Heyne ex Roth.shows 66.3% and stems with 52.3% moisture content. (Table:-3; Graph-1)

Ash Content in *Indigofera* species

The highest percentage of ash is found in stem of *Indigofera linifolia* (Linn.f.)Retz. i.e. 6.8% and lowest in leaves i.e. 5.4% and seeds with 5.8% ash content. In *Indigofera cordifolia* Heyne ex Roth. the highest percentage of ash is observed in stems i.e. 7.4% and 4.2% in leaves and 6.6% in seeds. (Table:-4; Graph-2)



Graph 1: Dry matter and Moisture Content in *I.linifolia and I. cordifolia*



Graph 2: Ash Content and Nitrogen Content in *I.linifolia and I. cordifolia*

Nitrogen content in *Indigofera* species

Nitrogen content in plants depends upon the nitrogen present in the soil. It is observed that each part of the plant species showed different amount of nitrogen content which is comparatively high. In both species of *Indigofera* the percentage of nitrogen in stems, leaves and seeds ranges from 2.6% to 5.2%. In *Indigofera linifolia* (Linn.f.)Retz. the seeds have high percentage of nitrogen i.e. 5.2% than stems (3.8%) and leaves (2.4). Similarly in *Indigofera cordifolia* Heyne ex Roth the seeds show high Nitrogen content i.e. 4.9% than stems (3.7%) and leaves (4.3%). Hence it is observed that species of *Indigofera* are good nitrogen fixer. (Table:-5; Graph-2)

Discussion:

Plants play an important role in health related issues as a medicine due to its medicinal properties. Plants have curative properties due to the presence of various complex chemical substances of different composition. The dry matter and moisture content was evaluated in the two species of *Indigofera* i.e. *I. cordifolia* Heyne ex Roth. and *I. linifolia* (Linn.f.)Retz. There were no significant differences between the two species.

Qualitative or quantitative determination of mineral elements present in plants is important because the concentration and type of minerals present must often be stipulated on the label of a food. The quality of many foods depends on the concentration and type of minerals what they contains, also play a very significant role against a variety of degenerative diseases and processes, they may also prevent and reduce injury from environmental pollutants and enhance the ability to work and learn, some minerals are essential to a healthy diet (e.g. Calcium, Phosphorus, Potassium and Sodium) where as some can be toxic (e.g. Lead, Mercury, Cadmium and Aluminium). The use of mineral element is found to have been developed and used widely to cure several health problems. The amount and composition of ash remaining after combustion of plant material varies considerably according to the part of the plant, age, treatment etc. The constituents of the ash also vary with time and from organ to organ. Ash usually represents the inorganic part of the plant (Tambe *et al*, 2012).

The ash content is a measure of the total amount of minerals present within a plant body; whereas mineral content is the measure of the amount of specific inorganic components present within a food such as Ca, Na, K and Cl. High mineral content retard the growth of certain microorganisms i.e. microbiological stability. Ash is the inorganic residue remaining after the water and organic matter have been removed by heating. This was done at high temperature using Muffle furnace (https://people.umass.edul). Ash was obtained from different plant parts. The Ash% was highest in *I. linifolia* (Linn.f.)Retz. and *I. cardifolia* Heyne ex Roth.. Ash % was found to be more in stems than other parts in both species (Table:-4; Graph-2). High ash content indicates that species of *Indigofera* are good source of inorganic minerals like Sulphur, Calcium, Magnesium Phosphorus and Nitrogen which are nutritionally important were found in reasonable amount in both species of *Indigofera* in all plant parts. High concentrations of these minerals could be of advantage. They have vital role to play in plant growth and development. The amount and composition of ash remaining after combustion of plant material varies considerably according to the part of the plant, age, cultural treatment etc. Thus, in a young leaf the ash may constitute approximately 5 per cent of the dry weight while in the mature leaf it may be 15 per cent. The ash content of the wood (of the order of 5 per cent) is usually much lower than that ofthe bark (up to 20 per cent) (Humphries, 1956).

It is observed from the earlier report the proximate analysis shows that the various parts of *Indigofera cordifolia* does not show great variation in moisture content. Therefore no microbes can attack this plant easily. The high ash content i.e. 3.4% and 3.2% indicates that the *Indigofera cordifolia* leaves and stem are good source of inorganic minerals. The percentage of dry matter content is more in leaves i.e. 30.33%. The seeds of *Indigofera cordifolia* are good source of nitrogen and that is approximately 3.85% of nitrogen, which is the higher concentration (Gudadhe *et al*, 2011). The moisture content was found to be higher in leaves of *Indigofera linifolia* collected from Amravati region of Maharashtra i.e. 57.5% than seeds and stem i.e. 27.2% and 52.9% respectively. The high ash content was reported in the same species i.e. 3.8% and 3.4% indicates that the *Indigofera linifolia* leaves and stems are good source of inorganic mineral (Gudadhe *et al*, 2011). The distribution of nitrogen in various parts of the plant was studied (Table:-5; Graph-2). According to the previous report on *Indigofera linifolia* and *I. cordifolia* collected from Amravati region of Maharashtra and present study it is observed that there is some differences occur in the dry matter and moisture content along with Ash and Nitrogen content. Therefore it may be concluded that some factors such as environmental, climatic as well as age factor of plants are responsible for the change in the percentage of Mineral constituents. The percentage of nitrogen was estimated by Micro-Kjheldal method and was found to be highest in seeds of all species of *Indigofera*, than leaves and stem hence they are good nitrogen fixers.

References:

Ahmad, S.A. (2003): Taxonomic and Pharmacognostic Studies of Some Local Unani Medicinal Plants. Ph.D. Thesis, Sant Gadge Baba Amravati University, Amravati.

Aobchey, P.; S. Supachock.; S. Phutrakul and S. T. Chen (2007): Simple Purification of Indirubin from Indiogofera tinctoria Linn. and Inhibitory Effect on Mcf – 7. Human Breast Cancer Cells. Ching Mai J. Sci Thailand 34(3): 329-337

Bazarbarua A. (2000): A textbook of Practical Plant Chemistry. S. Chand and Company Ltd. New Delhi.

Bhattacharyya, B.B., M. Johri (1998): Flowering Plants Taxonomy and Phylogeny, Narosa Publishing House, New Delhi.

Dallwitz, M. J., (1980): A general system for coding taxonomic description. Taxon, **29**: 41-164

Dallwitz, M. J.and C. B. Watson (2000): A general system for coding taxonomic description, Taxon, **29**: 41-164

Dhore, M.A.(2002): Flora of Amravati District With Special Reference to the Distribution of Tree Species, Ph.D Thesis, (1986): Pub.Amravati University, Amravati.

Dickinson, K. (2000): Metalosate@ Plant Nutrition News, Albion, **1 (3):**

Gudadhe, S. P. and Dhoran, V. S. and Nathar, V. N. **(2011**): Phytochemical Investigations on Indigofera cordifolia Hyene Ex Roth. Journal of Indian Botanical Society 90 (1&2):97-102.

Gudadhe, S. P. and Dhoran, V. S. and Nathar, V. N. **(2011):** Studies of Bioactive Constituents in Indigofera linifolia Retz. International Journal of Biochemical Sciences. 1(3): 30-35.

Howard, R. A. (1988): Flora of the Lesser Antilles, Leeward and Windward Islands. Dicotyledneous, Part 1. 4 Arnold Arboretum, MA: 673

Humphries E. C. (1956): Mineral Components and Ash Analysis. Modern Methods of Plant Analysis @Springer-Verlag Berlin Heidelberg pp: 468-502.

Hutchinson, J. (1964): The genera of flowering plants (Angiospermae):, Oxford University Press, Amen House, London, 1:400

Kadam V.B., Salve Sunanda B. and Wadikar M.S. (2014): Biochemical evaluation of ash value of some medicinal plants of genus Terminalia (Combretaceae): of Marathwada region in Maharashtra. Int. J. of Medicine and Pharmaceutical Research, 2(5): 776 – 780.

Miller, R. W. and C. R. Smith (1973): Seeds of Indigofera species: Their Contents of Amino Acids That May Be Deliterious J. Agri. Food chem., **21(5):**909-911.

Oser, B. L. (1976): Hawks Physiological Chemistry. 14th Ed. Tata Mc Graw Hill Publishing Co. Ltd. New York.

Ramawat, K. G. (2004): Biotechnology of Medicinal Plants Vitalizer and Therapactic. New Delhi.

Sadashivam S. and A. Manickam (2005): Biochemical Methods,IInd Edn, New Age International (P): Limited Publishers, New Delhi.

Siddiqui, A. T. S.U.A.; A. K. M. M. Hossain; M. M. Rahman and S. M. Rahman; S. M. Sayem (2007): Effect of phosphorus on growth and nodule formation of Indigo plant under old Himalayan Piedmont field condition. Int. J. Sustain. Crop Prod. 2(6): 21-24

Tambe S.S., Deore Shailaja, Fatima Sumia, Kadam V.B (2012): Determination of Ash Values of Some Medicinal Plants Of Marathwada Region In Maharashtra International Journal Of Pharmaceutical Research And Bio-Science.Vol-1 (3):337-346

Wallace T, (1943): The Diagnosis of Mineral Deficiencies in Plants by Visual Symptoms. Published by Majesty's stationary office, Bristol.

https://people.umass.edu/~mcclemen/581Ash&Minerals.html.

http://agropedia.iitk.ac.in/content/plant-nutrients-and-its-role

EFFECT OF SOIL CARBON SEQUESTRATION ON CLIMATE CHANGE MITIGATION

Sumana Balo

Department of Soil Science and Agricultural Chemistry,

Uttar Banga Krishi Viswavidyalaya, Pundibari,

Cooch Behar, West Bengal 736 165, India

Corresponding author E-mail: balosumanaagri@gmail.com

Abstract:

After the post green revolution era, continuous application of agrochemicals, and changes in land use pattern, cause although high production magnificantly but simultaneously creates deterioration of soil quality and standard atmospheric condition in a great extend. Emission of GHGs from agricultural land accounts for 28%, particularly by soils 12%. These increases CO_2 concentration in atmosphere from about 280 to more than 380 parts per million (ppm) over the last 250 years—is causing measurable global warming which has a significant impact on agriculture production in upcoming days. These losses of carbon in the form of CO_2 mainly from soil profile create a great concern on sequestration of carbon to combactalarming impacts of global warming. Various results show that a substantial amount of carbon can be accumulated by soil in the form of SOC through adaptation of Resources land management's practices including conversation agriculture techniques which has a pronounced effect on soil carbon sequestration process. Crop diversification can be an important component which can influence soil carbon pool by incorporation of different organic inputs. Stabilisation of SOC by means of physical, chemical, and biological means or their combinations enhances in storage of carbon leading towards improvement of soil physical and chemical structure which results in higher amount of plant available nutrients. So, healthy soils have a potential to produce healthy foods in one ways besides also contribute to accumulation of carbon as source of carbon sink from atmosphere that involves in achieving sustainable environment in near future.

Keywords: Soil organic carbon, Carbon Sequestration, GHGs, Climate change

Introduction:

'Carbon sequestration' is one of the most important concepts in studies of climate change (Krna and Rapson, 2013). Since CO_2 accounts for about 60% of greenhouse gas (GHG) emissions; reducing the net increase in atmospheric CO_2 concentration by 'C sequestration' can

be an effective mitigation strategy for climate change and for modulating anthropogenic emission of the global scale C cycle. Krna and Rapson (2013) defined 'endogenous C sequestration' as non-temporarily utilized biologic C fixed from the atmosphere is greater than the release of C to the atmosphere over a specified time period within a given system. It is a major concern that how long C must be sequestered in a system (i.e. land, soil) to usefully contribute to climate change mitigation (Mackey *et al.,* 2013). Smith *et al.* (2005) suggest that the "achievable potential" of GHG mitigation may be about 10–20% of the technical potential. The agriculture sector emitted 371.7 million tons of CO_2 eq. of which 13.84 million tons is CH_4 and 0.227 million tons is N_2O. Enteric fermentation constituted 61% of the total CO_2 eq. emissions from this sector and 20% of the emissions were from rice cultivation. Agricultural soils emitted 16% of the total CO_2 eq. emission from agriculture (INCCA, 2010). The remaining 3% of the emissions are attributed to livestock manure management and burning of crop residues in field.However, the term C sequestration has become very customary to imply a contribution to climate change mitigation. For this reason, C sequestration must slow or even reverse the increase in atmospheric concentration of CO_2. Thus, movement of C from one reservoir in the ecosystem to another is primarily termed accumulation, whereas an additional transfer of C from the atmosphere into a reservoir should be termed sequestration because of a genuine contribution to climate change mitigation (Powlson *et al.,* 2011).

Carbon (C) sequestration in soil refers to capture and storage of atmospheric CO_2 with pedosphere in a manner that also increases its mean residence time (MRT) and minimizes sinks of re-emission (Lal, 2007). Numerous objectives of soil C sequestration are:(i) off-setting anthropogenic emissions by fossil fuel combustion, cement production and deforestation, (ii) reducing net increase in atmospheric concentration of CO_2 (which reached 400 ppmv in 2013) and pool (800 PgC), (iii) improving soil organic C (SOC) concentration (and pool) to above the threshold level of 1.5–2.0%, (iv) restoring soil quality and its ecosystem functions and services, (v) improving water and nutrient retention capacity, (vi) enhancing use efficiency of inputs in soils of managed ecosystems, (vii) reducing risks of accelerated erosion and non-point source pollution (NPSP), (viii) creating climate-smart soils and agro-ecosystems, (ix) improving use efficiency of inputs, and strengthening soil's disease-suppressive characteristics, and (x) increasing and sustaining agronomic productivity, and advancing food and nutritional security. Because of having numerous co-benefits, there is a strong interest in the definition, concepts, experimental approaches, procedures of laboratory analyses, and methods of determining SOC sequestration rates which isdefined as, process of transferring CO_2 from the atmosphere into the soil of a land through plants itself, plant residues and other organic solids, which are stored and

retained in the unit as part of the soil organic matter (humus). Retention time of sequestered carbon in soil (terrestrial pool of carbon cycle) can range from short-term (immediately released back to the atmosphere) to long-term (prolong period) storage. Olson (2014) observed that the sequestered SOC processes should increasethe new SOC storage during and at the end of a study to above the previous pre-treatment baseline. Minister of Agriculture of France, Mr Stephane Le Foll, is proposing to UNFCCC-COP21 in Paris in December 2015 SOC sequestration at the rate of '4 per 1000' to offset anthropogenic emissions. It has been estimated that about 90% of the global technical mitigation potential in agriculture approximately 5,500–6,000 Mt CO2-eq. per year stems from mitigation in soils (Smith *et al.,* 2008). Mitigation measures that have been proposed including zero or reduced tillage, set-aside land, application of animal manure, extensification, that results in a shallower water table and control of grazing populations (e.g., Freibauer *et al.,* 2004; Singh and Lal 2005; Smith *et al.,* 2005) and may be concluded under the term 'soil carbon sequestration'. All mitigation measures which would be adoptable can be utilised depending upon the influence of institutional, educational, social, economic and political constraints, which may hamper agriculturally based efforts to mitigate CO_2 emissions. The biologically-mediated uptake and conversion of CO_2 to inert, long-lived, C-containing materials is popularly known as 'biosequestration' (U.S. Department of Energy, 2008). Biosequestration temporarily removes C from active cycling. Thus, 'C sequestration' can be defined as the uptake of C-containing substances and, in particular, CO_2 into another reservoir with a longer residence time (IPCC, 2007).

Sources of increase in atmospheric concentration of gases

The increase in atmospheric concentration of CO_2 by combustion of fossilfuel, however, occurred at the rate of 3.2 ± 0.1 Pg C/year, the absorption by the ocean was 2.3 ±0.8 Pg C/year and the uptake by an unknown terrestrial sink was 2.3±1.3Pg C/year (Prentice, 2001); Among the total 5 pools of carbon cycle the total soil C pool is four times the biotic (trees, etc.) pool and about three times the atmospheric pool. Land use change; decomposition of vegetation and mineralization/oxidation of humus or SOC are two components of estimated emissions of 136 ±55 Pg C. Jenny (1980) observed that among the causes which are responsible for CO_2 enrichment, highest ranks are accorded to the continuing burning of fossil fuels and the cutting of forests. The contributions of soil organic matter appear underestimated. The historic SOC loss has been estimated at 40 Pg by Houghton (1999) from 20^{th} century. SOC depletion can be restored. Further, improvements in quality and quantity of the SOC pool can increase agronomic production, enhance water quality, reduce sedimentation of reservoirs and waterways, and mitigate risks of global warming.

Impact of potential climate change on soil organic matter and soil quality

Change in Climate may affect soil moisture and temperature regimes of different ecosystem. It is predicted that a 3^0C increase in atmosphere temperature would cause an average altitudinal shiftof the vegetation belts of 500 m (Bottner *et al.,* 1995). Theoretically, an average rise in mean annual temperature of 1^0C is equivalent of an approximate poleward shift of vegetation zones by200 km (Ozenda and Borel, 1990). At the ecosystem level, the soil could affect vegetation through its influence on water availability, elemental cycling and soil temperature regime (Cheddadi *et al.,* 2001). Increase in soil temp exacerbates the rate of mineralization leading to a decrease in the SOC pool. Such decline in SOC increases the susceptibility of soil compaction, erosion, crusting and runoff.

Major sources of soil carbon reservoir

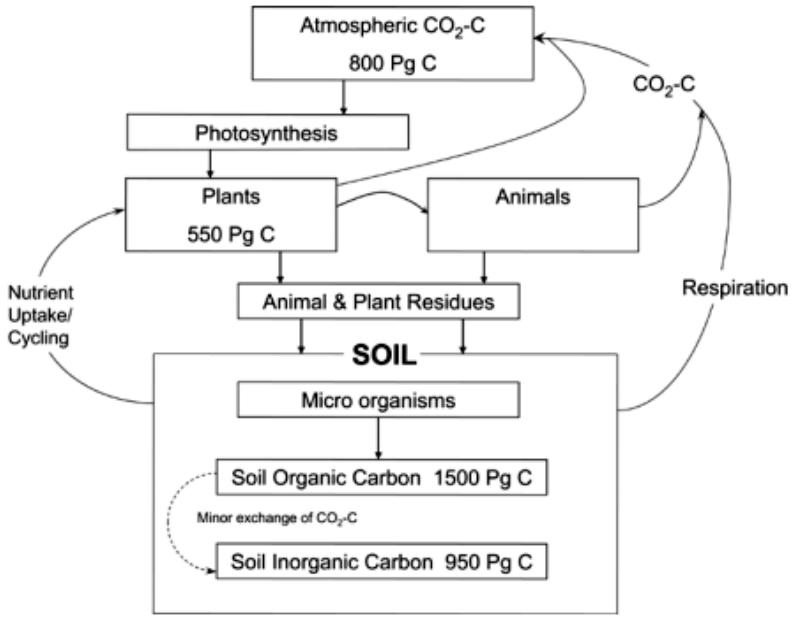


Figure 1: The terrestrial carbon cycle

Inputs of carbon (C) into the soil organic C (SOC) pool originate from the fixation of atmospheric CO_2-C through photosynthesis by plants into simple sugars, and subsequently into the more complex materials (i.e., cellulose and lignin), eventually deposited in their leaves, stems, and roots. Plant material and its organic C can be consumed by animals or become humified into soil organic matter (SOM), which contains SOC, through the action of microorganisms. Carbon storage as SOC is controlled by the soil environment and the quality of the organic matter in which the carbon resides. Decomposition is the biological conversion of organic matter into more oxidized constituents, including CO_2, which is released back to the atmosphere. Decomposition rates are affected by soil structure and by soil temperature and moisture conditions (Morgon *et al.,* 2010)

1. Soil Organic Matter (SOM)

Among the major sources for carbon sequestration, Soil organic carbon has a emmence practical importance, as most of the soil-sequestered carbon is basically stored as soil organic matter in soil system. The mass of soil organic C in the upper 1 m of soil is about 1200–1600 Pg C (Batjes and Sombroek 1997). On average, the soil contains about 2.5 times more organic C than the vegetation (650 Pg C) and about twice as much C as is present in the atmosphere (750 Pg C) (Baties, 1998). Major environmental factors which control the behaviour of organic matter in soil are moisture status, soil temperature, O_2 supply (drainage), soil acidity, soil nutrient supply, clay content and mineralogy etc. Niggli *et al.* (2009) calculated the sequestration potential of organic croplands to be 0.9–2.4 Gt CO_2 per year (which is equivalent to an average sequestration potential of about 0.2–0.4 t C per hectare and year for all croplands), which represents 15–47% of total annual agricultural GHG emissions.Besides this, Soil organic matter has positive effects on the water-capturing capacity of the soil. A higher water capturing capacity strengthens the resilience to droughts and reduces the risk of floods, which are both more likely to increase with climate change. Furthermore, soil organic matter enhances the nutrient buffer capacity and the microbial activity, both strengthening soil fertility (Scialabba *et al.,* 2010).

2. Peatland

In comparison with other ecosystem, peatlands are a small sink for carbon dioxide (Turunen *et al.,* 2002), a large source of methane (CH_4) (Huttunen *et al.,* 2003) and dissolved organic carbon (Aitkenhead and McDowell, 2000), and a huge pool of particulate organic carbon (Kremenetski *et al.,* 2003). Rates of carbon (C) sequestration (i.e., uptake of CO_2) and CH_4 emission depend strongly on height of the peatland surface above the water table (Alm *et al.,* 1997). The carbon sequestration process is pnly occurs when formation of new peat exceeds decay losses of all peat accumulated previously. Changes in the climatic water budget are likely to have large effects on peatland C sequestration process, mostly through acrotelm processes which controlls the rate at which litter is transformed into peat (Malmer&Walle ´n, 2004).

3. Forest

Forest ecosystems are important components of the global carbon cycle in world. The terrestrial part remove nearly 3 billion tons of anthropogenic carbon every year (3 Pg C year−1) through net growth, absorbing near about 30% of all CO_2 emissions from fossil fuel burning and net deforestation (Canadell *et al.,* 2007).

4. Ocean

The oceans play a critical role in capturing CO_2 from the atmosphere. Around 25% of all CO_2 emissions are absorbed by the ocean, making it one of the world's largest 'carbon sinks'. Carbon has been stored as bicarbonate ions in ocean which has received very little attention. The

alkalinity of the ocean increases naturally because of rock weathering in which > 1.5 moles of carbon are removed from the atmosphere for every mole of magnesium or calcium dissolved from silicate minerals (e.g., wollastonite, olivine, anorthite), and 0.5 moles for carbonate minerals (e.g., calcite, dolomite). These processes are mainly responsible for naturally sequestering 0.5 billion of CO_2 tons per year globally. This alkalinity is reduced in the ocean through carbonate mineral precipitation, which is almost exclusively formed from biological activity (Renforth, 2017). The biological pump drives maximum carbon storage in the deep ocean functionable through via gravitational settling of organic particles from surface waters (Philip *et al.,* 2019). Thus, the ocean's ability to sequester carbon from the atmosphere exerts an important control on global climate change.

Soil carbon sequestration

In order to mitigate climate change, it is necessary to reduce or retard the accumulation of GHGs in the atmosphere by increasing soil C sequestration and storage. Carbon sequestration generally refers to the medium and long-tenn (15-50 years) storage of C in the terrestrial ecosystems, in underground mainly as carbonates or in the oceans. The net amount of C sequestered at a site is the long-term balance between C uptake and C release. The level of organic C in a given soil depends on complex interactions of climate, soil physical, chemical, and biological processes under natural conditions, soil-forming factors largely determine SOM levels which increase rapidly with time initially as demonstrated in soil chronosequence studies (Goh *et al.,* 1976) and may then reach a maximum equilibrium level. It has been also observed that Virgin forests accumulate more SOC than nearby plantation forests (Goh and Heng, 1987). The decine in SOM level occurs when these forests are converted for agricultural uses due to disruption of plant C inputs adding to soil, decreased soil biological activity, soil inversion due to cultivation, and loss of high quality forest C with higher lignin content and more resistant C fractions (Murty *et al.,* 2002).

Conservation agriculture- a way to Soil carbon sink

Conservation agriculture (CA), comprising minimum soil disturbance, retention of crop residues and crop diversification, is widely used term for reducing soil degradation improving soil quality and increasing agricultural sustainability. Intensification of cropping systems with high above and belowground biomass (i.e., deep-rooted plant species) input may enhance CA systems for storing soil C relative to conventional tillage (Luo *et al.,* 2010). CA itself reduces the uses of tillage mechinaries thus, reduces the consumption of fossil fuel by 11.2% of the total energy input of the cropping system by increasing energy use efficiency of the system(Alluvione, 2011). Conservation agriculture provides many benefits, such as enhanced biodiversity and

microbial process throughout the soil surface, which contribute to increased water and nutrient use efficiency leading to sustainable crop production. Moreover, increases in SOC content increase crop yield and reduces yield variability. SOC accumulation not only sequestrates atmospheric CO_2, but also increases soil fertility and soil water holding capacity (Franzluebbers, 2002). Hence, healthy soils are becoming a key factor for developing sustainable crop production systems that are resilient to the effects of climate change.

Measures for carban sequestration and climate mitigation

1) Increase in SOC Content

It has potential for reducing GHG emission simultaneously restores soil health condition by improving physical, chemical and biological quality parameters of the soil (Lal *et al.*, 2011). Many mitigation options are found to be effective to promote SOC storage like reduction in soil erosion, conservation of soil moisture, crop diversification creates a link between climate change mitigation and adaptation efforts (Smith and Olesen, 2010).

2) Conservation Agriculture

As agriculture has profound contribution to GHG in atmosphere therefore, management of agricultural practice found to be cost effective strategy to compact climate change issues. Recommended management practices (RMPs) such as no tillage or minimum soil disturbance appear to promote C sequestration (West and Marland, 2002). Inputs of OC can be maximized by importing organic matter (OM) from other ecosystems, by incorporation of alarger fraction of the produced biomass to soil, or by increasing the primary production in the agroecosystem. All these measures results in a concomitant increase of C storage in the soil besides the improvement of other useful agronomic traits in plants and increases microbial activity in soil environments (Kallenbach and Grandy, 2011).

3) Carbon assimilation by Forest lands

Net carbon sequestration can also be achieved by increased forest carbon density, through both stand-scale management and landscape-scale strategies such as longer harvesting cycles or reduced disturbances. On a Vertisol soils of Ethiopia, Lulu and Insam (2000) observed positive impact of alley cropping (i.e., agroforestry) with Sesbania on the SOC pool.The expansion of the use of forest products that sustainably replace fossil-fuel CO_2 emissions and to increase forested land area through reforestation which also increase in forest carbon sink and reservoir can be managed to mitigate atmospheric CO_2 buildup phenomenon (Candell *et al.*, 2008).

5. Cover crop

Amomg the cover crop, leguminous cover crops enhances biodiversity, the quality of residue input and SOC pool. It is well established that ecosystems that posseshigh biodiversity absorb and sequester more C than those having low or reduced biodiversity. It has been found that legume-based cropping systems control the loss of C and N from soil. A beneficial effect of growing cover crops on enhancing SOC pool has been reported from Fullen and Auerswald (1998). Sainju et al. (2002) observed that practicing no till with hairy vetch can improve SOC content in soil surface.

6. Restoring degraded soils

Restoring degraded soils and ecosystems has a high potential for soil C sequestration. Most of the degraded soils have lost a large fraction of the SOC pool, which can be restored through adopting judicious land use management practices. Fullen (1998) observed that mean SOC content increased consistently and significantly on plots under the grass ley system at the rate of 0.78% in 4 years.

7. Nutrient management

Judicious nutrient management is crucial to SOC sequestration. In general, the use of organic manures and compost enhances the SOC pool more than application of the same amount of nutrients as inorganic fertilizers. Adequate supply of N along with other essential nutrients in soil can enhance biomass production under elevated CO_2 concentration. The potentiality of conservation tillage for sequestering SOC is greatly enhanced by soils, amended with organic manures (Hao *et al.,* 2002).

8. Irrigation

Judicious and cojoint application of irrigation water along with organic nutrients in a drought prone soil can enhance biomass production, increase in the amount of above ground and the root biomass that could be returned to the soil leading to improvement of SOC concentration. Irrigation can also enhance SOC concentrations in grassland area. An increasing concentration of SOC has been observed in Texas, where plots are treated with growing irrigated grain sorghum and wheat (Bordovsky *et al.,* 1999).

Conclusion:

Being the largest terrestrial reservoir of C to 3-m depth (4000 Pg), total soil C pool (both organic and inorganic) can be a source or sink of atmospheric CO_2 depending on land use and management. The rate of SOC sequestration depends on climatic condition, clay type, textural classes, depth of water time etc. The annual SOC sequestration potential is only 0.9 ± 0.3 Pg

C/year. The atmospheric concentration of CO_2 at the observed rate of 1990 (3.2 Pg C/ year) will continue to increase at the rate of 2.0–2.6 Pg C/year even with soil C sequestration. Though the potential of SOC sequestration is only a short-term strategy to mitigating anthropogenic enrichment of atmospheric CO_2, we need to develop a long term alternative solution for mitigating climate change issues in near future. But, Soil C sequestration is something that we cannot afford to ignore.

Reference:

Aitkenhead, J.A. and McDowell, W.H., 2000. Soil C: N ratio as a predictor of annual riverine DOC flux at local and global scales. Global biogeochemical cycles, 14(1): pp.127-138.

Alluvione, F., Moretti, B., Sacco, D., Grignani, C., 2011. EUE (energy use efficiency): of cropping systems for a sustainable agriculture. Energy 36, 4468–4481. https://doi. org/10.1016/j.energy.2011.03.075.

Alm, J., Talanov, A., Saarnio, S., Silvola, J., Ikkonen, E., Aaltonen, H., Nykänen, H. and Martikainen, P.J., 1997. Reconstruction of the carbon balance for microsites in a boreal oligotrophic pine fen, Finland. Oecologia, 110(3): pp.423-431.

Batjes, N.H. and Sombroek, W.G., 1997. Possibilities for carbon sequestration in tropical and subtropical soils. Global change biology, 3(2): pp.161-173.

Batjes, N.H., 1998. Mitigation of atmospheric CO2 concentrations by increased carbon sequestration in the soil. Biology and fertility of soils, 27(3): pp.230-235.

Bordovsky, D.G., Choudhary, M. and Gerard, C.J., 1999. Effect of tillage, cropping, and residue management on soil properties in the Texas Rolling Plains. Soil Science, 164(5): pp.331-340.

Bottner, P., Coûteaux, M.M. and Vallejo, V.R., 1995. Soil organic matter in Mediterranean-type ecosystems and global climatic changes: a case study—the soils of the Mediterranean Basin. In Global Change and Mediterranean-Type Ecosystems (pp. 306-325): Springer, New York, NY.

Boyd, P.W., Claustre, H., Levy, M., Siegel, D.A. and Weber, T., 2019. Multi-faceted particle pumps drive carbon sequestration in the ocean. Nature, 568(7752): pp.327-335.

Canadell, J.G. and Raupach, M.R., 2008. Managing forests for climate change mitigation. science, 320(5882): pp.1456-1457.

Cheddadi, R., Guiot, J. and Jolly, D., 2001. The Mediterranean vegetation: what if the atmospheric CO2 increased?. Landscape ecology, 16(7): pp.667-675.

Franzluebbers, A.J., 2002. Water infiltration and soil structure related to organic matter and its stratification with depth. Soil and Tillage research, 66(2): pp.197-205.

Freibauer, A., Rounsevell, M.D., Smith, P. and Verhagen, J., 2004. Carbon sequestration in the agricultural soils of Europe. Geoderma, 122(1): pp.1-23.

Fullen, M.A., 1998. Effects of grass ley set-aside on runoff, erosion and organic matter levels in sandy soils in east Shropshire, UK. Soil and Tillage Research, 46(1-2): pp.41-49.

Goh, K.M. and Heng, S., 1987. The quantity and nature of the forest floor and topsoil under some indigenous forests and nearby areas converted to Pinus radiata plantations in South Island New Zealand. New Zealand Journal of Botany, 25(2): pp.243-254.

Goh, K.M., Rafter, T.A., Stout, J.D. and Walker, T.W., 1976. The accumulation of soil organic matter and its carbon isotope content in a chronosequence of soils developed on aeolian sand in New Zealand. Journal of Soil Science, 27(1): pp.89-100.

Hao, Y., Lal, R., Owens, L.B., Izaurralde, R.C., Post, W.M. and Hothem, D.L., 2002. Effect of cropland management and slope position on soil organic carbon pool at the North Appalachian Experimental Watersheds. Soil and tillage research, 68(2): pp.133-142.

Houghton, R.A., 1999. The annual net flux of carbon to the atmosphere from changes in land use 1850–1990. Tellus B, 51(2): pp.298-313.

IPCC (2007): Climate change 2007: synthesis report. In: Core Writing Team, Pachauri RK, Reisinger A (eds): Contribution of working groups I, II and III to the fourth assessment report of the intergovernmental panel on climate change. IPCC, Geneva

Jenny, H., 1980. The soil resource: origin and behavior/[by] Hans Jenny. Ecological studies.

Kallenbach, C. and Grandy, A.S., 2011. Controls over soil microbial biomass responses to carbon amendments in agricultural systems: A meta-analysis. Agriculture, Ecosystems & Environment, 144(1): pp.241-252.

Krna, M.A. and Rapson, G.L., 2013. Clarifying 'carbon sequestration'. Carbon Management, 4(3): pp.309-322.

Lal, R., Delgado, J.A., Groffman, P.M., Millar, N., Dell, C. and Rotz, A., 2011. Management to mitigate and adapt to climate change. Journal of Soil and Water Conservation, 66(4): pp.276-285.

Lal, R.,2007. Carbon sequestration. Biotic and abiotic techniques of soil carbon sequestration, merits and limitation of each strategy, and policy implications. Philos Trans Roy Soc 363:815-830.

Luo, Z., Wang, E. and Sun, O.J., 2010. Can no-tillage stimulate carbon sequestration in agricultural soils? A meta-analysis of paired experiments. Agriculture, ecosystems & environment, 139(1-2): pp.224-231.

Mackey, B., Prentice, I.C., Steffen, W., House, J.I., Lindenmayer, D., Keith, H. and Berry, S., 2013. Untangling the confusion around land carbon science and climate change mitigation policy. Nature climate change, 3(6): pp.552-557.

Malmer, N. and Wallén, B., 2004. Input rates, decay losses and accumulation rates of carbon in bogs during the last millennium: internal processes and environmental changes. The Holocene, 14(1): pp.111-117.

Morgan, J.A., Follett, R.F., Allen, L.H., Del Grosso, S., Derner, J.D., Dijkstra, F., Franzluebbers, A., Fry, R., Paustian, K. and Schoeneberger, M.M., 2010. Carbon sequestration in agricultural lands of the United States. Journal of Soil and Water Conservation, 65(1): pp.6A-13A.

Murty, D., Kirschbaum, M.U., Mcmurtrie, R.E. and Mcgilvray, H., 2002. Does conversion of forest to agricultural land change soil carbon and nitrogen? A review of the literature. Global change biology, 8(2): pp.105-123.

Niggli, U., Fließbach, A., Hepperly, P. and Scialabba, N., 2009. Low greenhouse gas agriculture: mitigation and adaptation potential of sustainable farming systems. Ökologie&Landbau, 141, pp.32-33.

Olson, K.R., Al-Kaisi, M.M., Lal, R. and Lowery, B., 2014. Experimental consideration, treatments, and methods in determining soil organic carbon sequestration rates. Soil Science Society of America Journal, 78(2): pp.348-360.

Ozenda, P. and Borel, J.L., 1990. The possible responses of vegetation to a global climatic change. Scenarios for Western Europe, with special reference to the Alps. In Landscape-ecological impact of climate change. Proceedings of European Conference, Lunteren (pp. 221-249):

Powlson, D.S., Whitmore, A.P. and Goulding, K.W., 2011. Soil carbon sequestration to mitigate climate change: a critical re-examination to identify the true and the false. European Journal of Soil Science, 62(1): pp.42-55.

Prentice, I.C., Farquhar, G.D., Fasham, M.J.R., Goulden, M.L., Heimann, M., Jaramillo, V.J., Kheshgi, H.S., Le Quéré, C., Scholes, R.J., Wallace, D.W. and Archer, D., 2001. The carbon cycle and atmospheric carbon dioxide.

Raupach, M.R., Marland, G., Ciais, P., Le Quéré, C., Canadell, J.G., Klepper, G. and Field, C.B., 2007. Global and regional drivers of accelerating CO2 emissions. Proceedings of the National Academy of Sciences, 104(24): pp.10288-10293.

Renforth, P. and Henderson, G., 2017. Assessing ocean alkalinity for carbon sequestration. Reviews of Geophysics, 55(3): pp.636-674.

Sainju, U.M., Singh, B.P. and Yaffa, S., 2002. Soil organic matter and tomato yield following tillage, cover cropping, and nitrogen fertilization. Agronomy Journal, 94(3): pp.594-602.

Scialabba, N.E.H. and Müller-Lindenlauf, M., 2010. Organic agriculture and climate change. Renewable Agriculture and Food Systems, 25(2): pp.158-169.

Singh, B.R. and Lal, R., 2005. The potential of soil carbon sequestration through improved management practices in Norway. Environment, Development and Sustainability, 7(1): pp.161-184.

Smith, P. and Olesen, J.E., 2010. Synergies between the mitigation of, and adaptation to, climate change in agriculture. The Journal of Agricultural Science, 148(5): pp.543-552.

Smith, P., Andrén, O., Karlsson, T., Perälä, P., Regina, K., Rounsevell, M. and Van Wesemael, B., 2005. Carbon sequestration potential in European croplands has been overestimated. Global Change Biology, 11(12): pp.2153-2163.

Smith, P., Andrén, O., Karlsson, T., Perälä, P., Regina, K., Rounsevell, M. and Van Wesemael, B., 2005. Carbon sequestration potential in European croplands has been overestimated. Global Change Biology, 11(12): pp.2153-2163.

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C. and Scholes, B., 2008. Greenhouse gas mitigation in agriculture. Philosophical transactions of the royal Society B: Biological Sciences, 363(1492): pp.789-813.

Turunen, J., Tomppo, E., Tolonen, K. and Reinikainen, A., 2002. Estimating carbon accumulation rates of undrained mires in Finland–application to boreal and subarctic regions. The Holocene, 12(1): pp.69-80.

U.S. Department of Energy (2008): Carbon cycling and biosequestration: integrating biology and climate through systems science. Report from the March 2008 Workshop, DOE/SC-108, U.S. Department of Energy Office of Science.

West, T.O. and Marland, G., 2002. A synthesis of carbon sequestration, carbon emissions, and net carbon flux in agriculture: comparing tillage practices in the United States. Agriculture, Ecosystems & Environment, 91(1-3): pp.217-232.

PLANT TISSUE CULTURE: AN OVERVIEW

Kanchan Awasthi* and Ankita Yadav

Department of Botany,

Maharishi University of Information Technology, Lucknow, U. P.

*Corresponding author E-mail: kanchanawasthi1702@gmail.com

Abstract:

Micropropagation is a technique to produce genetically identical plantlets by using tissue culture method. Feasibility of photoautotrophic micropropagation has been shown in both herbaceous and woody plant species. Plant tissue culture also known as micropropagation because it involves rapid multiplication of small amount of plant material to produce more and more offsprings.

Introduction:

The planttissue culture is defined as culturing plant seeds, organs, explants, tissues, cells, orprotoplasts in synthetic genetic material that are chemically defined under sterile as wellcontrolled conditions of light, temperature, and humidity.

Micropropagation

Micropropagation refers to in vitro multiplication or plant reproduction under aseptic and natural conditions controlled to produce thousandsor millions of plants to be transferred to the field. Asexual reproduction is themultiplication of vegetative parts of plant.In vivo propagation of certain plants is done because, as they do not produce viable seeds e.g.bananas, grapes, figs, and chrysanthemum. Clonal propagation was successfully used for thedevelopment of apples, potatoes, tuberous and several ornamental plants

Micropropagation stages

Micropropagationis particularly a complex processinvolves 3 stages (I, II and III). Some authors add two additional categories (category 0 and IV) for more detailed understanding.

Stage 0: This is the first step of micro-propagation that involves the selection and growth of stock plants about 3 months under controlled conditions.

Stage I: In this phase, initiation andthe establishment of a proper culture is achieved. Selection of right or appropriate explant is very important. Explants that are used for micropropagation are organs, shoot tips and axillary buds. The selected plant is sterilized and washed before use.

Stage II: In this stage, the main function of the micropropagation occurs in the defined culture.Stage II mainly involves the multiplication of root and embryo formation from the explant.

Stage III: This phase involves the transfer of shoots to the culture medium for rapid development into shoots. Sometimes, shoots are planted directly into the ground to growroots. While handling a large number of species In vitro rooting of shoots is prefered.

Stage IV: This phase involves the plantation of plantlets in the soil.This is done by transferring stage III plants from the laboratory togreenhouse environment. In some plant species, stage III is skipped, and unrootedshoots are planted in pots.

The various applications of micropropagation are plant tissue in small quantity is sufficient to produce millions of clones in a year using micropropagation. It can take a long time to produce the same number of plants using common methods. The micropropagation process offers another good alternativein those species of plants that show resistance to convectional bulk propagation. Another way of propagation in bulk is mass micropropagation. Plants in large quantities can be produced in a short time.

This helps to save the endangered species and storage of the germplasm. This method can also be helpful for producing disease-free plants (meristem tip culture) . Increaseof in vitro stocks can be made at any time of the year. Also, a nursery can produce fruit, ornamental, and treespecies throughout the year. Through somatic embryogenesis the production of synthetic seeds are very common now-days.

Factors affecting Micropropagation:

With effective in vitroclonal propagation (micro propagation), development of a few traits is required.

1. The Genotype of the plant: The choice of rightgenotype of plant species (by experimentation) is needed for micropropagation. In general, plants with strong, vigorous growth are more suitable for micro-propagation.

2. Structure of plants: Plants (plant material) from the newly produced parts of the plant are much more effective than the old ones.

3. Cultural Media: The standard culturemedia of is suitable for plant tissue cultiureespeciallly in phase I and phase II. Addition of growth regulators (auxins and cytokinins) and changes inmineral composition is required in 3rd stage. This depends largely on the nature of the culture (meristem, bud etc.).

.**Embryo culture**

They are: (1) Culture of a mature embryo and (2) Embryo Rescue. Embryonic culture is in vitro development ofa mature or immature embryo with the ultimate goal of finding a healthy

plant.Generally, the term "embryo Culture" refers to the sexual produce embryo. There are two types of embryo culture - mature and immature embryonic culture (fetal rescue).

Somatic hybridization

The process of fusion of protoplast of somatic cells derived from different varieties or species of plants on a suitable nutrient medium to produce the somatic hybrids is called somatic hybridization.

For example pomato is the somatic hybrid obtained by the protoplast fusion of tomato and potato.

Applications:

Somatic hybridization has opened new potential for in vitro genetic modification of plants to improve the crops.

Application

1. Disease resistance: Several interspecific and inter-generic hybrids those are resistant to disease. Many diseases resistant genes (e.g., tobacco mosaic virus, potato X virus, club rot) can besuccessfully transferred from one species to another. For example, resistance has beenintroduced to tomatoes against diseases such as TMV, spotted wilt virus and insects pest.
2. Environmental tolerance: Genetics that is resistant to cold, frost and salt can be successfully introduced through somatic hybridization, e.g., introduction of coldtolerance gene of tomato.
3. Quality brands: Somatic hybrids for productionof high nicotine content and low erucic acid are produced.
4. Cytoplasmic malesterility: Modification of hybridization by means of cybridization has made it possibleto transmit cytoplasmic male infertility.

Limitations

Although somatic hybridization is a novel approach to plant biotechnology, there are several problems and limitations.

1. Somatic, hybridization does not always produce plantsgive fertile and viable seeds.
2. Regenerated plants obtained from somatic hybridization they are often variable due to somaclonal variability, chromosomal elimination, organelleseparation etc.
3. Protoplast culture is often associated with genetic instability.
4. There are limitations in hybrids selection options,
5. There is no certainty about the disclosure ofany specific character in somatic hybridization.
6. Somatic mixing between the twodiploids result in the formation of an undesirable amphidiploid.For this reason, haploid protoplasts are recommended for somatic hybridization.

Some other factors related to micropropagation

Cytokinin and Auxin effect on shoot development

In vitro culture of apical and axillary shoot bud explants on MS medium supplemented with auxin and cytokinin started to swell up after two weeks of culture. Shoot development from axillary shoot bud explants was faster (within four weeks) compared to apical shoot bud explants (after eight weeks). The difference of this growth shows that shoots produced from axillary shoot buds explants were higher compared to apical shoot bud explant. The production of shoot was increased as the concentration of BAP and Kinetin was increased. However, the number of shoot productions was reduced when the concentration of BAP was higher than a set level. Combination of cytokinin and auxincan give significant effects on shoot induction from apical or axillary shoot bud explants.

Multiple shoot induction in some plantswas achieved by culturing the shoot tip explants on MS medium supplemented with BAP (1) or when the lateral buds were cultured on Linsmaier and Skoog medium supplemented with BAP (2). Some study showed that further increase of BAP concentration will reduce the number of shoots induction and can cause necrosis, indicating an adverse effect of plant growth regulators beyond the optimal concentration. (4) Higher concentration of cytokinin beyond the optimum levels was also reported to cause necrosis and reduction in shoot formation during in vitro multiplication of musa sp. (5,6). Shoot development and multiple shoots induction by using various concentrations of BAP and NAA had been reported in several micropropagations of ornamental rhizomatic plants like *Alpinia purpurata* (7), *Costu spictus D. Don* (8), *Heliconia psittacorum* (9), and *Musa beccarii* (9). Present study showed that the use of axillary shoot bud explants in production and development of new shoots was more effective as compared to the using of apical shoot bud explants

Apex damage effects which induces microshoots formation

Stimulation of microshoots was obtained through elimination of apical dominance by shoot incision after 12 weeks culture. Two types of incision were applied in this method to determine the effects on the microshoots production. Microshoots were highly produced from the shoot bud that was longitudinally incised prior to culture.This result revealed that this method can be applied in mass propagation in order to increase the multiplication rate. Similar technique was used in stimulation of shoot branching in *Strelitzia sp.* and *Calathea ornate* .

The excision of an apex is important for mass propagation of valuable ornamental plants with a naturally low rate of multiplication like *Strelitzia* sp. and *Calathea* sp. Cronauer and Krikorian also reported the similar findings in induction of multiple shoots of two dessert banana clones and two plantain clones through excision of shoot tips. This phenomenon had been discussed by

Shimizu-Sato *et al.* as it was found that cytokinin will be induced by decapitation of the shoot apex and stimulate axillary bud outgrowth.

Addition of glutamine and activated charcoal for production of high quality plantlets

This is done to reduce necrosis problems through addition of glutamine, activated charcoal, or their combinations. Chlorophyll concentration in the leaf samples was varied depending on concentrations of glutamine and activated charcoal enriched in the medium. Addition of activated charcoal in MS medium was the most effective for production of shoots with dark green leaf and containing high concentration of chlorophyll pigments. However, the percentage of necrotic leaves was also increased with the increasing of activated charcoal concentration. Multiplication medium along with glutamine produced shoots with light green leaf and containing low concentration of chlorophyll pigments shows the lowest percentage of necrotic leaf. This revealed that exogenous amino acid from glutamine serves as nitrogen source for the synthesis of protein which is importantto reduce necrosis effect on the leaf. In addition, Zouine and Hadrami reported that exogenous supply of glutamine can possibly increase soluble storage protein inembryogenic cells of date palm suspension culture .Based on the overallresult, the high quality in vitro plantlets with dark green leaf and low percentage of necrotic leaf were observed on multiplication medium along with activated charcoal .Activated charcoal had been used to reduce explant browning problem in shoot tip culture for cryopreservation protocols in Rubusidaeus at some concentration in MS medium. The explant browning also could be overcome by growing embryos of *Dipterocarpus alatus* and *D. intricatus* initially on a filter paper bridge in liquid medium with activated charcoal to absorb the oxidized phenolic compounds. The induction of multiple shoots and rapid shoot elongation from the incised shoot buds in the Culture medium. The similar observations on induction and elongation of shoots have been reported in micropropagation studies such as cashew, eucalyptus, lilly, cotton, and yam.

Several studies have demonstrated that addition of activated charcoal alone or combined with auxin can promote roots induction from the mature in vitro plantlets. Apart from that,according to Eymaretal activated charcoal also can give signicant effect on in vitro nitrogen uptake in Lagerstroemia indicawhich demonstrated that the explants grown in medium with activated charcoal were capable of taking up both NO_3^- and NH_4^+.Similar results were showed where the multiplication medium along with glutamine and activated charcoal produced high quality of plantlets as in Culture medium.

Root Induction

Type and hormone concentration had significant relation with length, diameter, and number of roots. In general, roots were induced after three weeks of culture .Root number in both rooting media with NAA and IBA, respectively, was increased as the concentration of the auxin increased and started to decline when the concentration was higher than set level. Media with low

concentration of auxin produced longer roots. This shows that the root can be induced in plantlets in both types of auxins but the highest number of roots was produced in MS medium supplemented with NAA. Plantlet that cultured in media with several concentrations of NAA produced high quality of roots. Similar results were reported by Hamad *et al.* where media enriched with NAA were the best media for induction of adventitious roots in pineapple and produced tallest plantlets with high number of roots per shoot. Raihana *et al.* also demonstrated that MS medium supplemented with NAA gave the highest root number in micropropagation of *Curcuma mangga* from rhizome bud where the increasing of NAA could suppress the production of root. However, Loc and Yusuf *et al.* reported that MS medium supplemented with NAA could enhance roots induction in *Curcuma zedoaria* and *Boesenbergia rotunda*, respectively. Several studies demonstrated that most of the micropropagatedrhizomatic plants can produce roots in MS medium devoid of auxin with or without addition of activated charcoal like *Musa* sp., *Curcuma* sp. *Zingiber* sp., and *Heliconia* sp. studies suggested that these results might be due to the fact that some of rhizomatic plants can produce sufficient amount of auxin endogenously to initiate root induction.

Conclusion:

Plant tissue culture is a very important method which can be used to produce large quantity of plants with genetic modifications. Implication of auxincytokinin is also very effective in formation of shoot and root, which is proved to be very helpful during micropropagation. For the rapid multiplication of plants, micro-propagation is a refined and well adapted technique. Due to the fast speed of propagation it has a great profit-making potential, the high plant quality and the ability to produce disease-free plants. It is an art and science of plant propagation under in vitro conditions.

References:

Anu A., K. N. Babu, and K. V. Peter, Variations among somaclones and its seedling progeny in Capsicum annuum, Plant Cell, Tissue and Organ Culture,vol.76,no.3, pp.261-267 2004.

Barcel´o-Mu˜noz A., C. L. Encina, E. Sim´on-P´erez, and F. Pliego- Alfaro, Micropropagation of adult avocado, Plant Cell, Tissue and Organ Culture,vol.58, no1, pp.11-17 1999

Barrueto Cid L. P., A. C. M. G. Machado, S. B. R. C. Carvalheira, and A. C. M. Brasileiro, Plant regeneration from seedling explants of *Eucalyptus grandis x E. urophylla*, Plant Cell, Tissue and Organ Culture,vol.56,no.1, pp. 17-23 1999.

Boggetti B., J. Jasik, and S. Mantell, In vitro multiplication of cashew (*Anacardium occidentale* L.) using shoot node explants of glasshouse-raised plants, Plant Cell Reports,vol.18,no.6,pp.456-461 1999

Borges M., W. Ceiro, S. Meneses et al., Regeneration and multiplication of Dioscoreaalata germplasm maintained in vitro,PlantCell, Tissue and Organ Culture,vol.76,no.1,pp.87-90 2004

Cronauer S. S. and A. D. Krikorian, Multiplication of Musa from excised stem tips, Annals of Botany, vol.53, no.3, pp. 321-328 1884.

Eymar E., J.Alegre, M. Toribio, and D.Lopez-Vela, Effect of activated charcoal and -benzyladenine on in vitro nitrogen uptake by Lagerstroemia indica, Plant Cell, Tissue and Organ Culture, vol. 63 , no. 1 , pp. 57-65 2004

Hamad A. H. M., R. M. Taha, and S. Mohajer, In vitro induction and proliferation of adventitious roots in pineapple (*Ananas comosus* L.) cultivars of smooth cayenne and morris, Australian Journal of Crop Science,vol.7,no.7, pp.1038-1045 2013.

Hamirah M. N., H. B. Sani, P. C. Boyce, and S. L. Sim, Micropropagation of red ginger (*Zingiber montanumkoenig*), a medicinal plant, Asia-Pacic Journal of Molecular Biology and Biotechnology,vol. 18 ,no.1 pp.127-130 2010

Hazra S., A. V. Kulkarni, A. K. Banerjee et al., A rapid and simple method for in vitro plant regeneration from split embryo axes of six cultivars of cotton, Biologia Plantarum,vol.45,no.2, pp. 317-319 2002.

Jalil M., N. Khalid, and R. Y. Othman, Plant regeneration from embryogenic suspension cultures of Musa acuminatacv. Mas (AA), Plant Cell, Tissue and Organ Culture,vol. 75,no.3 ,pp.209-214 2003.

Jemmali A., N. Elloumi, C. Kevers, and J. Dommes, Morphological and hormonal characterisation of strawberry vitroplantsraised through axillary or stipular adventitious shooting, Plant Growth Regulation,vol.38,no.3,pp.273-278 2002.

Linington I. M., In vitro propagation of *Dipterocarpus alatus* and *Dipterocarpus intricatus*, Plant Cell, Tissue and Organ Culture,vol.27, no.1, pp. 81-88 1998

Nathan M. J., C. J. Goh, and P. P. Kumar, In vitro propagation of *Heliconia psitta* corumbud culture, Hort Science,vol.27,no.5, pp.450-452 1992

Negash A., K.Puite,J.Schaart,B.Visser,andF.Krens,In vitro regeneration and micro-propagation of enset from Southwestern Ethiopia, Plant Cell, Tissue and Organ Culture, vol.62,no.2,pp.153-158 2000.

Nguyen H. L., T. D. Doan, H. K. Tae, and S. Y. Moon, Micropropagation of zedoary (Curcuma zedoariaRoscoe)-a valuable medicinal plant, Plant Cell, Tissue and Organ Culture,vol.81,no. 1, pp. 119-122, 2005.

Nhut D. T., B. V. Le, S. Fukai, M. Tanaka, and K. T. T. Van, Effects of activated charcoal, explant size, explant position and sucrose concentration on plant and shoot regenerati on of Liliumlongiflorumvia young stem culture, Plant Growth Regulation, vol. □ 33□ , no. 1□ , pp. 59-65 2000

Raihana R., Q. Z. Faridah, A. A. Julia, A. H. A. Abdelmageed, and M.A.Kadir, Invitro culture of Curcuma manggafrom rhizome bud, Journal of Medicinal Plant Research,vol. 5,no.28,pp.6418-6422 2011

Salvi N. D., L. George, and S. Eapen, Plant regeneration from leaf base callus of turmeric and random amplified polymorphic DNA analysis of regenerated plants, Plant Cell, Tissue and Organ Culture,vol.66, no. 2,pp.113-119 2001

Shimizu-Sato S., M. Tanaka, and H. Mori, Auxin-cytokinin interactions in the control of shoot branching, Plant Molecular Biology, vol.69, no.4, pp.429-435 2009.

Stasolla C., N. Loukanina, H. Ashihara, E. C. Yeung, and T.A. Thorpe, Ascorbic acid changes the pattern of purine metabolism during germination of white spruce somatic embryos, Tre e Physiology, vol.21,no.6,pp.359-367 2001

Van de Pol P. A.and T. F. van Hell, Vegetative propagation of Strelitziareginae, Acta Horticulturae,vol. 226, pp. 581-586 1988.

Wang Q., J. Laamanen, M. Uosukainen, and J. P. T. Valkonen, Cryopreservation of in vitro-grown shoot tips of raspberry (RubusidaeusL.) byencapsulation-vitri□ cation and encapsulation-dehydration, Plant Cell Reports,vol.24, no.5, pp. 280-288 2005.

Wong W.C., M.Jalil, M.Ong-Abdullah,R.Y.Othman,andN. Khalid, Enhancement of banana plant regeneration by incorporating a liquid-based embryo development medium for embryogenic cell suspension, The Journal of Horticultural Science and Biotechnology,vol.81,no.3, pp.385-390 2006

Xiao W., X.Huang, Y. Chen, X. Dai, and J. Zhao, Plant regeneration from protoplasts of *Musa acuminata*cv. Mas (AA) via somatic embryogenesis, Plant Cell, Tissue and Organ Culture, vol.90,no.2,pp.191-200 2007.

Yusuf N.A., M.S.M.Annuar and N. Khalid, Efficient propagation of an important medicinal plant boesenbergia rotunda by shoot derived callus, Journal of Medicinal Plants Research , vol.5, no. 13, pp. 2629-2636 2011

Zouine J. and I. El Hadrami, Effect of ,-d, glutamine and BAP on embryogenic suspension culture of date palm (*Phoenix dactylifera* L.), Scientia Horticulturae,vol.112,no.2,pp.221-226 2007

STRATEGIES FOR UPSCALING PLANT SECONDARY METABOLITE PRODUCTION UNDER *IN VITRO* CULTURE SYSTEMS

Rajesh . S* and Radhamani. T

Centre for Plant Molecular Biology and Biotechnology,

Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

*Corresponding author E-mail: rajesh.s@tnau.ac.in

Abstract:

Plants are an integral part of our daily diet, and their nutritional value has been intensively studied for decades. They are distinguished by a significant range of chemical products, especially secondary metabolites (SMs) that have useful biological activities important to humans. The diversity of these substances is impressive, with hundreds of thousands of secondary metabolites identified in plants so far. Over exploitation of plants of therapeutic or industrial significance could lead to habitat destruction of certain plants and leads to endangering of plant species in wild. Plant tissue culture provides alternate strategy for non-destructive and sustainable production of secondary metabolites through cell cultures. This chapter describes various strategies for upscaling the secondary metabolite production under *in vitro* cultures.

Keywords: Plant cell cultures, Secondary metabolites, bioreactors, large scale production, PSMs

Introduction:

Plants are major sources of routine diet and the importance of its nutrient and phyto-constituents are widely analyzed for decades. Plants produce an array of low molecular weight compounds called as secondary metabolites, dubbed as Plant secondary metabolites (PSMs). These PSMs plays key role in the interaction of the plant with its environment, plant defense and pollination among others. These secondary metabolites are valuable for various uses and can be produced off the system through in vitro culture techniques. Various strategies are adopted for production of this high value compounds under tissue culture system and the production potential has been maximized that can be exploited on an industrial scale.

Strategies for up scaling secondary metabolite production:

Generally the process of the large scale production of secondary metabolites under *in vitro* culture from plant cells is complex in nature. A schematic work flow of strategies deployed

for plant secondary metabolite production is presented in Fig. 1 and involves the following aspects:

1. Selection of cell lines for high yield of secondary metabolites
2. Large scale cultivation of plant cells
3. Medium composition and effect of nutrients
4. Optimization of physical factors-Temperature, pH, Light and Oxygen
5. Elicitor induced production of secondary metabolites
6. Biotransformation using plant cell cultures
7. Hairy root cultures
8. Secondary metabolites release and analysis

1. Selection of cell lines for high yield of secondary metabolites:

The method for extracting secondary metabolites from plant cell cultures can be thought of as a multi-stage procedure. The first stage in this method is to choose a parent plant based on its molecular and biochemical properties, especially in terms of the high levels of desired metabolites. Generally any component of a plant can be used to produce callus tissue. But the success of callus development is dependent on the plant species and their attributes. Dicotyledons are more receptive to callus tissue induction than monocotyledons because woody plant calluses grow slowly in comparison to monocotyledons. Stems, leaves, roots, flowers, seeds, and any other plant parts can be utilized as explants, however younger and fresher explants are preferred.

1.1. Selection of plant species

The plant species are screened and selected based on metabolites present in it. Plants with high contents of the desired products for callus induction are selected to obtain high-producing cell lines.

1.2. Plant Genotype and Cultivar

One of the most important elements determining the biochemical status of plants and plant cell cultures is genetic potential. Environmental and physiological factors can influence the expression of genes involved in phytochemical production, although genetics is the most important component.

1.3. Obtaining rapid growing and mass productive cell lines

Inherent genetic and epigenetic instability in plant cell cultures is sometimes seen. Variability across cells frequently results in a steady decrease in production, which can be linked to genetic changes generated by culture mutations or epigenetic changes caused by physiological

circumstances. These unfavourable alterations can be reversed by selecting a desired cell population from a large number of heterogeneous ones.

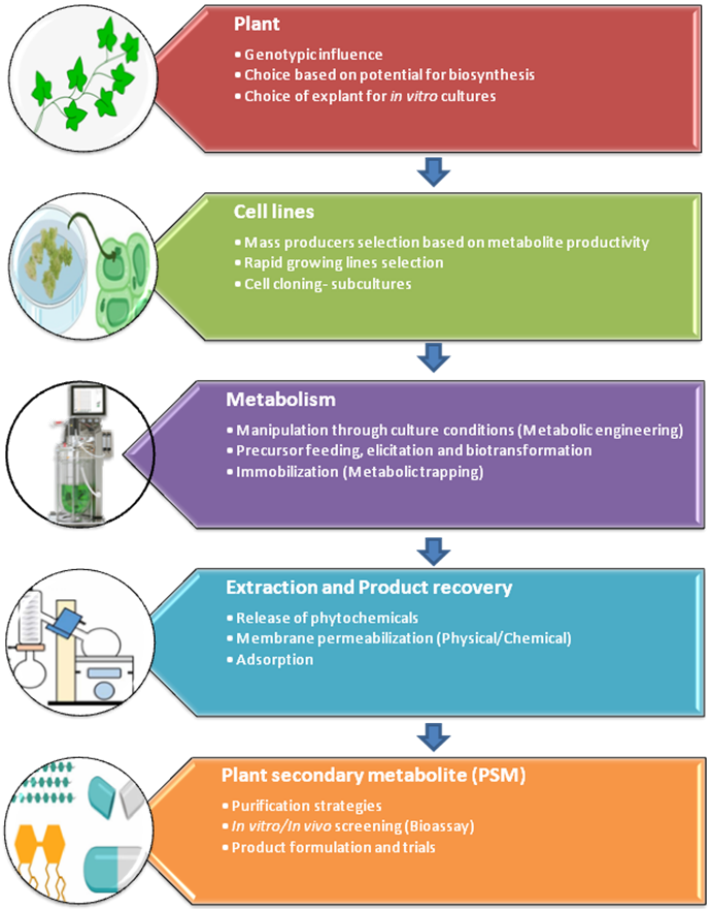


Figure 1: Scheme for Production of Secondary Metabolite in Plant Cell Cultures

1.4. Cell cloning

Individual plant cells do not necessarily have the same physiological features. To create highly productive cells, variability in biochemical activity within a population of cells has been used. It is a particularly effective method for increasing secondary metabolite levels. According to Kim and Chang (1990), the lack of particular enzymes is the most essential factor in plant cell cultures' failure to synthesize secondary metabolites. Once the cell line is chosen, investigation on cell growth, cell viability, pH changes carbon supply consumption, protein activity, organic phenomenon, target compounds accumulation are often applied. Extensive screening of a number of clones in *Lithospermum erythrorhizon* cultures resulted in a 13- to 20-fold increase in shikonin production (Kim and Chang, 1990). Number of cell aggregates can also be the key factor in producing a massive amount of biomass and bioactive compounds. In *Ficus deltoidea*, the flavonoid content of the cells increased as the aggregate size of the cells grew larger (Haida *et al*., 2019).

1.5. Protoplast fusion

Maximizing secondary metabolite production and accumulation in plant cultured cells necessitates the creation of new genotypes *via* protoplast fusion, but this requires the identification of genes encoding key enzymes in secondary metabolic pathways, as well as their expression once introduced into plant cells. High shikonin producing protoplasts were selected and converted into cell lines and cultured in suspension. A cell line with 1.8-fold productivity than parent line was obtained (Rao and Ravishankar, 1999).

1.6. Use of mutagens

Mutation strategies have been employed in order to obtain overproducing cell lines (Rao and Ravishankar, 1999). It entails subjecting a large population of cells to environmental stresses, and selection of only those cells that can resist the selection processes. Since plant cell cultures are diploid, they are of limited relevance to mutagenization. It is difficult to isolate overproducing cells from haploid cells that have been mutagenized. Since, secondary metabolites as well as enzymes are found inside haploid cells. Berlin *et al.* (1981) induced p-fluoro phenylalanine-resistant cell lines of tobacco cell cultures and found that, out of 31 resistant cell lines, five lines of *Nicotiana tabacum* and five lines of *Nicotiana glauca* accumulated higher levels of phenolics.

2. Large scale cultivation of plant cells:

Successful mass culture of plant cells with biomass productivity of over 1 g L^{-1} d^{-1} with moderate metabolite or protein levels is required for cell culture technology to be viable for commercial production of phytochemicals, which necessitates bioreactors that have been well designed and characterized for plant cell culture use.

2.1. Bioreactor

A bioreactor is a glass or steel vessel with probes with provisions for monitoring the pH, temperature, and dissolved oxygen in the culture, as well as the ability to sample cultures, add fresh medium, modify pH, air supply, mix cultures, and manage temperature without jeopardizing the culture's aseptic nature. This feature enables for precise control and monitoring of culture conditions in a bioreactor.

2.2 Stages in production of secondary metabolites using bioreactors

Initially, the callus of particular target genotype is chosen for cell suspension culture. Sub-culture is done every two weeks (15ml culture in 100 ml fresh medium, in a 250ml shake flask) by incubating at 25 ℃ at 150 rpm in a gyro-rotatory shaker. Normally, for 30L bioreactors, 100 ml cells are cultured in 1L medium of 2 L shake-flask. Bioreactor is prepared by

cleaning and assembling, checking any wear and tear, testing the pressure, temperature probe and dissolved oxygen content using appropriate probes before filling the vessel with the medium.

Sterilization of the bioreactor is done by passing superheated water from a thermo-circulator (130 ℃) and raising the temperature of the bioreactor to 121 ℃. The oxygen probe is connected to the controller and the bioreactor is incubated for about 3 days to detect any contamination. Inoculating cells (3 L) are transferred to a flask of 5 L with a needle and a stainless steel cover. After mixing, pre inoculation sampling is done. Once the cells are transferred, the needles are removed and covered with pre sterilized end cap. For sampling, 250 ml of sample is drawn every day to determine the dry weight, viability test (using Evans blue, Trypan blue, or phenosaphranin), total carbohydrate (using anthrone reagent or HPLC analysis), product, and pH. Some other parameters to be determined are the concentrations of phosphate, nitrate, and ammonia in the medium, cell number, and mitotic index. The growth rates of the cultures are determined using a linear regression analysis of a log-linear plot. The cells are harvested using low-speed centrifugation or filtering through Miracloth or a nylon bag in an ordinary domestic spin dryer can also be used to harvest 30 L of cells.

3. Medium composition and effect of nutrients:

It includes inorganic components, organics, and phytohormones. Changing of medium components is a very powerful way of enhancing the culture efficiency of plant cell cultures. Various types of media have been devised to culture the callus and cells in suspension. Murashige and Skoog (MS) is one of the most widely used media for plant tissue cultures. The B5 medium has lower quantities of inorganic nutrients than the MS medium.

3.1. Inorganic Components

Both nitrate (NO^-_3) and ammonium (NH^+_4) are sources of nitrogen in plant tissue culture media such as MS, LS, or B5. Nitrogen source is critical for alkaloids accumulation in *Holarrhena antidysenterica* plant suspension cultures, anthocyanin creation in *Vitis vinifera* cell suspensions, and shikonin production in *Lithospermum erythrorhizon* cell cultures. Higher ratios of NH^+_4 to NO^-_3 increased the production of berberine and ubiquinone, while lower levels of NH^+_4 and increasing levels of NO^-_3 stimulated the formation of shikonin and betacyanins. Capsaicin production in *Capsicum frutescens*, anthraquinones in *Morinda citrifolia*, and anthocyanin production in *Vitis* species were all improved when total nitrogen levels were reduced.

In plant cell cultures, phosphate increases cell proliferation but may have negative impact on secondary product accumulation. Cell development was slowed by higher K+ concentrations and under low K^+ levels; the cells stored more soluble sugar. Microelements are necessary for

plant growth and development in trace amounts and play a variety of roles. Iron is released slowly and continuously into the media *via* EDTA complexes.

The concentration of the carbon source has an impact on cell growth and secondary metabolite output in many circumstances. Sucrose levels at 2.5 and 7.5 % produced rosmarinic acid yields of 0.8 and 3.3 g L^{-1} in *Coleus blumei* medium, respectively (Qian *et al*., 2009). Manuhara *et al*. (2015) reported accumulation of maximum saponin content on MS medium with 5% sucrose in hairy-root culture of *Talinum paniculatum* (Jacq.) . The eurycomanone production of the cells is also promoted strongly (1.7 mg/g) from the cell suspension culture of *Eurycoma longifolia* (Nhan and Loc, 2017).

Vitamins like thiamine (vit. B1) and myo-inositol are considered necessary for plant cell development *in vitro*. Amino acids are a source of $NO^{-}{}_{3}$ and, like $NH^{+}{}_{4}$, their uptake causes the medium to become acidic. Glycine is most commonly used. Arginine, asparagine, aspartic acid, alanine, glutamic acid, glutamine, and proline are also employed. Casamino acid, peptone, yeast extracts, malt extracts, and coconut milk are some of the other supplements available. Depending on the type of culture being cultivated, liquid or solid media for plant cell culture *in vitro* can be utilized. Gelling agents should be considered appropriately. Agar is the most common gelling agent; its quality might vary from batch to batch and from supplier to supplier.

4. Effect of physical factors:

Physical conditions, such as light, temperature, and medium pH, have also been examined for their effect upon secondary metabolite accumulation in many types of cultures.

4.1. Temperature

A temperature of 17- 25 °C is normally used for induction of callus tissues and growth of cultured cells with varied levels for different plant species. At High temperature (35°C) the anthocyanin degradation and the inhibition of anthocyanin biosynthetic genes transcription (Mori *et al*.,2007) occurs in *Vitis vinifera* cv. Cabernet Sauvignon which reduces the total anthocyanin content to less than half of that in the control berries (25 °C). The low and high temperature shows a major difference in secondary metabolite accumulation in plants such as *Citrus sinensis* (Lo Piero, 2005) and *Rosa hybrida* (Dela *et al*., 2003).

4.2 Light

Plant cell cultures are also affected by the spectral quality, intensity, and duration of light irradiation. The stimulatory effect of light irradiation found on the synthesis of anthocyanins, vindoline, catharanthine, and caffeine (Sakamoto *et al*., 1994). Thus, light increased the formation of anthocyanin in cell cultures of *Daucus carota* and *Vitis vinifera*. Mulder-Krieger *et*

al. (1988) discovered that the composition of sesquiterpenes in callus cultures of *Marticaria chamomilla* was influenced by illumination. Caffeine production was increased by a factor of ten when *Coffea arabica* cell suspensions were illuminated. The greater antioxidant capability of SMs in plants can be activated by high light intensity (Arena *et al*. 2017). *Lactuca sativa* antioxidant phenolic molecule is more sensitive to monochromatic light than mixed light. Changing the light quality at a certain growth stage should be considered as strategic technique for increasing SM yield (Yang *et al*., 2018).

4.3 pH

The medium pH is usually adjusted between five and six, and pH extremes are avoided. The pH of the medium decreases during ammonia assimilation and increases during nitrate uptake. The slight difference in pH value can affect the overall production of biomass and bioactive compounds in cell suspension cultures. There was no production of biomass in media with pH 3.0 and pH 9.0; hence, both pH value treatments were not able to produce flavonoid (Haida *et al*., 2019).

4.4 Osmotic pressure

High osmotic potential in *Vitis vinifera* cell suspension cultures (Dodds and Roberts, 1985) leads to higher accumulation of anthocyanin. The sucrose or mannitol in the medium enhances the osmotic pressure and the accumulation of anthocyanins in *Vitis vinifera* culture increases to 1.5 times and reached 55 μg $cell^{-1}$.

5. Elicitor induced production of secondary metabolites:

Elicitation is considered the most practical strategy for increasing the production of desirable secondary metabolites from cells, organs and plant systems (Angelova *et al*., 2006; Namdeo, 2007). Biotic and abiotic elicitors are widely used for secondary metabolite production. The concentration, type and time course of elicitors greatly impact accumulation of secondary metabolites (Huang *et al*., 2021).

5.1. Elicitor concentration

Increased concentration of elicitors results in higher accumulation of secondary metabolites. Higher accumulation of ajmalicine in *Catharanthus roseus* cultures when treated with different concentrations of elicitor extracts of *Trichoderma viride*, *Aspergillum niger*, and *Fusarium moniliforme* were noted (Namdeo, 2007). High dosage of elicitor induces hypersensitive response leading to cell death, whereas optimum level was required for induction.

5.2. Duration of elicitor exposure

Catharanthus roseus cells produced three times more ajmalicine after 48 hours of treatment with *Trichoderma viride* extracts. Increased exposure time, on the other hand, resulted

in decrease of ajmalicine content (Asada and Shuler, 1989). The cell suspension culture of *G. sylvestre* was treated with MeJA and SA for 24 h, 48 h, and 72 h. With the 150 μM MeJA treatment, the maximum gymnemic acid production (135.41 ± 0.43 mg/g DCW) was recorded after 72 hours (Chodisetti *et al*., 2015). Apart from these factors, elicitor specificity, cell line or clone of microbial elicitor employed, presence of growth regulators, and ambient circumstances all influence elicitation efficiency. Secondary metabolites produced on elicitation from various plant species are listed in Table 1.

Table 1: Elicitors for production of secondary metabolite in plant cell cultures

Elicitors	Cell culture	Product
High electric field pulses	*Chenopodium ruburum*	Amaranthin
High hydrostatic pressure	*Chenopodium ruburum*	Amaranthin
Metal ions: Cu^{2+}, Cd^{2+}	*Vigna angularis*	Isoflavonoids
Ultrasound	*Panax ginseng*	Saponins
Agaropectin	*Lithospermum*	Shikonin
Chitosan	*Rubia tinctorum*	Anthraquinones
Fungal elicitor	*Ruta gravelones* *Morinda citrifolia*	Acridone expoxide Antraquinones
Jasmonic acid	*Viti vinifera*	Anthocyanins
Methyl jasmonate	*Nicotiana tabacum* *Coleus blumei*	Capsidiol, nicotine Rosmarinic acid, Taxol
	Andrographis paniculata	Andrographolide
	Taverniera cuneifolia	Glycyrrhizic acid
2,4-D	*Azadirachta indica*	Azadirachtin
	Catharanthus roseus	Catharathine
BAP + IAA	*Rauvolfia serpentina*	Serpentine
BA + NAA	*Stevia rebaudiana*	Stevioside
SA	*Rubia cordifolia*	Anthraquinone
	Glycyrrhiza glabra	Glycyrrhizin
Methyl salicylate	*Withania somnifera*	Withaniferin A
JA, MeJA	*Mentha piperita*	Rosmarinic acid

6. Biotransformation using plant cell cultures:

Biotransformation is the process of transforming organic compounds by cell culture into chemically distinct materials. Plant cells by biotransformation generate a variety of products. These reactions include reduction, oxidation, hydroxylation, acetylation, esterification, glucosylation isomerization, methylation, demethylation, epoxidation, etc. The advantages of biotransformation includes enhancement in the productivity of the desired compound and the production of novel compounds. Studies on biotransformation help to clarify the biosynthesis pathway, and catalysis can be conducted under mild conditions, reducing unwanted by-products, energy, safety, and costs.

7. Hairy root cultures:

Hairy roots cultures can be exploited for the biosynthesis of secondary metabolites. Hairy roots are developed as a result of infection by the plant pathogen *Agrobacterium rhizogenes* that upon infection transfers gene for hormone synthesis. This infective mechanism can be exploited for production of secondary metabolites in cell culture systems. Under *in vitro* conditions, hairy roots can grow faster and produce secondary metabolite as that of the parent plants. Several medicinal plants are exploited for secondary metabolite production through hairy root (HR) cultures (Kumar, 2015; Dhiman *et al*. 2018; Gutierrez-Valdes *et al*. 2020).

8. Secondary metabolites release and analysis:

8.1. Exudation

The vacuole stores a variety of chemicals produced by plants. In terms of product recovery costs, improving chemical transport from the vacuole to the culture medium would be quite beneficial. This could entail the development of new chemical or environmental agents to cause exudation. It may be important to investigate the physiological mechanisms of metabolite release from plant cells in order to use this strategy. The buildup of indole alkaloids in the vacuoles of *Catharanthus roseus* has been attributed to an ion-trap mechanism, in which the basic indole alkaloids are held in the acidic vacuole due to their positive charge at low pH, blocking diffusion through the tonoplast (Lee and Shuler, 2000).

8.2. Two-phase Systems

The existence of highly specialized structures including secretory and accumulatory features, such as oil glands, glandular trichomes, or a glandular epidermis, is most likely connected with the accumulation of secondary metabolites in cell cultures. These accumulation sites are absent in undifferentiated callus or suspension cultures. This is most likely the cause of the low yields of these chemicals in plant cell cultures. Even traces of secondary metabolites from the culture medium are accumulated in two-phase systems, eliminating any type of

feedback inhibition. By trapping flavour components in artificial accumulation sites, evaporation of the product into the gas phase can be reduced. The desired plant products can then be removed from the growth systems selectively. *In situ* recovery can concentrate the product, and downstream purification can be minimized if the product is selectively removed from the culture medium and cells.

8.3. Membrane Permeabilization

The majority of plant cell culture products are kept in vacuoles. Plasma membrane and tonoplast must be penetrated in order to release products from plant cell vacuoles. To start product release from cultivated plant cells, many approaches have been explored.

8.3.1. Chemical Permeabilization

In *Chenopodium ruburum*, cells were permeabilized with chitosan released Amaranthin into the culture medium in a time-dependent manner (Dornenburg and Knorr, 1997). *Beta vulgaris* cultures grew properly after this permeabilization treatment, attaining a maximum biomass concentration of 48 percent higher than non-permeabilized cultures after 14 days of culture. The maximal betacyanine content was only 25% lower in permeabilized cultures than in non-permeabilized cultures (Trejo-Tapia *et al*., 2007).

8.3.2. Physical Permeabilisation

High electric field pulses, high hydrostatic pressure, ultrasonic, and other physical forces can cause membrane permeabilization. Cell membrane permeability is reversible when the external electric field strength is equal to or slightly more than the critical value. This phenomenon is being used to get foreign DNA into cells that make cell fusion easier (Hashimoto *et al*., 1985; Biedinger *et al*., 1990).

Several reports on the successful production of plant secondary metabolites in cell suspension cultures, organ cultures are summarized and presented in Table 2, adapted from Wawrosch and Zotchev, 2021.

Table 2: Plant Secondary metabolite produced through *in vitro* cultures

Compound	Plant species	Product yield (DW)	Culture type
Camptothecin	*Ophiorrhiza mungos*	0.86 mg/g	Adventitious root culture
		1.12 mg/g	Cell suspension culture
Indole alkaloids	*Isatis tinctoria*	3.15 mg/g	Hairy roots
Berberine	*Argemone Mexicana*	ca. 1.3 mg/g	Shoot culture
Capsaicin	*Capsicum chinense*	2.87 mg/g	Cell suspension culture

Dihydrocapsaicin		1.03 mg/g	Cell suspension culture
Chlorogenic acid derivatives	*Gardenia jasminoides*	20.98 mg/g	Cell suspension culture
Cichoric acid	*Ocimum basilicum*	6.90 mg/g	Cell suspension culture
Flavonoids	*Oplopanax elatus*	53.87 mg/g	Adventitious root culture
Isoflavones	*Trifolium pretense*	23.53 mg/g	*In vitro* cultivated plant
Flavonoids (chrysin, wogonin and baicalein)	*Scutellaria bornmuelleri*	163.42 mg/g	Hairy roots
Rosmarinic acid	*Salvia leriifolia*	6.41 mg/g	Cell suspension culture
	Ocimum basilicum	22.53 mg/g	Cell suspension culture
Isoquercetin	*Ocimum basilicum*	3.72 mg/g	Cell suspension culture
Rutin	*Ocimum basilicum*	6.54 mg/g	Cell suspension culture
Lignans (total)	*Linum album*	122.73 µg/g	Hairy roots
Podophyllotoxin	*Linum album*	47 µg/g	Cell suspension culture
Lariciresinol diglucoside	*Linum usitatissimum*	11.9 mg/g	Adventitious root culture
Artemisinin	*Artemisia annua*	1.12 mg/g	Hairy roots
Bilobalide	*Ginkgo biloba*	78 mg/g	Cell suspension culture
Ursolic acid	*Lantana camara*	3.87 mg/g	Cell suspension culture
	Salvia fruticosa	10.77 mg/g	Cell suspension culture
Withanolides	*Withania somnifera*	14.2 mg/g	Cell suspension culture
Ginkgolide A / B / C	*Ginkgo biloba*	79 / 71 / 7.5 mg/g	Cell suspension culture
Ginsenosides	*Panax quinquefolius*	87.6 mg/L	Cell suspension culture
	Panax sikkimensis	222.2 mg/L	Cell suspension culture
	Panax ginseng	32.46 mg/g	Adventitious root culture
		2.62–9.04 mg/g	Cell suspension culture
Steviol glycosides (stevioside + rebaudioside A)	*Stevia rebaudiana*	92.58 mg/g	Adventitious root culture
Oleanolic acid glycosides	*Calendula officinalis*	52.52 mg/g	Hairy roots
Paclitaxel	*Taxus x media*	2.47 mg/g	Hairy roots
	Corylus avellana	404.5 µg/L	Cell suspension culture
		402.4 µg/L	Cell suspension culture
		3.2 µg/g	Hairy roots
L-Dopa	*Hybanthus enneaspermus*	12.64 mg/g	Adventitious root culture

8.4. Applications of plant secondary metabolites

PSMs have been utilized to treat a variety of human disorders in addition to cancer and microbial diseases. PSMs overcome multi-drug resistance in various gastrointestinal and breast

malignancies. Flavonoids, quinones, alkaloids, terpenoids, and a variety of other structurally varied compounds are examples of PSMs having anti-cancer activity. Artemisinin, is currently used in all major treatments for malaria, caused by Plasmodium parasite. Flavonoids (such as catechins from tea plants), tannins, terpenoids (such as essential oils from peppermint and lavender), and 18 alkaloids (reserpine, berberine) have all been discovered to exhibit antimicrobial activity in plants. Psoralens Plus Ultraviolet Radiation Therapy (PUVA), uses the phototoxic phenolic chemical psoralens (a furanocoumarin) to treat skin-related disorders (e.g. psoriasis, an autoimmune disorder, eczema by boosting skin sensitivity to UV light. PSMs are attractive targets for bioprospective activities aimed at identifying new products for the pharmaceutical/biomedical, agricultural, and bioproducts industries because of their extensive structural and functional diversity.

Conclusion:

Large scale production of plant secondary metabolites (PSMs) is possible through in vitro cultures are possible through plant, cell and organ culture. Individual reports on production of PSM in various culture systems and their promising yield could be considered based on informed decision for upscaling the production of secondary metabolites under industrial scales. Several factors including market prices, feasibility of the production of same product through chemical synthesis, mandated regulatory guidelines and consumer acceptance of the final product has to be considered. Plant metabolic engineering and use of microbial system as Biofactories for expression could also be envisaged for secondary metabolite production.

References

Angelova Z, Georgiev S, Roos W (2006) Elicitation of plants. Biotechnology & Biotechnological Equipment 20, 72–83.

Arena ME, Postemsky PD, Curvetto NR (2017) Changes in the phenolic compounds and antioxidant capacity of Berberis microphylla G. Forst. berries in relation to light intensity and fertilization. Sci. Hortic. 218, 63–71.

Asada M, Shuler ML (1989) Stimulation of ajmalicine production and excretion from *Catharanthus roseus*: effects of adsorption in situ, elicitors, and alginate immobilization. Appl Microbiol Biotechnol 30:475–481

Biedinger U, Youngman RJ, Schnabl H (1990) Differential effects of electrofusion and electropermeabilization parameters on the membrane integrity of plant protoplasts. Planta 180: 598-602.

Chodisetti B, Rao K, Gandi S, Giri A (2015) Gymnemic acid enhancement in the suspension cultures of Gymnema sylvestre by using the signaling molecules—methyl jasmonate and salicylic acid. *In Vitro Cellular & Developmental Biology-Plant*, *51*(1), 88-92.

Dela G, Or E, Ovadia R, Nissim-Levi A, Weiss, D, Oren-Shamir M (2003) Changes in anthocyanin concentration and composition in 'Jaguar' rose flowers due to transient high-temperature conditions. Plant Sci. 164, 333–340.

Dhiman N, Patial V, Bhattacharya A (2018). The current status and future applications of hairy root cultures. In *Biotechnological approaches for medicinal and aromatic plants* (pp. 87-155). Springer, Singapore.

Dodds JH, Roberts LW (1985) Experiments in plant tissue culture. Second edition. Cambridge University Press, New York, p 232

Dornenburg H, Knorr D (1993) Cellular permeabilization of cultured tissues by high electric field pulses or ultra high pressure for the recovery of secondary metabolites. Food Biotechnol 7:35–48

Gutierrez-Valdes N, Häkkinen ST, Lemasson C, Guillet M, Oksman-Caldentey KM, Ritala A, Cardon F (2020). Hairy root cultures—a versatile tool with multiple applications. *Frontiers in Plant Science*, *11*, 33.

Hashimoto H, Morikawa H, Yamoda Y, Kimura A (1985) A novel method for transformation of intact yeast cells by electroinjection of plasmid DNA. Appl. Microbiol. Biotechnol. 21: 336-339.

Huang P, Xia L, Zhou L, Liu W, Wang P, Qing Z, Zeng J (2021). Influence of different elicitors on BIA production in Macleaya cordata. *Scientific Reports*, *11*(1), 1-7.

Kim DJ, Chang HN (1990) Enhanced shikonin production from *Lithospermum erythrorhizon* by in situ extraction and calcium alginate immobilization. Biotechnol Bioengin 36(5):460–466

Knobloch KH, Berlin J (1980) Influence of medium composition on the formation of secondary compounds in cell suspension cultures of *Catharanthus roseus* L. Naturforschung 35:551–556

Lee CWT, Shuler ML (2000) The effect of inoculum density and conditioned medium on the production of ajmalcine and catharanthine from immobilized *Catharanthus roseus* cells. Biotechnol Bioengin 67:61–71

Lo Piero AR Puglisi I, Rapisarda P, Petrone G (2005) Anthocyanins accumulation and related gene, expression in red orange fruit induced by low temperature storage. J. Agric. Food Chem. 53, 9083–9088.

Manuhara YSW, Kristanti AN, Utami ESW, Yachya A (2015) Effect of sucrose and potassium nitrate on biomass and saponin content of Talinum paniculatum Gaertn. hairy root in balloon-type bubble bioreactor. Asian Pac J Trop Biomed. 5:1027–1032.

Mori K, Yamamoto NG, Kitayama M, Hashizume K (2007) Loss of anthocyanins in red-wine grape under high temperature. J. Exp. Bot. 58, 1935–1945.

Mulder-Krieger T, Verpoorte R, Barhein Svedsen A, Scheffer, J (1988) Production of essential oils and flavours in plant cell and Tissue cultures, A review, Plant cell Tissue and Organ Culture, 13: 85- 154

Namdeo AG (2007) Plant cell elicitation for production of secondary metabolites. Pharmacognosy reviews :1, http://www.phcogrev.com 69

Nhan NH, Loc NH (2017). Production of eurycomanone from cell suspension culture of Eurycoma longifolia. *Pharmaceutical biology*, *55*(1), 2234-2239.

Qian J, Guiping L, Xiujun L, Xincai H, Hongmei L (2009). Influence of growth regulators and sucrose concentrations on growth and rosmarinic acid production in calli and suspension cultures of Coleus blumei. *Natural product research*, *23*(2), 127-137.

Rao RS, Ravishankar GA (1999) Biotransformation of isoeugenol to vanilla flavor metabolites and capsaicin in freely suspended and immobilized cell cultures of *Capsicum frutescens*: study of the influence of *b*-cyclodextrin and fungal elicitor. Process Biochem 35:341–348

Sakamoto K, Iida K, Sawamura K, Hajiro K, Asada Y, Yoshikawa T, Furuya T (1994) Anthocyanin production in cultured cells of *Aralia cordata* Thunb. Plant Cell Tissue Org Cult 36(1):21–26

Trejo-Tapia G, Cuevas-Celis J, Salcedo-Morales G, Trejo-Espino H-L, Arenas-Ocampo ML, Jiménez- Aparicio A (2007) *Beta vulgaris* suspension cultures permeabilized with triton x-100 retain cell viability and betacyanines production ability: a digital image analysis study. Biotechnol Prog 23(2):359–363

Wawrosch C, Zotchev SB (2021) Production of bioactive plant secondary metabolites through in vitro technologies—status and outlook. *Applied Microbiology and Biotechnology*, *105*(18), 6649-6668.

Yang L, Wen KS, Ruan X, Zhao YX, Wei F, Wang Q (2018). Response of plant secondary metabolites to environmental factors. *Molecules*, *23*(4), 762.

Zobayed, S.M.A.; Afreen, F.; Kozai, T. Phytochemical and physiological changes in the leaves of St. John's wort plants under a water stress condition. Environ. Exp. Bot. 2007, 59, 109–116.

Advances in Plant Science Volume IV

ISBN: 978-93-91768-53-9

About Editors

Dr. Bhagwan D. Bulchandani completed his PhD from M.D.S. University, Ajmer, he has qualified NET-JRF and has been awarded Junior Research Fellowship by CSIR, New Delhi. Since last more than sixteen years, he has been involved rigorously in academic as well as administrative responsibilities in various capacities including Head, Department of Biotechnology for more than seven years at Sobhasaria Group of Institutions, Sikar, Rajasthan. Dr. Bulchandani has published several book chapters, research and review papers at national as well as international levels and has been on the editorial board of many reputed journals. A book NEET Biology, from Prabhat Publication, New Delhi is also credited to his contribution in academics. His research interests are Microbial Technology, Antibiotic Production, Genomics, Nanomedicine, Nutraceuticals, Probiotics, Secondary Metabolites, Green Technology to name a few. He has been the convener of two national conferences and has also published several articles on diverse topics in newspapers and magazines. Dr. Bulchandani also holds life membership of several professional societies.

Dr. Kavita Chahal is working as an Assistant Professor at Department of Botany, Government College Bichhua, Chhindwara, M. P. She obtained Ph. D in Microbiology; and has cleared CSIR-NET in Life Sciences and ASRB-NET in Agricultural Microbiology. She is an Editorial Board Member of American Journal of Life Sciences; International Journal of Current Science Research & Review; International Journal of Scientific Research in Biological Science; International Journal of Current Science Research and Review; Vidwan, an MHRD project (ID:175930), Active RMS member (ID: 116866) of International Journal of Creative Research Thoughts; and Member of Indian Academy of Sciences, Delhi. She has to her credit 24 Research papers, 9 Book chapters and 6 Books as an Editor.

Dr. Parashurama T. R (M.Sc., PGDMP., Ph.D) is Founder Secretary of Panchavati Research Academy for Nature (PRANA), Kalamaji, Central Western Ghats, Shimoga and Former Principal of Kumadvathi First Grade College, Shikaripura. He received Bachelor science (2003), Master of Science in Botany (2005) and Ph.D (2014) on Medicinal plant Pathology from Kuvempu University. His research interest includes medicinal plants, Ethno-botany, Pteridology, Phyto-chemistry and Plant Biodiversity. He was awarded JRF and SRF under DST, New Delhi sponsoring Project. He has published 34 research papers in National and International Journals, one book and two book chapters in national edited volumes. He has participated (36) in many National and international Seminars, Conferences and workshops with presented 11 papers. He contributed many significant new reports on medicinal plant diseases. He has excellent work experience in teaching, Administrative and research with various academic institutions and Universities.

Dr. Minal Shriram Patil is an Assistant Professor in the Department of Pharmacognosy, DCS's Annasaheb Ramesh Ajmera College of Pharmacy, Nagaon, Dhule (MS), INDIA. She has completed her Ph.D. from KBC North Maharashtra University, Jalgaon, Maharashtra. She has published more than 25 papers in reputed journals and presented more than 30 papers in national and international conferences. She received several awards and medals. Earlier she has delivered lectures on several topics in international and national conferences. She is also serving as Reviewer and Editorial member in reputed journals. She has guided UG and PG students in their project work. One International Book is also published with owe to her name.

Bhumi Publishing

ISBN 978-93-91768-53-9
9 789391 768539