RAMAKRISHNA MISSION VIVEKANANDA CENTENARY COLLEGE

TOPIC : " POLLEN BIOLOGY AND SEED DISPERSAL OF *Cleome viscosa* "

SUBMITTED BY : Subhradeep Sarkar

REGISTRATION NO. : A01 – 1112 – 114 – 001 –2019

COLLEGE ROLL NO. : 401

SEMESTER : V

DEPARTMENT : **Botany**

CONTENTS

❖ POLLINATION OBSERVED IN Cleome viscosa

Unique floral architecture of *Cleome viscosa* with bright yellow colour and production of ample pollen attracts a variety of insects. Insect foragers were found to visit all three flower morphs indiscriminately during 07:00–12:00 h, with peak activity during 08:00–09:00 h. *Cleome* sp. flowers are complete, bisexual, i.e., with functional male (androecium) and female (gynoecium), including stamens, carpels and ovary. Pollination is entomophilous i.e., by insects, or cleistogamy i.e., by self-pollination or allogamy i.e., by cross pollination.

The insects recorded were bees *(Apis dorsata*, *A. cerana, A. florea, Trigona iridipennis (Apidae), Halictus albescens , Nomia curvipes Nomia elliotii* and one unidentified bee (*Halictidae*), a fly *(Helophilus sp.)* and butterflies *(Pachliopta hector, Catopsilia pomona , C. pyranthe, Eurema hecabe, Pieris canidia, Cepora nerissa and Anaphaeis aurota, Acraea violae* and *Danaus chrysippus, Castalius romison* and *Chilades laius* **.** Though these insects visited both hermaphrodite and staminate flowers indiscriminately during early morning hence foraging was intense on large flowers. *A. dorsata* and *N. curvipes* visits for short durations while those of *H. albescens* and *N. elliotii* were of longer duration. The number of flowers foraged by them in a minute was also comparatively more. During the foraging activity, these insects shifted from one flower to another and ensured transfer of pollen. (Saroop and Kaul, 2015)

Bees and butterflies were the regular foragers during the entire flowering season. The bees foraged for both pollen and nectar, the fly forages only for pollen and butterflies only for nectar . These insects approached the flowers in upright position, landed on the petals and explores the flower for both pollen and nectar. While collecting pollen from the anthers, the bees comes in contact with their forehead and ventral side, result in pollen transfer from the anthers to their body. They show no difference in behavior between the stamens and the stigma and, hence, attempts to collect pollen from both sex organs were considered to be resulting in pollination.

In case of collection of nectar, the bees reaches to the nectaries and they comes in direct contact with their ventral as well as the dorsal surface with the stamens and stigma. This nectar probing behavior also contributes in pollination. The bees relatively spends more time per flower while collecting pollen, as compared to nectar collection. While collecting nectar, both bees and butterflies moves rapidly between flowers and plants . (Raju and Rani, 2016)

Light being a very important factor for induction and development of flowers in *Cleome viscosa* under low light (10–15 mol m−2 s−1 PFD) conditions, flowers as well as fruits developed properly. The shoots did not flower in the dark. (Rathore et. al)

❖ POLLEN GERMINATION AND POLLEN TUBE GROWTH

The pollen germination and pollen tube growth are important research material for morphological, physiological, biotechnological, ecological, environmental, evolutional, biochemical and molecular biological studies . Pollen tube elongation is a physiological process in which pollen tube navigates and responds to female tissues to release the sperm cell into the ovary for fertilization. Pollen tube extends entirely at the cell apex in form of an extreme polar growth, known as tip growth, producing uniformly cylindrical cells. Pollen tubes are excellent system for the study of polarized tip growth, cell movement, cell to cell communication, cell to cell recognition , signaling and importance of cytoskeleton in cell growth and differentiation in plants . Pollens normally germinate on stigma (Unal ,1986,1988) and the required environment for in vitro pollen germination is related to genetic composition and also the quality of nutrient reserves of pollen (Baker and Baker , 1979 ; Unal ,1986,1988)

❖ FRUIT SET

The fruit set was largely determined for hermaphrodite flowers of both the sizes and for a few pistillate flowers as well. The numbers before and after fruit formation were recorded and fruit set per plant (n=30) was estimated . The data were evaluated and average fruit set per plant was calculated. In addition, 20 flowers (n=2 per 10 plants) were emasculated, tagged and kept undisturbed for in vivo pollination. This was done to see if insects transfer pollen from one plant to another and ensure pollination. Another set of equal sample size were emasculated and bagged to check fruit set due to non pseudogamous apomixes. (Saroop and Kaul, 2015)

The fruit growth and development begins immediately after pollination and fertilization, during which the ovary elongates. The fruits mature within a week and change its color from green to brown. The natural fruit set is 98 % in SGF, 100 % in MGF and 99 % in LGF . The mature and dry fruits vary in length with each flower morph. The fruits produced from SGF are 5.30 ± 0.51 cm, those from MGF 6.30 ± 0.32 cm and those from LGF 7.40 ± 0.40 cm. The dry fruits are long-stalked, linear, hairy, sub - erect, and cylindrical capsules tapering at both ends, the upper end forms 3 mm long stipe. They dehisce from the tip to the base by the separation of false septum formed in ovary and release seeds into the air. The seeds are brown, sub - globose or orbicular with narrow cleft, 1–1.5 mm diameter, with strong cross-ribs and weak concentric ribs. The seed output per fruit varies with the flower morph: it is 89.80 ± 28.48 in SGF, 114.23 ± 19.80 in MGF and 152.61 ± 40.37 in LGF. Seed set rate varies with each flower morph: it is 90 % in SGF, 99 % in MGF, 94 % in LGF . Ovule arises as a mound of tissue on a parietal placenta and finally becomes campylotropous owing to the pronounced growth of the nucellus at the chalazal end. Ovules are bitegmic and crassinucellate. The inner integument develops first followed by the outer integument. The micropylar canal is formed by the pronounced growth of the outer integument over the inner and is zigzag . (Raju and Rani, 2016)

❖ SEED COAT

At first the outer and the inner integuments are bi-layered. Outer integument remains bi-layered even when it differentiates into testa. The outer layer of the testa becomes thick-walled while the cells of the inner layer enlarge and becomes vacuolated. The two-layered tegmen which arises from the inner integument subsequently becomes multilayered. The epidermal layer becomes conspicuous with rich cytoplasmic contents. During further development the outer epidermal cells at the micropylar end becomes vacuolated, enlarge finally and develop noticeable striations .The innermost layer of the tegmen becomes transformed into heavy walled sclereids. (Rao)

❖ SEED DISPERSAL

Seeds may be dispersed by autochory i.e., self-dispersal, anemochory i.e., wind dispersal, zoochory i.e., dispersal by birds or animals, anthropochory i.e., dispersal by humans. The seeds are dormant and germinate during rainy season. Seeds are minute and light in weight and disperse by wind during dry season and by rain water during rainy season. The plant reproduces exclusively by seed

❖ CLIMATE CHANGE FACTOR

The small leaved accessions can be differentiated from the large leaved accessions by having significantly more number of leaves and pods/plant and by having early flowering. Large leaved accessions have bore pods of significantly larger size than those in small leaved accessions. Heritability's of several characters were moderately high (number of pods/plant, seed yield/plant and days to flower).

The variances for all the thirteen characters of genotypes, seasons and genotypes x seasons interaction were significant. Among the different seasons : May to August (summer-monsoon -1), June to September (summer-monsoon-2), July to October (monsoon-autumn-1), August to November (monsoon-autumn-2)-on average bases the crops demonstrated better growth and seed productivity in the summer-monsoon-1 and -2 seasons (May to August and June to September) as compared to the monsoon-autumn-1 and -2 seasons (July to October and August to November). So harvest index was higher in the monsoon-autumn seasons. (Kumari et al, 2012)

❖ CONCLUSION

Cleome viscosa L. has several fascinating floral characters of both the evolutionary and functional importance. This article explains the prevalence of its variation in sex and size of flowers within the individual plants. This variation was further expressed differently over the two flowering months of July and August. Initially, i.e., in July, the frequency of these flowers was low; an increase occurs gradually and considerably in successive developing inflorescences during the month of August. Small sized flowers of all sex, differentiated in lesser number than their large counterparts. Staminodal tendency/stamen sterilization was almost equal among flowers of all sizes and sexes; and apparently increases with the age of the plant and the time of differentiation of the flower. Notwithstanding this, hermaphrodites of all types were produced in greater number. This marks that plants have a strong tendency towards hermaphroditism. That doesn't permit the plant's tendency to develop flowers of other sex expressions. Both the differentiation of staminate flowers and the hermaphrodite once commenced early. The subtending fruits signal seems to be the termination of pistil in the otherwise hermaphrodite flowers leads to a bulk of staminate flowers at the distal ends. (Swaroop and Kaul, 2014)

❖ REFERENCES

i. Plant Systematics : An Integrated Approach , Fourth Edition by Gurcharan Singh

ii. Aizen, M.A. & Feinsinger, P. 1994. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. – Ecology, 75: 330-351.

iii. Bhandari M M, Flora of the Indian Dessert, 1st ed. (Scientific, Jodhpur) 1978, 42.

iv. Aparadh, V.T. & Karadge, B.A. 2011. Microscopic pollen analysis of some selected Cleome species. – Trajectory, 19: 21-31.

v. Oommachan M, The Flora of Bhopal. 1st ed., J.K. Jain Brothers, Bhopal, 1977, 46.

vi. Nadkarni A K, The Indian Materia Medica, Vol. 1 (Popular Prakashan, Bombay), 1982, 351.

vii. Aparadh, V.T., Mahamuni, R.J. & Karadge, B.A. 2012. Taxonomy and physiological studies in spider flower (*Cleome* species): a critical review. – Pl. Sci. Feed, 2: 25-46.

viii. Asolkar, L.V., Kakkar, K.K. & Thakre, O.J. 1992. Second supplement to Glossary of Indian Medicinal Plants with Active Principles. PID, CSIR, New Delhi, p. 215.

ix. Duthie JF, Flora of the Upper Gangetic Plain and of the Adjacent Siwalik and Sub-Himalayan Tracts, 1st ed. Vol. 1 (Part I-II) (M/s. Bishen Singh Mahendra Pal Singh Dehra Dun), 1973, 50.

x. Maikhuri RK, Semwal RI, Rao KS, Nautiyal S and Saxena KG, Cleome viscosa, Capparidaceae: A weed or a cash crop?, Econ Bot, 2002, 54, 150.

xi. Aziz, S. & Shaukat, S.S. 2012. Population ecology of Cleome viscosa L., a desert summer annual. – Pakistan J. Bot,. 44: 1633-1638.

xii. A.J. Solomon Raju & D. Sandhya Rani , Sofia, 2016. Reproductive ecology of Cleome gynandra and Cleome viscosa (Capparaceae) : Phytologia Balcanica 22 (1): 15 – 28.

xiii. Anburaj J, Ravinder SC, Kuberan T, Sundaravadivelan C and Kumar P, Effects of plant growth regulators on callus induction from leaf explants of Cleome viscose, Res J Pharmac, Biol Chem Sci, 2010, 2, 576.

xiv. Maheswari J K, The Flora of Delhi, 1st ed, Council of Scientific and Industrial Research, New Delhi, 1976, 63.

xv. Rukmini C, Chemical, nutritional and toxicological evaluation of the seed oil of Cleome viscosa, Indian J Med Res, 1978, 67, 604.

xvi. Sudhakar M, Rao CV, Rao PM and Raju DB, Evaluation of antimicrobial activity of Cleome viscosa and Gamelina asiatica, Fitoterapia, 2006, 77, 47.

xvii. Saroop, S. and Kaul, V. (2011). Phenological events of Cleome viscosa L. growing in Jammu district. The International Journal of Plant Reproductive Biology 3(2): 161-164.

xviii. Singh, G., Ram, I.C. and Singh, D. (1991). Crop-weed competition studies in green gram and black gram. Tropical Pest Management, 37: 144-148.

xix. Williams, L.A.D., Vasques, E., Reid, W., Porter, R. and Kraus, W. (2003). Biological activities of an extract from Cleome viscosa L. (Capparaceae). Naturwissenschaften, 90: 468-472.

xx. Devi, B.P., Boominathan, R., Mandal, S.C. (2002). Evaluation of antidiarrhoeal activity of Cleome viscosa Linn. extracts in rats. Phytomedicine, 9(8): 739-742.

xxi. Reddy, C.N., Reddy, M.D., Devi, M.P. (2000). Efficency of various herbicides on weed control and yield of brinjal. Indian Journal of Weed Science, 32 (3 and 4).

xxii. Reddy, M.M., Vilatha, A.M. and Rao, L.J. (2007). Integrated weed management in pigeon pea (Cajanus cajan) and soybean (Glycine max) intercropping system on vertisol under rainfed conditions. Indian Journal of Agricultural Sciences 77(3): 177-178.

xxiii. Saroop, S. (2011). A preliminary study on seed to seed cycle of Cleome viscosa L. M.Phil. Dissertation, University of Jammu, Jammu

xxiv. Raju AJS, Ramana KV, Rao NG & Varalakshmi P, Monoecy and entomophily in Cardiospermumcanescens Wall. (Sapindaceae), a medicinally valuable herbaceous vine. Curr Sci, 101 (2011) 617.

xxv. Kaul V & Koul AK, Floral phenology in relation to pollination and reproductive output in Commelina caroliniana (Commelinaceae). Aust J Bot, 56 (2008) 59.

xxvi. Narbona E, Ortiz PL & Arista M, Functional andromonoecy in Euphorbia (Euphorbiaceae). Ann Bot, 89 (2002) 571.

xxvii. Strauss SY &Whittall JB, Non-pollinator agents of selection on floral traits. In: Ecology and Evolution of flowers (eds.Lawrence D Harder & Spencer CH Barrett, Oxford University Press, Oxford), 2006, 120.

xxviii. Solomon BP, Environmentally influenced changes in sex expression in an andromonoecious plant. Ecology, 66 (1985) 1321.

xxix. Chauhan S, Rana A, Sharma SB & Chauhan SVS, Reproductive biology of Cleome viscosa L. (Capparaceae). Ann For, 15 (2007) 343.

xxx. Goodwillie C, Kalisz S & Eckert CG, The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. Annu Rev Ecol Syst, 36 (2005) 47.

The study on use of ten popularly known herbal plants as anti-diarrheal medicines: a small survey.

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Dedicated to,

My parents & teachers

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I would like to express my special thanks of gratitude to all the faculty members of Department of Botany, Ramakrishna Mission Vivekananda Centenary College, Rahara, Kolkata for their able guidance and support in completing my project.

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Kiran Mandal

Date:

Table of contents:

1. Introduction

1.1. History of traditional medicine

Indian Traditional Medicine (ITM), the foundation of the age-old practice of medicine in the world, has played an essential role in human health care service and welfare from its inception. All traditional medicines have their local effects and are used extensively in West Asian nations like India, Pakistan, Tibet, and so forth, East Asian nations like China, Korea, Japan, Vietnam, and so forth, Africa, South, and Central America. This article is an attempt to illuminate Indian traditional medical service and its importance, based on recent methodical reviews. The World Health Organization (WHO) defines traditional medicine as health practices, approaches, knowledge, and beliefs incorporating plant, animal, and mineral-based medicines, spiritual therapies, manual techniques, and exercises, when applied solely or in combination treat, diagnose and prevent illness and help in maintaining well-being. Several countries in Africa, Asia, and Latin America are found to use traditional medicine (TM) in their primary health care system. A major portion of the African population uses traditional medicine for primary health care. Traditional medicine has maintained its popularity in all regions of the developing world and its use is rapidly spreading in industrialized countries. In China, for example, traditional herbal preparations account for 30%-50% of the total medicinal consumption. In Ghana, Mali, Nigeria, and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicines at home. A major portion of the population in developing countries does not have access to essential medicines. The implementation of safe and efficient TM/CAM therapies can hence become a key tool in increasing access to health care. Most of the countries have not officially recognized traditional medicine even though it exists and is popular and has been used very much in the last decade. As a result of which, education, training, and research in this domain lack due attention and support. The distinction along with the safety and efficiency of data on traditional medicine is very far from adequate to cater to the criteria needed in worldwide support to be used. One of the reasons for the lack of research data is due to health care policies.

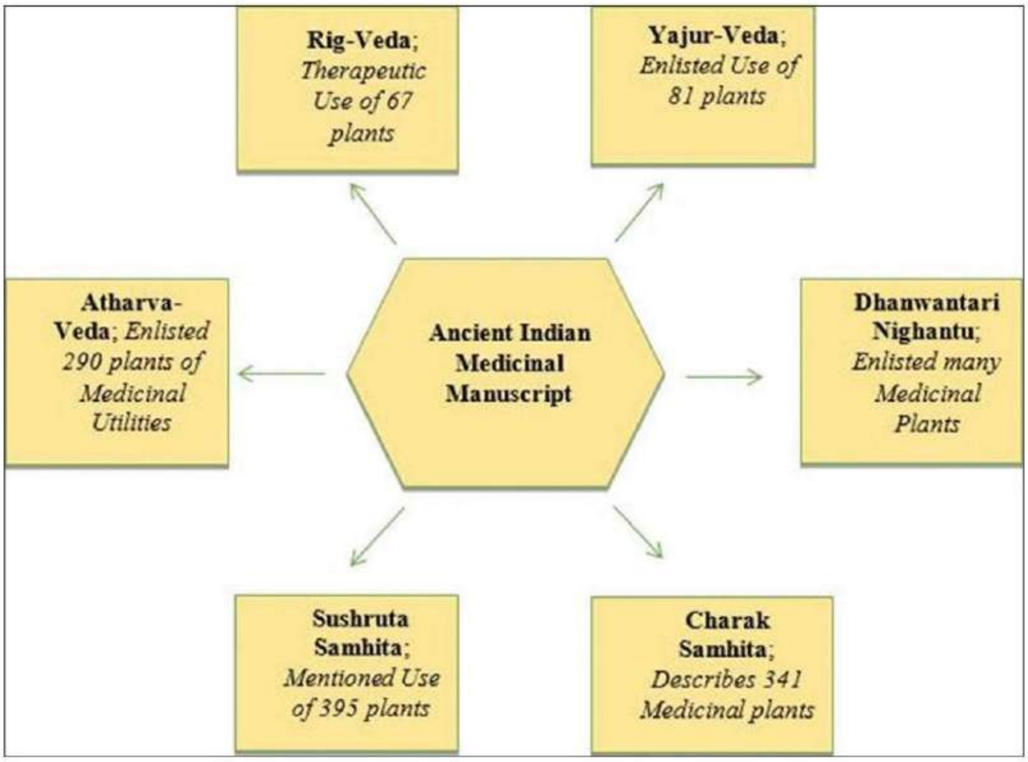


Figure 1. History of Indian traditional medicine (Asian J Pharm Clin Res, Vol 11, Issue 1, 2018, 421-426)

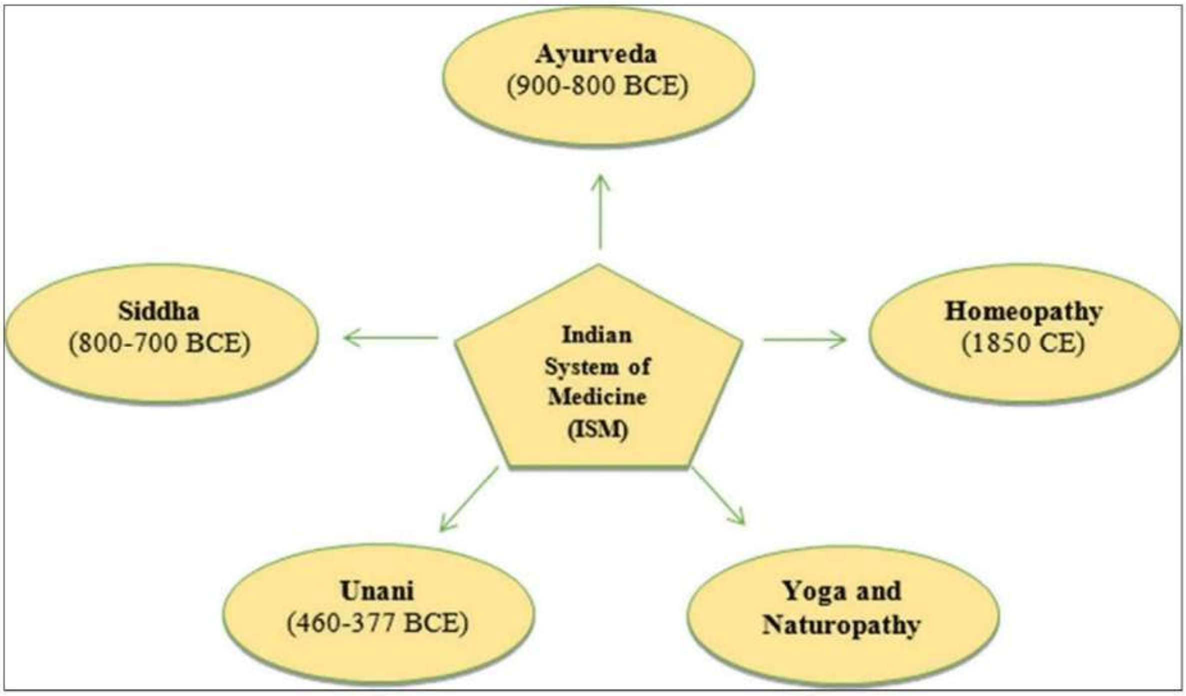


Figure 2. Popular types of traditional medicine practiced in India (Asian J Pharm Clin Res, Vol 11, Issue 1, 2018, 421-426)

1.2. Importance of traditional medicine

Considerable knowledge accumulated by the villagers and tribes on herbal medicine remains unknown to the scientists and urban people. Many plant species associated with the rural people are on the verge of disappearing and are on the vulnerable list. The impact of deforestation, urbanization, and modernization is shifting the rural people from their natural habitats, and their very knowledge, particularly concerning herbal drugs is slowly disappearing. Our immediate concern is to preserve this knowledge. Whatever knowledge exists today is mostly confined to the older generation. In this context, some approaches needed for the preservation and development of traditional knowledge are presented here, based on the author's experience in the ethno-medico-botanical survey for two decades.

i) **Contribution to primary health care:** About 30% of the people of the world's population regularly use traditional medicine. It is used in the control of health care and helps to identify the nature of illness by examination of the symptoms. We surely see that nowadays a major part of the world, including India, relies on traditional medicine.

ii) **Cost-effective:** It is one of the most famous traditions because it is less costly. Hence, it is affordable to the poor and downtrodden. These medicines are easily available in the market nowadays and as a result, they are accessible to the poor as well.

iii) **Fewer side effects:** Another important attribute of traditional medicine is that it has fewer side effects. That is why it has been widely used in India, Japan, Canada, Australia, France, and other countries.

1.3. Use of medicinal plants in diarrhea

Diarrhea is a leading cause of child and adult mortality in India. This paper deals with ethnomedicinal plants documentation and information which are used by the tribal of Majhgawan block of Satna district for the cure of diarrhea. The study revealed the use of 12 plant species by different tribes for the treatment of diarrhea. These plant species are enumerated alphabetically with their botanical name, vernacular name, family, plant parts used, and the way of using them medicinally. Holarrhena antidysenterica, *Curcuma amada*, *Ficus glomerata,* and *Butea monospermawere* are the plants used by more than one tribe for treating diarrheal conditions. *Holarrhena antidysenterica* was reported to be used by 4 different tribes of the region for the correction of diarrhea. Dominating tribes of the region Kol, Gond, and Mawasi utilized 50%, 33%, and 25% of the plant species reported to be used as a remedy of diarrhea in the region.

Table 1. List of ten popularly known plants used as anti- diarrheal medicines

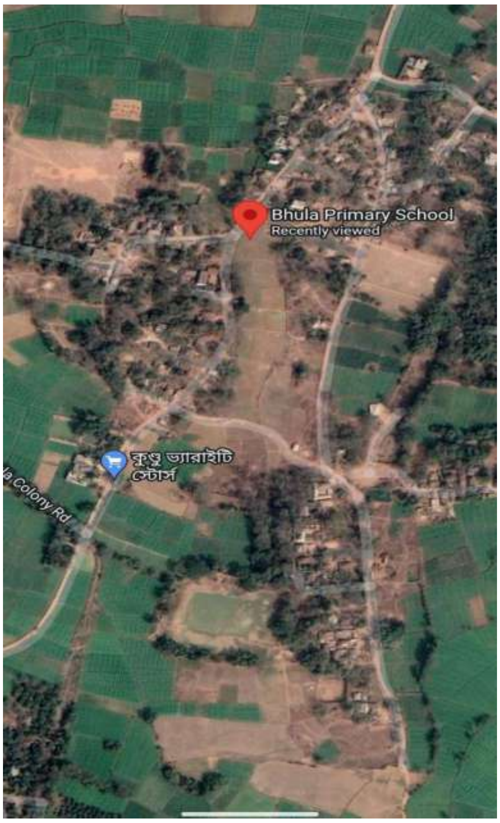
Serial No	Scientific Name	Common Name	Family
1.	*Asparagus racemosus*	Thanneervittaan Kizhangu	Liliaceae
2.	*Bauhinia purpurea*	Mandhaarai	Caesalpiniaceae
3.	*Borassus flabellifer*	Panai	Arecaceae
4.	*Carica papaya*	Pappaali	Caricaceae
5.	*Cissus quadrangularis*	Pirandai	Vitaceae
6.	*Cynodon dactylon*	Arugambull	Poaceae
7.	*Psidium guajava*	Common Guava	Myrtaceae
8.	*Gmelina arborea*	Gamhar	Lamiaceae
9.	*Acacia nilotica*	Gum arabic tree	Fabaceae
10.	*Vitex doniana*	Black plum	Lamiaceae

2. Materials and methods

2.1. Study area

Bansdiha (Pashchim Medinipur)

Nalbona (Pashchim Medinipur)



Bhula (Pashchim Medinipur)

Daldali (Pashchim Medinipur)

2.2. Methods

The survey was carried forward according to the documentation of ethnomedicinal plants documented by Savithramma et al., 2017

3. Results

The questionnaire forms are incorporated in original at the end.

4. Discussion

The medicinal properties of different plants have been reported and practiced since historic age. Many studies that have been carried out globally to proof their efficacy. Some findings have led to the production of plant-based medicines, but the examples are few. Most of the plant-based medicines used in recent days are in crude form. The active principles and specific mode of action in most of the cases are unknown. Many approaches are taken to investigate the role of plants and plant-based medicines in different common diseases. Most of the efforts are discreate and non-consistent. The detailed analysis with a specific plant and their compound in case-by-case manner is absent. Most of the herbal medicines used are based on knowledge of ethnobotany. The systematic assemblage of the traditional medicinal plants and common information center is missing. The repository of all sorts of information regarding medicinal plants is urgently necessary to develop the herbal medicine technology in the future. Detailed analysis and survey with the commonly used medicinal herbs are also necessary prerequisite. With the limited scope of this project ten very commonly used traditional medicinal plants used for curing diarrhea were analysed. During the analysis a series of questions were asked to the random participants. The questionnaire was formulated based on herbal medicine society. The plants analysed are, *Asparagus racemosus, Bauhinia purpurea, Borassus flabellifer, Carica papaya, Cissus* *quadrangularis, Cynodon dactylon, Psidium guajava, Gmelina arborea, Acacia nilotica, Vitex doniana.*

Name of the plant	Number of users (Out of 30)	Percentage of users	Number of male users	Number of female users	Percentage of male users	Percentage of female users
Psidium guajava	7	23	3	4	50	50
Vitex doniana	3	10	2	1	66.6	33.3
Asparagus racemosus	5	16	3	2	60	40
Acacia nilotica	6	20	2	4	28	72
Bauhinia purpurea	4	13	1	3	25	75
Borassus flabellifer	0	0	0	0	0	0
Carica papaya	5	16.6	3	2	60	40
Cissus quadrangulari s	3	10	2	1	66.6	33.3
Cynodon dactylon	5	16.6	3	2	60	40
Gmelina arborea	4	13.3	2	2	50	50

5. References

1. WHO. Legal Status of Traditional Medicine and Complementary/Alternative Medicine: A World Wide Review. Geneva 2001.

2. WHO. Fact Sheet, Traditional Medicine, Geneva, May 2003.

3. Bannerman RH, Burton J and Chien W. Traditional Medicine and Health Care Coverage. World Health Organization, Geneva, Switzerland 1993.

4. WHO. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine WHO/EDM/TRM/2000. 1, Geneva, Switzerland 2000.

5. Lambert J. Ethiopia: Traditional medicine and the bridge to better health. World Bank, available at: http://www.worldBank.rog/afr/ik/dfefault.htm.

6. Bhanot A, Sharma R, Noolvi MN. Natural sources as potential anti-cancer agents: A review. Int J Phytomed 2011;3:9-26.

7. Demain AL. Importance of microbial natural products and the need to revitalize their discovery. J Ind Microbiol Biotechnol 2014;41:185-201.

8. Katz L, Baltz RH. Natural product discovery: Past, present, and future. J Ind Microbiol Biotechnol 2016;43:155-76.

9. World Health Organization. WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems. WHO; 2004.

10. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol 2014;4:177.

11. Cragg GM, Newman DJ. Natural products: A continuing source of novel drug leads. Biochim Biophys Acta 2013;1830:3670-95.

12. Borchardt JK. The beginnings of drug therapy: Ancient mesopotamian medicine. Drug News Perspect 2002;15:187-92.

13. Huang KC. The Pharmacology of Chinese Herbs. 2nd ed. Boca Raton, FL: CRC Press; 1999.

14. Savithramma, Nataru, et al. "Documentation of ethnomedicinal information and antimicrobial validation of Thespesia populnea used by Yanadi tribe of Ganugapenta village, Chittoor district, Andhra Pradesh, India." Journal of intercultural ethnopharmacology 6.2 (2017): 158.

6. The questionnaire forms:

RAMAKRISHNA MISSION VIVEKANANDA CENTENARY COLLEGE

A PROJECT WORK ON

MINERALS IN PLANTS: BENEFICIAL AND NON-BENEFICIAL ROLES

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INTRODUCTION :-

The basic need shares by all the living organisms that is water, macromolecules and minerals are same. Mineral is a chemical compound, that has a well-defined chemical combination and which bears a specific crystalline structure. Minerals are abundant in nature and is produced by the natural geological processes on our Earth.

Minerals are largely found in the soil and food which is very important for functioning the living world i.e. plants and animals. Plants use inorganic minerals for their nutrition. Roots absorb mineral nutrients from soil water in ionic forms. Complex interactions that is weathering of rock minerals, decay of organic matter, animals and microbes takes place to form inorganic minerals in soil. There are many factors which influences the nutritional uptake of minerals by plants. Ions can be easily available to roots or could be tied up with other elements or soil itself. Plant nutrition is a term that takes into account that the interrelationship of mineral elements in the soil or soil less solution as well as their role in plant growth. It involves a complex balance of mineral elements essential and beneficial for optimum growth of plants.

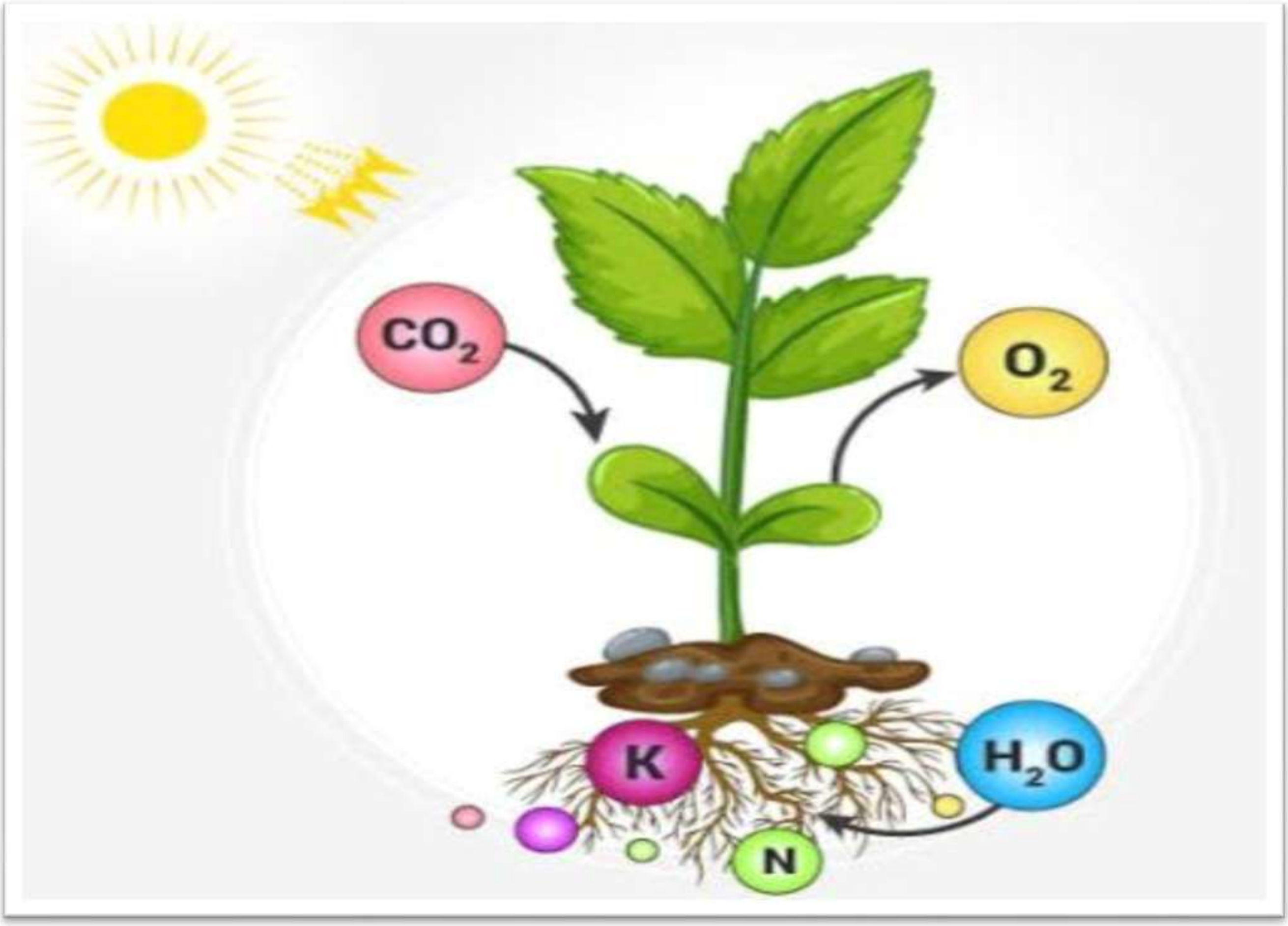

Figure 1

CLASSIFICATION OF PLANT MINERAL NUTRIENTS :-

Mineral nutrients are circulating among all the living organisms but they enter into the biosphere mainly through the root system of the plant. Plants spread their roots very deep into the soil in search of water and minerals. Though most of the time water is available near the root surface plants have to spread their roots deep for mineral uptake. Large surface area of roots helps them to absorb minerals in very low concentration. After absorbing the minerals plants translocate them into various parts of them.

Plants uptake minerals as their nutrients and they acquire Nitrogen, Potassium, and Phosphorus in ionic form. In nature there are plenty of minerals but plants only took 16 elements from nature. These 16 elements are obtained from the soil. These 16 elements are called "Essential Element". The term essential mineral element or mineral nutrients was coined by Arnon and Stout in 1939. They have concluded three criteria for an element to be considered as essential. These criteria are: 1. A plant must be unable to complete its life cycle in the absence of the mineral element.

2. The function of the element must not be replaceable by any other mineral element.

3. The element must be directly involved in plant metabolism.

These essential elements are very important in the structure or metabolism of plants and absence of these elements cause few abnormalities in plant growth and development. These essential elements are categorized into two types according to their relative concentration in plant tissue -

1. Macronutrients.

2. Micronutrients.

There is another term beneficial elements which can compensate the toxic effects of other elements or may be replace some essential elements to some extent. But this beneficial elements are not essential for all plants but for very few plants these beneficial elements are essential. A table of essential elements and their concentration in plant cell is given here in the next page.

A list of elements are tabulated in table form as follows:-

TABLE: 1

ELEMENT	CHEMICAL SYMBOL	CONCENTRATION IN DRY MATTER (% OR ppm)[a]	RELATIVE NUMBER OF ATOMS WITH RESPECT TO MOLYBDENUM
Obtained from water or carbon dioxide			
Hydrogen	H	6	60,000,000
Carbon	C	45	40,000,000
Oxygen	O	45	30,000,000
Obtained from the soil			
Macronutrients			
Nitrogen	N	1.5	1,000,000
Potassium	K	1.0	250,000
Calcium	Ca	0.5	125,000
Magnesium	Mg	0.2	80,000
Phosphorus	P	0.2	60,000
Sulfur	S	0.1	30,000
Silicon	Si	0.1	30,000
Micronutrients			
Chlorine	Cl	100	3,000
Iron	Fe	100	2,000
Boron	B	20	2,000
Manganese	Mn	50	1,000
Sodium	Na	10	400
Zinc	Zn	20	300
Copper	Cu	6	100
Nickel	Ni	0.1	2
Molybdenum	Mo	0.1	1

[a] The values of non-mineral elements (H,C,O) and the macronutrients are percentages (%). The value of micronutrients are shown in parts per million(ppm).

According to some researchers classification of essential elements into macronutrients and micronutrients is difficult to justify physiologically. Mengel and Kirkby in the year 2001 have proposed that the essential elements may be classified according to their biochemical role and physiological function. They proposed four groups into which the plant nutrients fit. These four basic groups are :

TABLE: 2

MINERAL NUTRIENT	FUNCTION
Group 1	Nutrients that are part of carbon compounds
Nitrogen (N)	It is constituents of amino acids, amides, proteins, nucleic acids, nucleotides, coenzymes, hexosamines, etc.
Sulfur (S)	It is the component of cysteine, cystine, methionine. It is also the constituents of lipoic acid, coenzyme A, thiamine pyrophosphate, glutathione, biotin, 5'-adenylylsulfate, and 3'-phosphoadenosine.
Group 2	Nutrients that are important in energy storage or structural integrity
Phosphorus (P)	It is the component of sugar phosphates, nucleic acids, nucleotides, coenzyme, phospholipids, phytic acid, etc. It has a key role in reactions that involve ATP.
Silicon (Si)	It is deposited as amorphous silica in cell walls. It also contributes to cell wall's mechanical properties including rigidity and elasticity.
Boron (B)	It complexes with maninitol, mannan, polymannuronic acid, and other constituents of cell walls. It is involved in cell elongation and nucleic acid metabolism.

Group 3	Nutrients that remain in ionic form
Potassium (K)	It is required as a cofactor for more than 40 enzymes. Principal cation in establishing cell turgor and maintaining cell electoneutially.
Calcium (Ca)	It is the constituents of the middle lamella of the cell walls. It is required as a cofactor by some enzymes involved in the hydrolysis of ATP and phospholipids. It acts as a second messenger in metabolic regulation.
Magnesium (Mg)	It is required by many enzymes involved in phosphate transfer. It is the constituents of the chlorophyll molecule.
Chlorine (Cl)	It is required for the photosynthetic reactions involved in O2 evolution.
Zinc (Zn)	It is the constituents of alcohol dehydrogenase, glutamic dehydrogenase, carbonic anhydrase etc.
Sodium (Na)	It is involved with the regeneration of phosphoenolpyruvate in C4 & CAM plants. It substitutes for potassium in some functions.
Group 4	Nutrients that are involved in redox reaction
Iron (Fe)	It is the constituent of cytochromes and nonheme iron proteins involved in photosynthesis, N2 fixation and respiration.
Manganese (Mn)	It is required for activity of some dehydrogenase, decarboxylases, kinases, oxidases and peroxidases. It is involved with other cation-

	activated enzymes and photosynthetic O2 evolution.
Copper (Cu)	**It is the component of ascorbic acid oxidase, tyrosinase, monoamine oxidase, uricase, cytochrome oxidase, phenolase, laccase, and plastocyanin.**
Nickel (Ni)	**It is the constituent of urease. In N2 – fixing bacteria it is the constituent of hydrogenase.**
Molybdenum (Mo)	**It is the constituent of nitogenase, nitrate reductase, and xanthine dehydrogenase.**

The above classification is arbitrary because many elements have several functional roles. For example, Manganese is listed in group 4 as a metal involved in several key electron transfer reactions although it is a mineral element that remains in ionic form which would place it in group 3. It is also found that several natural occurring elements like Aluminum, Selenium, Cobalt which are not essential elements are also accumulated in plant tissue. Plants contain Aluminum from 0.1-500 ppm and addition of low levels of Aluminum to a nutrient solution helps in plant growth (Marschner,1995).

In the genera of *Astragalus* , *Xylorhiza* , *Stanleya* ,many species accumulate Selenium. It is also seen that plants have no specific requirement for Selenium. Cobalt, a part of Cobalamin(Vitamin B12 & its derivatives), a component of various enzymes in nitrogen-fixing microorganisms is also required. Deficiency of Cobalt blocks the development and function of nitrogen-fixing root nodules. Plants that do not fix nitrogen, as well asnitrogen-fixing plants that are been given ammonium or nitrate, do not need cobalt.

Usually crop plants normally contain very less amount of such non-essential elements.

HYDROPONICS :-

It is a technique of growing plants with their roots deeply immersed in a nutritional solution without soil. It is also called "Solution Culture". The term "HYDROPONICS" was first used by William Frederick Gericke in the year 1937. In this process large volume of nutrient solution is required and we have to observe the nutrient concentration and pH of the solution. Sufficient amount of Oxygen (O_2) is also required and to supply of that Oxygen (O_2) vigorous bubbling of air through the solution is done.

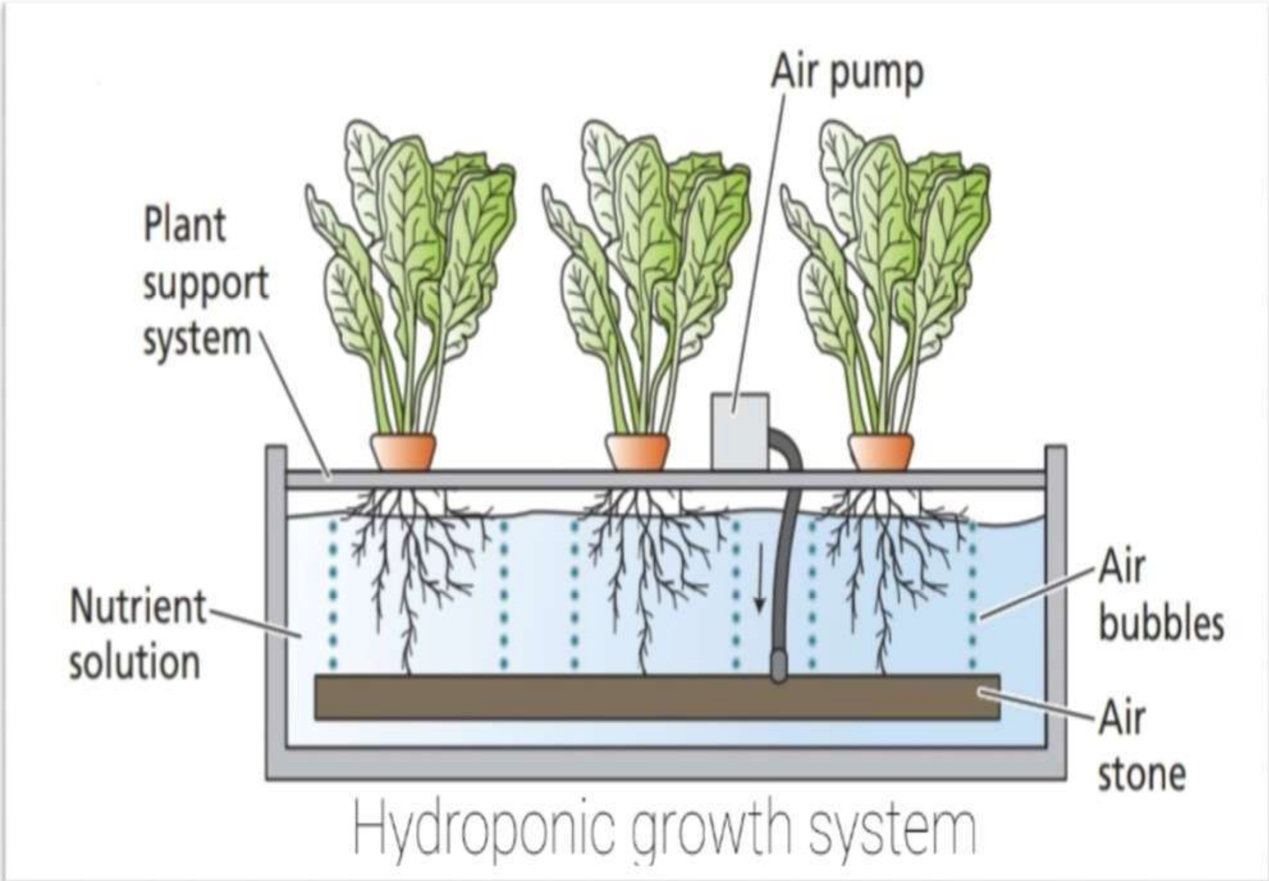

Figure 2: HYDROPONIC GROWTH SYSTEM

Plants commonly grown hydroponically are :-

1. Lettuce (*Lactuca sativa*)

2.Tomato (*Solanum lycopersicum*)

3. Cucumber (*Cucumis sativus*)

4. *Cannabis*

5.*Arabidopsis thaliana,* a model plant in plant science and genetics. In another form of hydroponic growth plants are grown in a supporting material such as sand, gravel, vermiculite, expanded clay. Nutrients are flashed through the supporting material and the old solution is removed by the process called leaching.

Figure 3: Lettuce

Figure 4: Tomato

Figure 5: Cucumber

Figure 6: Cannabis

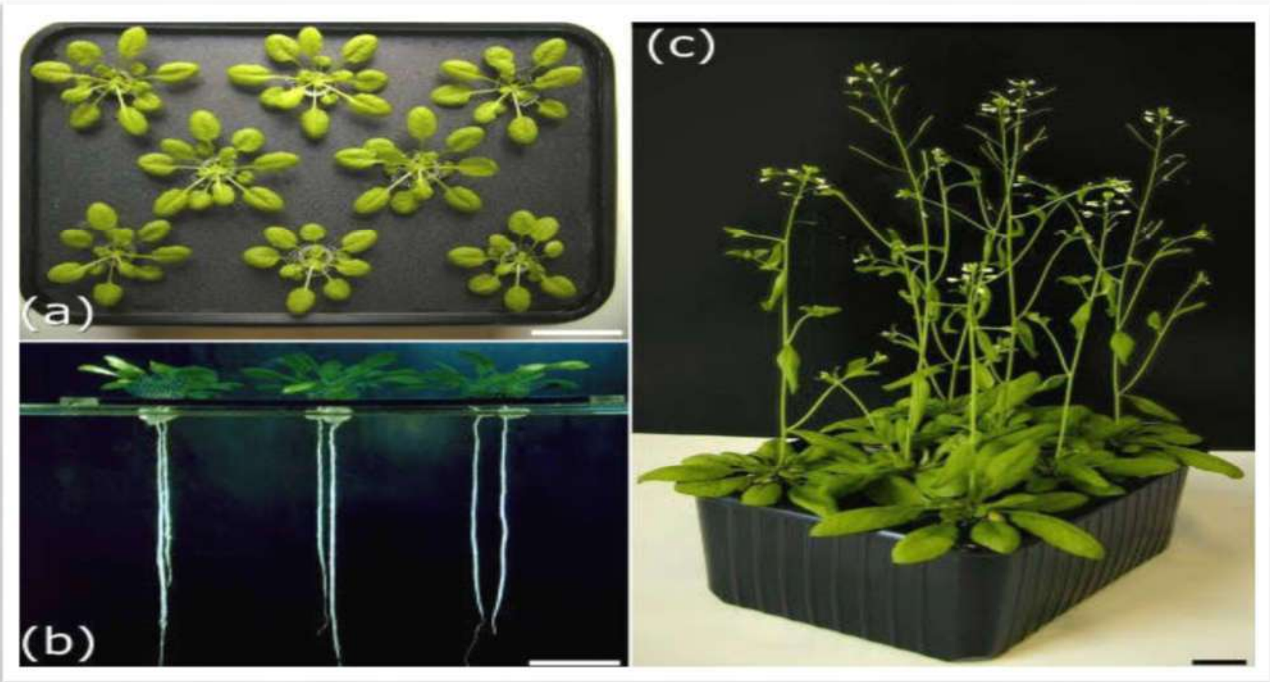

Figure 7: *Arabidopsis thaliana*

Except hydroponics there are few more techniques of growing plants without soil. These techniques are Nutrient film growth, Aeroponics, Ebb and flow system. These techniques are described below:

1. **NUTRIENT FILM GROWTH – It is also a type of hydroponic growth system. In this process plant roots lie on the surface of a trough and nutrient solutions flow in a thin layer along with the trough over the roots. This system ensures that the roots receive sufficient amount of Oxygen.**

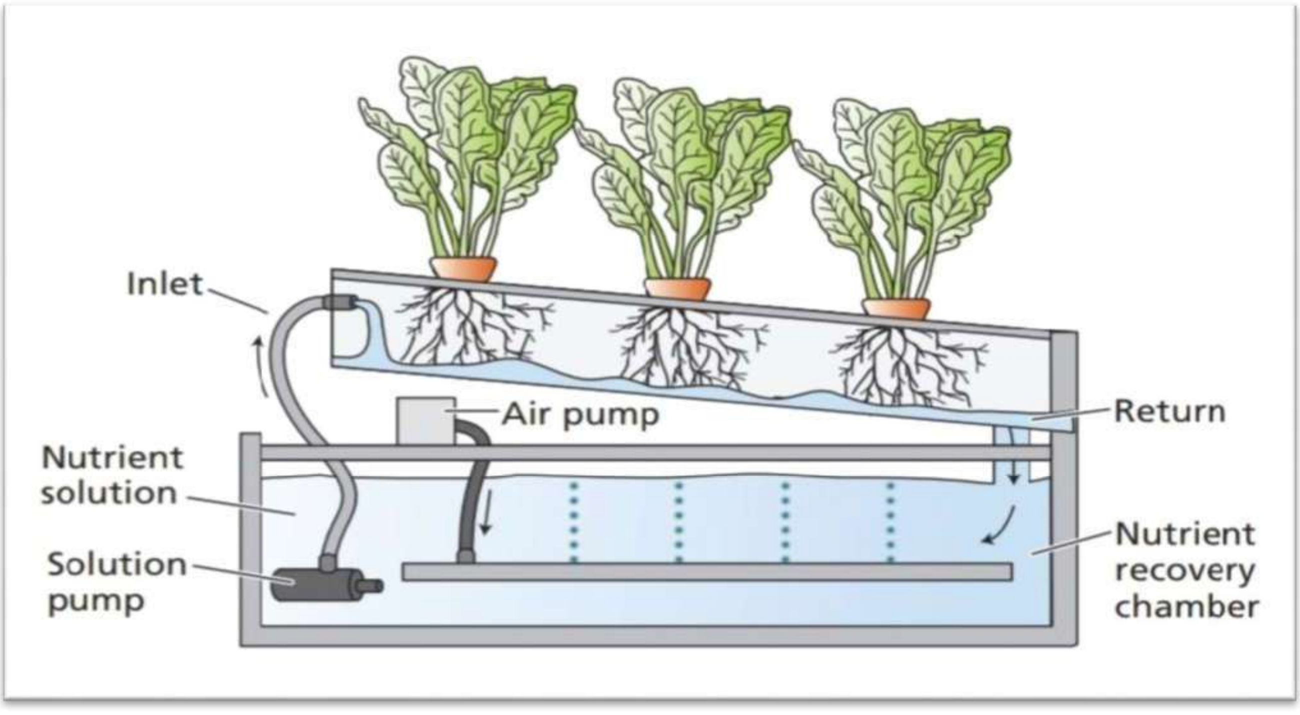

Figure 8: NUTRIENT FILM GROWTH SYSTEM

2. AEROPONIC GROWTH SYSTEM – **It is another type of hydroponic growth system. In this process plants are grown with their roots suspended in air and is spread with nutrient solution constantly. Aeroponics is the technique for future scientific investigation. In this process we can manipulate the gaseous environment around the roots. In this process higher level of nutrients are needed than hydroponics. But for some technical difficulties this process is not widespread.**

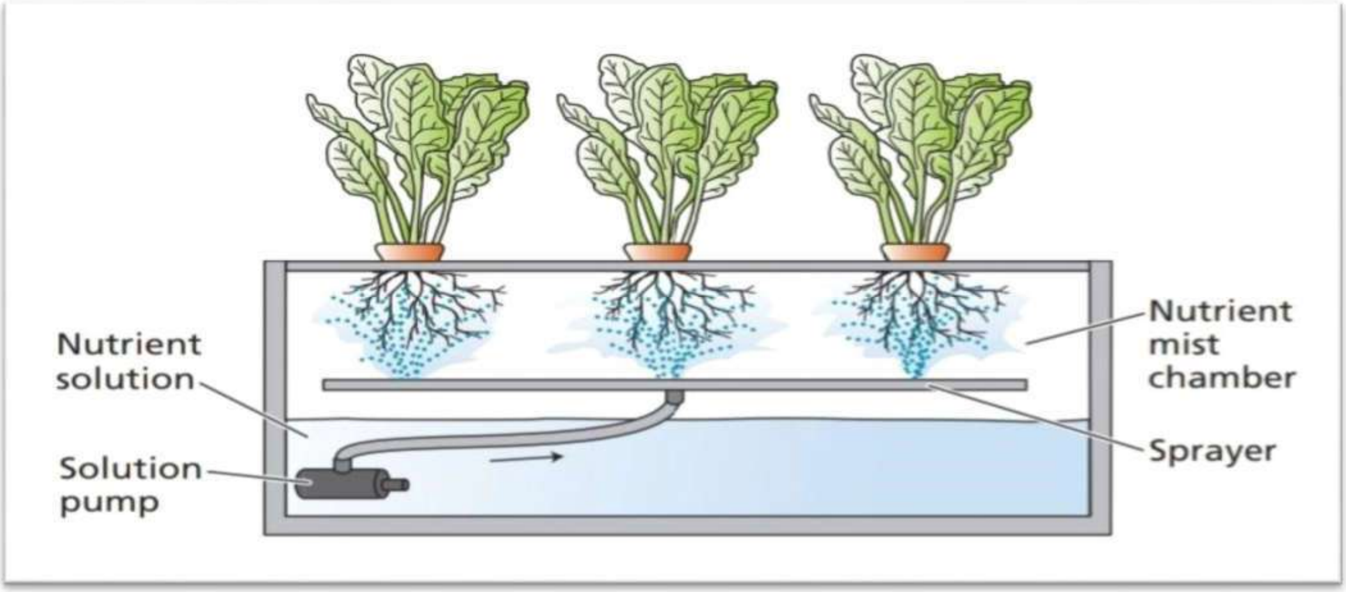

Figure 9: AEROPONIC GROWTH SYSTEM

3. Ebb and flow system – **It is also a type of solution culture. In this system the nutrient solution periodically rises to immerse plant roots and then reduces and exposing the roots to a moist atmosphere. Like aeroponic growth system ebb and flow system also need very high level of nutrients than hydroponics or nutrient film growth system .**

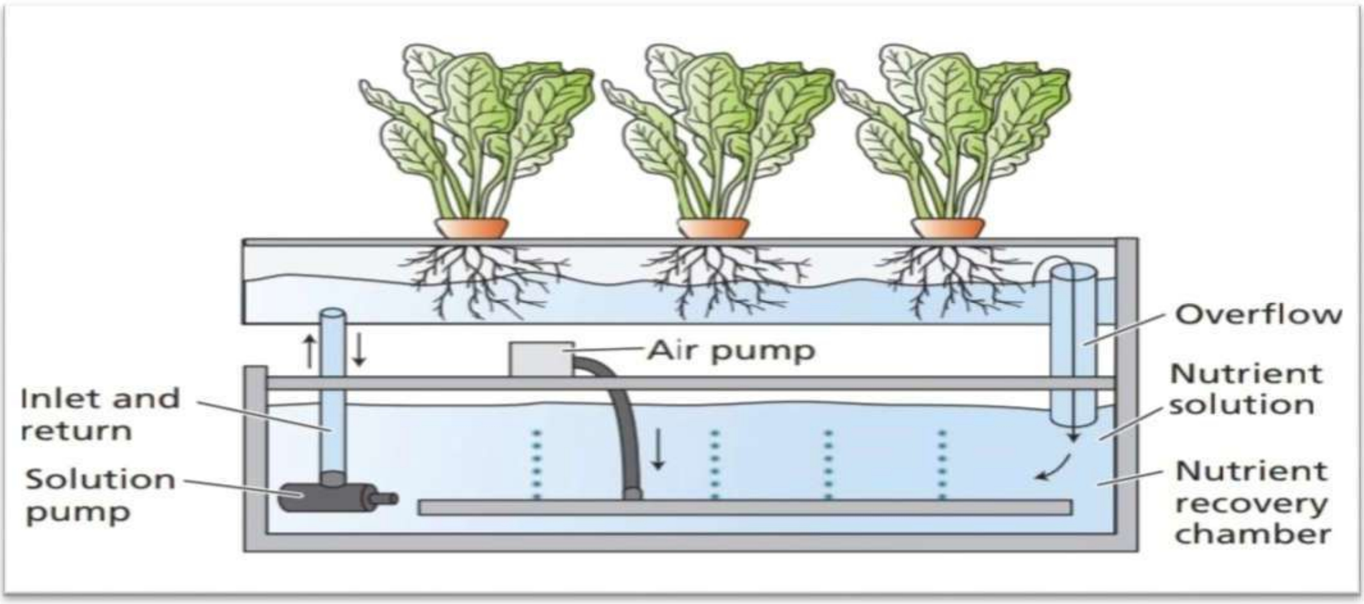

Figure 10: Ebb AND flow SYSTEM

METALLOENZYME:-

Enzymes are present in all plant cells and in seed it is also found in a greater variety. Most of the plant enzymes are intracellular in nature and they cannot diffuse through the cell wall.

In 1810, Planche showed that extracts of various plant roots contained oxidizing enzymes as they gave a blue color with guaiacum tincture. Guaiacum tincture is an alcoholic extract of guaiacum gum which is obtained from a species of guaiacum trees, which contains guaiaconic acid. Later several researchers work on this.

Metalloenzymes are enzyme proteins containing metal ions or metal cofactors which are directly bound to protein or enzyme bound to non-protein components. Roughly 30% of enzymes are metalloenzymes and they require metal ions for their activity. All metalloenzymes have one feature in common, that is the metal ion is bound to the protein with one labile coordination site. As with all the enzymes, the shape of the active site is crucial. The metal ion is usually located in a pocket whose shape fits the substrate. The metal ion catalyzes reactions that are difficult to achieve in organic chemistry. Here I will discuss about few metalloenzymes.

1.MANGANESE:-

Manganese (Mn) is an essential micronutrients in plant system. Biochemistry of manganese is very complex in the soil. It exists in three oxidation states – Mn(II),Mn(III),Mn(IV) in soil. Only the divalent form that is Mn^{2+} is available for plant to uptake and it activate several enzymes in plant cells. Plants can not uptake the other forms because Mn(II) is unstable and Mn(IV) forms highly insoluble oxides and precipitates. The concentration of Mn^{2+} in soil is dependent on particular soil pH and redox condition. When pH is high it reduces availability of Mn^{2+} by MnO_2 formation and reducing O_2 in soil air by flooding increases its availability.

Mn is a metal co-factor for approximately 6% of all known metalloenzymes. In plants most well studied Mn dependent enzymes are- 1. Mn-superoxide dismutase (Mn-SoD)

2. Oxalate oxidase (OxOx)

3. Mn cluster in photosystem II (PSII).

Biological chemistry of Manganese (Mn) in plants :

The most common coordination geometries of manganese in proteins is OCTAHEDRAL, SQARE-PYRAMIDAL, TRIGONAL-BIPYRAMIDAL and TETRAHEDRAL. The coordination geometries are given in the next page.

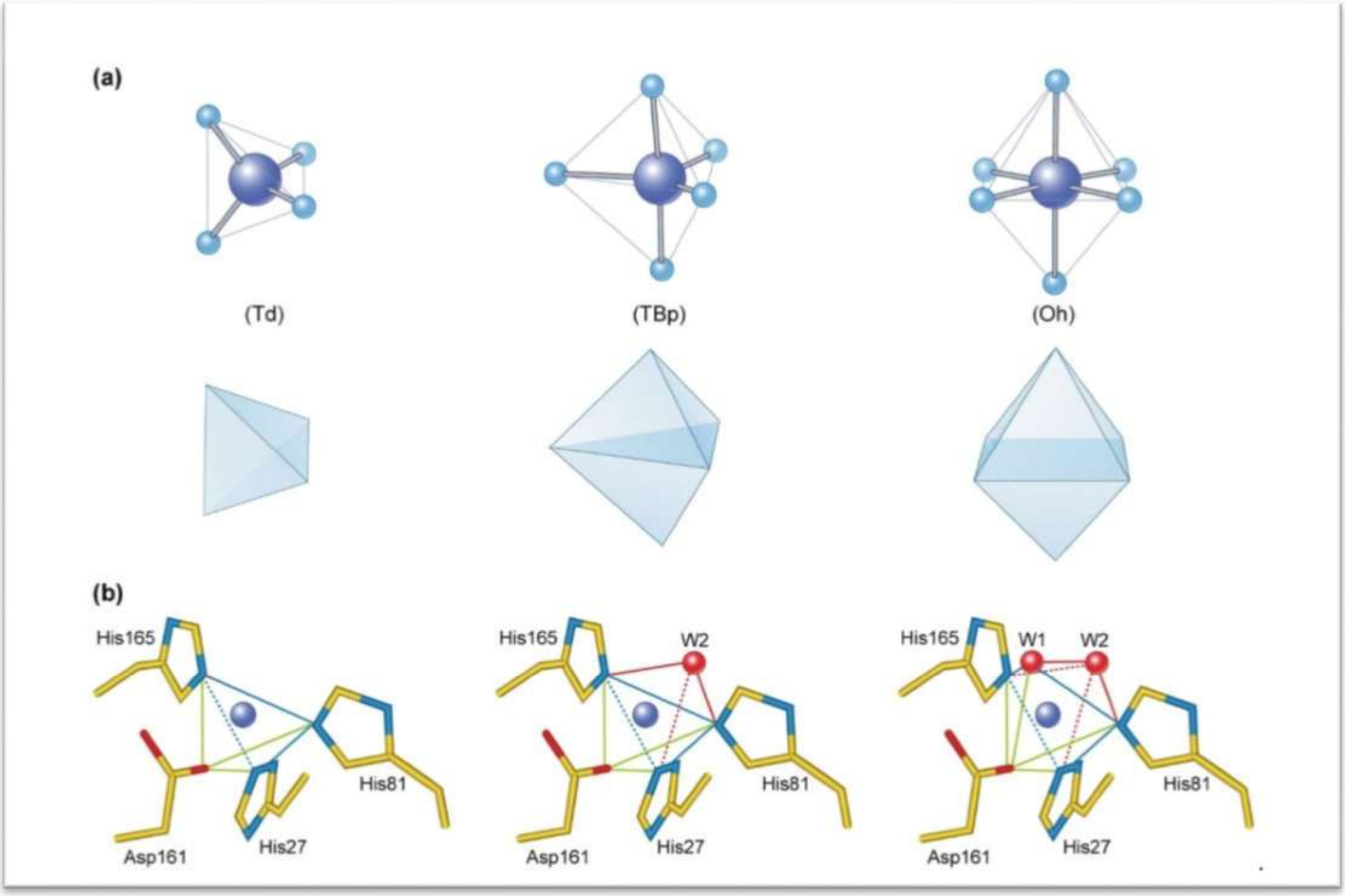

Figure 11: (a) The three dominating coordination geometries and coordination number observed in Mn (Purple sphere) containing metalloproteins are Tetrahedral (Td, n=4); Trigonal bipyramidal (TBp, n=5) and Octahedral (Oh, n=6). (b) The active site of Mn-SoD in the presence of Mn(Purple sphere), with and without water molecules (W1 and W2, red spheres) in the coordinating sphere. Three His residues (His 165,27 and 81) one Asp (161) coordinates Mn, and the involvement 0,1 or 2 water molecules determines the geometry of the Mn complex at the active site. Zero water produces Td, binding of W1 yields TBp, and binding of W1 and W2 yields Oh geometry.

Manganese in metalloenzymes :

We can find manganese as metalloenzymes in plants. Manganese is very important to activate several plant enzymes. Manganese has two major functions in enzyme i.e. 1.It acts as a lewis acid, for which manganese is similar with Magnesium, Cobalt, Zinc as functionally. 2. It acts as an oxidation catalyst for which it can be compared with Iron and Cooper which bears similar properties.

For majority of manganese containing metalloenzymes, Manganese is interchangeable with other divalent metal cataion. Mn(II) has a radius of approximately 0.78 angstrom, which is larger than Magnesium (0.66 angstrom) and smaller than Calcium (0.72 angstrom) but almost similar with Zinc (0.72 angstrom), Cooper (0.72 angstrom), Cobalt (0.74 angstrom).

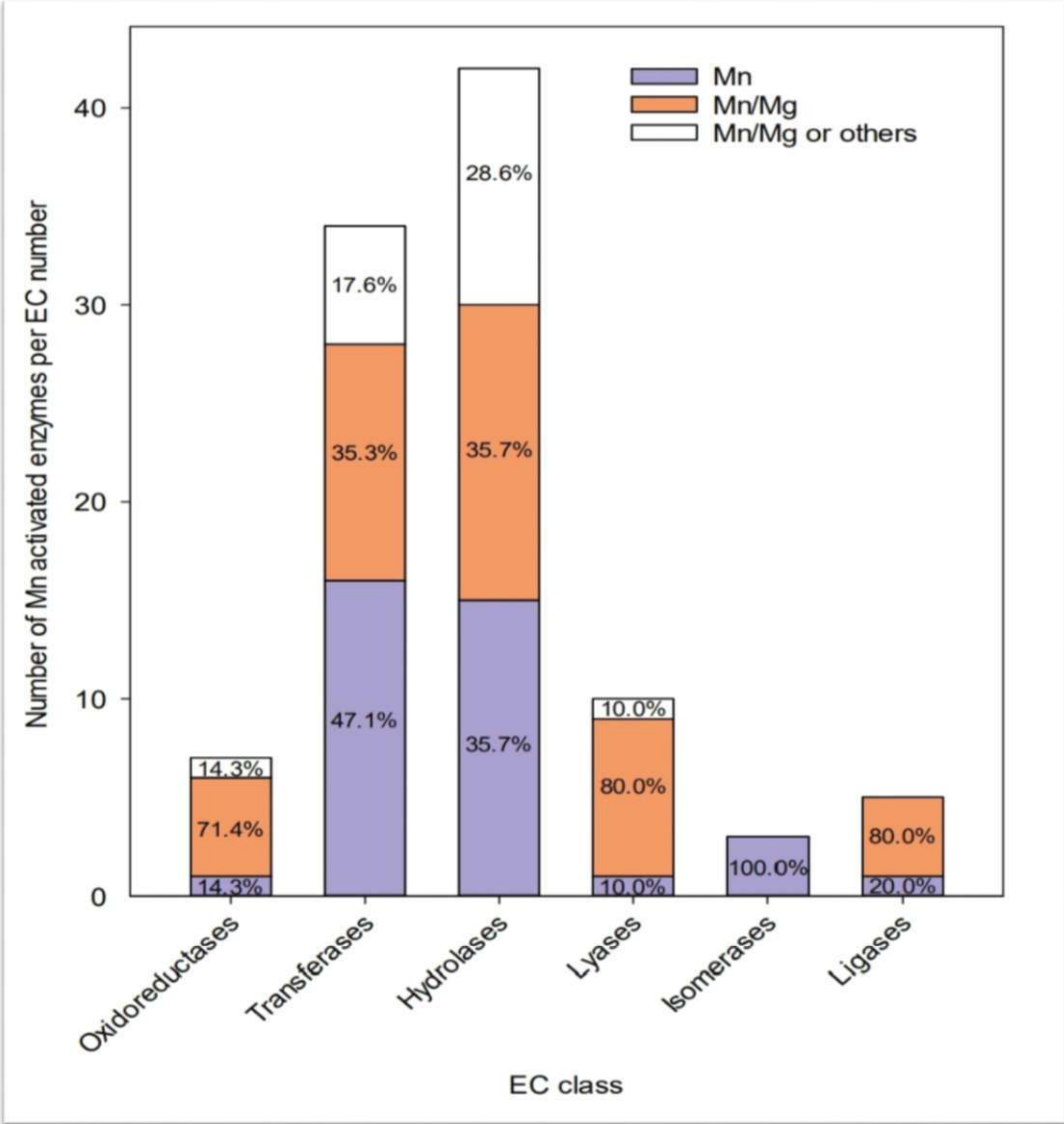

Figure 12: Distribution of manganese activated enzymes across the six Enzyme Classes(EC) together with relative frequencies(%) of the coordinated metal ion for each EC class.

Across the six enzyme classes, 44% of all manganese containing metalloenzymes can bind Magnesium (Mg) with different Mn/Mg stoichiometries. In leaf tissue, most manganese and magnesium is sequestered in the vacuoles and allocation to the chloroplast, golgi bodies, mitochondria where both metals serve key functions is tightly regulated at manganese and magnesium transporter level. In plants most well studied manganese dependent enzymes are 1. Mn-superoxide dismutase (Mn-SoD) , 2. Oxalate oxidase (OxOx) ,3. Mn cluster in photosystem II (PSII). These three enzymes are described shortly below.

1. Manganese in the active site of water oxidation in photosystem II

The most well studied manganese containing enzyme is Mn-cluster which is situated and surrounded by a protein matrix in photosystem II. PSII does a series of electron transfer reactions using solar energy.

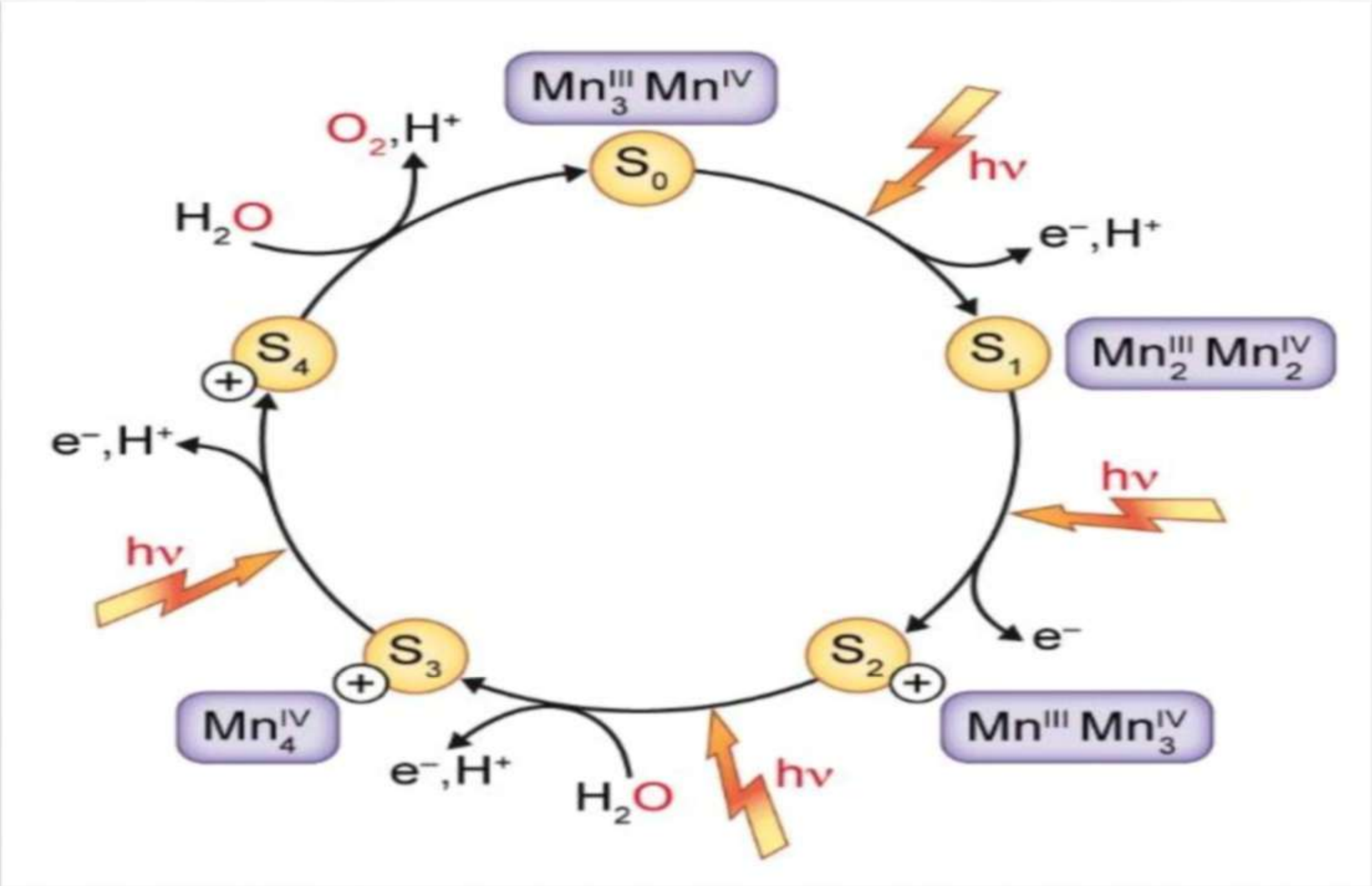

Figure 13: The Kok cycle showing the steps of oxygen evolving complex (OEC) of photosystem II (PSII) with details of S_i state (S_0 -S_4).

Light induced water oxidation requires 4 electrons and 4 protons to be striped from 2 H_2O molecules i.e. $2H_2O$ — $4H^+$+ $4e^-$+ O_2. This events are catalyzed by Mn-cluster of the OEC that cycles through different redox states known as S_i states.

2. Manganese dependent Superoxide Dismutase

Reactive Oxygen Species (ROS) are toxic byproducts of basic plant metabolism. ROS are extremely reactive with and harmful to all biomolecules, which causes damage of DNA and RNA, protein oxidation, lipid peroxidation. To counteract the effect of this plant cells have developed an anti-oxidative system to employ the ROS enzyme superoxide dismutase (SOD). SODs are a family of metalloenzymes which catalyzes the dismutation of superoxide radicals into molecular oxygen ($O2$) and hydrogen-peroxide ($H2O2$).

In plant cells ROS are primarily formed in chloroplast, mitochondria and peroxisomes. Based on prosthetic metal used by SOD, it is of three types i.e. 1.Fe-SOD, 2. Mn-SOD, 3. Cu-Zn-SOD, which are localized to the chloroplast; mitochondria and peroxisome; chloroplast and cytoplasm respectively.

Plant Mn-SOD is typically found in mitochondria. The active site of Mn-SOD is highly conserved and plant Mn-SOD share approximately 70% sequence similarities across a wide range of plant species. A study of green algae *Chlamydomonas* under Mn-deficit conditions showed a lossof Mn-SOD activity before PSII efficiency, suggesting a regulated intraorganellar supply of Mn to support PSII function in preference to Mn-SOD function in mitochondria. Thus prolonged Mn deficiency in plants is characterized by leaf necrosis.

3. Manganese dependent Oxalate oxidase

Oxalate oxidase (OxOx) is a manganese dependent germin enzyme, which catalyzes the oxygen dependent degradation of Oxalate by oxidation into two molecules of $CO2$ in a reaction that is coupled with the formation of $H2O2$. Most knowledge on OxOx in plants has derived from work in cereals.

The activity of OxOx is localized to the apoplast and exerts a dual role in the defense of pathogens by destroying fungal toxin, and promoting lignification. OxOx have a role in seed maturation and germination, where isoformy of germin have been reported as discrete makers.

Crystallographic assessment of OxOx shows that Mn is bound by the side chains of a 3 His cluster and 1 Glu residue, as well as two adjacent water molecules in an Octahedral metal complex. The active site of OxOx is situated in the core of the complex composed of two nearly structurally equivalent β-barrel domains of the monomer ,each contain Mn ion. Spectroscopic characterisation shows that the OxOx with the reduced Mn(II) form at the active center lacks activity but the activity is restored when the metal center is oxidised to either Mn(III) or Mn(IV) forms.

Several pathogens are believed to make use of Oxalate oxidase in pathogenesis, including *Sclerotinia sclerotoorum* (white mold) and *Erysiphaceae* (powdery mildew). The OxOx enzyme activity is detected in younger barley roots and in the leaves of protecting plants against the fungal toxin, the strategy of introducing oxalate degrading enzymes into plants. When OxOx gene from barley was introduced to oilseed rape it shows resistance to exogenously supplied oxalic acid, as a result of increased level of OxOx and enzyme activity.

2. Zinc:- Zinc is an essential plant micronutrient. It is widely available in soil and has a great importance for many crops. In plants we can see that zinc is not oxidized nor reduced. Many enzymes requires Zn ion (Zn^{2+}) for their activity and it may be required for chlorophyll biosynthesis. Many enzymes use zinc as co-factor like carbonic anhydrase, alcohol dehydrogenase, superoxide dismutase and RNA polymerase. Zinc is also required for synthesis of tryptophan, a very important amino acid requires for the synthesis of auxin Indole acetic acid. Thus zinc operates the control of plant growth and deficiency of zinc hampers the catalytic activity of the above mentioned enzymes and thus the metabolic pathways in which they are involved.

In plants zinc is only present and Zn(II) functions mainly as divalent cation in metalloenzymes and functions in the linkage of these enzymes to their corresponding substrate.

Biochemistry and physiology of Zinc:

Zinc is absorbed by most of the plants as a chelate and either via leaves or by roots. In long distance transport in the xylem, it is linked with organic acid or it present as a divalent cation and in floematic sap it is present in high concentration linked to low molecular weight organic solutes. The dynamics of Zn in the soil are similar to those of Cu and Fe, if pH <5 it is available in higher amount. Zinc is available in soil with pH 5-6. At higher pH it forms sparingly soluble compounds.

Zinc as a metallic co-factor:

An enzymatic co-factor is a non-enzymatic component that promotes the catalytic value of an enzyme. Almost one third of all enzymes requires ionic metals for their catalytic function. So ionic metals represent a substantial proportion of all the co-factors. Most of the trace nutrient metals share as a common characteristic a close involvement with enzymes. Many are active site components that link the enzymes to substrate.

Zinc is an ubiquitous and versatile of all metallic co-factor, Zn can act either structurally or catalytically. In Carbonic anhydrase, Zn works in a coordinated way with the CO2 substrate, in Carboxi peptidase it takes an active role in the breakage of peptide union. In another structural role, the Cu ,Zn-superoxide dismutase requires Zn to position the Cu atom in the channel accessed by the substrate.

Zinc is considered as an adapted metal because it acts as a divalent cation without special geometric preference. This is the characteristics that allows Zn to adopt to so many enzymatic environments. Zinc exists as Zn^{2+}, so it does not have redox properties. The electronic configuration of Zn^{2+} is [Ar] $3d^{10}$. For this configuration Zn complexes lack color and Zn behaves mainly as cation (Zn^{2+}) which is a good electron acceptor. Many enzymes use Zinc as a co-factor like 1. Carbonic anhydrase

2. Alcohol dehydrogenase

3. Tryptophan synthetase

4. Superoxide dismutase.

1.Carbonic anhydrase and photosynthetic activity

Carbonic anhydrase(CA) catalyzes the rapid conversion of carbon dioxide and water into bicarbonate ion(HCO_3^-) and a proton:

$$CO_2 + H_2O \longrightarrow HCO_3^- + H^+$$

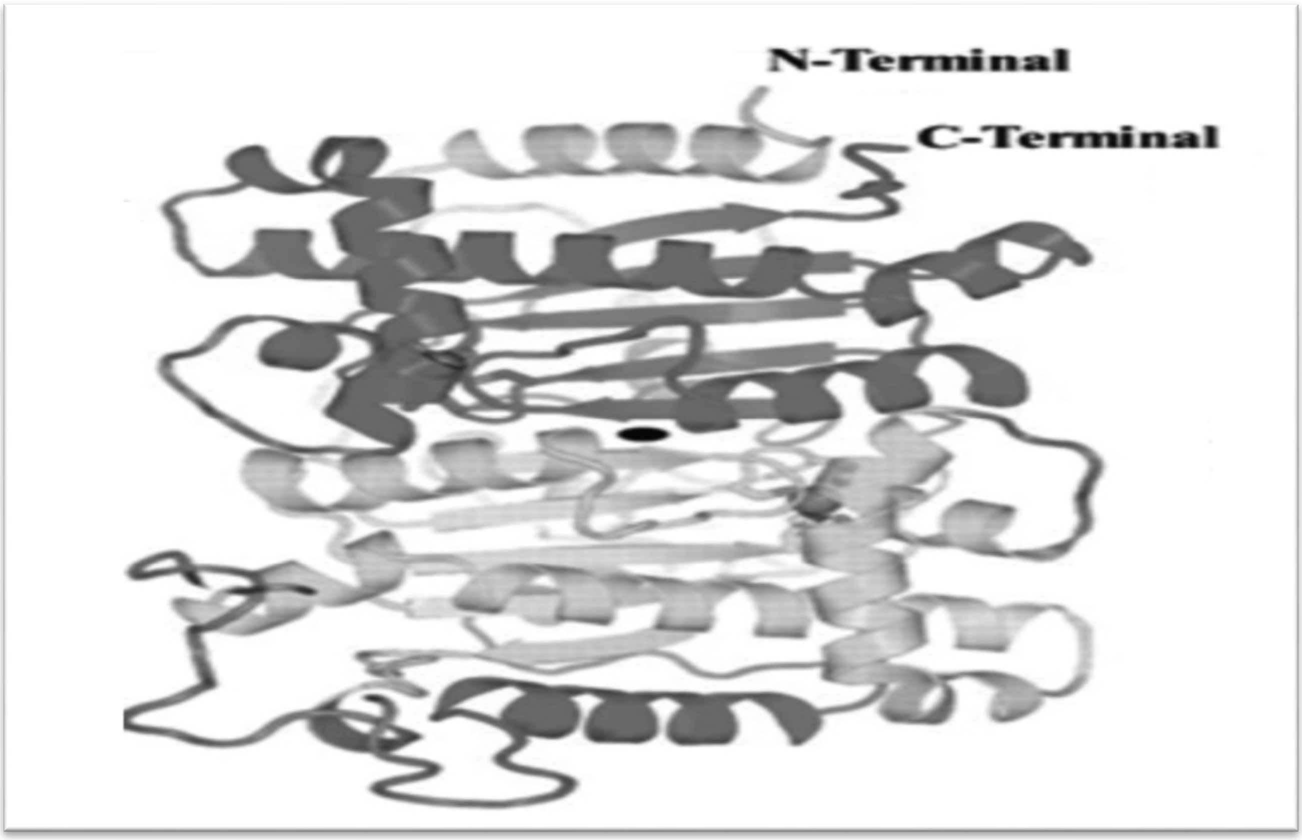

Figure 14: Structure of β-CA tetramar

It can be found in prokaryotes and in four families of higher plants being β Carbonic anhydrase. It is a metalloenzyme that requires Zn as a co-factor. It involves in various processes such as pH regulator, CO_2 fixation and stomatal closure. Carbonic anhydrase catalysis is performed by the metal ion, where Zn acts as a co-factor by its union to water to activate the catalytic site of the enzyme. In dicots, Carbonic anhydrase consists of six subunits and has a molecular weight of 180kDa and has six atoms of Zn per molecule. The enzyme is located in the chloroplast and in the cytoplasm.

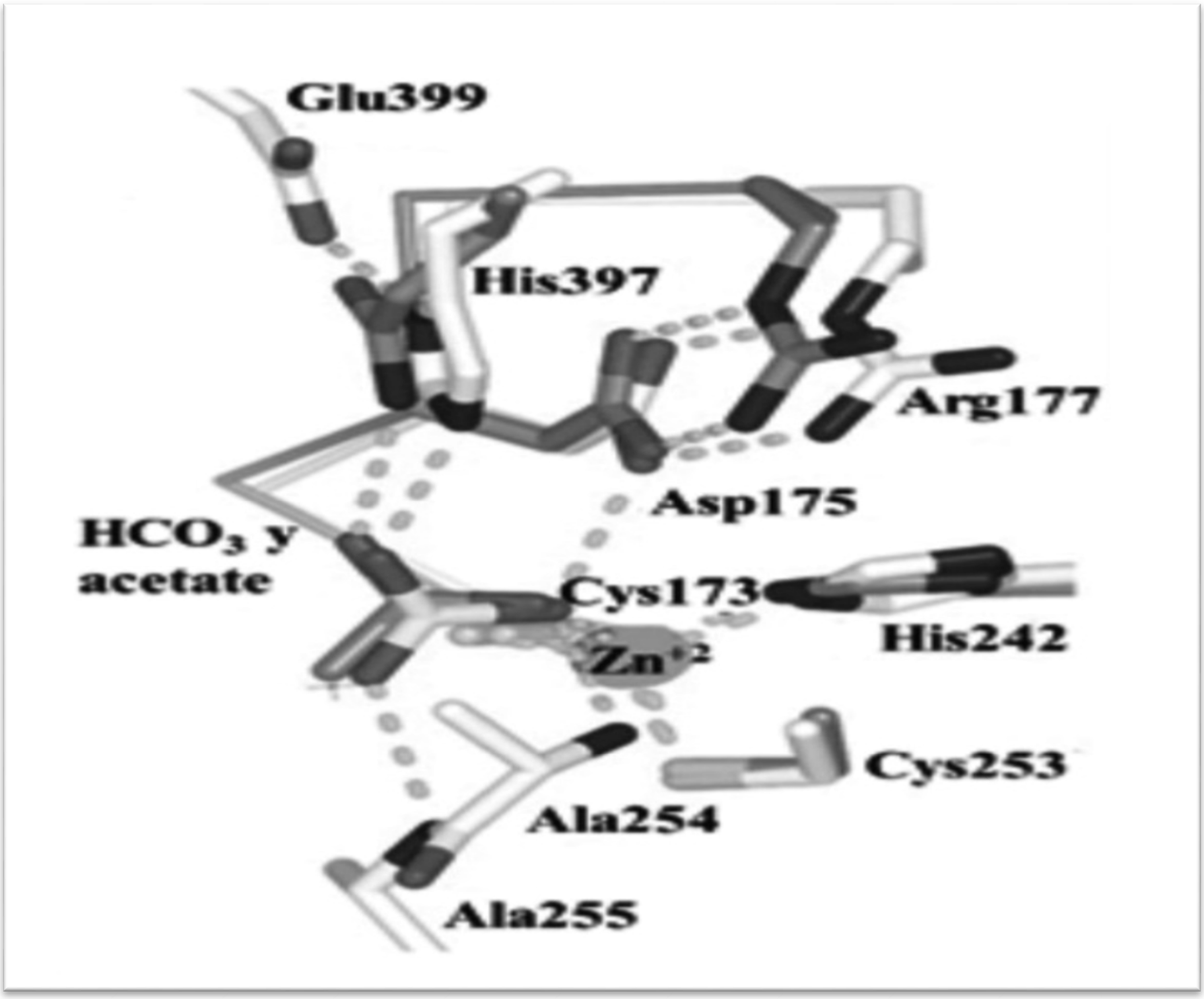

Figure 15: Characterization of active site metal cofactor Zn^{2+}

Among the enzymes participating in photosynthetic metabolism in a number of plant species, carbonic anhydrase is the only one whose activity varies with atmospheric CO2 concentration. Carbonic anhydrase functions in the fixation of photosynthetic CO_2 – i) In C3 plants, β-carbonic anhydrase helps to elevate CO_2 concentration in the chloroplast which is a substrate for the RuBisCO enzyme. This raises carboxylation so it can participate in C2

incorporation into carbohydrates during photosynthesis where only CO_2 can be used as a carbon source.

ii) CO_2 hydration toHCO_3^- is a substrate for carboxylase phosphoenolpyruvate in C4 and CAM plants.

iii) Carbonic anhydrase enables CO_2 distribution through the plasma membrane and the chloroplast.

2. Alcohol dehydrogenase and soil waterlogging

Alcohol dehydrogenase catalyzes the reversible oxidation of a wide variety of primary, secondary, and cyclic alcohols to their corresponding aldehydes and ketones. Alcohol dehydrogenase is an enzyme system located in the cytoplasm and widely distributed in many phyla that include organisms belonging to all kinds of classification of living things. They are dimers made up of subunits of around 374 amino acid residues and about 4okDa in weight. Each sub unit has two domains, a catalytic domain and a domain of union with enzyme and are separated with hydrophobic slit that forms the unity pocket to the substrate. This enzymes has two Zn atoms per molecule, one with catalytic function and the other with a structural function.

Alcohol dehydrogenase metabolizes the alcohol produced under anoxic conditions such as due to water logging. Water logging reduces the oxygen in soil and alter many physical, chemical, and biological processes which results into production of toxic substrate, CO_2 e.t.c.

3. Tryptophan synthetase and IAA synthesis

Tryptophan synthetase is activated by Zinc which regulates the synthesis of tryptophan and involved in the biosynthesis of indole 3-acetic acid (IAA). IAA requires the action of an enzyme activated by Zn as Zn deficiency reduces IAA and tryptophan is not synthesized.

4. Superoxide Dismutase and membrane integrity

Superoxide dismutase catalyzes the dismutation of superoxide and hydrogen peroxide. It is an important antioxidant. When superoxide dismutase is present in the plants Zn is associated with cooper (CuZnSOD) at the active site of the enzyme.

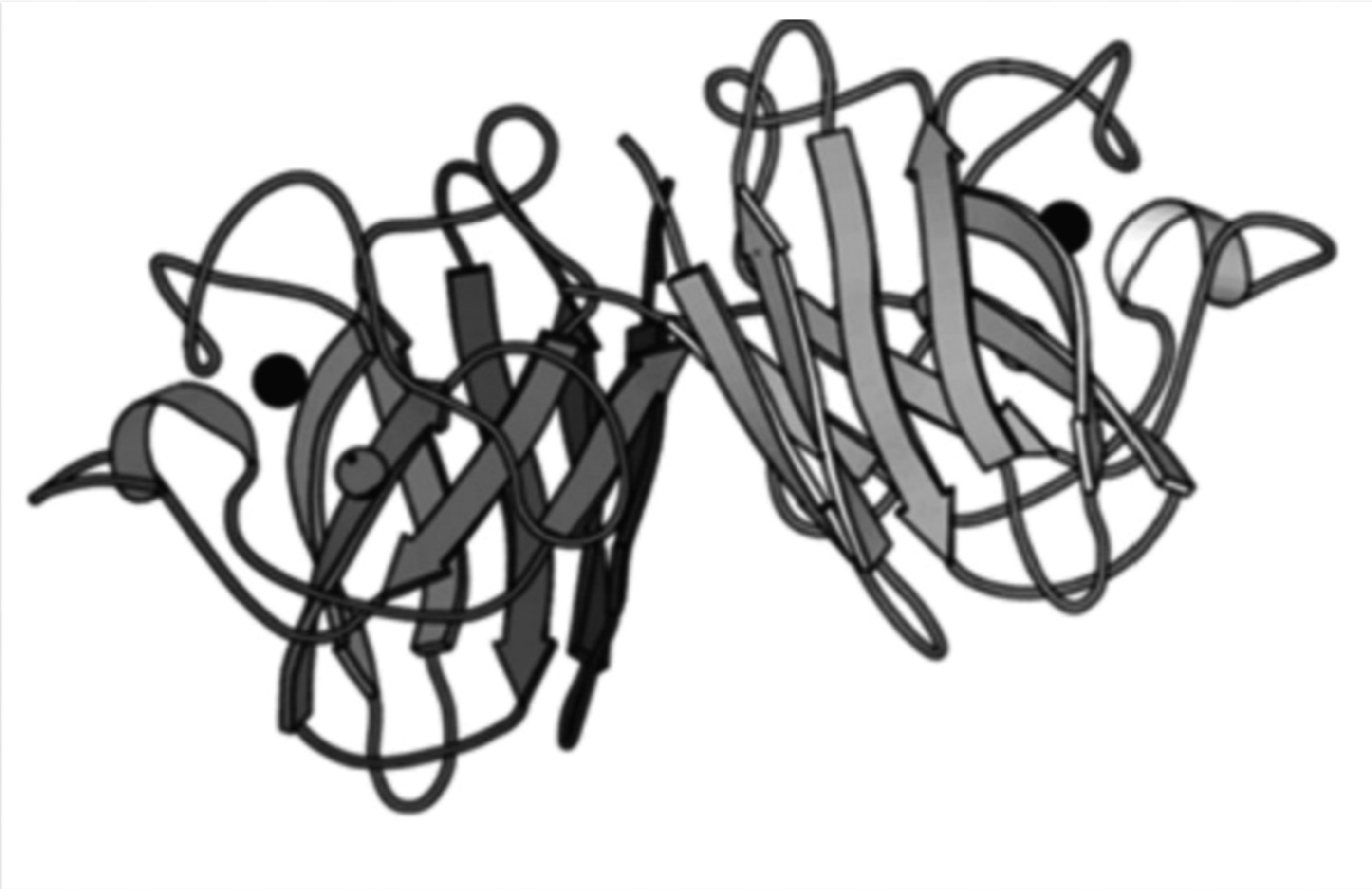

Figure 16: The metalloenzyme SOD is firmly attached to two metal cofactors. The outlining of the structure in sheet B of the SOD

Cooper atom represents the metallic - catalytic component, while Zn represents the structural one. The generation of toxic O2 radicals are controlled by Zinc as it interferes in NADPH oxidation as well as in removal of O2 radicals for its role in the enzyme CuZn-SOD. When a plant suffers from Zn deficiency O2 generation incresses usually and a rise in the permeability of the plasma membrane occurs as the toxic free O2 radicals break the double chains in pollyunsaturated fatty acid and phospholipids of membranes. The increase in permeability leads to the losses in sugars, amino acids and potassium and also the increase in lipid oxidation in leaves leads to chlorophyll destruction, necrosis and stunted growth as a product of IAA oxidation and particularly at higher radiation intensity (Cakmak,2000).

CONCLUSION:-

Minerals are very important for both plants and animals and we know it very well. Here in this project I have tried to cover the important roles of minerals in plants. Mineral elements are found naturally and plants utilize them by absorbing them with the help of their root system and this mineral elements helps in their various physiological processes including photosynthesis, metabolism e.t.c. This mineral elements are categorized in two classes i.e. Macronutrients and Micronutrients. These categories are based upon the level of nutrients needed by the plant. Plants can be also grown up without soil. The process of growing plants without soil is known as hydroponics. In this process of hydroponic growth system mineral nutrients are mixed with water and they are grown on it. Plant mineral nutrients are also part of the metalloenzymes. In the metalloenzyme the enzyme proteins are directly bound to protein or enzyme bound to non-protein components. Manganese, Zinc and few other plant mineral nutrients acts as metalloenzyme. These metalloenzymes are involved in various physiological processes like Photosynthesis, Metabolism. In plants deficiencies of these mineral nutrients shows various symptoms like Chlorosis(Yellowing of the leaves), Necrosis e.t.c. So it is very important for plants to survive in nature.

REFERENCE:-

1. Plant Physiology, fifth edition, Lincoln Taiz, Eduardo Zeiger
2. https://en.wikipedia.org/wiki/Mineral
3. https://images.app.goo.gl/A1syjtz8ydV8r83e9
4. https://vikaspedia.in/agriculture/crop-production/integrated-nutrient-management/what-is-plant-nutrition
5. https://images.app.goo.gl/EZpzGrS1A61hM5Af7
6. https://images.app.goo.gl/puHvyyR1yhKmBnB89
7. https://images.app.goo.gl/yXWDRbuzV4VCjUFQ9
8. https://images.app.goo.gl/DHvfWrgLXDwoBSex8
9. https://images.app.goo.gl/qLYtumJshUbnshkCA
10. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=plant+metalloenzymes&oq=plant+metallo#d=gs_qabs&u=%23p%3Duf8mUxlZYcAJ
11. https://en.wikipedia.org/wiki/Metalloprotein
12. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6843630/
13. https://www.google.com/url?sa=t&source=web&rct=j&url=https://www.interciencia.net/wp-content/uploads/2018/05/242-6131-OJEDA-43-04.pdf&ved=2ahUKEwik8-_53o72AhWGILcAHeKrB68QFnoECB0QAQ&usg=AOvVaw37vkd97Muysase6K3MOh-e

RAMAKRISHNA MISSION VIVEKANANDA CENTENARY COLLEGE

TOPIC : " GENETIC DIVERSITY AND PHARMACOLOGICAL ACTIVITIES OF *Cleome viscosa* "

SUBMITTED BY : SPANDAN HAZRA

REG NO. : A01 - 1112 - 114 - 005 - 2019 OF 2019-20

COLLEGE ROLL NO. : 406

SEMESTER : V

DEPARTMENT : BOTANY

CONTENTS

➢ INTRODUCTION

Cleome viscosa L., is an annual rainy season weed , which is universally distributed across the globe . Commonly known as ‘‘wild or dog mustard,’’. Cleome viscosa bears yellow flowers and long slender pods containing seeds . The genus *Cleome* is phylogenetic near relative of genus *Arabidopsis*, containing both C3 and C4 species (Marshall et al., 2007). *Cleome viscosa* is a C3 plant due to lack of Kranz anatomy and high CO2 compensation point (Sankhla et al., 1975**)**. The whole plant and its parts (leaves, seeds, and roots) are widely used in traditional system of Ayurvedic medicine to cure wounds, ulcers, inflammations, fever, bronchitis, diarrhoea and skin infections (Gupta et al., 2011). *Cleome viscosa* is highly effective in a wide spectrum of disease and reported to possess analgesic, antiemetic (Ahmed et al., 2011), anti-diarrhoeal (Devi et al., 2002), antipyretic (Devi et al., 2003) and antimicrobial (Bose et al., 2011) activities. *Cleome viscosa* seed extract have been used as a hepato protective agent (Chaudhary et al., 2010). *Cleome viscosa* leaves possess high amount of phenol and flavonoids. They are potent to antioxidant and free radical scavenging activities.

Plants bear flowers which shows vast intra-plant variation in both size (large and small) and sex (hermaphrodite, stamina and pistillate) (Saroop and Kaul, 2015). The average number of flowers per plant varies significantly and also their detailed structure and function varies. Both the plant and flower shows greater tendency towards hermaphroditism. The large and small sized flowers differed in their morphology and reproductive features; the former were significantly larger than the latter. Anthesis, anther dehiscence and stigma receptivity were coupled in all flower types. The structural closeness between stamens at two lengths and pistil further facilitated self-pollination. Each floral type contributed towards plant’s fitness in its own way. Hermaphrodite flowers exhibited both self and cross pollination. All these floral variations impart flexibility to the pollination system and provide fitness over the short flowering season. Two naturally growing populations of *Cleome viscosa* from Jammu, J & K, and India have been studied for floral variation at an intra-plant level and its role in life cycle. (Saroop and Kaul, 2014)

General Distribution in India: The species is most commonly found in tropical moist deciduous forests of Sikkim, Eastern Himalayas, West Bengal specifically in the districts of Paschim Bardhhaman, Birbhum and North 24 Parganas and also occurs in deciduous forests of Northern Circars, Khasi Hills of Meghalaya and regions of Upper Assam, Hills of Odisha, Nallamalai Hills and Western Ghats from South Kanara southwards (Troup, 1921). Outside India, it is found in various parts of Sri Lanka, USA, Nigeria, China, Ceylon, Pakistan, Africa, Malaysia, Java, China and Australia having varied distribution from plains to 6000 ft. above mean sea level. (https://indiabiodiversity.org/species/show/33047)

➢ FLOWER MORPHOLOGY

Flowers are pink, bracteates, ebracteolate, actinomorphic, bisexual, hypogynous, and protandrous and are in axillary racemes. Stamens are usually six in number, although five are not uncommon, in a single whorl with dithecous anthers. Ovary is bicarpellary, syncarpous, unilocular and is continued into a short solid style which ends in a bilobed glandular stigma. The wall of the ovary has epidermal glandular protrusions. The floral primordium becomes differentiated into a dome-shaped structure. Calyx initials are the first to appear and then the stamens after which the corolla initials arises. The sepals then differentiate and grow overarching the corolla displaying epidermal glandular outgrowths. The carpellary initials are last to be differentiated (Rao and Rao).

The inflorescence is a lax (1 - 4 flower per cm), corymbose, 30 cm long few-flowered raceme (13.72 ± 1.34). The flowers are also borne solitary in leaf axils. The flowers are bisexual and represent three flower morphs: Short Gynoecium Flowers (SGF), Medium Gynoecium Flowers (MGF) and Long Gynoecium Flowers (LGF); all three morphs occur on the same plant. The SGF consist of 4 mm long gynoecium, with 100 ovules and 7 mm long stamens, MGF has 8 mm long gynoecium, with 113 ovules and 8 mm long stamens, and LGF has 10 mm long gynoecium, with 162 ovules and 11 mm long stamens . The stamen number is 19.65 ± 3.39 in SGF, 17.35 ± 6.22 in MGF and 16.1 ± 4.99 in LGF. The production rate of these flower morphs is almost constant throughout the flowering phase; the percentage of SGF is 18–19 %, of MGF 60–62 % and of LGF 20–21 % (Raju and Rani, 2016)

All three flower morphs are pedicellate (20 mm long) and inverted-bell shaped, exposing the ovary and stamens. The pedicel is 20 mm long during flowering phase and 35–40 mm long during fruiting phase .The flower morphs are yellow and actinomorphic. The sepals are four, green but purple outside at the base, lanceolate, free but connate at the base, glabrous inside, glandular hairy outside, 5–6 mm long and 1–2 mm wide. The petals are four, yellow, free, oblong-spathulate, base cuneate with a 5 mm long claw at base, rounded at the tip, glabrous, 7–12 mm long and 3–5 mm wide. The stamens vary in number as mentioned above for all three flower morphs. They are free, glabrous, filaments almost filiform, anthers linear, green, and exserted and dithecous. The ovary is sessile, oblong-cylindric, glandular-pubescent, bicarpellary syncarpous, unilocular with numerous ovules on parietal placentation; the bicarpellary state of ovary is due to the development of a false septum during fruit development. The style is short (2–5 mm long), slender and extends into a capitate stigma. (Raju and Rani, 2016)

➢ PHARMACOLOGICAL ACTIVITIES

Several extraction of compounds with different types of solutions like aqueous , alcoholic , methanolic , ethanolic extracts are specifically used for the respective plant parts which includes roots , stems , leaves , fruits , seeds and other aerial parts . It exhibits various distinct pharmacological activites which includes anti-microbial activities , anti-diarrheal activities , psychopharmacological effects , anti-inflammatory , anti-pyretic and analgesic activities , gastro protective activities , insecticidal activities etc.

S.no	Pharmacological Activity	Reference
1.	Anti - microbial Activity	➢ Samy et. al , 1991 ➢ Mishra et. al , 1991 ➢ Williams et. al ,2003 ➢ Sudhakar et. al , 2006 ➢ Bose A et. al , 2010 ➢ Senthamilselvi et. al , 2012

		➢ Lakshmanan et. al , 2019
2.	Anti - diarrheal Activity	➢ Devi et. al , 2002 ➢ Tripathi et. al , 2010
3.	Anti – inflammatory Activity Anti – pyretic Activity Analgesic Activity	➢ Singh and West , 1991 ➢ Mandal et. al ,2003 ➢ Bose et. al , 2007 ➢ Bawankule et. al ,2008 ➢ Salawu et. al ,2008 ➢ Mina et. al ,2011
4.	Anti – helminthic Activity	➢ Mali et. al , 2007
5.	Gastro protective Activity	➢ Bhamarapravati et. al , 2003
6.	Immunomodulatory Effect	➢ Tiwari et. al , 2004 ➢ Bawankule et. al , 2007
7.	Insecticidal Activity	➢ Saxena et. al , 2000 ➢ Krishnappa and Elumali , 2013 ➢ Bansal et. al , 2014
8.	Hepatoprotective Activity	➢ Sengottuvelu et. al , 2007 ➢ Dixit et. al , 2009 ➢ Yadav et. al , 2010 ➢ Ramalingum et. al , 2016
9.	Psychopharmacological Effect	➢ Devi et. al , 2004 ➢ Cargg et. al , 2013 ➢ Griffin et. al ,2013
10.	Anti – cancer Activity	➢ Wagner et. al , 1986 ➢ Fotsis et. al , 1997 ➢ Kennedy et. al , 2001 ➢ Bala et. al , 2010 ➢ Bose et. al , 2011 ➢ Joseph et. al , 2014
11.	Anti – oxidant Activity	➢ Koppula et. al , 2011 ➢ Jane and Patil , 2012 ➢ Pallai et. al , 2013

12.	Anti – fungal Activity	➢ Tripathi et. al , 2010 ➢ Cragg et. al , 2013 ➢ Alele et. al , 2016
13.	Anti – arthritic Activity	➢ Havsteen et. al , 1983 ➢ Carevie et. al , 1988 ➢ Narendradhirakannan et. al , 2007
14.	Anti – depressant Activity	➢ Azam et. al , 2016
15.	Mutagenicity	➢ Polasa and Rukmini , 1987

➢ HYBRIDIZATION AND GENETIC DIVERSITY

For the estimation of genetic diversity, different molecular methods used such as biochemical assessment at protein level and DNA based techniques .This techniques have advantage over the classical morphological methods but biochemical assessment at protein level is very expensive as compared to the molecular investigation of DNA markers . Biochemical techniques which includes Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is a safe, simple, reliable and cheap method. The SDS-PAGE method is now extensively used as a biochemical technique to describe the genetic diversity in seed storage protein profile. Morphological description has a substantial role in the investigation of genetic polymorphism in crop plants. They are severely affected by ecological changes, which thereby complicates the analysis of inherited variation. (Muhammad et. al - Diversity, 2018)

All the genotype of the species shows the maximum allelic variation for leaf abaxial and adaxial surface with bright green (75%), and yellow green (75%) respectively, other leaves abaxial and adaxial surfaces were (25%) green and yellow green (26%) respectively. The majority of *Cleome viscosa* genotypes were (50%) yellow flowers while others were with (29%) white yellow colour and (21%) dull yellow. Most of the seeds were with black (46%)   (Muhammad et. al - Diversity, 2018)

➢ REFERENCES

1. Plant Systematics : An Integrated Approach , Fourth Edition by Gurcharan Singh
2. Aizen, M.A. & Feinsinger, P. 1994. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. – Ecology, 75: 330-351.
3. Bhandari M M, Flora of the Indian Dessert, 1st ed. (Scientific, Jodhpur) 1978, 42.
4. Aparadh, V.T. & Karadge, B.A. 2011. Microscopic pollen analysis of some selected Cleome species. – Trajectory, 19: 21-31.
5. Oommachan M, The Flora of Bhopal. 1st ed., J.K. Jain Brothers, Bhopal, 1977, 46.
6. Nadkarni A K, The Indian Materia Medica, Vol. 1 (Popular Prakashan, Bombay), 1982, 351.
7. Aparadh, V.T., Mahamuni, R.J. & Karadge, B.A. 2012. Taxonomy and physiological studies in spider flower (Cleome species): a critical review. – Pl. Sci. Feed, 2: 25-46.
8. Asolkar, L.V., Kakkar, K.K. & Thakre, O.J. 1992. Second supplement to Glossary of Indian Medicinal Plants with Active Principles. PID, CSIR, New Delhi, p. 215.
9. Duthie JF, Flora of the Upper Gangetic Plain and of the Adjacent Siwalik and Sub-Himalayan Tracts, 1st ed. Vol. 1 (Part I-II) (M/s. Bishen Singh Mahendra Pal Singh Dehra Dun), 1973, 50.
10. Maikhuri RK, Semwal RI, Rao KS, Nautiyal S and Saxena KG, Cleome viscosa, Capparidaceae: A weed or a cash crop?, Econ Bot, 2002, 54, 150.
11. Aziz, S. & Shaukat, S.S. 2012. Population ecology of Cleome viscosa L., a desert summer annual. – Pakistan J. Bot,. 44: 1633-1638.
12. A.J. Solomon Raju & D. Sandhya Rani , Sofia, 2016. Reproductive ecology of Cleome gynandra and Cleome viscosa (Capparaceae) : Phytologia Balcanica 22 (1): 15 – 28.
13. Anburaj J, Ravinder SC, Kuberan T, Sundaravadivelan C and Kumar P, Effects of plant growth regulators on callus induction from leaf explants of Cleome viscose, Res J Pharmac, Biol Chem Sci, 2010, 2, 576.
14. Maheswari J K, The Flora of Delhi, 1st ed, Council of Scientific and Industrial Research, New Delhi, 1976, 63.
15. Rukmini C, Chemical, nutritional and toxicological evaluation of the seed oil of Cleome viscosa, Indian J Med Res, 1978, 67, 604.
16. Sudhakar M, Rao CV, Rao PM and Raju DB, Evaluation of antimicrobial activity of Cleome viscosa and Gamelina asiatica, Fitoterapia, 2006, 77, 47.
17. Saroop, S. and Kaul, V. (2011). Phenological events of Cleome viscosa L. growing in Jammu district. The International Journal of Plant Reproductive Biology 3(2): 161-164.
18. Singh, G., Ram, I.C. and Singh, D. (1991). Crop-weed competition studies in green gram and black gram. Tropical Pest Management, 37: 144-148.

19. Williams, L.A.D., Vasques, E., Reid, W., Porter, R. and Kraus, W. (2003). Biological activities of an extract from Cleome viscosa L. (Capparaceae). Naturwissenschaften, 90: 468-472.
20. Devi, B.P., Boominathan, R., Mandal, S.C. (2002). Evaluation of antidiarrhoeal activity of Cleome viscosa Linn. extracts in rats. Phytomedicine, 9(8): 739-742.
21. Reddy, C.N., Reddy, M.D., Devi, M.P. (2000). Efficency of various herbicides on weed control and yield of brinjal. Indian Journal of Weed Science, 32 (3 and 4).
22. Reddy, M.M., Vilatha, A.M. and Rao, L.J. (2007). Integrated weed management in pigeon pea (Cajanus cajan) and soybean (Glycine max) intercropping system on vertisol under rainfed conditions. Indian Journal of Agricultural Sciences 77(3): 177-178.
23. Saroop, S. (2011). A preliminary study on seed to seed cycle of Cleome viscosa L. M.Phil. Dissertation, University of Jammu, Jammu
24. Raju AJS, Ramana KV, Rao NG & Varalakshmi P, Monoecy and entomophily in Cardiospermumcanescens Wall. (Sapindaceae), a medicinally valuable herbaceous vine. Curr Sci, 101 (2011) 617.
25. Kaul V & Koul AK, Floral phenology in relation to pollination and reproductive output in Commelina caroliniana (Commelinaceae). Aust J Bot, 56 (2008) 59.
26. Narbona E, Ortiz PL & Arista M, Functional andromonoecy in Euphorbia (Euphorbiaceae). Ann Bot, 89 (2002) 571.
27. Strauss SY &Whittall JB, Non-pollinator agents of selection on floral traits. In: Ecology and Evolution of flowers (eds.Lawrence D Harder & Spencer CH Barrett, Oxford University Press, Oxford), 2006, 120.
28. Solomon BP, Environmentally influenced changes in sex expression in an andromonoecious plant. Ecology, 66 (1985) 1321.
29. Chauhan S, Rana A, Sharma SB & Chauhan SVS, Reproductive biology of Cleome viscosa L. (Capparaceae). Ann For, 15 (2007) 343.
30. Goodwillie C, Kalisz S & Eckert CG, The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. Annu Rev Ecol Syst, 36 (2005) 47.

RAMAKRISHNA MISSION VIVEKANANDA CENTENARY COLLEGE

Isolation and culture of microalgae from freshwater.

COLLEGE ROLL NO – 407

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SEMESTER – 6TH SEMESTER

SUBJECT – PROJECT WORK

DEPARTMENT – BOTANY (HONS.)

CONTENTS PAGE NO.

A REVIEW ON ISOLATION AND CULTURE OF MICROALGAE FROM FRESH WATER

INTRODUCTION:

The word 'ALGAE' is derived from Latin word 'ALGA', which means seaweed. Algae are photosynthetic (aerobic-oxygenic photosynthesis) organisms because of having chlorophylls and they have simple reproductive structures. They are thallophytes which means lack of differentiate of stems, roots and leaves. They grow in moist habitat. Marine microalgae are unicellular, microscopic plant, which are floating and free living. Microalgae (including prokaryotic photosynthetic microorganisms such as cyanobacteria), as the primary producers of aquatic animals (fishes, shrimps & mollusks). The important microalgae are diatoms, dinoflagellets, green algae, blue-green algae or cyanobacteria etc. The main applications of most microalgae for aquaculture are related to their rich sources of vitamins, amino acids, essential fatty acids, polysaccharides etc. They have various applications in nutraceutical, pharmaceutical, cosmetics, biofuel production etc. Due to the above, the isolation and purification of microalgae are very crucial steps. The objective of algae isolation is to obtain axenic cultures, which means viable cultures of a single species without contaminants. The procedure of microalgae culture has below steps –

- Isolation of required species.
- Preparation of the suitable culture media.
- Maintenance of culture.
- Under controlled condition of light, aeration & temperature

The aim of working with micro-algal axenic cultures in research is to ensure that the results obtained from a study came directly from the microalgae species studied and not from a contaminant microorganism. Any contaminant can have an important economic impact, result in the loss of a batch, or severely modify the composition of the final product. Though freshwater microalgae have been extensively studied, in order to achieve the "green revolution" by "blue biotechnology," there are some areas which need to be focused (Barra et al. 2014) including (i) isolation, identification, and characterization of novel freshwater or marine microalgae strains, **(**ii**)** evaluation of nutritional and abiotic factor to enhance the growth and metabolite content, (iii) development of alternative culture technologies to upscale the biomass production while keeping the cost as low as possible, and (iv) development of PBR (photo-bioreactor) maintenance strategies to enhance the overall efficiency of the procedure. One important aspect is the selection of suitable cultivations strategy. Different cultivation methods are in practice for microalgae growth, including photoautotrophic (photosynthetic fixation in the presence of light), heterotrophic (carbon utilization in the absence of light), and mix-trophic (combination of both previously mentioned methods) (Shahid et al. 2017). Additionally, at mass cultivations, open pond system (natural ponds, lakes, lagoons or artificially designed shallow ponds) is preferred due to the ease of use and cost-effectiveness (Alam et al. 2015). Closed pond system is used when microalgae have to be grown in specific conditions as it offers more control over environmental parameters. Most preferred closed cultivation systems include flat-panel and tubular photo-bioreactors (PBR), while later one is most desirable for outdoor cultivation (Bibi et al. 2017). Hybrid cultivation system is utilize to combine the open and closed cultivation systems in order to achieve the maximum benefits of two methods while limiting the demerits (Shahid et al. 2017). In general, microalgae-based products are quite expensive due to extensive downstream processing (Alam et al. 2016). One possible solution in this regard is to focus on the bio refinery aspect of microalgae cultivation (Gill et al. 2013). In mixotrophic cultivation substrate cost can be reduced by integration of biomass production with wastewater treatment (Gill et al. 2016) as wastewater from various sources like domestic, dairy, piggery, etc. contain a high amount of essential nutrients. So, it is important to investigate the effect of nutrients on biomass growth and metabolite composition to exploit the full potential of the process (Chandra et al. 2016) [1-6].

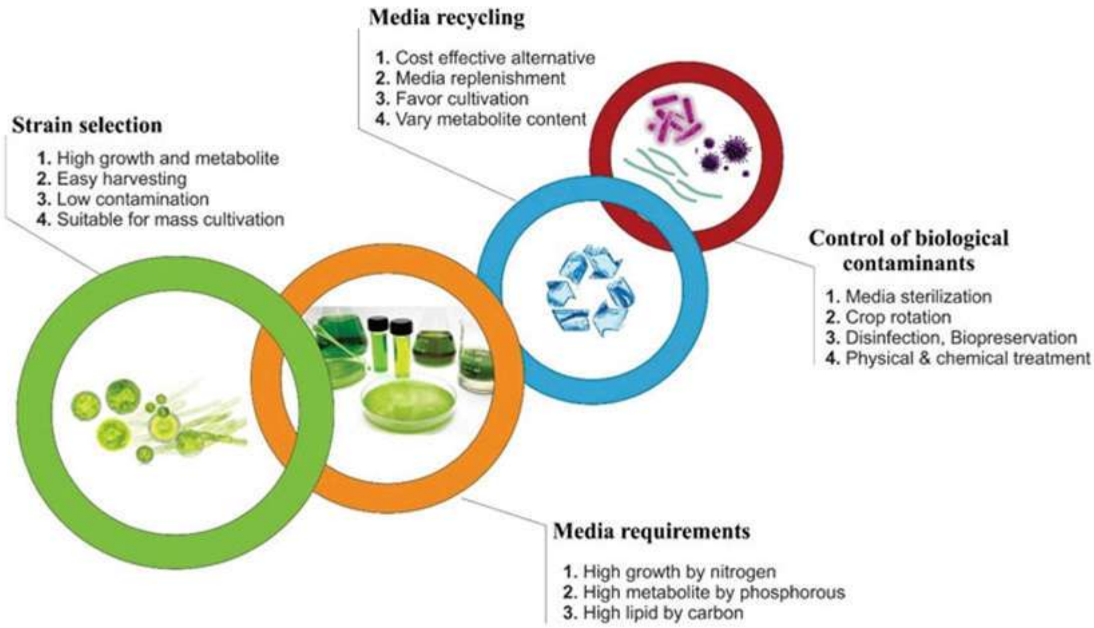

Fig. 1: Areas need to be focused on freshwater microalgae culture techniques

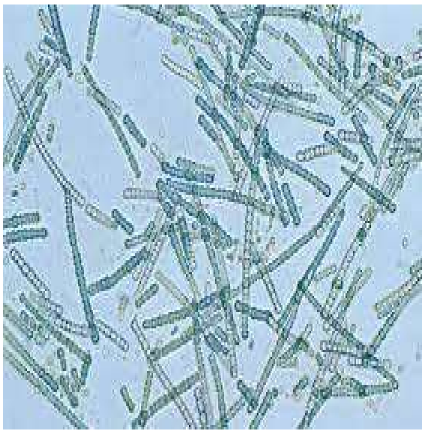
Fig. 2.1:- Cyanobacteria

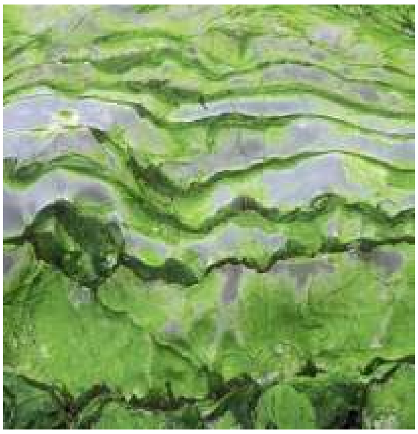
Fig. 2.2:- Green Algae


Fig. 2.3:- Diatom

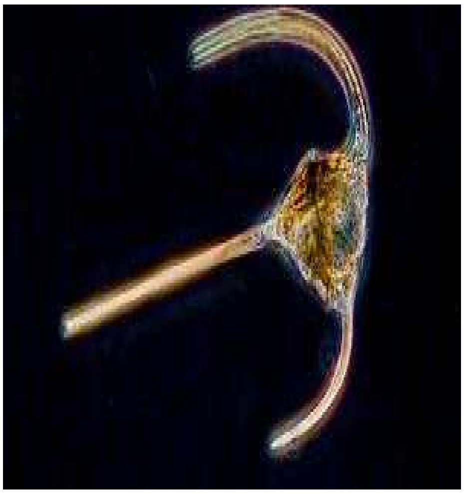
Fig. 2.4:-Dinoflagellate

PRE-ISOLATION TECHNIQUES:

These techniques will applied prior to isolation.

Collection:

The collection method is crucial for a successful establishment of an axenic culture. Damage of algal cells during collection leads to failure. When sampling an unknown environment or target organism, multiple collection methods should be addressed. Sampling techniques may include syringe sampling, scraping, brushing, and the inverted petridish method. However, the most reported sampling method is simply water collection from a water body. If the target species is usually found in-depth, a bottle or rosette sampler can be used. When sampling, it is fundamental to record factors such as light, water temperature, dissolved O_2 and CO_2, nutrient concentration, pH, and salinity to mimic these conditions at the laboratory and establish the location coordinates to replicate sampling if needed [3, 4, 8].

Enrichment of samples:

Once a sample is taken to the lab, adding nutrients to the natural sample will potentiate microalgal growth. Organic substances should be added in small amounts because they can cause undesirable bacterial proliferation. The enriched culture is incubated and examined every few days for the growth of the target species and then individual cells are isolated. Sometimes the target species grow successfully after isolation but die after several transfers, indicating that the culture medium lacks an element or compound or that the organism accumulates wastes that poison their environment [3]. Enrichment of samples should be targeted for different kinds of microalgae; for example, delicate algae and flagellates could be favored by enriching samples with ES enriched seawater; chrysophytes, chlorophytes, diatoms, and cryptophytes grow well under the addition of F/2 media to water samples. On the other hand, cyanobacteria growth is facilitated by adding BG11SW media [9]. This nutrient addition process will facilitate the growth of the targeted genera or species, easing the posterior purification techniques by favoring their growth. This will allow the number of microalgal cells to overthrow the number of fungi or bacterial cells, or even the cells of other microalgal species that are not of interest to the researcher.

Remotion Methods:

Microalgae usually secrete a heterogeneous matrix called extracellular polymeric substances (EPS), which mainly consists of polysaccharides, proteins, nucleic acids, and lipids. This way, algal cells interact with each other, mediate their adhesion to surfaces, and generate cell protection and energy storage reservoirs. Bacteria frequently attach and feed on this EPS layer. Vortexing, sonication, and surfactant treatment prior to isolation methods assist in detaching bacteria and increasing the separation efficiency. Detaching the bacteria from the EPS will increase the effectiveness of techniques such as filtration, centrifugation and antimicrobial treatment by allowing the proper separation of cells by their difference in size and density and making the bacteria more susceptible to the chemical agents added [6].

Filtration:

Filtration process utilizes a semipermeable membrane which can retain microalgae on the membrane while allowing the liquid media to pass through, leaving the algae biomass behind to be collected [10]. This method can harvest high concentration of cell from the medium and the varying pore size of the filter membrane enables the system to suit the need of different microalgae and are able to handle the more delicate species which are prone to damage due to shearing. However, this method is very prone to fouling and clogging and therefore requires frequent change of fresh filter or membrane that might contribute significantly to its processing cost. In view of this constraint, filter membrane using cheap and easily accessible material has been developed. Bejor and his colleagues [11] have successfully developed a filter membrane made out of stretch cotton which can achieve harvesting efficiency of 66–93%.

Table1: Various filtration methods and their respective target microalgae species and advantages.

Filtration techniques	Microalgae Species	Advantages	Disadvantages	References
Cross flow filtration	*Chlorella sp.*	High energy efficiency	Prone to membrane fouling and shearing of fragile materials	[12]
Axil vibration membrane filtration	*Chlorella pyrenoidosa*	Reduced membrane fouling	Require power-consuming pumping units	[13]
Polyactronitrile-based membrane filtration	*Scenedesmas* and *Phaeodactylum*	Reduced membrane fouling	Require power-consuming pumping units	[14]
Tilted membrane panel filtration	Wild microalgae strain	Reduction of membrane cost, and energy consumption	Membrane fouling	[15]
Ultrafiltration	*Dunaliella salina*	Less cell shearing, low energy and chemical consumption	High capital cost	[16]

Centrifugation:

Centrifugation operation separates microalgae cells from the culture media based on each component's density and particle size using centrifugal force. This technique has high cell harvesting efficiency, but the process is time consuming and energy intensive. Moreover, high gravitational force used in centrifugation might cause cellular damage making it unfavourable for certain applications since the sensitive nutrients might be lost. Several types of centrifugal systems have been used in the industry; these include disk stack centrifuges, perforated basket centrifuges, imperforated basket centrifuges, decanters, and hydro-cyclones [7].

MICROALGAE CULTIVATION:

Microalgae are capable to grow rapidly. Their high photosynthesis efficiency coupled with the ability to accumulate a large amount of bio products within their cells make them a suitable candidate to serve as industrial raw material. Besides, cultivation of microalgae does not require fertile land, a large quantity of freshwater, and herbicides and pesticide when compared to the other crop sand thus will not be competing for resources. Furthermore, cultivation of microalgae can even be performed using wastewater such as domestic sewage water and palm oil milling effluents which can assist in bioremediation of wastewater. Apart from wastewater treatment, cultivation of microalgae can also help with reduction of atmospheric carbon dioxide through photosynthesis, effectively contributing to the efforts of tackling greenhouse effect and global warming. Despite the benefits of microalgae cultivation, its developments are still plagued with various problems. For example, the low biomass production and the small size of cells when they are cultured in liquid medium render the harvesting process of microalgae very costly [7].

One of the ways to work around the short comings of usage of microalgae in the industry is by increasing their growth rate to compensate for their low cell density and difficulties in harvesting. Numerous equipment and technologies have been improved across the years to ramp up the production of microalgae. Although microalgae can be easily cultured in a highly controlled laboratory condition, however, it is still harder to ensure the high productivity of microalgae in large-scale production. An ideal microalgae culturing system should possess the characteristics, including: (i) adequate light source, (ii) effective transfer of material across liquid-gas barrier, (iii) simple operation procedure, (iv) minimal contamination rate, (v) cheap overall building and production cost, and (vi) high land efficiency. In general, microalgae culturing system can be broadly classified into two categories, which are the open pond and photobioreactor. Each system has its pros and cons [17].

Open pond:

Open pond cultivation of algae is mostly performed in so-called raceway ponds. Algae grow under daily light conditions and during growth carbon dioxide is taken up while produced oxygen is released from the pond. Additional carbon dioxide is injected at relevant positions in the system to enhance growth [18].

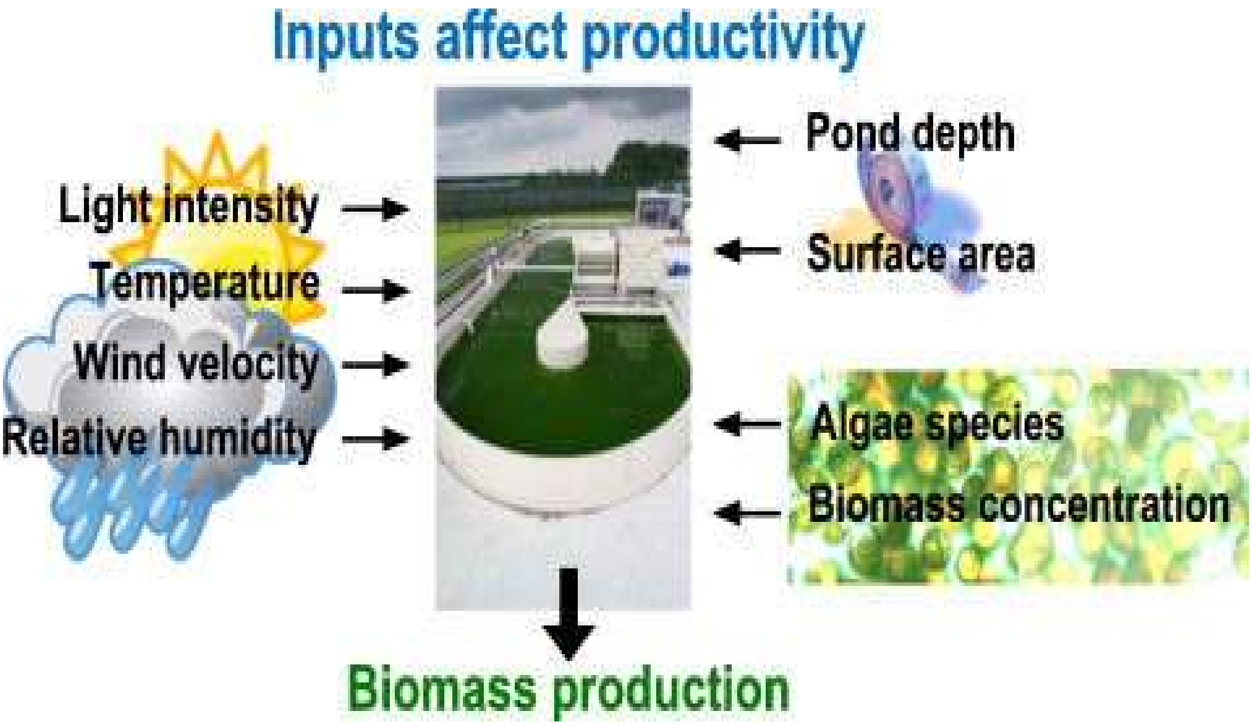

Fig. 3: Open pond microalgae production

Photobioreactor:

Tubular photobioreactors are the most common design of closed systems developed at industrial scale for microalgae cultivation (Torzillo and Zittelli, 2015, Zittelli et al., 2013). These systems are usually made of glass or plastic tubes in which the culture is circulated by pumps or air streams (airlift) [18].

Fig. 4: Closed or semi-closed photobioreactor

ISOLATION TECHNIQUES AND UNIALGAL CULTURE:

In most cases, these techniques will allow the establishment of a unialgal culture, free from other microalgae species, but that may or may not be contaminated with bacteria. Therefore, these methods do not usually ensure the axenicity of the microalgal cultures.

By agar-plating method:

For preparing the agar-medium, 1.5% agar is added to 1 liter of suitable medium this agar solution is sterilized in an autoclave for 15 minutes under 15 lbs pressure and 120ºC temperature. Now this medium is poured in sterilized Petri dishes and left for 24 hrs. In case of culture tubes, the medium is poured in 1/3 part in tubes and properly plugged with cotton before autoclaving [1].

Single cell isolation technique:

This method can be performed with a micropipette, a Pasteur pipette, or a glass capillary. The objective is to collect a single cell from the sample, deposit it in a sterile droplet of culture medium, pick up the cell again, and transfer it to a second sterile droplet. This process is repeated until a single algal cell can be placed into the final isolation vessel. Novel techniques for single-cell isolation include micromanipulation by employing stereomicroscope, microcapillary tubes, and optical tweezers, which greatly reduces labor and cell damage [6].

Dilution techniques:

The dilution technique has been used for many years and it is effective for organisms that are rather abundant in the sample but largely ineffective for rare organisms. The goal of the dilution method is to deposit only one cell into a test tube, flask, or well of a multiwell plate, thereby establishing a single-cell isolate (Kufferath 1928/29, Droop 1954, Throndsen 1978). If the approximate cell concentration is known, then it is easy to calculate the necessary dilution so that, on the basis of probability, a small volume (e.g., one droplet, 50 mL, 1 mL) contains a single cell. In practice, some contain more than one cell and others contain no cells at all. If the approximate cell number is unknown and cannot be immediately calculated (e.g., at a pond or aboard ship), then one can make repeated serial dilutions of 1:10 and between five and six repetitions is enough in most cases (i.e., six repeated 1:10 dilutions theoretically places a single cell into the final tube if the original sample had 106 cells·mL-1). The dilution technique is perhaps most often used when attempting to culture random algal species from field samples, often with the goal of discovering new species. The technique can be altered in several ways. First, the dilution can be made with culture medium, distilled water (freshwater organisms), filtered water from the sample site or some combination of these. The culture medium can be full strength for weedy organisms or very dilute for fastidious organisms. Second, an intensive effort can be made at the concentration at which single-cell isolation is expected (e.g., 10, 50 or 100 attempts). Commonly, one inoculates a large number of tubes from the last dilution, assuming that some single cells will die, some will contain two or more species, and some will receive no cells at all. Third, ammonium, selenium or another element can be added to some isolation tubes or cell walls to specifically select for species that require these nutrients. Similarly, one can incubate some isolates at one temperature or light regimen and others with different regimens. Axenic isolates aren't often obtained with the dilution technique, because bacteria usually more abundant than algae [3].

PURIFICATION METHODS FOR MICROALGAE AND OBTENTION OF AXENIC CULTURES:

Once a target species has been grown and isolated from other microalgae species, it is time to generate an axenic culture by eliminating any traces of bacteria or fungi that are commonly associated with micro-algal culture contamination. The use of the sterile technique and pre-sterilization of any material used during any of the steps of isolation, purification, sub-culture or escalation of microalgae, minimizes the risk of contamination and increases the success of axenic microalgae culture achievement. A laminar flow hood is recommended, but the sterile technique can still be performed in a laboratory room without one if precautions are taken. The sterility of materials should be checked and if there is any doubt, the material should be discarded. During purification, the main issue is to avoid killing the target species since algae are vulnerable to adverse environments. For microalgae, the addition of purification antibiotics or other selective agents is common.

Antibiotic treatment:

The main objective of antibiotic treatment is to reduce bacterial contamination of an algal culture to a number that will allow a small inoculum to transfer enough algal cells without viable bacteria. The efficacy and toxicity of the antibiotic treatment depend on the concentration and period of exposure and vary depending on the microalgae and bacteria species. Viable bacteria tend to decline drastically after 48 h of exposure to the microalgal culture to the antibiotic treatment. Then a small portion can be transferred to an adequate medium without antibiotics. Antibiotics will work in one of two ways: bactericidal (e.g., penicillin, vancomycin) will kill bacteria by interfering with the formation of the bacterial cell wall or bacteriostatic (e.g. chloramphenicol, tetracycline), which interferes with the bacterial metabolism but does not necessarily kill them. Theoretically, antibiotics will destroy all the contaminants without harming algal cells, but this is rarely achieved in practice. A well-studied and basic antibiotic treatment is 100/25/25 mg/L of penicillin, streptomycin, and gentamycin which is usually tolerated by most algae. Purification may also be achieved by the sequential transfer of the algal culture through a series of different antibiotics. This method has lower toxicity to the algae. Also, one antibiotic mix may be fatal to some bacteria but only suppress the growth of others, while using sequential antibiotics may kill the surviving bacteria [6].

Enzymatic treatment:

Obtaining axenic cyanobacteria culture using an antibiotic treatment usually has certain limitations and sometimes requires an alternative. Microalgae tend to be more resistant to lysozyme digestion than bacteria. Another option for generating an axenic culture is using a lysozyme base. Axenic cyanobacterial cultures have previously been obtained by treating cultures with the minimum lethal concentration for up to 90 min. Also, combinations of enzymatic treatment and antibiotics can lead to promising results. Tale (2014) used a combination of lysozyme (20µg/mL) and antibiotic mixture (cefotaxime-500µg/mL and tetracycline 50µg/mL) to obtain axenic cultures of the genera *Chlorella* and *Monoraphidium* [19].

GROWTH DYNAMICS:

The growth of micro-algae is differentiated into five phases. Growth denotes the increase in number beyond that present in the original inoculums [20]. Five distinct phases of growth are described below:-

THE LAG PHASE:

After the addition of inoculums to the above mentioned culture medium (Bristol Medium), the population remains unchanged temporarily. The cells at this point increase in size beyond their normal dimensions. Physiologically, they are very active and are synthesizing new protoplasm. The organisms are actually metabolizing, but there is a lag in cell division.

THE EXPONENTIAL PHASE:

The cells begin to divide steadily at a constant rate. Give optimum culture conditions, growth rate is maximal at this stage.

THE DECLINING GROWTH RATE PHASE:

Cell division slows down when nutrients, light, pH, carbon dioxide or other physical and chemical factors begin to limit growth.

THE STATIONARY PHASE:

Now at this point the exponential phase of growth begins to taper off after several hours (or days) in a gradual fashion. The population more or less remains constant for a time, perhaps as a result of complete cessation of division or the balancing of reproduction rate by an equivalent death rate.

THE DEATH PHASE:

The rate at which some cells die is faster than the rate of reproduction of new cells. The number of viable cells decreases geometrically. Cell density decreases rapidly and the culture eventually collapses.

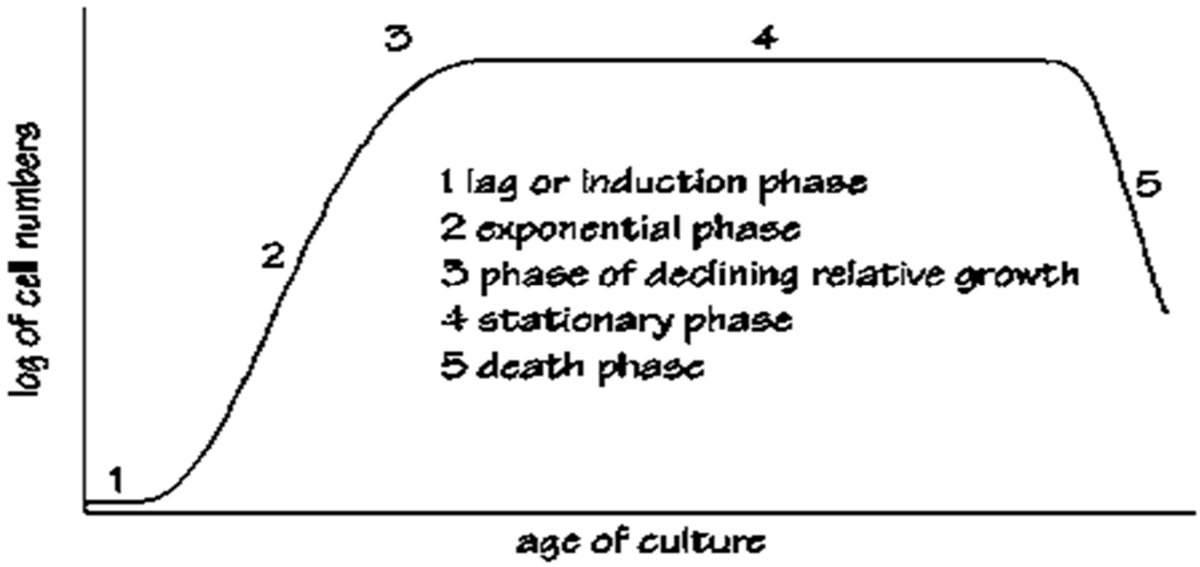

Fig. 5: Schematic diagram of algal growth phase

ENVIRONMENTAL FACTORS AFFECTING THE GROWTH: [21]

TEMPERATURE:

An organism's metabolic rate is affected by average temperature. For micro-algal growth, low temperatures are usually maintained in controlled rooms (18-23°C). Inoculate or transfer of algal starters must have been cultured previously in controlled rooms. Mass production should be done in early morning to prevent stress happened by sudden temperature increase. Other than growth, temperature factor also have an effect on the cell size and biochemical composition of microalgae. Normally, the ideal temperature range for micro-algal growth at optimal condition is between 20°C to 35°C.

LIGHT INTENSITY:

Principally, there are basic characteristics of light that influence biological plant growth, namely quantity, quality & duration of exposure. As with all plants, micro-algae photosynthesize, *i.e.* they assimilate inorganic carbon into organic matter. Light is one of the major energy input sources for the photosynthesis of microalgae. Thus, light quantity refers to the intensity of the illumination either from natural or artificial light. Expectedly, the growth of microalgae biomass in the culture is increased by increasing the light intensity until reach at saturation point where the photosynthesis rate is at the maximum level. However, too much light intensity exposure or oversaturation of light may lead to photo-inhabitation. Photo-inhabitation occurs due to the formation of reactive oxygen species which is harmful to microalgae cell and indirectly decrease the biomass productivity. Therefore, difference microalgae species and strain have difference requirement of light intensity for their growth at optimal condition.

PHOTOPERIOD:

Generally, photoperiod refers to light exposure in terms of duration with minimum and maximum are 0:24 hour and 24:0 hour, respectively. Photoperiod is as important as light intensity because it directly influences the efficiency of photosynthesis of microalgae in the culture. However, the optimum or ideal photoperiod of microalgae is depending on the species and strain. Other than that, the natural habitat of microalgae also may contribute to differences in required optimal photoperiod. Photoperiod is also important if the economic aspect is taken into account, since algae biomass is produced with the light from artificial sources.

SALINITY:

Another basic environmental factor that influences the growth of microalgae is salinity. Salinity refers to the presence of salt content in the water for microalgae grow. Expectedly, marine algae use or consume higher salinity concentration if compared to freshwater algae. Salinity is also a critical parameter to be tested as the presence of salinity may influence the growth of algae and biochemical composition of algae cell. Exposing algae to high salinity is harmful for freshwater algae cells, since it might transform their cell structure. Excess salinity inhibits photosynthesis process reduces the biomass productivity of microalgae. Therefore, the optimal salinity concentration acceptable to some microalgae species. Most microalgae are tolerant to salinity within 10psu-30psu except for Artic-seaice algae with a salinity of between 4psu-74psu. Mean while, *Dunaliella* sp. grew at optimamally when 25% NaC1 was added to the medium culture. Another study by Qin and Li on *Botryococcus braunii* Strain CHN 357 found that 0.15M of NaC1 was the greatest growth in the culture. In summary, different microalgae species require different concentrations of salinity for proper growth and biochemical composition.

pH:

The pH range for most cultured algal species is between 7 & 9, specifically the optimum range being 8.2-8.7. Complete culture collapse due to the disruption of many cellular processes can result from a failure to maintain an acceptable pH. The latter is accomplished by aerating the culture. In the case of high-density algal culture, the addition of carbon dioxide allows to correct for increased pH, which may reach limiting values of up to pH 9 during algal growth.

CARBON DIOXIDE:

Providing the algae with extra carbon, in the form of the gas carbon-dioxide (CO_2), will give much faster growth. CO_2 is supplied from compressed gas cylinders, and only a very little is needed (about half of one percent) in the air supplied to the culture. The CO_2 should be passed through a flow meter to ensure that the amount used will keep the pH of the culture between 7.8 and 8.0. The pH can be checked with indicator papers, which change colour with a change in pH, or with a pH meter. Both the air and the CO_2 should be filtered through an in-line filter unit of 0.3 microns to 0.5 microns before entering the culture, as this helps to prevent other, possibly contaminating, organisms from getting into the cultures.

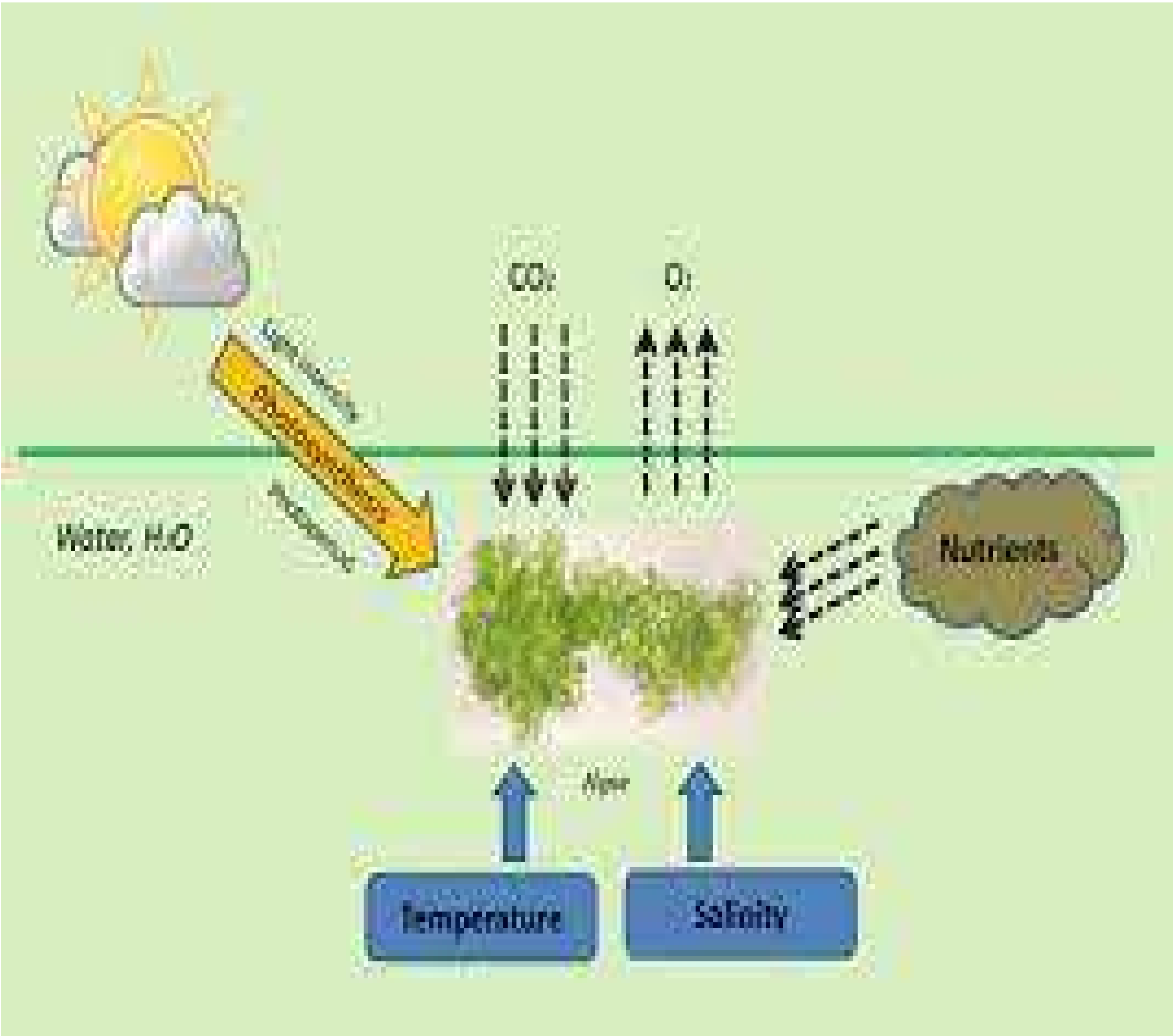

Fig. 6: Photosynthesis mechanism of microalgae influenced by light intensity, photoperiod temperature and saline.

CONTAMINATION CONTROL:

Despite using the sterile technique, contamination may occur. Therefore, the evaluation of contamination in any step of algal culture is essential for quality control. When a culture is presumably contaminated, several methods can help detect it. The simplest technique is microscopy, which allows to directly perceive the growth of contaminant microorganisms. This can be combined with the use of dyes that highlight undesired microorganisms. One of the disadvantages of microscopy is that it requires an experienced operator in order to obtain good results. Sterility tests can be performed by inoculating with the microalgae culture a 0.1% peptone agar or broth and incubating at a suitable temperature according to the suspected contaminant. This is usually satisfactory to observe bacterial or fungal growth. Malt extract is an alternative if fungi contamination is suspected. To test for air-borne contamination, a series of the test medium is exposed over specific periods, incubated, and observed. Variations of these simple tests can be used to test culture medium, vitamins, stocksolutions, pipettes, *etc*. Flow cytometry may also be used to detect contamination in cultures. However, as mentioned above, it is not cheap. Molecular techniques based on the polymerase chain reaction (PCR) have the advantage of detecting contamination even in low concentrations, and there are other new molecular alternatives to identify biological contaminants such as metagenomics, metatranscriptomics, and metaproteomics. Nonetheless, they are far more expensive than microscopy or sterility tests. These methods, with nearly endless modifications, can be performed to help evaluate sterile conditions. The tests must be carried out under conditions that show positive results when contamination exists and should not be considered as an absolute measure of sterility but as indicators of contamination. Different protocols and modifications are often required according to available materials and the goal of microalgae culture. As mentioned above, microalgae have applications in nutraceuticals, pharmaceuticals, cosmetics, biofuel production, etc. However, a bulk volume of microalgal biomass is needed to achieve its commercialization. Most methods for production are based on open ponds (which increase the chances of contamination from the environment) and closed photobioreactors. Open pond production has been successful only for a limited number of microalgae by applying very high salinity or high pH. Mass production of microalgae depends on large volumes of water. Therefore, it is unrealistic to adopt heat sterilization. Although water can be processed with bleach or filtered before mass cultivations, some pollutants usually remain. Closed photobioreactors reduce exposure of culture to the environment, giving better protection against pollutants. However, aeration is needed. Filtration with a microporousmembrane is generally adopted for air sterilization, but it is impractical to apply it to large volumes of air during masscultivation. Complete sterility is very difficult to achieve, even in closed photobioreactors. Therefore, it is essential to developtechniques for the timely identification of contaminants andachieve significant control. Temperature is a variable that could help controlcontamination. Incubating outside the temperature range ofcontaminant microorganisms may help reduce the risk of theirgrowth, keeping in mind the optimal temperature formicroalgal growth. Another factor that can be manipulated forthe benefit of culture control is using pH values that maysuppress contaminant growth but maintain the viablemicroalgae. Similarly, salinity can inhibit the development ofcontamination. Chemical agents can be applied to controlcontaminants, but this requires knowing the undesiredmicroorganism, the minimum inhibitory concentration of theagent to use, and the tolerance of the cultivated microalgae. Commonly used chemical agents are antibiotics, fungicides, pesticides, salts, aldehydes, peroxides, and ammonia or combinations. However, this alternative for contamination control is generally applied in the initial stages of cultivation (axenization processes). Another alternative for contamination control is the use of pulsed electric fields to selectively damagecells in the culture. One of the problems is that there is not enough information for biological control. Mechanisms known so far for contamination control have deficiencies and are not always effective as contamination may persist and lead tolower productivity, poor microbiological quality, or deviance from the intended composition. An alternative is the use of controlled closed and smaller systems. Although they have a higher economic impact on production, they allow for better control of any source of contamination and are recommended for feedstuffs or nutraceuticalapplications of biomass. Strain selection with resistance to biological pollutants is also an important factor to consider when mass production is intended. A greater understanding of how these biological pollutants interact with host microalgae and reasonable cultivation technology may lead to the development of effective control methods [6].

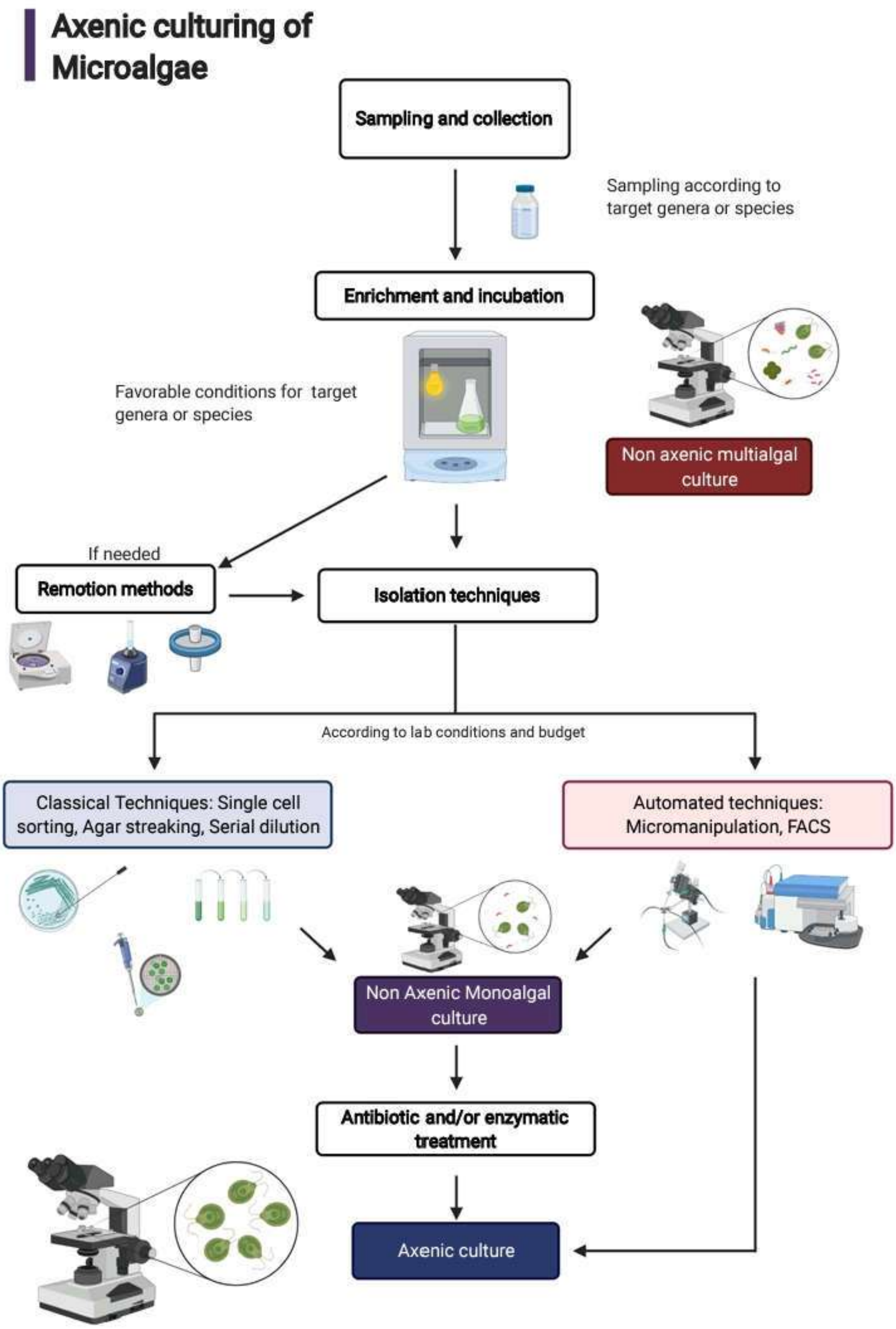

Fig. 7: General processing steps for axenic microalgae culture obtention.

CONCLUSION:

Based on the methodology reviewed, the best approach for microalgal isolation, obtention of axenic cultures, and maintenance depends on various factors. First, researchers must consider if there is a target species, in which case themethodology should be emphasized on sampling the correct environment, provide species requirements, and be aware of their weaknesses by using the appropriate media and avoiding purification and maintenance techniques that could be harmful. If the objective of isolation is to discover or study several species in a certain geographic location or sample, the approach will be completely different. Several sampling methods, media culture, and growth conditions should be applied. Once several genera have been identified, the criteria for isolating each must be applied. Another issue to be taken into account in microalgae isolation is the accessibility, resources, and lab facilities of the research institution. Classical techniques should beapplied. However, if the researcher is limited to resources for applying classical techniques, successful isolation, axenic culture establishment, and maintenance can be achieved without a major obstacle beyond the time consumed. Once the available techniques have been determined, a serial combination of them is recommended in order to achieve better results in the axenic microalgae culture. If the main goal is to culture contamination control should be taken into account. However, this is not an easy task, as strain selection, mass culture conditions, and periodical tests for contaminant microorganisms should be addressed previously to mass cultivation.

HIGHLIGHTED TOPICS:

- Microalgae isolation and purification
- Microalgae cultivation in open ponds and tubular photobioreactors
- Harvesting techniques like filtration, centrifugation
- Contamination control

REFERENCES:

1. Perumal P., Prasath B.B., Santhanam P., Ananth S., Devi A.S. and Kumar S.D., 2012. Isolation and culture of microalgae. In Workshop on advances in aquaculture technology (pp. 166-181).
2. Hyka P, Lickova S, Přibyl P, Melzoch K, Kovar K, 2013. Flow cytometry for the development of biotechnological processes with microalgae. Biotechnol ; 31(1): 2-16.
3. Andersen RA. Algal culturing techniques.Elsevier 2005.
4. Jahn MT, Schmidt K, Mock T, Plant Methods 2014. A novel cost effective and highthroughput isolation and identificationmethod for marine microalgae; 10(1): 26.
5. Fistarol GO, Hargreaves PI, Walter JM, et al. ApplPhycol 2018. Rapid isolation of culturable microalgae from a tropical shallow lake system ; 30(3): 1807-19.
6. Fernandez-Valenzuela S., Chávez-Ruvalcaba F., Beltran-Rocha J.C., San Claudio P.M. and Reyna-Martínez R., 2021. Isolation and Culturing Axenic Microalgae: Mini–Review. The Open Microbiology Journal, 15(1).
7. Tan J.S., Lee S.Y., Chew K.W., Lam M.K., Lim J.W., Ho S.H. and Show P.L., 2020. A review on microalgae cultivation and harvesting, and their biomass extraction processing using ionic liquids.Bioengineered, 11(1), pp.116-129.
8. Singh P, Gupta SK, Guldhe A, Rawat I, Bux F, 2015. Microalgae isolationand basic culturing techniques. Handbook of marine microalgae. Academic Press; pp. 43-54.
9. Barclay W, Apt K, 2013.Strategies for bioprospecting microalgae for potential commercial applications.Handbook of microalgal culture. Academic Press; pp. 69-79.
10. Al Hattab M, Ghaly A, Hammoud A, 2015. Microalgae harvesting methods for industrial production of biodiesel: critical review and comparative analysis. J Fundam Renewable Energy Appl.;5(2):1000154.
11. Bejor ES, Mota C, Ogarekpe NM, et al, 2013. Low-cost harvesting of microalgae biomass from water. Int J Dev Sustain.; 2(1):1–11.
12. Giménez JB, Bouzas A, Carrere H, et al, 2018. Assessment of cross-flow filtration as microalgae harvesting technique prior to anaerobic digestion: evaluation of biomass integrity and energy demand. Bioresour Technol.;269:188–194.

13. Zhao F, Chu H, Zhang Y, et al, 2017. Increasing the vibration frequency to mitigate reversible and irreversible membrane fouling using an axial vibration membrane inmicroalgae harvesting. J Membr Sci.;529:215–223.
14. Marbelia L, Mulier M, Vandamme D, et al, 2016. Polyacrylonitrile membranes for microalgae filtration: influence of porosity, surface charge and microalgae species on membrane fouling. Algal Res.;19:128–137.
15. Eliseus A, Bilad MR, Nordin NAHM, et al. Tilted membrane panel: a new module concept to maximize the impact of air bubbles for membrane fouling control in microalgae harvesting. Bioresour Technol. 2017;241:661–668.
16. Wenten I, Steven S, Dwiputra A, et al, 2017. From lab to full-scale ultrafiltration in microalgae harvesting. In Journal of Physics: Conference Series.. Bandung, Indonesia: IOP Publishing.
17. Jegathese SJP, Farid M, 2014;. Microalgae as a renewable source of energy: A niche opportunity. J Renewable Energy. 2014:1–10.
18. Narala R.R., Garg S., Sharma K.K., Thomas-Hall S.R., Deme M., Li Y. and Schenk P.M., 2016.Comparison of microalgae cultivation in photobioreactor, open raceway pond, and a two-stage hybrid system.Frontiers in Energy Research, 4, p.29.
19. Tale M, Ghosh S, Kapadnis B, Kale S, 2014. Isolation and characterization of microalgae for biodiesel production from Nisargruna biogasplanteffluent. BioresourTechnol; 169: 328-35.
20. Kazamia E., Riseley A.S., Howe C.J. and Smith A.G., 2014. An engineered community approach for industrial cultivation of microalgae. Industrial Biotechnology, 10(3), pp.184-190.
21. Gani P., Sunar, N.M., Matias-Peralta, H.M. and Apandi, N., 2019. An overview of environmental factor’s effect on the growth of microalgae. Journal of Applied Chemistry and Natural Resources, 1(2).

RAMAKRISHNA MISSION VIVEKANANDA CENTENARY COLLEGE

Ecological Aspect of Organic Farming

COLLEG ROLL: 408

REGISTRATION NUMBER : AO1-1112-114-007-2019

SEMESTER: 6th SEMESTER:

SUBJECT: PROJECT WORK

DEPARTMENT : BOTANY

CONTENTS

ORGANIC FARMING

INTRODUCTION:

Organic farming is an agricultural system that uses fertilizers of organic origin such as compost manure, green manure and bone meal and places emphasis on techniques such as crop rotation and companion planting. It originated early in the 20th century in reaction to rapidly changing farming practices. Certified organic agriculture accounts for 70 million hectares globally, with over half of that total in Australia. Organic farming continues to be developed by various organizations today. Biological pest control, mixed cropping and the fostering of insect predators are encouraged. Organic standards are designed to allow the use of naturally-occurring substances while prohibiting or strictly limiting synthetic substances. For instance, naturally-occurring pesticides such as pyrethrin and rotenone are permitted, while synthetic fertilizers and pesticides are generally prohibited. Synthetic substances that are allowed include, for example, copper sulfate, elemental sulfur and Ivermectin. Genetically modified organisms, nanomaterials, human sewage sludge, plant growth regulators, hormones, and antibiotic use in livestock husbandry are prohibited. Organic farming advocates claim advantages in sustainability, openness, self-sufficiency, autonomy and independence, health, food security, and food safety.

Organic agricultural methods are internationally regulated and legally enforced by many nations, based in large part on the standards set by the International Federation of Organic Agriculture Movements (IFOAM), an international umbrella organization for organic farming organizations established in 1972. Organic agriculture can be defined as "an integrated farming system that strives for sustainability, the enhancement of soil fertility and biological diversity while, with rare exceptions, prohibiting synthetic pesticides, antibiotics, synthetic fertilizers, genetically modified organisms, and growth hormones".[1-10]

HISTORY AND ORIGIN:

The first 40 years of the 20th century saw simultaneous advances in biochemistry and engineering that rapidly and profoundly changed farming. The introduction of the gasoline-powered internal combustion engine ushered in the era of the tractor and made possible hundreds of mechanized farm implements. Research in plant breeding led to the commercialization of hybrid seed. And a new manufacturing process made nitrogen fertilizer first synthesized in the mid-19th century affordably abundant. These factors changed the labour equation: there were almost no tractors in the US around 1910, but over 3,000,000 by 1950; in 1900, it took one farmer to feed 2.5 people, but currently the ratio is 1 to well over 100. Fields grew bigger and cropping more specialized to make more efficient use of machinery. The reduced need for manual labour and animal labour that machinery, herbicides, and fertilizers made possible created an era in which the mechanization of agriculture evolved rapidly.

Consciously organic agriculture (as opposed to traditional agricultural methods from before the inorganic options existed, which always employed only organic means) began more or less simultaneously in Central Europe and India. The British botanist Sir Albert Howard is often referred to as the father of modern organic agriculture, because he was the first to apply modern scientific knowledge and methods to traditional agriculture. From 1905 to 1924, he and his wife Gabrielle, herself a plant physiologist, worked as agricultural advisers in Pusa, Bengal, where they documented traditional Indian farming practices and came to regard them as superior to their conventional agriculture science. Their research and further development of these methods is recorded in his writings, notably, his 1940 book, An Agricultural Testament, which influenced many scientists and farmers of the day.

In Germany, Rudolf Steiner's development, biodynamic agriculture, was probably the first comprehensive system of what we now call organic farming. This began with a lecture series Steiner presented at a farm in Koberwitz (Kobierzyce now in Poland) in 1924. Steiner emphasized the farmer's role in guiding and balancing the interaction of the animals, plants and soil. Healthy animals depended upon healthy plants (for their food), healthy plants upon healthy soil, healthy soil upon healthy animals (for the manure). His system was based on his philosophy of anthroposophy rather than a good understanding of science. To develop his system of farming, Steiner established an international research group called the Agricultural Experimental Circle of Anthroposophical Farmers and Gardeners of the General Anthroposophical Society.

In 1909, American agronomist F.H. King toured China, Korea, and Japan, studying traditional fertilization, tillage, and general farming practices. He published his findings in Farmers of Forty Centuries (1911). King foresaw a "world movement for the introduction of new and improved methods" of agriculture and in later years his book became an important organic reference.

The term "organic farming" was coined by Walter James (Lord Northbourne), a student of Biodynamic Agriculture, in his book Look to the Land (written in 1939, published 1940).[10][11] In this text, James described a holistic, ecologically balanced approach to farming, "the farm as organism," basing this on Steiner's agricultural principles and methods. One year previously to his book's publication, James had hosted the first Biodynamic Agriculture conference in England, the Betteshanger Summer School and Conference, at which Ehrenfried Pfeiffer was the key presenter.

In 1939 James, Albert Howard, Ehrenfried Pfeiffer and George Stapleton joined at Farleigh to implement an experiment comparing Biodynamic, organic and chemical fertilization methods. "The Farleigh Experiment", had been planned since initial meetings in 1936 including ten participants. The experiment was cut short due to the fact that Biodynamic compost was not available until after the Betteshanger Summer School event, the disruption of the impending war, and lack of funding. Though inconclusive, this experiment was seen as providing impetus for the similar "Haughley Experiment" described below.

In 1939 Lady Eve Balfour, who had been farming since 1920 in Haughley Green, Suffolk, England, launched the Haughley Experiment. Lady Balfour believed that mankind's health and future depended on how the soil was used, and that non-intensive farming could produce more wholesome food. The experiment was run to generate data to test these beliefs. Four years later, she published The Living Soil, based on the initial findings of the Haughley Experiment. Widely read, it led to the formation of a key international organic advocacy group, the Soil Association.

In Japan, Masanobu Fukuoka, a microbiologist working in soil science and plant pathology, began to doubt the modern agricultural movement. In 1937, he quit his job as a research scientist, returned to his family's farm in 1938, and devoted the next 60 years to developing a radical no-till organic method for growing grain and many other crops, now know as natural farming , nature farming, 'do–nothing' farming or Fukuoka farming.

Technological advances during World War II accelerated post-war innovation in all aspects of agriculture, resulting in large advances in mechanization (including large-scale irrigation), fertilization, and pesticides. In particular, two chemicals that had been produced in quantity for warfare, were repurposed for peacetime agricultural uses. Ammonium nitrate, used in munitions, became an abundantly cheap source of nitrogen. And a range of new pesticides appeared: DDT, which had been used to control disease-carrying insects around troops, became a general insecticide, launching the era of widespread pesticide use. At the same time, increasingly powerful and sophisticated farm machinery allowed a single farmer to work larger areas of land and fields grew bigger.

In 1944, an international campaign called the Green Revolution was launched in Mexico with private funding from the US. It encouraged the development of hybrid plants, chemical controls, large-scale irrigation, and heavy mechanization in agriculture around the world.

During the 1950s, sustainable agriculture was a topic of scientific interest, but research tended to concentrate on developing the new chemical approaches. One of the reasons for this, which informed and guided the ongoing Green Revolution, was the widespread belief that high global population growth, which was demonstrably occurring, would soon create worldwide food shortages unless humankind could rescue itself through ever higher agricultural technology. At the same time, however, the adverse effects of "modern" farming continued to kindle a small but growing organic movement. For example, in the US, J.I. Rodale began to popularize the term and methods of organic growing, particularly to consumers through promotion of organic gardening.

In 1962, Rachel Carson, a prominent scientist and naturalist, published Silent Spring, chronicling the effects of DDT and other pesticides on the environment. A bestseller in many countries, including the US, and widely read around the world, Silent Spring is widely considered as being a key factor in the US government's 1972 banning of DDT. The book and its author are often credited with launching the worldwide environmental movement.

In the 1970s, global movements concerned with pollution and the environment increased their focus on organic farming. As the distinction between organic and conventional food became clearer, one goal of the organic movement was to encourage consumption of locally grown food, which was promoted through slogans like "Know Your Farmer, Know Your Food".

An early organic farmers' association, the Maine Organic Farmers and Gardeners Association was founded in 1971.

In 1972, the International Federation of Organic Agriculture Movements (IFOAM) was founded in Versailles, France and dedicated to the diffusion and exchange of information on the principles and practices of organic agriculture of all schools and across national and linguistic boundaries.

In 1975, Fukuoka released his book, The One-Straw Revolution, with a strong impact in certain areas of the agricultural world. His approach to small-scale grain production emphasized a meticulous balance of the local farming ecosystem, and a minimum of human interference and labour.

In the U.S. during the 1970s and 1980s, J.I. Rodale and his Rodale Press (now Rodale, Inc.) advocated for organic farming methods. The press's books offered how-to information and advice to Americans interested in trying organic gardening and farming.

In 1984, Oregon Tilth established an early organic certification service in the United States. In the 1980s, around the world, farming and consumer groups began seriously pressuring for government regulation of organic production. This led to legislation and certification standards being enacted through the 1990s and to date. In the United States, the Organic Foods Production Act of 1990 tasked the USDA with developing national standards for organic products, and the final rule establishing the National Organic Program was first published in the Federal Register in 2000·

In Havana, Cuba, the loss of Soviet economic support following the collapse of the Soviet Union in 1991 led to a focus on local agricultural production and the development of a unique state-supported urban organic agriculture program called organopónicos.

Since the early 1990s, the retail market for organic farming in developed economies has been growing by about 20% annually due to increasing consumer demand. Concern for the quality and safety of food, and the potential for environmental damage from conventional agriculture, are apparently responsible for this trend. [14-25]

METHODS:

Organic farming methods combine scientific knowledge of ecology and some modern technology with traditional farming practices based on naturally occurring biological processes. Organic farming methods are studied in the field of agroecology. While conventional agriculture uses synthetic pesticides and water-soluble synthetically purified fertilizers, organic farmers are restricted by regulations to using natural pesticides and fertilizers. An example of a natural pesticide is pyrethrin, which is found naturally in the Chrysanthemum flower. The principal methods of organic farming include crop rotation, green manures and compost, biological pest control, and mechanical cultivation. These measures use the natural environment to enhance agricultural productivity: legumes are planted to fix nitrogen into the soil, natural insect predators are encouraged, crops are rotated to confuse pests and renew soil and natural materials such as potassium bicarbonate and mulches are used to control disease and weeds. Genetically modified seeds and animals are excluded.

While organic is fundamentally different from conventional because of the use of carbon-based fertilizers compared with highly soluble synthetic based fertilizers and biological pest control instead of synthetic pesticides, organic farming and large-scale conventional farming are not entirely mutually exclusive. Many of the methods developed for organic agriculture have been borrowed by more conventional agriculture. For example, Integrated Pest Management is a multifaceted strategy that uses various organic methods of pest control whenever possible, but in conventional farming could include synthetic pesticides only as a last resort. [26 & 27]

CROP DIVERSITY:

Organic farming encourages crop diversity. The science of Agroecology has revealed the benefits of polyculture (multiple crops in the same space), which is often employed in organic farming. Planting a variety of vegetable crops supports a wider range of beneficial insects, soil microorganisms, and other factors that add up to overall farm health. Crop diversity helps environments thrive and protects species from going extinct. [28 & 29]

SOIL MANAGEMENT:

Organic farming relies more heavily on the natural breakdown of organic matter than the average conventional farm, using techniques like green manure and composting, to replace nutrients taken from the soil by previous crops. Farmers use a variety of methods to improve soil fertility, including crop rotation, cover cropping, reduced tillage, and application of compost. By reducing fuel-intensive tillage, less soil organic matter is lost to the atmosphere. This has an added benefit of carbon sequestration, which reduces greenhouse gases and helps reverse climate change. Reducing tillage may also improve soil structure and reduce the potential for soil erosion.

Plants need a large number of nutrients in various quantities to flourish. Supplying enough nitrogen and particularly synchronization, so that plants get enough nitrogen at the time when they need it most, is a challenge for organic farmers. Crop rotation and green manure (cover crops) help to provide nitrogen through legumes (more precisely, the family Fabaceae), which fix nitrogen from the atmosphere through symbiosis with rhizobial bacteria. Intercropping, which is sometimes used for insect and disease control, can also increase soil nutrients, but the competition between the legume and the crop can be problematic and wider spacing between crop rows is required. Crop residues can be ploughed back into the soil, and different plants leave different amounts of nitrogen, potentially aiding synchronization. Organic farmers also use animal manure, certain processed fertilizers such as seed meal and various mineral powders such as rock phosphate and green sand, a naturally occurring form of potash that provides potassium. In some cases pH may need to be amended. Natural pH amendments include lime and sulfur, but in the U.S. some compounds such as iron sulfate, aluminum sulfate, magnesium sulfate, and soluble boron products are allowed in organic farming.

Mixed farms with both livestock and crops can operate as ley farms, whereby the land gathers fertility through growing nitrogen-fixing forage grasses such as white clover or alfalfa and grows cash crops or cereals when fertility is established. Farms without livestock (stockless) may find it more difficult to maintain soil fertility, and may rely more on external inputs such as imported manure as well as grain legumes and green manures, although grain legumes may fix limited nitrogen because they are harvested. Horticultural farms that grow fruits and vegetables in protected conditions often rely even more on external inputs. Manure is very bulky and is often not cost-effective to transport more than a short distance from the source. Manure for organic farms' may become scarce if a sizable number of farms become organically managed. [30 & 31]

WEED MANAGEMENT:

Organic weed management promotes weed suppression, rather than weed elimination, by enhancing crop competition and phytotoxic effects on weeds. Organic farmers integrate cultural, biological, mechanical, physical and chemical tactics to manage weeds without synthetic herbicides. Organic standards require rotation of annual crops, meaning that a single crop cannot be grown in the same location without a different, intervening crop. Organic crop rotations frequently include weed-suppressive cover crops and crops with dissimilar life cycles to discourage weeds associated with a particular crop. Research is ongoing to develop organic methods to promote the growth of natural microorganisms that suppress the growth or germination of common weeds.

Other cultural practices used to enhance crop competitiveness and reduce weed pressure include selection of competitive crop varieties, high-density planting, tight row spacing, and late planting into warm soil to encourage rapid crop germination.[32-34]

LIVESTOCK:

Raising livestock and poultry, for meat, dairy and eggs, is another traditional farming activity that complements growing. Organic farms attempt to provide animals with natural living conditions and feed. Organic certification verifies that livestock are raised according to the USDA organic regulations throughout their lives. These regulations include the requirement that all animal feed must be certified organic.

Organic livestock may be, and must be, treated with medicine when they are sick, but drugs cannot be used to promote growth, their feed must be organic, and they must be pastured. Also, horses and cattle were once a basic farm feature that provided labour, for hauling and plowing, fertility, through recycling of manure, and fuel, in the form of food for farmers and other animals. While today, small growing operations often do not include livestock, domesticated animals are desirable parts of the organic farming equation, especially for true sustainability, the ability of a farm to function as a self-renewing unit. [35-37]

GENETIC MODIFICATIONS:

A key characteristic of organic farming is the exclusion of genetically engineered plants and animals. On 19 October 1998, participants at IFOAM's 12th Scientific Conference issued the Mar del Plata Declaration, where more than 600 delegates from over 60 countries voted unanimously to exclude the use of genetically modified organisms in organic food production and agriculture.

Although opposition to the use of any transgenic technologies in organic farming is strong, agricultural researchers Luis Herrera-Estrella and Ariel Alvarez-Morales continue to advocate integration of transgenic technologies into organic farming as the optimal means to sustainable agriculture, particularly in the developing world. Organic farmer Raoul Adamchak and geneticist Pamela Ronald write that many agricultural applications of biotechnology are consistent with organic principles and have significantly advanced sustainable agriculture.

Although GMOs are excluded from organic farming, there is concern that the pollen from genetically modified crops is increasingly penetrating organic and heirloom seed stocks, making it difficult, if not impossible, to keep these genomes from entering the organic food supply. Differing regulations among countries limits the availability of GMOs to certain countries, as described in the article on regulation of the release of genetic modified organisms.[38-39]

SUSTAINABILITY:

Organic farming is supposed to be environmentally friendly due to abandonment of external inputs such as mineral fertilizers or pesticides. Albeit conversion to organic farming frequently comes along with a decline in crop yields, proponents of organic farming emphasize the sustainability of that system particularly because of improving organic matter-related soil quality. Based on recent research on mechanisms driving soil organic matter turnover, however, it rather appears that low-input agro ecosystems may convert to smaller efficiency in terms of substrate use by heterotrophs which may affect soil organic matter storage in the long run. A compilation of field data confirms an inferior use efficiency in some organic soils and thus questions the claim of an overall sustainable use of the soil resource in organic farming systems. The desire for a sustainable agriculture is universal, yet agreement on how to progress towards it remains elusive. The extent to which the concept of sustainable agriculture has any operational meaning is discussed. Sustainability is considered in relation to organic farming a sector growing rapidly in many countries. The role of regulation and the use of synthetic agrochemicals, the desired degree of self reliance of agricultural systems, and the scale of production and trade in agricultural goods are all considered. The concept of sustainability lies at the heart of the debates that currently exist over the use of the planet's natural resources, yet there is no consensus on its meaning. Some have argued that, for example, organic farming and sustainable agriculture are synonymous, others regard them as separate concepts that should not be equated. The reason for the focus on organic agriculture is the rapid development of the organic sector in Europe and North America. This development has resulted in an EU average of 2.2% of agricultural land as organic, while in countries such as Austria and Sweden the figure is near 20%. USDA estimates that in the USA the value of retail sales of organic foods in 1999 was approximately $6 billion, while the number of organic farmers is increasing at a rate of about 12% per year. The area of organic and in-conversion land in the UK doubled between 1999 and 2000. Organic farming, as is discussed below, has a long history but its sudden elevation from relative obscurity merits a consideration of its development and nature. As noted above, the focus of the paper is predominantly European and North American, which is not to devalue the significance of developments in the Southern Hemisphere but rather to keep the paper reasonably focussed.

As the figures above on the development of the organic market indicate, the growth in consumer demand for environmentally friendly, “green” or chemical-free food products has led to an expansion in Europe and North America of organic registration schemes. These schemes are seen to guarantee that products are produced in a certain way, with a range of agricultural inputs prohibited. The effects of these schemes on producers, and the implications of an expansion of the world market in such goods are also discussed in the context of agricultural sustainability. This raises issues regarding the scale, productivity and organisation of a future sustainable agriculture.

Agricultural researchers and professionals widely recognise the importance of sustainable agriculture and the need to make this operational, i.e. to develop appropriate methods to measure sustainability of farming system. Consequently an increasing variety of evaluation methods for assessing the sustainability, notably at the farm's level, has been produced, but most often these methods are intended for conventional farming systems and therefore take little account of the specificity of organic farming. While it is conventional agriculture which raises more concerns over the adverse effects of cropping and farming systems such as water pollution by nitrates and pesticides, and gaseous emissions due to nitrogen inputs. But sustainability is not just a matter of treating adverse effects of productivism. Other factors outside the conventional system can lead to lack of sustainability. Despite not having the negative effects of productivism, organic farming can present lack of sustainability. The sustainability of organic farming systems needs therefore to be appreciated. However in fact, the sustainability of organic farming is addressed in the context of comparison between organic, integrated and conventional farming systems, using the same sets of indicators (Vereijken, 1997; Pacini et al., 2003). In France there are currently a dozen indicator-based methods used by professional actors and teaching, but none are specific to organic farming. This contrasts with the emphasis today in organic farming and its recent dynamics of development. Over the past fifteen years, organic farming has been in France a very strong development both at the production level and at the demand of organic products. In 1995, 3600 organic farms were farming roughly 130 000 ha, in 2008, it was 13 298 farms on 583 799 ha, (2,12 % of the total national Usable agricultural area). In the meantime, food processing and marketing companies using an organic label had grown from 700 to 7 398 (Agence Bio, 2009).

The gross market of organic products has the same dynamics; the average annual growth is more than 10% since 1999, whence the global food market was growing by 3%.

REGULATIONS:

Organic agriculture is defined formally by governments. Farmers must be certified for their produce and products to be labeled “organic,” and there are specific organic standards for crops, animals, and wild-crafted products and for the processing of agricultural products. Organic standards in the European Union (EU) and the United States, for example, prohibit the use of synthetic pesticides, fertilizers, ionizing radiation, sewage sludge, and genetically engineered plants or products. In the EU, organic certification and inspection is carried out by approved organic control bodies according to EU standards. Organic farming has been defined by the National Organic Standards of the U.S. Department of Agriculture (USDA) since 2000, and there are many accredited organic certifiers across the country. Although most countries have their own programs for organic certification, certifiers in the EU or the United States can inspect and certify growers and processors for other countries. This is especially useful when products grown organically in Mexico, for example, are exported to the United States.

ENVIRONMENTAL BENEFITS:

WATER:

In many agriculture areas, pollution of groundwater courses with synthetic fertilizers and pesticides is a major problem. As the use of these is prohibited in organic agriculture, they are replaced by organic fertilizers (e.g. compost, animal manure, green manure) and through the use of greater biodiversity (in terms of species cultivated and permanent vegetation), enhancing soil structure and water infiltration. Well managed organic systems with better nutrient retentive abilities, greatly reduce the risk of groundwater pollution. In some areas where pollution is a real problem, conversion to organic agriculture is highly encouraged as a restorative measure.

AIR AND CLIMATE:

Organic agriculture reduces non-renewable energy use by decreasing agrochemical needs (these require high quantities of fossil fuel to be produced). Organic agriculture contributes to mitigating the greenhouse effect and global warming through its ability to sequester carbon in the soil. Many management practices used by organic agriculture (e.g. minimum tillage, returning crop residues to the soil, the use of cover crops and rotations, and the greater integration of nitrogen-fixing legumes), increase the return of carbon to the soil, raising productivity and favouring carbon storage. A number of studies revealed that soil organic carbon contents under organic farming are considerably higher. The more organic carbon is retained in the soil, the more the mitigation potential of agriculture against climate change is higher. However, there is much research needed in this field, yet. There is a lack of data on soil organic carbon for developing countries, with no farm system comparison data from Africa and Latin America, and only limited data on soil organic carbon stocks, which is crucial for determining carbon sequestration rates for farming practices.

BIODIVERSITY:

Organic farmers are both custodians and users of biodiversity at all levels. At the gene level, traditional and adapted seeds and breeds are preferred for their greater resistance to diseases and their resilience to climatic stress. At the species level, diverse combinations of plants and animals optimize nutrient and energy cycling for agricultural production. At the ecosystem level, the maintenance of natural areas within and around organic fields and absence of chemical inputs create suitable habitats for wildlife. The frequent use of under-utilized species (often as rotation crops to build soil fertility) reduces erosion of agro-biodiversity, creating a healthier gene pool - the basis for future adaptation. The provision of structures providing food and shelter, and the lack of pesticide use, attract new or re-colonizing species to the organic area (both permanent and migratory), including wild flora and fauna (e.g. birds) and organisms beneficial to the organic system such as pollinators and pest predators. The number of studies on organic farming and biodiversity increased significantly within the last years. [41- 44]

LIMITATIONS:

Organic manure is not abundantly available and on plant nutrient basis it may be more expensive than chemical fertilizers if organic inputs are purchased. Production in organic farming declines especially during first few years, so the farmer should be given premium prices for organic produce. The guidelines for organic production, processing, transportation and certification etc are beyond the understanding of ordinary Indian farmer. Marketing of organic produce is also not properly streamlined. There are a number of farms in India which have either never been chemically managed / cultivated or have converted back to organic farming because of farmers' beliefs or purely for reason of economics. These thousands of farmers cultivating million acres of land are not classified as organic though they are. Their produce either sells in the open market along with conventionally grown produce at the same price or sells purely on goodwill and trust as organic through select outlets and regular specialized markets. These farmers may never opt for certification because of the costs involved as well as the extensive documentation that is required by certifiers.

PRODUCTIVITY:

Studies comparing yields have had mixed results. These differences among findings can often be attributed to variations between study designs including differences in the crops studied and the methodology by which results were gathered.

A 2012 meta-analysis found that productivity is typically lower for organic farming than conventional farming, but that the size of the difference depends on context and in some cases may be very small. While organic yields can be lower than conventional yields, another meta-analysis published in Sustainable Agriculture Research in 2015, concluded that certain organic on-farm practices could help narrow this gap. Timely weed management and the application of manure in conjunction with legume forages/cover crops were shown to have positive results in increasing organic corn and soybean productivity.

Another meta-analysis published in the journal Agricultural Systems in 2011 analyzed 362 datasets and found that organic yields were on average 80% of conventional yields. The author's found that there are relative differences in this yield gap based on crop type with crops like soybeans and rice scoring higher than the 80% average and crops like wheat and potato scoring lower. Across global regions, Asia and Central Europe were found to have relatively higher yields and Northern Europe relatively. [45- 47]

PROFITABILITY:

In the United States, organic farming has been shown to be 2.7 to 3.8 times more profitable for the farmer than conventional farming when prevailing price premiums are taken into account, Globally, organic farming is 22–35% more profitable for farmers than conventional methods, according to a 2015 meta-analysis of studies conducted across five continents.

The profitability of organic agriculture can be attributed to a number of factors. First, organic farmers do not rely on synthetic fertilizer and pesticide inputs, which can be costly. In addition, organic foods currently enjoy a price premium over conventionally produced foods, meaning that organic farmers can often get more for their yield.

The price premium for organic food is an important factor in the economic viability of organic farming. In 2013 there was a 100% price premium on organic vegetables and a 57% price premium for organic fruits. These percentages are based on wholesale fruit and vegetable prices, available through the United States Department of Agriculture's Economic Research Service. Price premiums exist not only for organic versus nonorganic crops, but may also vary depending on the venue where the product is sold: farmers' markets, grocery stores, or wholesale to restaurants. For many producers, direct sales at farmers' markets are most profitable because the farmer receives the entire markup, however this is also the most time and labour-intensive approach.

There have been signs of organic price premiums narrowing in recent years, which lowers the economic incentive for farmers to convert to or maintain organic production methods. Data from 22 years of experiments at the Rodale Institute found that, based on the current yields and production costs associated with organic farming in the United States, a price premium of only 10% is required to achieve parity with conventional farming. A separate study found that on a global scale, price premiums of only 5-7% were needed to break even with conventional methods. Without the price premium, profitability for farmers is mixed. For markets and supermarkets organic food is profitable as well, and is generally sold at significantly higher prices than non-organic food.[48-50]

EMISSIONS:

Researchers at Oxford University analysed 71 peer-reviewed studies and observed that organic products are sometimes worse for the environment. Organic milk, cereals, and pork generated higher greenhouse gas emissions per product than conventional ones but organic beef and olives had lower emissions in most studies. Usually organic products required less energy, but more land. Per unit of product, organic produce generates higher nitrogen leaching, nitrous oxide emissions, ammonia emissions, eutrophication, and acidification potential than conventionally grown produce. Other differences were not significant. The researchers concluded that public debate should consider various manners of employing conventional or organic farming, and not merely debate conventional farming as opposed to organic farming. They also sought to find specific solutions to specific circumstances.

A 2018 review article in the Annual Review of Resource Economics found that organic agriculture is more polluting per unit of output and that widespread upscaling of organic agriculture would lead cause additional loss of natural habitats.

Proponents of organic farming have claimed that organic agriculture emphasizes closed nutrient cycles, biodiversity, and effective soil management providing the capacity to mitigate and even reverse the effects of climate change and that organic agriculture can decrease fossil fuel emissions. "The carbon sequestration efficiency of organic systems in temperate climates is almost double (575–700 kg carbon per ha per year – 510–625 lb/ac/an) that of conventional treatment of soils, mainly owing to the use of grass clovers for feed and of cover crops in organic rotations."[51-53]

FOOD QUALITY AND SAFETY:

While there may be some differences in the amounts of nutrients and anti-nutrients when organically produced food and conventionally-produced food are compared, the variable nature of food production and handling makes it difficult to generalize results, and there is insufficient evidence to make claims that organic food is safer or healthier than conventional food. Claims that organic food tastes better are not supported by evidence.[54- 56]

CONCLUSION:

Organic farming is a production system which avoids or largely excludes the use of synthetically compounded fertilizers, pesticides, growth regulators, It also,

- Reduces Exposure To Harmful Chemicals.
- Consumes Less Energy.
- Reduces Nitrogen Run-Off Induced Pollution
- Facilitates Healthy Soil Formation.
- Combats the Effects of Global Warming.

REFERENCE:

1. Paull, John (2019). "Organic Agriculture in Australia: Attaining the Global Majority (51%)". Journal of Environment Protection and Sustainable Development.
2. "USDA Blog » Organic 101: Allowed and Prohibited Substances". blogs.usda.gov. Retrieved 6 April 2016.
3. Paull, John (2011) "Nanomaterials in food and agriculture: The big issue of small matter for organic food and farming", Proceedings of the Third Scientific Conference of ISOFAR (International Society of Organic Agriculture Research), 28 September – 1 October.
4. "USDA List of Allowed and Prohibited Substances in Organic Agriculture". USDA List of Allowed and Prohibited Substances in Organic Agriculture. USDA. 4 April 2016. Archived from the original on 28 December 2015. Retrieved 6 April 2016.
5. Arsenault, Chris. "Only 60 Years of Farming Left If Soil Degradation Continues". Scientific American. Retrieved 29 May 2016.
6. Coleman, Eliot (1995), The New Organic Grower: A Master's Manual of Tools and Techniques for the Home and Market Gardener (2nd ed.), pp. 65, 108,

7. Paull, John "From France to the World: The International Federation of Organic Agriculture Movements (IFOAM)", Journal of Social Research & Policy, 2010, 1(2):93-102.

8. Helga Willer, Julia Lernoud and Robert Home The World of Organic Agriculture: Statistics & Emerging Trends 2013, Research Institute of Organic Agriculture (FiBL) and the International Federation of Organic Agriculture Movements (IFOAM, 2013).

9. Paull, John (2011) "The Uptake of Organic Agriculture: A Decade of Worldwide Development", Journal of Social and Development Sciences, 2 (3), pp. 111-120.

10. "The World of Organic Agriculture - Statistics & Emerging Trends 2022" (PDF). FiBL and IFOAM. p. 11. Retrieved 20 February 2022.

11. Horne, Paul Anthony (2008). Integrated pest management for crops and pastures. CSIRO Publishing.

12. Stinner, D.H (2007). "The Science of Organic Farming". In William Lockeretz (ed.). Organic Farming: An International History. Oxfordshire, UK & Cambridge, Massachusetts: CAB International (CABI). Retrieved 30 April 2013.

13. Conford, P. (2001). The Origins of the Organic Movement. Glasgow, Great Britain: Floris Books.

14. Gieryn, T.F. (1999). Cultural Boundaries of Science: Credibility on the Line. Chicago, Il.: University of Chicago Press. pp. 233–335.

15. Joseph Heckman, A History of Organic Farming: Transitions from Sir Albert Howard's War in the Soil to the USDA National Organic Program

16. Yeshwant D. Wad, The Work At Indore

17. Gabrielle Howard had died while the Howards were still in India.

18. Vogt G (2007). Lockeretz W (ed.). Chapter 1: The Origins of Organic Farming. Organic Farming: An International History. CABI Publishing. pp. 9–30.

19. Lotter, D.W. (2003). "Organic agriculture" (PDF). Journal of Sustainable Agriculture. 21 (4): 59–128.

20. Kirchmann, Holger; Bergstrom, Lars (16 December 2008). Organic Crop Production - Ambitions and Limitations. Springer Science & Business Media. pp. 2

21. Paull, John (2013) "Koberwitz (Kobierzyce); In the footsteps of Rudolf Steiner'", Journal of Bio-Dynamics Tasmania, 109 (Autumn), pp. 7-11.

22. Paull, John (2013) "Breslau (Wrocław): In the footsteps of Rudolf Steiner", Journal of Bio- Dynamics Tasmania, 110:10-15.

23. Diver (1999), "Introduction" Archived 26 May 2011 at the Wayback Machine.

24. Paull, John (2011). "The secrets of Koberwitz: the diffusion of Rudolf Steiner's agriculture course and the founding of biodynamic agriculture". Journal of Social Research & Policy. 2 (1): 19–29. Archived from the original on 8 March 2016. Retrieved 6 March 2016.

25. Paull, John (2011). "Biodynamic Agriculture: The Journey from Koberwitz to the World, 1924-1938". Journal of Organic Systems. 6 (1): 27–41.

26. FiBL (2006) Use of potassium bicarbonate as a fungicide in organic farming Archived 11 January 2014 at the Wayback Machine

27. "Integrated Pest Management". U.S. Environmental Protection Agency. Retrieved 1 January 2013.

28. Fargione J, and D Tilman." Competition and coexistence in terrestrial plants". Pages 156-206 In U. Sommer and B Worm editors, Competition and Coexistence. Springer-Verlag, Berlin, Germany.

29. Crop diversity: A Distinctive Characteristic of an Organic Farming Method - Organic Farming; 15 April 2013

30. Watson CA, Atkinson D, Gosling P, Jackson LR, Rayns FW (2002 (PDF). Soil Use and Management. 18: 239–247. .

31. Gillman J. (2008). The Truth About Organic Farming.

32. Kathleen Delate and Robert Hartzler. 2003. Weed Management for Organic Farmers. Iowa State University Extension Bulletin 1883.

33. Staff, United Nations Conference on Trade and Development. Organic Standards Archived 6 June 2015 at the Wayback Machine

34. ^ Kremer, Robert J.; Li, Jianmei (2003). "Developing weed-suppressive soils through improved soil quality management". Soil & Tillage Research. 72 (2): 193–202

35. "Agricultural Marketing Service". www.ams.usda.gov. Retrieved 17 December 2018.

36. ^ FAO Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (Gl 32 – 1999, Rev. 1 – 2001) Archived 24 February 2021 at the Wayback Machine

37. "Home | Agricultural Marketing Service". www.ams.usda.gov.

38. Luis Herrera-Estrella; Ariel Alvarez-Morales (April 2001). "Genetically modified crops: hope for developing countries?". EMBO Reports. 2 (4): 256–258

39. Pamela Ronald; Raoul Adamchak (April 2018). Tomorrow's Table: Organic Farming, Genetics and the Future of Food. Oxford University Press.

40. Seufert, Verena; Ramankutty, Navin; Mayerhofer, Tabea (1 April 2017). "What is this thing called organic? – How organic farming is codified in regulations". Food Policy. 68: 10–20.

41. "USDSA Guidance Natural Resources and Biodiversity Conservation"(PDF). Agricultural Marketing Service, National Organic Program. United States Department of Agriculture. 15 January 2016. Retrieved 5 March2016.

42. (in French) Institut de recherche de l'agriculture biologique, 5 March 2016 at the Wayback Machine, second edition, September 2015 .
43. Hole, D.G.; Perkins, A.J.; Wilson, J.D.; Alexander, I.H.; Grice, P.V.; Evans, A.D. (2005). "Does organic farming benefit biodiversity?". Biological Conservation. 122 (1): 113–130.
44. Gabriel, Doreen; Roschewitz, Indra; Tscharntke, Teja; Thies, Carsten (2006). "Beta Diversity at Different Spatial Scales: Plant Communities in Organic and Conventional Agriculture". Ecological Applications. 16 (5): 2011–21.
45. Welsh, Rick (1999). "Economics of Organic Grain and Soybean Production in the Midwestern United States". Henry A. Wallace Institute for Alternative Agriculture. Policy Studies Report No. 13.
46. ^ Seufert, Verena; Ramankutty, Navin; Foley, Jonathan A. (2012). "Comparing the yields of organic and conventional agriculture". Nature. 485 (7397): 229–232.
47. ^ de Pont, Tomek; Rijk, Bert; van Ittersum, Martin K. (19 December 2011). "The crop yield gap between organic and conventional agriculture". Agricultural Systems. 108: 1–9.
48. Moyer, Jeff (2013). "Perspective on Rodale Institute's Farming Systems Trial". Crop Management. 12: 1–3.
49. Crowder, David W.; Reganold, John P. (16 June 2015). "Financial competitiveness of organic agriculture on a global scale". Proceedings of the National Academy of Sciences. 112 (24)
50. Greene, Catherine. "USDA Economic Research Service - Organic Prices". www.ers.usda.gov. Retrieved 25 March 2016.
51. "Organic farms not necessarily better for environment" (PDF). University of Oxford. 12 September 2004.

52. , H.L. Tuomisto, I.D. Hodge, P. Riordan & D.W. Macdonald Does organic farming reduce environmental impacts? - A meta-analysis of European research Authors' version of the paper published in: Journal of Environmental Management 112 (2012) 309-320

53. Meemken, Eva-Marie; Qaim, Matin (2018). "Organic Agriculture, Food Security, and the Environment". Annual Review of Resource Economics. 10 (1): 39–63

54. Barański, M; Srednicka-Tober, D; Volakakis, N; Seal, C; Sanderson, R; Stewart, GB; Benbrook, C; Biavati, B; Markellou, E; Giotis, C; Gromadzka-Ostrowska, J; Rembiałkowska, E; Skwarło-Sońta, K; Tahvonen, R; Janovská, D; Niggli, U; Nicot, P; Leifert, C (26 June 2014). "Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: a systematic literature review and meta-analyses". The British Journal of Nutrition. 112 (5): 794–811

55. Blair, Robert. (2012). Organic Production and Food Quality: A Down to Earth Analysis. Wiley-Blackwell, Oxford, UK.

56. Bourn D, Prescott J (January 2002). "A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods". Crit Rev Food Sci Nutr. 42 (1): 1–34

The study of remedial effects of ten popular herbal plants on common cold and fever: a small survey.

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Dedicated to,

All my teachers

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Sanajit Giri

Date:

Table of contents:

1. Introduction

1.1. History of Indian traditional medicinal plant

Ever since ancient times, in search of rescue for their disease, the people looked for the drug in nature. The beginning of the medical plants' use was instinctive, as in the case with the animal. The oldest evidence in form of written medicinal plants was used for drug (medicine) has been found in Nagpur in India, approximately 5000 years ago. They prepare 12 types of drugs from 250species of various kinds of plants, some of them alkaloids. In India holy books "Vedas" mentioned treatment with plants, which are commonly found in the country. Several spice plants used even today originated from India, name pepper, clove, ginger, etc. The medicinal plant is also called medicinal herbs: plants, including many now used as culinary herbs and species, have been used for medicinal purposes. Human settlements are often surrounded by weeds used as herbal medicines, such as nettle, dandelion, and chickweed. Humans are not alone in using herbs (medicinal plants) as medicine; some non-human primates, monarch butterflies, and sheep. The mushroom was probably used against whip worm. In ancient times, there were lots of medicinal plants including myrrh and opium are listed on clay tablets from around 300BC.From ancient times to the present medicinal/ayurvedic as documented in the Atharva Veda, the Rig Veda and the Sushruta Samhita have used hundreds of medicinal active plants/herbs and spices such as turmeric, which contains curcumin. The book remained the authoritative reference on herbalism for over 1500 years, into the 17thcentury.In India, plants have been used for medicinal purposes long before the ancient period. Evidence exists that Unani Hakins, Indian veds, and European and Mediterranean cultures were using herbs for over 4000 years as medicine. The traditional system of medicine continues to be widely practiced on many accounts. Population rises, inadequate supply of drug and development of resistance to the currently used drug for infectious of human elements. Medicinal plants such as Aloe, tulsi neem, turmeric, and ginger cure several common ailments. These are considered home remedies in many parts of the country. Medicinal plants are considered as rich resources of ingredients that can be used in drug development either in pharmacopeial, non-pharmacopeial, or a critical role in the development of human culture around the whole world. Over the past few decades, there has been a tremendous/ enormous increase in the use of herbal medicine; however, there is still an important lack of research data in this field. Therefore since 1999, WHO has been published three volumes of the WHO monographs/ thesis or dissertation on selected medicinal plants or herbs.

1.2. Importance of Indian traditional medicinal plant

There was so much importance of Indian traditional medicinal plant for so many decades and continue for so many decades also. So here, some importance of Indian traditional medicinal plants_

- ❖ Herbs such as black pepper, cinnamon, myrrh, and safflower are used for rehabilitation or comeback, swelling, boils, etc.
- ❖ Many herbs/ medicinal plants are used as blood purifiers to alter or change a long-standing condition by eliminating the metabolic toxin. These are also known as" blood cleansers".
- ❖ Some herbs are also played a core role in antibiotic properties. Tumeric is useful in inhibiting the growth of germs, harmful or injurious microbes, and bacteria. Tumeric is widely used as a home remedy to heal cuts and wounds.
- ❖ There were so many medicinal plants, which takes prevention against fever and allergy and production of heat caused by the condition, certain antipyretic herbs such as chirality, black pepper, and safflower are recommended by traditional Indian medicinal practitioners.
- ❖ Many medicinal plants are used to neutralize the acid produced by the stomach.

- In India, Indian sages were known to have remedies from plants that act against poisons from animal and snake bites.
- Some herbs like cardamom and coriander are recommended for their ambrosial qualities. Other aromatic herbs such as cloves and turmeric add a pleasant aroma to the food, thereby increasing the food qualities, taste, and smell of the meal.
- Medicinal plant ginger and clove are used in certain cough syrups. They are known for their linocuts or cough medicine property, which promotes the thinning and increasing the antibiotics properties of lungs, trachea, and bronchi.
- Vulnerable herbs like honey, turmeric, marshmallow, and licorice can effectively use a fresh cut and wound.
- Dietary supplements and herbal remedies are popular complementary or alternative products for people. These supplements are intended to supplement the diet and contain one or more dietary ingredients (vitamin, mineral, herbs, and other botanical, amino acid, or their substance).
- These botanicals are sold in many forms as fresh or dried products liquid or solid extract, tablets, capsules, powder, tea bags,and so forth.
- A particular group of chemicals or a single chemical may be isolated from a botanical and sold as a dietary supplement, usually in tablet or capsule form. An example is a phytoestrogen from soy products.
- Siddha system of medicine is believed as a brilliant achievement and symbol of Tamil culture which originated in southern parts of India. Siddha medicinewas invented from Dravidian culture and is grown in the time of Indus valley civilization.

- ❖ Currently, about 80% of antimicrobial immune-suppressive, cardiovascular and anti-cancer drugs are derived from plants source. More than 70% of entities among 177 anti-cancer drugs approved are based on natural products or mimetics.
- ❖ It was estimated that ayurvedic use 1200-1800 plants Siddha medicine include 500-900 plants, unani utilizes 400-700 medicinal plant and amchi medicine uses nearly 300 plants while folk healers of India use more than 7500 medicinal plants in different medicine. Three classical ayurvedic literature Charaka Samhita, Sushruta Samhita, and Astangatlti daya mentioned about 526,573 and 902 plants.

1.3. Use of Medicinal plants in common cold and fever

Medicinal plants are considered as a resource of ingredients that can be used in drug development either pharmaceutical or nonpharmaceutical or synthetic drugs. Moreover, some plants are considered an important source of nutrition, and as a result that they are recommended for their therapeutic values. So here we discuss Indian traditional medicinal plants which were given by me.

1. ***Ocimumtenuiflorum* (Tulsi):**

Tulsi is a well-known immunomodulatory herb that might improve the individual's ability to fight against the common cold. Tulsi had antimicrobial, antiallergic, and anti-inflammatory properties, therefore, preventing the inflammation of the nasal mucous membrane. Another importance of tulsi that gives relief from cough. Tulsi also prevents viral infection. It has anti-pyretic and diaphoretic activity.

2. ***Justicaadhatoda (Basak):***

Recurrent cough and congested throat can cause a lot of discomforts and prevent a peaceful good night sleep while making you feel exhausted. Being rich in anti-inflammatory, antibiotic properties. Bask holds high significance in treating the common cold, cough, and flu symptoms.

3. ***Aloe vera (Aloevera):***

   Aloe vera barbadensis miller compress is an effective alternative therapy in reducing body temperature in children with fever. It is recommended for midwives to apply this intervention to reduce body temperature significantly.

4. ***Phyllanthus emblicalimn (Amla):***

   Amla is known as a natural coolant and so it can make your symptoms of cold worse. It is believed to lower body temperature to a great extent.

5. ***Allium sativum (Garlic):***

   Garlic has shown promise as a treatment for preventing cold and flu.

   Garlic is alleged to have antimicrobial and antiviral properties that relieve the common cold, among other beneficial effects.

6. ***Nyctanthes arbor-tristis (Shiuli):***

   Shiuli is known for its medicinal properties can heal many diseases from arthritis, dry cough, cold and cough, fever to worms.During the transition season between monsoon and fall, many people catch a cold and are down with fever. In such a state shiuli juice consumes the situation to a great extent along with honey.

7. ***Jingiber officinale (Ginger):***

   Many people use ginger to help recover from cold or flu. However, the evidence supporting this remedy is mostly anecdotal.

Another way to treat a long fever is to use ginger. Ginger has antibacterial properties to make it effective against fever, cough, and common symptoms.

8. ***Tea plant (Camellia sinensis), Onion (Allium cepa), Oregano (Origanum vulgare)*** are also used in common cold and fever.

Table 1. List of ten popularly used plants to cure common cold and fever.

Serial No.	Scientific name	Common name	Family
1.	*Ocimumtenuiflorum*	Tulsi	Lamiaceae
2.	*Justicia adhatoda*	Basak, malaber nut	Acanthaceae
3.	*Aloe vera*	Aloe vera	Liliaceae
4.	*Phyllanthus emblicalinn*	Amla,indian gooseberry	Phyllanthaceae
5.	*Allium sativum*	Garlic	Amaryllidaceae
6.	*Nyctanthesarbor-tristis*	Shiuli, coral jasmene	Oleaceae
7.	*Zingiber officinale*	Ginger	Zingiberaceae
8.	*Camellia sinensis*	Tea plant, tea shrub	Theaceae
9.	*Allium cepa*	Onion	Apiaceae
10.	*Origanum vulgare*	Oregano	Lamiaceae

2. Materials and methods

Methods

The survey was carried forward according to the documentation of ethnomedicinal plants documented by Savithramma et al., 2017

Ocimum tenuiflorum **(Tulsi)**

https://www.britannica.com

Justicia adhatoda **(Basak)**

https://en.wikipedia.org

Aloe vera **(Aloevera)**

https://www.nccih.nih.gov

Phyllanthus emblica linn **(Amla)**

https://en.wikipedia.org

Allium sativum **(Garlic)**

https://www.alamy.com

Nyctanthes arbor-tristis **(Shiuli)**

https://in.pinterest.com

Zingiber officinale **(Ginger)**

https://gardenerspath.com

Camellia sinensis **(Tea Plant)**

https://www.nccih.nih.gov

Allium cepa **(Onion)**

https://www.masterclass.com

Origanum vulgare **(Oregano)**

https://www.thespruce.com

3. Results

The questionnaire forms are incorporated in original at the end.

4. Discussion

Herbal medicines are long been known for their curability. The use of medicinal plants increases day by day due to their easy, availability, affordability, accessibility and promising efficiency comparable to the other chemical medicines. The high cost and adverse effects of standard synthetic drug agents is also making herbal medicine a good choice presently. In this present study the effect of different herbal drugs on common cold and fever are analysed. In most of the cases the active principles as well as mode of action of the herbal drugs are unknown. Hence, the entire plants are used as crude drugs. Different plant parts e.g., leaves, roots, berk, seeds, flowers etc. are used as source of medicines. The pharmacological application of these drugs is mainly based on ethnobotanical knowledge and experiences. In the present study ten most popular plants were selected for the survey. The popularly used selected plants are, *Ocimumtenuiflorum, Justicia adhotoda, Aloe vera, Allium sativum, Nyctanthesarbor_tristis, Zingiber officinale, Allium cepa, Origanum vulgare, Camellia sinensis, Phyllanthus emblicalinn*. The common names and families are listed in table 1. To perform the survey target patients/ users are randomly selected in plain region(khardaha). The questionnaire to the targeted patients were prepared as per the standard guidelines provided by the herbal medicine society.

The data collected from the source have clearly noted that these selected herbal medicines are popular among the common people (Table 2). The clear indication towards inclination of herbal medicines is also observed among the peoples. The overall distribution of male and female patients using herbal medicines although present but the percentage of female users is more than that of male users. Many of the herbal plants are home grown and females are more inclined towards nature for their daily activities. This may lead them to use more herbal medicines than male. Another reason may be, in villages the exposure of females to the synthetic drugs and reach of doctors are limited. This may also lead them to use more traditional medicine than urban region. This is a small survey and needs more intensive study to find out actual scenario and future prospective of the herbal medicines. Although, this is impossible to conclude anything from this small-scale study but it surfaced a very interesting clue regarding popularities of alternative herbal drugs. The more detailed study on these topics would bring many information in future.

Table 2. Name of the plants and percentage of users.

Name of the plant	No. of users	Percentage of users of the plant	Male	Female	No. of male user cure	No. of female user cure	Percentage of male users	Percent age of female users
Ocimumtenu iflorum	5	16.67	5	-	5	-	10 0	-
Justicia adhotoda	5	16.67	2	3	2	3	40	60
Aloe *vera*	3	30	-	3	-	3		100
Allium sativum	3	30	1	2	1	2	33. 3	66.6
Nyctanthesar bor_tristis	5	16.67	2	3	2	3	40	60
Zingiber officinale	5	16.67	3	2	3	2	60	40
Allium cepa	2	6.67	1	1	1	1	50	50
Origanum vulgare	1	3.3	-	1	-	1	-	100
Camellia sinensis	-	-	-	-	-	-	-	-
Phyllanthus emblicalinn	1	3.3	-	1	-	1	-	100

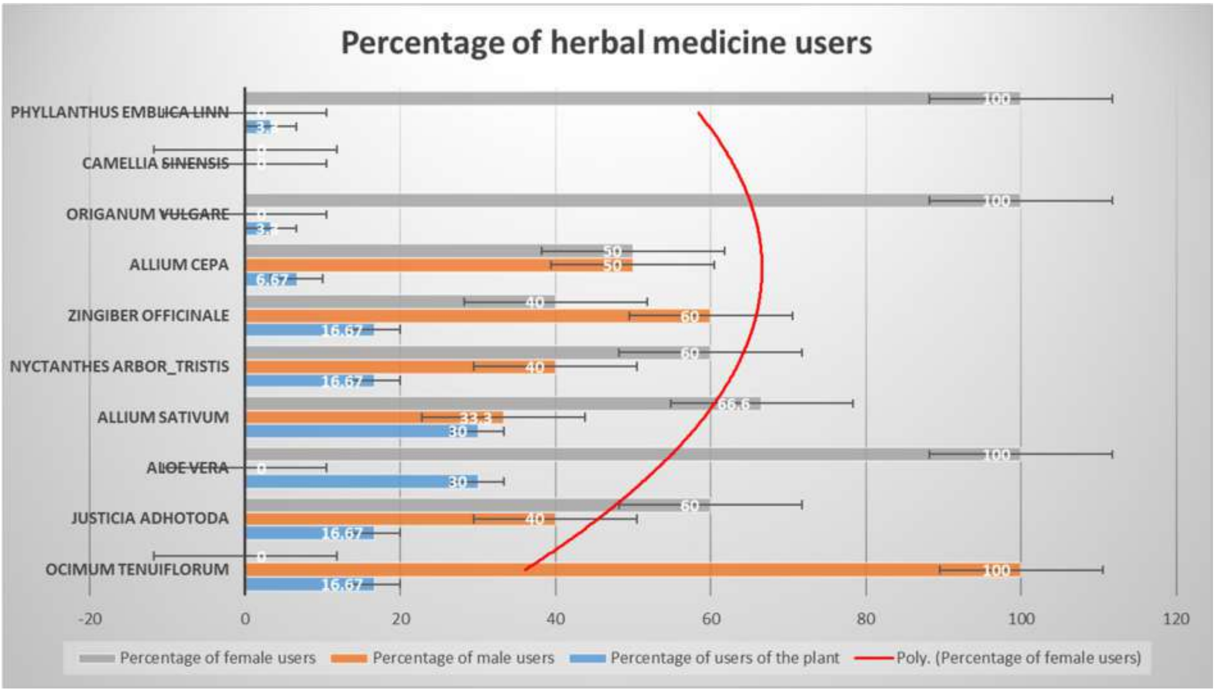

Figure 2. Percentage of herbal medicine users in common cold and fever.

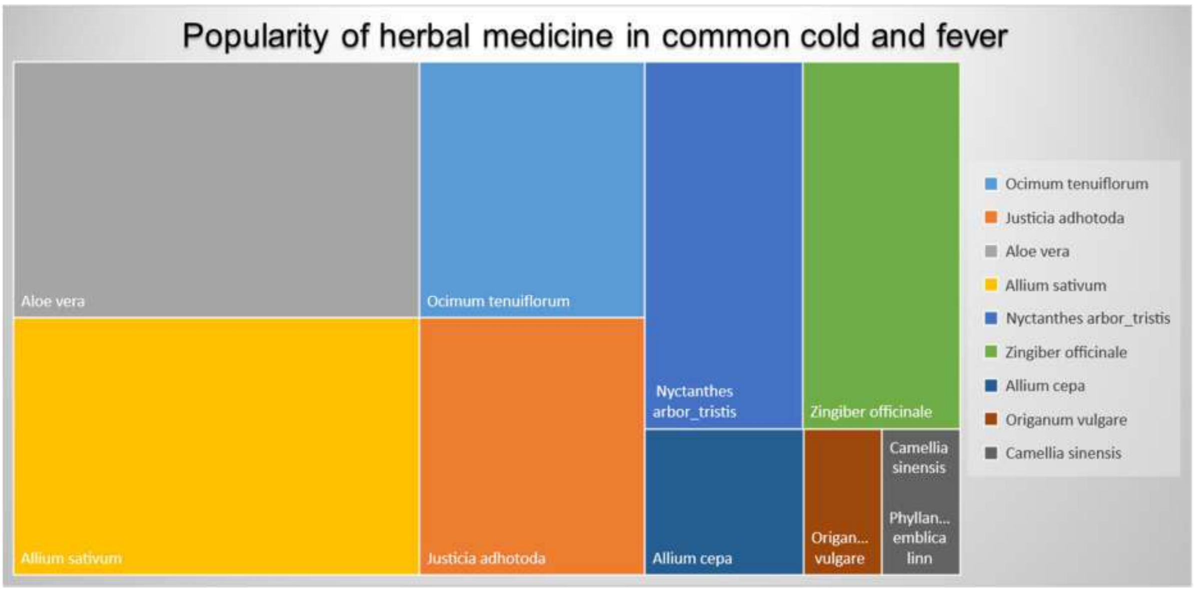

Figure 3. Popularity of ten selected herbal medicinal plants to cure common cold and fever.

5. References:

1. Abbink J (1995). Medicinal and ritual plants of the Ethiopian southwest:
2. an account of recent research. Indigenous knowl. Dev. Monitor, 3(2):
3. 6–8
4. Abebe D (1996). The development of drug research. Ethiopian Health
5. Nutr. Res. Institute News Lett., 1: 5-6.
6. Abebe D, Ayehu A (1993). Medicinal plants and Enigmatic health
7. practices of North Ethiopia. B. S. P. E. Addis Ababa, Ethiopia.
8. Addis G, Abebe D, Genebo T, Urga K (2002). Perceptions and practices
9. of modern and traditional health practitioners about traditional
10. medicine in Shirka District, Arsi Zone, Ethiopia. Ethiop J. Health Dev.,
11. 16(1): 19-29.
12. AFRO Technical Report Series (1976). African Traditional medicine,
13. Report of the Regional Expert Committee No. 1, pp. 3-4.
14. Akerele O (1993). Nature's medical botany does not throw it away.
15. World Health Forum, 14: 390-395.
16. Asfaw Z (1998). Conservation and Production of Traditional Medicinal
17. Plants in Home Gardens: The Case of Ethiopia: Addis Ababa University, Addis Ababa, Ethiopia.
18. Savithramma, Nataru, et al. "Documentation of ethnomedicinal information and antimicrobial validation of Thespesia populnea used by Yanadi tribe of Ganugapenta village, Chittoor district, Andhra Pradesh, India." Journal of intercultural ethnopharmacology 6.2 (2017): 158.

6. Scanned copies of the questionnaire forms

The study on use of ten popularly known herbal plants for immunomodulatory effect: a small survey.

Project submitted by:

Mr. Santanu Kar

B.Sc. Semester V

Department of Botany

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Kolkata- 700118

The study on use of ten popularly known herbal plants for immunomodulatory effect: a small survey.

Project submitted by:

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Dedicated to,

My parents & teachers

ACKNOWLEDGEMENT

I would like to express my special thanks of gratitude to all the faculty members of Department of Botany, Ramakrishna Mission Vivekananda Centenary College, Rahara, Kolkata for their able guidance and support in completing my project.

I would also like to extend my gratitude to the Prinicipal maharaj, Dr. Sw. Kamalasthananda and respected controller of examinations Sw. Vedanuragananda providing me with all the facility that was required and giving me opportunity to work.

Santanu Kar

Date:

Table of contents:

1. Introduction.

1.1. History of traditional medicine

Science has been advancing since ancient times. One of its branches is in medical science. Especially in the last century, medical science has made unbelievable progress all over the world. A lot of natural products (pharmaceutical, pesticides, herbicides) were discovered, which act like a warrior against various infections and diseases. These natural products are isolated from plants, animals, and microorganisms. Not only natural products but also several molecules like DNA, RNA, Proteins are also found naturally. These natural products are used in human welfare. One of seventeen megadiverse countries, India displays significant plant biodiversity. Various medicinal plants are found here. The records of Indian traditional medicine dates from before 5000 years. Data collection, saving, and use of traditional medicines are also be recorded. From ancient times, Indian people depends on traditional medicines, as primary health care. One of the best medical systems, used in health care is Ayurveda. The word Ayurveda is the aggregation of two Sanskrit words ‘Ayur’ means life and ‘Veda’ means knowledge. Ayurvedic medicine is one of India’s oldest treatment pathways. The primary system of this treatment consists of mainly two parts. One is ‘causes of illnesses and the other is ‘to make the patient more careful about the reason for illness. There are two classical texts of Ayurveda. One is Charaka Samhita which is focused on internal medicine and the other is Sushruta Samhita which is focused on surgery. Unani system of medicine is believed as a bright skill for health purposes. This system of medicine was derived in Greece and was circulated by Hippocrates, a famous physician during the 460 – 366 BC period. This system of medicine is created based on four conditions like hot, boiled, frosty, and dry.

Not only Ayurveda or Unani but there is also another kind of medical system is homeopathy. This is a therapeutic practice, which is used to the arrangement of substance whose effects is regulated to healthy persons similar to the appearances of the illness in the individual patient. In India, for 200 years, these homeopathic therapeutic methods are applied and now it is distributed throughout the world. A large number of people in India are dependent on Folk medicine. This type of medicine plays a vital role in the maintenance of health. Nearly 8000 plants species are used in folk medicine. Hence, this is a continuous process of improvement in Indian traditional medicine. Around 85% of Indian people depend on the traditional medicine system for fundamental medicinal worship.[Saikat Sen, Raja Chakraborty Journal of traditional and complementary medicine 7 (2), 234-244, 2017]

1.2. Importance of traditional medicine

In the last century, one of the greatest discoveries in medical science is medicine. Traditional medicine is used in many countries throughout the world. One of these countries where traditional medicine is an important part of health care in India. We know many natural products are used on the basis of traditional knowledge which is an important source of food, health care, and income. Traditional medicines have various importance like (contributions to primary health care, the cost-effective, side effect is less).

- **Contributions to primary health care**: It is proved that one-third of the world's population regularly used traditional medicine. Traditional medicine is the aggregation of skills, study's, practices, and pieces of knowledge based on experiences and beliefs. Traditional medicines are used in the control of health care and identification of the nature of an illness or other problem by examination of the symptoms, the release of physical and mental depression, improvement, and prevention of disease. Nowadays, major populations of the world, including India, are dependent on traditional medicine as primary health care.

- **Cost effective**: one of the reasons for the most popularized traditional medicine is cost-effective. So, people from a poor and middle-class family, can use traditional medicine affordable. Traditional medicines are available in local medicine shops, that's why people can purchase them easily.
- **Side effect is less**: Another important attribute of traditional medicine is, side effects less. Properly prescribed traditional medicine can have very few harmful side effects. So, traditional medicines are considered as fewer side effects than their efficacy That's why traditional medicine has been widely used in India, China, Korea, Japan, Australia, Canada, Belgium, France, and other countries.

1.3. Use of medicinal plants as traditional immunomodulation drugs

A strong immune system is required to protect our body against fungus, bacteria, viruses and avoid illness. Medicinal plants are a great source of medicine. Medicinal plants are the main pillar of traditional medicines, that enhance the immune response. More than 3 billion peoples in less developed countries take advantage of traditional medicine regularly. Kalmegh is a very popular medicinal plant that is used for various therapeutic purposes like antimalarial activities, anti-diabetic activities, antidepressant activities, and immunomodulatory activities that enhances the immune response in our body. Aloe vera is packed full of immune-boosting polysaccharides which help the immune system to behave properly. Brahmi boosts immunity by nourishing the body with important nutrients and antioxidants that help in warding off infections and diseases.

The main components of turmeric are curcuminoids. It is used for various treatments like cough, diabetic wounds, hepatic disorders, etc. Garlic contains several health-promoting properties like sulphur-containing metabolites (allicin and its derivatives), which increase our body's immune response. Ginger contains a bioactive compound, gingerol, which has an inflammatory effect. It has antibacterial, antifungal, antiseptic, and antiviral activities that's why it promotes a healthy immune system. Cloves are sweet and aromatic spices. They have also been used in traditional medicine. It contains various important nutrients (vitamins, minerals). It has several functions to enhance immune response such as (protect against cancer, can kill bacteria, improving liver health, etc). Black pepper is considered as the sovereign of spices. Its active compounds have a role in boosting white blood cells, which, our body uses to fight off invading bacteria and viruses. This spice contains many antibacterial and anti-inflammatory properties that help keep infections away and are also known to provide relief from uneasiness produced due to wounds and swellings. Amrita is a medicinal plant that is full of antioxidants and helps to release toxins from the body. It can reduce fever and ease the symptoms of life-threatening fevers like dengue, and malaria. Its active compounds increase our body's immunity. Spearmint is a medicinal plant that contains nutrients (vitamins, iron, manganese), an antioxidant, anti-inflammatory agent that reduced symptoms of asthma, improves cold symptoms, and protects our body by enhancing immune response.

Table 1. List of ten popularly known plants used as immunomodulation

Serial No	Scientific Name	Common Name	Family
1.	***Andrographis paniculata***	Kalmegh	Acanthaceae
2.	***Bacopa monnieri***	Water hyssop, Brahmi	Plantaginaceae
3.	***Aloe vera***	Aloe vera	Liliaceae
4.	***Curcuma longa***	Turmeric	Zingiberaceae
5.	***Allium sativum***	Garlic	Amaryllidaceae
6.	***Syzigium aromaticum***	Clove	Myrtaceae
7.	***Zingiber officinale***	Ginger	Zingiberaceae
8.	***Piper nigrum***	Black pepper	Piperaceae
9.	***Tinospora cordifolia***	Amrita, guduuchii	Menispermaceae
10.	***Mentha spicata***	Spearmint	Lamiaceae

2. Materials and methods

2.1. Study area

Moyna(East Medinipur)

Egra (East Medinipur)

Bhagabanpur (East Medinipur)

Jakpur(West Medinipur)

2.2. Methods

The survey was carried forward according to the documentation of ethnomedicinal plants documented by Savithramma et al., 2017.

3. Results

The questionnaire forms are incorporated in original at the end.

4. Discussion

Medicinal plants have been used in healthcare since long ago. Studies have been carried out globally to verify their efficacy and some of the findings have led to the production of plant-based medicines. Although many works were conducted to find out efficacy of these alternative medicines but still the mode of action of most of these drugs are unknown. The active principles of these medicinal plants must be isolated, quantified and instantiated thoroughly to decipher their actual roles. There many plant species existed in the society and almost every plant of them exhibited some sorts of medicinal properties. These repertoires of compounds are able to revolutionize the medical biotechnology. The contemporary synthetic and chemical drugs possess tremendous side effects and toxicity to the human health. The rapid function, easy availability and non-existence of competitive alternative compelled consumers and practitioners to use and recommend these drugs. Recently the awareness of drug toxicity increases among common people and patients are more inclined and show eagerness in utilization of alternative herbal medicines. This brings emerging perspectives in the field of herbal technology and is an interesting approach to counteract drug-based toxicity. Immunomodulation is an essential practice among traditional medical practices and ethnopharmacology. The use of different plants and plant products as immunomodulatory drugs are very common even in the high prevalence age of synthetic chemical drugs. The use of traditional medicine is mainly based on ethnobotanical and ethnopharmacological knowledge and experiences those people gather with time. Hence, it is prerequisite as well as essential to collect field data to find out how these herbal plants and their medicinal values have affected people in social welfare. In the present small survey ten commonly used herbal plants in modulation of immunity was selected e.g., *Andrographis paniculate, Bacopa monnieri, Aloe vera, Curcuma longa, Allium sativum, Syzigium aromaticum , Zingiber officinale,* *Piper nigrum, Tinospora cordifolia, Mentha spicata*. The survey was conducted in the district of East Medinipur and small region of west Medinipur . The target population was selected randomly and asked some questions for the survey. The questionnaire was formulated according to the guidelines provided by the herbal medicine society. The data showed most of the plants those selected for the study were known to the people except, *Tinospora cordifolia*, *Mentha spicata*. The distribution of male and female users are more or less equal throughout the data set. The use of *Syzigium aromaticum, Zingiber officinale, Piper nigrum, Bacopa monnieri,* and *Aloe vera* were surprisingly high among male than female users. On the other hand, use of *Andrographis paniculate*, *Allium sativum* were high among females. *Curcuma longa* is popular equally among both males and females. With limited scope of the project the small data demonstrated that the popularity of some herbal medicines as immunomodulators are really high among people but some are completely unknown to them. The proper utilization and awareness is necessary to popularize these drugs among people for universal use. Commercialization of these medicinally important plants can also facilitate people to know more about these drugs. Although, small survey can not bring any conclusive remarks but this study may provide a foundation for more consorted research in this filed. The application of different cutting-edge biotechnological approaches is also necessary to investigate chemical spectrum of these herbal drugs and find out specific mode of action of those compounds.

Table 2. Name of the plants and percentage of users.

Name of the Plant	Number of users (Out of 30)	Percentage of user	Number of male users	Number of female users	Percentage of male Users	Percentage of female Users
Andrographis paniculata	4	13.3	2	2	50	50
Bacopa monnieri	3	10	2	1	66.6	33.3
Aloe vera	5	16.6	3	2	60	40
Curcuma longa	4	13.3	2	2	50	50
Allium sativum	3	10	1	2	33.3	66.6
Syzigium aromaticum	3	10	2	1	66.6	33.3
Zingiber officinale	5	16.6	3	2	60	40
Piper nigrum	3	10	2	1	66.6	33.3
Tinospora cordifolia	0	0	0	0	0	0
Mentha spicata	0	0	0	0	0	0

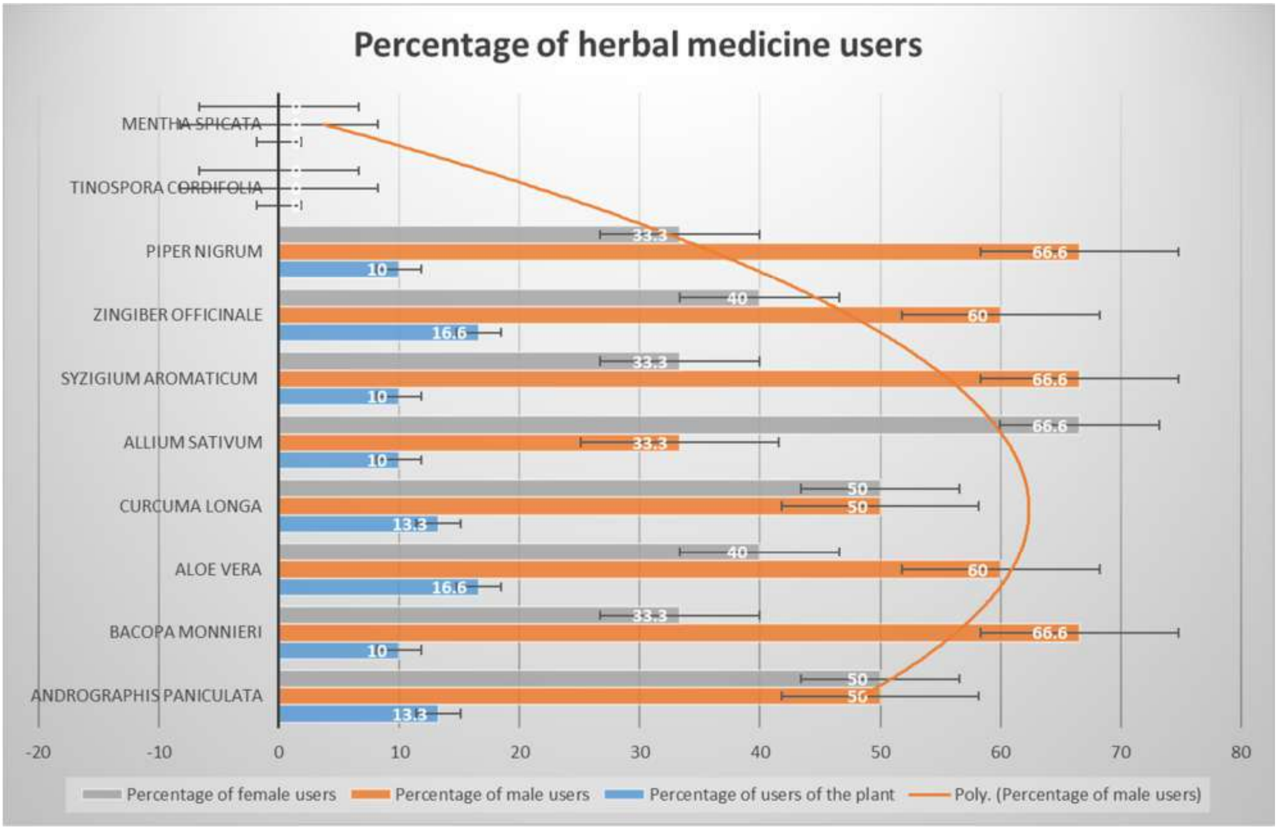

Figure 1. The percentage users of the ten commonly used herbal medicines as immunomodulators.

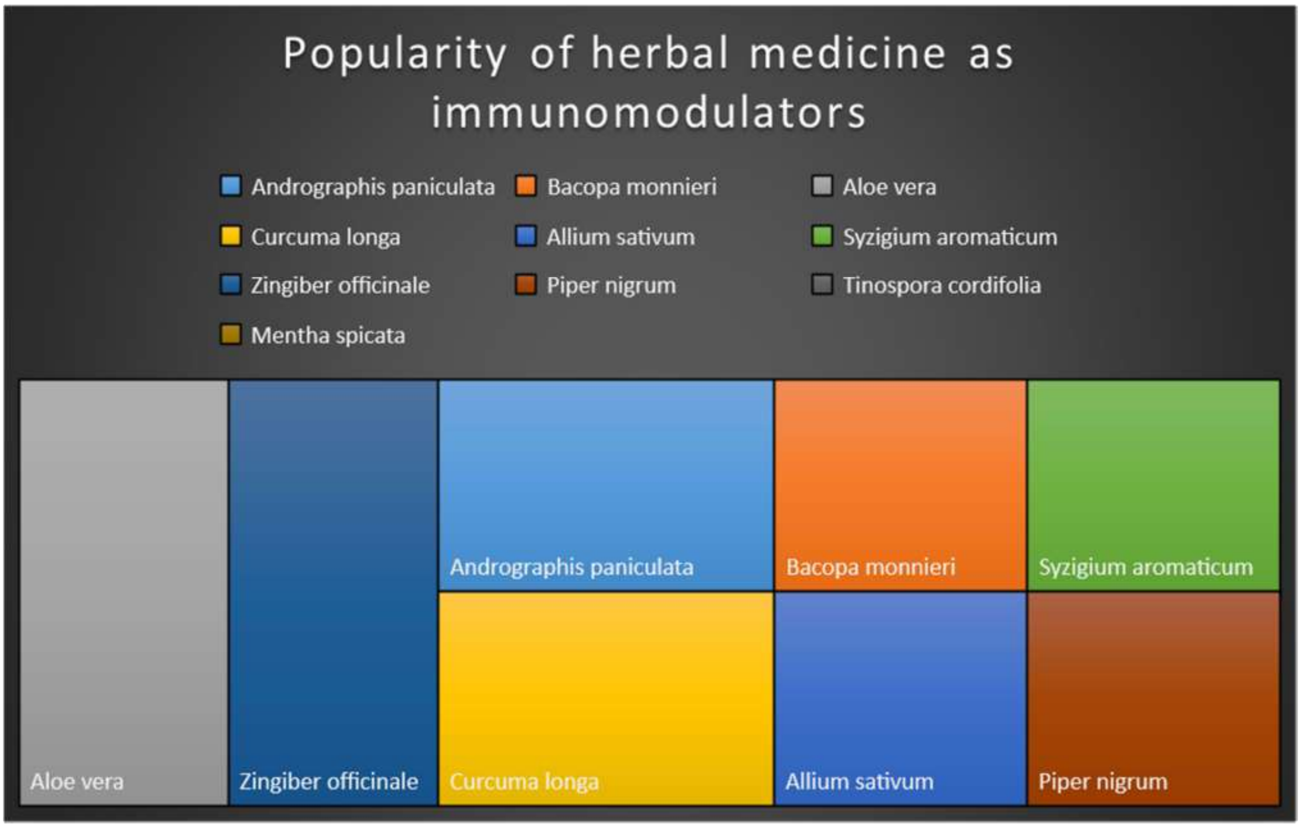

Figure 2. The popularity of ten commonly used herbal medicines for immunomodulation.

5. References:

1.Saikat Sen, Raja Chakraborty

Journal of traditional and complementary medicine 7 (2), 234-244, 2017

2.Yogini S Jaiswal, Leonard L Williams Journal of traditional and complementary medicine 7 (1), 50-53, 2017

3.Fatemeh Jamshidi-Kia, Zahra Lorigooini, Hossein Amini-Khoei

Journal of herbmed pharmacology 7 (1), 2018

4.Vetrivel Umashankar, Sanjay H Deshpande, Harsha V Hegde, Ishwar Singh, Debprasad Chattopadhyay

Frontiers in medicine 8, 2021

5. Ayurvedic Pharmacopoeia of India. Government of India, Ministry of Health and Family Welfare. Part I. Vol. III. New Delhi, India:

Department of Indian Systems of Medicine and Homoeopathy; 2001b.

6.Ayurvedic Pharmacopoeia of India. Government of India, Ministry of Health and Family Welfare. Part I. Vol. II. New Delhi, India: Department

of Indian Systems of Medicine and Homoeopathy; 2001a.r

7.Mukherjee PK, Wahile A. Integrated approaches towards drug development from ayurveda and other Indian system of medicines.

8.Prasad LV. Indian system of medicine and homoeopathy. In: Chaudhury RR, Rafei UM, editors. Traditional Medicine in Asia. Chaudhury RR,

Rafei UM, editors. Traditional Medicine in Asia

9.Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and medicine from natural products. Molecules 2016;21:559.

10.Dev S. Ancient-modern concordance in ayurvedic plants: examples. In: Development of Plant-Based medicines: Efficacy and Safety.
Dordrecht: Springer Netherlands; 2001 p. 47-67.
11.Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol
2014;4:177.
12.Thillaivanan S, Samraj K. Challenges, constraints and opportunities in herbal medicines e a review. Int J Herb Med. 2014;2:21e24.
13. Lim MK, Sadarangani P, Chan HL, Heng JY. Complementary and alternative medicine use in multiracial Singapore. Complement Ther
Med 2005;13:16-24.
14. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules 2016;21:559.
15. Sen S, Chakraborty R, De B. Challenges and opportunities in the advancement of herbal medicine: India's position and role in a global context. J Herb Med.2011;1:7e75.
16. Savithramma, Nataru, et al. "Documentation of ethnomedicinal information and antimicrobial validation of Thespesia populnea used by Yanadi tribe of Ganugapenta village, Chittoor district, Andhra Pradesh, India." Journal of intercultural ethnopharmacology 6.2 (2017): 158.

6. The questionnaire forms:

RAMKRISHNA MISSION VIVEKANANDA
CENTENARY COLLEGE

Name: Rohit Ghara

Roll No: 411

Registration No : A01-112-114-010-2019

Subject : Botany (Hons)

Semester : V

Project Topic : Role of Microalgae in Wastewater treatment

Project Guider: Dr. Harishankar Dey.

Academic Year: 2021-2022

INTRODUCTION:

Developing country like India, due to the increasing population and rapid industrialization,the amount of wastewater generated day by day. So presently water pollution is one of the most critical environmental problems. For wastewater treatment there are various conventional methods , but they are very costly and not economical. Nowadays, some new green technical methods of wastewater treatment are being introduced to resolve the problems related to the conventional methods. During the last 50 years Biological wastewater treatment systems with microalgae have gained importance and now it is widely accepted that microalgal based wastewater treatment systems are more effective than conventional wastewater treatment systems. Cultivation of Microalgae in wastewater for wastewater treatment, pollution control and production of energy from microbial biomass is nowadays common treatment method.. Microalgae have become significant organisms for biological treatment of wastewater. Microalgae based treatment is one of the good treatment for solving the environmental problems such as global warming, the increase of ozone hole and climate changed as it able to consume high quantity of carbon dioxide in Photo-synthesis process to produce oxygen and glucose. Microalgae have the ability to remove nutrients, heavy metals, organic and inorganic toxic substances and other impurities present in the wastewater by using the sunlight, CO2, and various nutrients. The main advantage of using algal system is that it absorbs solar radiation in the form of energy in its chloroplast cell and takes CO2 along with nutrients from wastewater to synthesis their biomass and produce oxygen. The released oxygen from microalgae is enough to meet the most aerobic bacterial requirements while metabolizing the residual organics in the treated wastewater. Algae also release a large amount of organic compounds that can be digested in aqueous system. The bacteria, in turn constitute an essential source of CO2 required for algal growth, stimulate the release of vitamins & organic growth factors and change the pH of the supporting medium for algal. wastewater as their energy requirement for growth and the simultaneous removal of pollutants. The microalgae could also grow in the arid environment and highly saline water. Microalgae's are also able to grow, assimilate, and resist toxic conditions in wastewater that make it's a versatile microorganism, for treating such wastewater in various field. The harvested microalgae biomass could be used to produce carbon neutral fuels, high value pigments, fish and animal feed, biofertilizers, bioplastics and carbon dioxide mitigation in a cyclic manner. A number of studies have reported successful cultivation of several species of microalgae such as Chlorella.

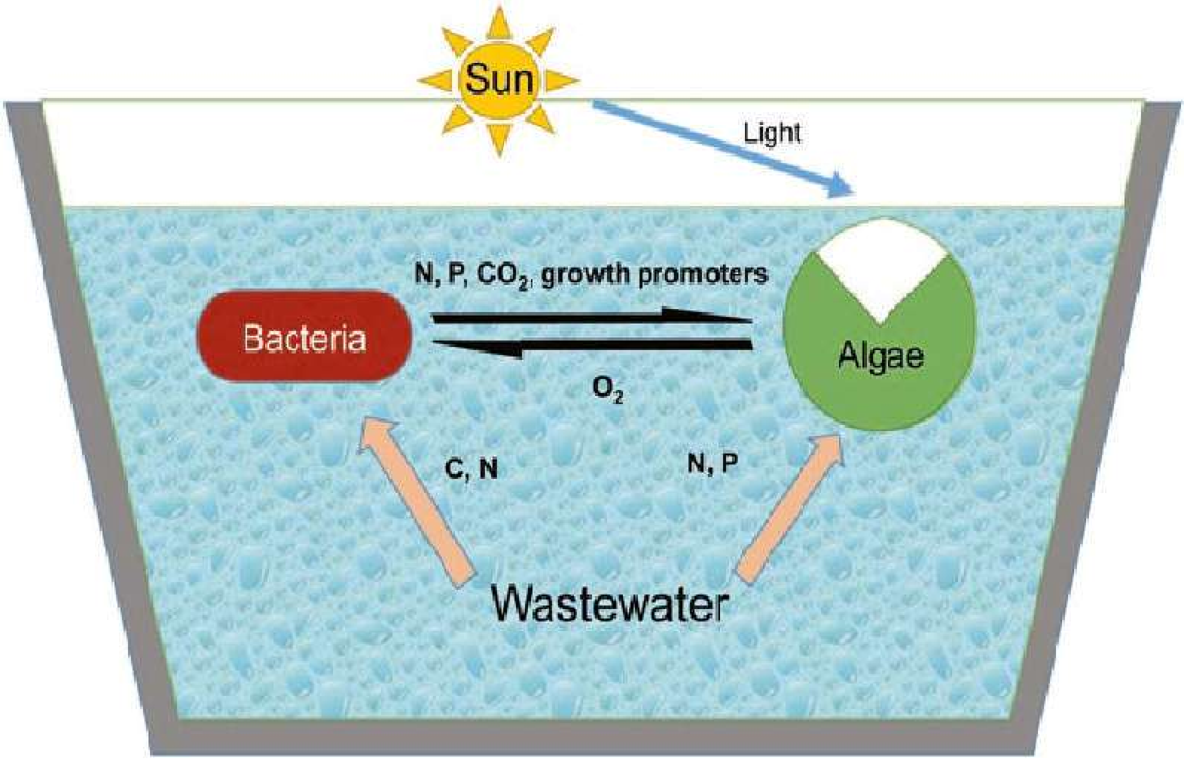

Figure : Waste Water Treatment By Microalgae

REVIEW OF THE LITERATURE

The various study conducted to identify the treatment of wastewater using microalgae discussed below:

One work showed that there are important benefits to be derived from integrating algal production systems with nutrient-rich waste streams. The energy resulting from algae will play a significant role in providing energy security while important services such as water treatment can be significantly achieved by these systems. It also shows that by the end of 14-day batch culture was removed 94% ammonia, 89% TN and 81% TP with the help of algae.

The another work investigate the treatment efficiencies of the Algae based sewage treatment plant located in Mysore. The study showed moderate treatment levels with 60% total COD removal, 50 % of filterable COD removal, 82% of total BOD removal, and 70%of filterable

BOD removal. The nitrogen removal efficiency was less. However, a rapid reduction in the suspended solids after a higher euglenoid growth indicates particulate carbon removal by algal ingestion. by Mahapatra et al, (2013)

Another studied on Integrated Bacillus sp. immobilized cell reactor and Synechocystis sp. algal reactor for the treatment of tannery wastewater with CAACO reactor. The effluent after treatment through primary clarifier, chemo autotrophs immobilized cell reactor known as CAACO reactor and removed BOD5,COD, TOC, VFA and sulphide, respectively, by 96 %, 87 %, 83 %, 71 %, and 100 %. The residual organics in treated tannery wastewater was further treated in Synechocystis sp. inoculated algal batch reactor. The algal pond was able to discharge treated wastewater of characteristics BOD5, 20± 7 mg/l; COD, 167±79 mg/l; and TOC, 78±16 mg/l conforming to the discharging standards prescribed by pollution control agencies. The cumulative percentage removal of BOD5, COD, TOC, VFA, and sulphide in the present investigation were 98 %, 95 %, 93 %, 86 %, and 100 %, respectively. by Sekaran et al (2013)

Another study evaluate the growth of green algae Chlorella sp. on wastewaters sampled from four different points of the treatment process flow of a local municipal wastewater treatment plant and how well the algal growth removed nitrogen, phosphorus, chemical oxygen demand (COD), and metal ions from the wastewaters. The study showed average specific growth rates in the exponential period were 0.412, 0.429, 0.343, and 0.948 day−1 and removal rates of NH4–N were 74-82 %, phosphorus 83- 90% and 50- 83.0 % COD were removed for four different types of wastewater. It was also found that metal ions, especially Al, Ca, Fe, Mg, and Mn in concentrate, were removed very efficiently. by Wang et al (2010)

Another work, investigate Nutrient removal by the integrated use of high rate algal ponds and macrophyte systems in China. The study showed percentage removals for COD were 54.5% in “winter” (8 days) and 44.5% in “summer” (4 days). The mean annual removal of COD was only about 50%. However, the percentage removal of dissolved COD compared to the total COD in the influent was about 73%. Indeed, the HRAP produced an effluent with a low concentration of dissolved COD (about 60 mg/l), but the total COD may be high due to algal biomass. The mean removal performances were about 50% for COD and phosphorus, 75% for TKN and 90% for

NH4-N. This system was especially efficient in removing ammonia from wastewater. by Chen et al, (2003)

Another studied on nitrogenous wastewater treatment by activated algae and given that it is possible to remove TKN between 69.5%-93.4%, NHJ-N between 77%-98% and urea between 53% to cent per cent for nitrogen loadings between 0.1 g/L day-0.37 g/L day. The major NHJ-N removal mechanism was the stripping of ammonia in the range between 52%-66%. It was possible to achieve nitrification at all SRT's and corresponding COD/TKN ratios. The COD removal was found to average between 89.5%-97.7% for the organic loadings between 0.5 g/L-day to 0.68 g/L-day.by Gupta, (1985)

COMPOSITION OF TYPICAL WASTEWATER

Watercourses receive pollution from many different sources, which vary both in strength and volume. The composition of wastewater is a composition of the life styles and technologies practiced in the producing society (Gray, 1989). It is a complex mixture of natural organic and inorganic materials as well as man-made compounds. Three quarters of organic carbon in sewage are present as carbohydrates, fats, proteins, amino acids, and volatile acids. The inorganic constituents include large concentrations of sodium, calcium, potassium, magnesium, chlorine, sulphur, phosphate, bicarbonate, ammonium salts and heavy metals (Tebbutt, 1983; Horan, 1990; Lim et al., 2010). Different sources of pollutants include discharge of either raw or treated sewage from towns and villages; discharge from manufacturing or industrial plants; run-off from agricultural land and leachates from solid waste disposal sites these sites of pollution have problems so that a solution is sought (Horan, 1990). Scarcity of water, the need for energy and food are forcing us to explore the feasibility of wastewater recycling and resource recovery (De la Nou¨ e and De Pauw, 1988).

MICROALGAE COMPOSITION OF SEWAGE

Wastewater environment is an ideal media for a wide range of microorganisms specially bacteria, viruses and protozoa. The majority is harmless and can be used in biological sewage treatment but sewage also contains pathogenic microorganism, which are excreted in large numbers by sick individuals and a sympotic carrier. Bacteria which cause cholera, typhoid and tuberculosis; viruses which cause infectious hepatitis; protozoa which cause dysentery and the eggs of parasitic worms are all found in sewage (Glynn Henery, 1989; Shaaban et al., 2004). The efficiency of disinfecting sewage is generally estimated by the extent of removal of total coliform organisms (Sebastian and Nair, 1984).

MICROALGAE FOR WASTEWATER TRETMENT

SELECTION OF STRAIN

Over 3000 strains of microalgae were identified[1] ; the characteristics of these algae strains would vary, and so would be their wastewater treatment potential. However, the major factors of selecting a suitable strain or a mix-consortia for wastewater treatment would be

(i) characteristics of wastewater, (ii) the desired level of treatment efficiency required and the cost and energy requirement of biomass harvesting

THE NECESSITY OF PRETREATMENT OF WASTEWATER

Wastewater could contain several compounds at elevated concentrations that inhibit biological (e.g., microalgal) growth [2]. Similarly, the turbidity and pH of wastewater could inhibit microalgal growth [3]. Physicochemical pretreatment of wastewater would improve the conditions for microalgal growth and reduce wastewater's strength [4,5]. Lin et al. (2017) adopted a three-step strategy for efficient treatment of textile industry water (TWW): adsorption of toxic compounds by granular activated carbon, followed by anaerobic digestion to produce electricity and reduce the load of the TWW before cultivating microalgae in the partially treated TWW [6] Although ammonium is the most preferred nitrogen source for microalgal growth, the ammonium tolerance limit for microalgal strains was reported as high as 1000 _mol NH4-N L 1 [7]. A pretreatment would be required to reduce the ammonium concentration in wastewater, or wastewater should be diluted to a tolerable limit where the selected strain could grow efficiently [8]. For several wastewater types (e.g., metal, mining, paper, oil, and grease), electrocoagulation was used as a pretreatment step, which could effectively remove various chemical additives, turbidity, pathogens [9–11]. Microalgal biomass cultivation in such pretreated wastewater could also reduce residual nutrients and turbidity from the treated wastewater [4].

MECHANISM OF TREATING WASTEWATER

NUTRIENTS UPTAKE

The treatment of wastewater having low nitrogen to organic carbon ratio (C/N) is challenging. In such wastewater, supplementation of organics is often practiced to improve bacterial nutrient removal efficiency as a source of energy. On the contrary, microalgae could utilize sunlight, the soluble inorganic carbon dioxide, nitrogen, and other nutrients to increase their cell numbers while treating wastewater. Depending on the strain type, microalgal cellular nitrogen content could range from 3–10% [12,13]. A variety of inorganic (e.g., ammonium, nitrate, nitrite, atmospheric nitrogen) and organic (e.g., urea, glycine, etc.) forms of nitrogen could be assimilated by microalgal/cyanobacterial strains, although the efficiency would again vary among strains and growth conditions. From a cost perspective, microalgal removal of phosphorus from wastewater could be a superior choice over chemical precipitation and engineered wetland based phosphorus removal [14,15]. Microalgae could also selectively consume nitro and amino groups from different aromatic compounds (e.g., aminonaphthalenes and nitrobenzonates) as nitrogen source— thereby reducing the toxicity of the original pollutants [16].

METALS ADSORPTION

High concentrations of heavy metals in wastewater could prohibit microalgal photosynthesis[17]. Nevertheless, microalgae efficiently concentrate the metal pollutants both internally and externally and could be adopted for metal removal from wastewaters [18]. Microalgal cells require several metals (i.e., Fe, Mn, Cu, Co, Zn, and Mo) in trace amounts for their growth requirements. However, microalgae are also capable of concentrating on various heavy metals (e.g., Cd, Hg, Ni, Zn, Fe, Cu, Pb, Cr, etc.) through different mechanisms various heavy metals (e.g., Cd, Hg, Ni, Zn, Fe, Cu, Pb, Cr, etc.) through different mechanisms [18]. The cell walls of microalgae are comprised of carbohydrates and polysaccharides that could have multiple negatively charged (amino, hydroxyl, carboxyl, pyruvate, sulfide, phosphate, etc.) groups as active sites for adsorbing positively charged metal cations [19]. Such extracellular adsorption of heavy metals occurs very fast till a saturation level is reached Heavy metals could also get transported through the cell membrane inside the cells, thereby reducing their concentrations in wastewater [20]. Several microalgae could release various polysaccharides, also known as exopolysaccharides (EPS), into the growth media [21]. Negatively charged groups within EPS molecules are also capable of absorbing metals [22]. Both living and dead cells of microalgal are capable of metal removal, although the metal removal efficiencies for living cells are higher compared to dead cells [23]. The removal efficiencies of several metals (e.g., Cd, Cr, Cu, Pb, Hg, Ni, and Zn) by selected microalgae are listed in Table

		Operating Condition				
Metals	**Strain**	**Initial conc.** (mg/L)	**pH**	**Temp.** (oC)	**Time** (Hour)	**Removal Efficiency(%)**
Cadmium	Chlorella sp.	100	7.4	25	24	33-41
Chromium	Chlorella vulgaris	227	0.5-5	24	6	50.7-80.3
Copper	Spirulina maxima	56.6	7.96	26-28	240	94.9
Lead	Chlorella sp.	1-50	8.1-8.6	28	288	66.3
Mercury	Chlorella vulgaris	10-200	1-8	35	0.17-0.35	34.21-93

Table Source : Internet

ORGANIC REMOVAL

There are mainly three mechanisms (i.e., biodegradation, consumption, biosorption) that the microalgae could utilize to remove the organics from wastewater. Microalgal cell walls could have several polymers group that could offer potential sorption sites for organic pollutants; however, the removal of organics by microalgal absorption was rather low [24,25]. Although most of the microalgae are photosynthetic in nature, a group of microalgae could utilize various organics in either mixotrophic or heterotrophic mode, and this would allow mixing organic-rich stream (e.g., wastewater from food processing, glycerol from biodiesel plant, etc.) other wastewater for combined treatment and improving the biomass and lipid yield [26,27]. Apart from bioaccumulation, microalgae, either as monoculture or consortia, could transform organic pollutants (e.g., phenolics, petroleum hydrocarbons, polychlorinated bisphenyls, etc.) to other less toxic and non-toxic compounds, or even completely mineralized products (i.e., CO_2) [28–31].

SYMBIOTIC RELATIONSHIP

Microalgae could also participate, in a symbiotic relationship with bacteria or fungi, to degrade organics [32]. As a byproduct of the photosynthesis process, microalgae produce oxygen, which could be utilized by aerobic bacteria for the mineralization of organics to carbon dioxide; microalgae would utilize this CO_2, and the process would be repeated, ultimately treating wastewater [33]. On the other hand, bacteria produce several useful compounds such as Vitamin B12, indole-3-acetic acid, Microalgae could also participate, in a symbiotic relationship with bacteria or fungi, to degrade organics [32]. As a byproduct of the photosynthesis process, microalgae produce oxygen, which could be utilized by aerobic bacteria for the mineralization of organics to carbon dioxide; microalgae would utilize this CO_2, and the process would be repeated, ultimately treating wastewater [33]. On the other hand, bacteria produce several useful compounds such as Vitamin B12, indole-3-acetic acid, siderophores to promote microalgal growth. At times, bacteria could also regenerate or fix several inorganic nutrients (e.g., iron, nitrogen) and organics (e.g., D-glucose, Na-acetate) that are not available to microalgae for utilization [34]. Bacterial release of polysaccharides and proteins could also assist the bioflocculation of microalgae biomass [35].

PASSIVE REMOVAL OF CONTAMINANTS AT ELEVATED Ph

As early as 1970, it was observed that microalgae flocculated in the waste stabilization pond, specifically on warm and sunny days when the CO_2 was depleted, and pH increased [36]. Therefore, microalgae could also passively assist in removing the pollutant from wastewater. As the microalgae grow, the soluble carbonate would be consumed— giving rise to the culture pH, in case there would be no additional precipitates (e.g., various metals, phosphorus)—facilitating their removal form.

C, N and P RATIOS IN DIFFERENT DIFERENT WASTE STREAM

A significant influence to the microalgal treatment performance is the composition of the wastewater. In order to grow and function, micro-algae require three primary nutrients: carbon, N and P (Falkowski and Raven, 2007). The adaptation of these nutrients is strongly affected by the overall composition of nutrients that are available in the cultivation medium (Kapdan and Aslan, 2008). Nutrient utilization rates by micro-algae are closely associated with their growth, and a limited supply of a primary nutrient can significantly reduce their growth rate (Xin et al., 2010a, 2010b; Al Ketife et al., 2017). In this context, to ensure optimal nutrient removal efficiency from cultivation medium, an optimal ratio of nutrients that is reflective of the micro-algal elemental stoichiometry needs must be present. Further to this, trace amounts of micronutrients, such as calcium, magnesium, potassium, manganese, silica, zinc, iron and others are essential and generally abundantly available in wastewater (Falkowski and Raven, 2007; Borowitzka and Moheimani, 2013). In a comparative study, (Wang et al. (2010) found that a Chlorella sp. had a higher average specific growth rate with a concomitant improved efficiency in inorganic N and P removal from PSW, compared to STE. The removal capacity of the micro-alga from PSW was 68.5% TN and 90.6% TP, and from STE50.8% TN and 4.96% TP. Moreover, a56.5% decline in COD was recorded from the PSW, while in the STE an increase of 22.7% was reported, indicating that oxidisable carbon matter was being excreted by the micro-algae.

Summary of major nutrient removal efficiencies by algal cultivation [Wang et al., 2010]

Algae Species	Wastewater Treatment	N(%)	P(%)	C(%)	Retention time

Algal-bacterial symbiosis	Settled domestic sewage	92	74	97%BOD 87%COD	10h
Chlorella pyrenoidosa	Settled domestic sewage	93.9	80	NA	13 days
Cyanobacteria	Secondary treated domestic swine wastewater	95	62	NA	1day
Chlorella vulgaris	Diluted pig slurry	54–98	42–89	98%Bod	4-5 days

CONCLUSION:

The study shows the capability of microalgae to uptake the carbon, nitrogen, phosphorus and heavy metal, and microalgae have a potential for the treatment of wastewater for various types of effluent. Sewage and industrial wastewater is naturally enriched in nutrient that can be used for algal growth. It also observed that the major factors that effecting algal growth and treatment efficiency are carbon dioxide and light from all other factors.

SUMMARY

Algae can be used in wastewater treatment for a range of purposes, including;

1. reduction of BOD

2. removal of N and/or P

3. inhibition of coliforms

4. removal of heavy metals

The high concentration of N and P in most wastewaters also means these wastewaters may possibly be used as cheap nutrient sources for algal biomass production. This algal biomass could be used for:

1. methane production

2. composting

3. production of liquid fuels ((pseudo-vegetable fuels)

4. as animal feed or in aquaculture and production of fine chemical

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REFERENCE :

1.Microalgae and wastewater treatment

Treatment of Wastewaters by Microalgae and the Potential Applications of the Produced Biomass—A Review

Hareb Al-Jabri, Probir Das *, Shoyeb Khan , Mahmoud Thaher and Mohammed AbdulQuadir

2.Microalgae and wastewater treatment

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3.wastewater treatment- A Review
Seyedeh Fatemeh Mohsenpour a, Sebastian Hennige b, Nicholas Willoughby a, Adebayo Adeloye c, Tony Gutierrez

4.Acclimation of microalgae to wastewater Environment

Olumayowa Osundeko1,2, Andrew P. Dean1, Helena Da vies1 and Jon K. Pittman1,3

5.Cai, T.; Park, S.Y.; Li, Y. Nutrient recovery from wastewater streams by microalgae: Status and prospects. Renew. Sustain. Energy
Rev. 2013, 19, 360–369. ..……[7]

6.Sheehan, J. A Look Back at the U.S. Department of Energy's Aquatic Species Program—Biodiesel from Algae. Program 1998, 3281–249………[1]

7.Tyagi, R.D.; Couillard, D. Toxic effects of inhibitors in biological wastewater treatment processes. Can. J. Chem. Eng. 1988, 66, 97–106……… [2]

8.Das, P.; AbdulQuadir, M.; Thaher, M.; Khan, S.; Chaudhary, A.K.; Alghasal, G.; Al-Jabri, H.M.S.J. Microalgal bioremediation of petroleum-derived low salinity and low pH produced water. J. Appl. Phycol. 2018, 31, 435–444. …………..……[3]

9. Chen, R.; Liu, Y.; Liao, W. Using an environmentally friendly process combining electrocoagulation and algal cultivation to treat high-strength wastewater. Algal Res. 2016, 16, 330–337. ……………..[4]

10. Chen, R.; Li, R.; Deitz, L.; Liu, Y.; Stevenson, R.J.; Liao,W. Freshwater algal cultivation with animal waste for nutrient removal and biomass production. Biomass Bioenergy 2012, 39, 128–138……………..[5]

11. Lin, C.-Y.; Nguyen, M.-L.T.; Lay, C.-H. Starch-containing textile wastewater treatment for biogas and microalgae biomass production. J. Clean. Prod. 2017, 168, 331–337. ..……[6]

12. Teasdale, P.R. Removing ammonium from water and wastewater using cost-effective adsorbents: A review. J. Environ. Sci. 2018, 63, 174–197. ……..……..[8]

13. Experimental investigation of chemical oxygen demand and turbidity removal from cardboard paper mill effluents using combined electrocoagulation and adsorption processes. Environ. Prog. Sustain. Energy 2012, 31, 361–370. ……………..……..[9]

14. Symonds, E.M.; Cook, M.M.; McQuaig, S.M.; Ulrich, R.M.; Schenck, R.O.; Lukasik, J.O.; Van Vleet, E.S.; Breitbart, M. Reduction of nutrients, microbes and personal care products in domestic wastewater by a benchtop electrocoagulation unit. Sci. Rep. 2015, 5, 9380. ...…….[11]

15.Adamakis, I.D.; Lazaridis, P.A.; Terzopoulou, E.; Torofias, S.; Valari, M.; Kalaitzi, P.; Rousonikolos, V.; Gkoutzikostas, D.; Zouboulis, A.; Zalidis, G.; et al. Cultivation, characterization, and properties of Chlorella vulgaris microalgae with different lipid contents and effect on fast pyrolysis oil composition. Environ. Sci. Pollut. Res. 2018, 25, 23018–23032. ………………..[12]

16. Premalatha, M. Characterization of micro algal biomass through FTIR/TGA/CHN analysis: Application to Scenedesmus sp. Energy Sources Part A Recover. Util. Environ. Eff. 2015, 37, 1–8………[13]

17. Pizarro, C.; Mulbry, W.; Blersch, D.; Kangas, P. An economic assessment of algal turf scrubber technology for treatment of dairy manure effluent. Ecol. Eng. 2006, 26, 321–327. [14]

18. Christenson, L.; Sims, R. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol. Adv. 2011, 29, 686–702. ….. [15]

19. Luther, M. Degradation of different substituted aromatic compounds as nutrient sources by the green alga Scenedesmus obliquus. In Proceedings of the Dechema Biotechnol Conference 4,Weinheim, NY, USA, 28–30 May 1990; pp. 613–615…..[16]

20. Suresh Kumar, K.; Dahms, H.U.; Won, E.J.; Lee, J.S.; Shin, K.H. Microalgae—A promising tool for heavy metal remediation. Ecotoxicol. Environ. Saf. 2015, 113, 329–352. ………[17]

21. Suresh Kumar, K.; Dahms, H.U.; Won, E.J.; Lee, J.S.; Shin, K.H. Microalgae—A promising tool for heavy metal remediation. Ecotoxicol. Environ. Saf. 2015, 113, 329–352… …….[18]

22. Lau, P.S.; Lee, H.Y.; Tsang, C.C.K.; Tam, N.F.Y.; Wong, Y.S. Effect of Metal Interference, pH and Temperature on Cu and Ni Biosorption by Chlorella Vulgaris and Chlorella Miniata. Environ. Technol. 1999, 20, 953–961. …..…….[19]

23. Blaby-Haas, C.E.; Merchant, S.S. The ins and outs of algal metal transport. Biochim. Biophys. Acta Mol. Cell Res. 2012, 1823, 1531–1552. …. …….[20]

24. Gaignard, C.; Laroche, C.; Pierre, G.; Dubessay, P.; Delattre, C.; Gardarin, C.; Gourvil, P.; Probert, I.; Dubuffet, A.; Michaud, P. Screening of marine microalgae: Investigation of new exopolysaccharide producers. Algal Res. 2019, 44, 101711……… [21]

25. Torres, M.A.; Barros, M.P.; Campos, S.C.G.; Pinto, E.; Rajamani, S.; Sayre, R.T.; Colepicolo, P. Biochemical biomarkers in algae and marine pollution: A review. Ecotoxicol. Environ. Saf. 2008, 71, 1–15………….. [24]

26. Wang, L.; Xiao, H.; He, N.; Sun, D.; Duan, S. Biosorption and Biodegradation of the Environmental Hormone Nonylphenol By Four Marine Microalgae. Sci. Rep. 2019, 9, 5277. …….[25]

27. Choi, Y.K.; Jang, H.M.; Kan, E. Microalgal Biomass and Lipid Production on Dairy Effluent Using a Novel Microalga, Chlorella sp. Isolated from DairyWastewater Biotechnol. Bioprocess. Eng. 2018, 23, 333–340. ………..[27]
28.

28.Kobayashi, H.; Rittmann, B.E. Microbial removal of hazardous organic compounds. Environ. Sci. Technol. 1982, 16, 170A–183A. ………….[28]

29.Delrue, F.; Álvarez-Díaz, P.D.; Fon-Sing, S.; Fleury, G.; Sassi, J.F. The environmental biorefinery: Using microalgae to remediate wastewater, a win-win paradigm. Energies 2016, 9, 132. [31]

30.Subashchandrabose, S.R.; Ramakrishnan, B.; Megharaj, M.; Venkateswarlu, K.; Naidu, R. Consortia of cyanobacteria/microalgae and bacteria: Biotechnological potential. Biotechnol. Adv. 2011, 29, 896–907. ..…….[32]

31. Ramanan, R.; Kim, B.-H.; Cho, D.-H.; Oh, H.-M.; Kim, H.-S. Algae–bacteria interactions: Evolution, ecology and emerging applications. Biotechnol. Adv. 2016, 34, 14–29. and bacteria: Biotechnological potential. Biotechnol. Adv. 2011, 29, 896–907…….. [33]

32.Fuentes, J.L.; Garbayo, I.; Cuaresma, M.; Montero, Z.; González-Del-Valle, M.; Vílchez, C. Impact of microalgae-bacteria interactions on the production of algal biomass and associated compounds. Mar. Drugs 2016, 14, 100……………. [34]

33. Wang, H.; Laughinghouse, H.D.; Anderson, M.A.; Chen, F.;Willliams, E.; Place, A.R.; Zmora, O.; Zohar, Y.; Zheng, T.; Hill, R.T. Novel bacterial isolate from permian groundwater, capable of aggregating potential biofuel-producing microalga Nannochloropsis oceanica IMET1. Appl. Environ. Microbiol. 2012, 78, 1445–1453. ……..[35]

34. Golueke, C.G.; Oswald, W.J. Surface Properties and Ion Exchange in Algae Removal. J. Water Pollut. Control. Fed. 1970, 42, R304–R314…..[36]

RAMAKRISHNA MISSION VIVEKANANDA CENTENARY COLLEGE

A PROJECT WORK ON WATER TRANSPORT IN PLANTS

SUBMITTED BY- SUBHAM CHATTERJEE

REGISTRATION NO.- A01-1112-114-012-2019

COLLEGE ROLL NO.- 413

SEMESTER- V

DEPARTMENT - BOTANY

:::ROOT:::

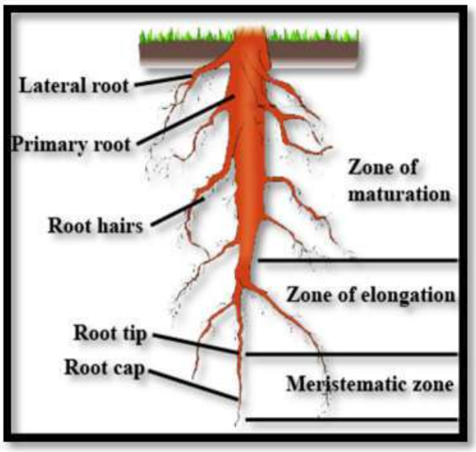
DEFINITION- The root is the descending axis of the plant ,which normally develops under the ground level, but may sometimes it develops from any other part of the plant body.

In case of all the vascular plants roots are the main important underground part. Root generally penetrates inside the soil and serves the functions and absorption. Roots are usually cylindrical and non-green in colour because of lack of chlorophyll. The growing part of the root(root tip)is protected by a cellular layer , called calyptra. This part is mainly responsible for plant growth. Roots are also help plants to stable at one place. It anchor the plant in the soil. Root is usually positively geotropic (i.e. grows downward into the soil) and positively hydrotropic (i.e. grows towards the source of water) but negatively phototropic (i.e. grows away from sunlight).

It mainly differentiate from stem by lacking of leaf scares and buds. Root has also root cap and branches like the stem . But The root branches originates from the internal tissue not the buds. However , not all the plants have their roots under the ground . Some of the plants growing their root in the upward direction, above the ground. These roots help the plants for minerals and water uptake .

If we see the anatomy of roots, it directly reflects to its origin. Root evolved in the seed plant and ferns depends upon the pressure of land environment and the plant size. Plants have specialized organ i.e, root , that help them survive and reproduce in a great diversity of habits. Major organs of most plants includes root, stems, leaves.

Roots are important organs in all vascular plants. Most of the vascular plants have two types of roots; primary roots that grow downward, and secondary roots that branch towards outside . Basically, root is a part of plant that absorbs water and nutrients from the environment , stores energy for the plant and anchoring the plant into the ground



Fig; root structure

❖TYPES OF ROOTS AND ROOT SYSTEM:

The roots are mainly of three types ; tap root(normal or main) and adventitious root and fibrous root. The description of these roots are described as follows in detail ;

i)TAP ROOT:- It is the root system that develops from the radicle and continues as the primary root (tap root) which gives off lateral roots. It is large, central and dominant type. Typically, a taproot is somewhat straight and very thick, is tapering in shape, grows directly downward.

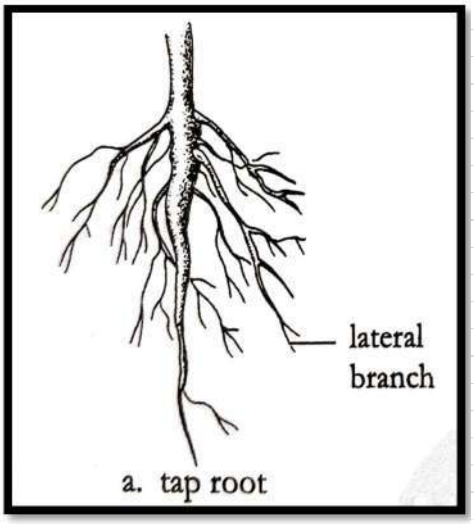
- **Characteristics of Tap roots:-**
  - Primary Prominent in dicot plant.
  - roots or tap roots grow and becomes stout.
  - Secondary and tertiary roots are grow from primary roots.
  - Primary roots grows long and thick while others are small.
  - The main root of the tap root system ,grows vertically downward.

- As the taproot grows straight down, it can penetrate deep into the soil and obtain more nutrients and minerals.
- Plants with a taproot system generally have leaves with reticulate venation.
- It generally arise from the radicle of germination embryo.

So, tap roots are somewhat straight and very thick, tapering in shape, grows directly downward. In Dicots, one or the two divisions of flowering plants, start with a taproot. Branches of secondary roots undergoes further division and form tertiary roots. The primary roots along with its branches forms primary or tap root system. Taproots are generally grow more deeply into the soil. There is an another type of root ,called lateral root, which originates from the pericycle, branch off from the tap root ,and subsequent lateral roots can branch off other lateral roots. The taproot is also good at anchoring the plant into the soil so preventing them from being blown over in windy environments. A taproot is when there is one main root that grows straight down deep into the soil. It only has very few lateral roots that develop, grow this main root. In the tap root system , the main root is largest and longest and the lateral branches are shorter than the main root .There is one main root with branches arising in an acropetal manner. The roots are grow in acropetal succession, that is younger roots are present at the growing end of the tap root and the older roots presents near the base of the stem. The main or primary root persists throughout the life of the plant. A taproot penetrates deep into the soil for the search of water, because of this feature tap roots are also help the plant to anchor into the soil. In some cases tap roots can from a storage organ for foods like, carrots, radish, beetroot and turnips. Tap roots can reach water at great depth.

Types of tap root:-

Tap root system is of two types— deep feeder and surface feeder. Deep feeder tap root system has an elongated long tap root . which usually penetrates the deeper layers of the soil. Deep feeders have long network of tap roots. The deep feeder root system is also known as racemose root system.



In surface feeders the tap root does not elongate very much. The secondary roots spread to a greater extent, mostly horizontal near the soil surface. Such a system is also named as cymose tap root system. The cymose or surface feeder tap root system of some annual plants consists of thin fibrous roots.

- **Modifications of tap root:-**

The tap roots became swollen and fleshy with the stored food. There is several types of modifications in tap root system. The tap root Depending upon various characteristics , tap roots are modified in various way, like conical roots, fusiform roots, tuberous roots etc. In some plants, the roots are changed their shape and structure to modify themselves for various purpose. They became modified to perform various functions. These modified roots are not only for the function of absorption and conduction of water and minerals, these modified tap roots has many other types of function. The tap roots are modified for various plant purpose , some of them are for plant support , storage of plant food and also for the respiration. Also the roots are get modified to maintain the physiological and mechanical strength of the plant.

The taproots are modified into the following –

I)storage of food.

ii) carry out of N2 – fixation or nodulated roots.

iii) for respiration

I)Storage of food:-

The storage roots can be defined as, the tap root which are capable to store food. In some plants, sometimes the roots are get a fleshy structure due to the absorption of foods, as minerals, water etc. The main purpose of this type of modified root is that they can store food, prepared by plants inside them. So, this food is utilized by the plants for nutritional purpose during uncomfortable conditions of plants . Then , this food is also known as reserve food of plants.

In storage roots, the cells of primary tap root or the main root cells swell and start accumulating food particles, whereas the secondary and tertiary roots remains thin . the upper stem is reduced and disc-shaped in the beginning and bears radicle leaves.

Depending upon their shape, storage roots are categorised into four types, these are –

a)Conical type of storage root,
b)Fusiform type of storage root,
c)Napiform type of storage root,
d)Tuberous type of storage root.

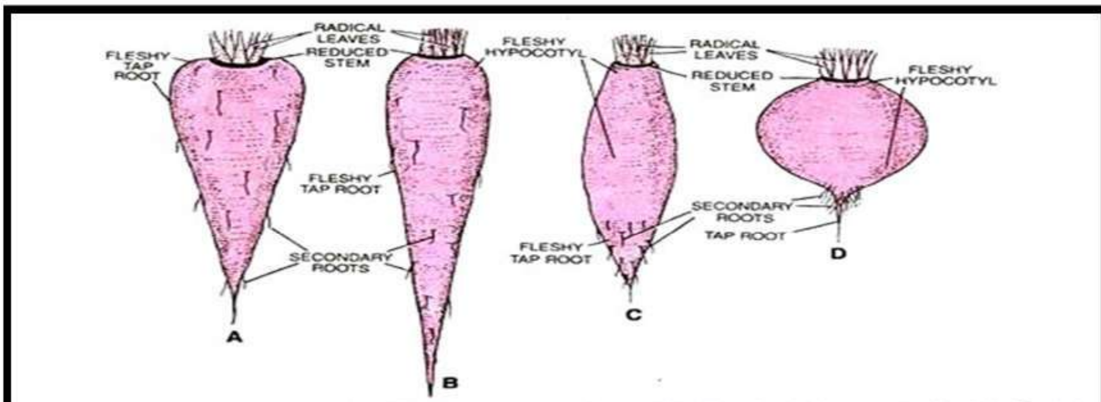


Fig. 5.6. Fleshy tap roots. A, conical fleshy tap root of Carrot (**Gaajar**). B, fusiform root of Indian Radish (**Mooli**). C, fusiform root of European Radish. D, napiform fleshy tap root of Turnip (**Shalgam**).

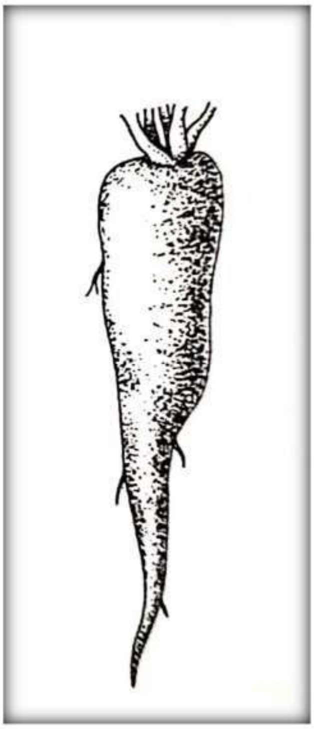
Source of fig: google (link in references).

a)conical type of storage roots :-

In this system of tap root system, the main root or the primary root starts swelling from the base of the stem and gradually reduces towards the apex of the root. This roots are broad at the base and conical at the apex. Conical shaped root has one swollen end and a tapered end in both opposite direction . conical shaped roots are slight fleshly in nature. The fleshy tap root resembles the cone. Not only, the primary root , in this type of roots , many secondary and tertiary roots are also visible. Many thread like structure are called as secondary and tertiary root. These secondary and tertiary roots arise throughout the length of the conical fleshy taproot. It forms a single, dominant , straight axis that penetrates the soil directly downwards to a considerable depth without dividing and form which other lateral roots sprout .

Example- *Daucus carota* (carrot plant).

Fig;



Fig; Carrot

Fig; conical root

(source- image taken from field ,captured by me)

(source- link in reference section)

b)fusiform type of storage roots :-

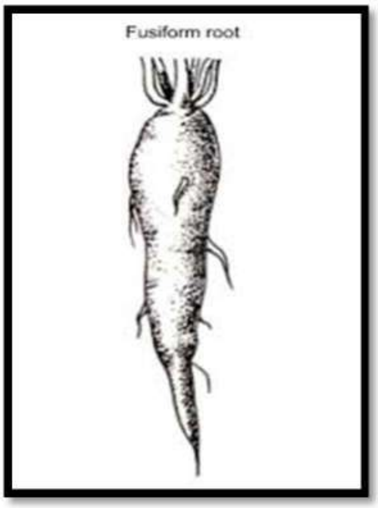
In this system of tap root, the primary root is tapered at both the ends. This type of root is swollen in the middle and tapering towards both ends forming spindle shaped structure. Fusiform/this type of roots are thicker in the middle and tapered on both ends. In this type roots both hypocotyl and roots are also helps in storage of foods. They also help to get oxygen for transpiration. Half of the fleshy part of this type of root is derived from the hypocotyl. The ends of this roots being the base of the stem and apex of the root. These roots are also known as stilt roots. They grow obliquely downwards and penetrate the soil . the primary function, is to provide support of the plant. It appears like a spindle, thickest from the middle and narrow from the ends. The main difference between conical and fusiform tap root is, in conical roots tapering occurs at only one end. , while in fusiform roots tapering is present at both the ends.

Example- **Indian Radish (***Raphanus* *sp.)..*

Its fusiform roots consists of swollen hypocotyl near the base and

Swollen taproot in the rest of the region.

Fig;



Fig; fusiform root

fig; Indian Radish(*Raphanus sp.)*

(link in the reference section)

C) Napiform type of storage roots:-

In this type of tap root , the total roots are mainly fleshy in nature. This roots are appears like a sphere. It is very broad at the top and also the bottom portion. In this root the upper part, and are sharply tapering towards the tip of the root. The swellings of the taproot occurs from the base of the stem, but does not reduce gradually . The taproots are tapers at the base . The fleshy part of this root is mostly derived from the hypocotyl. The main difference between the fusiform and napiform is, napiform type of roots is nearly globular or spherical in outline.

Example; German Turnip (*Brassica oleracea):* Most of the swollen part of the turnip is hypocotyl. The taproot appears at the tapered end of the turnip where secondary, tertiary roots are also present.

Beet root(*Beta vulgaris*).

Fig;

Fig; napiform root(turnip)

(link in the reference section)

fig; napiform(beet root)

(link in the reference section)

D) Tuberous type of storage roots:-

Basically tuberous root is tuber like root without buds or scale leaves. There are the storage taproots which do not attain any particular shape. Each tuberous roots has the capability of producing a new plant, but usually the tuberous roots grow in a tangled mass of roots and stem. Tuberous roots , a true root so thickened as to resemble a tuber , but bearing no buds or eyes. Tuberous roots is basically a storage roots. Tuberous roots are found in a number of plants including asparagus, airplane plant, dahlia, daylilies, peonies, some irises, sweet potato, taro, and many others. These types of roots are bulbous type.

Example; 4 O' clock plant *(Mirabilis jalapa).*

Fig;

Fig; tuberous root

(link in the reference section)

fig; *mirabilis jalapa*

(link in the reference section)

II)Carry out of Nitrogen fixation- nodulated root:-

Nodulated roots are the type of taproots that are irregularly swollen. The primary, secondary, tertiary roots are swollen, called root nodules or nodulated roots. These nodulated roots are consisting of millions of nitrogen fixing bacteria of genus *Rhizobium*.

The nitrogen which is freely available in the atmosphere that cannot be utilized by plants directly. This *Rhizobium* helps the plant to collect the nitrogen and utilize of it. The *Rhizobium* which is present in the root nodule take the nitrogen gas from the atmosphere and convert it into organic compounds of nitrogen. And this process is known as nitrogen fixation. Actually, the bacteria convert nitrogen gas to nitrate , which can be easily absorbed by roots of plants.

Mainly in legumes (pea family), the secondary or primary taproot bear root nodules. In this nodules the *Rhizobium* bacteria stay. They help to take nitrogen and this nitrogen is fixed by this bacteria in the exchange of food and shelter. Here, we see a perfect mutualism between the bacteria and plants.

Some non-leguminous plant plants also contains root nodules for nitrogen fixation. Not only the *Rhizobium,* but also some plants use a actinobacteria, called *Frankia* as a nitrogen fixator.

Example:- some leguminous root nodular plants-------------------

1)*Pisum sativum*- Pea.

2)*Cicer arietinum*- gram/chana.

3)*Glycine max*- Soyabean.

4)*Trigonella foenum-graecum*- Methi

Some non-leguminous root noduler plants--------------

1)*Parasponia sp.*

Figures:

Fig; root nodule(*Pisum sativum*) (link in the reference section)

fig; root nodule(*Cicer arietinum*) (link in the reference section)

Fig; root nodule*(Glycine max.)* (link in the reference section)

fig; root nodule*(Trigonella foenum-graecum)* (link in the reference section).

III)For Better Respiration:-

Many plants are growing in saline, swamps, marshy, and salt lakes, they are also known as halophytes. These plants are also known as hydrophytes. In these type of plants, we see, many roots are come out of the ground and grow in a vertically direction. These type of roots are known as pneumatophores. Pneumatophores are the aerial taproots which come out from the soil and help the plant to breathe. For this reason , they are also known as respiratory roots.

These plants are generally grow in mangrove or swamps. The soil of these type of places are clayey and sticky . For this type of soil , the soil blocked the air to pass through it. So, these pneumatophores are came out of the soil for better respiration. The roots appear like conical spikes. They occur in large numbers near the trunk. They grow vertically in upward direction opposite the gravity, they show negative geotropism. The surface of these root contains very small pore-like structures, known as lenticels or pneumathodes. These pneumathodes help plants in the exchange of gases. The remaining surface of the root is covered by cork. Also they are outside of the soil, they are also non-green and non-photosynthetic. Pneumatophores are mainly found in mangrove plants.

Fig;

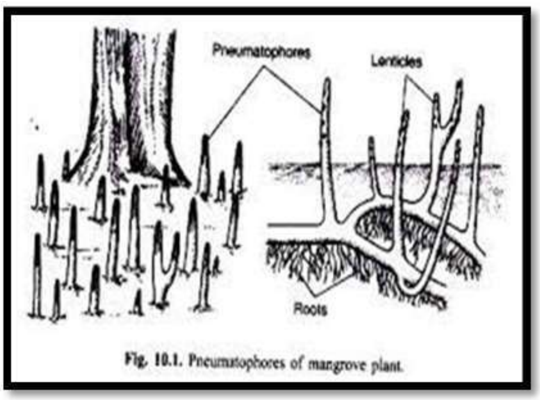


Fig. 10.1. Pneumatophores of mangrove plant.

Fig; mangrove plants

(link in the reference section)

ii) Fibrous root:-

firstly, the fibrous root is the opposite of tap root. It is moderately branched root. Usually, it is thin in nature. This type of roots are thread like and these are originate from the base of the stem . Also, this type of roots has no prominent central axis and that branches in all direction. The main root does not grow in too deep. They are mainly found in monocot plants and ferns. Many trees begin its life with a taproot but in its maturity stages, it becomes wide- spreading fibrous root . This type of roots are mainly horizontally spreaded. Only few of them are vertical and deeply anchoring into the ground. This fibrous root system look like a mat made out of roots . Fibrous roots grow fairly close to the surface of the ground. On the other hand, fibrous roots are bushy roots in which thin, moderately branching roots grow from the stem. Fibrous roots are mainly found in monocots and ferns.

Fibrous root system, which helps combat erosion by anchoring the plants to the top layer of the soil. The roots is the fibrous roots system are morphological similar in contrast to the roots in the tap root system in which a thin, short root arises from a single , thick root .

Types of fibrous root system-

i)Fleshy fibrous root- This type of fibrous root are of three types.

- Tuberous root- e.g, *Ipomoea batatas.*
- Fassiculated root- e.g, *dahlia*, *asparagus officinatum.*
- Annulated root – e.g, *cephaelis ipecacuanha.*

ii) Stilt roots- *saccharum officinarum, zea mays.*

iii) Reproductive roots- These types of roots develop buds on them and take part in vegetative propagation. These buds later under favorable conditions developed into a separate new plant.

e.g, *Dahlia*, *Ipomoea batatas.* Etc.

- **Functions of fibrous roots:-**

➢ This type of roots are extremely useful in erosion control.
➢ They keep the plant attached to the soil firmly.
➢ This roots can absorbs more water and nutrient from the soil.

Figures,

Fig; Fibrous root .

(link in the reference section)

Iii) ADVENTITIOUS ROOTS:-

Adventitious roots are a type of plant roots that form any non-root tissue and are produced both during normal development. A root growing from a location other than the underground, descending portion of the axis of a plant, as from a stem or leaf. These type of roots are arises from parts of the plant other than the radicle. Actually, these roots are mainly arise from then injured roots, internodes, etc. In this system of root, a number of roots develop at a single point. Adventitious roots are generally seen growing from aerial parts of the plants.

Modifications of Adventitious Roots:- the roots are modified for many types for mechanical support and perform many other vital functions.

- **Modifications for storage of foods**-
  - Tuberous root- *Ipomoea batatus.*
  - Fassiculated root- *Dahlia.*
  - Moniliform root- *Portulaca grandiflora.*
  - Annulated root- *Cephaelis ipecacuanha.*
  - Nodulose root- *Cucurma longa.*
- **Modifications for provide mechanical support/strength-**
  - Prop or pillar formation- *Ficus bengalensis.*(Banyan tree).
    Rhizophora sp. (Mangrove plants).
  - Stilt roots- *Saccharum officinarum* (Sugarcane).
    Zea mays (Maize).
  - Climbing roots- *Tecoma stans* with *Piper betle.*

Functions of Adventitious roots-

- ✓ This type of roots provide the mechanical support to the plant.
- ✓ Adventitious roots are still roots, and therefore, their main function is the absorption of minerals and water.
- ✓ The adventitious roots can also extend through the soil and help plants to maintain their body position better.

Figures;

Fig; Adventitious roots..

(link in the reference section)………

❖ Movement of water transport in plants:-

Most plants obtain the water and minerals they need through their roots. The water absorbed by the root hairs is translocated upwards through the xylem. The water after being absorbed by the roots is distributed to all parts of the plants (excess of which is lost through transpiration). the water has to move upward through the stem. This upward movement of water is called as Ascent of sap. The water moves into the plant through a pathway like, soil-roots-stems-leaves-air. Water enter the root by separate paths which eventually converge in the stele, or central vascular bundle in roots. This water also evaporate from the plants through transpiration. This helps to movement the water through xylem. Water from the roots is pulled up by this tension. At night, when stomata close and transpiration stops, the water is held in the stem and leaf by the cohesion of water molecules to each other as well as the adhesion of water to the cell walls of the xylem vessels and tracheids. This is known as cohesion-tension theory of ascent of sap.

The theories of Ascent of sap are— I) Vital force theory.

2) Root Pressure theory.

3) Theory of Capilarity.

4) Cohesion-tension theory.

These techniques helps water to move into the plants. Water passes through the xylem . water taken from soil by root hairs to take more waters and nutrients from the soil. The phloem and xylem tissue are the main parts through which water moves in plant.

➢ Vital force Theory-

The intimate association of xylem tracheary elements to the living xylem parenchyma cells have lead some scientist to think that the water in the xylem vessels and tracheids is ascended due to the vital activities of the living xylem parenchyma cells that surround these tracheary elements.

Although there is no direct evidences about this theory, but some scientist , sir Godlewski (1884) and Sir J.C.Bose (1923) are the proponent of this theory. Not only these scientist , some other scientist also work on this theory.

Sir Godlewski (1884) has proposed a theory on this topic, the theory is known as 'RELAY- PUMP THEORY'. According to his theory , also vessels and tracheids acted as reservoir and water is lifted by the pumping action of parenchyma cells . And also showed that if the lower portion of a branch is killed, the above leaves also affected in few days.

After that , Sir Jagadish Chandra Bose (1923), proposed an another theory in this regard. This theory is also known as 'PULSATION THEORY OF ASCENT OF SAP' . The theory believes that the innermost cortical cells of the root absorb water from the outer side and pump the same into xylem channels. He observed a pulsatory activity in the inner most cortical cells and suggested that this pulsatory movement actually helps the water to lift the water in xylem elements. Molish (1928) also supported Bose's theory and observed pulsatory activity of many type of living cells.

This theory has got a limited acceptance because ascent of sap cannot be stopped even after killing all the living cells.

➢ ROOT PRESSURE THEORY-

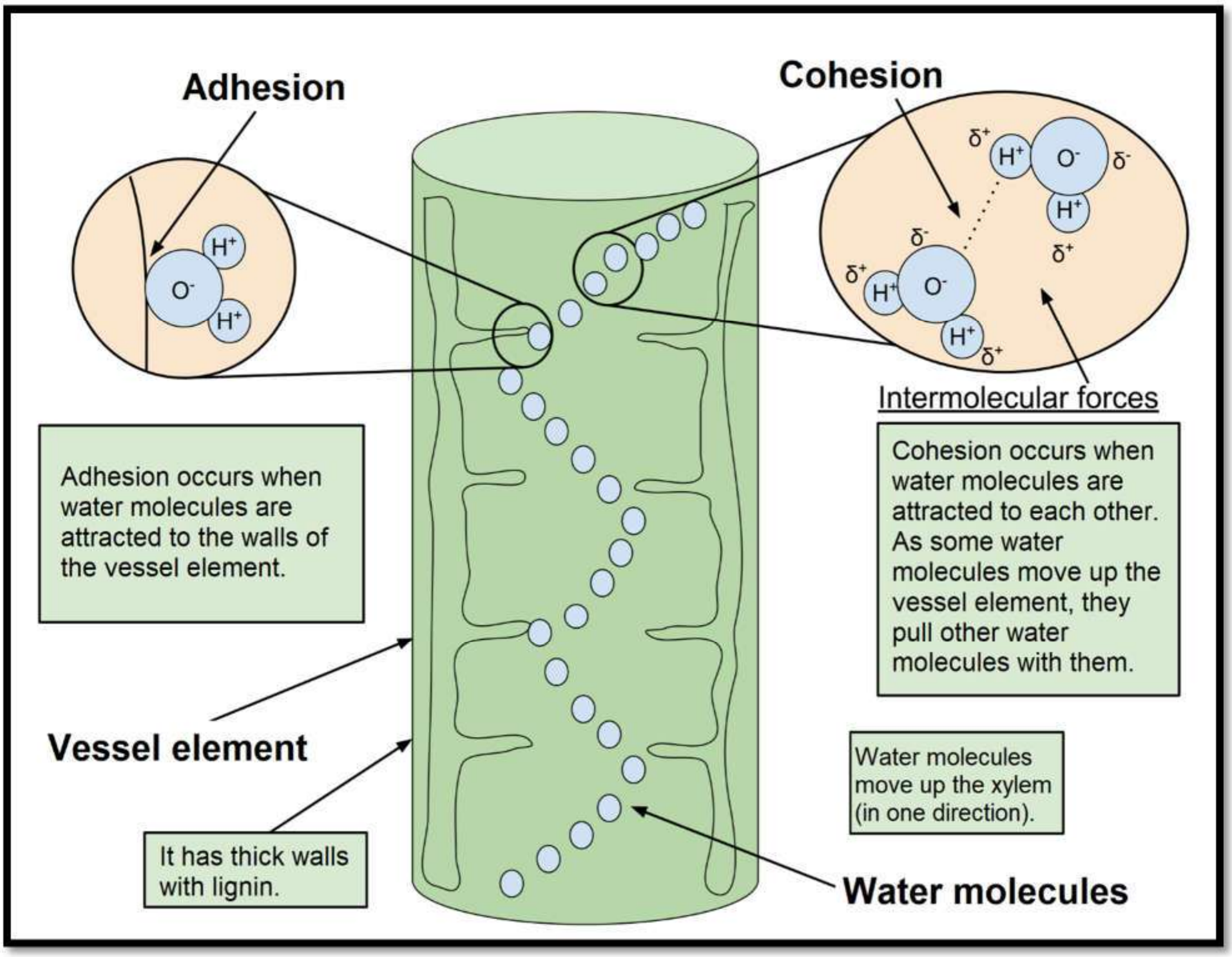
If the stem of a well-watered plant is cut above the soil surface , xylem sap bleeds out of the cut end which may persists for several hours, indicating the presence of positive pressure in the xylem. This pressure is developed from the root , and known as root pressure. Root pressure has its basis in the structure of roots and the active uptake of mineral salts from the soil. The endodermal cells of the root is provided with a impermeable suberin bands on their radial and transeverse walls. These bands are called Casparian bands. These bands create a barrier between the cortex and the stele as a result of which water can only move in and out of the stele through the membranes of the endodermal cells and through the plasmodesmatal connections. Pressure has not been detected in m species. However, root pressure is seen to devel in some herbaceous species like, tomato colocasia etc and also in some woody speas: the spring when sap moves up to the devel buds. Existance of root pressure is evidenced in the phenomenon of guttation in these herbans plants. Root pressure is a positive pressure that develops in the xylem sap of the root of some plants. It is a manifestation of active water absorption. Root pressure is observed in certain seasons which favour optimum metabolic activity and reduce transpiration. Root pressure is retarded or becomes absent under conditions of starvation, low temperature, drought and reduced availability of oxygen. There are three view points about the root pressure development. These are – Osmotic, Electro-osmotic, non-osmotic. The normally observed root pressure is generally low which is unable to raise the sap to the top of trees. Root pressure is generally observed at night when evapotranspiration is low. It may be helpful in re-establishing continuous water chains in xylem which often break under enormous tension created by transpiration.

➢ THEORY OF CAPILARITY-

According to this theory , water rises in the tracheary element due to capillary rise . capillary rise depends upon the interaction of some forces like adhesive force of vessel wall, surface tension due to cohesive forces between water molecules and the force of gravity. Water rises in tubes of small diameter, kept in vessel having water, due to force of surface tension or adhesion and cohesion. Water similarly rises up in the walls of xylem channels due to adhesion. Cohesive force present amongst water molecules pulls the water upwards through the xylem channels. The upward movement will continue till the forces of adhesion and cohesion are balanced by the downward pull of gravity. Capillarity occurs only when base of the tube dips in container having water. Xylem vessels are not directly connected with soil water. Rise due to capillarity will increase when the lumen of vessels is less. Tall plants should, therefore, have narrow vessels as compared to smaller plants. The truth is, however, reverse. Capillarity cannot operate in plants having tracheids due to the presence of end walls.

➢ COHESION-TENSION THEORY-

According to the cohesion-tension theory**,** transpiration is the main driver of water movement . Water from the roots is ultimately pulled up by this tension. Negative water potential draws water from the soil into the root hairs, then into the root xylem. Cohesion and adhesion draw water up the xylem. In 1895, the Irish plant physiologists H. H. Dixon and J. Joly proposed that water is pulled up the plant by tension (negative pressure) from above. As we have seen, water is continually being lost from leaves by transpiration. Dixon and Joly believed that the loss of water in the leaves exerts a pull on the water in the xylem ducts and draws more water into the leaf. he answer to the dilemma lies the cohesion of water molecules; that is the property of water molecules to cling to each through the hydrogen bonds they form**.**



::::CONCLUSION:::

Water is a vital element for plant. Plant takes up water through their roots and transport it to the leaf through stem. Roots are various types like, tap root, fibrous root, adventitious roots. Basically, plant takes up water by root hairs so that it absorb more water to uptake. Plant takes up and transport water through various theories. Water transport from root to shoots to leaf to maintain water balance in its body. This water help plants to photosynthesis and respiration. Root takes up water by root pressure . This water go through the plant by various process, like root pressure, capillarity, embolism, guttation, cavitation, etc. water uptake process is discovered by many scientists. Sir J.C.Bose has pulsation theory, Diction & Jolley has Cohesion-Adhesion theory like that.

:::REFERENCES:::

1) PLANT PHYSIOLOGY AND DEVELOPMENT BY 'TAIZ AND ZEIGER', 6TH EDITION.....

2) INTRODUCTION TO PLANT PHYSIOLOGY BY WILLIAM G. HOPKINS AND NORMAN P.A.HUNAR, 4TH EDITION....

3) LEHNINGER PRINCIPLES OF BIOCHEMISTRY BY DAVID L. NELSON & MICHEAL M.COX , 7TH EDITION....

4) PLANT PHYSIOLOGY BY MUKHERJI & GHOSH,

5) ADVANCED BOTANY BY SANJEEV PANDEY, (VOLUME-1)....

6) https://images.app.goo.gl/qZ9krRpN2krFQSkM6

7) https://images.app.goo.gl/uKvcAPzUTXGtWnGSA

8) https://images.app.goo.gl/9T9GmRAgaC5KkDrd9

9) https://images.app.goo.gl/BWoCJ4Ga7sYBWn9y5

10) https://images.app.goo.gl/j7d49E1QZj4EQSeJA

11) https://images.app.goo.gl/8nUGdfdbkFw5XHQD7

12) https://images.app.goo.gl/mnVEENcu7fobtgtk7

13) https://images.app.goo.gl/Uey3b1k71vVZ5puz5

14) https://images.app.goo.gl/zVVKNVqY41GaJqGX6

15) https://images.app.goo.gl/LgfYXp8RoBh6LNoEA

16) https://images.app.goo.gl/CXRNKoSCYVwy48xBA

17) https://images.app.goo.gl/vLT6wUuDuoXmodu69

18) https://images.app.goo.gl/zPqNCXukvpvN45UQ6

19) https://images.app.goo.gl/oic6pEoFp9J9LDiE8

20) https://images.app.goo.gl/qwuFKqmHz1mKSnPh6

21) https://images.app.goo.gl/gRuxbZcSBc9kmmab8

22) https://images.app.goo.gl/cNPwmVe3Q69WVvJ59

23) https://images.app.goo.gl/u3HVUpx9MePWabrN9

24) https://images.app.goo.gl/kugoy7VAQWNp8hcH6

25) https://images.app.goo.gl/nxB1DQkeBTA2Kw8K6

26) https://images.app.goo.gl/nxB1DQkeBTA2Kw8K6

27) https://images.app.goo.gl/EtjnNZSQNS7Skk86A

28) https://images.app.goo.gl/PSHpkNmrxweyeWgn7

RAMKRISHNA MISSION VIVEKANANDA CENTENARY COLLEGE, RAHARA.

A PROJECT REPORT ON :–

MENDELISM IN LIGHT OF BIOSTATISTICS.

Name :– Sourav Kumar Dutta

Registration no:– A01-1152-114-014-2019

College roll no :- 415

Semester:- VI

Department:– Botany.

MENDELISM IN LIGHT OF BIOSTATISTICS

Introduction:-

Genetics and Biostatistics these two subjects are very closely related with each other.

Definition of genetics:-

Genetics is a branch of biology. Genetics is the study of genes, genetic variation and heredity of organism. The study of the structure and function of the gene is called genetics.

Genetics is the study of genes. A part of DNA which synthesis polypeptide or RNA ,is called gene.

Genetics is important for us in all fields of life e.g.-

- **It plays a important role in medicine and pharmaceutical industry.**
- **It is important in study of genetic disorders.**
- **In study of all biological subjects, like evolution, developmental biology, molecular biology , taxonomy, ecology, behaviour of animal and plants, physiology, biotechnology etc. requires an understanding of genetics.**

DIVISIONS OF GENETICS:-

Genetics consists of four major sub disciplines-

- **Transmission genetics**
- **Molecular genetics**
- **Population genetics**
- **Quantitative genetics**

—(according to Peter J Russell, iGenetics,3rd edition, 2020).

DEFINITION OF BIOSTATISTICS:–

‘Statistics’ is derived from the Latin word ‘status'. The study of statistical methods and procedures like collection, classification, presentation, analysis and interpretation of numerical data and make inference from all this is called statistics.

The father of modern Statistics is Sir Ronald Aylmer Fisher.

- **The term Biostatistics joined two fields of study– Bio(it stands for biology) and statistics.**

The application of statistical methods to different fields of biology including human biology, medicine, public health, agriculture, veterinary, microbiology and genetics, called Biostatistics. It is also known as Biometry.

Statistics is important in research field:–

- **All research are incomplete without the statistics. All result in the research need to be statistically validated.**
- **Statistics needs for design of experiments.**
- **For deriving logical conclusions from the data.**
- **It needs for deriving single values from a group of variables.**

The father of Biostatistics was Sir Francis Galton and father of Biometry was Walter Weldon .

Biostatistics is very important in different fields of genetics like population genetics, applied genetics etc. It is also important in Molecular Biology, Biochemistry, Physiology, Endocrinology, Agriculture, Forestry, Ecology, Environmental Management, Pharmacology and Medicine industry etc.

Biostatistics in genetics:–

The birth of genetics is based on the statistical use by Mendel to analyse the result of F1 and F2 crosses . In Mendelian genetics the statistical methods that generally applied are chi–square or probability test.

- **Chi–square tests are used to determine the deviation between observed and expected frequencies of phenotype in a new generation. It can also use when the outcome of a cross confirms to the same results of Mendel's laws.**

History of Genetics and work of Mendel:–

The father of Genetics was Gregor Johan Mendel .

Mendel was born in July 22 ,1822, Heinzendorf, Silesia, Austrian Empire (now in Hynčice, Czech Republic). In 1845 ,Mendel was admitted to Augustinian monastery in Brno. He graduated from seminary. In 1851 to 1853 he went for further study at university of Vienna. After 2 years of study in Vienna, he came to Brno and then he taught at school and started his experimental works with pea plants.

In the time of 1856 to 1863 he began breeding experiments of pea and presented his work at meetings of the Brno Natural Science Society in 1865. Mendel's paper was published in 1866 . He died on 6th January 1884 in Brno ,Czech Republic. But in his life span nobody seemed to be noticed that Mendel had discovered the basic principles of inheritance .

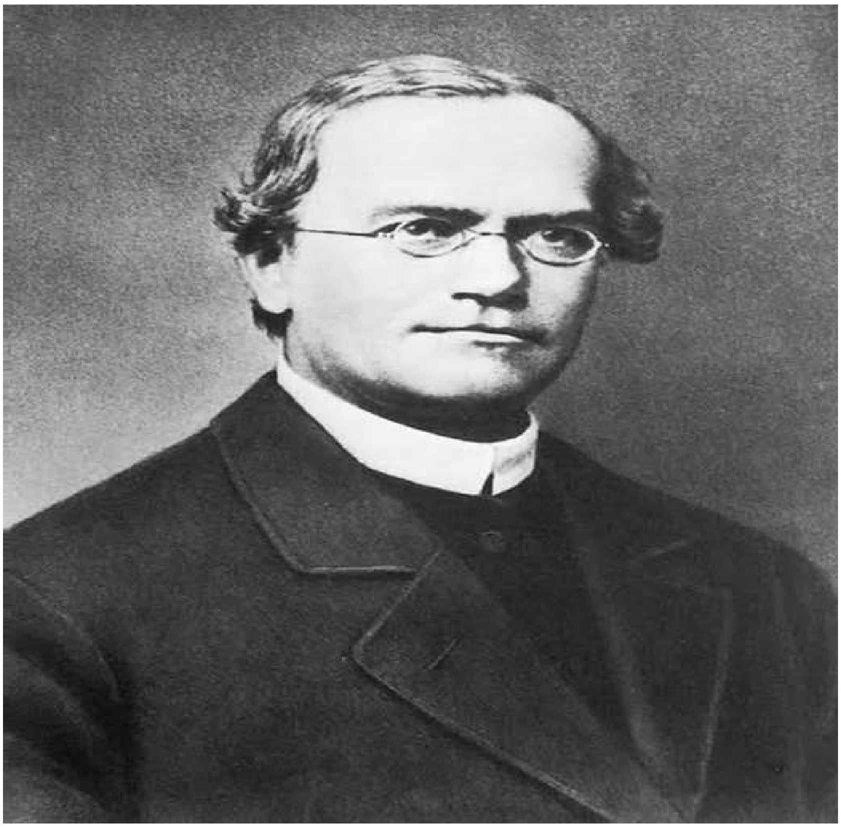
But in nearly 1900 three Botanist — Hugo De Vries, Erich Von Tschermak and Carl Correns – started similar experiments with plants independently and arrived at conclusions like those of Mendel's work. Then the scientific community gives attention to Mendel's theory.

Mendel's success:-

Mendel's approach was effective for several reasons:-,they are–

- **His choice of experimental subject was Pea plant (*Pisum sativum)*, that showed clear advantage for genetic investigation.**
- **The Pea plant is very easy to cultivate . Mendel had the monastery garden ,so he was easily study about this .**

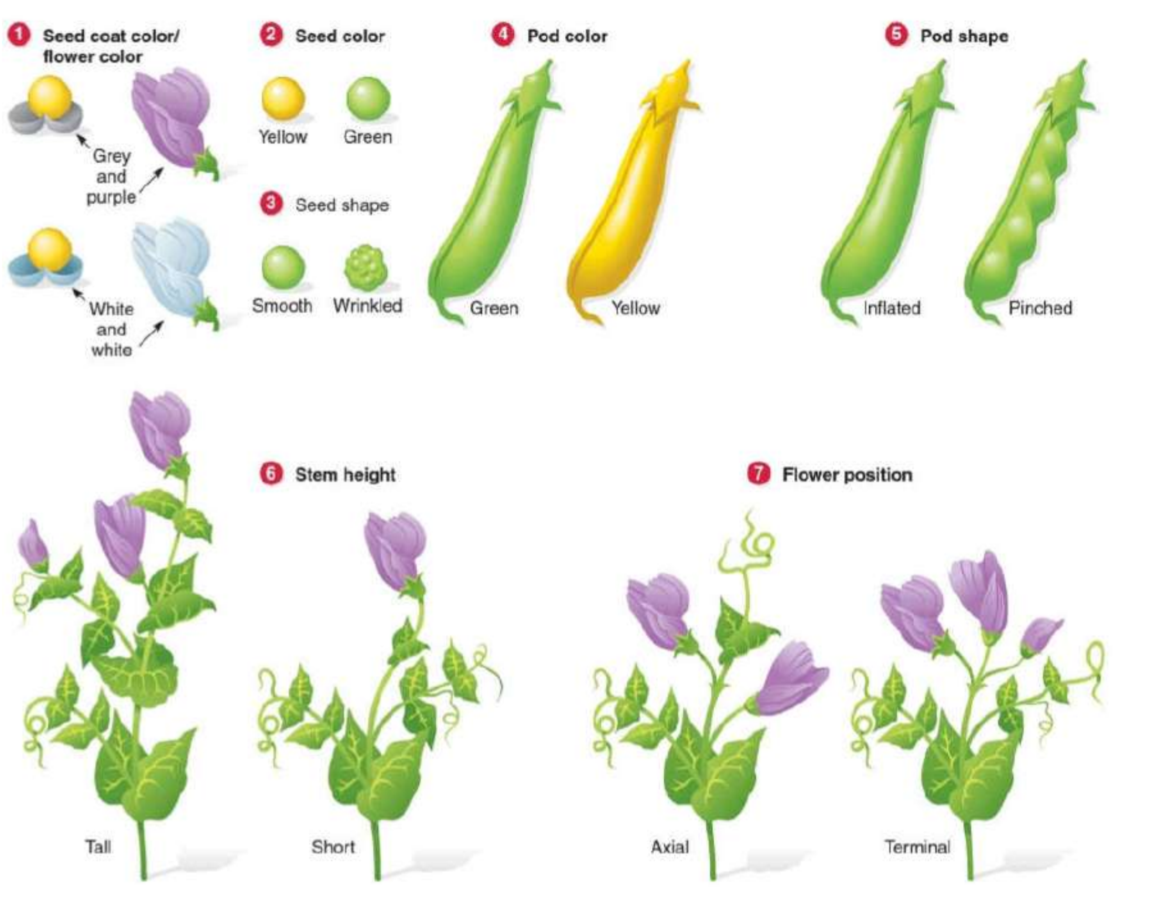
- Peas grows quickly. Peas completing entire generation in a single growing season.
- Peas also produce many seeds . Mendel planted this seeds and then he detected meaningful mathematical ratios in the traits from the progeny.



(Figure:1.picture of Gregor Johan Mendel)

Peas have large number of varieties that were available to Mendel was crucial for him , because these varieties differed in various charters and these varieties were genetically pure.

- Mendel selected 7 pairs of traits to study in his experiment (This 7 pairs of traits shown in figure 2).



(Figure:-2. 7 pairs of traits in Mendel experiment.)

(This image is taken from iGenetics by Peter J Russell, 3rd edition ,2020)

- **<u>Lastly</u>, Mendel was successful because his experimental approach and result was based on mathematics. Many earlier investigators who just described the results of crosses but Mendel approached mathematical hypothesis that was based on his observations and then he conducted additional crosses to test his hypothesis. He kept every records , number of progeny and then he calculated ratios of different types.**

MENDEL'S LAWS AND EXPLANATIONS:–

Self fertilization (selfing)cross between male gametes(pollen) and female gametes(eggs) from same individual.

Cross fertilization is the fusion of male gametes from one individual and female gametes from the other individual.

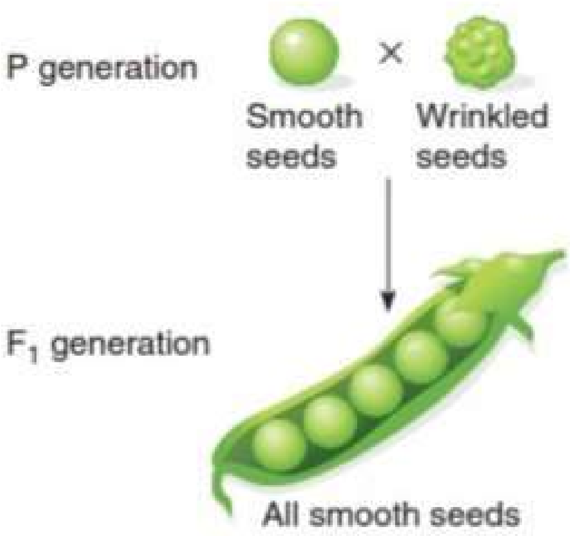
In time of crossing, if the trait under investigation remained unchanged from parent to offspring for many generations, such strains are called true-breeding or pure breeding strains.

MONOHYBRID CROSSES AND MENDEL'S PRINCIPLE OF SEGREGATION:–

- The parental generation is called the P generation.
- The progeny of the p mating is the first filial generation or F1 generation.
- Crosses between F1 generation produce F2 generation or second filial generation.
- Interbreeding between the offspring of each generations produce F3, F4 ,F5 ....and so on generations.

Monohybrid crosses was first performed by Mendel between true breeding strains of peas . The peas have alternative forms of a single trait . When he crossed between pure breeding smooth seed peas and wrinkled seed peas ,this result all smooth seeds. This cross is done in two ways —smooth female × wrinkled male and wrinkled female and smooth male , this two ways are called Reciprocal cross. But the result is same in both cases, smooth seeds are produced after the cross.

In F1 progeny seeds are all smooth, they exactly resembled only one of the parents in this cross rather than being a mixture of both parental phenotypes . In F1 generation the all offspring of pure- breeding parents are same , this phenomenon referred to as the principle of uniformity in F1(given in picture:-3).

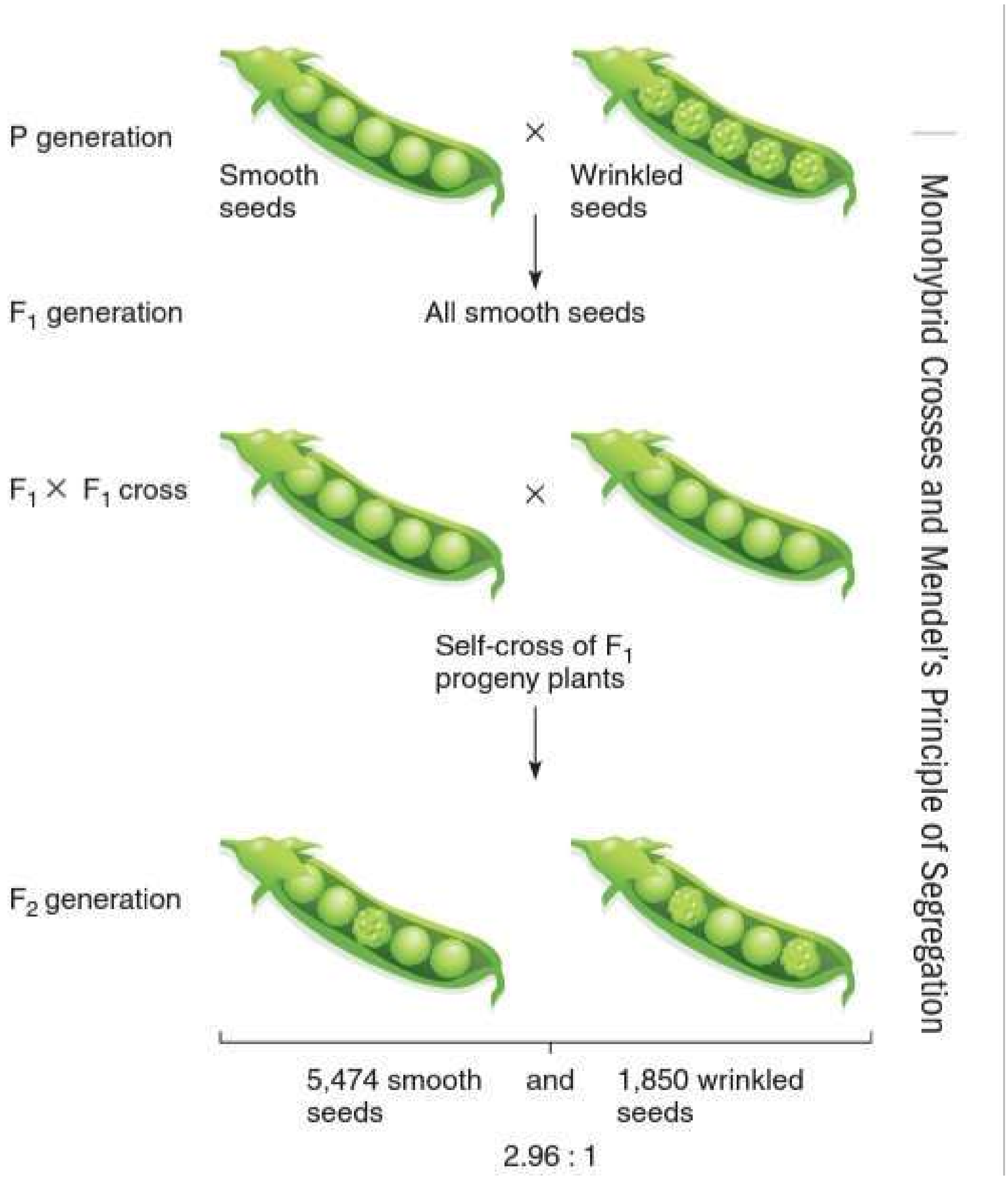


(Picture 3:- all the F1 progeny seeds were smooth .

Picture 3 Reference:-the picture is taken from the book iGenetics by Peter J. Russell ,3rd edition)

Next Mendel allowed the F1 plants to self fertilize to produce the F2 seeds. But here in F2 generation the both seeds type smooth and wrinkled appeared and both type could be found in the same pod(given in picture :-4).

Mendel found that 5,474 were smooth and 1,850 were wrinkled seeds. The ratio between smooth and wrinkled is very close to 3:1(the given data of seed number is taken from the book iGenetics by Peter J. Russell 3rd edition).



(Picture 4:– F2 generation of Mendel's monohybrid experiment.

Picture 4 Reference:-the picture is taken from the book iGenetics by Peter J. Russell , 3rd edition.

In F1 generation just one parental type of seed produced phenotypically but the other trait is not produced. But when the F1 seeds are pollinated the resulting F2 generation produced both parental types in their phenotype.

But how it is possible that one trait in P generation disappear and then reappear in F2?

- **The answer is:-**

Mendel concluded that the particular factors determined the alternative traits like smoothness or wrinkledness of the seeds. These factors were transmitted from one generation to another generation through gametes (we now know factors are known by gene). Each factor have two alternative forms (now call alleles) , both of them specified one of the traits . Here in this example one allele that results in a smooth seed and another allele that results in wrinkled seeds.

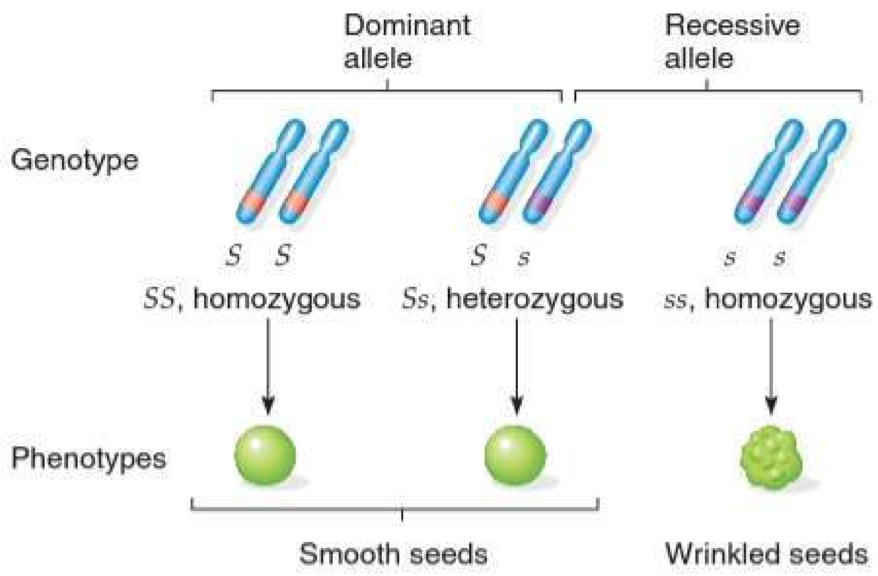
Mendel said that a true breeding strain of peas must contain a pair of identical factors. In modern terms this means ,peas are diploid that have two copies of each gene on a pair of homologous chromosomes.

In F1 generation both alternative forms are present because one strain come from male parent (pollen grains) and the other from female parent (eggs) . But only one of trait was seen in the F1 generation because the expression of the other trait was missing or masked by the visible trait ; this masking is called dominance.

Cross between smooth seed ×wrinkled seed , the seed are all smooth in F1 generation. Here the allele for smoothness is dominant over the wrinkled allele and the smooth seed trait is considered to the dominant trait and the allele associated with it is called dominant allele. The wrinkled is recessive to smooth . The wrinkled seed trait is called recessive trait and the allele is called recessive allele.

Use of symbols:-

In this example we use the symbol ' S ' to the allele for smoothness and the symbol ' s ' to the allele for wrinkledness. The capital letter is used for dominant allele and small letter for recessive allele . Using these symbols ,we denote the genotype of the particular organism . SS denote smooth seed and ss denote wrinkled seed . SS and ss both are homozygous for that gene (picture :-5). Ss is heterozygous (picture:-5). But Ss also denote smooth seed because here S gene was dominant.

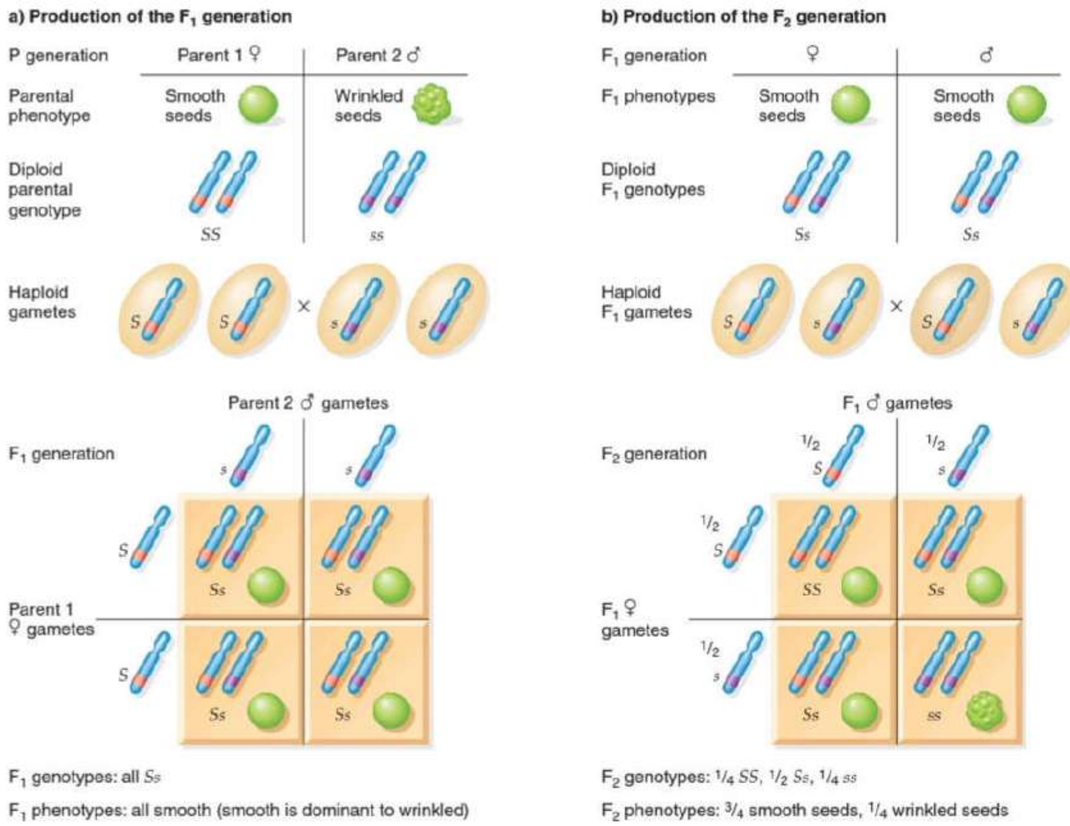


(Picture 5:- Dominant and recessive alleles of a gene for seed shape in peas.

Reference:- this picture is taken from iGenetics ,3rd edition, by Peter J. Russell.)

The F1 and F2 generation of cross between smooth seed and wrinkled seeds is given below. Here SS and Ss is smooth seeds and ss is wrinkled seed .

(Picture 6:- production of F1 and F2 generation in Mendel's monohybrid cross.



Picture 6 Reference:- the picture is taken from iGenetics by Peter J. Russell, 3rd edition.)

Mendel also analysed the behaviour of the six other pairs of traits. But the same results were obtained. The seven sets(picture :- 7)of crosses ,he made the following general conclusions about his data:-

1. The results of Reciprocal crosses were always the same .
2. All the F1 progeny resembled one of the parental strains , indicating the dominance of one allele over the other .
3. In the F2 generation, the parental trait that had disappeared in the F1 generation reappeared . Furthermore the trait seen in the F1 (the dominant trait) was always found in the F2 at

about three times the frequency of the other trait (the recessive trait).

(Reference of the given three conclusions given above :- iGenetics by Peter J . Russell, 3rd edition.)

Picture 7 is given below. From this box we know about all the seven characters of peas monohybrid crosses result .

Character[a]	F_1	Dominant	F_2 (Number) Recessive	Total	F_2 (Ratio) Dominant : Recessive
Seeds: smooth versus wrinkled	All smooth	5,474	1,850	7,324	2.96:1
Seeds: yellow versus green	All yellow	6,022	2,001	8,023	3.01:1
Seed coats: grey versus white[b]	All grey	705	224	929	3.15:1
Flowers: purple versus white	All purple				
Flowers: axial versus terminal	All axial	651	207	858	3.14:1
Pods: inflated versus pinched	All inflated	882	299	1,181	2.95:1
Pods: green versus yellow	All green	428	152	580	2.82:1
Stem: tall versus short	All tall	787	277	1,064	2.84:1
Total or average		14,949	5,010	19,959	2.98:1

[a]The dominant trait is always written first.
[b]A single gene controls both the seed coat and the flower color trait.

(Picture 7:– Mendel's results in crosses between plants differing in one of seven characters.

Reference of picture 7:- iGenetics by Peter J. Russell, 3rd edition.)

THE PRINCIPLE OF SEGREGATION:–

Mendel proposed his first law , the principle of segregation:-

Recessive traits ,which are masked in the F1 from a cross between two breeding strains, reappear in a specific proportion in the F2. In modern terms this means that the two members of a gene pairs segregate from each other during the formation of gametes in meiosis(**the law of segregation is taken from iGenetics by Peter J. Russell, 3rd edition**).

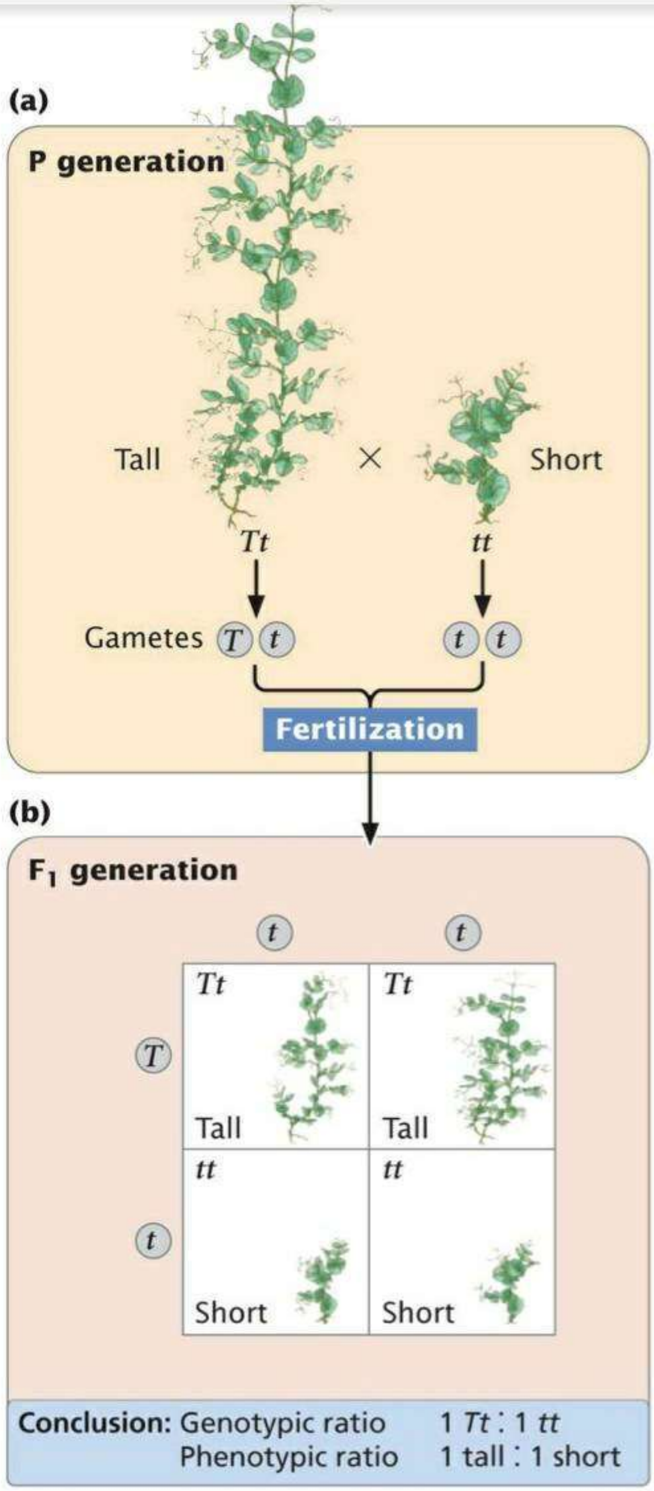
In between the half gametes are carry one allele and the other half carry the other allele. From the Mendel's first law we know that the members of a pair of alleles segregate during meiosis. Every offspring have one allele from each parent. In meiosis anaphase 1 the homologous pairs of chromosome separate from each other.

PREDICTING THE OUTCOMES OF GENETICS CROSSES:–

For predict the outcome of crosses between plants with different phenotypes :-first we create Punnett square and then use Probability rules to predict the results of crosses.

The Punnett square:–

- The Punnett square was developed by English Geneticist Reginald C. Punnett in 1917.
- A Punnett square is a square diagram that is used to predict the genotypes of a particular cross or breeding experiment (the definition is taken from:– https://en.m.wikipedia.org/wiki/Punnett_square)
- Each cell in Punnett square contains an allele from each of corresponding gametes . Fusion of those gametes produced the genotype of the progeny .Diagram of Punnett square is given below in picture 8.



(Picture 8:- the Punnett square can be used to determine the results of a genetic cross.

Reference of picture 8 :- Genetics A Conceptual Approach, international sixth edition by Benjamin A. Pierce).

PROBABLITY AS A TOOL IN GENETICS:-

Probability expresses the likelihood of the occurrence of a particular event(definition is taken form Genetics A Conceptual Approach, international sixth edition by Benjamin A. Pierce).

Two rules of Probability are useful for predicting the ratios of offspring produced in genetic crosses.

1. The Multiplication Rule:–
   Multiplication rule, that the probability of two or more independent events occurring together is calculated by multiplying their independent probability.
2. The Addition Rule:-
   The second rule of probability was Addition rule which , states that the probability of any one of two or more mutually exclusive events is calculating by adding the probabilities of these events.
   (Definition of Multiplication rule and Addition rule is taken from Genetics A Conceptual Approach, international sixth edition , by Benjamin A . Pierce.)

Use of Multiplication Rule and Addition Rule :-

Example:- The probability that a child will be a boy is ½ or 0.5 , similarly , the probability that the child will be a girl is ½ .

- The probability that both children in a family with two children will be girls is ¼. By the Multiplication Rule ,the probability of the first and second being girl is= ½×½=¼.
- By Addition Rule the probability of having two boy or two girls is =¼+¼ =½.

Genetic Symbols:–

1. **Capital letters are used for wild type allele and small letters are used to designate recessive allele.**
   Example - **Tall height (T)**
   Short height (t)
2. **Two or three letters may be used for a single allele.**
   Example- **The recessive allele for heart shaped leaves in cucumbers is designated as hl .**
3. **Sometimes superscripts and sub scripts are used to distinguish between genes –Lfr1 and Lfr2 represent dominant mutant alleles at different loci that produce lacerate leaf margins in *Opium poppies*.**

The use of Test cross:–

A cross between an individual expressing dominant phenotype with a homozygous recessive individual is called Test cross(Picture :-9).

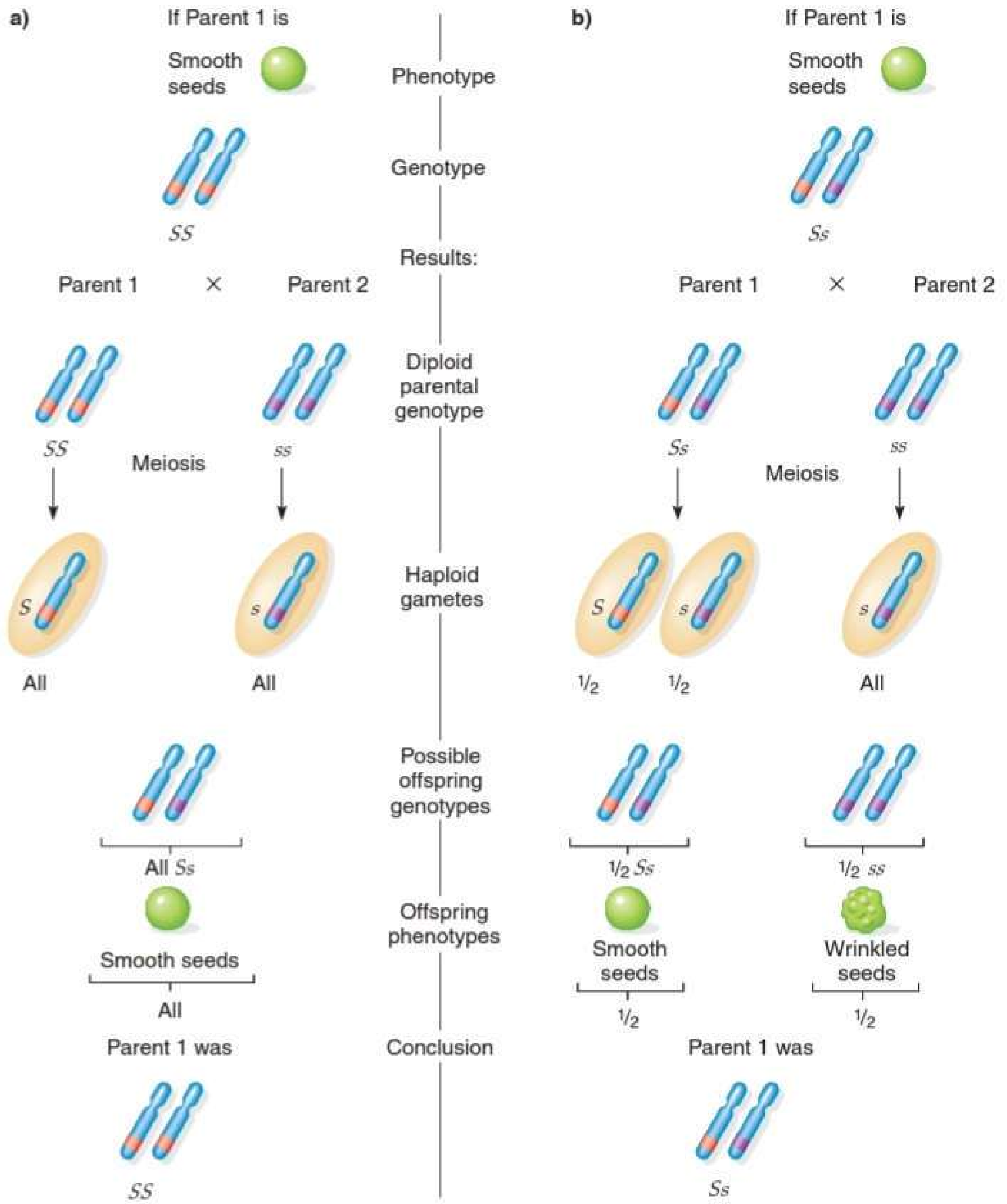
The test cross was very useful to determine whether a plant with the dominant phenotype was homozygous or heterozygous .

Conclusion:-

If a plant with a dominant trait is test crossed and all the progeny are dominant phenotype, then we said that , the plant must have been homozygous for dominant allele and if the progeny had a 1:1 ratio of dominant: recessive phenotypes then we said , the plant must have been heterozygous.

Picture 9 is given below. Picture 9 shows the test cross experiment result.

Picture (9)



a)
If Parent 1 is
Smooth seeds
Phenotype
Genotype
SS
Results:
Parent 1
×
Parent 2
Diploid parental genotype
SS
Meiosis
ss
S
s
Haploid gametes
All
All
Possible offspring genotypes
All Ss
Offspring phenotypes
Smooth seeds
All
Parent 1 was
Conclusion
SS
b)
If Parent 1 is
Smooth seeds
Ss
Parent 1
×
Parent 2
Ss
ss
Meiosis
S
s
s
1/2
1/2
All
1/2 Ss
1/2 ss
Smooth seeds
Wrinkled seeds
1/2
1/2
Parent 1 was
Ss

(Picture 9:- determining the genotypes of the F2 generation smooth seeds (parent1) by testcrossing plants grown from the seed with a homozygous recessive wrinkled strain.

Reference of picture 9:- iGenetics by Peter J. Russell, 3rd edition .)

Loss of function Mutations:–

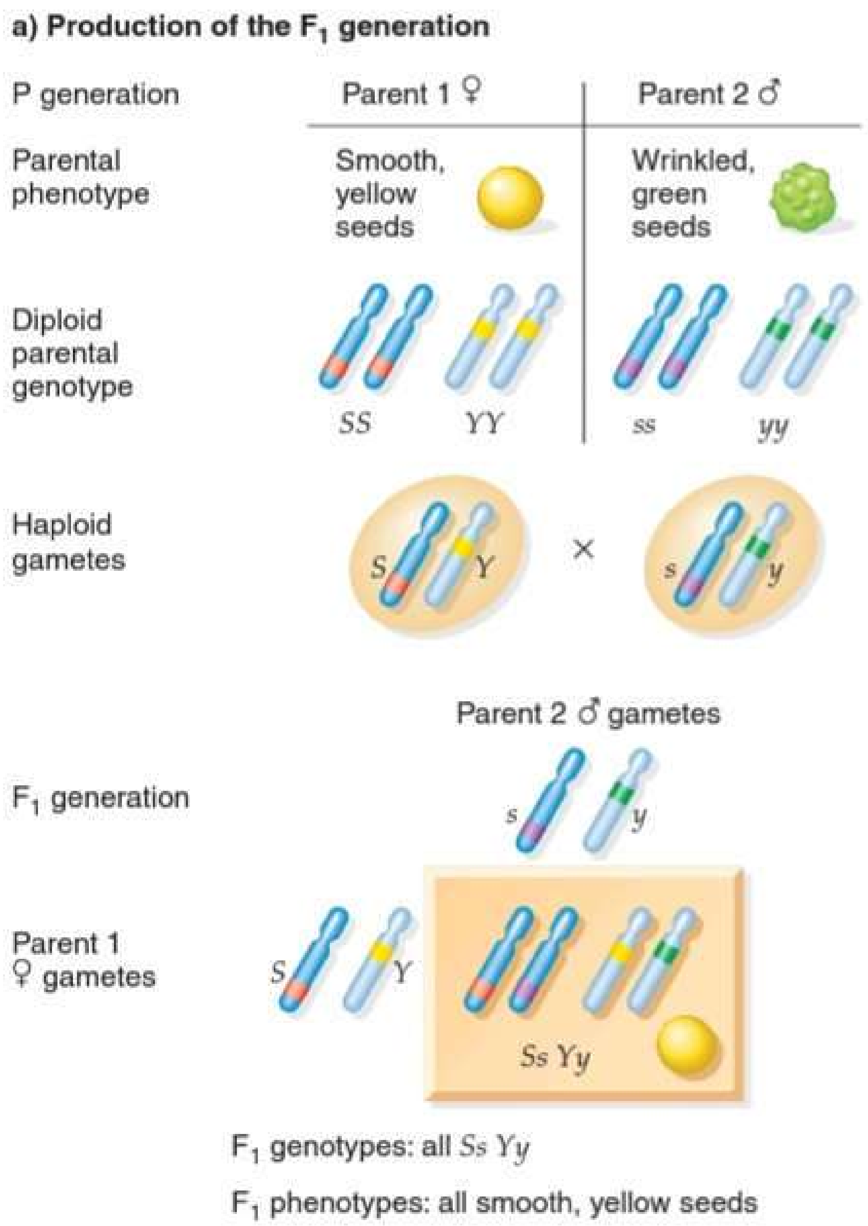
Wild type alleles encode a product for a particular biological function . If a mutation in the gene causes the protein product of the gene to be absent , partially functional or non functional and then the associated biological function is lost or decreased significantly . Such mutations are called Loss of Mutations. The recessive alleles are non functional because they don't produce functional product . In heterozygous individual the one wild type allele is sufficient to produce enough protein to allow the normal phenotype.

A mutation that results in no protein or a protein with no function is known as a null mutation.

DIHYBRID CROSS AND MENDEL'S PRINCIPLE OF INDEPENDENT ASSORTMENT:–

Mendel crossed varieties of peas that differed in two characteristics is known as a dihybrid cross.

Mendel analyzed a number of dihybrid crosses but he obtained the same result. From these experiments, he proposed his second law ,the Principle Of Independent Assortment , which states that the factors for different pairs of traits assort independently of one another. In modern terms, this means that pairs of alleles for genes on different chromosomes segregate independently in the formation of gametes. (The law is taken from iGenetics by Peter J Russell ,3rd edition.)



Figure(10 a):–F1 generation in Mendelian dihybrid cross.

(Reference :–this picture is taken from iGenetics by Peter J Russell, 3rd edition.)

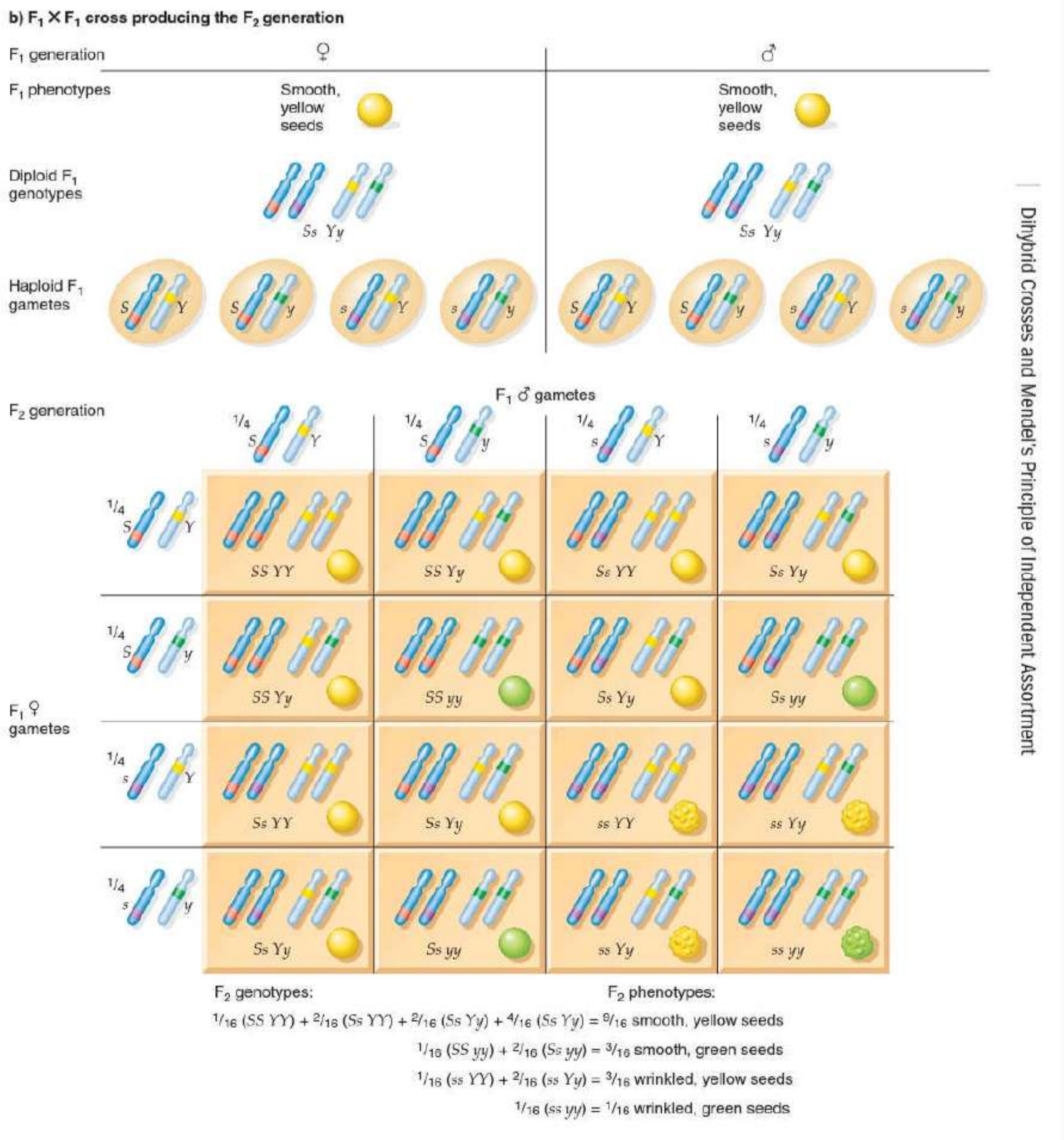


Figure (10 b):– **F2 generation in Mendelian dihybrid cross.**

(Reference:–the picture is taken from iGenetics by Peter J Russell , 3rd edition.)

Example:–The pair of traits for seed shape:– smooth (S) and Wrinkled (s) and The pair of traits for seed colour :– Yellow (Y) and Green (y).

Mendel made crosses between true breeding smooth, yellow plants(SSYY) and wrinkled, green plants (ssyy). He got the result shown on figure (10 a and 10 b).

All the F1 seed from this cross were smooth and yellow. The F1 seeds are heterozygous (SsYy) for both pair of alleles at two different loci . This type of individuals are called dihybrids and a cross between two of these dihybrids of the same type is called dihybrid cross.

Mendel self-pollinated the dihybrid F1 plants which produce the F2 generation. In F2 generation have two possible outcomes (figure 10b):-

i)The alleles for seed shape and seed colour in the original parents would be transmitted together to the progeny. Here the phenotypic ratio of smooth , yellow: wrinkled, green was 3:1. Here the gametes are two (SY and sy).

ii)The other possibility was, the inheritance pattern of the alleles are independent of one another. One allele of 1st gene inherited with another anyone allele of the 2nd gene . In this case the phenotypic ratio was:–

9 smooth and yellow

3 smooth and green

3 wrinkled and yellow

1 wrinkled and green

Here the gametes are SY, Sy, sY and sy .

Here the phenotypic ratio =9:3:3:1.

And the genotypic ratio=1:2:2:4:1:2:1 :2:1.

In Mendel's experiment the phenotypic ratio was 9:3:3:1. So the ratio was result of independent assortment of the two pairs of alleles for two genes into the gametes . This happens because the random fusion of the gametes occur.

In every dihybrid cross Mendel was close to 9:3:3:1 ratio. In case of smooth, yellow and wrinkled, green cross he counted 315 smooth, yellow ; 108 smooth, green; 101 wrinkled, yellow and 32 wrinkled, green seeds. It is very close to the predicted ratio 9:3:3:1.(the calculations and numbers are taken from iGenetics by Peter J Russell, 3rd edition.)

Branch diagram of dihybrid crosses:–

Branch diagram is a convenient way to organizing all the combinations of the characteristics (this definition is taken from Genetics A Conceptual Approach by Benjamin A Pierce , international sixth edition).

A branch diagram can be used to calculate the expected ratios of phenotypic or genotypic classes. Monohybrid and dihybrid in both cases branch diagram is used.

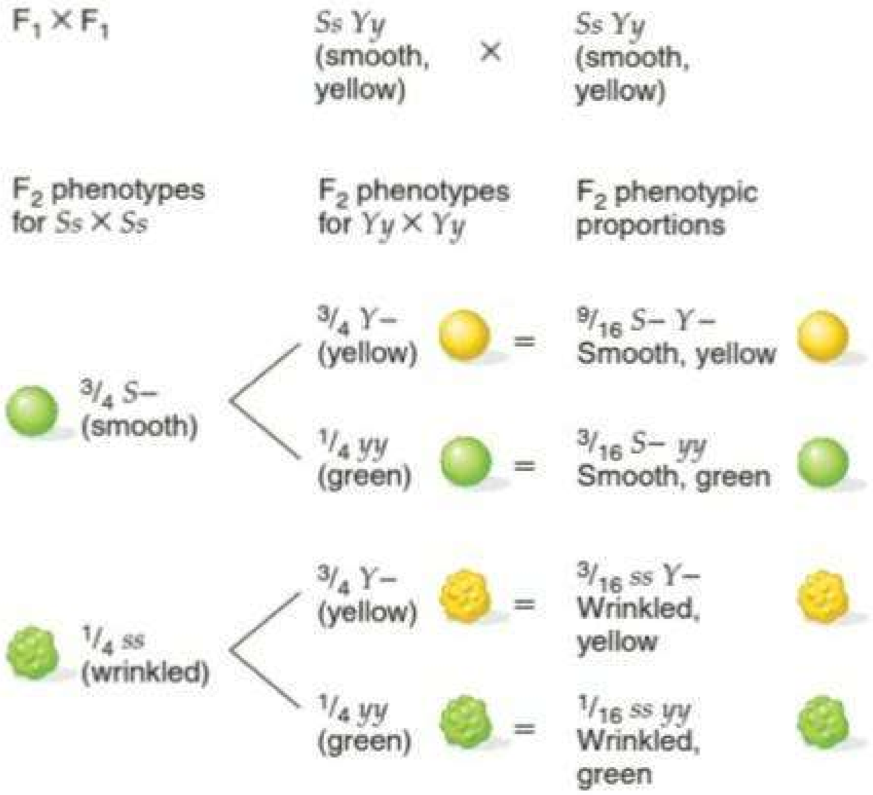


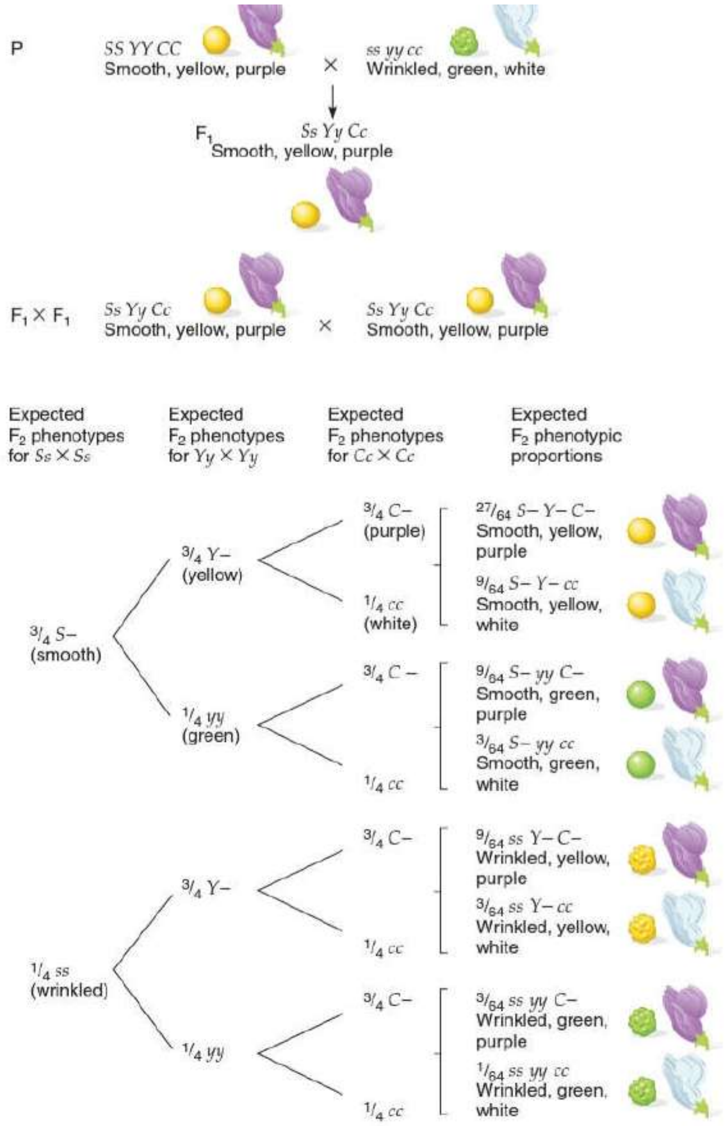
Figure 11:–**Branch diagram of dihybrid cross in F2 generation.**

(The picture is taken from iGenetics by Peter J Russell, 3rd edition.

In this figure the S– means that ,phenotypically seeds are smooth and Geno typically SS or Ss and Y– means that phenotypically the seeds are yellow and Geno typically YY or Yy.

Trihybrid Crosses:–

Mendel also confirmed his laws by Trihybrid crosses. Here the F2 generation phenotypic and genotypic ratio are predicted by the same way used in case of Dihybrid . Here the phenotypic ratio is 27:9:9:9:3:3:3:1.



Branch diagram derivation of the relative frequencies of the eight phenotypic classes in the F_2 of a trihybrid cross.

Figure 12:–branch diagram of Trihybrid of cross.

(The picture is taken from iGenetics by Peter J Russell, 3rd edition.)

The figure shows the branch diagram deviation of the relative frequencies of the eight phenotypic classes in the F2 of a Trihybrid cross.

Modified Mendelian ratios :-

One gene never acts by itself in controlling of an individual phenotype. For controlling phenotype of an individual need highly complex and integrated patterns of molecular reactions that all are under gene control.

When a single phenotype is controlled by interaction between alleles of two or more genes , the phenomenon called Epistasis. In Epistasis one gene masking the phenotypic expression of another gene.

A gene that masks the expression of another gene is called epistatic and a gene whose expression is masked called hypostatic.

Epistasis ratios are the cause of variation in Mendelian ratio.

Different Epistasis ratios are:–

1. **Dominant epistasis ratio :– 12:3:1**
2. **Recessive epistasis ratio:– 9:3:4**
3. **Duplicate genes with cumulative effect:– 9:6:1**
4. **Duplicate recessive epistasis (or complimentary epistasis):– 9:7**
5. **Duplicate dominant epistasis:– 15:1**
6. **Dominant and recessive epistasis:–13:3**

Table (1) shown the different types of Mendelian and epistasis phenotypic ratios. Table 1 is given below—

No of classes	Types	Ratios
A. Mendelian Ratios	1. **monohybrid ratio in F2 generation.**	**3:1**
	2. **Monohybrid ratio in test cross.**	**1:1**
	3. **Dihybrid F2 generation ratio.**	**9:3:3:1**
	4. **Dihybrid test cross ratio.**	**1:1:1:1**
B. Epistasis ratio–	1. **Dominant epistasis ratio**	**12:3:1**
	2. **Recessive epistasis ratio**	**9:3:4**
7	3. **Duplicate genes with cumulative effects.**	**9:6:1**
	4. **Duplicate recessive** 5. **epistasis.**	**9:7**
	6. **Duplicate dominant epistasis.**	**15:1**
	7. **Dominant and recessive epistasis.**	**13:3**

Chi-square Test and its significance in Genetics:–

If we have observation data (o) of sample characters then we try to make conclusions :– the observed and the expected data are similar or not. If the observed and expected data are same then we say that the experiment is statistically correct.

For this hypothesis testing, we need Chi-square test.

Chi-square test is used as a test of significance when data is expressed in frequencies or in terms of percentages (the definition is taken from Biostatistics by Veer Bala Rastogi , 3rd revised edition.).

The symbol of Chi-square is χ2 . The Chi-square test is also called Pearson's Chi-square test.

Chi-square is a non parametric test . It is apply only in case of qualitative data .

Test of Significance:–

It is a procedure for comparing data ,observed data with hypothetical data or theoretical data.

When two or more hypothesis or assumptions are based on a population ,it becomes necessary to test which of the assumptions are right or to what extent? This testing is called test of significance. Test of significance is two types . They are –

1. **Null hypothesis(h0):–**

   Here the dataset does not follow the hypothetical or expected data.

2. **Alternative hypothesis (h1):–**

   It is just the opposite of null hypothesis.

Chi-square test is calculated **by the following formula:–**

$X^2 = \sum(O-E)^2/E$

Here , x^2=Chi-square

O= observed value

E=expected value

Different examples of Chi-square testing is given below:–

Sample 1:–

No of classes	Sample character	Observation no(o)
1.	Small seed	46
2.	Large seed	18

Here ,total no of seeds = 46+18=64.

Null hypothesis:–

The given dataset doesn't follow the Mendelian monohybrid test cross ratio 3:1.

Alternative hypothesis:–

The given dataset follows the Mendelian monohybrid ratio 3:1.

Table 2 shows the calculation of sample 1. Table 2 is given below –

No of classes	Sample character	Observed no (O)	Expected no(E)	O–E	$(O-E)^2$	$(O-E)^2/E$
1.	Small seeds	46	48	−2	4	4/16=0.25
2.	Large seeds	18	16	2	4	4/48=0.083

Here the degree of freedom (df)=n–1=2–1=1.

So the Chi-square value :–

$X^2=\sum(O-E)^2/E$

Or, $X^2=0.25+0.083$

Or, $X^2=0.333$

The Chi-square value is smaller than the probability table value at $p \leq 0.05$ at 1 degree of freedom. Hence the variation is insignificant and the null hypothesis does not stands true .

Hence the Alternative hypothesis is true and follows the Mendelian monohybrid ratio 3:1.

Sample no 2:–

No of classes	Sample character	Observation no(O)
1.	Small seed	46
2.	Large seed	34
	Total seed	80

Null hypothesis:- **the given dataset does not follow the duplicate recessive epistasis 9:7.**
Alternative hypothesis:–
The given dataset follows the duplicate recessive epistasis 9:7.

The table 3 shows the chi-square calculation of sample2. The table is given below –

No of classes	Sample character	Observed no	Expected no	(O–E)	(O-E)^2	(O-E)^2/E
1.	**Small seed**	**46**	**45**	**1**	**1**	**1/45**
2.	**Large seed**	**34**	**35**	**–1**	**1**	**1/35**

Here the degree of freedom is (df)= 2–1=1.
So the Chi-square value –
X^2=1/45+1/35
Or, X^2=0.051

The Chi-square value is smaller than the probability table value at $p \leq 0.05$ at 1 degree of freedom. Hence the variation is insignificant and null hypothesis does not stands true.

Hence the Alternative hypothesis is true and follows the duplicate recessive epistasis ratio 9:7.

Sample 3:–

No of classes	Sample character	Observation no(O)
1.	**Long brown seed**	**35**
2.	**Long orange seed**	**9**
3.	**Short brown seed**	**14**
4.	**Short orange seed**	**6**
	Total seed	**64**

Null hypothesis:– The given dataset does not follow the Mendelian dihybrid ratio 9:3:3:1.

Alternative hypothesis:– **The given dataset follows the Mendelian dihybrid ratio 9:3:3:1.**

Table 4 shows the calculations of sample 3. The table is given below –

No of classes	Sample character	Observed no (O)	Expected no(E)	Deviation (O-E)	$(\text{Deviation})^2$ $(O\text{-}E)^2$	$(O\text{-}E)^2/E$
1.	**Long brown seed**	**35**	**36**	**–1**	**1**	**1/36=0.02**
2.	**Long orange seed**	**9**	**12**	**–3**	**9**	**9/12=0.75**
3.	**Short brown seed**	**14**	**12**	**2**	**4**	**4/12=0.33**
4.	**Short orange seed**	**6**	**4**	**2**	**4**	**4/4=1**

Here the degree of freedom (df) is =4–1=3.
So, Chi-square value is –
$X^2=\Sigma (O-E)^2/E$
Or, $X^2=0.02+0.75+0.33+1$
Or , $X^2=2.1$

The Chi-square value is smaller than the probability table value at $p \leq 0.05$ at 3 degree of freedom. Hence the variation is insignificant and the null hypothesis is does not stands true.

Hence the Alternative hypothesis is true and follows the Mendelian dihybrid ratio 9:3:3:1.

CONCLUSION:–

In modern days statistics is used in all field of science subjects. Without statistics it is impossible do research.

Genetics and biostatistics these two subjects are very closely related with each other.

Mendel was given two laws on the basis of his experiments with peas . The laws are:– the principle of Segregation and the principle of Independent Assortment . For predict the outcome of crosses between plants with different phenotypes :– first we create Punnett square and the use of probability rules to predict the result of crosses. In case of genetics two probability rules are generally used , additional rule and Multiplication rule. Genetic symbols are used to define different types of genes and their alleles. In Mendel's monohybrid cross F2 generation phenotypic ratio is 3:1 and dihybrid cross F2 generation phenotypic ratio is 9:3:3:1 and Trihybrid phenotypic ratio is 27:9:9:9:3:3:3:1.

But in nature organisms are not maintain Mendel's ratio all time because one gene never acts by itself in controlling of an individual phenotype. For controlling phenotype of an individual need highly complex and integrated patterns of molecular reactions that all are under gene control. When a single phenotype is controlled by interaction between alleles of two or more genes, the phenomenon called epistasis. There are several types of epistasis ratio present .

Chi-square test is very important for calculating the relation between expected and observed data in genetics. Chi-square test is used as a test of significance when data is expressed in frequencies or in terms of percentages. By using Chi-square test formula we compare between observed data with theoretical data . Without the help of biostatistics the calculation of genetics is impossible.

REFERENCES:–

1. iGenetics A Molecular Approach by Peter J Russell 3rd edition.
2. Genetics A Conceptual Approach by Benjamin A. Pierce , sixth edition.
3. Concepts of Genetics by Klug, Cummins, Spencer and Palladino ; eleventh edition.
4. Genetics by P.S.Verma and V.K. Agarwal (S. Chand and Company LTD.).
5. Biostatistics by Veer Bala Rastogi, 3rd revised edition.
6. https://images.app.goo.gl/dv9G3FLFJhgZJgje8
7. https://en.m.wikipedia.org/wiki/Punnett_square
8. https://www.sciencedirect.com/

RAMAKRISHNA MISSION VIVEKANANDA CENTENARY COLLEGE

TOPIC : **"PHENOLOGY, CHROMOSOME NUMBER AND POLLEN PHYSIOLOGY OF** *Cleome viscosa*"

SUBMITTED BY : *Suman Paul*

REG NO. : *A01 - 1152 - 114 - 016 - 2019 OF 2019-20*

COLLEGE ROLL NO. : *417*

SEMESTER : V

DEPARTMENT : BOTANY

CONTENTS

❖ *PHENOLOGY AND FLOWERING SEASONS*

It is an erect branching annual weedy herb, which grows up to 1 meter in full sunlight, in swamp soils at roadsides, in waste places, and in open places. The plant appears during early monsoon. Abundant vegetative growth occurs by July and flowering occurs during August-November based on the extent of rainfall. The plant forms pure stands in certain areas and grows as scattered individuals along with other plants, such as *Cleome gynandra* (Capparaceae), *Boerhaavia diffusa* (Nyctaginaceae), *Euphorbia hirta* , *Phyllanthus niruri* , *Sida cordata* (Malvaceae) (Raju and Rani , 2016)

The floral characters are similar for all three flower morphs, unless specified. The mature buds begin to open at 02:30 h by showing slits between petals and after half an hour, the stamens protrude through the slits. After a short while, anthesis is complete and the petals reflex, exposing the stamens and stigma. The anthesis occurs during 03:00–04:00 h and anther dehiscence occurs by longitudinal slits simultaneously. The stigma is receptive to pollen two hours after anthesis and ceases its receptivity by noontime of the same day. During receptive period, it is shiny and glittery against sunlight. The pollen output per flower in all three flower morphs slightly varied at initial, peak and final phase of flowering. Among these morphs, the SGF morphs produce the highest number of pollen grains followed by MGF and LGF morphs, and the same trend is evidenced throughout the flowering season. (Raju and Rani, 2016)

Cleome viscosa requires a mean annual temperature of 14 ºC (lower limit) and 28 ºC (upper limit). Mean annual Rainfall 300 mm (lower limit) and 2000 mm (upper limit). Soil reaction – acidic – neutral; Soil texture -- light – medium. (Raju and Rani, 2016)

❖ *POLLEN MORPHOLOGY AND PHYSIOLOGY*

Pollen grains, being the male reproductive unit and the carrier of male genetic material in the higher plants play a vital role in fertilization and assist successful post fertilization events. High yield generally depends on viable pollen grains. Pollen fertility and viability have a supreme importance in plant reproduction. So pollen fertility, viability, is basic aspect for the improvement of plant before going to successful breeding programme. Pollen grains are simple structure of plant cells and pollen tube formation is a good and appropriate model of growth and development (Taylor and Helper, 1997). Thus pollen germination and pollen tube growth are important research material for morphological, physiological, biotechnological, ecological, environmental, evolutional, biochemical and molecular biological studies. (Ottavio et al., 1992). Pollen tube elongation is a lively process in which pollen tubes navigates and respond to female tissues to accomplish their mission of delivering the sperm cells for fertilization. Pollen tube extents exclusively at the cell apex via an extreme form of polar growth, known as tip growth, producing uniformly set cylindrical cells (Cheung, 2001). Pollen tubes are excellent system for the study of polarized tip growth, cell movement, cell to cell communication, cell to cell recognition and signalling in plants. In recent years, pollen germination and pollen tube development are used as materials for determining the importance of cytoskeleton in cell growth and differentiation (Ma et al., 2000). Pollens normally germinate on stigma (Unal, 1986, 1988) and the required environment for in vitro pollen germination is related to genetic composition and also the quality of nutrient reserves of pollen (Baker and Baker, 1979; Unal, 1986, 1988). During the past few years pollen tube growth in vitro becomes a popular model system for cell biology studies in plant cell (Moutinho et al., 2001). The present investigation is aimed to study the effect of sucrose and boric acid at various concentrations separately and in combinations and salts of Calcium, Magnesium and Potassium on in vitro pollen germination of *Cleome viscosa* Linn., a medicinally (Khare, 2007) important plant belonging to the family Capparidaceae, popularly known as wild mustard. (Dey and Mondal et.al)

The pollen grains are identical and possess similar characters in all three flower morphs. They are monads, triangular, yellow, slightly sticky, prolate, tricolpate, lobate, with veiculate ornamentation and tectum reticulated. The pollen grain size varied with each flower morph, it is 19.80 ± 4.20µm in SGF, 27.45 ± 5.23µm in MGF and 23.62 ± 5.71µm in LGF. The pollen-ovule ratio varies depending on the number of ovules produced and the number of pollen grains produced per flower. The ratio is the highest in SGF and the lowest in LGF morphs; almost the same trend exists in the blooming season. (Raju and Rani, 2016)

In all three morphs, the pollen contains six essential amino acids and nine non-essential amino acids. The essential amino acids are threonine, methionine, lysine, histidine, arginine, and tryptophan. The nonessential amino acids include alanine, amino-butyric acid, aspartic acid, cysteine, cystine, glycine, hydroxyproline, proline, and serine. The total protein content per 1 mg of pollen is 90.45 µg in all three flower morphs. All three flower morphs are nectariferous and the nectar is secreted in traces or minutely by the nectar glands situated at the flower base around the ovary. The flowers present nectar by the time the flower opens and is covered by the hairs around the ovary. The petals fold back by noon on the day of anthesis, enclosing the stamens and stigma. The closure of petals facilitates the contact between the stamens and stigma and effects autogamy. The flowers remain in that state, wither away the next day and drop off on the 3rd day. (Raju and Rani, 2016)

Pollen count depends upon flowers of different sizes and sexes. Highest production was found in large hermaphrodite (100857.9±4233.5) followed by large staminate (86018.6±4356.5) flowers and their respective small (37984.3±4566.7 and 36971.3±4502.5) counterparts. One way ANOVA on the log transformed pollen counts revealed the significant differences between large and small counterparts of hermaphrodite ($F(1,28)= 45.75$; $P <0.05$) and staminate ($F(1,18)= 35.33$; $P <0.05$) flowers . In pistillate flowers, pollen production was the least (3799.11±752.129) and non-viable.

The transection of a young anther lobe consists of a group of microspore mother cells surrounded by inner tapetum, one middle layer, endothecium and outer epidermis. Cells of the tapetum are uninucleate at first but later become binucleate and are of the glandular type. The epidermis finally becomes stretched and wavy; endothecium exhibits fibrous thickenings; middle layer and tapetum become disorganized. The tetrads of microspores formed by the meiotic divisions of microspore mother cells are usually tetrahedral, but occasionally intersect and isobilateral in arrangement. The triporate pollen-grains have a thick smooth exine all over except at the pores where only a thin intine is seen. Most of the mature pollen-grains remain uninucleate and two-nulceate grains also occur, though very rare. (Saroop and Kaul, 2014)

❖ CHROMOSOME NUMBER

Cleome viscosa is an erect yellow-flowered herb. Ten bivalents were observed at diakinesis and metaphase I stages. Meiosis was normal, and there was 90% pollen fertility. The same haploid count was reported in the species previously by Janaki Ammal (1933) and

Balamani et al. (1981); but Raman and Kesavan (1963) have reported n = 24. (Koshy et. al, 1983).

❖ CONCLUSION

In summary, in the present study the potential of *Cleome viscosa*, an annual herbaceous weed plant, was evaluated for it to be developed into a short duration North East India over four summer-monsoon and monsoon-autumn seasons identified an accession which produced high yield of seeds from its crops of 13 to 15 weeks in the monsoon-autumn season. Collectively the accessions demonstrated considerable genetic variability in agronomic characters thus indicating possibilities of selection breeding in C. viscosa for developing superior cultivars.

❖ REFERENCES

1. Plant Systematics : An Integrated Approach , Fourth Edition by Gurcharan Singh
2. Aizen, M.A. & Feinsinger, P. 1994. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. – Ecology, 75: 330-351.
3. Bhandari M M, Flora of the Indian Dessert, 1st ed. (Scientific, Jodhpur) 1978, 42.
4. Aparadh, V.T. & Karadge, B.A. 2011. Microscopic pollen analysis of some selected Cleome species. – Trajectory, 19: 21-31.
5. Oommachan M, The Flora of Bhopal. 1st ed., J.K. Jain Brothers, Bhopal, 1977, 46.
6. Nadkarni A K, The Indian Materia Medica, Vol. 1 (Popular Prakashan, Bombay), 1982, 351.
7. Aparadh, V.T., Mahamuni, R.J. & Karadge, B.A. 2012. Taxonomy and physiological studies in spider flower (Cleome species): a critical review. – Pl. Sci. Feed, 2: 25-46.
8. Asolkar, L.V., Kakkar, K.K. & Thakre, O.J. 1992. Second supplement to Glossary of Indian Medicinal Plants with Active Principles. PID, CSIR, New Delhi, p. 215.
9. Duthie JF, Flora of the Upper Gangetic Plain and of the Adjacent Siwalik and Sub-Himalayan Tracts, 1st ed. Vol. 1 (Part I-II) (M/s. Bishen Singh Mahendra Pal Singh Dehra Dun), 1973, 50.
10. Maikhuri RK, Semwal RI, Rao KS, Nautiyal S and Saxena KG, Cleome viscosa, Capparidaceae: A weed or a cash crop?, Econ Bot, 2002, 54, 150.

11. Aziz, S. & Shaukat, S.S. 2012. Population ecology of Cleome viscosa L., a desert summer annual. – Pakistan J. Bot,. 44: 1633-1638.
12. A.J. Solomon Raju & D. Sandhya Rani , Sofia, 2016. Reproductive ecology of Cleome gynandra and Cleome viscosa (Capparaceae) : Phytologia Balcanica 22 (1): 15 – 28.
13. Anburaj J, Ravinder SC, Kuberan T, Sundaravadivelan C and Kumar P, Effects of plant growth regulators on callus induction from leaf explants of Cleome viscose, Res J Pharmac, Biol Chem Sci, 2010, 2, 576.
14. Maheswari J K, The Flora of Delhi, 1st ed, Council of Scientific and Industrial Research, New Delhi, 1976, 63.
15. Rukmini C, Chemical, nutritional and toxicological evaluation of the seed oil of Cleome viscosa, Indian J Med Res, 1978, 67, 604.
16. Sudhakar M, Rao CV, Rao PM and Raju DB, Evaluation of antimicrobial activity of Cleome viscosa and Gamelina asiatica, Fitoterapia, 2006, 77, 47.
17. Saroop, S. and Kaul, V. (2011). Phenological events of Cleome viscosa L. growing in Jammu district. The International Journal of Plant Reproductive Biology 3(2): 161-164.
18. Singh, G., Ram, I.C. and Singh, D. (1991). Crop-weed competition studies in green gram and black gram. Tropical Pest Management, 37: 144-148.
19. Williams, L.A.D., Vasques, E., Reid, W., Porter, R. and Kraus, W. (2003). Biological activities of an extract from Cleome viscosa L. (Capparaceae). Naturwissenschaften, 90: 468-472.
20. Devi, B.P., Boominathan, R., Mandal, S.C. (2002). Evaluation of antidiarrhoeal activity of Cleome viscosa Linn. extracts in rats. Phytomedicine, 9(8): 739-742.
21. Reddy, C.N., Reddy, M.D., Devi, M.P. (2000). Efficency of various herbicides on weed control and yield of brinjal. Indian Journal of Weed Science, 32 (3 and 4).
22. Reddy, M.M., Vilatha, A.M. and Rao, L.J. (2007). Integrated weed management in pigeon pea (Cajanus cajan) and soybean (Glycine max) intercropping system on vertisol under rainfed conditions. Indian Journal of Agricultural Sciences 77(3): 177-178.
23. Saroop, S. (2011). A preliminary study on seed to seed cycle of Cleome viscosa L. M.Phil. Dissertation, University of Jammu, Jammu
24. Raju AJS, Ramana KV, Rao NG & Varalakshmi P, Monoecy and entomophily in Cardiospermumcanescens Wall. (Sapindaceae), a medicinally valuable herbaceous vine. Curr Sci, 101 (2011) 617.
25. Kaul V & Koul AK, Floral phenology in relation to pollination and reproductive output in Commelina caroliniana (Commelinaceae). Aust J Bot, 56 (2008) 59.

26. Narbona E, Ortiz PL & Arista M, Functional andromonoecy in Euphorbia (Euphorbiaceae). Ann Bot, 89 (2002) 571.
27. Strauss SY &Whittall JB, Non-pollinator agents of selection on floral traits. In: Ecology and Evolution of flowers (eds.Lawrence D Harder & Spencer CH Barrett, Oxford University Press, Oxford), 2006, 120.
28. Solomon BP, Environmentally influenced changes in sex expression in an andromonoecious plant. Ecology, 66 (1985) 1321.
29. Chauhan S, Rana A, Sharma SB & Chauhan SVS, Reproductive biology of Cleome viscosa L. (Capparaceae). Ann For, 15 (2007) 343.
30. Goodwillie C, Kalisz S & Eckert CG, The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. Annu Rev Ecol Syst, 36 (2005) 47.

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MICROBIAL BIOFERTILIZER

CONTENTS

INTRODUCTION

According to the Food and Organization (FAO) of the united state nations , the populations of the world is expected to increase to more than nine billion by 2050, a third more people to feed than today. It is therefore, necessary to dramatically increase agriculture production by managing the rhizosphere in a relatively short period to ensure food security . Some factors are necessary to meet this goal , including the right environmental conditions and availability of fertile soil conditions that are becoming rarer with time . From the middle of the 20^{th} century until date, chemical fertilizers have helped in feeding the world's populations. This has been done through the provision of the required nutrients , such as Phosphorus (P), Nitrogen (N) and potassium (K) to plants . About 53 billion tones of NPK fertilizers are used yearly to supplement the number of nutrients needed for plant growth and yield performance. Unfortunately only a small percentage of these nutrients are used by plants, while a greater percentages is precipitated by metal cations present in the soil. Moreover, the extensive and inappropriate use of chemical fertilizers results in environmental issues that are a major concern to farmers, furthering the arguments for the introduction of agriculture practice that do not harm the environment. Scientists everyday have begun to direct their interests towards ensuring agrarian sustainability using beneficial soil microorganisms instead of chemical fertilizers and pesticides.

TYPES OF MICROBIAL BIOFERTILIZER

Biofertilizers are grouped into different types on the basis of their functions and mode of action. The commonly used biofertilizers are nitrogen fixer(N-fixer), potassium solubilizer (K-solubilizer), phosphorus solubilizer(P-solubilizer) ,andplant growth promoting rhizobacteria (PGPR). The presence of bacteria in the soil depends upon the physical and chemical properties of the soil, organic matter, and phosphorus contents, as well as cultural activities. However, nutrient fixation and plant growth enhancement by bacteria are key components for achieving sustainable agriculture goals in the future. Microbes also facilitate various nutrient cycles in the ecosystem.

Different types of microbial biofertilizers are given below……..

(i) Nitrogen fixing biofertilizer

(ii) Phosphorus solubilizing biofertilizer

(iii) Phosphorous mobilizing biofertilizer

(iv) Potassium solubilizing biofertilizer

(v) Potassium mobilizing biofertilizer

(vi) Micronutrients

(vii) Plant growth promoting biofertilizer.

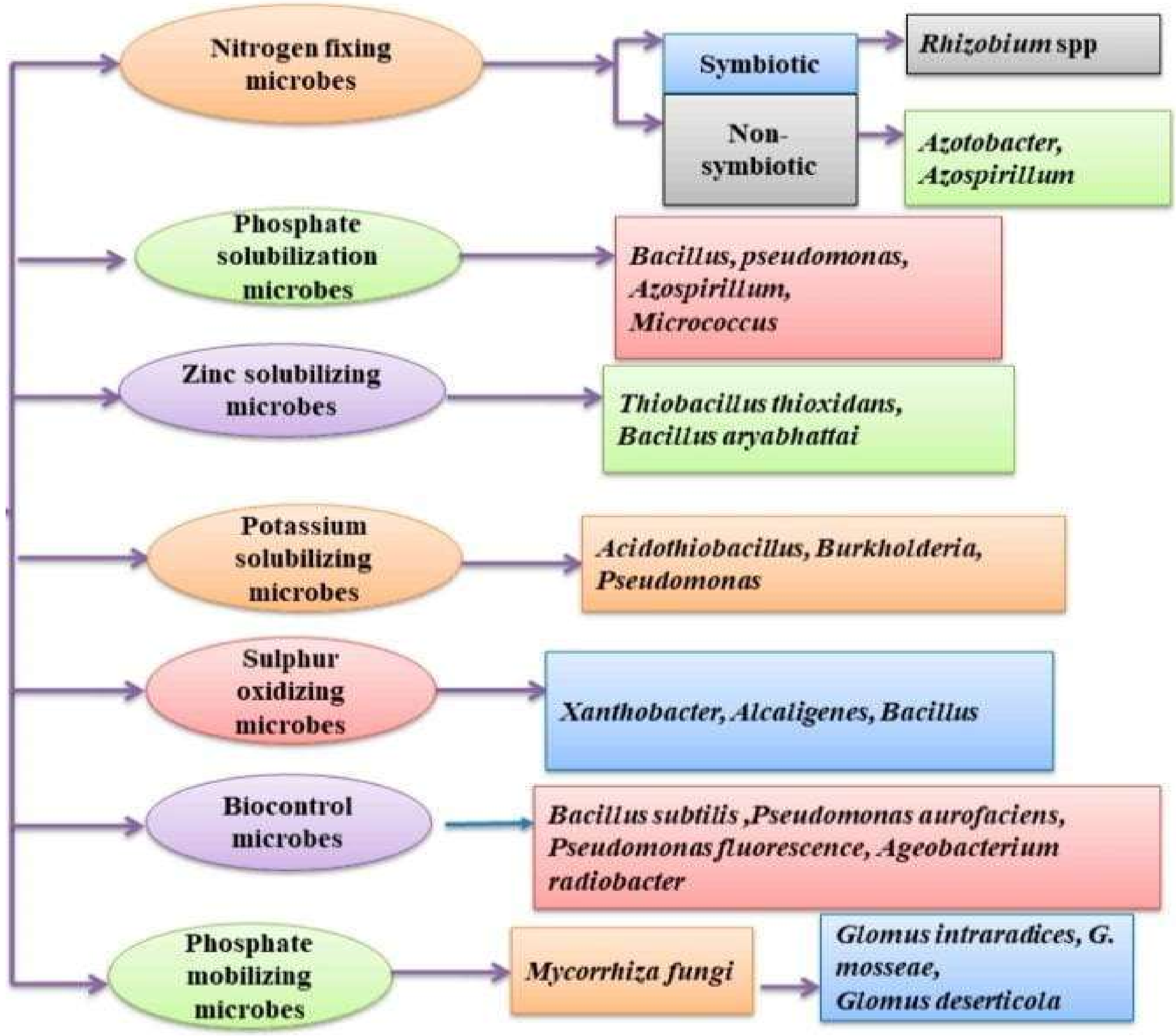

Figure 1. Different types of biofertilizers.

NITROGEN FIXING BIOFERTILIZER

Nitrogen is the most limiting nutritional factor for plant growth. The atmosphere contains about 80% of the nitrogen in free state, but most of the plants cannot utilize atmospheric nitrogen. A specialized group of microbes are required to fix this nitrogen and make it available to the plant. These microorganisms are known as biological nitrogen fixers (BNFs). They transform the inert N2 into plant-usable organic form. Nitrogen fixation can provide 300–400 kg N/ha/yr and increase the crop yield by 10–50%. In plants, up to 25% of total nitrogen comes from N-fixation. The roots of plants release substances into the soil, which support colonization and nitrogen fixation by bacteria in the rhizosphere of plants. They can efficiently substitute for chemical fertilizers to a varied extent, thus reducing the chemical load from the environment. A rough approximation of such substitution are given below in the table. They are grouped into free-living bacteria, blue-green algae, and symbionts, such as Rhizobium, Frankia, and Azolla. The N2-fixing bacteria associated with legumes include Rhizobium, Mesorhizobium, Azorhizobium, Bradyrhizobium, Sinorhizobium, and Allorhizobium and those with non-legumesinclude Achromobacter, Alcaligenes, Arthrobacter, Acetobacter, Azomonas, Beijerinckia, Clostrid-ium, Bacillus, Enterobacter, Erwinia, Desulfovibrio, Derxia, Corynebacterium,Campylobacter,Herbaspirillum, Klebsiella, Lignobacter, Mycobacterium, Rhodospirillum, Rhodo-pseudomonas, Xanthobacter, Mycobacterium, and Methylosinus. Although many genera are isolated from the rhizosphere, mainly members of Azospirillum and Azotobacter genera have been widely tested to increase yield of legumes and cereals under field condition. The main N2-fixing bacteria are described below.

Biofertilizers	Substitutes/H/Year
Rhizobium	50-100 kg N
Azolla	9.2-18.4 kg N
Azospillum	27.6 kg N
Blue Green Algae	24.8-29.9 kg N
Frankia	89.7 kg N

Table 1. Substitution of nitrogen by biofertilizers.

THE RHIZOBIUM

Rhizobium belongs to the bacterial family Rhizobiaceae and is the best example of symbiotic nitrogen fixation. It can fix N2 in legumes as well as in non-legume crops. Rhizobium has been shown to fix up to 300 kg N/ha/year in different legume crops. The bacteria infect the legume root and form nodules, within which they reduce molecular nitrogen to ammonia, which is utilized by the plant to produce proteins, vitamins, and other nitrogen-containing compounds. Thus, these root nodules act as factories of ammonia production. Rhizobium species improve the growth of non-legumes by inducing changes in root morphology and growth physiology. The Rhizobium application increased crop growth by improving plant height, seed germination, leaf chlorophyll, and N content. Seed inoculation of rice with different strains of Rhizobium at graded levels of N increased straw yield by 4% to 19% and rice grain yield by 8% to 22%. Rhizobium, Bradyshzodium,Sinorhizobium, Azorhizobium, and Mesorhizobium are collectively called rhizobia. They can act directly by fixing nitrogen or influencing plant hormones or indirectly by decreasing the inhibitory effects of pathogens. Rhizobium is commonly used in agronomic practices to ensure adequate nitrogen and have potential to replace chemical N fertilizer, Rhizobium strains and concluding that growth and yield parameters were significantly enhanced by rhizobium application in comparison to the control.

AZOTOBACTER

Azotobacter is a free-living, nitrogen-fixing diazotrophic bacterium; it plays an important role in the nitrogen cycle because of its various metabolic functions. Azotobacter has the ability to produce vitamins like thiamine and riboflavin. It belongs to the family Azotobacteriaceae and is used as a biofertilizer for all non-leguminous plants, especially rice, cotton, vegetables, sugarcane, sweet potato, and sweetsorghum. Azotobactor fixes almost 30 kg/N/year; it is mainly commercialized for sugarcane crop, as it increases the cane yield by 25–50 tons/hectares and sugar content by 10–15%. Azotobacter is present in both alkaline and acidic soils. A. chroococcum is the most prevalent species found in the soil, but other species like A. vinelandii, A. insignis, A. beijerinckii, and A. macrocytogenes are also found. Eklund et al. (2013) demonstrated that the presence of A. chroococcum in the rhizosphere of cucumber and tomato was correlated with increased growth and germination of seedlings. Another study showed inoculation with A. chroococcum caused a significantly increase in plant growth compared to control. Azotobacter also produces antifungal compounds and antibiotics that inhibit the growth of several pathogenic fungi in the root zone and help prevent seedling mortality. The major limiting factor for the proliferation of Azotobacter is the presence of reduced amount of organic matter in the soils; consequently, the rhizoplane lacks Azotobacter cells.

AZOSPIRILLUM

This bacterium is also essential, as it fixes a considerable amount of nitrogen in the soil. It is associated with the rhizospheric region and fixes up to 20–40 kg N/ha in non-leguminous plants, such as cereals, millets, oilseeds, cotton, and sorghum. It mostly forms a symbiotic association with plants. Several studies have shown the potential of Azospirillum for crop improvement. Azospirillum-inoculated wheat seedlings developed good water status; fresh weight was higher from inoculated seeds than from non-inoculated seeds. Somers et al. showed that A. *brasilense* could synthesize phenyl acetic acid (PAA), an auxin-like molecule with anti-microbial activity. It is demonstrated that the co-inoculation of A. *lipoferum* and B. *megaterium* provided balanced nitrogen and phosphorus nutrition to the plant and produced a higher yield than inoculation with only Azospirillum.

ANABAENA AZOLLAE

It is a symbiotic bacterium and used to fix the atmospheric nitrogen, mostly in rice .It is always associated with the free-floating fern known as Azolla. Azolla leaves contain 4–5% Nitrogen and 0.2–0.4% , quickly decompose, and provide the nitrogen to the plant. The Azolla-Anabaena system contributes 1.1 kg N/ha/day; one crop of Azolla provided 20–40 kg N/ha to the rice crop in about 20–25 days. Azolla is used as a biofertilizer in many countries, such as Vietnam, China, Thailand, and the Philippines. Another benefit of using this bio-fertilizer is its metal tolerance ability; thus, can be applied to the heavy metal-polluted areas .

BLUE-GREEN ALGAE (CYANOBACTERIA)

Nitrogen-fixing cyanobacteria are the most widespread N2 fixers on the earth. Cyanobacteria or blue green algae are a diverse group of prokaryotes consisting of Nostoc, Anabaena, Oscillatoria, Aulosira, and Lyngbya. They play an important role in nourishing the soil with nitrogen and supply vitamin B complex and growth-promoting substances like auxins, indole acetic acid, and gibberellic acid, which accelerate plant growth. They fix 20–30 Kg/N/ha in submerged rice fields and increase crop yield by 10–15% when applied at 10 Kg/ha. Reportedly, N availability to plants was increased by the application of cyanobacteria in agriculture , particularly in the rice fields. Cyanobacteria have been shown to enhance seed germination, shoot and root growth, and yield of wheat and rice. In rice fields, blue-green algae can fix 25–30 kg N/ha/season. In a study, the effect of the exudates of the cyanobacterial strains were tested on the growth parameters of *Sorghum durra* and *Helianthus annuus*. Shoot length was increased to about 120–242% as compared to control with various other positive effects. Strain showed potential for releasing bioactive compounds and enhanced the plant growth and yield. In another study, rice inoculation with cyanobacteria isolated from rice field showed the positive effects simultaneously on rice plant and soil properties. Application of cyanobacteria as biofertilizers is useful for economically weak farmers who are unable to invest in costl chemical fertilizers. Cyanobacterial biofertilizers can be used for a variety of biomes and environments.

PHOSPHATE SOLUBILIZING & MOBILIZING BIOFERTILIZER

Plant contains about 0.2% of phosphorus on a dry weight basis, and it is an essential nutrient for plant growth and development. Compared to other macro nutrients, phosphorus is so far the least mobile nutrient available to plants under most soil conditions. Microorganisms are needed to convert the insoluble forms of phosphate to the soluble forms. Several bacteria and a few fungi species are involved in the phosphate solubilizing process. The phosphate-solubilizing bacteria (PSB) convert the insoluble phosphate, such as HPO4 and H2PO4, into the soluble form by using different mechanisms, including the production of organic acids, chelation, and ion exchange reactions. Among the microbial populations, phosphate - solubilizing bacteria account for 1–50%, whereas 6 of 20 fungi account for only 0.1–0.5% of phosphate-solubilizing activities. The PSB can release metabolites, such as organic acids, having hydroxyl and carboxyl groups that chelate the cation bound to the phosphate and convert it to the soluble form, which is utilized by the plants. The secreted acids also reduce the pH of the soil and dissolve the bound phosphate to make it available to the plants. Along with the organic method, microorganisms also use the proton-extrusion mechanism to solubilize the phosphate. The PSB provide the phosphate as well as other trace elements, such as Fe and Zn, ultimately enhancing the plant growth. They also synthe size the enzyme that kills the pathogens, thus protecting the plant from diseas. Phosphate-mobilizing microbes can mobilize the immobile forms of phosphorous. They transfer and mobilize the insoluble phosphate from soil layers to the root cortex. *Arbuscular mycorrhiza* is an example of phosphate-mobilizing fungi, in which fungi penetrate the roots and increase the surface area of roots, stimulate metabolic processes, and absorb the nutrients into the roots. Reportedly, phosphorus-solubilizing bacteria (PSB) sometimes act as phosphate mobilizers.

MYCORRHIZA

It is a symbiotic association between the host plant and a certain group of fungi. It is arguably the most important symbiosis on earth. This association provides the essential nutrients to the plant, mostly phosphorous and growth hormones, which promote plant growth. They also increase the surface area of roots to increase the absorption of nutrients from the soil and provide resistance to plants against plant pathogens. The hyphae of fungi absorb the insoluble phosphorus and convert it into the solubilized form, which is taken up by the plant and, in return, the plant provides shelter and other nutrients to the fungi. These fungi are ubiquitous in geographical distribution and are associated with all crops.

ENDOMYCORRHIZA OR VAM FUNGI

Vesicular arbuscular mycorrhiza (VAM) is the symbiotic association between certain phycomycetous fungi and angiosperm roots. These symbiotic soil fungi colonize the roots of approximately 80% of plant families. They enhance the transfer of nutrients from the soil into the root system via specialized structures known as vesicles and arbuscules. This association provides many benefits to the plant. The fungal hyphae enhance the uptake of phosphorous and other nutrients as well as increase the root and shoot length. They also help the plant to uptake a large amount of water from the roots. VAM can potentially increase plant tolerance to various biotic and abiotic stresses and could replace the fertilizer requirements of trees and reduce the needs of current levels of chemical fertilizers. VAM fungi could contribute to more than two fold increased acquisition of the less mobile nutrients like P, S, Ca, Mg, Zn, and Cu from the rhizosphere. Six genera of fungi have been shown to form mycorrhizal associations: Glomus, Acaulospora, Gigaspora, Sclerocystis, Entrophospora, and Scutellospora. Co-inoculation treatment of VAM fungi, Glomus fasciculatum with Bradyrhizobium sp. + Pseudomonas striata or Penicillium variable, significantly increased the nutrient uptake and plant yield.

POTASSIUM SOLUBILIZING AND MOBILIZING BIOFERTILIZERS

Potassium (K) is the second most abundant and important plant nutrient after nitrogen and phosphorus. Although K is an abundant element in the soil, only 1–2% is available to plants, whereas the rest is present as mineral K that cannot be taken up by plants. Therefore, a continuous K replenishment of soil solution is required. It plays a vital role in the growth and development of plants. If not supplied in adequate quantity, the plants will grow slowly, have poorly developed roots, and produce small seeds and low yields. It has been reported that a wide range of bacterial and fungal strains use various mechanisms, including the production of acids, chelation, acidolysis, and exchange reactions to solubilize the insoluble K into soluble forms. Examples of potassium- solubilizing biofertilizer include Bacillus spp. and Aspergillus niger. Arthrobacter spp. Cladosporium, and Sphingomonas aminobacter with varying potential for K solubilization B. edaphicus and B. mucilaginosus are known to improve solubilization as well as mobilization when inoculated in soil, improved the oil content and groundnut biomass by 35.4% and 25%, respectively, along with enhanced K and P availability. Recently, a study has shown that a potassium-solubilizing strain Bacillus pseudomycoides enhanced K uptake in tea plants in the mica waste-treated soil by increasing potassium availability. Another strain Bacillus cereus significantly increased the plant height, shoot dry weight, and branches number by about 15%, 26%, and 27%, respectively, compared to the control. Some fungi like Aspergillus spp., and Penicillium spp. Also have potential to solubilize and mobilize K from organic and inorganic sources. Thus, role of K solubilizers is significant for ensuring the regular supply of K to crop plants. These also exert positive impact on the availability of other essential nutrients to the soil, and thus play an important role in maintaining soil sustainability.

SULFUR OXIDIZING BIOFERTILIZERS

Sulfur as a micronutrient is also required by the plants. It has been reported that sulfur plays a key role in improving certain biological and physical properties of the soil. Sulfur is famous for soil buffering from high pH values. Previous studies have shown that sulfur also promotes the efficiency of nitrogenous and phosphorus fertilizers and increases the efficiency of crops to uptake micronutrients. An example of sulfur-oxidizing microbe is Thiobacillus spp.; Thiobacillus thioparous and T. thioxidans can oxidize sulfur to plant usable sulfates that helpin nourishment of plants. A recent study has shown that inoculation of Thiobacillus along with elemental sulfur increases the oxidation of elemental sulfur, resulting in increased nutrients availability in soil and consequently increased plant growth. Sulfur compounds, especially in reduced form, significantly pollute the environment. Sulfur oxidizing bacteria also play significant role in environment protection by biological elimination of sulfur pollution.

ZINC SOLUBILIZING BIOFERTILIZER

Zinc is one of the essential micronutrients required at relatively small concentrations (5–100 mg/kg) in tissues for the growth and reproduction of plants. Zinc deficiency is very common in soil that results from the increased application of fertilizers in an imbalanced manner, intensive agriculture, and poor soil health. It is estimated that by 2025, Zinc deficiency may increase from 42% to 63% if the contributing reasons are neglected. Zinc is involved in the synthesis of growth hormones. Zinc deficiency in plants leads to retarded shoot growth, reduced membrane integrity and reduced leaf size, chlorosis, and increased susceptibility to

light, heat, and fungal infections and affects grain yield, root deve-lopment, pollen formation, and water uptake and transport. Zinc deficiency can lead to yellowing of leaves and stunted growth in wheat. Consuming zinc-deficient wheat can lead to zinc deficiency in humans as well. Zinc deficiency is considered the fifth most important human related death in less developed countries. Therefore, addressing Zn deficiency in agriculture is getting top priority among other minor nutrients. Microbial inoculants have been identified to solubilize the complex form of zinc in soil. Mycorrhiza, Saccharomyces spp., and several genera of rhizobacteria such as Pseudo monas spp. and Bacillus spp. are reported to increase Zn availability in soil. These microbes solubilize the zinc by chelated ligands and oxidoreductive systems. These bacteria also produce phytochromes, antibiotics, vitamins, and antifungal substances, and help the plant in many aspects. In a study, rice plants inoculated with a suitable combination of Zn solubilizing bacterial strains increased the growth attributes and rice yield and were found more efficient in acquiring Zn from the soil as compared to non-inoculated plants. Biofertilizers containing Zn solubilizing bacteria have been reported to boost up the maize production.

PLANT GROWTH PROMOTING RHIZOBACTERIA

A group of free-living rhizosphere bacteria that colonize plant roots and exert a beneficial effect on plant growth are referred to as PGPR. They act as biofertilizers by promoting growth and development of plants, facilitating biotic and abiotic stress tolerance, and helping in the mineralization of the soil by decomposing organic matter. Inoculation of PGPR imparts various beneficial effects to the plant. They increase the tolerance of plant to drought, salinity , and biotic stress. They enhance the seed germination and soil fertility and promote growth by producing phyto-hormones including Auxins, IAA, ethylene, gibberellin, etc.. They can modulate plant secondary metabolites and bioremediation of heavy metals and pollutants. PGPR includes member of several genera, e.g- *Agrobacterium, Arthrobacter, Alcaligenes, Azotobacter, Acinetobacter, Actinoplanes. Bacillus, Frankia, Pseudomonas, Rhizobium, Micrococcus Streptomyces, Xanthomonas, Enterobacter, Cellulomonas, Serratia, Flavobacterium, Thiobacillus*, etc.

MICROBIAL BIOFERTILIZER USED FOR SPECIFIC CROPS ARE GIVEN BELOW :-

Biofertilizer	Function	Crops
Rhizobium (symbiotic)	Fixes 200–300 kg N/ha/year	Pea, pulses legumes, cow pea, green gram, black gram, groundnut, soyabean, berseem, wheat, jowar, bajra, maize
	Increases yield up to 10–30%	
	Maintain soil fertility	
Azotobacter	Supplies 20–40 kg N/ha/year	Mustard, sunflower, banana, sugarcane, grapes, papaya, watermelon, tomato, chilly, lady finger, coconut
	Promote growth substances such as vitamins, IAA, gibberellic acids.	
	Increase yield up to 10–15%	
	Maintain soil fertility	
Azospirillum	Fixes 20–160 kg N/ha /year	Rice, sugarcane, millet, wheat, sorghum, bajra
	Increase water and mineral uptake	
	Production of plant hormones	
	Enhance root growth	
	Increase crop yield	
Blue-green algae	Fixes 20–40 kg N/ha/year	Rice
	Promote growth substances such as vitamins, IAA, Gibberelic acids	

Table 2 : Biofertilizer used for specific crops.

Biofertilizer	Function	Crops
Azolla	Fixes 30–60 kg N/ha/year	Rice
	Used as green manure	
Arbuscular mycorrhizal fungi (symbiotic)	Increase root absorption area for nutrient access	Soybean, wheat, and corn
	Fixes phosphate	
	Increase crop yield	
Pseudomonas	Production of siderophores and plant hormones	Potato, reddish, sugar beat
	Fixes phosphate	
	Increase crop yield	
Bacillus spp.	Solubilize the phosphate and fix the nitrogen in soil	Many vegetables and fruits
	Synthesis of growth hormones	
	Production of antibiotics	
	Increase crop yield	

Table 3 : Different types of biofertilizer used for specific crops.

SOME MYCORRHIZAL FUNGI THAT ARE USED AS BIOFERTILIZER

Mycorrhizal Fungi	Plants	Effect on Plant
Glomus versiforme *Glomus mosseae*	Tomato	Promotes growth and yield under water stress and more efficient conditions
Glomus etunicatum	Maize	Improves chlorophyll content and nutrient uptake in maize
Acaulospora lacunosa	Strawberry	Enhances nutrient uptake in strawberry
Rhizophagus irregularis	Wheat	Improves tolerance to stress, enhances plant growth, and increases seed yield
R. irregularis	Maize	Enhances tolerance to salt stress, improves growth parameters
G. mosseae and *G. geosporus*	Strawberry	Enhances growth and improves its tolerance to water stress
Rhizophagus irregularis	Tomato	Protects plants against pathogens (*Sclerotinia sclerotiorum*) and improves nutrient uptake in plants
Glomus deserticola	Snapdragon	Increases the total dry matter, chlorophyll content and improves Snapdragon tolerance to water stress
Glomus spp. and *Mortierella* spp.	Seashore mallow	Increases shoot and root weight under salt stress
Glomus versiforme	*Mentha arvensis* L.	Increases dry weight and improves nutrient uptake in salt stress conditions

Table 3 : Micorrhizal fungi used as biofertilizer.

FLOW CHART FOR PRODUCTION OF BIOFERTILIZER

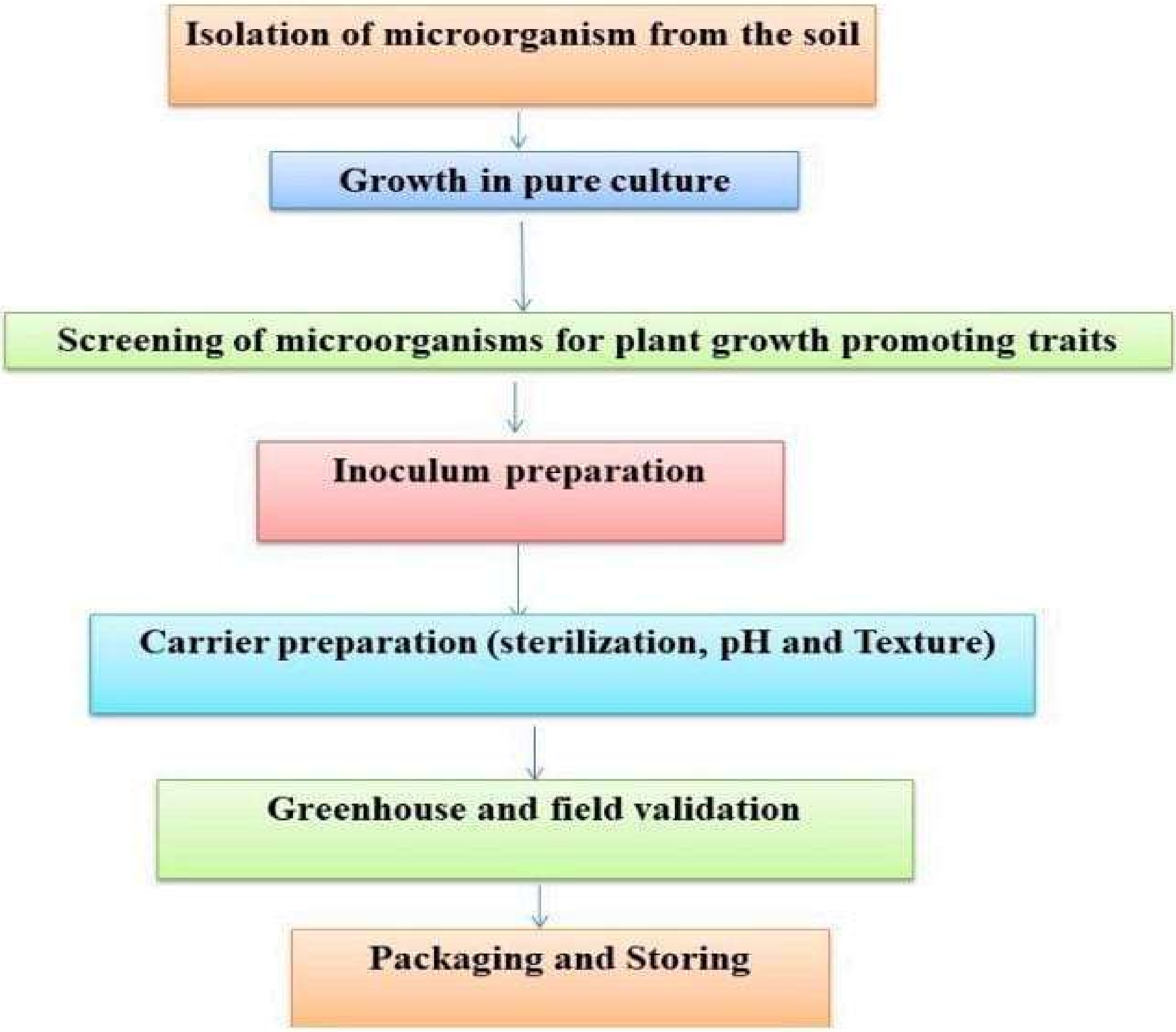

Figure 2. production of biofertilizer.

CONCLUSION

The use of microbial biofertilizers as a key to modern agriculture is fundamental, based on its renewable , low cost ,and eco-friendly potential in ensuring sustainable agriculture. Importantly, the applications of biofertilizer as an integral component of agriculture practice in promoting plant yield has gained more traction recently in meeting the demand of food production of the world populace.

In conclution, over dependence on the use of chemical fertilizers has encouraged industries to produce chemicals that are toxic to human health . Thus, causing ecological imbalances. These drawbacks are combined with a high cost of production that is beyond the means of many farmers in the developing world. The applications of biofertilizer is eco-friendly, relatively inexpensive, non toxic, and possesses the significant potential to increase plant yield. Thus, the functions of plant growth-promoting microorganisms and the applications of biofertilizer made from viable microbial strains to the field bodes well for successful management of the rhizospere for sustainable agriculture.

REFERENCES

1. Berg, G. Plant–microbe interactions promoting plant growth and health: Perspectives for controlled use of microorganisms in agriculture. Microbiol. Biotechnol. 2010,
2. Mishra, P.; Dash, D. Rejuvenation of biofertilizer for sustainable agriculture and economic development. Consilience 2014,
3. Brahmaprakash, G.; Sahu, P.K. Biofertilizers for sustainability. J. Indian Inst. Sci. 2012,
4. Etesami, H.; Emami, S.; Alikhani, H.A. Potassium solubilizing bacteria (KSB): Mechanisms, promotion of plant growth, and future prospects A review. J. Soil Sci. Plant Nutr. 2017,
5. Parikh, S.J.; James, B.R. Soil: The foundation of agriculture. Nat. Educ. Knowl. 2012,
6. Choudhury, A.; Kennedy, I. Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production. Biol. Fertil. Soils 2004,
7. Kizilkaya, R. Nitrogen fixation capacity of Azotobacter spp. strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. J. Environ. Biol. 2009,
8. Kamran, S.; Shahid, I.; Baig, D.N.; Rizwan, M.; Malik, K.A.; Mehnaz, S. Contribution of zinc solubilizing bacteria in growth promotion and zinc content of wheat. Front. Microbiol. 2017,
9. Bhosale, H.; Kadam, T.; Bobade, A. Identification and production of zotobacter vinelandii and its antifungal activit against Fusarium o sporum. J. Environ. Biol. 2013.