Society of Green
World for Sustainable
Environment

• Volume 13
• Number 1
• January - June 2023

Print ISSN 2319-2186
Online ISSN 2322-0996
www.biotechtoday.in
NAAS Accredited
A Peer Review Journal

*International Journal of Biological Sciences*

# BIOTECH TODAY

Society of Green World for Sustainable Environment
35, Akshardham, Roorkee Road, Modipuram, Meerut - 250110 (U.P.)
Mob. : +91-9412472292
E-mail - biotechtoday@gmail.com

NAAS Rating : 3.94
Indexed & Abstracted with:
Google Scholar, Summon
Proquest & CNKI Scholar
Ebsco Discovery

*Internationally marketed by:*
IndianJournals.com
A Product of Diva Enterprises Pvt. Ltd.
*www.Indianjournals.com*

An International Journal of Biological Sciences

# Biotech Today

(A Peer Reviewed Journal)



**Volume 13** **Number 1** **January-June 2023**

## Published and Printed By

**Prof. RS. Sengar**
35, Akshardham, Roorkee Road, Modipuram, Meeut-250110 (U.P.)
**E-mail :** biotechtoday@gmail.com, Mob.: 9412472292, 7906045253
**Website:** www.biotechtoday.in Printed by: Society of Green World For Sustainable Environment, Meerut (U.P.) India

## Online Submission of Manuscripts

The Journal has web fully enabled manuscript submission and peer review system with easy log-in tracking procedures available at **www.indianjournals.com** and **www.biotechtoday.in** any correspondence relate to submission, publications and permission and any other matters. Concerning the Journal should be addressed to the editor or the editor-in chief.

An International Journal of Biological Sciences

# Biotech Today

Peer Review Journal

Volume 13 Number 1 January-June 2023

## In This Issue Page No.

## REVIEW AND RESEARCH ARTICLE

An International Journal of Biological Sciences

# Biotech Today

(A Peer Reviewed Journal)

| Volume 13 | Number 1 | January-June 2023 |
|---|---|---|

## Society of Green World for Sustainable Environment (SGWSE)

**(Registered Under Societies Registration Act 1860)**

### Aims and Objectives

The Society (SGWSE) has been functioning with following aims and objectives:

- To constitute a forum at international and national level for bringing together individuals and organization involved in agriculture and biological science activities.
- To develop international research/development linkages and disseminate up-to-date technologies in the field of agriculture and biological science.
- To promote and undertake research and development and extension service in the field of agriculture and biological science.
- To explore new areas in agriculture, biological research, biotechnology crop cultivation technologies, development activity and logistics management.
- To propagate utilization on non-conventional and renewable sources in agriculture and biological research.
- To develop purely organic package for growing and cultivation of crops.
- To develop technology of in vivo propagation of important crops.
- To offer recognition and awards to professional groups and individual for attainment of excellence in the field of agriculture, biological science and Biotechnology.
- To interact with government agencies, scientific organization and NGOs to promote and protect interest of agriculture and biological science researcher.

To organize symposia, seminar, and workshops and bring out timely publication(s) to meet the objectives of the society. An International Journal of Biological Sciences ''Biotech today'' is the official journal of Society of Green World for have been receiving research articles from all over the world. Concerned libraries from all over the World have been subscribing Biotech Today has come to be a leading international journal with its unique quality and standard. We have been covering current references on different crops or biological sciences activities and technologies along with addresses of corresponding authors, so that interested person could contact them as per their own interest biotech Today is being indexed in Google Scholar, Summon Proquest and CNKI Scholar, EBSCO Discovery. The society requests al the cientists/industrialists/technologists engaged in biological science and agriculture to contribute their research findings to Biotech Today and also to become members of the society with their active participation so that the aims and objectives of the society could be fulfilled in their true perspectives.

## Executive Council



**For membership and other activities of SGWSE, Please write to:**

35, Akshardham, Roorkee Road, Modipuram, Meeut-250110 India
Mob.: 09412472292, **E-mail:** biotechtoday@gmail.com

**Biotech Today (January-June 2023) : 13 (1) : 05 - 13**

DOI : 10.5958/2322-0996.2023.00001.7

**RESEARCH ARTICLE**

# The Impact of Climate Change on Vegetable Production

**Tazeem Fatma Jafri[*1], Khursheed Hussain[1], Anil Kumar[2], Sumati Narayan[1], Mohd Faruk[*3], Harshavardhan M. Totawar[1], Arizoo[1] and Sonika Jaryal[4]**

**Received**: 27 September, 2022, **Revised**: 16 November, 2022, **Accepted**: 10 December, 2022, **Published**: 1 January 2023

## Abstract

Climate change is causing notable consequences for the cultivation of vegetables. It is leading to alterations in temperature and precipitation patterns, which subsequently affects the growth and output of vegetable crops. These changes can result in both favorable and adverse outcomes, contingent on specific circumstances and the implementation of adaptation strategies. Extreme weather events such as droughts, floods and storms are becoming more frequent and severe due to climate change. These events can inflict damage on crops, disrupt planting schedules and reduce overall vegetable production. Additionally, elevated temperatures can adversely affect the quality of vegetables by impacting their taste, texture, and nutritional composition. Variations in rainfall can cause soil waterlogging and drought, which, in turn, can trigger root diseases and nutrient deficiencies. Prolonged droughts can stifle growth and reduce crop yields. Increasing temperatures and erratic climate patterns intensify the threat of pests and diseases, leading to increased pesticide usage, elevated production expenses and potential declines in yield and quality. Shortages of water resources and declines in biodiversity can impact both crops and overall agricultural productivity. Climate change has significant effects on ecosystems, particularly concerning pollination services and their adverse impact on vegetable crops that rely on insect pollinators, resulting in reduced fruit set and lower yields. Addressing these challenges is essential for the sustainable future of vegetable production. Farmers and researchers are actively investigating adaptation strategies to mitigate the impact of climate change on vegetable farming. These strategies include the development of crop varieties that can withstand drought and heat, enhanced irrigation methods, the adoption of agroforestry techniques and sustainable soil management practices. Precision agricultural technology, along with the use of greenhouses and controlled-environment farming, offers consistent growth conditions for vegetables.

**Keywords:** Climate changes, crop quality, fruit set, vegetable production, precision farming

## Introduction

Vegetables are essential sources of the micronutrients required for healthful diets. Vegetables are high in potassium, which helps to maintain healthy blood pressure, dietary fibre, which lowers blood cholesterol levels and may reduce the risk of heart disease, folate (folic acid), which helps prevent birth defects, vitamin A, which supports healthy eyes and skin and vitamin C, which not only supports healthy teeth and gums but also helps the body absorb iron. The importance of vegetables for the security of food and

[1]Division of Vegetable Science, SKUAST-K, Shalimar
[2]Division of Vegetable Science, ANDUAT Ayodhya, UP
[3]Division of Vegetable Science, ICAR-IARI, New Delhi
[4]Division of Fruit Science, SKUAST-K
*Corresponding Author: tazeemraza01@skuastkashmir.ac.in

nutrition is becoming more widely acknowledged producing vegetables is a crucial part of farm diversification methods and offers a promising economic prospect for reducing rural unemployment and poverty in developing nations. The most accessible source of vitamins and minerals for human health is found in vegetables.

The World Vegetable Center ("WorldVeg") is a global public research organisation that is solely dedicated to boosting vegetable production and consumption. The Asian Vegetable Research and Development Center (AVRDC), which was established in 1971 by a number of East and Southeast Asian nations, the United States and the Asian Development Bank, today conducts research and development projects in Asia and Africa.

In order to meet nutrition goals, the World Health Organization and the Food and Agricultural Organization of the United Nations recommend a daily diet of at least 400 g of fruit and vegetables per person. However, consumption rates significantly below this level are typical in many nations. The number of undernourished individuals is increasing, with close to 770 million people in 2020, 118 million more than in 2019 and about 160 million more than in 2014. The answer may lie in increasing local vegetable production to increase accessibility and availability. Vegetable gardens can increase the security of food and nutrition, bring in more money and promote better health.

Vegetable production is impacted by climate change worldwide. Changing weather patterns and a changing climate have put agricultural productivity at jeopardy due to extremes in temperature and increased rainfall variability. Yet, depending on the level of climatic change, geographical location, and agricultural production method, its nature and effects change. In general, vegetables are more vulnerable to environmental extremes like high temperatures and soil moisture stress. A significant greenhouse gas, $CO_2$, has an impact on vegetable crop growth and development as well as the prevalence of insect pests and diseases. Crop failures, low yields, declining quality, and an increase in pest and disease issues are frequent in climate change conditions, making vegetable agriculture unprofitable. Vegetables are essential for guaranteeing food and nutritional security, but because they are so perishable and their costs spike during emergencies like droughts or floods, the poor cannot afford them. Small and marginal farmers, especially those that rely heavily on vegetables, may be more affected by climate change.

## Effects of Climate Change on Vegetable Crops

Vegetable crops are susceptible to climatic change, like other agricultural products. Vegetables are often vulnerable to environmental extremes, therefore, high temperatures are the main causes of low yields and will be made worse by climate change. Water has a significant impact on vegetable yield and quality, drought circumstances significantly lowered vegetable productivity, and tomatoes in particular are one of the vegetable crops that are most susceptible to excessive water. The main problems restricting the maintenance and growth of vegetable yield would be rising temperatures, decreased irrigation water supply, flooding, and salinity. The yield of vegetable crops would decline as a result of global climatic change, particularly because of unpredictable high temperatures and inconsistent rainfall patterns. (Youssef *et. al.* 2016) stated that environmental conditions have a detrimental impact on the production of tomatoes. Thakur and Jahn claimed that persistently poor weather conditions and climatic changes brought on by an increase in temperature, unpredictable rainfall, and more This is a discussion of some of the significant environmental stresses that influence the

yield of vegetable crops. The increased frequency of illnesses and the water demand are all expected to have an impact on the output of different vegetable crops. According to Adeniyi (2013), one of the most significant elements determining agricultural productivity is rainfall.

## Influence of Temperature

Since many physiological, biochemical, and metabolic processes in plants are temperature-dependent, variations in daily mean maximum and minimum temperatures are the primary influence of climate change that negatively effects vegetable output. In tropical and arid regions, where high temperatures are common, vegetable production is impacted. High temperatures have a substantial impact on the morphological, physiological, biochemical and molecular responses of the plant, which affects the plant's growth, development and yield.

## Disorders of vegetable crops caused by temperature Fluctuation

| Crop | Disorder | Caused Factor |
|---|---|---|
| **Asparagus** | high fibre in the spheres and stalks<br>Asparagus Feathering Lateral branch growth | High temperature (>32°C) |
| **Carrot** | Low carotene content | Temperature > 20° C |
| **Cauliflower** | Blindness<br>Buttoning<br>Riceyness | Temperature fluctuation |
| **Cole crops, Lettuce** | Tip burn | High temperature |
| **Potato** | Greening<br>Chilling Injury | Sunburn<br>low temperatures (-1 to -2 °C). |
| **Tomato** | Puffiness<br>Cat face<br>Unfruitfulness | Low or high temperatures.<br>high or low temp at fruit set.<br>Temperature fluctuation |

High temperatures can severely affect tomato yield by decreasing fruit set and smaller fruit size, lower-quality fruits. Post-pollination in pepper, high temperatures reduced fruit set but had no negative effects on pistil or stamen viability, indicating that fertilisation is vulnerable to high temperature stress. High temperatures also affect the red coloration of developing chilli fruits by causing flower drop, ovule abortion, poor fruit set and fruit drop. Temperature increases in cucumber negatively affect sex expression, blooming, pollination and fruit setting. When temperatures are extremely hot, cucumber flowers drop off early. Fruits become bitter when cucumber plants are subjected to heat stress during the fruit development stage.

## Influence of extreme weather events

### (1) Drought

A prolonged period of exceptionally low rainfall, especially one that has a negative impact on growing or living conditions, is referred to as a "drought" (Allaby, 1989). In general, drought stress happens when the amount of water that is accessible in the soil is decreased and the surrounding environment results in a constant loss of water through transpiration or evaporation. Being by definition a succulent product, vegetables typically contain more than 90% water (AVRDC, 1990). Water thus has a significant impact on vegetable yield and quality, and drought circumstances significantly lower vegetable productivity. Extreme water stress may stop photosynthesis, disrupt metabolism and ultimately cause plant death (Jaleel *et al*., 2008). Drought stress has been proven to be a crucial limiting factor during the early stages of plant growth and establishment. It has an impact on both expansion and elongation growth (Anjum *et al*., 2003). Drought impacts reverse osmosis, which is the loss of water from plant cells, and raises the salt concentration in the soil. This decreases the yield of most vegetables by increasing water loss in plant cells and inhibiting several physiological and biochemical activities like photosynthesis and respiration. Drought conditions have a detrimental impact on the seed germination in vegetable crops such as onions and okra, as well as on the sprouting of potato tubers.

Potatoes are extremely drought-sensitive. Reduced tuber yield might also be a result of moderate water stress. Drought circumstances cause leafy plants like amaranthus, palak, and spinach to lose water, which lowers their quality (AVRDC, 1990). Drought stress also affects chilli, which results in yield losses of up to 50–60%. Despite high levels of foodgrain and horticultural production, India's food price inflation grew and became more erratic from 2019 to 2021. The price of vegetables, particularly tomatoes, onions, and potatoes, contributes significantly to this instability. Large prediction mistakes in inflation are the result of such instability (RBI, 2020).

**(2) Flooding**

Agricultural disasters caused by rainfall-induced flooding and related waterlogging are common around the world and frequently inflict significant harm to crop productivity. Abiotic stress caused by the submersion affects crops in a variety of ways in addition to the direct rushing effect, such as diminished light availability, oxygen depletion and altered chemical properties of soil. The sum of all these physical and chemical changes can significantly slow down crop development and production as well as crop stand. Water fills the soil pores that were previously occupied by oxygen in water-logged soils. Such soils are deficient in oxygen. Plants growing there experience reduced growth and survival due to this $O_2$ deficit. Flood-sensitive plants, such as tomato, soybean and sunflower, perish in the standing water. The majority of vegetable crops are quite vulnerable to flooding and there is little genetic diversity for this trait. Flooded crops, particularly tomato plants, build up endogenous ethylene that harms the plants (Drew, 1979). Increased formation of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) in the roots is encouraged by low oxygen levels. Tomatoes typically respond to waterlogged conditions by rapidly developing epinastic growth of their leaves and the involvement of ethylene buildup has been suggested. Stomatal closure is frequently brought on by flooding, primarily in C3 plants. These plants' water flow is decreased as a result. As a result of the decreased root permeability, leaves also get dehydrated. In the end, the reduced water flow from the roots to the shoots causes leaf wilting.

The transfer of toxic compounds (acetaldehyde/alcohol) created under anaerobic circumstances in the roots as well as the amounts of plant growth regulators (PGRs) carried from the roots to shoots via transpiration stream are the causes of these alterations in leaves, shoots or roots. The roots have lower quantities of endogenous PGRs like Gibberellin (GA) and cytokinins (CK). As a result, the shoots' ABA and ethylene levels have increased, which leads to stomatal closure and an earlier beginning of senescence, respectively.

**Effect of flooding stress on the endogenous levels of PGRs and their effect on plants**

| S.N. | Level of PGR in plants | Effects on plants under water logging |
|---|---|---|
| 1. | Reduced Auxins | Causes "Hypertrophy" (Enlargement or contraction of cells in the stem's cortex leads to the expansion or constriction of the stem base) |
| 2. | Decreased GA3 | Causes stem elongation and cell expansion to be reduced. |
| 3. | Decreased CK | Causes senescence to begin sooner and assimilate partitioning to the sinks to be slower. |
| 4. | Increased ABA | Cause stomatal closure, leads to a cascading impact on the rates of gas exchange during photosynthesis, respiration and transpiration. This closure results in the efflux of potassium ions (K+) from the guard cells, diminishes ion transport due to reduced transpiration rates and hampers starch production in the guard cells, ultimately leading to the closure of stomata. |
| 5. | Increased Ethylene | Causes "Epinasty" in leaves, which is an uneven growth of leaves caused by more cell elongation on the upper side of the leaf than the bottom side; this causes plant senescence and hypertrophy. |

Moreover, it has been noted that under flooding stress, auxin levels are decreased and aminocyclopropane-1-carboxylic acid (ACC), a precursor to the production of ethylene, is raised.

## Impact of rising atmospheric carbon dioxide levels

The main components of the Earth's atmosphere are nitrogen (78.1%), oxygen (20.9%) and a trace quantity of carbon dioxide (0.031%). Changes in the atmospheric $CO_2$ concentration can affect the growth and physiological behaviour of plant tissues. Net photosynthesis, biomass output, protein, sugar, and organic acid content, stomatal conductance, firmness, seed yield, light, water and nutrient utilisation efficiency and plant water potential are all affected by increased atmospheric $CO_2$. The ripening of tomato fruit is prevented by the high ambient $CO_2$ level. This inhibition results from the suppression of genes associated with ripening, which is likely related to the stress effect of high $CO_2$. Potato plants grown in high $CO_2$ environments may initially have higher photosynthetic rates, but as $CO_2$ concentrations rises, the photosynthetic rates will decrease. Chlorophyll content in leaves has reportedly decreased due to the higher
$CO_2$ concentration, especially later in the growing season following tuber commencement.

## Changes in pest and disease patterns

Global warming and climate change are two of the most talked-about topics in today's society and are of considerable significance to agriculture. Alterations in precipitation patterns, the rise in atmospheric $CO_2$ levels and higher temperatures significantly affect both agricultural production and insect pests in agriculture. Climate changes can have various consequences on insect pests. These effects include the potential expansion of their geographical range, increased survival rates during winter, a higher number of reproductive generations, altered synchronicity with host plants, shifts in species interactions, heightened risk of invasive pests during migration, increased incidence of insect-transmitted plant diseases and decreased effectiveness of biological control measures, particularly those involving natural predators and parasites. As insects are poikilothermic organisms, their body temperature is determined by the surrounding environment. Hence, it's likely that temperature is the most crucial environmental factor affecting insect behavior, distribution, growth, and reproduction. The intricate physiological consequences resulting from rising temperatures and elevated $CO_2$ levels can profoundly influence the dynamics between agricultural crops and insect pests. As a result of climate change, new ecological niches are created, giving insect pests the chance to settle, spread and move between different geographic regions. Farmers should be ready to confront emerging and more severe pest challenges in the years ahead due to the impact of climate change. The geographic range of insects would probably increase (particularly in the north). Few pests will become more common as a result of improved overwintering survival rates and the capacity to produce additional generations. There will most certainly be an increase in plant illnesses spread by insects as well as invasive pest species that may colonise new places more easily. The diminished effectiveness of biological control agents or natural enemies, is another unfavourable effect that climate change might have and this could pose a significant challenge for future pest management programmes. We run a considerable risk of suffering substantial financial losses and a threat to human food security if climate change variables create ideal conditions for insect infestation and crop destruction.

## Changes in vegetable crop yields and quality

Changing weather patterns and a changing climate have affected agricultural output by introducing extremes in temperature and increasing the variability of rainfall. The main issues affecting the performance of agriculture, notably vegetable crops, are climate change and its variability. Short growing seasons, which will negatively affect growth and development in particular due to terminal heat stress and lower water availability, are anticipated to result in a reduction in fruit and vegetable production. Rainfall variability and a decline in the number of wet days will have the most effects on agriculture that relies on rain. Vegetable production methods are now subject to additional restrictions as a result of the increased uncertainties and hazards caused by the issue of climate change and climate variability.

## Mitigating the effects of climate change on vegetable crops

Adoption of effective and efficient measures is the sole means of reducing the negative effects of climate change on vegetable production, specifically with regard to their productivity, quality and yield. The most effective adaptation strategies for minimising the effects of climate change include changing the sowing date, using efficient technologies like drip irrigation, soil and moisture conservation measures, managing fertilisers through fertigation, using grafting techniques using plant regulators, protected cultivation and enhancing pest management. An alternative approach to harnessing plant diversity to address climate change involves grafting a vulnerable scion cultivar onto a resilient rootstock. There are several agronomic methods available, including mulching, organic farming, carbon-sequestering cropping systems and agroforestry, which offer diverse opportunities for alleviating the impacts of climate change on vegetable farming.

## Vegetable varieties with various stress tolerance released in India for cultivation

| Crop | Variety | Abiotic stress tolerance |
|---|---|---|
| Tomato | Pusa Sheetal | Tolrant to low temperature (Fruit setting occurs at night temperatures as low as 8°C) |
| | Pusa Hybrid 1 | Fruit set up to 28°C (high) night temperature |
| | Pusa Sadabahar | Fruit set at both low (6°C) and high (30°C) night temperature |
| | Sabour Suphala | Tolerant to salt, during the seed germination phase. |
| | Arka Vikas | Tolerant to moisture stress |
| Eggplant | SM-1, SM-19 and SM-30 | Tolerant to drought |
| | Pragati and Pusa Bindu | Tolerance to salt |
| Okra | Pusa Sawani | Salinity tolerant |
| Musk melon | Jobner 96-2 | Tolerant to High soil pH |
| Spinach beet | Jobner Green | High soil pH (up to 10.5) tolerant |
| Cucumber | Pusa Barkha | Tolerant to high temperature |
| | Pusa Uday | Suitable for throughout the year |
| Bottlegourd | Pusa Santusthi | Hot and cold set variety |
| Onion | Hisar-2 | Tolerant to salinity |
| Carrot | Pusa Kesar | Tolerant to high temperature |
| Radish | Pusa Himani | Grown throughout the year |
| Sweet potato | Sree Nandini | Drought tolerant |
| Potato | Kufri Surya | Heat tolerant up to 25°C night temperature |
| | Kufri Sheetman and Kufri Dewa | Frost tolerant |
| Cassava | H-97 and Sree Sahya | Tolerant to Drought |

The utilization of protected farming techniques and advanced post-harvest technology can also play a pivotal role in surmounting the challenges posed by climate change. It's feasible to predict the potential effects of climate change on the production of

vegetable crops using weather forecasting models and growth simulation models. These models also assist in formulating the necessary adaptation measures.

## Conclusion

The process of climate change never stops. Better diagnosis can be made by having a general understanding of the effects of climate change and this is the primary strategy for taking more mitigation measures. The sensitivity of the climate, which is negatively impacted by both the natural environment and human activity, needs to be recognised. According to earlier research, environmental aberrations such floods, droughts, salinity, high temperatures, etc. that are caused directly or indirectly by anthropogenic factors, such as these, have the greatest impact on the scale of climate change. Apart from the transition in cropping seasons, growth and yield patterns, the situation with pests and diseases and insect pollination behaviour. It is abundantly obvious that there is a serious threat in the near future, prompting warnings about future food and nutrition crises, employment and livelihood security difficulties and poverty. Although the effects of climate change cannot entirely be eliminated, they can be minimised with sincere involvement.

## Referance


Abewoy, D, (2018). Review on Impacts of Climate Change on Vegetable Production and its Management Practices. Adv Crop Sci Tech 6: 330.

Adeniyi, A, (2013). Impact of Climate on Productivity of Selected Crops I Ilorin, Kwara State, Nigeria. *Ilorin Journal of Business and Social Sciences* 15: 59-66.

Anjum, F., Yaseen, M., Rasul, E., Wahid, A. and Anjum, S. (2003). Water stress in barley (*Hordeum vulgare* L.). Effect on morphological characters. *Pakistan Journal of Agricultural Sciences,* 40: 43-44.

Arora, S. K., Partap, P. S., Pandita, M. L. and Jalal, I. (1987). Production problems and their possible remedies in vegetable crops. *Indian Horticulture*. 32(2): 2-8.

AVRDC. (1990). Vegetable Production Training Manual. *Asian Vegetable Research and Training Center*. *Shanhua*, Tainan. PP. 447.

Bhardwaj, ML, (2012) *Effect* of climate change on vegetable production in india in vegetable production under changing climate scenario.

Bindi, M., Hacour, A., Vandermeiren, K., Craigon, J., Ojanpera, K., Sellden, G., Hogy, P., Finnan, J. and Fibbi, L. (2002). Chlorophyll concentration of potatoes grown under elevated carbon dioxide and/or ozone concentrations. *European Journal of Agronomy*. 17(4): 319–335.

Burke, JI., Finnan, JM., Donnelly, A. and Jones, MB. (2001). The effects of elevated concentrations of carbon dioxide and ozone on potato (Solanum tuberosum L.) yield. *Agriculture and Food Development authority*, Carlow, Ireland. Pp. 1-19.

Caulfield F., Bunce JA. (1994) Elevated atmospheric carbon dioxide concentration affects interactions between Spodoptera exigua (Lepidoptera: Noctuidae) larvae and two host plant species outdoors. *Environ. Entomol.* 23:999–1005.

Erickson, AN, Markhart, AH, (2012). Flower developmental stage and organ sensitivity of bell pepper (*Capsicum annuum* L.) to elevated temperature. *Plant Cell and Environment* 25: 123-130.

FAO (2020) Climate Related Transboundary Pests and Diseases. (accessed on 19 December)Available online: http://www.fao .org/3/a-ai785e.pdf.

Hare J. D. (1992) Effects of plant variation on herbivore-natural enemy interactions. In: Fritz R.S., Simms E.L., editors. *Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics*. 1st ed. University of Chicago Press; Chicago, IL, USA: pp. 278–298.

Hazra P, Samsul, HA., Sikder D and Peter KV, (2007). Breeding tomato (Lycopersicon esculentum Mill) resistant to high temperature stress. *International Journal of Plant Breeding* 1: 31-40.

Holmer, Robert. (2013). Importance of Vegetables

for Food and Nutrition Security in a Rapidly Urbanising World.

Jaleel, CA., Manivannan, P., Lakshmanan GM A., Gomathinayagam, M. and Panneerselvam R. (2008). Alterations in morphological parameters and photosynthetic pigment responses of Catharanthus roseus under soil water deficits. *Colloids and Surfaces B: Biointerfaces,* 61: 298-303.

Jefferies, RA., and Mackerron, DKL. (1993). Responses of potato genotypes to drought. II. Leaf area index, growth and yield. *Annals of Applied Biology*. 122:105–112.

Khalid E. and Ibrahim A, (2021) Climate Change Impact on Vegetable Crops and Potential for Adaptation: A Review, *Hortscience Journal of Suez Canal University*,10(1):13-30.

Kishor, V. and Shekhar, H. (2022). Extreme Weather Events and Vegetable Inflation in India 57°, 44-45.

Kocmánková E., Trnka M., Juroch J., Dubrovský M., Semerádová D., Možný M., Žalud Z., Pokorný R. and Lebeda A. (2010) Impact of climate change on the occurrence and activity of harmful organisms. *Plant Prot.* Sci. 45:S48–S52.

Kumar, SN., Aggrwal PK., Rani S., Jain S., Saxena R. and Chauhan N. (2011). Impact of climate change on crop productivity in Western Ghats, coastal and northern regions of India. *Current Sciences,* 101:332-341.

Malhotra SK, Srivastava AK (2015). Fertiliser Requirement of Indian Horticulture. *Indian Journal of Fertilisers* 11: 16-25.

Malhotra SK, Srivastva, AK, (2014). Climate smart horticulture for addressing food, nutritional security and climate challenges. In: Srivastava AK (ed) *Shodh chintan scientific articles,* ASM Foundation, New Delhi,: 83-97

Naik, PS., Singh, M. and Ranjan, JK. (2017). Impact of Climate Change on Vegetable Production and Adaptation Measures. In: Minhas, P., Rane, J., Pasala, R. (eds) *Abiotic Stress Management for Resilient Agriculture*. Springer, Singapore.

Pena, R., and Hughes, J. (2007). Improving Vegetable Productivity in a Variable and Changing Climate. SAT e journal.4 (1):1- 22.

Pepijn, Schreinemachers, Emmy, B., Simmons, Marco CS., Wopereis, (2018), Tapping the economic and nutritional power of vegetables, *Global Food Security*, 16: 36-45.

Prasad BVG. and Chakravorty S.(2015), Effects of Climate Change on Vegetable Cultivation - *A Review, Nature Environment and Pollution Technology An International Quarterly Scientific Journal*, 14: 923-929.

Devi PA., Dass P. and Jhilki J (2017). Effect of Climate Change on Vegetable Production- A Review. *Int. J. Curr. Microbiol. App. Sci.* 6(10): 477-483.

Roth SK. and Lindroth RL. (1995). Elevated atmospheric $CO_2$: Effects on phytochemistry, insect performance and insect-parasitoid interactions. *Glob. Chang. Biol.* 1: 173–182.

Rothan, C., Duret, S., Chevalier, C. and Raymond, P. (1997). Suppression of Ripening-Associated Gene Expression in Tomato Fruits Subjected to a High CO, Concentration. *Plant Physiology*. 114: 255-263.

Schmidhuber, J. and Tubiello, FN. (2007) Global food security under climate change. *Proc. Natl. Acad. Sci.* USA 104: 19703–19708.

Skendžiæ S, Zovko M, Živkoviæ IP, Lešiæ V, and Lemiæ D. (2021) The Impact of Climate Change on Agricultural Insect Pests. *Insects*. 12(5):440.

Somarribaa E, Cerdaa R, Orozcoa L, Cifuentesa M and Dávila H (2013) Carbon stocks and cocoa yields in agroforestry systems of central america. *Agriculture Ecosystems and Environment* 173: 46-57.

Sun, L., Chen, S.; Chao, L. and Sun, T. (2007). Effects of flooding on changes in Eh, pH and speciation of cadmium and lead in contaminated soil. Bull. Environ. *Contam. Toxicol*. 79, 514–518.

Tirado MC, Clarke R, Jaykus LA, McQuattersGollop A and Frank JM (2010). Climate change and food safety: A review. *Food Research International* 43: 1745-1765.

Venkateswarlu B, Shanker, AK. (2012) Dryland agriculture bringing resilience to crop production under changing climate. In: Crop

Stress and its Management: Perspectives and *Strategies. Springer, Netherlands,* 19-44.

Vervuren, P., Blom, C.W.P.M., De-Kroon, H.(2003). Extreme flooding events on the Rhine and the survival and distribution of riparian plant species. *J. Ecol.* (2003), 91: 135–146.

Youssef, MS., Mira, MM., Renault, S. Hill, R.D. and Stasolla, C. (2016). Phytoglobin expression influences soil flooding response of corn plants. Ann. Bot. 118: 919–931.

**Biotech Today (January-June 2023) : 13 (1) : 14 - 16**

DOI : 10.5958/2322-0996.2023.00002.9

**RESEARCH ARTICLE**

# Investigating Protein-Ligand Interactions for Crystal Structure of NSP15 Endoribonuclease from SARS CoV-2 against Caffeine

**Keshav Singh [*], Vivek Kumar Srivastava [**] and Ashish Shukla [**]**

**Received**: 06 September, 2022, **Revised**: 08 November, 2022, **Accepted**: 6 December, 2022, **Published**: 1 January 2022

## Abstract

"SARS-CoV-2, the new corona virus that caused the COVID-19 pandemic, has complicated origins and important ramifications. It most likely started in bats, maybe via an intermediary host, and quickly developed, giving rise to many viral strains. (Florian, 2022) Global health and economies are impacted by a wide range of clinical symptoms caused by COVID-19, ranging from minor to severe. This study investigates the binding capability of SARS-CoV-2 against caffeine. In response to this unprecedented global threat, it seeks to improve our understanding of the virus in order to develop stronger pandemic preparedness and response tactics. (Mustafa, 2020) This work offers valuable insights for combating SARS CoV-2 and improving treatment strategies by knowing potential anti- SARS CoV-2 agents specifically against Crystal Structure of NSP15 *Endoribonuclease* from SARS CoV-2. (Takahiko, 2020)

**Keywords:** Bioinformatics, evolution, Hypothesis, Phylogenomics, Tree.

## Introduction

SARS-CoV-2, or Severe Acute Respiratory Syndrome Coronavirus 2, is a highly contagious virus responsible for the COVID-19 pandemic. It belongs to the Coronavirus family and primarily spreads through respiratory droplets. The virus's genetic makeup consists of single-stranded RNA, and it infects human cells by binding to ACE2 receptors. SARS-CoV-2 causes a wide range of symptoms, from mild respiratory issues to severe pneumonia and can lead to fatal outcomes, especially in vulnerable populations. Effective preventive measures include vaccination, mask-wearing and social distancing. The global response to SARS-CoV-2 has led to unprecedented public health challenges and significant scientific research to combat the pandemic. The urgent need for effective, safer and more accessible anti- SARS CoV-2 drugs is evident, necessitating innovative approaches to drug design and development.

This research focuses on a crucial aspect of anti- SARS CoV-2 development: understanding and harnessing protein-ligand interactions. Such interactions are at the core of drug action, as they determine the binding affinities, binding modes and ultimately, the efficacy of drug candidates. By comprehensively investigating these interactions, we aim to contribute to the development of new, potent and targeted anti-SARS CoV-2 agents.

## Material and methods

Protein and ligand selection

Drug development, protein-protein interaction analysis and numerous other areas of molecular research have all been transformed by molecular docking, a computer

*Assistant Professor, **Professor, Department of Biotechnology Raja Balwant Singh Engineering Technical Campus, Bichpuri, Agra

***Corresponding Author:** Keshav037@skuastkashmir.ac.in

technique used to predict the binding patterns and affinities of small molecules or ligands with target proteins. This potent tool is crucial in the creation and development of innovative therapeutic medicines as well as in the molecular understanding of basic biological processes. However, a vital first step—the choice of an appropriate protein structure—heavily influences the accuracy and dependability of docking experiments. (Shengyou, 2015)

The landscape of protein-ligand interactions is extremely complicated and diverse and choosing the appropriate protein structure is crucial to gaining biologically pertinent information. (Olivier, 2010) Multiple factors are taken into account during the selection process, including the protein conformation chosen (for example, based on crystallographic structures, homology models or experimental data), the inclusion of pertinent cofactors and ligands and the consideration of post-translational modifications or mutations that may affect the conformation and binding affinity of the binding site. In this study, we examine the numerous parameters and the crucial role of protein selection in docking investigations.

## Molecular docking

With broad applications in drug development and structural biology, molecular docking is a crucial computational technique for predicting the binding interactions between proteins and small molecules. Our study offers a concise but thorough approach for carrying out protein molecular docking, including essential procedures and recommended practices to guarantee solid and trustworthy outcomes.

Preparation of the ligand and protein, which includes active site definition and shape optimization, is the first step in the procedure. The creation of a binding site grid directs docking algorithms, which investigate ligand conformations and rank binding poses using a variety of search techniques and scoring systems. To effectively predict the action of ligands, flexibility and conformational sampling are essential.

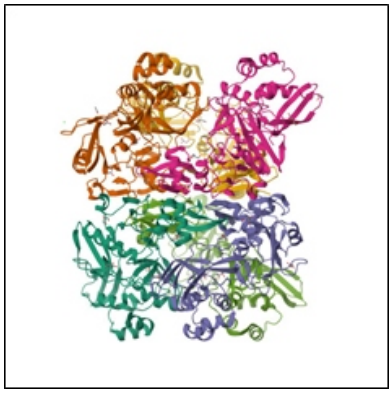


**Fig**: - **Crystal Structure of NSP15 *Endoribonuclease* from SARS CoV-2**

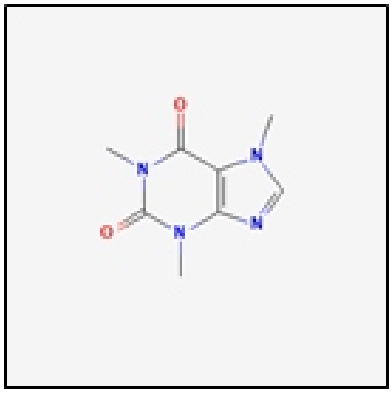



**Fig:- 2D structure of Caffeine**

Crystal Structure of NSP15 Endoribonuclease from SARS CoV-2 structure was obtained from PDB database and the Caffeine was obtained from PubChem (ID 2519) (NCBI, 2023)

## Result

During the molecular docking simulations, the results obtained demonstrate interactions yet less promising between the ligand and target protein, suggesting potential binding affinity and confirming the feasibility of further in-depth studies for drug discovery and protein-Ligand interaction analysis.

**Table:1- RMSD Table for binding between Crystal Structure of NSP15 *Endoribonuclease* from SARS CoV-2 and Caffein**

| Rank | Sub Rank | Run | Binding Energy | Cluster RMSD | Reference RMSD |
|---|---|---|---|---|---|
| 1 | 1 | 4 | - 4.69 | 0.00 | 66.62 |
| 2 | 1 | 7 | -4.19 | 0.00 | 80.19 |
| 2 | 2 | 1 | -4.16 | 0.27 | 80.34 |
| 3 | 1 | 3 | -4.10 | 0.00 | 79.89 |
| 3 | 2 | 8 | -4.08 | 1.24 | 79.82 |
| 4 | 1 | 10 | -4.10 | 0.00 | 81.33 |
| 4 | 2 | 2 | -4.08 | 1.57 | 80.68 |
| 5 | 1 | 5 | -4.05 | 0.00 | 80.40 |
| 6 | 1 | 6 | -3.98 | 0.00 | 55.08 |
| 7 | 1 | 9 | -3.96 | 0.00 | 91.53 |

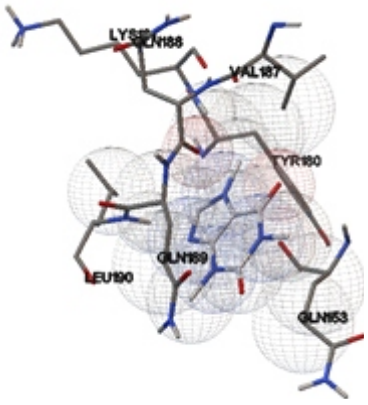



**Fig:- Interaction between NSP15 *Endoribonuclease* from SARS CoV-2 and Caffeine**

## Conclusion

The study provides a clear road map for protein molecular docking, particularly for Crystal Structure of NSP15 Endoribonuclease from SARS CoV-2 assisting researchers in rapidly and effectively using this potent technology in their structural biology and drug development projects. Researchers may improve the repeatability and dependability of their docking investigations by the knowledge of molecular interactions and hastening the process of developing new drugs.

## References

Florian. K. (2020) SARS-CoV-2 vaccines in development. *Nature* 586: 516-527.

Mustafa, H., Kilic, S. and Saraç. F. (2020) Coronaviruses and sars-cov-2. (9) *Turkish journal of medical sciences* 50: 549-556.

Takahiko, K., Platt, D. and Parida. L. (2020) Variant analysis of SARS-CoV-2 genomes. *Bulletin of the World Health Organization* 98, 7: 495.

Di, W. Wu, T. Liu, Q. and Yang. Z. (2020) The SARS-CoV-2 outbreak: what we know. *International journal of infectious diseases* : 44-48.

Wenjie, C., Tian, Y., Li, Z. Zhu, J. Wei, T. and Lei. J. (2021) Potential interaction between SARS-CoV-2 and thyroid: a review." *Endocrinology* 162 (3) : bqab004.

Kumar, V., Jung, and Liang. PH (2013) Anti-SARS coronavirus agents: a patent review (2008–present). *Expert opinion on therapeutic patents* 23 (10): 1337-1348.

Sheng-You. H. (2015) Exploring the potential of global protein–protein docking: an overview and critical assessment of current programs for automatic ab initio docking. *Drug discovery today* 20: 969-977.

Olivier, S, Eulalie L. Pinto, Villoutreix, BO. Perahia, D. and Miteva MA.. (2010) How to choose relevant multiple receptor conformations for virtual screening: a test case of Cdk2 and normal mode analysis. *European Biophysics Journal* 39: 1365-1372.

Jeffrey JG., (2006) High-resolution protein–protein docking. *Current opinion in structural biology* 16: 183-193.

Nataraj SP., Syed, K. and Tuszynski. J. (2017) Software for molecular docking: a review. *Biophysical reviews* 9: 91-102.

McConkey, Brendan J., Sobolev, V. and Edelman. M. (2002) The performance of current methods in ligand–protein docking. *Current Science* 845-856.

Csaba, H. and van der Spoel. D. (2006) Blind docking of drug-sized compounds to proteins with up to a thousand residues. *FEBS letters* 580 (5): 1447-1450.

National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 2519, Caffeine. Retrieved September 16, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Caffeine.

**Biotech Today (January-June 2023) : 13 (1) : 17 - 22**

DOI : 10.5958/2322-0996.2023.00003.0

**RESEARCH ARTICLE**

# Optimization of Pectinase and Cellulase Production from Microorganisms Isolated from Agriculture Waste

**Aditya Pathak, Akriti Gaurav, Pankaj Chauhan, Neelesh Kapoor and R. S. Sengar**

**Received**: 05 August, 2022, **Revised**: 12 October, 2022, **Accepted**: 4 November, 2022, **Published**: 1 January 2023

## Abstract

*Bacillus subtilis* and *Pseudomonas fluorescens* were isolated as the pectinase and cellulase-producing bacteria from agricultural waste. Optimization of the fermentation medium for maximum pectinase and cellulase production was carried out. The culture conditions like pH, temperature, inoculum age and raw substrate were optimized. The optimum conditions found for pectinase and cellulase production were 37°C at pH 7 with agricultural substrate stimulates the production of pectinase and cellulase. Among bacteria, *Pseudomonas fluorescens* is the best cellulase and *Bacillus subtilis* is the best pectinase producer.

**Keywords**: *Bacillus subtilis, Pseudomonas fluorescens, pH.*

## Introduction

The term "Agricultural waste" refers to undesirable trash generated as a result of agricultural activity. Agricultural solid wastes are usually produced from the agricultural activities involving preparation, production, processing and consumption of agricultural produce, livestock and their products. Due to issues with the lack of facility and the electrical supply, farming operations generate agricultural waste. Human population increases day by day and growing human population has made higher agricultural production necessary. It has been estimated that agricultural output has increased by more than three times over the past 5 decades. According to the Indian Ministry of New and Renewable Energy, India produced 500 million tons of agricultural waste, out of which 92 metric tons were burned each year that causes severe environmental pollution by producing a large amount of greenhouse gases (Jain *et al*., 2020). The major components of these residues are cellulose, pectin, and lignin. Cellulose is a linear polymer made of D-glucose that is joined by 1,4-glucoside linkages (Lynd *et al.,* 2002). Enzymatic hydrolysis is a key method for turning agricultural, wastes into valuable products. Agricultural wastes including wheat straw, rice bran, sugarcane bagasse, vegetables wastes are the cheapest and most readily available natural carbon sources for the manufacture of industrially significant enzymes. Different kind of microbes from agricultural wastes have been used to manufacture countless enzymes that have a wide range of uses in industrial operations for food, medicine, textile and dye usage. It is quite possible to decrease manufacturing costs and increase the usage of enzymes for industrial applications by using agricultural wastes (Bharathiraja *et al.,* 2017). A significant amount of the need for industrial enzymes worldwide is met by microbial cellulases and pectinases which are used in a variety of industries. To increase the production and lower the cost of cellulase and pectinase processing processes, superior

College of Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

*Corresponding Author: aditya.adityapathak99@gmail.com

bioprocesses are being created. Cellulose is the principal constituent of the cell wall of most terrestrial plants. These form the structurally strong framework in the cell walls. Despite a worldwide and enormous utilization of natural cellulosic sources, there are still abundant quantities of cellulosic sources and there are still abundant quantities of cellulose containing raw materials and waste products that are not exploited or which could be used more efficiently. The problem in this respect is, however, to develop processes that are economically profitable. Complete hydrolysis of the enzyme requires synergistic action of 3 types of enzymes, namely, cellobiohydrolase, endoglucanase or carboxymethylcellulase (CMCase), and beta-glucosidases. Cellulase acts collectively to hydrolyse cellulose from agricultural waste to produce simple glucose units (Bhat *et al.,* 2000). This cellulose-degrading enzyme can be used, for example, in the formation of washing powders, extraction of fruit and vegetable juices, and starch processing (Camassola *et al.,* 2007).

Along with pectin other polysaccharides such as cellulose and xylan type polysaccharides strengthen the structure of cell-walls in the flesh of fruits (Beatriz and Fabrice 2012). Pectinases are the group of enzymes that catalyzes the degradation of pectic substances through de-esterification reactions (Pedrolli *et al.,* 2009). Fruit waste is the best source of pectin. In recent years, bacteria have emerged as a significant source of pectinolytic enzymes, producing a variety of sets of enzymes that aid in the general breakdown of pectin substrates. There are different low-cost substrates like rice straw, fruit waste and sugarcane molasses which is used for cost-effective production of the cellulase and pectinase enzyme by fermentation techniques. The present work was carried out to optimize the nutritional and environmental parameters for improving cellulose production by microorganisms.

## Materials and Methods

**Sample Collection:** A total of 24 samples comprising agricultural waste, decaying agricultural waste and fruit waste were collected from different regions of Meerut and Ghaziabad for the isolation of cellulase and pectinase producing microbial strains. The samples were transported to the Bioremediation Laboratory, Division of Plant Biotechnology, College of Biotechnology, SVPUA and T, Meerut in properly labelled plastic bags.

**Enrichment of the Agriculture wastes sample:** In a sterile beaker containing 100 grams of samples, 1 gram of cellulose and 5ml of water were added to the agricultural waste, and 1 gram of pectin and 5 ml of water were added to the fruit waste.

### Isolation and Screening of Microorganisms

One gram of pectin-enriched agricultural waste from each collection site were pooled and homogenized in sterile distilled water and 10-fold serial dilutions were prepared. 1 ml aliquots from each dilution were inoculated by spread plate method on to the sterile petriplates containing yeast extract pectin (YEP) medium with pH 7.2, containing pectin 2.5gm and yeast extract 5.0 gm at 37°C for 24 hours (Oumer *et al.,* 2018). The clearing areas of the medium after the addition of Logule's iodine solution were used to classify pectinase secretion producers and the colony with maximum zone diameter was preceded for further studies.

One gram of cellulose-enriched agricultural waste from each collection site were pooled and homogenized in sterile distilled water and 10-fold serial dilutions were prepared. 1 ml aliquotes from each dilution were inoculated by spread plate method on to the sterile petriplates containing CMC agar media with pH 7.2 containing $NaNO_3$ 0.2 gm, $K_2HPO_4$ 0.05 gm, $MgSO_4.7H_2O$ 0.02 gm, $MnSO_4.H_2O$

0.002 gm, $FeSO_4.7H_2O$ 0.002 gm, $CaCl_2.2H_2O$ 0.002 gm, CMC (1%) 1 gm, Agar 2gm, Distilled water 100 ml at 37 °C for 24 hours. To visualize the hydrolysis zone, the plates were flooded with an aqueous solution of 0.1% Congo red for 15 min and washed with 1M NaCl (Apun *et al.,* 2000) and the colony with maximum zone diameter was preceded for further studies. Pure cultures were sub cultured onto slant media and maintained for identification and enzyme studies.

Besides, a more quantitative assay method was used to determine the cellulose activity of the selected bacterial isolate in liquid medium. The cellulase activity of each culture was measured by determining the amount of reducing sugars liberated by using a DNS method (Miller *et al.,* 1959). A bacterial isolate with the highest activity was selected for optimization of Pectinase and cellulase production.

**Bacterial Identification.** The bacterial isolates were presumptively identified by means of morphological examination and some biochemical characterizations. The parameters investigated included colonial morphology, gram reactions, endospore formation, catalase production, VP reaction, indole production, starch hydrolysis, citrate utilization, and gelatine hydrolysis. The results were compared with Bergey's Manual of Determinative Bacteria (Gibbons *et al.,* 1974).

**Enzyme Production Medium.** Production medium contained (g/L) glucose 0.5 gm, peptone 0.75 gm, FeSO4 0.01 gm, $KH_2PO4$ 0.5 gm, and MgSO4 0.5 gm. Ten millilitres of medium were taken in a 100mL conical flask. The flasks were sterilized in autoclave at 121°C for 15 min, and after cooling, the flask was inoculated with overnight grown bacterial culture. The inoculated medium was incubated at 37°C in shaker incubator for 24 h. At the end of the fermentation period, the culture medium was centrifuged at 5000 rpm for 15 min to obtain the crude extract, which served as enzyme source.

**Enzyme Assay**. Cellulase and Pectinase activity was measured following the method of Miller (Janani *et al.,* 2011; Miller *et al.,* 1959). Briefly, a reaction mixture composed of 0.2 mL of crude enzyme solution plus 1.8 mL of 0.5% carboxymethyl cellulose (CMC) in 50 mM sodium phosphate buffer (pH 7) was incubated at 37°C in a shaking water bath for 30 min. The reaction was terminated by adding 3 mL of DNS reagent. The colour was then developed by boiling the mixture for 5min. OD of samples was measured at 575 nm against a blank containing all the reagents minus the crude enzyme.

**Process Optimization for Maximum Pectinase and Cellulase Production**

**pH.** Flasks with broth containing the optimum concentration of substrate and carbon source are taken and the pH of the broth is adjusted to 6.0, 6.5, 7.0 .7.5, 8.0, 8.5 and 9.0 in different flasks using 1N HCl and 1N NaOH and sterilized. The cultures are inoculated and incubated at particular temperature. At the end of incubation period, the cell-free culture filtrate is obtained and used as enzyme source.

**Temperature.** Production medium at pH 7.0 was inoculated with overnight grown selected bacterial strain. The broth was incubated at different temperatures from 28, 37, 40, 45 and 50 °C for 24 h. At the end of incubation period, the cell-free culture filtrate is obtained and used as enzyme source.

**Inoculum age:** Production medium at pH 7.0 was inoculated different inoculum age in the range of 18h, 24h, 36h and 48h for cellulase and pectinase enzyme production by selected strains. The broth was incubated at 37°C for 24 h. At the end of incubation period, the cell-free culture filtrate is obtained and used as enzyme source.

**Agricultural Waste Material**. To find out the suitability of agricultural waste as substrate for enzyme production, different

substrates, that is, rice straws, wheat straws, fruits wastes and sugarcane molasses are taken in the growth medium under submerged condition. The enzyme activity is measured after 24 h for enzyme production.

**Results and Discussion:** Pectinase and Cellulase-producing bacteria were isolated from agricultural waste. Based on the morphological and biochemical characteristics, the isolates were identified as *Pseudomonas fluorescens and Bacillus subtilus*

**Effect of pH.** Both isolates were allowed to grow in media of different pH ranging from 6.0 to 9.0. Maximum pectinase and cellulase activity were observed in medium at pH 7.0 in case of *Pseudomonas fluorescens and Bacillus subtilis,* (Figure 1). This result was in correlation with the finding of other workers for different *Bacillus subtilis* strains

(Bhagat *et al.,* 2021).

**Effect of Incubation Temperature.** Enzyme activity recorded at different temperatures revealed that all the four bacteria yielded maximum pectinase and cellulase production at 37°C (Figure 2). The temperature was found to influence extracellular enzyme secretion, possibly by changing the physical properties of the cell membrane. Optimum temperature for maximum growth of *Bacillus subtilis* was 37°C. These results are nearly close to those of Hoa *et al.,* 2013 who found that the enzyme produced by *Pseudomonas fluorescence* was activated at 35°C showing the optimum temperature at 35°C.

**Effect of inoculum age on enzyme production.** In our study, we also calculated the effect of inoculum age on enzyme production. From the different inoculum ages, such as 18, 24, 36 and 48 hours, we observed that 24 hrs was the best age of inoculums for both cellulase and pectinase activity.

**Effect of raw substrates on enzyme production.** The pectinase activity of *Bacillus subtilis* and cellulase activity for *Pseudomonas fluorescens* were higher with fruit waste and sugarcane bagasse respectively. With an appropriate substrate concentration, *Bacillus subtilis* produces maximum pectinase.

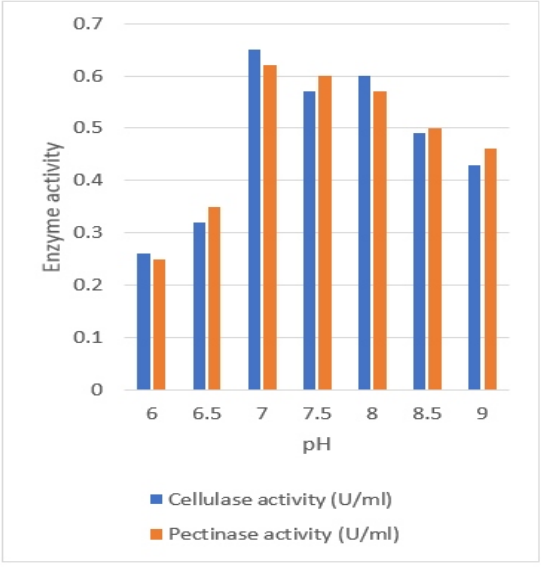



**Fig 1: Effect of pH on cellulase and pectinase activity.**

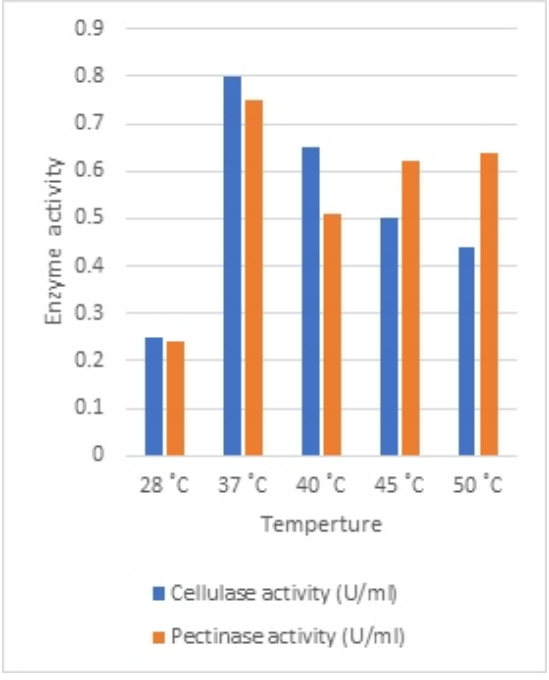



**Fig 2: Effect of temperature on cellulase and pectinase activity**.

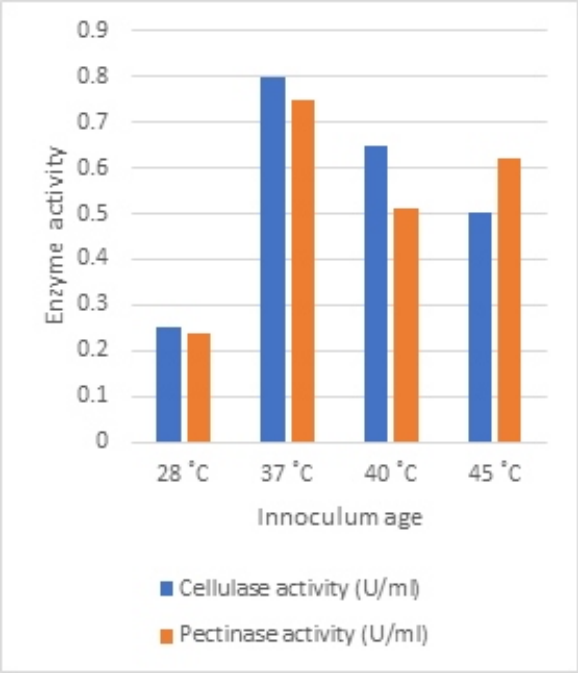



**Fig 3: Effect of inoculum age on cellulase and pectinase activity.**

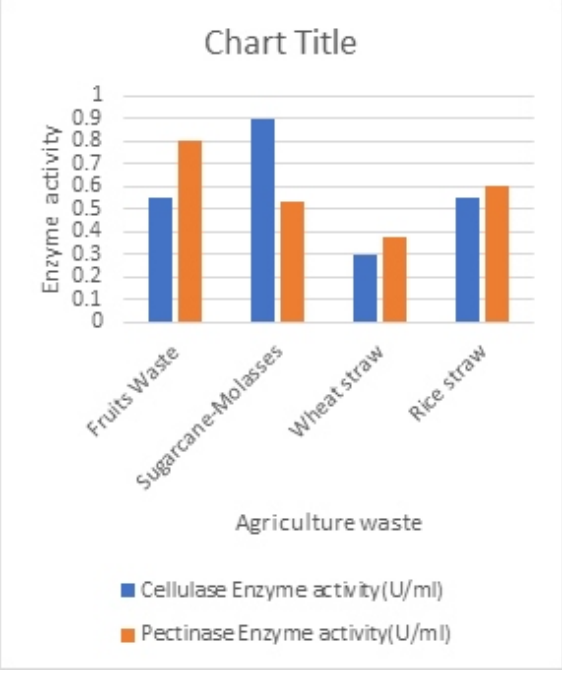



**Fig 4: Effect of raw substrate in cellulase and pectinase activity**

## Conclusion

The aim of the present work was to isolate and identify a pectinase and cellulase producer from agriculture waste. *Bacillus subtilis* and *Pseudomonas fulorescens* produced maximum yield of pectinase and cellulases respectively. The optimum temperature and pH were determined as 37°C and 7 pH and 24 hr inoculum age of culture. After optimization, the mass production was carried in one litre of optimized media at 37°C for 24 hrs at a pH of 7 on a rotary shaker at 200 rpm. Bacteria, which have high growth rate as compared to fungi, good potential to be used in cellulose production. However, the application of bacteria in producing pectinase and cellulase is not widely used.

Pectinase and Cellulase yields appear to depend on a complex relationship involving a variety of factors like inoculum size, pH value, temperature and substrate, and so forth (Immanuel *et al.,* 2006).

Further studies were in progress in the purification and application of cellulase in different commercial fields. The purified pectinase and cellulase can be used for various purposes in detergent industries, food industries and pharmaceutical industries. The high activity and stability of pectinase and cellulase enzymes between neutral to alkaline pH and high temperature will be of use in various industrial and biotechnological applications.

## References

Apun, K., Jong, BC. and Salleh, MA. (2000). Screening and isolation of a cellulolytic and amylolytic Bacillus from sago pith waste. *The Journal of general and applied microbiology*, 46(5): 263-267.

Beatriz B, and Fabrice V. (2012). Enzymatic liquefaction of cell-walls from kent and tommy atkins mango fruits. *Int J Food Sci Nutr Eng* 2(5): 76–84

Bhagat, SA., and Kokitkar, SS. (2021). Isolation and identification of bacteria with cellulose-degrading potential from soil and optimization of cellulase production. *Journal of Applied Biology and Biotechnology*, 9(6): 154-161.

Pedrolli, DB., Monteiro, AC., Gomes, E., and

Carmona, E C. (2009). Pectin and pectinases: production, characterization and industrial application of microbial pectinolytic enzymes. *Open Source.*

Jansová, E., Schwarzová, Z., and Chaloupka, J. (1993). Sporulation and synthesis of extracellular proteinases in Bacillus subtilis are more temperature-sensitive than growth. *Folia microbiologica*, 38(1), 22–24

Miller, GL. (1959). Study Of Variables. *Analytical Chemistry*, *31*(3): 426-428.

Gibbons, NE., and Buchanan, RE. (Eds.). (1974). *Bergey's manual of determinative bacteriology*. Williams and Wilkins company.

Hoa, BT., and Hung, PV. (2013). Optimization of nutritional composition and fermentation conditions for cellulase and pectinase production by Aspergillus oryzae using response surface methodology. *Internationl Food Research Journal*, 20(6): 3269.

Immanuel, G., Dhanusha, R., Prema, P., and Palavesam, AJIJ. (2006). Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. *International Journal of Environmental Science and Technology*, *3* :25-34.

Jain, R., and Naik, SN. (2020). Sustainable Management of Agricultural Waste in India. *Handbook of Solid Waste Management: Sustainability through Circular Economy*, 1-26.

Janani, LK., Kumar, G., and Rao, KB. (2011). Production and Optimization of Pectinase from Bacillus sp. MFW7 using Cassava Waste. *Asian Journal of Biochemical and Pharmaceutical Research*, 1(2): 329-336.

Lynd, LR., Weimer, PJ., Van Zyl, WH., and Pretorius, IS. (2002**).** Microbial cellulose utilization: fundamentals and biotechnology. *Microbiology and molecular biology reviews*, 66(3): 506-577.

Camassola, M., and Dillon, AJP. (2007). Production of cellulases and hemicellulases by Penicillium echinulatum grown on pretreated sugar cane bagasse and wheat bran in solid-state fermentation. *Journal of Applied Microbiology*, 103(6): 2196-2204.

Bhat, M. (2000). Cellulases and related enzymes in biotechnology. *Biotechnology advances*, *18*(5): 355-383.

Miller, GL. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical chemistry*, *31*(3): 426-428.

Oumer, OJ., and Abate, D. (2018). Screening and molecular identification of pectinase producing microbes from coffee pulp. *BioMed research international*,

John, SE. (1996). "An Industrial Application of Cellulases", Journal of Biotechnology and Bioengineering. Press syndicate of the University of Cambridge, Third Edition. 68-83.

Wu, M., Zhang, L., Li, D., Wang, Y., Zhang, Z., and Mao, Z. (2008). Conditions Study of Cellulase and Acid Protease Production during the Process of Solid-state Fermentation of Flaxseed Meal. In *2008 Providence, Rhode Island, American Society of Agricultural and Biological Engineers.*

**Biotech Today (January-June 2023) : 13 (1) : 23 - 30**

DOI : 10.5958/2322-0996.2023.00004.2

**RESEARCH ARTICLE**

# Zinc induced Oxidative stress and hepatoarchietectural changes in fresh water fish *Channa punctatus*

**Abha Trivedi [*1], Jumman Bakhasha [2], Kamlesh K. Yadav [3] , Vaishnavi Saxena [4], Neeti [5] and Swaika Yadav [6]**

**Received**: 14 July, 2022, **Revised**: 18 October, 2022, **Accepted**: 22 November, 2022, **Published**: 1 January 2023

## Abstract

Numerous biological functions in living creatures involve the significance of Zinc due to its participation in various metabolic activities. It is advantageous when present in traces but becomes noxious when exists in copious amounts. The present study was conducted to assess the deleterious effects of Zinc on fish *Channa punctatus* exposed to the maximum permissible limit (5 mg/l) and two-fold of the maximum permissible limit (10 mg/l) for 15, 30 and 45 days. ROS levels were significantly ($p<0.05$) elevated in a dosage- and duration-dependent manner. Increased ROS resulted in the raised oxidative stress that was specified by the unusual activities of anti-oxidant biomarkers. The activities of enzymatic biomarkers (SOD, CAT and GR) were significantly ($p<0.05$) increased while the activity of non-enzymatic biomarker (GSH) was significantly ($p<0.05$) reduced in treated fish. Zinc intoxication also resulted in severe damage to the histology of the liver. For the reason that information about Zinc toxicity on and above the maximum permissible limit in the Rohilkhand region was not available, the present study was outlined to fill up the prevailing research gap.

**Keywords:** Zinc, ROS, oxidative stress, histopathology, *C. punctatus*

## Introduction

Zinc ($Zn^{+2}$) is essential for the physiological functioning of all living organisms in small quantities but it is toxic in higher concentrations . It enters into the water bodies through anthropogenic activities viz., alloying, mining, electroplating, ceramics, and mostly through industrial effluents. Higher concentrations of zinc decline aquatic fauna and flora. In the aquatic food chain, fishes are at the top of the trophic level and respond quickly under xenobiotic stress. Respiratory anguish, due to damage to the gill or accumulation of heavy mucous is one of the evident symptoms in fish acutely exposed to $Zn^{+2}$. This desperately affects fish in addition to those who consume them indirectly or directly through the food chain . $Zn^{+2}$ causes loss of appetite, reduced growth, and immunological abnormalities in fish . It is used as an active element of several enzymes for many metabolic reactions, it is toxic when present in elevated concentrations .

Zinc-exposed fish can lead to various genotoxicological, and biochemical abnormalities and bioaccumulation in the kidney and liver which are the primary sites of biotransformation with excretion and detoxification respectively . Many studies have worked on the adverse effect of Zinc accumulation in many tissues of fish exposed to elevated concentrations of Zinc . Further, zinc arbitrated reactive oxygen species (ROS)

[1]Toxicogenomics Laboratory, Department of Animal Science, M.J.P.Rohilkhand University, Bareilly-243006, India.

[3]Department of Zoology, Government Degree College, Unnao-209801, India

*Corresponding Author: abha14sep@skuastkashmir.ac.in

cause lesions viz., pyknosis, hypertrophy, vacuolization, and inflammation in liver tissues of the test fish.

Zinc expounded the adverse effects on the cytoplasmic proteins of skeleton cells in fish . Prolonged exposure to Zinc generates oxidative stress by overproduction of ROS through diminishing of equilibrium dynamics of oxidative metabolism . Oxidative stress and damage of biomolecules, viz., DNA and protein leading to physiological agitation in heavy metal exposed fish has been already documented . Despite elevated concentration and chronic exposure of zinc being predictable in the aquatic body on account of established industrial operations, only less information is available regarding genotoxicological and biochemical anomalies in the Rohilkhand region in fish *Channa punctatus*.

Fish loaded with elevated amounts of zinc are discomfort for human consumption. To understand the eco-toxicological mechanism of zinc mediated level of ROS, oxidative stress in terms of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and glutathione reductase (GR), histopathological lesions and bioaccumulation in the liver of fish *C. punctatus*.

In the present investigation, freshwater, edible fish *Channa punctatus* has been chosen as a test model because in the aquatic food chain fishes occupy the highest trophic level, and are quite susceptible to sudden changes in the physico-chemical characteristics of water. Therefore, they are good bio-indicators for aquatic pollution. They are economically, biologically, and ecologically important. Moreover, they are highly susceptible to even a low concentration of toxicants.

## Materials And Methods:

**Fish acclimatization and experimental design:** Test fish *C. punctaus* (34 ± 3.0 g; 14.7 ± 1.0 cm), were caught by local fishermen from natural habitats of Rohilkhand region and brought to the laboratory in the well-aerated large containers. Thorough washing of fish was carried out with tap water and for the elimination of external infections (if any were present), fish were treated with 0.05% $KMnO_4$. Fish were transferred to glass aquaria according to their weight which was 4 g fish per liter of water were used during acclimatization as per guidelines of OECD, 2019. Pellets of fish food (Perfect Companion Group Company Limited, Thailand) were used to feed the fish two times, morning and evening in a day, during the period of acclimatization . The nurturing of fish throughout the complete experiment in the laboratory was done by standard protocols (APHA *et al*., 2012). After 10d acclimatization, 120 acclimatized fish were randomly divided into three groups (one control 'GI' and two treated groups, GII and GIII). Three replicates of each group having 10 fish. For GII, the exposure concentration was the maximum permissible limit (PL) of 5 mg/L according to the Central Pollution Control Board (CPCB), New Delhi, United States Environmental Protection Agency (USEPA), and Bureau of Indian Standards (BIS) and for GIII double of PL 10 mg/L. For all sets, GI, GII, and GIII fish were loaded for 15, 30, and 45 d of exposure duration. After each exposure period, fish were euthanized with 0.01% diethyl ether before sacrificing them for the evaluation of ROS in erythrocytes, GSH level, the activity of SOD, CAT, and GR in the tissues of the liver, and histopathological alterations in the Liver of the test animal.

**Test chemical:** Test chemical, $ZnSO_4.7H_2O$, cas no. 7446-20-0 of Himedia, Maharashtra, India was procured from the local dealer of Bareilly district.

**ROS measurement in fish erythrocytes:** To examine the ROS level in fish erythrocytes, a fluorescent dye, 2´, 7´-dichlorodihydrofluorescein (20 ìM, DCFH-DA; Sigma Aldrich, USA) was used. Fish blood was kept to be incubated with DCFH-DA for 30 min. The preparation of slides was done after incubation in the dark. Afterward, the

measurement of the intracellular fluorescence was carried out by using Fluorescence (Floid™ cell imaging station, Life Technologies) at excitation and emission wavelengths 482nm and 532nm, respectively with 10/40X magnification of the objective lens. Image J software (version 1.50, USA) to quantify the fluorescent intensity.

**Estimation of biochemical parameters in the liver:**

**Cell lysate:** After each exposure period, fish were dissected and liver tissue was taken out. Tissue samples were homogenized in homogenization buffer (HB) in the ratio of 1:10 w/v. The samples were centrifuged at 3000 rpm for 10 minutes at 4°C. 500ul Lysis buffer, 10 l of Dithiothreitol (DTT), and Phenylmethanesulfonyl fluoride (PMSF) were added and the complete suspension was kept at 30 min for incubation. Afterward, collecting cell lysate after centrifugation at 4°C for 15 min was executed at the rate of 12,000 rpm, and assessed the antioxidant enzymes. All the antioxidant enzymes were measured by the UV-VIS spectrophotometer (Shimadzu, UV-1900i) against blank.

**Superoxide-dismutase (SOD):** method was performed for the estimation of SOD in the liver. The constituents of the reaction mixture were 1.2 mL sodium pyrophosphate buffer (0.052 mM, pH 8.3), 0.1 mL phenazine methosulphate (PMS), 0.3 mL (1M) nitroblue tetrazolium chloride (NBT), and 0.2 mL of sample and incubate for 5 min at 36 °C. further incubation was done by 0.2 mL (1M) nicotinamide adenine dinucleotide (NADH) and the addition of glacial acetic acid, after incubation. The activity of SOD is measured in Units/min/mg of protein.

**Catalase(CAT):** CAT activity was measured by the method of . The reaction mixture was made by 1 mL of 20 mM $H_2O_2$ and 1.88 mL of 50 mM sodium phosphate buffer (pH 7.0) with 100 l of homogenate. CAT activity was done based on assessing the decomposition rate of $H_2O_2$ for the duration of 1 min at the absorbance of 240nm.

**Reduced glutathione (GSH):** GSH estimation was done by With some modifications. The process of assessing GSH in sample tissues like the gill and muscle includes the reaction of tissues with DTNB (5,5 dithio, 2-nitrobenzoic acid), also known as action and as a result of this reaction compound is produced that shows the absorbance of light at 412nm. A mixture of DTNB and T.E buffer was put into an incubator for incubation with 100 l of tissue homogenate. Absorbance was taken at 412nm.

**Glutathione reductase(GR):** GR activity was done by the method of . The mixture of 600 l of buffer (0.1 M potassium phosphate + 0.5 mM EDTA + 0.1 mM KCl; pH 7.5), 100 l of 0.1 mM NADPH, 100 l of $H_2O$ was taken and the supernatant of gill and muscle tissues was added in 100ul quantity separately in the mixture for reaction and incubate for 5 min at 37°C. Reaction initiated with the addition of 100 l of 1mM GSSG. Absorbance was taken at 340nm and measured in Units/min/mg.

**Histopathological alteration in the liver tissue:** After a 45d exposure period liver tissue was taken out and washed with distilled water. Fixation of tissues was done in 10% neutral buffered formalin (NBF) solution for 48 h. To remove the moisture content, tissues were executed with a graded series of ethanol and cleaned with xylol. Subsequently, to obtain blocks, the liver was inserted in paraffin wax. Blocks were kept overnight at room temperature. To get sections (1mm) of the tissue sample, the instrument used was a Microtome (YSI062 Yorco Precision Rotary Microtome, India). Hematoxylin stain was applied for 1 min to do the counterstaining, and eosin stain was used for the duration of 2 min. The mounting of sections was done in DPX after they were processed properly. Afterward, an oil immersion microscope (Nikon Corporation K-12,432) with an

objective lens of 10/40X magnification was used for capturing the photographs.

**Data analysis:** The significance ($p < 0.05$) was analyzed with a one-way analysis of variance (ANOVA) with Tukey's post hoc test using SPSS software (version 20.0, SPSS Company, Chicago, USA) for each result. All the data are presented as mean ± standard error mean (S.E.M.).

## Results

### Physiochemical parameters

In the test medium of all the groups GI, GII, and GIII, the physicochemical parameters viz., pH 7.29, temperature (°C) 25.33, dissolved oxygen (DO) 7.43mg/L, alkalinity 72.10mg/L $CaCO_3$, and hardness 78.21mg/L $CaCO_3$ were recorded before and after completion of the exposure period.

**Estimation of ROS generation:** The level of ROS in erythrocytes of fish was found significant ($p<0.05$) in Zn-exposed groups. The maximum elevation of ROS production was to be found in G III at 45 dyas compared to the control. Microphotograph (Fig. 1a) showed the differences in ROS level and the quantitated levels of ROS in blood cells of test fish were shown in Fig. 2b.

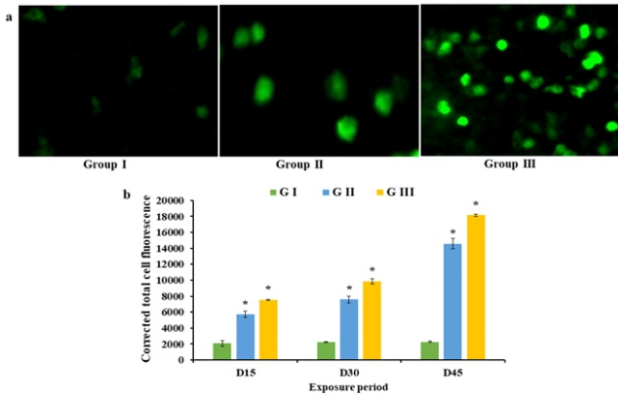



**Fig 1a Microphotograph showing the fluorescent intensity of ROS level was measured by DCFH-DA dye (b) showed the corrected total cell fluorescence induced by Zn. (mean ±SD, n=3 fish which were taken in triplicates). (* symbol represents the significant ($p<0.05$) difference from the control).**

**$Zn^{+2}$ induces super-oxide dismutase (SOD):** There was a significant ($p<0.05$) increased SOD activity in the liver with increasing concentration of $Zn^{+2}$ as compared to the control group. The maximum elevation of SOD activity was found in group III at 45d in fig-2a.

**$Zn^{+2}$ induces catalase activity (CAT):** There was a significant ($p<0.05$) increased activity in the liver of catalase with increasing concentration of $Zn^{+2}$ as compared to the GI. The maximum activity of catalase was found to be high in group III at 45d in fig-2b.

**$Zn^{+2}$ diminishes non-enzymatic antioxidant defense:** Fig -2c indicates the contents of GSH in the fish liver. There was a significant ($p<0.05$) decrease in the GSH content in increasing the concentration of $Zn^{+2}$ as compared to the GI. The minimum level of GSH activity was found in GIII at 45d.

**$Zn^{+2}$ alters the glutathione reductase (GR) activity:** Fig. 2d indicates the GR activity in the liver of fish *C. punctatus*. There was a significant ($p<0.05$) increase in GR activity in increasing the concentration of $Zn^{+2}$. The highest elevation was found in GIII at 45d.

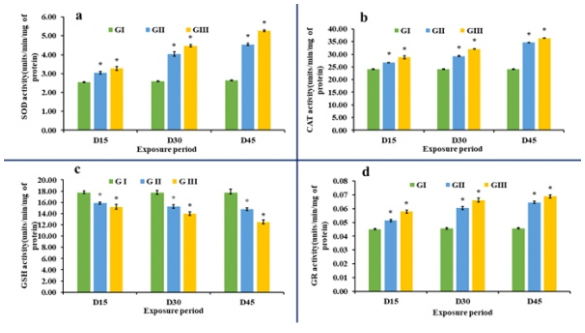



**Fig- 2 The activities of SOD, CAT, GSH, and GR in control and Zn-exposed groups in the liver of fish *C. punctatus* for 15, 30, and 45 d exposure periods. (mean ±SD, n=3 fish which were taken in triplicates). (* symbol represents the significant ($p<0.05$) difference from the control).**

**$Zn^{+2}$ affects the histological architecture of the liver:** Zinc-exposed test fish *C. punctatus* shows histological alterations in the liver after 45d exposure period. Fig.3 represents the histological findings of the liver of fish for control and exposed groups. No damages were found in GI, whereas pyknosis (PN), vacuolization (V), inflammation (In), and necrosis (N) were found in treated groups. Maximum deformities were found in GIII as compared to the control.

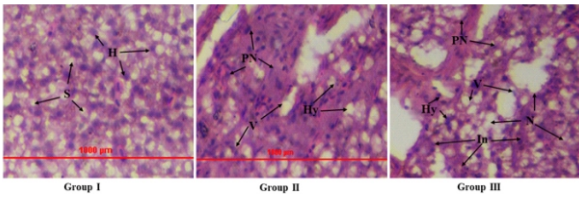



**Fig 3- Microphotographs of untreated fishes showing normal hepatocytes (H) and sinusoids (S) in the liver. Fishes exposed to Zn showed vacuolization (V), pyknotic nuclei (PN), inflammation (In), and hypertrophy (Hy) in the liver.**

**Table 1. The statistical study of histological alteration in the liver of Zn exposed groups at 45d in fish *C. punctatus*.**

| Parameters | Group I | Group II | Group III |
|---|---|---|---|
| Vacuolization (V) | - | 1x | 3x |
| Pyknotic nuclei (PN) | - | 1x | 4x |
| Inflammation (In) | - | 2x | 3x |
| Hypertrophy (Hy) | - | 1x | 2x |
| Necrosis | - | 2x | 3x |

None -, mild 1x, moderate 2x, strong 3x, 4x, high 4x

## Discussion

Zinc is the most critical and extremely important active element that is needed for the many metabolic reactions and growth of organisms. This micronutrient, however, shows toxicity when it is used in the highest concentration in vital organisms. It can become unsafe when it reaches the maximum permissible limit. The unpleasant effects can be evaluated by biochemical and molecular studies conducted on many vital organs of the fish. Among various aquatic creatures, *C. punctatus* is a fish that can show an accumulation of different heavy metals in its vital tissues. The liver, considered the most vital and bio-transformatory site of metals, where they develop numerous harmful effects. The present study examined the oxidative stress in the liver of fish exposed to zinc. The most consumable part of the fish body is muscle but it is not a good indicator of whole-body contamination like the liver because the liver is the most bio-transformatory site of the body which provides a more immediate evaluation of the current environmental levels of contamination.

ROS is paramount for the processes of physiology and cell signaling. Elevated ROS production and oxidative stress are pernicious to normal physiology. Zinc can raise ROS production in the liver . We initiated further studies on the generation of ROS levels, and the stimulation state of different antioxidant enzymes viz., CAT, SOD, GSH, and GR in the liver. In the liver, significantly ($p<0.05$) increased levels of ROS were observed. It has been recorded that other heavy metal intoxication boosts ROS production in the liver of mice . Similarly, ROS levels were found to be elevated in the liver of *C. punctatus*, when treated with dichlorvos and zinc.

SOD, CAT is the main baseline of defense against ROS. SOD converts superoxide radicals to $H_2O_2$ and CAT catalyzes this $H_2O_2$ to $H_2O$ and $O_2$. SOD-CAT have been regarded as crucial enzymes for stress tolerance and an increment in SOD-CAT activities is an indication of elevated oxidative stress tolerance in stressed organisms. This study showed that with time SOD-CAT activities increased continuously also studied an increase in SOD-CAT activity under copper stress in fish *C. punctatus* and three-spined stickle back.

Additionally, have similarly depicted the histological changes as the end-point marker for the assessment of harmful potential and hazard evaluation of heavy metals in the liver.

The present study shows histopathological alterations, viz., Vacuolization (V), Pyknosis (Py), Inflammations (In), Necrosis (N), and Hypertrophy (Hy) in the liver of the zinc-exposed groups GII and GIII (Subburaj *et al*., 2015; Radhakrishnan and Hemalatha, 2010).

## Conclusion

The outcome of the present study confirms that ROS and oxidative stress are induced by the maximum permissible limit and two times PL. To date, the mechanism of Zinc-induced oxidative damage and molecular damage in the liver tissue is not well clarified. In the present study decorative a comprehensive profile of zinc-induced toxicological manifestations in fish of extensive availability. A major outcome of this investigation includes zinc-induced oxidative stress in the prime site of biotransformation-liver in fish which leads to histopathological alterations when exposed to 5 mg/L and 10 mg/L. Therefore, results suggest that zinc accumulates in fish tissues through contaminated river water and might be transferred to humans via food chains. So, it needs to assess the water quality of the river and more research work is needed to study on gene level at the Rohilkhand region.

## Acknowledgment

We recorded our candid thanks to the Department of Higher Education, Research and Development, Government of Uttar Pradesh, India, for providing Research and Development project 2021-2022 vide grant no. 108/2021/2585/sattar-4-2021-4(28)/2021. We are also thankful to the Head, Department of Animal Science, M J P Rohilkhand University, Bareilly (243006) for providing laboratory facilities.

1. **Conflict of Interests:** The author declares that there is no conflict of Interest.
2. **Data availability:** In the present manuscript, given data was generated during the study.

## References

Aebi, H. (1984). Catalase *in vitro. Methods in Enzymology*, 105(C): 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

Avci, A., Kaçmaz, M., and Durak, I. (2005). Peroxidation in muscle and liver tissues from fish in a contaminated river due to a petroleum refinery industry. *Ecotoxicology and Environmental Safety,* 60(1): 101–105. https://doi.org/10.1016/j.ecoenv.2003.10.003

APHA, AWWA, WEF, (2012). Standard Methods for the Examination of Water and Wastewater, 22nd ed. APHA 800 I Street, NW, Washington, DC 20001-23710.

Awasthi, Y., Ratn, A., Prasad, R., Kumar, M., Trivedi, A., Shukla, J. P., and Trivedi, S P. (2019). A protective study of curcumin associated with Cr6+ induced oxidative stress, genetic damage, transcription of genes related to apoptosis and histopathology of fish, *Channa punctatus* (Bloch, 1793). *Environmental Toxicology and Pharmacology,* corred, 103209. https://doi.org/10.1016/j.etap.2019.103209

Awasthi, Y., Ratn, A., Prasad, R., Kumar, M., and Trivedi, S. P. (2018). An in vivo analysis of Cr6+ induced biochemical, genotoxicological and transcriptional profiling of genes related to oxidative stress, DNA damage and apoptosis in liver of fish, Channa punctatus (Bloch, 1793). *Aquatic Toxicology*, 200, 158–167. https://doi.org/10.1016/j.aquatox.2018.05.001

Burress, M. (1975). Development and Evaluation of On-site Toxicity Test Procedures for Fishery Investigations. *Investigation in Fish Control*, 68, 0–8.

Carlberg, I. and Mannervik, B. (1985). Glutathione reductase. *Methods in Enzymology,* 113(C): 484–490. https://doi.org/10.1016/S0076-6879(85)13062-4

Ganesan, J., and Karuppasamy, R. (2015). Pattern of Zinc Accumulation in Different Tissues of Freshwater Fish, Channa Punctatus (Bloch.) Under Long-Term Exposure. *International Journal of Modern*

*Research and Reviews,* 3(1): 595–598. http://journalijmrr.com/wp-content/uploads/2015/03/IJMRR-145.pdf

Gioda, CR., Lissner, LA., Pretto, A., da Rocha, J. BT., Schetinger, MRC., Neto, J. R., Morsch, VM., and Loro, VL. (2007). Exposure to sublethal concentrations of Zn(II) and Cu(II) changes biochemical parameters in Leporinus obtusidens. *Chemosphere,* 69(1):170–175. https://doi.org/10.1016/j.chemosphere.2007.04.008

Jaric, I., Višnjic-Jeftic, željka, Cvijanovic, G., Gacic, Z., Jovanovic, L., Skoric, S., and Lenhardt, M. (2011). Determination of differential heavy metal and trace element accumulation in liver, gills, intestine and muscle of sterlet (Acipenser ruthenus) from the Danube River in Serbia by ICP-OES. *Microchemical Journal,* 98(1), 77–81. https://doi.org/10.1016/j.microc.2010.11.008

Kumar, M., Gupta, N., Ratn, A, Awasthi, Y., Prasad, R., Trivedi, A., and Trivedi, SP. (2020). Biomonitoring of Heavy Metals in River Ganga Water, Sediments, Plant, and Fishes of Different Trophic Levels. *Biological Trace Element Research,* 193(2): 536–547. https://doi.org/10.1007/s12011-019-01736-0

Kumar, M., Ratna, A., Prashad, R., Trivedi, SP., Sharma, Y. K., and Shukla, A. K. (2015). Assessment of zinc bioaccumulation in fish Channa punctatus exposed chronically. Global Journal of Bio-Science and Biotechnology, 4(4): 347–355.

Kumar, M., Singh, S., Dwivedi, S., Trivedi, A., Dubey, I., and Trivedi, SP. (2022). Copper-induced Genotoxicity, Oxidative Stress, and Alteration in Transcriptional Level of Autophagy-associated Genes in Snakehead Fish Channa punctatus. *Biological Trace Element Research,* 0123456789. https://doi.org/10.1007/s12011-022-03301-8

Liu, H., Guo, H., Jian, Z., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X. and Zhao, L. (2020). Copper Induces Oxidative Stress and Apoptosis in the Mouse Liver. Oxidative *Medicine and Cellular Longevity*, 2020. https://doi.org/10.1155/2020/1359164

Lushchak, VI. (2011). Environmentally induced oxidative stress in aquatic animals. Aquatic Toxicology, 101(1): 13–30. https://doi.org/10.1016/j.aquatox.2010.10.006

Moron, MS., Depierre, JW., and Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. BBA - *General Subjects,* 582(1), 67–78. https://doi.org/10.1016/0304-4165(79)90287

Radhakrishnan, MV., and Hemalatha, S., (2010). Sublethal toxic effects of cadmium chloride to liver of freshwater fish Channa striatus (Bloch.). *Am. J. Toxicol. Sci.* 2: 54–56.

Ratn, A., Awasthi, Y., Kumar, M., Singh, SK., Tripathi, R., and Trivedi, SP. (2017). Phorate induced oxidative stress, DNA damage and differential expression of p53, apaf-1 and cat genes in fish, Channa punctatus (Bloch, 1793). *Chemosphere,* 182, 382–391. https://doi.org/10.1016/j.chemosphere.2017.05.008

Ratn, A., Prasad, R., Awasthi, Y., Kumar, M., Misra, A., and Trivedi, SP. (2018). Zn2+ induced molecular responses associated with oxidative stress, DNA damage and histopathological lesions in liver and kidney of the fish, Channa punctatus (Bloch, 1793). *Ecotoxicology and Environmental Safety*, 151:10–20. https://doi.org/10.1016/j.ecoenv.2017.12.058

Sanchez, W., Palluel, O., Meunier, L., Coquery, M., Porcher, JM., and Aït-Aïssa, S. (2005). Copper-induced oxidative stress in three-spined stickleback: Relationship with hepatic metal levels. *Environmental Toxicology and Pharmacology,* 19(1): 177–183. https://doi.org/10.1016/j.etap.2004.07.003

Singh, D., Nath, K., Trivedi, SP., and Sharma, YK. (2008). Impact of copper on haematological profile of freshwater fish, Channa punctatus. *Journal of Environmental Biology*, 29(2): 253–257.

Subburaj, A., Francis, T., Elakkanai, P., and Jayakumar, N., (2015). Sub-lethal effect of zinc induced histopathological alterations in the gill, liver and kidney of goldfish, (Carassius auratus). *Ind. J. Vet. Anim. Sci. Res.* 44: 25–35.

Trivedi, SP., Ratn, A., Awasthi, Y., Kumar, M., and Trivedi, A. (2021). In vivo assessment of dichlorvos induced histological and biochemical impairments coupled with expression of p53 responsive apoptotic genes in the liver and kidney of fish, Channa punctatus (Bloch, 1793). *Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology*, 245(November 2020): 109032. https://doi.org/10.1016/j.cbpc.2021.109032

Wang, J., Xiao, J., Zhang, J., Chen, H., Li, D., Li, L., Cao, J., Xie, L., and Luo, Y. (2020). Effects of dietary Cu and Zn on the accumulation, oxidative stress and the expressions of immune-related genes in the livers of Nile tilapia (Oreochromis niloticus). *Fish and Shellfish Immunology,* 100(March):198–207. https://doi.org/10.1016/j.fsi.2020.03.012

Wood, CM. (2011). Silver. *In Fish Physiology* (Vol. 31, Issue PART B). Elsevier Inc. https://doi.org/10.1016/S1546-5098(11)31001-1

Yadav, KK., and Trivedi, SP. (2009). Sublethal exposure of heavy metals induces micronuclei in fish, Channa punctata. *Chemosphere,* 77(11): 1495–1500. https://doi.org/10.1016/j.chemosphere.2009.10.022

**Biotech Today (January-June 2023) : 13 (1) : 31 - 40**

DOI : 10.5958/2322-0996.2023.00005.4

**RESEARCH ARTICLE**

# Watercore and fruit quality of apple cultivars as influenced by harvesting time and calcium sprays

**Farooq Ahmad Khan*, Amit Kumar, Sumati Narayan, Faheemullah Khan and Astha**

**Received**: 14 September, 2022, **Revised**: 27 November, 2022, **Accepted**: 11 December, 2022, **Published**: 1 January 2023

## Abstract

The present investigation was carried out at division of Basic Sciences and Humanities, Faculty of Horticulture, SKUAST-K, Shalimar during the year 2019 to 2021 to determine the role of harvest maturity and pre-harvest calcium chloride sprays on incidence and severity of watercore and fruit quality thereof. Two varieties of apple viz., Red Delicious (V1) and Silver Spur (V2) were foliar sprayed with $CaCl_2$ (0.0-S1), 0.5 (S2), 1.0 (S3) and 1.5% (S4) at 14, 7 and 0 days before expected dates of fruit harvest (M). Fruits were harvested 1 week after foliar spray that made 3 harvesting dates (M1, M2 and M3). Fruits were stored in CA at 3±1°C. Each treatments replicated thrice. After 2-monts of storage, fruits were analyzed for their watercore incidence and physico-chemical quality. Data indicated that V2 was more susceptible to watercore 8.2%) compared to V1 with 3.6 % watercore. Maximum and minimum watercore incidence was noted with late (M3) and early (M1) with measured values of 9.0 and 2.7 percent, respectively. Foliar spray of 1.0 percent CaCl2 was proved as most appropriate in reducing the watercore of apple fruits.

**Key words:** Apple, mass density, physiological disorders, sorbitol, thermography, watercore

## Introduction

Watercore is an internal physiological disorder in apples that can cause the tissue of the apple to appear translucent and allow the intercellular spaces to be filled with a sugar-water solution." (Fidler *et al*., 1973; Khan *et al*., 2021). The water-soaked appearance of watercore affected fruits results from the accumulation of sorbitol-rich liquid in the intercellular spaces of the apple tissue (Serban *et al*., 2019; Tanaka *et al*., 2020; Liu *et al*., 2021; Khan *et al*., 2021). As the name implies, this disorder commonly manifests itself around the core and the primary vascular bundles (Watkins and Mattheis, 2019; Khan *et al*., 2021), but is not restricted to this area, and in severe stages symptoms may be externally visible (Fig 1a and b) It is usually associated with advanced fruit maturity (Wang *et al*., 2023), and recent tests indicate there may be a high ratio of nitrogen to calcium (Khan *et al*., 2007; Khan and Shahid, 2013). Low night temperatures in fall often hasten fruit maturity and promote watercore development. The intensity of watercore disorder varies from year to year, variety to variety, location to location and even with canopy position of the tree (Khan *et al*., 2021; Wang *et al*., 2023). The disorder increases fruit susceptibility to internal breakdown (IB) caused by low oxygen ($O_2$) and/or high carbon dioxide ($CO_2$) concentrations (Argenta *et al*., 2002; Khan *et al*., 2021.

Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar-190025, Distt. Srinagar (J&K);

[1]RVSKVV, Gwalior (M.P.)

*Corresponding Author: drkhan_387@skuastkashmir.ac.in

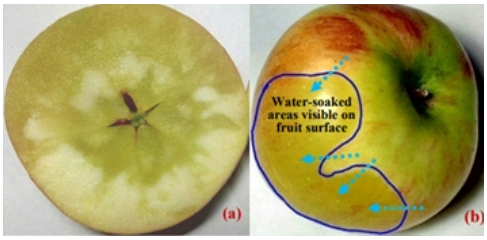



**Fig 1. Internal and external symptoms of watercore in apple (Khan *et al*., 2021)**

The leading theory for watercore development is that high temperature and high light makes the cell membranes leaky and cool nights slow down the uptake of sorbitol from the cell wall space into the parenchyma cells (Marlow and Loescher, 1984; Khan *et al*., 2021). It is hypothesized that the sorbitol translocation to the fruit is faster than its assimilation perhaps due to an inability of the apple tissue to convert sorbitol to fructose. The browning and breakdown caused due severe watercore is possibly the result of reduced gas diffusion in the affected tissue that may involve an accumulation of ethanol and acetaldehyde (Khan *et al*., 2021). Watercored apples may be accepted and even preferred by some people due enhanced sweet flavour, but in general such fruit are difficult or impossible to store for any length of time must be diverted to the less profitable processing industry (FAO, 2018; Khan *et al*., 2018). As such, watercore poses a serious problem for extended marketing seasons that emphasizes the necessity for controlling watercore as well as it's non-destructive detection before storage. Earlier researches indicated that presence of liquid in the intercellular air spaces increases the specific gravity of watercored fruits. As such mass density separation may be the least expensive and non-destructive method for separating the watercored and healthy fruits (Khan *et al*., 2007; Khan *et al*., 2018; Khan *et al*., 2021). However, this method requires modification depending on the cultivar, fruit size and temperature fluctuations.

The present investigation was performed to examine the contributions of harvest maturity to watercore development in apple and its management using calcium chloride as pre-harvest spray. Attempt was also made to standardize the protocol for detection of watercore before storage of fruits.

## Materials and Methods

The present investigation was carried out at division of Basic Sciences and Humanities, Faculty of Horticulture, SKUAST-K, Shalimar during the year 2020 and 2023. Two varieties of apple viz., Red Delicious and Silver Spur were selected as study material. Different concentrations of calcium chloride ($CaCl_2$) viz., 0.0, 0.5, 1.0 and 1.5 were foliar sprayed at 14, 7 and 0 days before optimum dates of fruit harvest that was determined on the basis of days after full bloom (Khan *et al*., 1998). Fruits were harvested at one week before optimum harvest date, at optimum maturity and one week after optimum maturity. Pre-cooling of fruits was done immediately after harvest to remove the field heat. Pre-cooled fruits of both the varieties were kept in 15kh wooden boxes and stored in controlled atmosphere (CA) storage at 3±1°C for two months. There were three replications of each treatment and each fruit box served as one sample unit. After two months of storage fruits were brought out of CA and 20 fruits from each box were selected randomly and cut them to observe the incidence of watercore. Fruits were also analyzed for severity of watercore on the basis of 1-4 scale (modified from Neuwald *et al*., 2010) where in 1 represents fruit without watercore and 4 is equivalent to 50% or more of flesh involvement. To separate the watercored apples from healthy ones, sampled fruits of both the varieties were dipped in a solution having specific gravity of 0.9 (Fig. 2). Fruits settled in the bottom of the solution were considered as watercored while as fruits hanging in the solution were categorized as healthy. Fruits were also analysed for their firmness, TSS and acidity. Data were tabulated and analysed statistically using analysis of variance technique as described by

Cochran and cox (1969). Software package used for analysis was INDOSTAT.

**Fig. 2 Separation of healthy and watercored fruits using a liquid with 0.9 specific gravity**

## Results and Discussion

Data with respect to the individual effects of variety, harvesting time and $CaCl_2$ spray (Fig. 3) indicated that incidence of watercore was higher with Silver Spur variety (8.2%) as compared to lower watercore incidence (3.6%) with Red Delicious apple. Data also indicate that late harvested fruits are more likely to develop watercore (9.0%) compared to timely harvest (6.2%) with lesser chance of watercore incidence (2.7%) in early harvested fruits. However, application of $CaCl_2$2 as foliar spray one week before harvest found effective in reducing the watercore development in apple fruits. Spray of 1.5% $CaCl_2$ exhibited the minimum incidence of watercore (4.2%) followed by 1.0% $CaCl_2$ (4.6%). However, both the treatments were found at par with each other. Two-way interactions of the treatments differed significantly (Fig. 4) with each other. Interactions of variety and CaCl2 (V×S) showed watercore incidence was highest with V2×S1 (11.9%) followed by V2×S2 (9.9%), V2×S3 (5.8%) and V2×S4 (5.2%) where as all the interactions of V1 with S showed lesser than 5.0 percent watercore with minimum incidence (3.2%) recorded in V1×S4 interaction. Among different treatments of harvesting time and $CaCl_2$ spray (M×S) the highest incidence of watercore (12.8%) was evidenced with M3×S1 followed by M3×S2 (9.6%) against the minimum watercore incidence (2.1%) observed with M1×S4 interaction. Interactions of variety and harvesting time (V×S) indicated that variety Silver Spur with late harvesting (V2×M3) produced maximum watercored fruits (11.6%) while variety Red delicious with early harvesting (V1×M1) gave minimum watercore (1.6%) incidence. Data with regard to 3-way interaction (Table 1) of the treatments showed that V2×M3×S1 produced highest watercored fruits (17.3%) followed by V2×M3×S2 (13.6%) compared to the least watercore incidence (1.5-1.6%) recorded with the interaction of Red delicious variety with early maturity under all CaCl2 treatments (V1×M1×S).

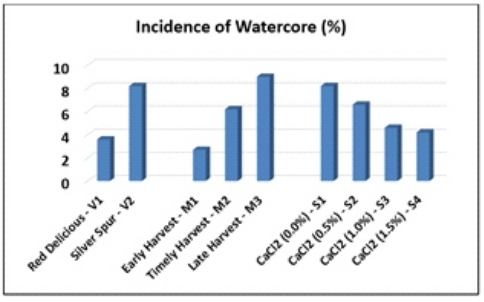



**Fig. 3 Individual effects of treatments on incidence of watercore in apples** ($CD_{0.05}$=0.03)

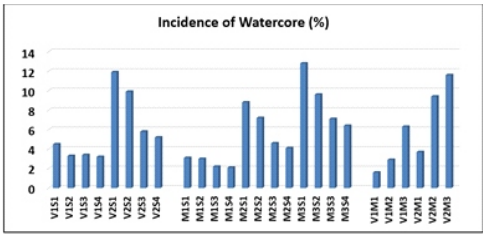



**Fig. 4 First order interaction effects of treatments on incidence of watercore in apples ($CD_{0.05}$=0.03)**

**Table 1: Effect of harvest maturity and $CaCl_2$ spray on watercore development (% incidence) in apple cultivars**

| Treatments | V1 | | | V2 | | |
|---|---|---|---|---|---|---|
| | M1 | M2 | M3 | M1 | M2 | M3 |
| S1 | 1.6 | 3.8 | 8.2 | 4.6 | 13.8 | 17.3 |
| S2 | 1.5 | 2.7 | 5.6 | 4.5 | 11.7 | 13.6 |
| S3 | 1.6 | 2.6 | 6.1 | 2.8 | 6.6 | 8.1 |
| S4 | 1.5 | 2.6 | 5.4 | 2.7 | 5.6 | 7.4 |
| C.D. (P=0.05) | 0.02 | | | | | |

Varietal differences in the incidence and intensity of watercore has also been reported by earlier workers (Yamada *et al.*, 2005; Khan

*et al*., 2021). Conforming to our findings, other experimental evidences also suggest that watercore incidence of apple increased with the delay of harvesting time (Bowen and Watkins, 1997; Wang *et al*., 2023).

Data with respect to individual effects of treatments on severity of watercore differed significantly (Fig. 5) wherein Silver Spur (V2) greater severity of watercore (2.7) compared to Red Delicious (V1) apple fruits (1.5). Among different harvesting times, late harvesting (M3) showed more severity of watercore (3.3) followed by timely harvesting (M2) and early harvesting (M1) with severity score values of 2.6 and 2.1, respectively. Among different $CaCl_2$ treatments maximum watercore severity was noted with S1 (2.7) followed by S2 (2.2) against the least watercore severity in S3 and S4 (1.8). First order interactions of different

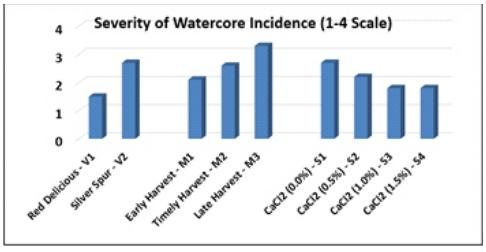



**Fig. 5. Individual effects of treatments on severity of watercore in apples ($CD_{0.05}$=0.07)**

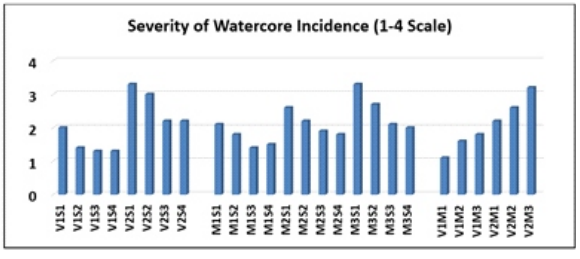



**Fig. 6. First order interaction effects of treatments on severity of watercore in apples ($CD_{0.05}$=0.07)**

treatments showed significant variations with respect to severity of watercore (Fig. 6).

Amongst variety and $CaCl_2$ spray (V×S) interactions V2×S1 exhibited greater severity of watercore (3.3) followed by V2×S2 (3.0) and V1×S1 (2.0) compared to least severity of watercore (1.3) observed with V1×S3 and V1×S4. Talking about the interactions of harvesting time and $CaCl_2$ spray (M×S), data clearly indicate that M3×S1 exhibited highest watercore severity (3.3) followed by M3×S2 and M2×S1 with severity scale value of 2.7 and 2.2, respectively against the least severity scale of 1.4 recorded with M1×S3. Watercore severity among the variety and harvesting time (V×M) showed that V2×M3 exhibited more severity (3.2) followed by V2×M2 (2.6) against the least severity score of 1.1 recorded with V1×M1 followed by V1×M2 with severity score of 1.6. Second order interactions of the treatments (Table 2) indicate that V2×M3×S1 resulted in maximum watercore severity of 4.0 followed by V2×M3×S2 (3.6) against the least watercore severity (1.0) recorded with V1×M1×S2, V1×M1×S3 and V1×M1×S4.

**Table 2: Effect of harvest maturity and calcium chloride spray on severity of watercore (1-none to 4-severe) scale development in apple cultivars**

| Treatments | V1 | | | V2 | | |
|---|---|---|---|---|---|---|
| | M1 | M2 | M3 | M1 | M2 | M3 |
| S1 | 1.5 | 2.0 | 2.5 | 2.6 | 3.2 | 4.0 |
| S2 | 1.0 | 1.5 | 1.8 | 2.5 | 2.8 | 3.6 |
| S3 | 1.0 | 1.4 | 1.6 | 1.8 | 2.3 | 2.6 |
| S4 | 1.0 | 1.4 | 1.4 | 2.0 | 2.2 | 2.5 |
| C.D. (P=0.05) | 0.06 | | | | | |

Incidence and severity of watercore in apple has also been reported to vary with cultivars (Bhat and Khan, 2010; Cebulj *et al*., 2021). Deficiency or low level of fruit calcium has been reported to associated with physiological disorders including incidence of watercore. Our result is also supported by Buccheri *et al*., 2020. Conforming to our findings, other experimental evidences also suggest that watercore incidence of apple increased with the delay of harvesting time (Bowen and Watkins, 1997; Wang *et al*., 2023). Reports on reduction in watercore incidence and severity through foliar spray of calcium are also available in literatures (Conway *et al*., 2002; Wang, 2018).

Individual effects of different treatments

showed significant variation with respect to fruit firmness (Fig. 7) and Red Delicious apple (V1) exhibited higher fruit firmness (19.1 lb/inch$^2$) compared to Silver Spur (17.4 lb/inch$^2$). Similarly, early harvested fruits showed higher fruit firmness value (18.8 lb/inch$^2$) compared to late harvested ones (17.8 lb/inch$^2$). If we talk about the individual effects of $CaCl_2$ spray there was an evidence of increased fruit firmness in $CaCl_2$-treated 1fruits ()18.1-18.6 lb/inch$^2$) compared to untreated ones (17.8 lb/inch$^2$). Significant differences in fruit firmness value were observed with respect to the 2nd order interactions of individual treatments (Fig. 8). Among the variety and $CaCl_2$ interactions (V×S), V1×S2 and V1×S3 recorded the highest fruit firmness value of 19.5 lb/inch2 compared to minimum fruit firmness value of 18.5 lb/inch2 recorded with V1×S1. Among the harvesting time and $CaCl_2$ interactions (M×S), M1×S3 resulted in maximum fruit firmness (19.1lb/inch$^2$) while as M3×S1 recorded the least fruit firmness (17.2lb/inch$^2$). Considering the interaction effects of variety and harvesting time (V×M) it can be stated that V1×M1 recorded the highest fruit firmness (19.7 lb/inch2) followed by V1×M2 (19.1 lb/inch2) while as the minimum fruit firmness value (17.0 lb/inch$^2$) was recorded with V2×M3 followed by a fruit firmness value of 17.5 lb/inch$^2$ with V2×M2. Considering the second order interactions of different treatments (Table 3) it can be inferred that variety Red delicious in combination with early harvest and $CaCl^2$ spray of 1.0% (V1×M1×S3) resulted in highest fruit firmness value (20.1 lb/inch$^2$) that was also at par with fruit firmness value (20.0 lb/inch2) recorded with V1×M1×S4. However, variety Silver Spur in combination with late harvesting and $CaCl_2$ spray of 0.0% (V2×M3×S1) recorded the least value of fruit firmness (15.5 lb/inch$^2$) and was also at par with V2×M3×S2 with fruit firmness value of 16.6 lb/inch$^2$.

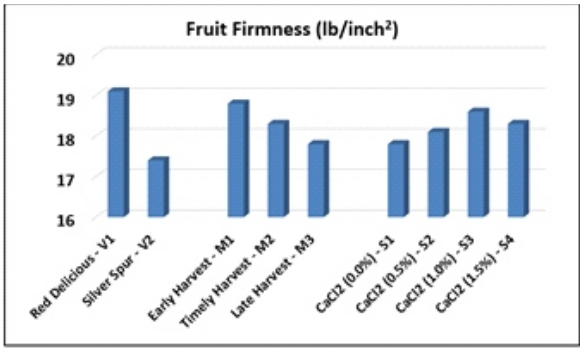



**Fig. 7 Individual effects of treatments on fruit firmness in apples ($CD_{0.05}$=1.13)**

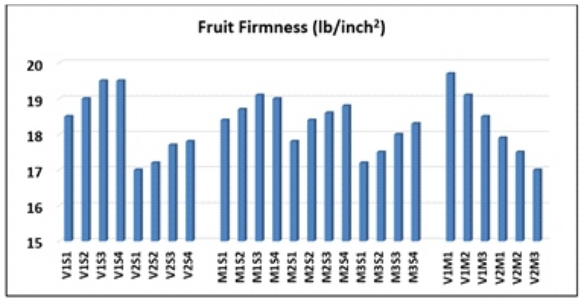



**Fig. 8 First order interaction effects of treatments on fruit firmness in apples ($CD_{0.05}$=1.05)**

**Table 3: Effect of harvest maturity and calcium chloride spray on fruit firmness (lb/inch$^2$) of apple cultivars**

| Treatments | V1 | | | V2 | | |
|---|---|---|---|---|---|---|
| | M1 | M2 | M3 | M1 | M2 | M3 |
| S1 | 19.3 | 18.5 | 17.8 | 17.5 | 17.1 | 16.5 |
| S2 | 19.5 | 19.0 | 18.4 | 17.8 | 17.3 | 16.6 |
| S3 | 20.1 | 19.5 | 18.8 | 18.1 | 17.7 | 17.2 |
| S4 | 20.0 | 19.5 | 19.0 | 18.0 | 18.0 | 17.5 |
| C.D. (P=0.05) | 0.82 | | | | | |

Significant difference in fruit firmness due to varieties has also been supported by earlier research (Kumar *et al.*, 2018; Li *et al.*, 2023). Variation in fruit firmness due to altered date of harvest has also been reported by Mohebi *et al.*, (2017) wherein early harvested fruits showed higher fruit firmness compare to later harvests. Application of CaCl2 as foliar spray in improving fruit firmness has been well established facts due to it's indispensable role in cell wall rigidity (Lanauskas and Kvikliene, 2006; Chandel *et al.*, 2019).

Individual effects of variety, harvesting time and $CaCl_2$ spray on total soluble solids (TSS) content of apple presented in Fig. 9 indicate that Red Delicious (V1) cultivar had lower TSS value (11.6°Brix) compared to a higher value of TSS (13.5°Brix) recorded with Silver Spur (V2) variety. So far as harvesting time is concerned, the TSS value of late harvested fruits (M3) was highest (13.1°Brix) as compared to the lowest TSS value (12.1°Brix) of early harvested fruits (M1). Talking about $CaCl_2$ spray, the highest TSS (13.1°Brix) was recorded with $CaCl_2$ 0.0% (S1) followed by CaCl2 0.5% (S2), CaCl2 1.0% (S3) and $CaCl_2$ 1.5% (S4) with TSS value of 12.7, 12.3 and 12.1°Brix, respectively. Total soluble solids (TSS) content of the fruits under the influence of first order interactions was found as significant (Fig. 10). Among the V×S interactions, V2× S1 recorded the highest value of TSS (14.1°Brix) followed by V2× S2 (13.7°Brix) against the minimum TSS value of 11.1°Brix recorded with V1× S4. Considering the data regarding the interactions of M× S, it may be stated that M3× S1 recorded the highest TSS value (13.8°Brix) followed by M3× S2 (13.3°Brix) compared to the minimum TSS value of 11.7° Brix recorded with M1× S4. Data on interaction of V×M suggest that V2× M3 recorded the highest value of TSS (14.0° Brix) followed by V2× M2 (13.5° Brix) against the minimum TSS value of 11.1° Brix recorded with V1× M1. Information on effect of three-way interactions of different treatments viz., V×M×S (Table 4) indicated that V2×M3×S1 recorded the highest value of TSS (14.7°Brix) followed by V2×M3×S2 with TSS value of 14.7°Brix against the minimum value of TSS (10.7° Brix) recorded with V1×M1×S4 followed in ascending order by V12×M1×S3 with recorded TSS value of 11.0° Brix.

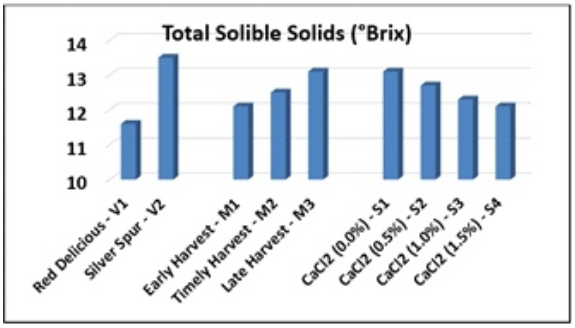



**Fig. 9 Individual effects of treatments on fruit total soluble solids in apples ($CD_{0.05}$=1.57)**

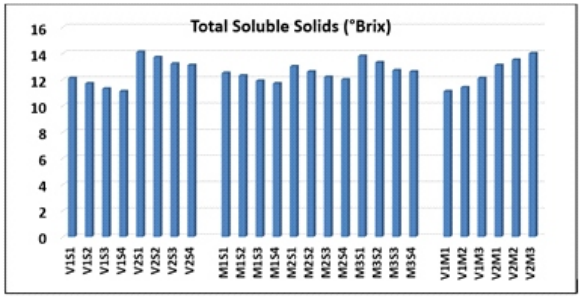



**Fig. 10 First order interaction effects of treatments on fruit total soluble solids in apples ($CD_{0.05}$=1.43)**

**Table 4: Effect of harvest maturity and calcium chloride spray on fruit TSS (°Brix) of apple cultivars**

| Treatments | V1 | | | V2 | | |
|---|---|---|---|---|---|---|
| | M1 | M2 | M3 | M1 | M2 | M3 |
| S1 | 11.4 | 12.0 | 12.8 | 13.6 | 14.0 | 14.7 |
| S2 | 11.2 | 11.5 | 12.4 | 13.3 | 13.7 | 14.1 |
| S3 | 11.0 | 11.2 | 11.7 | 12.8 | 13.2 | 13.6 |
| S4 | 10.7 | 11.0 | 11.6 | 12.7 | 13.0 | 13.5 |
| C.D. (P=0.05) | 1.31 | | | | | |

Significant difference in TSS contents among apple cultivars has been reported through earlier studies (Jan *et al*., 2012; Damyar *et al*., 2015; Li *et al*., 2023). Increased TSS with delayed harvesting of apple fruits has also been reported by earlier researchers (Kvikliene *et al*., 2011; Mohebi *et al*., 2017; Chalise and Giri, 2019). Dris and Niskanen (1999) also observed a decreased TSS in apple fruits due to $CaCl_2$ spray. However, our findings regarding $CaCl_2$ spray and fruit TSS seems contradictory to few earlier reports (Ganai *et al*., 2018; Chandale *et al*., 2019).

Information regarding individual effects of variety, harvesting time and $CaCl_2$ spray on fruit acidity content are presented in Fig. 11. Perusal of the data indicate that Silver Spur variety (V2) was more acidic (0.41%) than a lower value of fruit acidity (0.30%) recorded with Red Delicious apple (V1). Fruit acidity was also higher (0.41%) in early harvesting (M1) compared to the acidity value of timely harvesting (M2) (0.37) and late harvesting (M3) (0.31). However, no significant difference in acidity was recorded with respect to $CaCl_2$ sprays. Information regarding fruit acidity differed significantly with respect to first order interaction of treatments (Fig 12). Among V×S interactions, the highest acidity value (0.42%) was recorded with V2×S3 and V2×S4 which were also statistically at par with V2×S1 and V2×S2 with recorded values of 0.41 and 0.40%, respectively. Considering the interaction of harvesting time and $CaCl_2$ spray (M×S), data further explained that M1×S4 and M1×S3 recorded the highest acidity (0.42%) that was also at par with M1×S2 with recorded values of 0.40 percent. However, M3×S1 interaction recorded the least acidity value (0.30%) that was also at par with M3×S2, M3×S3 and M3×S4 with recorded acidity value of 0.31 percent. Among the interaction of variety and harvest time (V×M), V2×M1 resulted in highest acidity value (0.46%) that was statistically at par with V2×M2 against the lowest acidity value of 0.27% recorded with V1×M3 interaction. Information regarding second order interaction of various treatments presented in Table 5 clarified that V2×M1×S4 produced highest fruit acidity (0.48%) followed by V2×M1×S2 with observed acidity value of 0.45 percent. Interaction of V2×M1×S4 and V2×M1×S2 were also at par with V2×M1×S3 and V2×M1×S1, respectively. The lowest fruit acidity (0.23%) was however, recorded with V1×M3×S1.

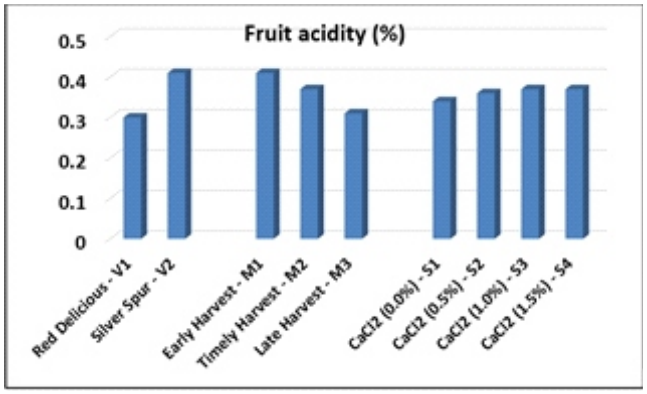



**Fig. 11 Individual effects of treatments on fruit acidity in apples ($CD_{0.05}$=0.03)**

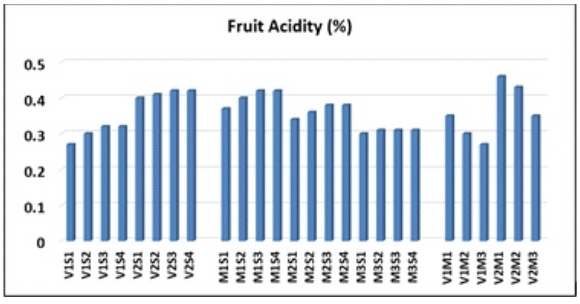



**Fig. 12 First order interaction effects of treatments on fruit acidity in apples ($CD_{0.05}$=0.01)**

**Table 5: Effect of harvest maturity and calcium chloride spray on fruit titratable acidity (%) of apple cultivarsq**

| Treatments | V1 | | | V2 | | |
|---|---|---|---|---|---|---|
| | M1 | M2 | M3 | M1 | M2 | M3 |
| S1 | 0.31 | 0.27 | 0.23 | 0.43 | 0.40 | 0.37 |
| S2 | 0.35 | 0.30 | 0.25 | 0.45 | 0.41 | 0.36 |
| S3 | 0.36 | 0.32 | 0.28 | 0.47 | 0.44 | 0.34 |
| S4 | 0.36 | 0.31 | 0.30 | 0.48 | 0.45 | 0.32 |
| C.D. (P=0.05) | 0.02 | | | | | |

Jia *et al*. (2018) communicated that apple fruit acidity is genetically diversified by natural variations in three hierarchical epistatic genes: MdSAUR37, MdPP2CH and MdALMTII. Decreased TSS with delayed harvesting of apple fruits has also been reported by earlier researchers (Chalise and Giri, 2019). Our finding regarding harvesting time and fruit acidity conform the findings of Mohebi *et al*. (2017). Dris and Niskanen (1999) also reported that preharvest $CaCl_2$ sprays

increased fruit titratable acidity and decreased the incidence of physiological storage disorders of some apple cultivars.

Fruit specific of healthy and watercored fruits were estimated and information are presented in Figure 13. Data clearly indicate that watercored fruits showed higher specific gravity (1.00) compared with low specific gravity (0.79) of healthy fruits. This also indicate that watercored and healthy fruits may be separated using a solution having specific gravity value of 0.9. Akihiro Itai also suggested that the increased specific gravity of watercored fruits may be exploited to separate the healthy fruits from water core affected fruits.

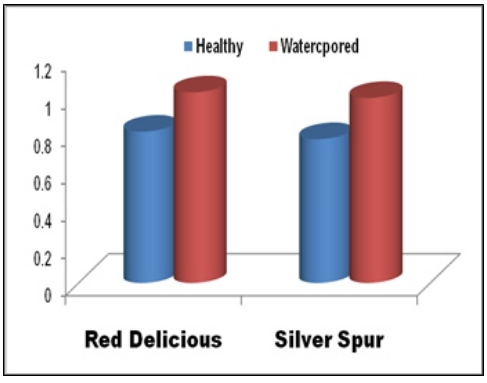



**Fig 13. Specific gravity of healthy and watercored fruits of Red Delicious and Silver spur fruits of apple**

## Conclusion

Apple cultivars vary in their susceptibility to watercore incidence and severity and harvest maturity plays an important role in occurrence of watercore in apple fruit. Foliar spray of calcium chloride one week before expected harvest found beneficial in reducing the watercore disorder in apple cultivars. Therefore, it may be recommended that harvesting of watercore-susceptible apple should be done at optimum physiological maturity and foliar spray of Calcium chloride @1.0% should also be done just 1 weeks before harvest to further reduce the incidence of watercore in apples.

## References

Arzani, K., Nock, JF. and Watkins, CB. (2016). Watercore incidence in Red Delicious apple in relation to $I_{AD}$ values: effect of harvest date and Harvista and ReTain applications. *ASHS-2016*, *Annual Conference* – August 8-11, *Atlanta, Georgia*. In: 30303, USA.

Argenta, LC., X. Fan, and JP. Mattheis. (2002). Impact of watercore on gas permeance and incidence of internal disorders in 'Fuji' apples. *Postharvest Biology and Technology,* 24: 113-122.

Bhat, SA. and Khan, FA. (2010). Physiological disorders of apple and pear. Training Compendium-Advanced Technologies for postharvest management of food crops (editors, MY Ghani, FA Khan and SA Bhat), Organized by Division of PHT, SKUAST-Kashmir, March 25-27, 2010. 88-96.

Bowen, JH. and Watkins, CB. (1997). Fruit maturity, carbohydrate and mineral content. relationships with watercore in 'Fuji' apples. *Postharvest Biology* and Technology, 11 (1): 31-38.

Buccheri, M., Caramanico, R., Ughini, V., Grassi, M. and Vanoli, M. (2020). Watercore in 'Pomella Genovese' apples: quality characteristics and antioxidants. Advances in Horticultural Science, 34 (1S): 312.

Chalise, B. and Giri, RK. (2019). Determination of optimum harvesting date for 'Royal Delicious' apple (Malus domestica Borkh.) at Jumla, Nepal. International Journal of Advanced Research in Biological Sciences, 6 (12): 131-139.

Cebulj, A., Petkovsek, MM., Lucaciu, CR., Veberic, R., Marinovic, S., Kolarek, M., Hutabarat, OS., Faramarzi, S., Rattei, T., Molitor, C., Hudina, M., Haselmair-Gosch, C., Halbwirth, H. and Slatnar, A. (2021). Alteration of the phenylpropanoid pathway by watercore disorder in apple (*Malus* × *domestica*). Scientia Horticulturae, 17:110438. https://doi.org/10.1016/j.scienta.2021.110438.

Chandale, JS., Sharma S. and Verma P. (2019). Effect of calcium sprays on fruit quality and shelf-life of apple (*Malus* × *domestica*). The *Indian Journal of Agricultural Sciences*, 89 (9): 1539–42. https://doi.org/10.56093/ijas.v89i9.93521.

Cochran, WG. and Cox, GM. (1959). Experimental Designs (Second Edition) John Wiley & Sons- New York.

Conway, WS., Sams, CE. and Hickey, KD. (2002). Pre- and postharvest calcium treatment of apple fruit and its effect on quality. *Acta Horticulturae*, 594: 413-419. https://doi.org/10.17660/ActaHortic.594.53.

Damyar, S., Dastjerdi, R. and Soleimani, L. (2015). Conference: Proceedings of the International Scientific Conference devoted to the 90th anniversary since the formation of the Institute for Fruit Growing

Dris, R. and Niskanen, R. (1999). Calcium chloride sprays decrease physiological disorders following long-term cold storage of apple. *Plant Foods for Human Nutrition*, 54(2):159-71.

FAO. (2018). Preventing post-harvest losses in the apple supply chain in Lebanon. www.fao.org/publications, ISBN 978-92-5-130022-0.

Fidler, JC., Wilkinson, BG. and Edney, KL. (1973). The biology of apple and pear storage. *Commonwealth Agricultural Bureaux, Farnham* Royal.

Ganai, NA., Rasool, K., Rather, GH., Ahmed, Z. and Saleem, T. (2018). Fruit characteristics of apple cv. Red Delicious as influenced by foliar spray of calcium chloride and boric acid. *International Journal of Current Microbiology and Applied Sciences*, 7 (3): 546-552.

Itai, A. (2015). Watercore in fruits. In: Abiotic Stress Biology in Horticultural Plants, Y. Kanayama, A. Kochetov (eds.), *Springer* Japan. 127-145.

Jan, I., Rab, A., Sajid, M., Ali, A. and Shah, ST. (2012). Response of apple cultivars to different storage durations. *Sarhad Journal of Agriculture*, 28 (2): 219-225.

Jia, D., Shen, F., Wang, Y., Wu, T., Xu, X., Zhang, X. and Han, Z. (2018). Apple fruit acidity is genetically diversified by natural variations in three hierarchical epistatic genes: MdSAUR37, MdPP2CH and MdALMTII. Plant Journal, 95(3):427-443. doi: 10.1111/tpj.13957.

Kevers, C., Pincemail, J., Tabart, J., Defraigne, J. and Dommes, J. (2011). Influence of cultivar, harvest time, storage conditions and peeling on the antioxidant capacity and phenolic and ascorbic acid contents of apples and pears. *Journal of agricultural and food chemistry* 59: 6165-6171.

Khan, FA., Ahmed, I. and Khan, FU. (1998) Maturity assessment in apple. *Indian Farmer Times,* 16(5): 17-1 9.

Khan, FA., Bhat, MY., I.M, Khan, FU. and Khalil, A. (2007). Physiological disorders of apple and pear. In: Post-Harvest management of Horticultural Crops (eds: MA Mir *et al*.). *Agrotech Publishing Academy, Udaipur*.

Khan, FA., and Shahid A. (2013). Physiological disorders of fruits and their management. In *Ecofriendly Management of Plant Diseases* (Editors: Dr Shahid Ahmad and Dr Udit Narayan), *Daya Publishing House, New* Delhi. Pp. 131-155.

Khan, FA., Bhat, SA. and Narayan, R. (2018). Physiological problems in apple and their management, pp. 315-326. In: Apple Production and Value Chain Analysis (Eds. Nazeer Ahmad, Shabir Ahmad Wani and W. M. Wani) Astral International Pvt. Ltd., New Delhi.

Khan, FA. Lone, SA., Kumar, A., Bhat, SA., Moinuddin, Salma, U, Fasil Fayaz and Saima Fayaz. (2021). Watercore – a physiological disorder of apple related to imbalance carbohydrate metabolism in fruit tissues, *SKUAST Journal of Research*, 23 (1): 1-10.

Kumar, P., Sethi, S., Sharma, RR., Singh, S., Saha, S., Sharma, VK., Verma, MK. and Sharma, SK. (2018). Nutritional characterization of apple as a function of genotype. *Journal of Food Science and Technology,* 55 (7): 2729–2738. https://doi.org/10.1007/s13197-018-3195-x.

Kvikliene N, Kviklys D, Valiuskaite A, Viskelis P, Uselis N, Lanauskas J and Buskiene L.

(2011). Effect of harvest date on fruit maturity, quality and storability of 'Lodel'apples. *Journal of Food, Agriculture & Environment* 9: 210-213.

Lanauskas, J and Kvikliene, NK. (2006). Effect of calcium foliarv application on some fruit quality characteristics of 'Sinap Orlovskij' apple. *Agronomy Research*, 4 (1): 31-36.

Li, X., Qiu-fang, SU., Jian, R., Whang, Z., Fu, J., Guo, J., *Yang, H. and Zhao, Z.* (2023). Comparison of cell wall changes of two different type apple cultivars during fruit development and ripening. *Journal of Integrative Agriculture* (In Press): https://doi.org/10.1016/j.jia.2023.07.019.

Li, Z., Yang, *H., Fang, W., Huang, X., Shi, J.* and *Zou, X.* (2023). Effects of variety and pulsed electric field on the quality of fresh-cut apples. *Agriculture,* 13: 929. https://doi.org/10.3390/ agriculture13050929.

Liu, X., Fan, HM., Liu, DH., Liu, J., Shen, Y., Zhang, J., Wei, J. and Wang, CL. (2021). Transcriptome and metabolome analyses provide insights into the watercore disorder on 'Akibae' pear fruit. *International Journal of Molecular Sciences*, 22: 4911.

Marlow, GC., and W.H. Loescher. (1984). Watercore. *Horticultural Reviews,* 6:189-251.

Neuwald, DA., Kitteman, D. and Streif, J. (2010). Watercore dissipation in Fugi apples at different holding temperatures. In: C. Nunes, ed., *Environmentally Friendly* and *Safe Technologies for Quality of Fruits* and Vegetables. Universidade Do Algarve.

Mohebi, M., Babalar, M., Askari, MA., Talae, A., and Ahmadi, A. (2017). Effects of harvest date on apple fruit quality at harvesting and after cold storage. Journal of Postharvest Technology, 5 (2): 29-36.

Rehman, MU., Rather, GH., Gull, Y., Mir, M.M. and Umar, I. (2017). Effect of 1-naphthaleneacetic acid and calcium chloride on preharvest drop and quality of 'Red Delicious' apples. *Canadian Journal of Plant Science*, 97 (5): https://doi.org/10.1139/cjps-2016-0359.

Serban, C., McCormick, RJ., Hanrahan, I. and Neuwald, DA. (2019). Fruit canopy position and crop load influence on watercore severity in 'Fuji' apples. *Acta Horticulturae*, 1242: 899-904

Tanaka, F., Hayakawa, F. and Tatsuki, M. (2020). Flavor and texture characteristics of 'Fuji' and related apple (*Malus domestica* L.) cultivars, focusing on the rich watercore. *Molecules*, 25, 1114

Wang, H., Yuan, J., Liu, T., Chen, L., Ban, Z., Duan, L., Wang, L., Tang, Y., Li, X. and Li, J. (2023). Fruit canopy position and harvest period affect watercore development and quality of the 'Fuji' apple cultivar fruit. Scientia Horticulturae, 311: 111793.

Watkins, CB. and Mattheis, JP. (2019). Temperate fruits – apples. In Postharvest Physiological Disorders in Fruits and Vegetables, Sergio Tonetto de Freitas and Sunil Pareek (eds), CRC Press, *Taylor and Francis Group*.

Wojcik, P., Skorupinska, A. and Filipczak, J. (2014). Impacts of preharvest fall sprays of calcium chloride at high rates on quality and 'Conference' pear storability. *Scientia Horticulturae*, 168: 51-57.

Yamada, H., Kamio, M. and Amano, S. (2005). Varietal differences in susceptibility to early or high temperature-induced watercore in apples. *Journal of the Japanese Society for Horticultural Science,* 74 (2): 115-120.

**Biotech Today (January-June 2023) : 13 (1) : 41 - 46**

DOI : 10.5958/2322-0996.2023.00006.6

**RESEARCH ARTICLE**

# Understanding the Impact of Terminal Heat Stress on Wheat: Physiological, Molecular, and Agronomic Perspectives

**Prafulla Kumar[1*], Ravindra Kumar[1], Sushmita[2] and R. S. Sengar[1]**

**Received**: 8 August, 2022, **Revised**: 11 October, 2022, **Accepted**: 26 October, 2022, **Published**: 1 January 2023

## Abstract

Terminal heat stress (THS) is a significant abiotic stress that severely affects the growth, development, and yield of wheat, one of the most important cereal crops worldwide. As global warming continues to intensify, the incidence and severity of THS are expected to increase, posing a major challenge to food security. Therefore, understanding the mechanisms underlying THS tolerance in wheat is essential for the development of climate-resilient cultivars and sustainable agriculture. This review article provides a comprehensive overview of the recent advances in our understanding of THS response in wheat at the physiological, molecular, and agronomic levels. The physiological effects of heat stress on wheat are discussed, including the impact on plant growth, photosynthesis, and grain development. Molecular mechanisms of heat stress tolerance are also described, including gene expression and protein synthesis. Additionally, agronomic strategies to mitigate the impact of terminal heat stress on wheat are explored, including breeding for heat tolerance, changes in planting dates, and irrigation management. Furthermore, we review the current strategies for improving THS tolerance in wheat, including conventional breeding, marker-assisted selection, and genetic engineering. Overall, this review article aims to provide a valuable resource for researchers, agronomists, and breeders interested in developing climate-resilient wheat cultivars and sustainable agricultural practices under THS conditions.

**Keywords:** Wheat, terminal heat stress, reproductive stage, grain yield, physiological responses, molecular responses, agronomic strategies.

## Introduction

Wheat is a crucial cereal crop that is cultivated worldwide, providing a source of food and livelihood for millions of people (FAOSTAT, 2021). However, wheat production is hampered by several biotic and abiotic stresses, including heat stress (Prasad *et al*., 2019). Terminal heat stress is a significant constraint to wheat production, particularly in arid and semi-arid regions, as it occurs during the reproductive stage of wheat (Wassmann *et al*., 2009). The problem is compounded by the rising frequency and intensity of heat waves due to climate change (IPCC, 2021). The impact of terminal heat stress on wheat physiology is extensive, affecting grain yield, quality, and biomass accumulation (Almeselmani *et al*., 2006). At the molecular level, it causes changes in gene expression, protein synthesis, and metabolic pathways, which are regulated by different signaling pathways and transcription factors such as heat shock proteins and reactive oxygen species (Bita and Gerats, 2013; Kumar *et al*.,

[1]Division of Plant Biotechnology, College of Biotechnology, SVPUA&T, Meerut

[2]Department of Agronomy, College of Agriculture, SVPUA&T, Meerut

*Corresponding Author: prafull19kumar@gmail.com

2013). Additionally, terminal heat stress affects the timing of growth stages, the duration of the grain filling period, and the efficiency of water and nutrient use (Tattaris *et al*., 2016).

In recent years, researchers have made significant progress in understanding the mechanisms underlying wheat's response to terminal heat stress through different techniques, including physiological, molecular, and agronomic approaches (Haque *et al*., 2017; Wang *et al*., 2018; Wahid *et al*., 2019). However, a comprehensive review of these perspectives is still required. This book chapter aims to provide a comprehensive overview of the impact of terminal heat stress on wheat from the physiological, molecular, and agronomic perspectives. The chapter will also highlight the challenges and opportunities for mitigating the negative impact of terminal heat stress on wheat production. Ultimately, the chapter aims to provide a roadmap for future research aimed at developing heat-tolerant wheat varieties and improving wheat production in the face of climate change.

## Physiological Responses to Terminal Heat Stress

The physiological responses of wheat to terminal heat stress are complex and multifaceted involving various physiological processes and biochemical pathways (Semenov *et al*., 2014; Prasad *et al*., 2017; Pradhan *et al*., 2020). The effects of terminal heat stress on wheat physiology can be categorized into two main categories, direct and indirect effects. High temperatures, which can cause various structural and functional damages to plant cells and tissues, are the direct effects of terminal heat stress. These damages include the denaturation of enzymes and other proteins, membrane lipid peroxidation, and disruption of photosynthesis, respiration, and other metabolic processes. Terminal heat stress can cause a reduction in biomass accumulation, leaf area, photosynthesis, and transpiration rates at the whole-plant level. It can also accelerate the rate of senescence, leading to early plant death, reduced yield, and poor grain quality (Almeselmani *et al*., 2006; Wahid *et al*., 2007). Indirect effects of terminal heat stress on wheat physiology are mainly due to changes in plant water relations, which can worsen the negative effects of high temperatures. High temperatures can increase plant water demand and transpiration rates, leading to water deficit and a reduction in soil water content. Water stress can further worsen the negative effects of terminal heat stress, causing a reduction in biomass accumulation, leaf area, photosynthesis, and yield (Semenov *et al*., 2014; Pradhan *et al*., 2020).

In response to terminal heat stress, The wheat plant can activate a variety of physiological and biochemical mechanisms in response to terminal heat stress to mitigate or prevent damage and maintain cell and tissue integrity. These mechanisms include the production of protective molecules such as heat shock proteins, antioxidants, and osmolytes, which can prevent protein denaturation, scavenge reactive oxygen species, and maintain cellular turgor and osmotic balance. (Wang *et al*., 2019; Zhang *et al*., 2014; Sivakumar *et al*., 2014) Wheat plants can also alter their growth and development by changing their phenology, morphology, and resource allocation in response to terminal heat stress. For example, they may accelerate their flowering and grain filling processes to avoid peak heat stress periods, reduce leaf area to minimize water loss and heat load, or allocate more resources to root growth to improve water and nutrient uptake. (Pradhan *et al*., 2020; Tariq *et al*., 2021) The complex and dynamic physiological responses of wheat to terminal heat stress illustrate the plant's ability to cope with high temperatures and maintain productivity. Understanding these mechanisms and

interactions is critical for developing effective strategies to enhance wheat's heat tolerance and improve its adaptation to changing climatic conditions.

## Molecular Responses to Terminal Heat Stress

Molecular responses to terminal heat stress in wheat involve the activation of various signaling pathways and the upregulation of specific genes involved in heat stress tolerance. These molecular response is evident in changes in gene expression, protein synthesis, and enzyme activity. (Almeselmani *et al*., 2006; Guo *et al*., 2018) One of the most important molecular responses to terminal heat stress in wheat is the activation of the heat shock response (HSR). The HSR is a complex signaling pathway that is activated by various stresses, including heat stress, and involves the upregulation of heat shock proteins (HSPs). HSPs act as molecular chaperones to stabilize and protect other proteins in the cell. (Wang *et al*., 2019). Apart from HSR, other signaling pathways are also activated in response to terminal heat stress in wheat, including the mitogen-activated protein kinase (MAPK) pathway, the calcium signaling pathway, and the reactive oxygen species (ROS) signaling pathway. These pathways are essential for mediating stress responses and can lead to changes in gene expression and protein synthesis. (Mittal *et al*., 2012; Guo *et al*., 2018).

At the molecular level, Terminal heat stress in wheat can cause changes in gene expression at the molecular level, with specific heat stress-responsive genes being upregulated or non-essential genes being downregulated. A number of heat stress-responsive genes in wheat have been identified, including those that are involved in HSP synthesis, antioxidant defense, and stress signaling (Wang *et al*., 2018; Zhang *et al*., 2019). HSP genes like HSP101, HSP90, and HSP70 have been found to be crucial in protecting wheat plants against heat stress (Wang *et al*., 2018). Along with changes in gene expression, molecular responses to terminal heat stress in wheat can also be characterized by changes in enzyme activity. For instance, heat stress can lead to the activation of enzymes that are involved in ROS detoxification, such as superoxide dismutase (SOD) and catalase (CAT) (Kumar *et al*., 2013). Furthermore, heat stress can cause changes in the activity of enzymes involved in carbohydrate metabolism, such as amylase and sucrose synthase (Ghaffari *et al*., 2016). In order to improve heat stress tolerance in this important crop, it is critical to understand the molecular responses to terminal heat stress in wheat. By identifying key signaling pathways and genes involved in heat stress tolerance, researchers can develop new breeding strategies, management practices, and molecular tools that can enhance wheat productivity under high temperature conditions.

## Agronomic Responses to Terminal Heat Stress

Agronomic responses to terminal heat stress in wheat are critical for ensuring optimal yield and quality of wheat production. Several agronomic strategies can be employed to mitigate the effects of terminal heat stress on wheat crops. One such strategy is the choice of planting time. Planting wheat earlier in the season can help the crop avoid the peak heat stress period, thereby reducing the likelihood of significant yield losses (Barnabás *et al*., 2008). The selection of heat-tolerant cultivars is also an essential agronomic strategy for minimizing the impact of terminal heat stress on wheat production. Heat-tolerant cultivars are characterized by their ability to maintain high yields under conditions of high temperature stress. (Reynolds *et al*., 2012). Optimizing irrigation scheduling is another agronomic strategy for mitigating the effects of terminal heat stress. (Jalota *et al*., 2019)

Adequate and timely irrigation can help maintain soil moisture and reduce the negative impact of heat stress on wheat growth and development. Implementing precision farming techniques, such as precision irrigation and fertilization, can also be beneficial in reducing the impact of heat stress on wheat crops. (Liu *et al*., 2015). Crop residue management is another agronomic strategy that can help mitigate the effects of terminal heat stress on wheat production. Crop residues left in the field after harvest can help reduce soil surface temperatures, maintain soil moisture, and improve soil structure, all of which can help reduce the negative impact of heat stress on wheat crops. (Singh *et al*., 2020). In conclusion, agronomic strategies can play a crucial role in mitigating the effects of terminal heat stress on wheat production. Planting time, cultivar selection, irrigation scheduling, precision farming techniques, and crop residue management are some of the key agronomic strategies that can help minimize the impact of heat stress on wheat crops. Implementing these strategies in a comprehensive and integrated manner can lead to more robust and resilient wheat production systems that can better withstand the challenges posed by terminal heat stress.

## Conclusion

Terminal heat stress is a significant challenge for wheat production globally, and its impact is projected to worsen with climate change. This review provides a comprehensive summary of the physiological, molecular, and agronomic aspects of terminal heat stress in wheat. It emphasizes the complex mechanisms involved in heat stress tolerance and the critical role of genetic diversity in the development of heat-tolerant cultivars. Physiological research has demonstrated that heat stress affects a range of physiological processes, such as photosynthesis, respiration, water relations, and nutrient uptake. It also causes reactive oxygen species to accumulate and alters the plant's hormonal balance, leading to reduced growth, yield, and quality. Molecular research has identified several genes and pathways involved in heat stress tolerance, including heat shock proteins, transcription factors, and antioxidant enzymes. Advances in genomics and molecular breeding technologies offer promising opportunities for developing heat-tolerant cultivars with improved yield and quality. Agronomic practices such as adjusting sowing time, crop management, and irrigation can mitigate the negative impact of heat stress on wheat production. However, the effectiveness of these practices may depend on various factors, including the severity and duration of heat stress, soil and climatic conditions, and crop genotype. In conclusion, interdisciplinary research involving plant physiology, molecular biology, genetics, and agronomy is essential for developing effective strategies to enhance wheat production under changing climatic conditions. This review highlights the significance of understanding the physiological, molecular, and agronomic aspects of terminal heat stress to address this global challenge.

## References

Almeselmani M., Deshmukh PS. and Sairam R. K. (2006). High temperature stress tolerance in wheat genotypes: Role of antioxidant defense enzymes. *Acta Physiol. Plant,* 28(3): 365–371.

Almeselmani, M., Deshmukh, PS., Sairam, RK., Kushwaha, SR. and Singh, TP. (2006). Protective role of antioxidant enzymes under high temperature stress. *Plant Science,* 171(3): 382-388.

Barnabás, B., Jäger, K. and Fehér, A. (2008). The effect of drought and heat stress on reproductive processes in cereals. *Plant, Cell and Environment*, 31(1): 11-38.

Fahad, S., Hussain, S., Saud, S., Hassan, S. and Chauhan, BS. (2016). Wheat production in a changing climate: Mitigation and adaptation strategies. *Food Energy Security,* 5(3): 185-214.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. and Basra, SMA. (2011). Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development,* 31(4): 853-879.

Food and Agriculture Organization of the United Nations. (2020). FAOSTAT database. http://www.fao.org/faostat/en/#data/QC

Ghaffari, MR., Nakhoda, B. and Razmjoo, J. (2016). Effect of terminal heat stress on yield and yield components of different wheat cultivars. *Acta Agriculturae Slovenica*, 107(1): 75-83.

Guo J., Shi G., Guo X., Zhang L. and Xu W. (2018). Transcriptome analysis reveals molecular mechanisms of heat tolerance in wheat. *Int. J. Genomics,* doi: 10.1155/2018/6794351

Jalota, SK., Kaur, S., Singh, M. and Sidhu, HS. (2019). Performance of wheat under irrigation scheduling and heat stress conditions. *International Journal of Current Microbiology and Applied Sciences,* 8(6): 1436-1444.

Kumar, S., Beena, AS. and Awasthi, R. (2013). Heat stress induced oxidative damage and metabolic changes in buffalo (*Bubalus bubalis*) oocytes and their prevention by antioxidant supplementation in vitro. *Journal of Assisted Reproduction and Genetics*, 30(10): 1399-1405.

Liu, W., Zhang, L., Zhang, W., Cao, Y. and Chen, X. (2015). Variable-rate irrigation improves yield and water use efficiency of winter wheat under sprinkler irrigation in northwestern China. *Agricultural Water Management,* 148: 171-178.

Mittal, D., Madhyastha, DA., Grover, A. and Grover A. (2012). Heat shock response in plants: A complex game with chaperones and more than twenty heat stress transcription factors. *J. Biosci,* 37(2): 457–477.

Pradhan, GP., Prasad, PVV., Fritz, AK., Kirkham, M. B. and Gill, B. S. (2020). Effects of terminal heat stress on grain growth and quality of wheat. *Scientific Reports,* 10(1): 1-13.

Pradhan, GP., Prasad, PVV., Fritz, AK., Kirkham, M.B. and Gill, BS. (2020). Effects of high night temperature and water stress on flowering and grain-filling in wheat. *Plant Growth Regul,* 92: 139–152.

Pradhan, GP., Prasad, PVV., Fritz, AK., Kirkham, M. B. and Gill, B. S. (2012). Effects of drought and high temperature stress on synthetic hexaploid wheat. *Functional Plant Biology*, 39(3): 190-198.

Prasad, PVV., Djanaguiraman, M., Perumal, R. and Ciampitti, IA. (2017). Impact of high temperature stress on floret fertility and individual grain weight of wheat. *Journal of Agricultural Science,* 155(02): 239-249.

Prasad, PVV., Pisipati, SR., Momèiloviæ, I. and Ristic, Z. (2011). Independent and combined effects of high temperature and drought stress during grain filling on plant yield and chloroplast EF-Tu expression in spring wheat. *Journal of Agronomy and Crop Science*, 197(6): 430-441.

Qin, D., Wu, H., Peng, H., Yao, Y. and Ni, Z. (2008). Differential gene expression and expression pattern analysis of heat shock transcription factors in wheat (*Triticum aestivum* L.) under different abiotic stresses. *Biochemical and Biophysical Research Communications*, 375(1): 30-35.

Reynolds, MP. and Ortiz-Monasterio, JI. (2013). Challenges to wheat production in dry environments: *Global wheat production environments (Eds. J. I. Ortiz-Monasterio, J. L. McNab, and H. Govaerts). Springer.*

Reynolds, MP., Pierre, CS., Saad, ASI., Vargas, M., Condon, AG. and Acevedo, E. (2012). Heat stress and crop production in developing countries: impacts and adaptation. *Crop Stress Management and Global Climate Change,* 111-138.

Semenov, MA., Stratonovitch, P., Alghabari, F. and Gooding, MJ. (2014). Adapting wheat in Europe for climate change. *Journal of Cereal Science*, 59(3): 245-256.

Singh, R., Singh, Y., Singh, V., Singh, R., Kumar, A. and Kumar, S. (2020). Impact of crop residue management on soil health, water-use efficiency and productivity of wheat under terminal heat stress. *Scientific Reports,* 10(1): 1-14.

Sivakumar, P., Sharmila, P., Saradadevi, R.,

Joseph, R. and Chakravarthi, DVN. (2014). Physiological and biochemical responses of wheat genotypes under terminal heat stress. *J. Agron. Crop Sci,* 200: 402–413.

Slafer, GA. and Savin, R. (1994). Source-sink relationships and grain mass at different positions within the spike in wheat. *Field Crops Research,* 36(1): 1-16.

Tahir, M., Bakhsh, A., Tariq, M. and Aziz, T. (2016). Effect of planting time on yield and yield components of wheat (*Triticum aestivum* L.) under heat stress conditions. *Pure and Applied Biology,* 5(3): 676-684.

Tari, I., Dancsó, P., Bónis, P., Ökrész, L., Toldi, O. and Csiszár, J. (2020). Physiological and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences,* 21(12): 4323.

Tariq, M., Nawaz, A., Shahbaz, M., Ashraf, U., Alamzeb, S., Nawaz, F. and Shah, T. M. (2021). Heat stress in wheat (*Triticum aestivum* L.): Physiological, biochemical, and molecular aspects. *J. Plant Growth Regul*, 40: 596–617.

Wahid, A., Gelani, S., Ashraf, M. and Foolad, M. R. (2007). Heat tolerance in plants: An overview. *Environmental and Experimental Botany,* 61(3): 199-223.

Wani, SH. and Sah, SK. (2014). Mitigation of drought stress in plants: Recent advances. *Plant Stress Physiology,*open source.

Wang, M., Xu, Q., Yuan, Q., Xu, H., Kong, L., and Liu, Y. (2019). Transcriptomic analysis of wheat seedling leaves during the heat stress response. *Int. J. Mol. Sci,* 20(2): 325.

Wang, X., Chen, S., Shi, X., Liu, D., Zhao, P., Lu, Y. and Ma, C. (2018). HSP101 functions as a specific molecular chaperone in vivo and in vitro to maintain chlorophyll metabolism in Arabidopsis. *Plant Physiology and Biochemistry*, 125: 69-78.

Wang, Y., Lu, Y., Chen, Q., Yu, S., Huang, L. and Zhang, Y. (2019). Proteomic analysis of heat stress response in wheat leaves using a iTRAQ-based comparative proteomic approach. *Cereal Res. Commun,* 47: 593–602.

Xin, Z., Velikova, V., Shi, K., Gao, J., Liang, Z. and Zou, Z. (2011). Distinctive responses of wheat superoxide dismutases to light and heat stresses. *Journal of Plant Physiology*, 168(16): 2090-2097.

Zhang, J., Jia, W., Yang, J. and Ismail, AM. (2014). Role of antioxidant enzymes in response of wheat seedlings to high temperature and NaCl stress. *Afr. J. Biotechnol*, 11: 3385–3392.

Zhang, Q., Ma, C., Zhao, J., Zhao, P., Liu, D. and Zhu, G. (2019). Transcriptome dynamics of wheat during grain development under high temperature stress. *Frontiers in Plant Science*, 10: 1088.

**Biotech Today (January-June 2023) : 13 (1) : 47 - 52**

DOI : 10.5958/2322-0996.2023.00007.8

**RESEARCH ARTICLE**

# Modulation of Morphological and Biochemical Attributes in *Stevia rebaudiana via* Foliar Application of Salicylic Acid

**Sanjeev Kumar Maurya[1,2], Alok Srivastava[2] and Sanjay Kumar Garg[2*]**

**Received**: 28 September, 2022, **Revised**: 26 October, 2022, **Accepted**: 16 November, 2022, **Published**: 1 January 2023

## Abstract

This research aimed to assess how the foliar application of salicylic acid influences the physiological and biochemical traits of *Stevia rebaudiana*. The investigation encompassed various parameters including plant height, branch and leaf counts, fresh and dry leaf biomass, chlorophyll and carotenoid levels, along carbohydrate and protein contents. Multiple concentrations of salicylic acid – 50, 100, and 150 mg/l – were applied through foliar spraying, while a control group received no salicylic acid treatment. Results derived from the experiment highlighted that Stevia plants treated with 100 and 150 mg/l levels of salicylic acid exhibited increased plant height, branch and leaf numbers, and higher yields of fresh and dry leaves compared to the non-treated control group. Furthermore, the application of salicylic acid positively impacted chlorophyll, carotenoid, protein, and carbohydrate contents in *Stevia* leaves. The application of salicylic acid not only enhanced both the quantitative and qualitative attributes of the plant but also led to improved performance during its growth cycle. These findings hold substantial significance across various agricultural contexts.

**Keywords:** Salicylic acid, *Stevia rebaudiana*, *Physiological and biochemical, Growth.*

## Introduction

The rising global demand for medicinal and aromatic plants underscores the importance of cultivating these species worldwide (Bahamin et al., 2013; Fathi and Bahamin, 2018). One such plant of significance is Stevia rebaudiana, a member of the Asteraceae family, renowned for its intense sweetness derived from its leaves, commonly employed for culinary and medicinal purposes (Karimi *et al.*, 2017). Originating from South American countries like Paraguay, Argentina, and Brazil, this perennial herb reaches up to a meter in height. Its leaves are a treasure trove of sweetening glycosides, including stevioside, rebaudioside A-F, steviolbioside, and dulcoside A, with sweetness levels around 300 times higher than sucrose. These compounds have found applications in managing conditions such as diabetes, dental issues, obesity, hypertension, and even cancer, showcasing a versatile range of benefits (Lemus-Mondaca *et al.*, 2012; Mandal *et al.*, 2015). From moderating blood sugar in type II diabetes to reducing hypertension, these compounds offer a wide array of health advantages (Bayraktar *et al.*, 2016). With its remarkable sweetness and therapeutic attributes, Stevia acts as a viable sugar substitute (Garzi *et al.*, 2019; Gerami *et al.*, 2019; Bahari Saravi *et al.*, 2021).

Stevia exhibits adaptability to various climates, thriving particularly well in warm and subtropical conditions. It flourishes in well-drained soil with consistent watering

[1]Department of Botany, Constituent Government College, Puranpur, Pilibhit – 262122

[2]Department of Plant Science, M. J. P. Rohilkhand University, Bareilly – 243006

*Corresponding Author: gargskplantscience@gmail.com

(Maurya and Garg, 2021). Due to poor seed germination, propagation is commonly achieved through stem cuttings or tissue culture techniques, with the plant thriving under both full and partial sunlight exposure (Maurya and Garg, 2020). However, factors like genetic makeup, environmental stress, and nutrient availability can constrain crop productivity. Environmental stresses have been shown to elevate the accumulation of secondary metabolites such as alkaloids, flavonoids, and essential oils (Bahamin *et al*., 2021). Research indicates that only a small fraction of global agricultural land remains free from these stresses (War *et al*., 2011). Under such circumstances, the application of exogenous plant growth regulators like auxins, gibberellins, cytokinins, and salicylic acid have been explored to enhance plant growth and productivity.

Salicylic Acid (SA) is a natural phytohormone with a significant role in plant growth, development, and defense mechanisms. Belonging to the class of plant growth regulators or hormones, this phenolic compound is naturally synthesized by plants in small amounts, yet exerts substantial influence over various aspects of their life cycle (Gharib and Hegazi, 2010). It's important to note that the effects of salicylic acid on plant growth vary depending on factors such as plant species, SA concentration, growth conditions, and the specific pathways activated in response to SA. While favorable outcomes can arise in certain growth aspects, elevated concentrations or imbalances can lead to adverse effects. Scientists continue to delve into the intricate interactions between salicylic acid and plant growth, aiming to better understand its role and potential applications in agriculture. The impact of salicylic acid on plant growth is complex and contingent, regulating physiological processes such as ethylene production, development, flowering induction, stomatal closure, and defense against pathogens and pests (EL-Tayeb, 2005; Erfani *et al*., 2022). This study investigates the effects of foliar application of various concentrations of salicylic acid on the growth and biochemical responses of *Stevia rebaudiana*. The potential of salicylic acid to enhance growth by stimulating cell division and elongation is highlighted, with its application holding promise for improved growth and development across different conditions (Maleki *et al*., 2022).

## Materials and Methods

An experimental investigation was undertaken within the premises of the Department of Plant Science at M.J.P. Rohilkhand University in Bareilly. The core objective of this study was to analyze the effects of foliar application of salicylic acid (SA) on Stevia plants. Bareilly, located at coordinates 79.5°E longitude and 28.5°N latitude, rests at an elevation of 172.21 meters above sea level. The prevailing climate in Bareilly falls under the humid subtropical category, characterized by warm and dry summers as well as cold winters. During the summer season, average maximum temperatures range from 42 to 43°C, while average minimum temperatures in winter range from 6.4 to 6.8°C. Annual average precipitation in Bareilly is approximately 787.16 mm and relative humidity ranges from 87 to 100 percent between July and February, gradually decreasing to 50 percent by the first week of May.

The experimental site features a sandy clay loam soil composition. Stevia planting materials were procured from CIMAP Pantnagar in Uttarakhand, India. Experimental plots measuring 1×1 meters were established, with plant spacing set at 30×30 cm and initial light irrigation provided. The study comprised multiple treatment groups, including three salicylic acid dosages (referred to as SA1, SA2, and SA3, with application rates of 50, 100, and 150 mg L-1,

respectively), alongside a control group (C) that received no SA treatment. Foliar application of SA was administered to Stevia plants 15 days after transplantation. Subsequent SA treatments were applied at 30, 45, and 60 days after transplantation using foliar application. A range of growth parameters were evaluated, encompassing plant height, branch and leaf counts, as well as measurements of fresh weight (FW) and dry weight (DW). These assessments were conducted on five randomly selected plants from each replication, and observations were recorded 70 days after transplantation. Additionally, chlorophyll content (as per Arnon, 1949) and carotenoid content (following Ikan, 1969) were determined using fresh leaves. Simultaneously, carbohydrate levels (according to Morris *et al*., 1948) and protein content (as determined by Lowry *et al*., 1951) were analyzed using dry leaves.

## Results and discussion

Table 1 illustrates that the utilization of various SA treatments yielded notably elevated values in plant height, branch count, and leaf count when compared to the control group in Stevia plants. Strikingly, the SA3 treatment demonstrated the most significant increase in branch count (a substantial 49% rise), while plant height and leaf count experienced their most noteworthy improvements through the SA2 treatment, with increments of 24% and 33% respectively. Additionally, the table demonstrates that applying different concentrations of SA led to higher fresh and dry leaf weights in Stevia plants in comparison to the control group. Notably, Stevia plants treated with the SA2 regimen displayed the most substantial weight augmentation, enhancing leaf fresh weight and dry weight by up to 30% and 32% respectively compared to control plants. Treatment SA3 also exhibited enhanced fresh and dry weights with increases of 26% and 28% respectively when compared to the control, albeit slightly less than the SA2 treatment. These results align with findings from a similar experiment on Patriot Chrysanthemums where SA application boosted fresh weight (Mashhadian *et al*., 2012). The enhancement of dry weight due to SA application has been consistently observed in various studies. Mady (2009) noted an increase in leaf dry weight following SA application in tomatoes. Chandra *et al*. (2007) also corroborated our findings by reporting similar dry weight enhancements in several cowpea cultivars.

SA treatments resulted in heightened chlorophyll and carotenoid content within fresh Stevia leaves, as depicted in Figure 1. However, the distinction between the effects of SA2 and SA3 treatments was not pronounced. The SA2 treatment elevated chl a, chl b, and total chl content by approximately 41%, 37%, and 40% respectively, while the SA3 treatment increased these values to around 42%, 41%, and 42% respectively, in comparison to the control.

The current study also demonstrated that applying varying concentrations of SA led to increased protein and carbohydrate content compared to the control, as shown in Figure 2. The most substantial increase of 39% in protein content was observed with the SA3 treatment in comparison to the control. In carbohydrate estimation, all SA concentrations elevated carbohydrate content compared to the control, but the SA3 treatment (18% increase) showed a slight decrease in carbohydrate content when compared to the SA2 treatment (20% increase). Importantly, SA application significantly enhanced carbohydrate and protein content, with the increase demonstrating a positive correlation with the applied SA concentration.

The amplified effects observed across different parameters following SA application can be attributed to the bio-regulatory impacts of this compound on plant physiological and biochemical processes. These effects encompass ion uptake, cell division, cell elongation, cell differentiation, photosynthetic efficiency, protein and carbohydrate synthesis, and a range of enzymatic activities (Raskin, 1992; Blokhima *et al*., 2003; El-Housini *et al*., 2014). Our experimental findings are consistent with those of Khan *et al*. (2003) in corn and soybean, as well as Magda *et al*. (2013) in barley. El–Mergawi and Abdel-Wahed (2004) noted that the response to SA varies among plant species due to genotype variation. Abdel–Wahed *et al*. (2006) also observed enhanced vegetative growth with the application of SA (3mM) in yellow maize plants.

## Conclusion

Diverse positive effects were noted in the morphological, physiological, and biochemical aspects of Stevia plants when exposed to different levels of SA treatment. Throughout this study, the application of distinct concentrations of SA via foliar spraying exhibited enhanced growth and augmented biomass in Stevia plants. At the biochemical level, the presence of SA contributed to heightened levels of key photosynthetic pigments including chl a, chl b, total chlorophyll, and carotenoids. Notably, protein content demonstrated substantial elevation in the dry leaves, accompanied by a noticeable increase in carbohydrate content. Given these comprehensive enhancements, it is recommended to explore the use of exogenous foliar SA application as a strategy to enhance the commercial production of Stevia leaf biomass.

**Table 1: Effect of salicylic acid on physiological parameters (per plant)**

| Treatments | Plant Height (cm) | Branch Number | Leaf Number | Leaf Fresh Weight (g) | Leaf Dry Weight (g) |
|---|---|---|---|---|---|
| Control (C) | 57.62 ± 3.26 | 26.6 ± 1.85 | 273.4 ± 12.31 | 51.14 ± 2.22 | 12.75 ± 0.64 |
| SA1 | 61.72 ± 3.05 | 31.2 ± 1.72 | 300.6 ± 09.58 | 55.60 ± 2.26 | 14.00 ± 0.53 |
| SA2 | 71.26 ± 2.30 | 38.0 ± 1.41 | 363.2 ± 11.67 | 66.30 ± 2.04 | 16.84 ± 0.52 |
| SA3 | 70.52 ± 3.06 | 39.6 ± 1.74 | 351.0 ± 11.88 | 64.53 ± 1.94 | 16.35 ± 0.61 |

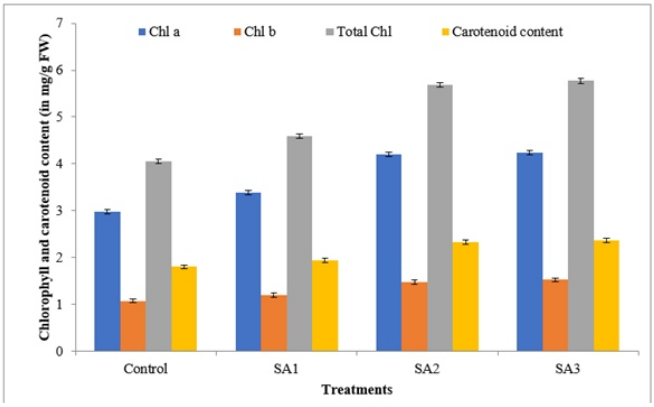



**Fig 1: Effect of salicylic acid on chlorophyll and carotenoid content in fresh stevia leaves.**

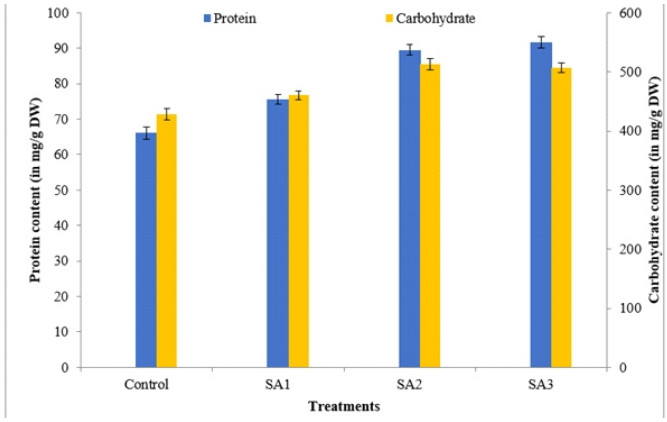



**Fig 2: Effect of salicylic acid on protin and carbohydrate content in dry stevia leaves.**

## References

Abdel-Wahed, MSA., Amin, AA. and Rashad EM. (2006). Physiological effect of some bioregulators on vegetative growth, yield and chemical constituents of yellow maize plants. *World J. of Agric. Sci*., 2(2): 149-155.

Arnon DI. (1949). Copper enzymes in isolated Chloroplasts. Polyphenols oxidase in *Beta vulgaris*. *Plant Physiol*., 29: 1-15.

Bahamin, S., Koocheki, A., Nassiri Mahallati, M. and Behashti S. (2021). Effect of nitrogen and phosphorus fertilizers on yield and nutrient efficiency indices in maize under drought stress. *Environmental Stresses in Crop Sciences*, 14(3): 675-690.

Bahamin, S., Parsa, S. and Ghoreishi, S. (2013). The Examination of Effects of Growth Stimulating and Salinity Bacteria on the

Characteristics of *Mentha spicata* leaves. *International Journal of Agronomy and Plant Production*. 4(9): 2119-2125.

Bahari, SH., Gholami, A. Pirdashti, H. Baradaran, M., and Asghari, HR. (2021). Growth and physiological response of *Stevia rebaudiana* Bertoni to the coexistence of endophytic fungi and polyamine spermidine foliar application under saline conditions. *Environmental stresses in Agricultural Sciences*, 14(1): 241-263.

Bayraktar, M., Naziri, E., Akgun, IH., Karabey, F., Ilhan E., Akyol B., Bedir E. and Gurel A. (2016). Elicitor-induced stevioside production, *in vitro* shoot growth, and biomass accumulation in micropropagated *Stevia rebaudiana*. *Plant Cell, Tissue and Organ Culture*, 127: 289-300.

Blokhina, O., Virolainen, E. and Fagerstedt, KV. (2003). Antioxidants, oxidative damage and oxygen deprivations stress (A review). *Ann. Bot*., 91: 179-194.

Chandra, A., Anand, A. and Dubey, A. (2007). Effect of salicylic acid on morphological and biochemical attributes in cowpea. *Journal of Environmental Biology* 28(2): 193-196.

El-Housini, EA., Ahmed, MA., Hassanein, MS. and Tawfik MM. (2014). Effect of Salicylic Acid (SA) on growth and Quality of Stevia (*Stevia rebaudiana* Bert.) under Salt Stress. *American-Euresian J. Agric. & Environ. Sci.*, 14(4): 275-281.

El-Mergawi, RA. and Abdel–Wahed, MSA. (2004). Diversity in salicylic acid effects on growth criteria and different indole acetic acid forms among faba bean and maize. *Egypt. J. Agron*., 26: 49-61.

El-Tayeb, MA. (2005). Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant Growth Regu* 45: 215-224.

Erfani S., Rezaei, M., Farahvash F. and Mahmoudjanlo, M. (2022). The effect of nano potassium fertilizer, potassium sulfate and salicylic acid on physiological characteristics of *Calendula officinalis* L. under water stress. *Journal of Iranian Plant Ecophysiological Research*, 17(65): 66-85.

Fathi, A. and Bahamin S. (2018). The effect of irrigation levels and foliar application (zinc, humic acid and salicylic acid) on growth characteristics, yield and yield components of roselle (*Hibiscus sabdariffa* L.). *Environmental Stresses in Crop Sciences*, 11(3): 661-674.

Garzi, A., Omidi, H. and Bostan, A. (2019). Effect of *Stevia rebaudiana* seed priming treatments with salicylic acid, iron and zinc on some germination traits and photosynthetic pigments under drought stress. *Iranian Seed Research*, 6(2): 125-135.

Gerami, M., Hamdian, A. and Akbarpour, V. (2019). Investigation of the effect of putrescine and salicylic acid on some physiological and antioxidant properties of *Stevia rebaudiana* B. in salinity conditions. *Journal of Crop Breeding*. 11(29): 40-54.

Gharib, FA. and Hegazi, AZ. (2010). Salicylic Acid ameliorates germination, seedling growth, phytohormone and enzymes activity in Bean (*Phaseolus vulgaris* L.) under cold stress. *J. Am. Sci*., 6: 1-6.

Ikan R. (1969) Natural Products, a Laboratory Guide. Academic Press, New York, USA. 101.

Karimi, R, Vahedi M, Pourmazaheri, H. and Balilashaki, K. (2017). Biotechnological approaches in *Stevia rebaudiana* and its therapeutic applications. *Adv. Biomed. Pharm* 4(1): 31-43.

Khan W., Prithiviraj, B. and Smith DL. (2003). Photosynthetic response of corn and soybean to foliar application of salicylates. *J. Plant Physiol* 160-485.

Lemus-Mondaca R., Vega-Gálvez A., Zura-Bravo L. and Ah-Hen, K. (2012). *Stevia rebaudiana* Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. *Food Chemistry*. 132: 1121-1132.

Lowry, OH., Rosebrough, NJ., Farr, AL. and Randall, RJ. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem*., 193: 265-275.

Mady, MA. (2009). Effect of Foliar Application with Salicylic Acid and Vitamin on Growth and Productivity of Tomato (*Lycopersicon esculentum*, Mill.) Plant. *Journal of*

*Agricultural Science*, 34: 6735-6746.

Magda, AF., Shalaby, MA., Ahmed, MSA., Abdallah and El-Housini EA. (2013). Physiological role of salicylic acid in improving growth and productivity of barley (*Hordeum vulgare* L.) under sandy soil conditions. *Middle East J. of Agric. Res*., 2(2): 68-75.

Maleki M., Sobhanian H., Yazdanpananah E. and Maleki, A. (2022). The effect of salicylic acid on the yield of vegetative organs and active ingredients of Stevia (*Stevia rebaudiana* Bertoni) under drought stress. *Journal of Plant Environmental Physiology*. 66(2): 89-107.

Mandal, S., Upadhyay, S., Singh VP. and Kapoor, R. (2015). Enhanced production of steviol glycosides in mycorrhizal plants: A concerted effect of arbuscular mycorrhizal symbiosis on transcription of biosynthetic genes. *Plant Physiology and Biochemistry*, 89: 100-106.

Mashhadian, NV., Tehranifar, A., Bayat, H. and Selahvaezi Y. (2012). Salicylic and citric acid treatments improve the vase life of cut Chrysanthemum flowers. *Journal of Agriculture, Science and Technology*. 14, 879-887.

Maurya, SK. and Garg SK. (2020). Morphological and biochemical responses to boron and zinc fertilizers in *Stevia rebaudiana*. *Plant Archives*, 20(1): 344-348.

Maurya, SK. and Garg SK. (2021). Morphological and biochemical changes in *Stevia rebaudiana* in response to macronutrients and micronutrients application. *Biochemical and Cellular Archives*, 21(2). 4621-4626.

Morris LD. (1948). Quantitative determination of carbohydrate with Dreywood's anthrone reagent. *Science*, 107: 254-255.

Raskin, I. (1992). Role of salicylic acid in plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol*. 43: 439-463.

War AR., Paulraj MG., War MY. and Ignacimuthu S. (2011). Jasmonic acid-mediated-induced resistance in groundnut (*Arachis hypogaea* L.) against *Helicoverpa armigera*. *Journal of Plant Growth Regulation*. 30: 512-523.

**Biotech Today (January-June 2023) : 13 (1) : 53 - 59**

DOI : 10.5958/2322-0996.2023.00008.X

**RESEARCH ARTICLE**

# Varietal evaluation of Gerbera under shade net condition in Prayagraj agroclimatic condition

**Purva Chaturvedi[1], Devi Singh[2] and C. John Wesley[3]**

**Received**: 20 September, 2022, **Revised**: 26 November, 2022, **Accepted**: 16 December, 2022, **Published**: 1 January 2023

## Abstract

Gerberas are widely cultivated and have a significant market value due to their popularity as ornamental plants and their traditional medicinal uses. The global gerbera market includes various segments, such as cut flowers, potted plants and medicinal products. Therefore, present investigation was carried out with title at the Department of Horticulture, Naini Agricultural Institute, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj, Uttar Pradesh during the Winter 2022-23 to determine the performance of different varieties of gerbera for its growth and flowering. Under this experiment, overall, 8 varieties were used comprising of variety Shveen, Petali, Livia, Hiami, Deepti, 17026, Alcochate and Breakdance. The current study found that variety Hiami performed better in terms of characters like plant height at 30, 60 and 90 DAP (18.13, 21.20 and 23.77 cm respectively); early for days to first flower bud emergence (39.43 DAP); days from bud to flowering (9.97 days); number of days for flowering from planting (54.53 DAP); number of days for peak flowering (58.17 DAP); maximum number of buds (10.63 buds); stalk length (64.77 cm); diameter of flower (9.33 cm) and yield per 200 $m^2$ (11693 flowers). Variety Deepti performed better for parameters like number of leaves at 30, 60 and 90 DAP (7.53, 10.37 and 12.63 leaves respectively); plant spread at 30, 60 and 90 DAP (18.77, 26.13 and 35.30 cm respectively); Vase life (8.80 days); second highest for yield per 200 $m^2$ (10263 flowers).

**Keywords**: *Gerbera, Varieties, stalk length, yield.*

## Introduction

Gerbera (*Gerbera jamesonii* Hook.) came into dictionary of floriculture after it was discovered by pre-Linnean botanist, Gronovious but it received its fortunate name in honour of German naturalist, 'Traugott Gerber' who travelled in Russia in 1743. It belongs to family Asteraceae and is suitable both for export and domestic market, because of its potential to withstand long transportation. It is a diploid species with somatic chromosome number 2n=50. The modern gerbera arose from *G. jamesonii* hybridized with *G. viridifolia* and possibly other species. It is commonly known as 'Transvaal Daisy', 'Barberton Daisy' or 'African Daisy' and is a small group of temperate and tropical Asian and African perennial herbs.

The plants are stemless and tender perennial herbs, leaves are radical, petioled, lanceolate and deeply lobed. They produce very attractive flower heads. Flower head is solitary; many flowered, with conspicuous ray florets in one or two rows. Based on flower head types or forms they are grouped into single, double, and semi double cultivars. The flower stalks are long, thin, hollow and leafless. This

[1]PG Scholar, Department of Horticulture, NAI

[2]Associate Professor, Department of Horticulture, NAI

[3]Associate Professor, Centre for geospatial technologies, VIAT Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj (U.P.).

*Corresponding Author: @gmail.com

characteristic has popularized gerbera and is in great demand in market for preparation of bouquets.

The consumers' preference changes with time. Hence, crop improvement is the need of the time to sustain the availability of desirable cultivars. Improvement through selection depends upon the variability existing in the available genotypes, which may be either due to different genetic constitution of cultivars or variations in the growing environments. Gerbera is a vegetatively propagated crop through suckers on commercial scale and selection is an easy method for varietal improvement in it. Selection is effective only when the observed variability in the population is heritable in nature. Genetic variance, heritability and other genetic parameters are reported to be subject to fluctuations with changing environments (Lal *et al*., 1982).

Netherland is the largest producer of gerbera in the world, accounting for over 78% of the global production. According to the Food and Agriculture Organization (FAO) of the United Nations, Netherland produced over 213,000 metric tons of gerbera in 2021. According to the Indian Council of Agricultural Research (ICAR), gerbera is primarily cultivated in the states of Karnataka, Maharashtra, Tamil Nadu, and West Bengal. It is grown in both open fields and under protected cultivation, such as polyhouses and shade nets. The area under production of gerbera in India is estimated to be around 6.42 thousand hectares and production is 2.65 million metric tons. Karnataka ranks first in gerbera production followed Maharashtra and Tamil Nadu. In Uttar Pradesh, area under production is 0.56 thousand hectares with production of 0.73 million metric tons. (Source: NHB, Ministry of Agriculture & Farmers Welfare, Government of India, 2021-22).

Germplasm is the basic material with a breeder to initiate his crop improvement programme. It consists of genetic variability for quantitative and qualitative traits. A proper understanding of classification of cowpea germplasm for qualitative and quantitative traits may serve as useful guidelines for plant breeders for selection and improvement of the crop. Yield is a complex character and depends upon number of component characters which are quantitatively inherited. As such before launching any breeding programme, a thorough knowledge of the nature and magnitude of genetic variability and extent of association between yield and other components is essential. Evaluation of genotypes to assess the existing variability is considered as preliminary step in any crop improvement programme. Information on the magnitude of variation in the available genetic material and the part played by the environment on the expression of plant characters are prime importance for the appraisal of the magnitude of possible improvement.

Varietal evaluation is a crucial process in the world of gerbera cultivation. It allows growers and researchers to compare different gerbera varieties, observing their growth rates, flower size, colors and overall health under similar conditions. By identifying the best-performing varieties, growers can choose the most suitable ones for specific purposes such as cut flower production, potted plants or landscaping, ultimately leading to increased productivity and profitability. Moreover, the evaluation helps in determining disease and pest resistance, enabling growers to select varieties better suited for their local conditions, thus reducing the need for chemical treatments and production costs. Additionally, the assessment of flower quality aids in ensuring market acceptance, as superior attributes like size, shape, color and longevity become apparent. Not only does varietal evaluation contribute to crop improvement, but it also fosters sustainability and resource management by identifying

varieties that require fewer inputs, promoting environmentally friendly practices and conserving valuable resources in gerbera farming.

The performance of each cultivar varies with the region, season and other growing condition. However, the concept of protected cultivation of gerbera is new. The success of hitech floriculture depends on the selection of proper varieties. Considering the commercial importance of this crop, there is a prime need for identification of a suitable cultivar for specific region. The promising varieties can be assessed for their stability across the environment. Hence, this study is being conducted to identify the suitable gerbera cultivar under protected cultivation with respect to flower yield, quality of flower and its important traits for plains of Prayagraj. Uttar Pradesh.

## Material and Methods

The present investigation was done to understand the performance of different varieties for plant and floral growth and yield of Gerbera. The investigation was carried out at Horticultural Research Farm (HRF), Department of Horticulture, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Prayagraj during Winter Season 2022. Observations were recorded at different growth parameters like plant height, number of leaves, plant spread, flowering parameters like number of days taken for first flower bud emergence, number of buds per plant, stalk length and yield parameters like yield per 200 $m^2$. The data were statistically analysed by the method suggested by Fisher and Yates, 1963. The different varieties used for varietal evaluations were $V_1$ (Shveen); $V_2$ (Petali); $V_3$ (Livia); $V_4$ (Hiami); $V_5$ (Deepti); $V_6$ (17026); $V_7$ (Alcochate) and $V_8$ (Breakdance).

## Results and Discussion

### A) Vegetative Parameters

**Plant height and Number of leaves** (Table 1)

The maximum plant height at 90 DAP (23.77 cm) was observed in variety Hiami followed by variety Livia with 21.60 cm. Minimum plant height at 90 DAP (16.00 cm) was observed in variety Petali. The improved performance of one variety of gerbera under a shade net compared to other varieties can be attributed to its specific genetic traits and adaptability. This variety may possess characteristics that enable it to thrive in reduced light conditions, promoting elongated and controlled growth. Its inherent ability to efficiently utilize available light resources allows it to maintain optimal photosynthesis and growth rates under shade. Additionally, this variety might have a natural inclination towards vertical growth, which is further enhanced in the sheltered environment provided by the shade net. As a result, it exhibits superior plant height compared to other varieties in such conditions. Similar findings were reported by Sil *et al*., (2017); Singh *et al*., (2017) and Deepa *et al*., (2019) in gerbera.

The maximum number of leaves at 90 DAP (12.63 leaves) was observed in variety Deepti followed by variety Hiami with 12.47 leaves. Minimum number of leaves at 90 DAP (8.77 leaves) was observed in variety Alcochate. The enhanced performance of one gerbera variety under a shade net compared to others in terms of leaf production can be attributed to its genetic predisposition for shade tolerance and resource allocation. This variety might have evolved mechanisms that maximize leaf development and photosynthetic efficiency under reduced light conditions. Its genetic traits likely prioritize leaf growth over other competing processes, resulting in a higher leaf count. Moreover, the shade net creates a more favourable microclimate, optimizing light

distribution and reducing stress on the plant, allowing this variety to allocate more energy towards leaf production, ultimately leading to a greater number of leaves. Similar findings were reported by Jhangde *et al*., (2019); and Akhtar *et al*., (2019) in Gerbera.

**Plant spread (Table 1)**

The maximum plant spread at 90 DAP (35.30 cm) was observed in variety Deepti followed by variety Breakdance with 33.33 cm. Minimum plant spread at 90 DAP (26.40 cm) was observed in variety Alcochate. The superior performance of one gerbera variety under a shade net concerning plant spread can be attributed to its genetic characteristics and growth behaviour. This specific variety may possess traits that promote lateral growth and branching, allowing it to take advantage of available space more effectively. The shade net provides a controlled environment that encourages horizontal expansion without excessive competition for light and resources. Additionally, the reduced light intensity may trigger specific genetic responses in this variety, leading to a broader canopy and denser foliage. As a result, the shade net fosters ideal conditions for this gerbera variety to exhibit better plant spread compared to others. Similar findings were reported by Jhangde *et al*., (2019) and Maitra *et al*., (2020) in gerbera and Kumar *et al*., (2020) in Marigold.

**B) Floral parameter**

**Days for first flower bud emergence, Days from bud to flowering and Number of days for flowering from planting (Table 1)**

The minimum days for first flower bud emergence (39.43 days) was observed in variety Hiami followed by variety Livia with 56.67 days. Maximum days for first flower bud emergence (114.03 days) was observed in variety Petali. The minimum days from bud to flowering (9.97 days) was observed in variety Hiami followed by variety 17026 with 13.53 days. Maximum days from bud to flowering (15.23 days) was observed in variety Petali. The minimum number of days for flowering from planting (54.53 days) was observed in variety Hiami followed by variety Livia with 70.83 days. Maximum number of days for flowering from planting (127.20 days) was observed in variety Petali. The improved performance of one gerbera variety under a shade net concerning early bud emergence, days from bud to flowering and number of days for flowering from planting can be attributed to its inherent genetic traits and shade tolerance. This specific variety might have evolved mechanisms that enable it to initiate bud development even in lower light conditions. The shade net creates a more favourable microenvironment, maintaining stable temperatures and reducing stress on the plant. This enhances the early activation of flowering genes in the variety, resulting in faster bud initiation and development. Additionally, its natural adaptability to shade allows it to thrive in such conditions, leading to earlier bud emergence compared to other varieties that may require higher light intensity. Similar findings were reported by Deepa *et al*., (2019); Akhtar *et al*., (2020); Maitra *et al*., (2020) in gerbera and Pani *et al*., (2020) in Rose.

**Number of buds per plant and Number of days for peak flowering (Table 1)**

The maximum number of buds per plant (10.63 buds) was observed in variety Hiami followed by variety Deepti with 9.33 buds. Minimum number of buds per plant (4.60 buds) was observed in variety Petali. The superior performance of one gerbera variety under a shade net in terms of producing more buds per plant can be attributed to its genetic predisposition and adaptability to reduced light conditions. This particular variety may have evolved traits that promote prolific bud formation even in lower light intensities. The shade net creates a controlled environment,

which reduces stress and creates optimal conditions for bud development. The variety's genetic makeup likely prioritizes reproductive growth, leading to a higher bud formation rate. Additionally, the reduced competition for light and resources under the shade net further enhances the allocation of energy towards bud production, resulting in more buds per plant compared to other varieties. Similar findings were reported by Singh *et al*., (2017) in Gerbera.

The minimum number of days for peak flowering (58.17 days) was observed in variety Hiami followed by variety Livia with 74.50 days. Maximum number of days for peak flowering (131.13 days) was observed in variety Petali. The superior performance of one gerbera variety under a shade net concerning an early peak in flowering can be attributed to its genetic predisposition and adaptability to reduced light conditions. This specific variety might have evolved traits that promote rapid flowering onset and synchronization. The shade net creates a controlled environment, providing consistent light levels and reducing stress on the plant. As a result, the variety's genetic makeup is triggered to initiate and accelerate the flowering process, leading to an early and concentrated peak in flower production. Additionally, the reduced competition for light and resources under the shade net further enhances its ability to reach an early peak in flowering compared to other varieties. Similar findings were reported by Deepa *et al*., (2019); Akhtar *et al*., (2020) in gerbera.

**Stalk length, diameter of flower, vase life and yield per 200 $m^2$ (Table 1)**

The maximum stalk length (64.77 cm) was observed in variety Hiami followed by variety Shveen with 58.00 cm. Minimum stalk length (49.87 cm) was observed in variety Petali. The maximum diameter of flower (9.33 cm) was observed in variety Hiami followed by variety Livia with 8.67 cm. Minimum diameter of flower (7.47 cm) was observed in variety Shveen. The better performance of one gerbera variety under a shade net, resulting in greater stalk length and flower diameter, can be attributed to its specific genetic traits and adaptability to reduced light conditions. This particular variety may possess characteristics that promote elongated stem growth, enabling it to reach for available light. The shade net provides a controlled environment that fosters vertical growth without excessive competition for light. As a result, the variety allocates more energy to stalk development, leading to longer stems compared to other varieties that might require higher light intensities for elongation. Additionally, the shade net's sheltered environment reduces the risk of physical damage and allows uninterrupted stem growth. Similar findings were reported by Sil *et al*., (2017); Deepa *et al*., (2019); Akhtar *et al*., (2020) in gerbera.

The maximum Vase life (8.80 days) was observed in variety Deepti followed by variety Alcochate with 7.93 days. Minimum Vase life (4.43 days) was observed in variety Breakdance. The better performance of one gerbera variety under a shade net, resulting in more vase life, can be attributed to its genetic traits and adaptability to reduced light conditions. This specific variety may possess characteristics that promote better water uptake and nutrient retention in its stems and flowers. The shade net provides a controlled environment, reducing stress and transpiration, which helps preserve the flower's freshness and vitality. Additionally, the variety's natural tolerance to shade enables it to sustain optimal flower health for a longer duration. As a result, it exhibits an extended vase life compared to other varieties that might require higher light intensities and experience quicker wilting. Findings were in accordance with earlier findings of Singh *et al*., (2017) ; Jangde *et al*., (2019); Akhtar *et al*., (2020); Maitra *et al*., (2020) in gerbera.

The maximum yield per 200 $m^2$ (11693

flowers) was observed in variety Hiami followed by variety Deepti with 10263 flowers. Minimum Yield per 200 $m^2$ (5060 flowers) was observed in variety Petali. The better performance of one gerbera variety under a shade net, resulting in more yield per 200 $m^2$, can be attributed to its genetic traits and adaptability to reduced light conditions. This specific variety may possess characteristics that promote better water uptake and nutrient retention in its stems and flowers. The shade net provides a controlled environment, reducing stress and transpiration, which helps preserve the flower's freshness and vitality. Additionally, the variety's natural tolerance to shade enables it to sustain optimal flower health for a longer duration. As a result, it exhibits an extended yield per 200 $m^2$ compared to other varieties that might require higher light intensities and experience quicker wilting. Findings were in accordance with earlier findings of Singh *et al*., (2017); Jangde *et al*., (2019); Akhtar *et al*., (2020); Maitra *et al*., (2020) in Gerbera.

**Table 1 : Performance of different varieties for vegetative and floral parameters studied for Gerbera.**

| Variety Notation | Variety details | Plant height (cm) [90 DAP] | No of leaves [90 DAP] | Plant spread (cm) [90 DAP] | Days for first flower bud emergence (DAP) | Number of buds per plant | Days from bud to flowering (days) | Number of days for flowering from planting (DAP) | Number of days for peak flowering [DAP] | Stalk length (cm) | Diameter of flower (cm) | Vase life (days) | Yield per 200 $m^2$ (Flowers) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $V_1$ | Shveen | 20.47 | 9.87 | 30.87 | 58.20 | 5.63 | 14.13 | 75.03 | 78.80 | 58.00 | 7.47 | 6.43 | 6193 |
| $V_2$ | Petali | 16.00 | 12.13 | 32.00 | 114.03 | 4.60 | 15.23 | 127.20 | 131.13 | 49.87 | 8.00 | 4.70 | 5060 |
| $V_3$ | Livia | 21.60 | 11.40 | 32.60 | 56.67 | 5.50 | 14.13 | 70.83 | 74.50 | 55.23 | 8.67 | 6.63 | 6050 |
| $V_4$ | Hiami | 23.77 | 12.47 | 30.90 | 39.43 | 10.63 | 9.97 | 54.53 | 58.17 | 64.77 | 9.33 | 7.90 | 11693 |
| $V_5$ | Deepti | 16.50 | 12.63 | 35.30 | 62.87 | 9.33 | 14.90 | 77.80 | 81.13 | 50.70 | 8.17 | 8.80 | 10263 |
| $V_6$ | 17026 | 17.13 | 10.87 | 30.67 | 84.03 | 8.40 | 13.53 | 96.13 | 99.97 | 52.93 | 8.30 | 8.03 | 9240 |
| $V_7$ | Alcochate | 17.77 | 8.77 | 26.40 | 59.20 | 7.47 | 14.73 | 73.97 | 77.47 | 56.73 | 8.27 | 7.93 | 8217 |
| $V_8$ | Breakdance | 18.33 | 10.37 | 33.33 | 81.20 | 6.47 | 14.43 | 95.30 | 100.90 | 57.00 | 8.40 | 4.43 | 7110 |
| 'F' test | S | S | S | S | S | S | S | S | S | S | S | S | S |
| S.E. (m) ± | | 1.37 | 0.15 | 1.71 | 4.34 | 0.25 | 0.37 | 5.28 | 5.13 | 3.12 | 0.32 | 0.28 | 1.67 |
| C.D. at 5% | | 2.95 | 0.32 | 3.67 | 9.31 | 0.53 | 0.80 | 11.32 | 10.99 | 6.69 | 0.69 | 0.57 | 3.54 |
| C.V. | | 8.88 | 1.65 | 6.65 | 7.66 | 4.20 | 3.29 | 7.71 | 7.15 | 6.87 | 4.73 | 4.78 | 2.53 |

SE Standard Error; CD coefficient of dispersion; CV coefficient of variance.

## Conclusion

The current study found that variety Hiami performed better in terms of characters like plant height at 30, 60 and 90 DAP (18.13, 21.20 and 23.77 cm respectively); early for days to first flower bud emergence (39.43 DAP); days from bud to flowering (9.97 days); number of days for flowering from planting (54.53 DAP); number of days for peak flowering (58.17 DAP); maximum number of buds (10.63 buds); stalk length (64.77 cm); diameter of flower (9.33 cm) and yield per 200 $m^2$ (11693 flowers). Variety Deepti performed better for parameters like number of leaves at 30, 60 and 90 DAP (7.53, 10.37 and 12.63 leaves respectively); plant spread at 30, 60 and 90 DAP (18.77, 26.13 and 35.30 cm respectively); Vase life (8.80 days); second highest for yield per 200 $m^2$ (10263 flowers).

## References


Akhtar, M D., Singh, D., and Fatmi, U. (2020). Varietal evaluation of Gerbera (*Gerbera jamesonii*) under naturally ventilated polyhouse condition in Prayagraj. *International Journal of Current Microbiology and Applied Sciences*, 9(10), 2633-2639

Deepa, MS., Sudarshan, GK., and Keerthishankar, K. (2019). Evaluation of Gerbera (*Gerbera jamesonii*) cultivars for growth, flowering and yield under different growing structure. *International Journal of Chemical Studies*, 7(1): 1138-1140.FAOSTAT (2020). www.fao.org/faostat/en/data/CC. Food Supply- Crops Primary Equivalent. Visited on 15/12/2022.

Fisher, RA. and Yates, F. (1967). The Design of Experiments: Statistical Principles for Practical Applications. *New York*: Hafner Publishing Company.

Jangde, T., Sharma, G., Jangde, B., and Banjara, N. C. (2019). Evaluation of Gerbera (*Gerbera jamesonii*) cultivars under naturally ventilated polyhouse in Chhattisgarh plain. *Journal of Pharmacognosy and Phytochemistry*, 8(3), 2112-2114.

Kumar, R., Prasad, VM., and Singh, D. (2021). Varietal evaluation of Chrysanthemum (*Dendranthema grandiflora* L.) under Prayagraj agro-climatic conditions. *The Pharma Innovation Journal*, 10(12), 245-248.

Kumar, V., Singh, RS., Pal, M., Ojha, MD., Singh, AP., Verma, RK., and Singh, PK. (2020). Varietal performance of Marigold (*Tagetes spp*.) under sub-tropical condition of Bihar. *Journal of Pharmacognosy and Phytochemistry*, 9(3): 922-924.

Lal, SD., Seth, JN.; Yadav, JP. and Danu, NS. (1982). Genetic variability and correlation studies in rose. *Programming Horticulture*., 14(4): 234-236.

Maitra, S., Shankar, T., Sairam, M., and Pine, S. (2020). Evaluation of Gerbera (*Gerbera jamesonii* L.) Cultivars for Growth, Yield and Flower Quality under Protected Cultivation. *Indian Journal of Natural Sciences*, 10(60), 20271.

NHB (2022). National Horticultural Board, Ministry of Agriculture and Farmers Welfare, *Government of India*, 2021-2022.

Pani, PK., Saravanan, SS., and Bahadur, V. (2020). Varietal Evaluation of Hybrid Tea Rose under Prayagraj Climatic Condition. *International Journal of Current Microbiology and Applied Sciences*, 9(10), 3571-3576.

Sil, M., Sarkar, MM., Raghupathi, B., and Mondal, S. (2017). Varietal Evaluation of Gerbera (*Gerbera jamesonii*.) Grown in a Polyhouse. *International Journal of Current Microbiology and Applied Sciences*, 6(7), 810-814.

Singh, B., Pinder, R., Srivastava, R., and Yadav, H. (2017). Varietal Evaluation of Gerbera Cultivars Under Shade Net Conditions. *Chemical Science Review and Letters*, 6(21), 500-504.

Singh, P., Bhardwaj, A., Kumar, R., and Singh, D. (2017). Evaluation of Gerbera Varieties for Yield and Quality under Protected Environment Conditions in Bihar. *International Journal of Current Microbiology and Applied Sciences*, 6(9), 112-116.

**Biotech Today (January-June 2023) : 13 (1) : 60 - 62**

DOI : 10.5958/2322-0996.2023.00009.1

**RESEARCH ARTICLE**

# Impact assessment of Magnesium in the Ground Soil and Suspended Particulate Matter in the ambient air of Kumaun region of Lesser Himalayas

**Ashok Kumar Raghav**

**Received**: 16 October, 2022, **Revised**: 22 November, 2022, **Accepted**: 8 December, 2022, **Published**: 1 January 2023

## Abstract

A high concentration of magnesium, also known as a secondary soil nutrient, along with suspended particulate matter (SPM), can have adverse effects on soil quality. This study aims to assess the impact of magnesium in the soil and suspended particulate matter in the air around the hills of magnesite mining areas. Additionally, it seeks to determine how mining coupled with a manufacturing unit located in this area posed a challenge to the pristine environment of these hills. The $Na_2CO_3$ fusion method was employed to analyse ground samples of soil, rocks, and suspended solids. Laboratory findings revealed that the presence of magnesium and suspended particulate matter was primarily limited to immediate vicinity of the mine area.

**Key Words:** Magnesite, Soil, Suspended Particulate Matter, Emissions, Rock, Aerosol and Air.

## Introduction

In environmental contexts, the presence of base cations such as magnesium carries significant importance. Their deposition has a profound influence on surface pH levels, notably contributing to increased alkalinity. This, in turn, acts as a buffering agent, effectively neutralizing the adverse effects of acidity stemming from sulphur and nitrogen emissions. It is for this reason these emissions are of interest, rather than their negative impacts upon human health or ecosystems.

Traditionally, it was believed that the primary source of base cations in the atmosphere stemmed from dust particles resulting from soil erosion. However, a closer examination of concentration patterns in both air and precipitation has shed light on substantial emissions originating from urban and industrial sources, challenging earlier assumptions.

Soil typically encompasses magnesium within the range of 0.05% to 0.5% of its total composition, yet only a fraction of this magnesium is readily available for plant absorption. This accessible magnesium exists within the soil solution and binds to the exchange sites found in clays and organic matter, referred to as "exchangeable magnesium," like how potassium behaves. However, in contrast to potassium, magnesium exhibits limited mobility in transitioning from non-exchangeable to exchangeable forms.

Elevated levels of magnesium in the soil can raise concerns. In certain regions, the consistent application of magnesian limestone over numerous years has led to an accumulation of magnesium in the soil. Conversely, in other areas, naturally occurring high soil magnesium content can be attributed to the parent material.

Broadly speaking heightened soil magnesium concentrations typically do not impede crop growth but can potentially hinder the uptake of potassium. Consequently, when soil

APG Shimlr University, Shimla, India

*Corresponding Author: Ashok_raghav@gmail.com.in

magnesium levels are deemed excessively high, it is presumed that this elevation is the result of magnesium-containing lime applications.

Suspended particulates, also referred to as atmospheric aerosol particles, atmospheric particulate matter, particulate matter (PM), or suspended particulate matter (SPM), consist of minuscule solid or liquid particles suspended in the atmosphere. Some of these particles, such as dust, dirt, soot, or smoke, are substantial or dark enough to be visible to the naked eye. The term "aerosol" typically encompasses the mixture of particles and air, rather than just the particulate matter itself. These particulates can originate from natural sources or human activities, and their presence has repercussions on climate and precipitation, which can have adverse effects on human health beyond direct inhalation.

**Estimation of Mg in Soil and Rock and Suspended Solids:**

The Magnesium in the ground samples of rock and soils was determined by $Na_2CO_3$ fusion method as described by Robinson (1945) and Heald (1969). The acidified extract was made to a suitable volume and the Mg was determined by EDTA titration method using EBT indicator as described by Cheng and Bray (1951). The total Mg in the suspended solids was estimated by adopting the $Na_2CO_3$ fusion method.

The concentration of magnesium was estimated to determine its source characteristics with respect to nature and anthropogenic activities like mining operations. In all the four samples of soil, magnesium ore and suspended particulate matter were collected from sites shown in Fig.2.

The site I is located at a distance of 200 meters from the plant near village Kafligair/Sinduri. The site II is located at 1Km from the plant near village Har Khola. The site III is at distance of 3 km from the plant, while site IV is at 5 km towards Takula.

While the sample of magnesite ore was derived from Block no. VI., the standard soil sample was extracted from Hawalbagh (Almora) for elemental analysis of Magnesium.The sampling site is shown in Fig.1.

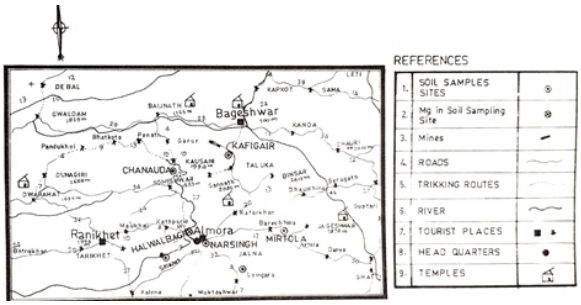



**Fig.1. Soil sampling sites and sites for concentration of Mg in soil**

The data were recorded in the month of December. It was assumed that emissions from the factory would have deposited on the ground soil in due course of time depending on the local micrometeorological conditions. In all the four samples of soil, magnesium ore and suspended particulate matter were collected from sites shown in Fig.2.

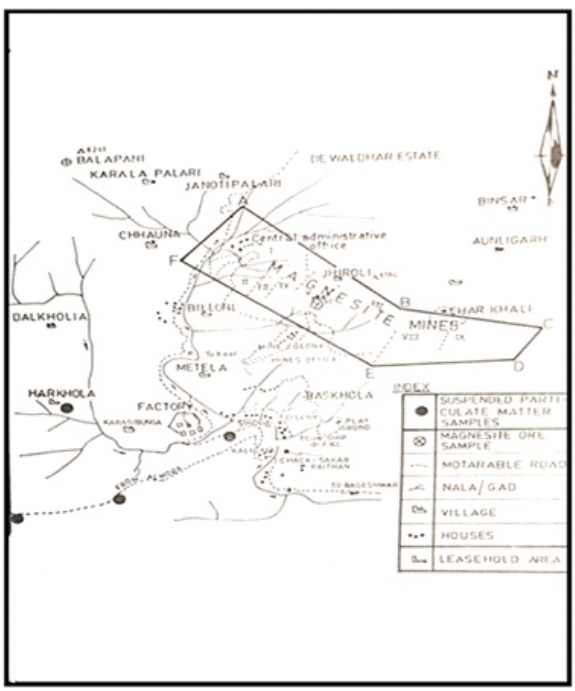



**Fig.2. Sites of Suspended Particulate Matter and Magnesite Ore**

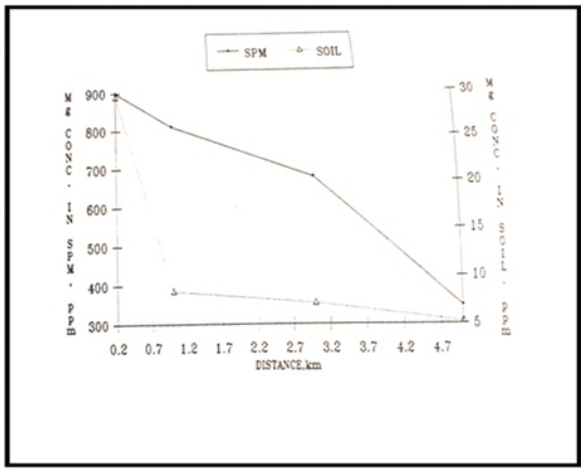



**Fig.3. Concentration of Mg in Suspended Particulate Matter and Soil with respect to distance from Plant**

## Observations

**Table1. Concentration of Magnesium in Suspended Particulate Matter (SPM), Soils near plant, Standard soil, and Magnesite ore**

| Concentration of Magnesium (ppm) | | | | |
|---|---|---|---|---|
| Sampling Site | Distance Km. | SPM | Soils near plant | Standard Soil Magnesite Ore |
| I | 0.2 | 900 | 29556 | |
| II | 1.0 | 810 | 8540 | |
| III | 3.0 | 675 | 7200 | |
| IV | 5.0 | 345 | 5250 | |
| Hawalbagh (Almora) | NR | NR | 5100 | |
| Block No. IV | NR | NR | NR | 245297 |

NR = Not Recorded

## Results and discussion

The analyzed samples revealed that there is a steep decline in the concentration of magnesium in the soil with increasing distance from the plant to a level corresponding with its normal content in the soil as is observed from the standard soil sample of Hawalbagh. The magnesium content of soil varies from a fraction of 1 percent in sandy soils of humid regions to many times more than this amount in clay soils of arid regions and it constitutes about 2 percent of earth crust (Kanwar, 1977). The highest concentration of magnesium in the soil as well as in SPM was recorded in sample I and II. The magnesium in soil sample I and II was observed as 29556 ppm and 8540 ppm, respectively. Similarly, the concentration of magnesium in SPM was recorded to be 900 and 810 in sample I and II, respectively with a much lower value of 675 and 345 in sample III and IV, respectively. Fig.3 explains the concentration of Mg in Suspended Particulate Matter and Soil with respect to distance from Plant

## Conclusion

It is evident from the observed data that emissions from the plant are not dispersed far and wide. But emissions are confined to the area around factory only. The concentration of magnesium in the soil decreases with increasing distance from the plant to a level corresponding with its normal content in the soil corresponds to Hawalbagh. Thus, the presence of magnesium concentration in the soil and in the dispersal of SPM in air is confined to the peripheral area of the plant. No adverse effect on the health of the people living in the vicinity of the plant was ever reported by the workers and local populace.

## Acknowledgement

The author is extremely thankful to the Director, Defence Agricultural Research Laboratory (DARL) district Nainital for providing Lab assistance and technical support.

## References

Cheng, KL. and Bray, RH. (1951). Determination of Ca and Mg in soil and plant material. *Soil Sci*., 72: 449-458.

Kanwar, JS. (1977). Soil Fertility Theory and Practice. ICAR. New Delhi.

Heald, WR. (1969). Calcium and Magnesium. IN: Methods of Soil Analysis, Part II. Am. Soc, Agron. Inc. Wisconsin, USA, pp. 999-1010.

Robinson, WO. (1945). The fusion analysis of soils. *Soil Sci*., 59:7-11.

**Biotech Today (January-June 2023) : 13 (1) : 63 - 66**

DOI : 10.5958/2322-0996.2023.00010.8

**RESEARCH ARTICLE**

# *Eucalyptus Globulus* Essential Oil: A Promising Antifungal Agent for *Candida albicans*

**Surya Pratap Singh[1], Shivani Singh[2] and R.S. Sengar[*]**

**Received**: 17 September, 2021, **Revised**: 26 November, 2021, **Accepted**: 6 December, 2021, **Published**: 1 January 2022

## Abstract

*Candida albicans,* a prominent fungal pathogen, poses a significant threat to human health, particularly in immunocompromised individuals. The rise of drug-resistant strains has underscored the need for alternative antimicrobial agents. Essential oils derived from aromatic plants have gained attention for their potential antimicrobial properties. This study explores the antimicrobial activity of *Eucalyptus Globulus* essential oil (EO) against *Candida albicans*. The EO was tested against *Candida albicans* strains through various in vitro assays, including Zone of inhibition (ZOI), minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), Our findings revealed that *Eucalyptus Globulus* EO exhibited potent *antifungal activity against Candida albicans*. In conclusion, this study highlights the significant antimicrobial potential of *Eucalyptus globulus* essential oil against *C. albicans*. These findings emphasize the importance of further research to harness the therapeutic potential of essential oils in combatting fungal infections. *Eucalyptus* Essential Oil has gained attention due to its potential antimicrobial properties, including antifungal activity. This study aims to assess the effectiveness of Eucalyptus EO at different concentrations against *C. albicans*

Keywords: Candida albicans, Essential Oil, *Eucalyptus Globulus,* anti-microbial assay

[1]Department of Agronomy, J.S University Shikohabad (U.P.)

[2]Department of Agriculture Biotechnology, S.V.B.P.U.A.&T, Meerut

[*]**Corresponding Author:** @gmail.com

## Introduction

*Eucalyptus globulus* is a flowering tree in the Myrtaceae (Myrtle) family. Throughout human history, it has been utilised for thousands of years. The *Eucalyptus* genus. They have around 700 distinct species and forms been successfully introduced on a global scale. Eucalyptus is a type of tree. Native to Australia and Tasmania, as well as Africa America ranges from tropical to temperate. (Hayat *et al*., 2015) Essential oils (EOs) are colourless liquids that contain predominantly aromatic and naturally occurring volatile organic components found throughout the plant, including seeds, flowers, peel, stem, bark, and every part of the plant (Jugreet *et al*., 2020). The utilisation of medicinal plants is an age-old practise in which humans attempted to better their health by utilising natural resources. The beginning of the use of plants as a treatment resource the study of diseases was empirical, with the idea that plants could be a source of sickness. an alternative to medical care (Jugreet *et al*., 2020). *Candida* strains spp. resistance to antifungal treatment has grown in recent decades, causing alarm amongst medical professionals (Petrovska *et al*., 2012). *Eucalyptus* is frequently utilised as an anaesthetic, expectorant, anti-inflammatory, and antiseptic (Bhattacharjee, 2016). They are renowned in medicine for possessing antibacterial, antifungal, and anti-inflammatory qualities, as well as analgesic properties via their essential oils. Due to their biological qualities, essential oils from the genus Eucalyptus are also used in the

pharmaceutical and cosmetics industries. The oil of eucalyptus and leaves are utilised in medicine. Eucalyptus oil is utilised as an anti-microbial agent in a variety of creams, soaps, and toothpastes Laboratory investigations have demonstrated that eucalyptus oil contains anti-bacterial and anti-fungal compounds. The leaves of eucalyptus also have antibacterial properties. (Bacher *et al*., 2011). *C. albicans* is a pleomorphic fungus that can be yeast or filamentous with hyphae formation. This morphological change capacity is known as polymorphism because of the production of germ tubes and the subsequent development of this form, which is also capable of producing chlamydospores. The two forms are associated to the infectious process, and the yeast form has a stronger ability to spread to human beings, while the hyphae can enter host phagocytic cells (Mulyaningsih *et al*., 2010). (Barbosa *et al* 2018) *C. albicans* is the most prevalent cause of candidiasis and an opportunistic infection. The study of secondary metabolites of medicinal plants, such as essential oils and plant extracts, in recent years has revealed that most essential oils derived from herbs contain anti-microbial and antifungal effect (Bokaeian *et al*., 2010). (Goodar *et al*, 2017) The aim of this study was to evaluate the effects of eucalyptus leaves EO in treatment of *C. albicans* infection.

## Material and Methods

Fresh leave of *Eucalyptus Globulus* were collected from Sardar Vallabhbhai Patel University of Agriculture and Technology Meerut. YPD agar and broth were procured from Himedia India.

## Test Organism

The *Candida albicans* were procured from Microbial Type Culture Collection (MTCC) Chandigarh. The Cultures of C. albicans were maintained in their appropriate agar slants at 4°C.

## Isolation of Essential oil:

Water distillation was performed on a portion 200 g of dried and finally pulverised plant material for 3.5 hours using a Clevenger-type apparatus as indicated by European Pharmacopoea. The essential oil was collected and dried over anhydrous sodium sulphate before being stored in an amber vial at 4°C until analysis. The yield was determined considering the dry weight of the sample.(Barbosa *et al*., 2018).

## Preparation of Inoculum

Three or four *c. albicans* isolated colonies were injected in 2 mL of YPD broth and cultured until the broth growth was similar to the WHO-recommended Mac-Farland criterion (0.5%).

## Essential oil antimicrobial Assay

## Zone of inhibition Assay

The antimicrobial activity of eucalyptus leaves Essential oil was tested using agar well diffusion method against Candida *Albicans*. A sterile cotton swab was used to smear the freshly created inoculum all over the surface of the YPD plate. Four wells of 6mm diameter were bored in the medium with a sterile cork-borer of 6mm diameter and labelled properly. Different concentrations of the *Eucalyptus Globulus* EO 1, 5,10, 20% were introduced into the respective wells. The agar plates were incubated at the appropriate temperature for the growth of *C. albicans* (typically 35-37°C) for 24 to 48 hours. After incubation, the plates were examined for the presence of zones of inhibition around the wells. The diameter of each zone was measured using a scale in mM (Goodarzi *et al*., 2018).

## Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal concentration (MFC)

The broth dilution method was used to determine the Minimum inhibitory

concentration (MIC) against the *Candida albicans*. The MIC experiments were carried out utilising various concentrations of essential oils, including 1mg/ml, 5mg/ml, 10mg/ml and 20mg/ml. Add different concentrations of the essential oil to separate wells of the microplate containing the *Candida albicans* culture. Incubate the microplate at a controlled temperature for a specific period (commonly 24 hours). Visually inspect the wells for fungal growth. The MIC is the lowest concentration of the essential oil at which there is no visible growth or significantly reduced growth compared to the control (wells without the essential oil). Transfer samples from the wells that showed no visible growth (or significantly reduced growth) to fresh growth medium without the essential oil. Incubate the transferred samples for an additional period (commonly 24-48 hours). Examine the transferred samples for regrowth. The MFC is the lowest concentration at which there is no regrowth of *Candida albicans* on plate

## Results and Discussion

The study on the effects of *Eucalyptus globulus* essential oil against *Candida albicans* yielded significant results, which are discussed below:

**Zone of Inhibition**: In our study, we observed a distinct zone of inhibition when *Eucalyptus Globulus* essential oil was tested against *Candida albicans*. The data clearly demonstrates a concentration-dependent effect of Eucalyptus Essential Oil against Candida albicans. As the EO concentration increased, the size of the inhibition zones also increased. This suggests that Eucalyptus EO possesses potent antifungal properties against this pathogenic yeast. At the lowest concentration tested (1%), Eucalyptus EO still exhibited a notable zone of inhibition, measuring 7 mm. This indicates that even at a relatively low concentration, the EO has some inhibitory effect on *Candida albicans* growth. The most significant inhibition was observed at the highest concentration (20%), with a remarkable 27 mm zone of inhibition. This concentration likely provides a sufficiently high dose of EO compounds to effectively inhibit fungal growth. The antifungal activity of Eucalyptus EO is attributed to its constituents, such as eucalyptol and terpinen-4-ol, which have known antimicrobial properties (Strasburg, 1996). The result is shown in table 1:

MIC (Minimum Inhibitory Concentration): In our experiment, we determined the MIC for E. globulus essential oil against C. albicans. This concentration was found to be 1mg/mL. This indicates that at this concentration, the essential oil effectively prevents the growth of C. albicans.

MFC (Minimum Fungicidal Concentration): In our study, we determined the MFC for Eucalyptus Globulus essential oil against C. albicans. This concentration was found to be 5mg/ml. This suggests that at the MFC, the essential oil not only inhibits the growth of C. albicans but also has a fungicidal effect, effectively killing the yeast.

These findings indicate that *E. Globulus* essential oil possesses strong antifungal properties against *C. albicans*. The zone of inhibition, MIC, and MFC values provide evidence of its efficacy in inhibiting and killing this pathogenic yeast. This is particularly promising in the context of developing natural and alternative treatments for C. albicans infections, which are often associated with antibiotic resistance issues. Further research may be necessary to explore the mechanisms by which *E. Globulus* essential oil exerts its antifungal effects and to assess its safety and efficacy in clinical settings. Nevertheless, these initial results suggest that this essential oil holds promise as a potential therapeutic agent against *C. albicans* infections.

## Conclusion

In conclusion, our study demonstrates that

Eucalyptus essential oil has significant antifungal activity against *Candida albicans*. These findings provide a foundation for further research into the development of eucalyptus-based antifungal therapies. However, additional studies are needed to validate these results in vivo and assess the safety and clinical potential of Eucalyptus essential oil as an antifungal agent.

**Table1: Antifungal activity of *Eucalyptus Globulus* essential oil against *C.albicans***

| *Eucalyptus Globulus* E.O (%) | Zone of Inhibition (mm) |
|---|---|
| 1 | 7 |
| 5 | 12 |
| 10 | 18 |
| 20 | 27 |

## References


Hayat, U, J cslani, M.I., Rehmar, R., and Nadeem, F. (2015) *A Review on Eucalyptus globulus: A new perspective in therapeutics. Int. J. Chem. Biochem. Sci* 8: 85-91.

Jugreet, B. Sharmeen,, (2020) Chemistry, bioactivities, mode of action and industrial applications of essential oils. *Trends in Food Science & Technology* 101 (2020): 89-105.

Jugreet, BS., Suroowan, S., Rengasany, RK., and Mahomoodally, MF. (2020). Chemistry, lioactivities, mode of action and industrial applications of essential oils. Frends in food science and Technology, 101 : 89-105.

Petrovska, Biljana B. (2012) Historical review of medicinal plants usage. Pharmacognosy reviews 6.11; 1.

Bhattacharjee, Partha. (2016) Epidemiology and antifungal susceptibility of Candida species in a tertiary care hospital, Kolkata, India. Current medical mycology 2.(2): 20.

Bachir, Raho G., and Mechaal Benali,(2012). Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus against Escherichia coli and Staphylococcus aureus. Asian Pacific journal of tropical biomedicine* 2.9: 739-742.

Mulyaningsih, S., Sporer, F., Reichling, J. and Wink, M. Anti bacterid activity of essential oils from Eucalptus and of selected components against multi-duy iesistant bacterial pathoges. Pharma ceutical biology 49(9); 893-899.

Bokaeian, MG. Nakhace, A., Moodi. B., and khazaci, HA. (2010) Eucalyptus globulus (eucalyptus) treatment of candidiasis in normal and diabetic rats. *Iranian Biomedical Journal* 14.3 121.

Barbosa, JP., de Oliveira, TR., Puppin, D., Teixeira, AL., Boni, GC., de Feiria, SNB., and Höfling, JF. (2018). Anti-Candida activity of essential oils from Eucalyptus species. a preliminary study. *Oral Health*. 8(3) : 1-5.

Goodarzi, Hedieh and Mohsen, (2017) "Separation of 1, 8-cineol from essentil oil of eucalyptus globulus and synthesis of new derivatives and antifungal activity of against candida albicans. *Biochemical & Cellular Archives* 17.(2).

Strasburg: Council of Europe. 3rd ed., (1996). Anonymous. *European Pharmacopoeia*; pp. 121–2.

Yasmeen, Farhat, *et al. Cedrus deodara* (Deodar) and *Zanthoxylum armatum* (Timur) evaluated as antimicrobial and antioxidant agents. *Journal of Pharmaceutical Sciences and Pharmacology* 2.2 (2015): 110-118.

Yasmeen, F., Rauf, T., Babar, M., Ali, S., and Andleets Cendrus deodara (Deodar) and Zanthoxylum armathunt (Timur) evaluated as antimicrobial and anti oxident agent. Journal ofd pharmaceutical Science and pharmocology, 2 (2) ; 110-1

Nezhad FM, Zeigham H, Mota A, Sattari M, Yadegar A. (2009) Antibacterial activity of eucalyptus extracts on methicillin resistance *Staphylococcus aureus. Res J Biol Sci*.; 4(8):905–908

**Biotech Today (January-June 2023) : 13 (1) : 67 - 71**

DOI : 10.5958/2322-0996.2023.00011.X

**RESEARCH ARTICLE**

# Optimization of parameters for biotic production of citric acid

**Shilpi Singh**

**Received**: 15 September, 2022, **Revised**: 19 November, 2022, **Accepted**: 08 December, 2022, **Published**: 1 January 2023

## Abstract

Increasing cost of production and global demand for citric acid is driving research towards optimizing process conditions to yield very high quantity of the organic acid using abundant cheap substrates and selected microorganisms. Citric acid is produced mainly by submerged aerobic fermentation using fungal strain of *Aspergillus niger*. The present paper reports the study of process parameter such as selection of Cheap raw material and its percent dilution, pH, temperature and incubation period under the influence of some citric acid producing fungus such as : *Aspergillus fischeri* SS-20, A. *foetidus* SS-21. *A. aculeatuas* SS-22, *A. carbonarius* SS- 23 and *A. wentii* SS-24 has been assessed for their citric acid producing capacity. Among the fungal species, *A. wentii* SS-24 is known as a potential producer of citric acid. It has been found that production of citric acid by A. wentii SS-24 proceeds best when molasses solution 25% (w/v) is allowed to ferment for 11 days of incubation period at 35°C temperature by maintaining pH value of fermentation medium to 2.1 along with some other nutritional ingredients required by the fungus *A. wentii* SS-24. Therefore, utilization of low-cost sugar industry bioproduct molasses which serve as suitable substrate for optimization of citric acid production is advocated because of their advantages such as income generation, reduction in environmental problems and public health hazards associated with it.

**Key words:** Citric acid fermentation, molasses, *A. wentii* SS-24)

## Introduction

Citric acid is scientifically known as 2-hydroxy-1, 2, 3-propanetricarboxylic acid). Joint FAO/WHO Expert Committee on Food Additives have given approval for global recognition of citric acid as a generally regarded as safe (GRAS) organic acid considering its wide application in the pharmaceutical and food industries (Varshney, 2016; Dutta *et al*., 2019). Citric acid can also be described as a biochemical product usually obtained by fermentation (Oladele *et al*., 2015). Since global demand for citric acid has exceeded natural citric acid supply, the use of biotechnological fermentation processes has become imperative (Kishore and Reddy, 2011). Cane molasses, beet molasses and glycerol have been utilized by researchers for the purpose of producing reasonable quantity of citric acid (Soccol *et al*., 2006). Different types of fruits contain citric acid which gives fruit a sour taste (Khairan *et al.,* 2019). In recent times, the use of fruit waste to produce citric acid is increasingly becoming more attractive to researchers because it is capable of reducing the cost of producing the organic acid (Varshney, 2016; Dutta *et al*., 2019, Leelawati Kumari 2018). The use of banana and plantain peels fermented by *Aspergillus niger* to produce citric acid was successfully carried out by Khairan *et al*., 2019; Chukwuemeka *et al.* (2019). In a recent study, Urak *et al.* (2014)

Department of Chemistry, Doranda College, Ranchi-834002, Jharkhand

*Corresponding Author: ssinghchemru@skuastkashmir.ac.in

reported the use of *Yarrowia lipolytica* for citric acid production. Also, Hesham *et al.* (2020) suggested the use of *Candida tropicalis* for commercial production of citric acid since it is more advantageous than *A. niger* which is a widely used fungus for that purpose.

Therefore, optimizing these variables is aimed at achieving best performance which will cause an upsurge of citric acid yield in large quantity. From 2008 the citric acid demand was found to be increasing by 5% yearly. (Thiruvengadam Shankar and Thangvel Sivakumar (2016)

### *Estimation of citric acid*

Determination of the concentration of citric acid present in the culture filtrate was carried out by means of titration. Ten millilitre (10 ml) culture broth was withdrawn and 3 drops of phenolphthalein was added as an indicator (Imandi *et al.*, 2007; Khosravi and Zoghi, 2008). Exactly 0.1M NaOH was titrated against 10 ml (equivalent to 10g) culture broth until the end-point was reached when the change in colour was noted. The quantity of NaOH used was read off as titre and the value was recorded.

% Citric acid = Normality × Volume of NaOH × Equiv. wt. of CA × Dilution factor Weight of Sample (g) × 10 ml

Parametric determination of citric acid production by *A. wentii* SS-24 virtually as important to the success of an industrial fermentation as is the selection of an organism to carry out the fermentation. Medium supplies nutrients for growth, energy, building of cell substances and biosynthesis of fermentation products. A poor selection of medium components can effect cellular growth and little if any yield of fermentation products. The optimization of parameters like concentration of selected raw material, hydrogen ion concentration, temperature and incubation period of the fermentation medium can partially or fully influence the types and ratios of products from among those for which a microorganism has biosynthetic capability. Thus, parametric determination of citric acid production by *A. wentii* SS-24 is very critical.

In the present communication autharess has confined her study to optimize the parameters for maximum production of citric acid by fermentation using the fungal strain of *A. wentii* SS-24

### Experimental Medium

The composition of the production medium for each fermentor flask containing 100 mL production medium is as below :

Molasses: 25% (w/v) $NH_4NO_3$: 0.75 , $KH_2PO_4$ : 0.75%, $MgSO_4.7H_2O$ : 0.75%, pH : 2.1

The pH of the medium was adjusted to 2.1 by adding requisite amount of lactic acid and it was ascertained by a pH metre.

### Preparation of the culture medium

In order to retain metabolic activity and to maintain the growth of the selected strain of fungus, i.e; *A. wentii* SS-24 it was fresh cultured periodically. Different micro-oganisms require different nutrient materials. Thus, culture medium vary in form and composition, depending on the specific species to be cultivated. The culture media for the maintenance of *A. wentii* SS-24 is as follows :

**Sucrose :** 0.200 g, Malt-extract : 0.50 g, **Yeast-extract :** 0.50 g, Peptone : 0.50 g, **Agar-Agar:** 0.80 g, Distilled water : 100 ml, **pH** : 2.0

The pH of the culture medium was adjusted to 2.0 by adding requisite amount of KCl- HCl buffer solution.

The above content was transferred in a 250 ml flat bottom conical flask and was made upto 100 ml by adding requisite amount of distilled water. The flask was then tightly plugged with non-absorbent cotton wool plugs.

### Sterilizations

The growth and production media were sterilized in an autoclave maintained at 15 lbs steam pressure for 30 min.

**Strain :** *A. wentii* SS-24 was used in the present study. The strain was procured from NCL, Pune,India.

**Assay methods:** Evaluation of citric acid formed (Imandi *et al*., 2007; Khosravi and Zoghi, 2008) was made titrimetrically.

**Age of the inoculum:** 50 hours old.

**Quantum of the inoculum:** 0.05 ml fungal suspension of *A. wentii* SS-24.

**Molasses concentration :** 10%, 12%, 14%, 16%, 18%, 20%, 22%, 25%, 27% and 30%.

**Temperature** (in°C) : 10, 15, 20, 25, 30, 35, 40, 45, 50, 55

**Incubation period:** 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 days.

**pH :** 1.5, 1.6, 1.8, 2.0, 2.1, 2.2, 2.4, 2.6, 2.8, 3.0

## Results and Discussion

The data recorded in the table-1 shows that different carbohydrates have been used and it has been observed that molasses under trial has much importance because it is cheap and easily available. It is used because when left open and unused it causes pollution to our environment.

The data recorded in the table-2 shows that a 25% molasses solution serves as an optimum substrate solution for the production of citric acid. It is obvious that higher concentration of molasses interferes with the production of citric acid producing enzyme activity of *A. wentii* SS-24 and inhibits the yield of citric acid.

The results recorded in the table-2 show that *A. wentii* SS-24 attains its best activity when the pH of the medium is maintained at 2.1. In much acidic medium, *A. wentii* SS-24 failed to give significant yield of citric acid. However, at pH 2.1 and onwards the yield of citric acid has been found discouraging and insignificant.

It is obvious from the table-2 that *A. wentii* SS-24 shows its best activity at the temperature 35°C. Lower temperature of the experiment at 10 to 30°C caused discourasing yield of citric acid. Higher temperatures, i.e; 40°C and onwards also deactivated the enzymatic system of citric acid fermentation process and the yield of citric acid was very much insignificant.

It is also obvious from the result that an incubation period of 11 days is most favourable for citric acid production by *A. wentii* SS-24. No increase in the yield of citric acid has been observed even after incubation period of 13 to 19 days.

**Table - 1 Effect of different carbohydrate sugars on production of citric acid by *A. wentii* SS-24**

| S.No. | Carbohydrates | Yield of Citric acid* in g/100 ml. |
|---|---|---|
| 1 | Arabinose | 1.612 |
| 2 | Rhamnose | 1.479 |
| 3 | Xylose | 2.278 |
| 4 | Glucose | 4.290 |
| 5 | Fructose | 5.425 |
| 6 | Galactose | 3.315 |
| 7 | Sorbose | 1.795 |
| 8 | Lactose | 3.275 |
| 9 | Sucrose | 6.383 |
| 10 | Maltose | 5.695 |
| 11 | Starch | 1.263 |
| 12 | Inuline | 1.311 |
| 13 | Dextrine | 1.563 |
| 14 | Raffinose | 1.483 |
| 15 | Mannitol | 3.291 |
| 16 | Molasses** | 7.890 |

*Each value represents mean of three observations

**Optimum yield of citric acid (from source, i.e. 25% molasses) 1-7 monosaccharides, 8-10. Disaccharides ,11-14 Polysaccharides.

Molasses has been employed as a source of carbohydrate for citric acid production-25% molasses solution corresponds approximately, 10.5 gm approx of fermentable sugar, Experimental deviation + 1.5 to 2.5%.

**Table – 2 Effect of concentration of molasses substrate, pH, temperature and incubation period on parametric determination for citric acid by *A. wentii* SS-24**

| % of Molasses | pH | Temp. in (°C) | Incubation period in days | Corresponding yield of citric acid* in g/100ml | | | |
|---|---|---|---|---|---|---|---|
| 10 | 1.5 | 10 | 1 | 2.318 | 3.334 | 3.778 | 0.886 |
| 12 | 1.6 | 15 | 3 | 3.620 | 3.916 | 5.165 | 0.998 |
| 14 | 1.8 | 20 | 5 | 4.403 | 5.416 | 6.730 | 5.763 |
| 16 | 2.0 | 25 | 7 | 5.385 | 6.112 | 6.918 | 6.217 |
| 18 | 2.1** | 30 | 9 | 5.473 | 7.982*** | 7.380 | 7.560 |
| 20 | 2.2 | 35** | 11** | 6.178 | 6.915 | 7.839*** | 8.112*** |
| 22 | 2.4 | 40 | 13 | 6.457 | 5.110 | 6.650 | 6.318 |
| 25** | 2.6 | 45 | 15 | 7.659*** | 4.413 | 5.005 | 5.863 |
| 27 | 2.8 | 50 | 17 | 6.338 | 3.440 | 4.116 | 4.230 |
| 30 | 3.0 | 55 | 19 | 4.119 | 2.115 | 3.334 | 2.115 |

*Each value represents mean of three trials.

**Optimum values of molasses solution, pH, temperature and incubation period.

*** Optimum yield of citric acid.

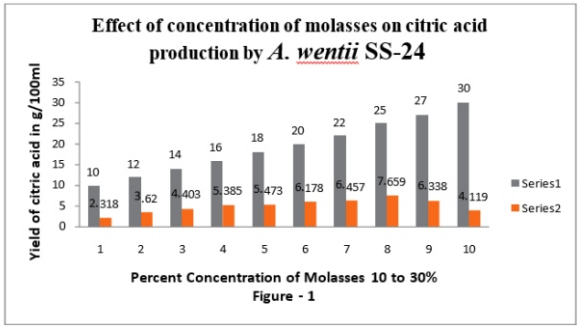


Figure - 1

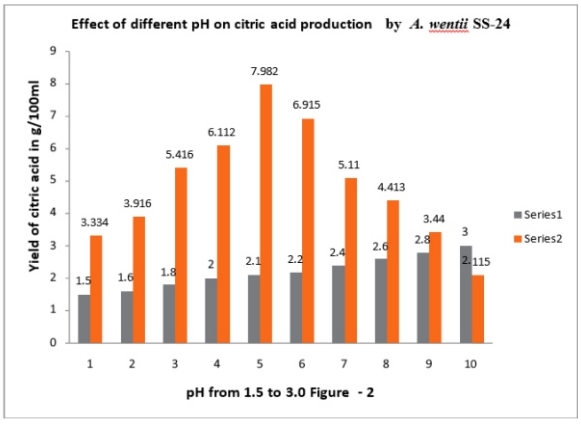
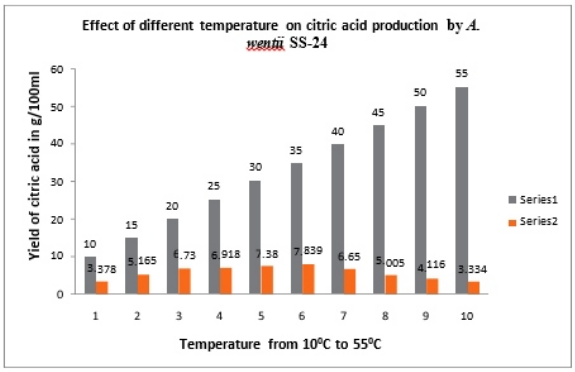
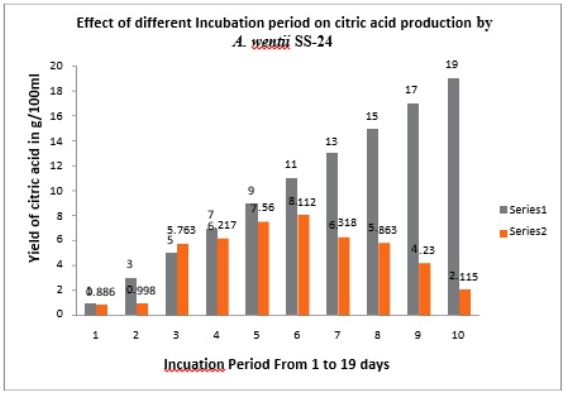


Figure - 2




## References

Chukwuemeka, C., Ethel, OC., Kalu, A.D., and Chukwuma, N. (2019). Citric acid production by *Aspergillus niger* using banana and plantain peels. *GSC Biological and Pharmaceutical Sciences*, 08(02): 015-021.

Dutta, A., Sahoo, S., Mishra, RR., Pradhan, B., Das, A., and Behera, BC. (2019). A comparative study of citric acid production from different agro-industrial wastes by *Aspergillus niger* isolated from mangrove forest soil. *Environmental and Experimental Biology*, 17: 115-122.

Imandi, SB., Bandaru, VVR., Somalanka, SR., and Garapati, HR. (2007). Optimization of medium constituents for the production of citric acid from by product glycerol using Doehlert Experiment Design. *Enzyme and Microbial Technology*, 40: 1367-1372.

Khairan, K., Makstum, A. and Yulvizar, C. (2019). Utilization of banana peel waste for citric acid production by *Aspergillus niger*. IOP Conference Series: *Earth and Environmental Science*, 364: 012005, 1-7. DOI: 10.1088/1755-1315/364/1/012005

Khosravi, DK. and Zoghi, A. (2008). Comparison of pre-treatment strategies of sugar cane bagasse: experimental design for citric acid production. Bioresource Technology, 99, 6986-6993.

Kishore, KA. and Reddy, GV. (2011). Optimization of operating variables in the fermentation of citric acid using response surface methodology. 2nd International Conference on Chemical Engineering and Applications, 23: 108-113.

Leelawati (2018). Parametric determination of conditions for bioproduction of citric acid *J. Chemtracks* 20(122): 97-102.

Oladele, KO., Siew, QY., Zakry, FAA., Lan, JC., and Ling, TC. (2015). Overview of citric acid production from *Aspergillus niger*. *Frontiers in Life Science*, 8(3), 271 – 283.

Soccol, CR., Vandenberghe, LPS., Rodrigues, and C., Pandey, A. (2006). New perspectives for citric acid Production and application. *Food Technology and Biotechnology*, 44(2): 141-149.

Thiruvengadam, and Thangvel (2016). Optimization of Citric acid production using *A. niger* isolated from the leaf litter soil of Sathuragiri Hills, *Universal Journal of Microbiology Research*, 4 (4) : 79 - 87.

Urak, S., Yeniay, O., and Karasu-Yalcin, S. (2014). Optimization of citric acid production from a carrot juice-based medium by Yarrowia lipolytica using response surface methodology. Annals of Microbiology, 65(2), 639-649.

Varshney, AS. (2016). Production, comparative and quantitative analysis of citric acid by *Aspergillus niger* using food waste as a substrate. *Journal of Experimental Food Chemistry*, 2(4), 1-6.

**Biotech Today (January-June 2023) : 13 (1) : 72 - 84**

DOI : 10.5958/2322-0996.2023.00012.1

**REVIEW ARTICLE**

# Discovery, metabolism and importance of auxins and cytokinins: The plant growth regulators

**Akanksha Singh[1], R S Sengar[2]*, Shailendra Pratap Singh[3], Vikas Singh[4], Shalani Gupta[5], Neelesh Kapoor[6], Mukesh Kumar[7], Vaishali[8], Manoj Kumar Yadav[9] and Reshu Chaudhary**

**Recieved**: 12 October, 2022, **Revised**: 04 November, 2022, **Accepted**: 10 December, 2022, **Published**: 1 January 2023

## Abstract

Auxins and cytokinin are two fundamental classes of plant growth regulators that play crucial roles in various aspects of plant development and physiology. This article provides an overview of the discovery, metabolism, and importance of auxins and cytokinin in plant growth and development. Auxins, initially discovered in the early 20th century, are primarily known for their involvement in regulating cell elongation, tropisms, apical dominance, and root development. Indole-3-acetic acid (IAA) is the most prominent naturally-occurring auxin. Auxins are synthesized in young tissues, including shoot apices and developing seeds, and are transported polarly within plants. Cytokinin, discovered later than auxins, is essential for cell division, shoot formation, leaf senescence, and overall plant growth. Adenine derivatives, such as zeatin, kinetin, and isopentenyl adenine, are commonly found in cytokinin. Like auxins, cytokinins are synthesized in actively dividing tissues, primarily in root tips and developing embryos. Understanding the metabolic pathways and signaling mechanisms of auxins and cytokinin has paved the way for their practical applications in agriculture, horticulture, and plant biotechnology. The precise manipulation of auxin and cytokinin levels through exogenous application or genetic engineering has been utilized to enhance crop productivity, control plant growth and development, and improve stress tolerance in plants.

Keywords: Auxins, cytokinin, plant growth regulators, discovery, metabolism, plant development, physiological responses, crop improvement.

## Introduction

Auxins and cytokinins are essential plant hormones that regulate various aspects of plant growth and development. They play fundamental roles in processes such as cell division, elongation, differentiation, tropisms, and organogenesis. Understanding the functions and interactions of auxins and cytokinins is crucial for comprehending the mechanisms underlying plant growth and for developing strategies to manipulate plant growth and development for agricultural purposes. Auxins are a class of plant hormones primarily responsible for cell elongation and differentiation. The most well-known and abundant naturally occurring auxin is indole-3-acetic acid (IAA). Auxins are synthesized in young and actively dividing tissues, including shoot apices, young leaves, and developing seeds. They are involved in numerous processes such as apical dominance, root development, phototropism, gravitropism, and lateral organ formation. Auxins are also involved in responses to environmental stimuli, such as light and touch. The polar transport of auxins allows for their distribution

[1-9]Department of Agriculture Biotechnology, College of Agriculture, Sardar Vallabh Bhai Patel University of Agriculture and Technology, Meerut, 250110 Uttar Pradesh.

[10] Dept of Bitechenology, KVS Cos, SVSU, meerut

***Corresponding Author:** sengerbiotech7@gmail.com

throughout the plant, regulating growth and development in a coordinated manner (Lavy and Estelle, 2016). Cytokinins are plant hormones that primarily regulate cell division, shoot formation, and overall plant growth. They promote cell division and influence various developmental processes, including shoot meristem activity, leaf senescence, nutrient mobilization, and apical dominance. Cytokinins are primarily synthesized in actively dividing tissues, such as root tips, developing embryos, and germinating seeds. The biosynthesis, degradation, and conjugation pathways of cytokinins contribute to their precise regulation and influence the balance between cell division and differentiation. Auxins and cytokinins interact synergistically or antagonistically to control various aspects of plant growth and development. The ratio and distribution of auxins to cytokinins play critical roles in determining cellular processes, tissue differentiation, and organogenesis (Xiong and Jiao, 2019). The crosstalk between auxin and cytokinin signaling pathways involves complex molecular mechanisms, including the activation or repression of target genes, modulation of transcription factors, and regulation of protein stability. These interactions and signaling pathways allow for the fine-tuning of plant growth and responses to environmental cues. The knowledge gained about auxins and cytokinins have paved the way for their practical applications in agriculture and biotechnology. Manipulating auxin and cytokinin levels through an exogenous application, genetic engineering, or plant tissue culture techniques have been used to improve crop yields, control plant growth, and development, enhance stress tolerance, and propagate plants through tissue culture techniques. In conclusion, auxins and cytokinins are key plant hormones that regulate diverse aspects of plant growth and development. Their intricate interactions and signaling pathways contribute to the coordination of cellular processes, tissue differentiation, and organogenesis (Dharmasiri *et al*., 2005). Understanding the roles and mechanisms of auxins and cytokinins provides a foundation for harnessing their potential in agriculture, horticulture, and biotechnology to address challenges related to crop production and plant growth control.

## Discovery of Auxins

The discovery of auxins can be attributed to the pioneering work of Charles Darwin and his son Francis Darwin in the late 19th century. Their studies focused on the responses of plants to light and gravity, particularly phototropism and gravitropism. In their experiments, Darwin observed that when plants were exposed to unilateral light, the stem of the plant would bend towards the light source. They hypothesized that a substance called "influence" was responsible for this growth response. This influence was later identified as auxin. In the early 20th century, the Dutch scientist Frits Went made significant contributions to the discovery and understanding of auxins. Went's work involved investigating the phenomenon of phototropism in coleoptile tips (the protective sheath covering the emerging shoots of plants). He towards the light. Went conducted a series of experiments to isolate and characterize this substance. He extracted it from the coleoptile tips and found that it could induce phototropic responses in other parts of the plant. He named this substance "auxin," derived from the Greek word "auxin" meaning "to grow." Indole-3-acetic acid (IAA) was identified as the major naturally occurring auxin by several researchers in subsequent years. This discovery was facilitated by advancements in analytical techniques and the development of more sensitive methods for auxin detection and quantification (Jacobs, 1979). The research conducted by the Darwin, Frits Went, and other scientists laid the foundation for our understanding of auxins

and their role in plant growth and development. Since then, further research has expanded our knowledge of auxins, including their biosynthesis, transport, signaling pathways, and interactions with other hormones. Today, auxins are recognized as essential plant growth regulators involved in a wide range of processes, including cell elongation, tropisms, apical dominance, root development, and tissue differentiation. The discovery of auxins has had a profound impact on plant biology and has led to applications in agriculture, horticulture, and biotechnology (Masuda and Kamisaka, 2000).

## Types and structures of auxins

There are several types of auxins, both naturally occurring and synthetic, that have been identified and studied for their roles in plant growth and development. Some of the commonly known auxins include:

1. Indole-3-acetic acid (IAA): IAA is the most abundant naturally occurring auxin in plants. It plays a crucial role in various processes such as cell elongation, apical dominance, root development, and phototropism.
2. Indole-3-butyric acid (IBA): IBA is another naturally occurring auxin found in plants. It is involved in root initiation, root growth, and adventitious rooting in cuttings.
3. Naphthaleneacetic acid (NAA): NAA is a synthetic auxin commonly used in plant research and horticulture. It is chemically similar to IAA and has similar effects on plant growth and development.
4. 2,4-Dichlorophenoxyacetic acid (2,4-D): 2,4-D is a widely used synthetic auxin in agriculture. It is known for its selective herbicidal properties, targeting broadleaf weeds while sparing most monocotyledonous plants.
5. 1-Naphthylacetic acid (NAA): NAA is a synthetic auxin that is frequently used in tissue culture and micropropagation techniques. It promotes root development and callus formation.
6. 3-Indolebutyric acid (IBA): IBA is a synthetic auxin commonly used in horticulture, especially for rooting hormone treatments. It promotes adventitious root formation in cuttings and grafting.

It is important to note that different auxins may have varying degrees of activity and effectiveness depending on the plant species, application method, and concentration used. The choice of auxin often depends on the specific desired outcome or application in plant research, horticulture, or agricultural practices (Gomes and Scortecci, 2021).

Auxin molecules are normally derived from the amino acid tryptophan. These types of amino acids have a six-sided carbon ring, which is attached to the five-sided carbon ring. The difference between the auxin molecule and the tryptophan is based on what is attached to the ring. To create a common auxin IAA molecule, two enzymes are needed to act on tryptophan first, an aminotransferase removes nitrogen and hydrogen from the side-chain attached to the 5-sided ring. After that decarboxylase enzyme removes the carboxyl group, leaving COOH. A chloride ion attached to the six-sided ring and IAA is born. Most auxins are some derivations of this molecule (Calderon-Villalobos *et al*., 2010).

## Biosynthesis of auxins

The biosynthesis of auxins occurs through various pathways in plants, involving both tryptophan-dependent and tryptophan-independent pathways. Here is an overview of the biosynthesis pathways of auxins:

A. Tryptophan-dependent pathway: The tryptophan-dependent pathway is the major route for auxin biosynthesis in plants.

i. Tryptophan (Trp) is an amino acid precursor for auxin biosynthesis. It can be synthesized de novo by plants or obtained from the environment.

ii. Tryptophan is converted to indole-3-pyruvic acid (IPA) through a series of enzymatic reactions. This pathway involves enzymes such as TAA (tryptophan aminotransferase) and YUC (YUCCA) proteins.

iii. Indole-3-pyruvic acid (IPA) is then converted to indole-3-acetaldehyde (IAAld) by the action of the enzyme YUC. This step is a rate-limiting step in auxin biosynthesis.

iv. Indole-3-acetaldehyde (IAAld) is further oxidized to indole-3-acetic acid (IAA), the most abundant naturally occurring auxin, through the action of aldehyde dehydrogenase enzymes (e.g., ALDH10A).

B. Tryptophan-independent pathways: Apart from the tryptophan-dependent pathway, auxins can also be synthesized via tryptophan-independent pathways, especially under stress or specific developmental conditions.

i. The indole-3-butyric acid (IBA) pathway: In this pathway, IBA is synthesized from a fatty acid precursor called indole-3-butyryl-CoA. IBA can then be converted to IAA through IBA-to-IAA conversion enzymes.

ii. Other tryptophan-independent pathways: Some plants have alternative pathways for auxin biosynthesis, which involve intermediates like tryptamine, phenylalanine, and tyrosine. These pathways are not as well characterized as the tryptophan-dependent pathway (Leopold, 2022, Menon *et al.*, 2022).

It is important to note that the biosynthesis of auxins can be regulated by various factors, including developmental cues, environmental conditions, and hormonal signals. Additionally, the biosynthesis pathways of auxins can vary among different plant species, tissues, and developmental stages (Teale *et al.*, 2006; Mano and Nemoto, 2012).

Understanding the biosynthesis pathways of auxins is essential for unraveling the regulation of auxin levels in plants and developing strategies to modulate auxin biosynthesis for agricultural and biotechnological applications.

## Auxin transport

Auxin transport refers to the movement of the plant hormone auxin from one part of the plant to another. This transport is essential for establishing auxin gradients and distributing auxin to various tissues and organs, where it plays a role in regulating growth and development. Auxin transport can occur in two main directions: acropetal transport (from the base of the plant towards the shoot apex) and basipetal transport (from the shoot apex towards the base of the plant). The movement of auxin is primarily facilitated by specialized proteins called auxin efflux carriers or auxin transporters, with the PIN (PIN-FORMED) proteins being the most well-known and studied (Lomax *et al*, 1995).

i. Polar Transport: Auxin transport is polar, meaning it occurs in a directional manner within the plant. This polarity is established by the asymmetric distribution of PIN proteins in the plasma membrane of plant cells. PIN proteins actively transport auxin out of cells, allowing it to move from cell to cell in a unidirectional manner.

ii. Efflux Carriers: PIN proteins are localized either at the basal (bottom) or apical (top) ends of cells, creating a polarized auxin flow. PIN efflux carriers at the basal end transport auxin out of cells, while those at the apical end facilitate auxin entry into cells. This asymmetric distribution of PIN proteins helps establish concentration gradients of auxin.

iii. Auxin Gradient Formation: The polar transport of auxin, combined with its active efflux and influx at specific locations, leads to the formation of auxin gradients within plant tissues. These gradients play a crucial role in various developmental processes, such as cell elongation, organ initiation, and tropic responses. For example, in phototropism, the asymmetric distribution of auxin due to light exposure leads to differential growth on the shaded and illuminated sides of the plant, resulting in bending towards the light source.

Regulation: The transport of auxin is tightly regulated by multiple factors. One important mechanism is the feedback regulation between auxin and the expression and localization of PIN proteins. Auxin can

influence the expression and localization of PIN proteins, which, in turn, affects the direction and intensity of auxin transport. Additionally, other hormones and environmental stimuli, such as light and gravity, can modulate auxin transport and contribute to the regulation of plant growth and development (Friml, 2003).

Overall, auxin transport plays a critical role in establishing auxin gradients, coordinating plant development, and regulating growth processes. The polarized movement of auxin-mediated by PIN proteins allows plants to respond to environmental cues and orchestrate their growth and adaptation.

**Importance of PIN proteins in auxin transport**

PIN proteins are a class of membrane proteins that play a central role in auxin transport within plants. They function as auxin efflux carriers, actively transporting auxin out of cells or facilitating its movement between cells. PIN proteins are essential for establishing auxin gradients and directing auxin flow during various developmental processes (Køeèek *et al*, 2009).

Here are some key features and functions of PIN proteins in auxin transport:

Distribution and Polar Localization: PIN proteins are localized in the plasma membrane of plant cells, and their distribution is often polarized. Depending on the tissue and developmental stage, PIN proteins can be found at different locations within the cell, such as the basal (bottom), apical (top), or lateral sides. This polar localization of PIN proteins determines the directionality of auxin transport.

PIN-FORMED (PIN) Family: PIN proteins belong to the PIN-FORMED family, which consists of several members (e.g., PIN1, PIN2, PIN3, etc.) with distinct expression patterns and roles in auxin transport. Different PIN proteins have specific localization patterns and are involved in different aspects of plant development and tropic responses.

Asymmetric Auxin Transport: PIN proteins actively mediate the efflux of auxin out of cells or its influx into cells. The polarized distribution of PIN proteins creates an asymmetry in auxin transport, allowing auxin to move directionally from cell to cell. PIN proteins at the basal end of cells transport auxin out, while those at the apical end facilitate auxin entry into cells.

PIN-Mediated Auxin Gradients: The polar transport of auxin mediated by PIN proteins helps establish concentration gradients of auxin within tissues and organs. These gradients are crucial for various processes, including cell elongation, organ initiation, vascular tissue differentiation, and tropic responses (e.g., phototropism, gravitropism). The directional movement of auxin, guided by the polarized distribution of PIN proteins, influences the differential growth and developmental responses observed in plants.

Regulation of PIN Localization: The localization of PIN proteins can be dynamically regulated in response to developmental and environmental cues. For example, the localization of PIN proteins can be influenced by auxin itself through feedback regulation. High auxin levels can induce the internalization of PIN proteins from the plasma membrane, reducing auxin efflux. Additionally, other factors, such as phosphorylation and interaction with other proteins, can also modulate the localization and activity of PIN proteins.

Understanding the role of PIN proteins in auxin transport is crucial for unraveling the mechanisms of plant growth and development. The polarized movement of auxin mediated by PIN proteins provides the spatial information necessary for plants to respond to their environment, regulate their growth, and coordinate various physiological processes (Adamowski and Friml, 2015; Mellor *et al.*, 2022).

## Auxin signaling

Auxin signaling refers to the complex process by which cells perceive and respond to the presence of the plant hormone auxin. Auxin signaling plays a pivotal role in regulating various aspects of plant growth and development, including cell elongation, tissue differentiation, apical dominance, lateral root formation, tropisms, and vascular development. The signaling pathway involves a series of molecular events that allow cells to interpret and respond to auxin concentrations.

Here is an overview of auxin signaling:

1. Auxin Entry into the Cell: Auxin molecules enter plant cells through various influx carrier proteins located in the plasma membrane.
2. Intracellular Pool: Once inside the cell, auxin molecules establish an intracellular pool, allowing them to move within the cytoplasm.
3. Auxin Efflux Carrier Proteins: Auxin efflux carrier proteins, such as PIN proteins, facilitate the export of auxin from the cytoplasm to the extracellular space or neighboring cells.
4. Auxin/IAA Proteins: Auxin molecules can bind to Auxin/Indole-3-Acetic Acid (IAA) proteins, which regulate the availability and transport of auxin.
5. Auxin Receptors: Auxin binds to specific receptors, primarily from the TIR1/AFB family, forming an auxin/receptor complex.
6. Auxin/Receptor Complex: The auxin/receptor complex interacts with other proteins, leading to the degradation of Aux/IAA proteins and the release of Auxin Response Factors (ARFs).
7. Activation/Inhibition of Auxin Response Factors: The release of ARFs allows them to regulate the expression of auxin-responsive genes by either activating or inhibiting their transcription.
8. Transcriptional Regulation of Auxin-Responsive Genes: ARFs bind to specific DNA sequences called auxin response elements (AuxREs) within the promoters of target genes, thereby controlling their expression levels.
9. Auxin-Mediated Responses: The transcriptional regulation of auxin-responsive genes ultimately leads to various auxin-mediated responses in plants, including cell elongation, differentiation, tropisms, apical dominance, and many others.

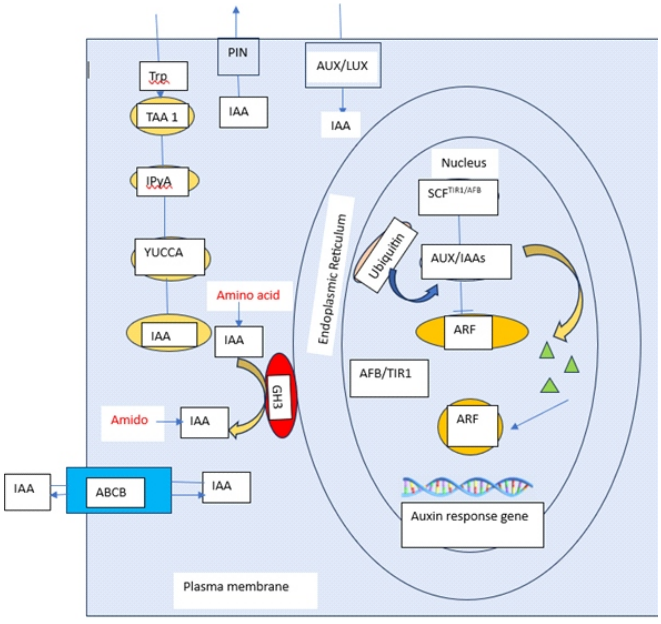



*Figure: A diagrammatic representation of auxin signaling.*

IAA is biosynthesized from Trp via IPyA by TAA1 and YUCCA in the TAA/YUC pathway. IAA is transported by AUX1/LAX1, PIN, and ABCB proteins. The auxin-induced GH3 enzyme converts active IAA to inactive IAA-amido. Auxin induces ubiquitination and degradation of Aux/IAA proteins via $SCF^{TIR1/AFB}$. Degradation of the Aux/IAA repressor recovers ARF activity to activate transcription of auxin-responsive genes (Paciorek and Friml, 2006; Morffy and Strader, 2022).

Auxin polar transport depends on three transport proteins: the import carrier protein AUX/LAX (AUXIN1/LIKE-AUX1) family, the export carrier protein PIN (pin-formed) family, and the carrier protein ABCB/MDR/PGP (ATP binding cassette B/Multidrug-resistance/p-glycoprotein) family with both import and export functions (Leyser, 2018).

## Role of auxin in plant growth and development

Auxin plays a crucial role in plant growth and development. It regulates various processes, including cell elongation, differentiation, tropisms, organogenesis, and apical dominance. Here are some key roles of auxin in plant development:

i. **Cell Elongation:** Auxin promotes cell elongation, especially in shoots. It loosens the cell wall, allowing cells to expand and elongate, contributing to the plant's growth of stems, leaves, and other aerial parts.

ii. **Tropisms:** Auxin controls plant responses to environmental stimuli, such as phototropism (growth towards light), gravitropism (response to gravity), and thigmotropism (response to touch). It regulates differential growth on different sides of the plant, enabling it to bend and grow towards or away from the stimuli.

iii. **Apical Dominance:** Auxin synthesized in the apical meristem (the growing tip of a shoot) suppresses the growth of lateral buds below it, maintaining apical dominance. This ensures that the plant's energy is primarily directed towards upward growth, promoting verticality.

iv. **Root Development:** Auxin is essential for root development and branching. It promotes the initiation and elongation of roots, as well as the formation of lateral roots. Additionally, auxin redistributes within the root to establish the gravitropic response, helping roots grow downward.

v. **Vascular Tissue Differentiation:** Auxin is involved in the differentiation of vascular tissues, including the xylem and phloem. It regulates the formation of vascular bundles, which transport water, nutrients, and sugars throughout the plant.

vi. **Leaf and Fruit Development:** Auxin influences leaf development by promoting leaf initiation and expansion. It also plays a role in fruit growth and development, including fruit setting, seed development, and fruit ripening.

vii. **Flower Development:** Auxin is involved in various stages of flower development, including the formation and positioning of floral organs. It promotes the initiation and growth of floral primordia, controls floral organ identity, and regulates flowering time.

viii. **Senescence and Abscission:** Auxin regulates the senescence (aging) and abscission (shedding) of plant organs. It promotes senescence in older leaves and triggers the abscission process, allowing plants to shed leaves, flowers, or fruits when necessary.

Overall, auxin acts as a versatile plant hormone that coordinates and regulates various aspects of plant growth and development, ensuring proper morphology and adaptation to the environment (Zažímalová *et al*., 2014; Mishra *et al.*, 2022).

## Discovery of cytokinin

The discovery of cytokinins as plant hormones can be attributed to a collaborative effort between several scientists, primarily Carl Miller and Folke Skoog. Here is a brief overview of the discovery of cytokinins:

In the early 1950s, researchers were studying the growth-promoting properties of coconut milk on plant tissue cultures. Carl Miller, a biochemist at the University of Wisconsin, and his student James Bonner were investigating the effects of coconut milk on tobacco tissue culture growth. They found that coconut milk stimulated the growth and division of cells. In 1955, a new compound named kinetin was isolated from old or autoclaved (but not fresh) DNA from herring sperm and calf thymus. In 1955, Miller presented his findings at a symposium where he met Folke Skoog, a plant physiologist from Sweden. Skoog was intrigued by Miller's results and proposed collaborating on further research. Miller and Skoog began investigating the active compounds present in coconut milk responsible for the growth-promoting effects. They discovered that a class of compounds called cytokinins was responsible for stimulating cell division and growth in plant tissues (Hluska *et al.*, 2021).

In 1956, Miller and Skoog published their groundbreaking paper titled "Chemical Regulation of Growth and Organ Formation in Plant Tissue Cultures in Vitro," in which they described the identification and characterization of cytokinins. They isolated and purified a compound called kinetin from coconut milk, which they determined to be a potent cytokinin.

The term "cytokinin" was coined to describe these growth-promoting compounds, derived from the Greek word "cyto," meaning cell, and "kinin," referring to their ability to promote cell division. The discovery of cytokinins had a significant impact on plant biology and tissue culture research. It provided insights into the complex regulatory mechanisms of plant growth and development and opened up new possibilities for manipulating plant growth in vitro. For their groundbreaking work on cytokinins, Carl Miller and Folke Skoog received numerous accolades and recognition within the scientific community. Their discovery laid the foundation for further research on cytokinins and their role in plant growth and development (Werner *et al*., 2001).

## Types and structures of cytokinins

Several types of cytokinins have been identified in plants, both naturally occurring and synthetic. Here are some commonly known types of cytokinins:

i. **N6-isopentenyl adenine (iP):** iP is one of the most abundant naturally occurring cytokinins in plants. It is involved in promoting cell division and shoot formation.

ii. **Zeatin (Z):** Zeatin is another naturally occurring cytokinin that is widely distributed in plants. It plays a significant role in promoting cell division, shoot initiation, and chloroplast development.

iii. **Dihydrozeatin (DHZ):** DHZ is a derivative of zeatin and exhibits similar cytokinin activity. It is involved in regulating cell division and shoot development.

iv. **N6-(2-isopentenyl) adenine (2iP):** 2iP is a synthetic cytokinin that is commonly used in plant research and tissue culture. It has similar biological activities to iP and is often used to enhance shoot proliferation and axillary bud growth.

v. **Benzyladenine (BA):** BA is a synthetic cytokinin that is widely used in plant tissue culture and horticulture. It promotes cell division, shoot formation, and lateral bud growth.

vi. **Meta-Topolin (mT):** Meta-Topolin is a synthetic cytokinin with similar biological activities to naturally occurring cytokinins. It is often used in tissue culture and micropropagation to induce shoot formation and enhance multiplication rates.

## Biosynthesis of cytokinins

The biosynthesis of cytokinins in plants involves complex enzymatic pathways and the interplay of various precursor molecules. The biosynthetic pathways can differ between plants, tissues, and developmental stages, but the general steps and key enzymes involved are outlined below:

i. **Adenylate Isopentenyltransferase (IPT) pathway:** The primary pathway for cytokinin biosynthesis is the Adenylate Isopentenyltransferase (IPT) pathway. The initial step involves the conversion of adenosine 5'-phosphate (AMP) into isopentenyladenosine 5'-monophosphate (iPMP) through the action of the enzyme adenylate isopentenyltransferase (IPT). iPMP is further converted to isopentenyladenosine (iPA) by the enzyme phosphoribohydrolase.

ii. **Tryptophan-dependent pathway:** In certain plant species, cytokinins can be synthesized from tryptophan, an amino acid. Tryptophan is converted to indole-3-pyruvic acid (IPA) via a series of enzymatic reactions, including tryptophan aminotransferase (TAA) and cytochrome P450 enzymes. PA is then converted to isopentenyladenine (iP) through the action of adenine phosphoribosyltransferase (APT) and adenine isopentenyltransferase (AIMT).

iii. **Other pathways:** Apart from the IPT pathway and tryptophan-dependent pathway, there are alternative pathways for

cytokinin biosynthesis that involve different precursor molecules. In the aromatic cytokinin pathway, phenylalanine and tyrosine are used as precursors for cytokinin synthesis, leading to the production of phenyl urea cytokinins. In the cytokinin glucoside pathway, cytokinins are first conjugated with glucose to form cytokinin glucosides. These glucosides can be subsequently hydrolyzed to release active cytokinins (Wybouw and De Rybel, 2019).

## Cytokinin transport

The transport of cytokinins involves both long-distance and short-distance movement, which is crucial for the coordination of various physiological processes. Here are the main aspects of cytokinin transport:

1. **Long-distance transport:** Long-distance transport refers to the movement of cytokinins over significant distances within the plant, typically through the xylem or phloem vessels.

i. **Xylem transport:** Cytokinins can be transported upward through the xylem vessels from the roots to the shoots. This transport occurs through the transpiration stream, where water and dissolved substances are pulled up from the roots to the shoots due to the transpiration process. Cytokinins can move passively with the water flow in the xylem sap.

ii. **Phloem transport:** Cytokinins can also be transported through the phloem, which carries sugars and other nutrients from source tissues (e.g., mature leaves) to sink tissues (e.g., developing shoots or roots). The precise mechanisms of cytokinin movement through the phloem are not fully understood, but it is thought to involve active transport processes.

2. **Short-distance transport:** Short-distance transport of cytokinins occurs within tissues and between adjacent cells. It allows cytokinins to be distributed to specific target sites and regulate local physiological processes.

i. **Cellular efflux carriers:** Cytokinins can be exported out of cells through specific efflux carriers, including members of the PIN-FORMED (PIN) protein family. These transporters facilitate the directional movement of cytokinins across the plasma membrane, allowing them to be transported to neighboring cells.

ii. **Vascular parenchyma cells:** Within tissues, cytokinins can move through vascular parenchyma cells. These cells are involved in the radial transport of substances across different tissues and play a role in distributing cytokinins within a plant.

iii. **Apoplastic transport:** Cytokinins can also move through the apoplast, which refers to the extracellular space between cells. This transport mechanism allows cytokinins to diffuse through the cell walls and reach adjacent cells (Perilli *et al*., 2010).

The transport of cytokinins is highly regulated, and its direction and intensity are influenced by factors such as tissue-specific expression of transporters, hormonal crosstalk, and developmental cues. The coordination of long-distance and short-distance transport mechanisms ensures that cytokinins reach their target sites and elicit the desired physiological responses throughout the plant. Mainly, three kinds of cytokinin transporters have been reported to date: purine permeases (PUP), equilibrative nucleoside transporters (ENT), and G subfamily ATP-binding cassette (ABCG) transporters (Durán-Medina *et al*., 2017).

Here are some examples of cytokinin transporters and their functions:

1. **ATP-binding cassette (ABC) transporters:** ABCB family: ABCB transporters, also known as multidrug resistance (MDR) proteins, have been implicated in cytokinin transport. They are involved in cytokinin efflux from cells, contributing to long-distance transport and the establishment of cytokinin gradients within plant tissues.

2. **Cytokinin-specific binding proteins:** Arabidopsis thaliana histidine kinase receptors (AHKs): AHKs are cytokinin receptors that not only perceive cytokinins but also play a role in cytokinin transport.

They can function as cytokinin influx carriers, facilitating the uptake of cytokinins into cells.

3. **PUP transporters:** PUP1/PUP2: PUP1 and PUP2 (Purine Permease 1 and 2) are members of the PUP transporter family involved in cytokinin transport. They are responsible for the uptake and efflux of cytokinins across cellular membranes, enabling the movement of cytokinins between cells or compartments.
4. **PIN-FORMED (PIN) proteins:** PIN proteins are auxin efflux carriers, but they are also involved in cytokinin transport. They play a role in the directional movement of cytokinins across plasma membranes, contributing to their distribution within tissues.
5. C**ytokinin oxidase/dehydrogenase (CKX):** CKX enzymes, while not transporters themselves, play a crucial role in regulating cytokinin levels and transport indirectly. They degrade cytokinins, converting them into inactive forms and thereby controlling their availability and distribution within plant tissues.
6. **Cellular influx and efflux carriers:** In addition to the specific transporters mentioned above, there may be other cellular influx and efflux carriers involved in cytokinin transport. These carriers facilitate the movement of cytokinins into and out of cells, regulating their distribution and concentration gradients.
7. **Xylem and phloem transport systems:** Cytokinins can move through the xylem and phloem vessels, utilizing the existing transport systems for water, nutrients, and assimilates. The movement of cytokinins through these vascular tissues is likely facilitated by specific carriers or interactions with other molecules.

## Cytokinin signaling

Cytokinin signaling is a complex process that involves the perception of cytokinins by receptors, activation of downstream signaling pathways, and regulation of gene expression to elicit specific physiological responses. Here is a general overview of the cytokinin signaling process:

a. **Cytokinin Perception:** Cytokinin perception begins with the binding of cytokinins to specific receptors located on the plasma membrane. In Arabidopsis thaliana, the primary receptors are members of the Arabidopsis Histidine Kinase (AHK) family, such as AHK2, AHK3, and AHK4. Cytokinin binding induces a conformational change in the receptor complex, leading to the autophosphorylation of the receptor and subsequent activation.

b. **Signal Transduction:** Activated cytokinin receptors phosphorylate specific histidine residues within their own protein structure. The phosphorylation event triggers the transfer of the phosphate group to a conserved aspartate residue in a downstream response regulator protein, such as Arabidopsis Response Regulator (ARR) proteins. Phosphorylated ARR proteins act as transcription factors that regulate gene expression.

c. **Transcriptional Regulation:** Phosphorylated ARR proteins can act as either positive or negative regulators of gene expression depending on the specific ARR protein and the target gene.

- Positive regulators (Type-A ARR proteins) typically activate gene expression by binding to specific DNA sequences known as cytokinin response elements (CREs) present in the promoter regions of target genes.
- Negative regulators (Type-B ARR proteins) can repress gene expression by interacting with other transcription factors or by inhibiting the activity of positive regulators.

d. Gene Expression: Target genes regulated by cytokinin-responsive transcription factors include those involved in cell division, shoot development, leaf senescence, chloroplast development, and other processes related to cytokinin responses. Cytokinin signaling leads to the upregulation or downregulation of these target genes, ultimately influencing plant growth, development, and physiological responses.

e. Crosstalk with other Signaling Pathways: Cytokinin signaling interacts with other hormonal and signaling pathways, such as auxin, gibberellins, and abscisic acid.

Crosstalk between these pathways allows for the integration of multiple signals to fine-tune plant responses.

The cytokinin signaling process is tightly regulated and coordinated to ensure appropriate responses to cytokinin concentrations and timing. The precise molecular mechanisms and components of cytokinin signaling may vary among plant species, tissues, and developmental stages. Continued research is needed to unravel the intricate details of cytokinin signaling and its interactions with other signaling pathways, contributing to a deeper understanding of plant growth and development (Kieber and Schaller, 2018).

Cytokinin signaling involves the regulation of gene expression through the activation or repression of specific genes. Several key genes and gene families are involved in cytokinin signaling pathways. Here are some of the important genes involved in cytokinin signaling:

1. **Arabidopsis Histidine Kinase (AHK) family:** AHK2, AHK3, AHK4: These receptor genes are part of the Arabidopsis Histidine Kinase family and function as cytokinin receptors, perceiving cytokinin signals and initiating downstream signaling cascades.
2. **Arabidopsis Response Regulator (ARR) family:**

a. **Type-A ARR genes (e.g., ARR4, ARR5, ARR6, ARR7):** These genes encode transcription factors that act as positive regulators of cytokinin signaling. They are involved in activating downstream target genes in response to cytokinin signals.

b. **Type-B ARR genes (e.g., ARR1, ARR2, ARR10):** These genes also encode transcription factors but function as negative regulators of cytokinin signaling. They can repress the activity of Type-A ARR genes or interact with other transcription factors to inhibit gene expression.

3. **Cytokinin-Oxidase/Dehydrogenase (CKX) family:** CKX genes (e.g., CKX1, CKX2, CKX3): CKX genes encode enzymes involved in the degradation of cytokinins. They regulate cytokinin levels and influence the duration and strength of cytokinin signaling by converting active cytokinins into inactive forms.
4. **Cytokinin Response Factors (CRFs):** CRF genes (e.g., CRF1, CRF2, CRF3): CRF genes are involved in the downstream transcriptional regulation of cytokinin-responsive genes.
5. They act as transcription factors and mediate cytokinin-induced gene expression.
6. **Arabidopsis Two-Component Signaling (TCS) pathway genes:** HP (Histidine Phosphotransfer): Genes encoding proteins involved in the histidine phosphotransfer signaling cascade, such as AHPs (Arabidopsis Histidine Phosphotransfer Proteins), which function as intermediates in transmitting cytokinin signals from receptors to downstream response regulators.
7. **Auxin Response Factors (ARFs):** Some ARF genes, which are primarily associated with auxin signaling, have been found to be regulated by cytokinin signals as well. Crosstalk between cytokinin and auxin signaling pathways involves the regulation of ARF genes (Hwang *et al*., 2012).

It is important to note that the specific genes involved in cytokinin signaling may vary among plant species. Additionally, the expression and function of these genes can be influenced by factors such as tissue specificity, developmental stage, and environmental conditions. Studying the roles and interactions of these genes in cytokinin signaling pathways is essential for understanding the molecular mechanisms underlying cytokinin responses and their impact on plant growth, development, and physiology (Wong *et al.*, 2015).

## Role of Cytokinin in plant growth and development

The major roles of cytokinins in plant growth and development:

1. Cell Division and Meristem Activity: Cytokinins promote cell division and stimulate the activity of shoot and root meristems. They contribute to the formation and maintenance of meristematic tissues,

which are responsible for continuous growth and development in plants.

2. Shoot and Leaf Development: Cytokinins influence shoot development, stimulating shoot elongation, branching, and the formation of lateral shoots. They also play a role in leaf expansion and leaf initiation. Higher levels of cytokinins can result in increased shoot growth and the development of more branches.
3. Root Growth and Development: Cytokinins regulate root growth and development, promoting lateral root formation and inhibiting primary root elongation. They contribute to the balance between shoot and root growth by modulating root-to-shoot communication and resource allocation.
4. Chloroplast Development and Photosynthesis: Cytokinins influence chloroplast development and the maintenance of chloroplast function. They regulate the expression of genes involved in chlorophyll biosynthesis and photosynthetic machinery, affecting photosynthetic efficiency and plant productivity.
5. Delaying Leaf Senescence: Cytokinins can delay leaf senescence, the natural aging process of leaves. They maintain leaf greenness, delay chlorophyll degradation, and prolong the functional lifespan of leaves. This can enhance the overall plant productivity and nutrient remobilization.
6. Reproductive Development: Cytokinins are involved in various aspects of reproductive development, including flower initiation, floral organ formation, and fruit development. They influence the transition from vegetative to reproductive growth, promote flower bud differentiation, and regulate the balance between vegetative and reproductive organs.
7. Apical Dominance and Bud Activation: Cytokinins play a role in apical dominance, which inhibits the growth of lateral buds in favor of the main apical bud. They can release the dormancy of lateral buds and promote their activation, leading to branching and shoot proliferation.
8. Stress Response and Nutrient Uptake: Cytokinins are involved in plant responses to abiotic and biotic stresses. They regulate stress-related gene expression, and enhance plant tolerance to drought, salinity, and other environmental stresses. Cytokinins also influence nutrient uptake and assimilation, affecting plant nutrient status (Werner and Schmülling, 2009; Wu *et al.,* 2021).

## Acknowledgment

I am very thankful to the Division of Plant Biotechnology, college of Biotechnology Sardar Vallabhbhai Patel University of Agri. And technology Meerut for providing me with such facility so that I am able to write this review paper. The authors would like to express their profound appreciation to Dr. R.S. Sengar for his expert input and opinion that have shaped this paper and placed it in a better context.

## References

Adamowski, M., and Friml, J. (2015). PIN-dependent auxin transport: action, regulation, and evolution. *The Plant Cell*, 27(1), 20-32.

Arnao, M. B., and Hernández-Ruiz, J. (2020). Is phytomelatonin a new plant hormone? *Agronomy*, *10*(1): 95.

Calderon-Villalobos, L I., Tan, X., Zheng, N., and Estelle, M. (2010). Auxin perception—structural insights. *Cold Spring Harbor perspectives in biology*, *2*(7): a005546.

Dharmasiri, N., Dharmasiri, S., Weijers, D., Lechner, E., Yamada, M., Hobbie, L. and Estelle, M. (2005). Plant development is regulated by a family of auxin receptor F box proteins. *Developmental cell*, *9*(1), 109-119.

Durán-Medina, Y., Díaz-Ramírez, D., and Marsch-Martínez, N. (2017). Cytokinins on the move. *Frontiers in Plant Science*, *8*, 146.

Friml, J. (2003). Auxin transport—shaping the plant. *Current opinion in plant biology*, *6*(1), 7-12.

Gomes, GLB., and Scortecci, KC. (2021). Auxin and its role in plant development: structure, signalling, regulation and response mechanisms. *Plant Biology*, *23*(6): 894-904.

Hluska, T., Hlusková, L., and Emery, RN. (2021). The Hulks and the Deadpools of the cytokinin universe: A dual strategy for cytokinin production, translocation, and signal transduction. *Biomolecules*, *11*(2), 209.

Hwang, I., Sheen, J., and Müller, B. (2012). Cytokinin signaling networks. *Annual review of plant biology*, *63*, 353-380.

Kieber, JJ., and Schaller, GE. (2018). Cytokinin signaling in plant development. *Development*, *145*(4), dev149344.

Skùpa, P., Libus, J., Naramoto, S., Tejos, R., Friml, J., and Zažímalová, E. (2009). The PIN-FORMED (PIN) protein family of auxin transporters. *Genome biology*, 10(12), 1-11.

Lavy, M., and Estelle, M. (2016). Mechanisms of auxin signaling. *Development*, *143*(18), 3226-3229.

Leopold, AC. (2022). *Auxins and plant growth*. Univ of California Press.

Leyser, O. (2018). Auxin signaling. *Plant Physiology*, *176*(1), 465-479.

Lomax, TL., Muday, GK., and Rubery, PH. (1995). Auxin transport. *Plant hormones: physiology, biochemistry, and molecular biology*, 509-530.

Mano, Y., and Nemoto, K. (2012). The pathway of auxin biosynthesis in plants. *Journal of Experimental Botany*, *63*(8): 2853-2872.

Masuda, Y., and Kamisaka, S. (2000). Discovery of auxin. In *Discoveries In Plant Biology: Volume III* (pp. 43-57).

Mellor, NL., Vo, U., Ware, A., Janes, G., Barrack, D., Bishopp, A., ... and Band, L. R. (2022). Systems approaches reveal that ABCB and PIN proteins mediate co-dependent auxin efflux. *The Plant Cell*, *34*(6): 2309-2327.

Menon, N., Richmond, D., Rahman, MR., and Menon, BR. (2022). Versatile and facile one-pot biosynthesis for amides and carboxylic acids in E. coli by engineering auxin pathways of plant microbiomes. *ACS Catalysis*, *12*(4), 2309-2319.

Mishra, BS., Sharma, M., and Laxmi, A. (2022). Role of sugar and auxin crosstalk in plant growth and development. *Physiologia plantarum*, *174*(1) : e13546.

Morffy, N., and Strader, LC. (2022). Structural aspects of auxin signaling. *Cold Spring Harbor Perspectives in Biology*, *14*(1): a039883.

Paciorek, T., and Friml, J. (2006). Auxin signaling. *Journal of Cell Science*, *119*(7) : 1199-1202.

Perilli, S., Moubayidin, L., and Sabatini, S. (2010). The molecular basis of cytokinin function. *Current opinion in plant biology*, *13*(1) : 21-26.

Skoog, F. (2019). A personal history of cytokinin and plant hormone research. In *Cytokinins* (pp. 1-14). CRC Press.

Teale, WD., Paponov, IA., and Palme, K. (2006). Auxin in action: signaling, transport and the control of plant growth and development. *Nature reviews Molecular cell biology*, *7*(11) : 847-859.

Werner, T., and Schmülling, T. (2009). Cytokinin action in plant development. *Current opinion in plant biology*, *12*(5) : 527-538.

Werner, T., Motyka, V., Strnad, M., and Schmülling, T. (2001). Regulation of plant growth by cytokinin. *Proceedings of the National Academy of Sciences*, *98*(18): 10487-10492.

Wong, WS., Tan, SN., Ge, L., Chen, X., and Yong, JWH. (2015). The importance of phytohormones and microbes in biofertilizers. *Bacterial metabolites in sustainable agroecosystem*, 105-158.

Wu, W., Du, K., Kang, X., and Wei, H. (2021). The diverse roles of cytokinins in regulating leaf development. *Horticulture Research*, *8*.

Wybouw, B., and De Rybel, B. (2019). Cytokinin–a developing story. *Trends in plant science*, *24*(2) : 177-185.

Xiong, Y., and Jiao, Y. (2019). The diverse roles of auxin in regulating leaf development. *Plants*, *8*(7) : 243.

Zažímalová, E., Petrášek, J., and Benková, E. (Eds.). (2014). *Auxin and its role in plant development* (pp. 2675-2688). Vienna: Springer.

Biotech Today (January-June 2023) : 13 (1) : 85 - 96

DOI : 10.5958/2322-0996.2023.00013.3

REVIEW ARTICLE

# A Review on Biological Biochemical Elucidations: Techniques and Technologies

**Shivanshu Garg[1], Pooja Nain[2*] and Himanshu Punetha[3]**

**Received**: 21 September, 2022, **Revised**: 05 November, 2022, **Accepted**: 09 December, 2022, **Published**: 1 January 2023

## Abstract

To understand a plant, animal, bacteria, fungi and virus through biochemical means involves rapid but accurate measurements of the biochemicals concerned. Nonetheless, gene to protein true type case scenarios have been lacking in most of the non-understood studies of these molecules occurring naturally. To generate potential of their use of and to involve these in current research, important techniques have been enlisted here so as to overcome the knowledge lag behind instrumentation based research. The application advantages of these are enormous ranging from field of physics, physiology, pediatrics, pharmaceuticals to plant biology. The makers have been set for biochemical analysis and the relative points of difference have been mapped throughout the existing biological world, only possible by means of these selective techniques and technologies, of whose mention becomes a landmark of biochemical studies there within. This review article comprises short, to the point; the methodology and advantage application of modern era technologies to provide discrete yet research applicable portion of the concerned techniques and technologies.

**Keywords:** Instruments, Analysis, Biochemicals, Compounds, Molecules, Metabolites

[1,3]Department of Biochemistry, CBSH-GBPUA&T, Pantnagar, Uttarakhand-263145, India

[2]Department of Soil Science, College of Agriculture, GBPUA&T, Pantnagar, Uttarakhand-263145, India

*__Corresponding Author:__ nainpooja6@gmail.com

## Bioreactors

A bioreactor creates a controlled environment in which biological, biochemical, and biomechanical criteria for created products can be met. A bioreactor is a device or system designed to support a biologically active environment. The synthesis of important biotechnological products from natural and genetically modified cell systems requires sophisticated and sound bioreactor design with unique performance characteristics. Various types of bioreactors can be classified based upon oxygen and stirring requirement, operation mechanism, microbial growth and process requirement. Bioreactor models used in bioprocesses include stirred tank reactor, bubble column, airlift, packed bed, fluidized bed, membrane and photo-bioreactors. The bioreactor performance can be measured by studying about $k_La$ (volumetric mass-transfer coefficient). Rate law for bioreactor is given by Monod equation. Bioreactor types, their design, and features needs to be studied for biomolecules production, such as biomass, enzymes, organic acids, fragrance compounds, spores, mushroom production, pigments, antibiotics, and others. Mammalian cell culture, vegetable cell culture, and photo-bioreactors for algal culture are examples of recent bioreactor applications. Therefore, a good bioreactor design should focus on increased productivity, validation of desired parameters, and cost-effective production of consistent and higher-quality products (Jaibiba *et al* 2020; Muniraj *et al*., 2019; Singhal *et al*., 2018; Singh *et al*., 2014 and Spier *et al*., 2011).

## Western Blotting

Blots are techniques for transferring nucleic acids or proteins onto Acarrier like nylon or nitrocellulose membranes. In many instances, the molecules are transferred onto the blotting membrane after gel electrophoresis whereas other times the samples are directly added onto the membrane. Western blot is used forspecific identification and characterization of proteins . Western blotting is also known as immunoblotting, because an antibody isused to specifically detect its antigen. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) separated proteins are electrophoretically transferred to a polyvinylidene fluoride (PVDF) membrane which is then incubated with specific antibodies, then developed to show the protein of interest. Other blotting techniques (i.e.Southern blot, Northern blot and Eastern blot) employ same principles but are used to analyze DNA, RNA and post-translational protein modifications. Some applications of this technique are; qualitative detection of single proteins and protein-modifications, medical diagnostics, e.g., in the HIV test or used as the definitive test for Bovine spongiform encephalopathy i.e, Mad cow disease. (Sawhney and Singh, 2014; Towbin *et al*., 1979; Wilson and Walker, 2010)

## Agarose Gel Electrophoresis

Agarose gel electrophoresis is the most effective way of separating DNA fragments of varying sizes. Agarose is isolated from the seaweed genera Gelidium and Gracilaria, and consists of repeated agarobiose subunits. To separate DNA using Agarose gel electrophoresis, the DNA is loaded into pre-cast wells in the gel and a current is applied. The phosphate backbone of the DNA molecule is negatively charged, therefore when DNA samples are loaded from the cathode, DNA fragments will migrate to the positively charged anode. The gel is rinsed gently in a solution of ethidium bromide and then viewed under ultraviolet light (300 nm wavelength). DNA molecules are separated on the basis of size within an Agarose gel in a pattern such that the distance traveled is inversely proportional to the log of its molecular weight. Both vertical as well as horizontal types of apparatus for casting of Agarose gels are available. Some applications of this technique are; Estimation of the size of DNA molecules, Analysis of PCR products, separation of restricted genomic DNA prior to Southern analysis (Sawhney and Singh, 2014; Thimmaiah, 2006 and Wilson *et al*., 2010).

## Thin Layer Chromatography

Thin layer chromatography is a technique used for the separation of mixtures of chemical compounds on a glass slide coated with a thin layer of adsorbent material such as silica gel.The separation relies on the relative affinity of compounds towards the stationary phase and mobile phase. The behavior of an individual compound in TLC is characterized by a quantity Known as R*f*(Relative front). TLC is a quick, sensitive, and inexpensive technique used to determine the number of components in a mixture, verify the identity and purity of compounds, monitor the progress of a reaction, determine the solvent composition (Nikalje, 2017).

## Paper Electrophoresis

Electrophoresis is a process in which, when a charged molecule is placed under the influence of electric field, then the charged molecule moves towards the opposite charge bearer electrode. One type of electrophoresis is paper electrophoresis in which an unknown protein or amino acid in a sample is separated on the basis of their charge. In this form of electrophoresis, the sample is applied as a circular point or spot on a strip of Whatman filter paper of cellulose acetate paper moistened with the buffer solution. The ends of the paper are immersed in separate reservoirs containing buffer and in which the electrodes are fitted. Upon passing electric

current, the ions in the sample migrate towards oppositely charged electrodes at characteristic rates. This method is suitable for separation of low molecular weight compounds such as amino acids, small peptides and nucleotides. This technique is divided into two types on the basis of placing the paper either horizontal, which is called horizontal electrophoresis and placing the paper in vertical position and it is called vertical electrophoresis. Some applications of this technique are; distinguishing amino acids and various proteins, haemoglobin abnormalities and diagnosing the sickle cell anaemia disease. However, this technique has become old due to its innovative modification throughout the time. At present, this process has changed from paper to gel electrophoresis and to fully automatic protein analyzer or amino acids analyzer machines; such as; Aracus- amino acid analyzer and K9840 protein analyzer that can analyze in 5-10 minutes per sample which is far better than classical paper electrophoresis (David *et al*., 2006; Wilson *et al*., 2018).

**Nuclear Magnetic Resonance Spectroscopy (NMR)**

Nuclear magnetic resonance (NMR) is a spectroscopic technique that detects the energy absorbed by changes in the nuclear spin state. The application of NMR spectroscopy includes the study of proteins and nucleic acids that provides unique information on the dynamics and chemical kinetics of these systems. One important feature of NMR is that it provides information over an exceptionally wide range of time scale, ranging from seconds to Pico-seconds. In addition, NMR can also provide atomic level structural information of proteins and nucleic acids in solution. NMR provides a method of obtaining structural information if the molecule cannot be crystallized. The continuous wave (CW) and the Fourier transformation (FT) spectrometer are two types of NMR spectrometers. Continuous wave spectrometers analyze the sample at a constant frequency of light while applying a sweeping magnetic field to achieve nuclear resonance. Fourier transform spectrometer analyzes the sample which involves exciting the full NMR spectra by utilizing short monochromatic radio frequency pulses. In the case study, primary metabolites in aqueous extract of mulberry (*Morus alba* L.) leaves were characterized by using proton nuclear magnetic resonance ($^1$H-NMR) spectroscopy. With the convenience of resonance assignment, GABA together with the other 10 primary metabolites was simultaneously identified and quantified in $^1$H-NMR spectrum (Nelson and Weaver, 1964; Wang *et al*., 2017; Watanable *et al*., 2002 and Wishart *et al*., 1992).

**The Immunoassay Techniques: ELISA, EIA and RIA**

Immunoassays are the bio-analytical methods to detect and quantify the target molecules in biological samples with the aid of the specificity of an antigen-antibody reaction. The use of these methods is frequently used in clinical diagnostics, drug discovery, and food testing. There are several types of immunoassay techniques including; Fluoro immune assays, Chemiluminescence immunoassays, Counting immunoassays, Enzyme-linked Immunosorbent Assay (ELISA) and Radioimmunoassay (RIA). ELISA follows the principle of capturing target antigen (or antibody) in the sample using specific antibody (or antigen), as well as the concept of enzyme-substrate reaction for detection and quantification of target molecule. The enzyme is used as a labeling molecule and enzyme activity is measured colorimetrically after the addition of substrate. Once the substrate is added, the enzyme modifies the substrate into a colored product. The enzyme activity is measured by recording the light absorption of the colored

product via microplate reader at 450nm. The commonly used enzymes for labeling are Horseradish Peroxidase and Alkaline Phosphatase. Depending on the antigen-antibody combination, there are 4 types of ELISA: Direct ELISA, Indirect ELISA, Sandwich ELISA and Competitive ELISA. The other technique RIA uses the concept of radioactivity. The labeling molecules used in RIA are radioisotopes. The commonly used radioisotopes for labeling are $I^{125}$, $C^{14}$ and $H^{3}$. In this assay, a known amount of antigen is labelled and mixed with a known amount of antibody directed against that antigen. When the sample containing the antigen of interest is added, the non-radioactive antigens are substituted for the radioactive antigens. After removal of the non-fixed antigens by washing, it is possible to measure the ratio between the radioactivity present in the supernatant and that of the standards. It is thus possible to determine the amount of antigen present in the sample. The instruments used to measure radioactivity are scintillation counters and GM counters. A comparison was done between ELISA and RIA by measuring the hormone cortisol in 9 ewes, 18 lactating Holstein cows and 12 growing heifers. It was observed that ELISA proved to be better approach than RIA as it gave better data output and less error, when compared to RIA. As the result was observed, it was concluded that lactating cows showed higher vulnerability to thermal stress than the growing heifers (Darwish, 2006; Gan and Patel, 2013; Grange *et al*., 2014; Lequin, 2005 and Nejad *et al*., 2019).

## Autoradiography and Geiger-Muller Counter

A Geiger-Muller counter is a device used for the detection and measurement of á particles, â particles, ã rays and X-rays. It consists of a pair of electrodes that have a high voltage and is surrounded by a gas usually Argon or Helium. The instrument consists of a GM tube and a counter. The GM tube is cylindrical, with a fine tungsten wire stretched along its axis. When a radiation enters the tube, it ionizes the gas and the ions formed are attracted to the electrode and electric current is produced. Scale counts the current pulses and obtains a count. It is used in applications such as radiation dosimetry, radiological protection, experimental physics and nuclear industry.Autoradiography is a bio-analytical technique used to visualize the distribution of radioactive labeled substance with radioisotope in a sample. It can also be used for quantitative estimation by using densitometer. In this technique, a radioactive substance is put in direct contact with a thick layer of photographic emulsion having silver halide crystals. The silver halide crystals are exposed to radiation which converts silver halide into metallic silver forming dark spots. The location of dark spots indicates the position where radioactive substance is concentrated. An autoradiograph is produced. This technique is used to determine the cell or tissue localization of a radioactive substance, in radio-pharmaceutical research, find site and performance of a targeted drug, investigate various properties of DNA. The autoradiography is used for characterization of high affinity ã-hydroxybutyric acid binding sites in mammalian brain tissue. Results showed the digital autoradiograms that enabled quantification and localization of radioligand binding in the brain tissue. In another study, GM counter is used for validating the efficiency of the beta counting system using slit height adjustment. The study showed that the efficiency of the counting system will be higher when the sample was placed on the height of the slit that was closer to the detector (Caro and Van, 1962; Ghosh, 2021; Greim-Krey *et al*., 2019; Sivasalianathan *et al*., 2017 and Wilson *et al*., 2018).

## Capillary Electrophoresis and 2-D Electrophoresis

Electrophoresis is an important technique for separation of charged molecules utilizing electric field. The two-dimensional (2-D) gel electrophoresis separates proteins by molecular charge and molecular size. This technique separates protein in two steps according to two independent properties, the first is isoelectric focusing and the second is SDS-PAGE. Both dimensions can be done on same SDS-PAGE apparatus and up to ten samples can be run on simultaneously using one gel. Its applicability and adaptability were enhanced because of introduction of immobilized pH gradient stripe, as they give good reproducible result and handling become easy. Another important and advanced technique using principle of electrophoresis is capillary electrophoresis (CE), which is a rapid and versatile electrophoretic technique having several applications. The primary element of a basic CE instrument includes a narrow glass capillary, buffer system and electrodes connected to a high voltage power supply. As electrophoretic separation is carried out in capillary tubes, it offers the possibilities of rapid and automated analysis of minute volume of complex mixture with high resolution and sensitivity. It gives faster results, and a large range of detection methods like optical detection and electrochemical detection are available. In the case study, CE is used to determine the citrate ion concentration in blood plasma in which sample is taken from hemodialysis patient in another case study in which detection of protein carbonyl derivatized with biotin-hydrazide by 2-D gel electrophoresis from which increase in their carbonyl content is determined (Morzunova, 2006; Polyakova *et al*., 2018; Suzuki and Honda, 1998 and Tamizi and Jouyban, 2015).

## Ion Exchange Chromatography

Ion exchange chromatography separates ions and polar molecules based on their affinity to the ion exchanger. It works on almost any kind of charged molecule—including large proteins, small nucleotides, and amino acids. The two types of ion chromatography are anion-exchange and cationexchange. Cation-exchange chromatography is used when the molecule of interest is positively charged, whereas anion-exchange chromatography is used when the molecule of interest is negatively charged. Their common components are: pump, injector, column, and detector. Successful packing of the column is an important aspect of ion chromatography. Stability and efficiency of a final column depends on packing methods, solvent used, and factors that affect mechanical properties of the column. The chromatogram obtained depicts peaks of the fractions eluted out of the column. Ion exchange chromatography has wide range of applications in industry, protein purification, water analysis and quality control. In a study partial purification of crude enzyme 'lipase' produced from *Pseudomonas aeruginosa* was carried out by ammonium sulphate fractionation, gel filtration chromatography G-50 and ion exchange chromatography with DEAE cellulose. Characterization studies indicated that enzyme showed highest activity at 55°C and pH9. In another study a potential de-hairing alkaline protease enzyme was purified and characterized using Ion Exchange Chromatography from *Bacillus cereus* LS2B. The SDS-PAGE of all the fractions was performed in which visualization of protein bands from fraction number 14 to 24 have very high density dominated by protein bands with a molecular weight of 20 kDa (Cummins *et al*., 2017; Ganokar *et al*., 2018; Junaidi *et al*., 2018 and Rathore and Pinky, 2012).

## Electron Microscopy: SEM and TEM

An electron microscope uses a beam of electrons to examine objects on a very fine scale. As electrons have smaller wavelength,

they can achieve higher magnification and higher resolution. Resolution provided by electron microscope is 1000x, higher than light microscopes. The two main types of electron microscopes are Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) differ in their operating styles as the former scans the surface of the organism while the latter gives image of the internal composition of the cell. Their common components are electron gun, electromagnetic lenses, specimen holders, image producing and recording system. Sample preparation is the most important step for processing of electron microscope. Diffraction pattern obtained from the micrographs can detect whether the material is in mono crystal or poly crystal form along with the information that molecule is made up of large or small particles. Electron microscope is having wide range of applications in various fields like industrial, chemical, biosciences and disease diagnosis. In a case study, morphological analysis of an entomopathogenic fungus *B. bassiana* was done by exposing it to heavy metals and dyes that were harmful for human health. Visible changes in the morphology of the fungus caused by Pb(II) inside the cell were observed. The results clearly depicted *B. bassiana* as potential dye and heavy metal remediation candidate (Gola *et al*., 2018; Hall, 1954; Lodish *et al*., 2008 and Ul-Hamid, 2018).

## Zeta Sizer: Instrumentation and Applications

Zeta sizer is particle analyzer which can measure particle size, mass, zeta potential. It uses laser (light amplification stimulated emission of radiation) to form interfere fringe pattern and any motion that causes change in fringe pattern is detected. Two types of scatterings: Rayleigh scattering and Mie scattering are used. Instrument uses dynamic light scattering (DLS) to measure Brownian motion and applies Stroke-Einstein equation to get hydrodynamic diameter of the particle. Static light scattering (SLS) uses Reyliegh equation and Debye plot to get the average mass of particle. Zeta potential is the potential present at the slipping plane. This plane is the interface which separates mobile fluid from fluid that remains attached to the surface. It is measured by the electrophoretic mobility in a 'U' shaped vessel; movement of particles in the applied electric field causes the change in fringe pattern, which is detected. Henry's equation is used to get zeta potential of the particle in accordance to their electrophoretic mobility. Instrument has applications in many fields such as bioscience and pharmaceuticals, paints, inks, coatings, nanomaterials and many more. In a case study, investigation of bovine serum albumin aggregation upon exposure to silver(I) and copper(II) metal ions using Zetasizer was done, the research showed how different concentrations of silver and copper ions caused agglutination and change of zeta potential of Bovine Serum Albumin (Alhazmi *et al*. 2021; Aljamali, 2015; Caravalho *et al*., 2018; Wu *et al*., 2019 and Soleimanifard *et al*., 2019).

## Ultracentrifuge and Its Types

Centrifugation is a process that uses the centrifugal force to separate and purify mixtures of particles in a liquid medium. The smaller the particles, the higher the G (Gravitational forces generated by machine)-forces required for the separation. It is a key technique for isolating and analyzing cells, sub-cellular fractions, supra-molecular complexes and, with higher G-force instruments or 'ultra'-centrifuges (up to 60,000 revolutions per minute corresponding to ~ 200 000×g) isolated macromolecules such as proteins or nucleic acids. These high-speed devices require a vacuum to avoid overheating of samples. Analytical ultracentrifugation (AUC) is a powerful method for the quantitative analysis of macromolecules in solution by ultracentrifuge. AUC has broad applications for the study of bio-macromolecules in a wide range of solvents and over a wide range of solute concentrations.

Three optical systems are available for the analytical ultracentrifuge (absorbance, interference, and fluorescence) that permits precise and selective observation of sedimentation of particles in real time. As compared to other methods, in AUC, samples are characterized in their native state under biologically relevant provided solution conditions. Because the experiments are performed in free solution, no complications arise due to non-interactions with matrices or surfaces. Moreover, being nondestructive, samples may be recovered for further tests following AUC. While analytical ultracentrifugation is mainly concerned with the study of purified macromolecules or isolated supra-molecular assemblies, preparative centrifugation methodology is devoted to the actual separation of tissues, cells, sub-cellular structures, membrane vesicles and other particles of biochemical interest.

The Sedimentation Velocity (SV) and Sedimentation Equilibrium (SE) are two experimental types of AUC done by varying conditions in respect to rotor speed, types of centrepieces, loading concentrations releasing different and wide nature of results. In the present case study, SV and SE experiments of AUC revealed the interactions (first in kind) between VanAtype-VanS Histidine Kinase system found in bacteria and Vancomycin - the antibiotic marked as last resort of defence against hospital infections.

The neutralization of developed antibiotic resistance by gram-positive bacteria against Vancomycin is in trial phase by conjugation of poly-cationic peptides to Vancomycin (Creeth and pain, 1967; Pjillips-Jones *et al*., 2017 and Harding and Winzor, 2001).

## IR Spectroscopy

Infrared spectroscopy is the measurement of the interaction of infrared radiation with matter by absorption spectra. It is used to study and identify chemical substances or functional groups in solid, liquid, or gaseous forms. The characteristic vibrational absorption bands used for the identification of chemical structures as well as methods for quantitative measurement of chemical composition, for the detection of the samples requires specific instruments called spectrophotometer, a common laboratory instruments that uses this technique is a Fourier transform infrared (FTIR) spectrometer. Infrared spectroscopy exploits the fact that molecules absorb specific frequencies that are characteristics of their structure. These absorptions are resonant frequencies, i.e. the frequency of the absorbed radiation matches the frequency of the bond or group that vibrates. The energies are determined by the shape of the molecular potential energy surfaces, the masses of the atoms, and the associated vibronic coupling. frequencies can be absorbed otherwise it will be transmitted, mainly absorption of the IR radiation or light which gives peaks at different wave no. in IR spectrum. Infrared (IR) spectroscopy has the potential to provide biochemical information without disturbing the biological sample. Consequently, the spectroscopic study of biological cells and tissue is an active area of research .Analysis of functional groups present in the spiny lobsters (*Panulirus homarus*, *Panulirus polyphagus* and *Panulirus versicolor*) muscle tissue by using Fourier Transform Infra-Red (FTIR) Spectroscopy method have been determined using IR transmission and elemental analysis by the FTIR (Chatwal, 2010; Kommuri *et al*., 2018; Silverstein and Bassler, 1964).

## Affinity Chromatography

Affinity chromatography separates proteins on the basis of a reversible interaction between a protein (or a group of proteins) and a specific ligand coupled to a chromatography matrix. The technique offers high selectivity, hence high resolution, and usually high capacity for the protein(s) of interest. Purification can be in the order of several thousand-fold and recoveries of active

material are generally very high. Affinity chromatography is unique in purification since it is the only technique that enables the purification of a biomolecule on the basis of its biological function or individual chemical structure. A particular ligand is chemically immobilized or "coupled" to a solid support so that when a complex mixture is passed over the column, the molecules having specific binding affinity to the ligand become bound. After other sample components are washed away, the bound molecule is eluted from the support, resulting in its many fold purification from the original sample. The diversity of the antibody-antigen interaction and our potential to manipulate the characteristics of the interaction has created many uses of antibodies or antibody fragments. Immunoaffinity chromatography utilizes antigen or antibody as ligand to create highly selective media for affinity purification. The technique has immense importance in separation, analysis, or characterization of chemicals and biochemicals. A biomimetic ligand is a synthetic compound that can be tailored to mimic natural biological recognition motifs or to interact with key surface-exposed residues on target proteins. Biomimetic ligand FYE-ABI (Phenylalanine-Tyrosine-Glutamate and 5-amino-benzimidazole) was designed, that mimic Protein A-antibody interaction. Hybrid ligand with a tripeptide group (FYE) and a hydrophobic charge-induction group (ABI) was designed by molecular simulation. FYE-ABI had high selectivity for hIgG, achieving high purity (93.9%) and high recovery rate (88.9%) under elution pH of 4.5 (Anusha and Sirisha, 2018; Labrou, 2014; Rodriguez *et al*., 2020 and Urh *et al*., 2009).

## Circular Dichroism and Optical Rotatory Dispersion

Circular dichroism (CD) is a method of measurement of the difference in absorbance of two components i.e. left and right of circularly polarized light. The circularly polarized light is obtained by passing a monochromatic light through a photoelastic modulator tube. Due to inherent property of a chiral compound a difference in the absorbance arises which is measured by CD machine. Many a times much preferred ellipticity (è) is used as measured count. The graphs obtained after passing the light through sample are compared with standard graphs for á helix, â sheet and random coil. Optical Rotatory Dispersion (ORD) measures the ability of an optically active compound to rotate the plane of polarized light. It consists of a monochromator, a polarizer and an analyser. A plane polarized light is incident on an optically active compound. Light when viewed from the analyser end is rotated by the rotational angle (á). Plot of á and the wavelength (ë) gives ORD graph. The Cotton Effect arises due presence of absorbtive band in vicinity of CD-ORD curves of a substance. Three nanosize monoamido amino derivatives of alginic acid (extracted from the Indian seaweed species, *Sargassum tenerrimum*) and agarose (polysaccharides of marine origin) are synthesized agrose succinate half ester-hexamethylene monoamido (AEAm), cyclohexanemonoamido (AECAm), and alginic-acid-based monoamido amino acid (Alg-EDA). CD-ORD spectrophotometry was used to study their protein-mimicking functions by interaction with Salmon testes DNA and bovine serum albumin (BSA) in varying pH regime (Chadar *et al*., 2019; Crabbe, 1972; Greenfield, 2000 and Miles *et al*., 2021).

## Fluorescence Spectroscopy

Fluorescence spectroscopy is a type of electromagnetic spectroscopy that measures fluorescence from a molecule based on its fluorescent properties. Fluorescence is a type of luminescence caused by photons exciting a molecule, raising it to an electronic excited state. Here, the electrons quickly relax

to the lowest available energy state. Once this state is achieved and after a fluorescence lifetime, the electrons will relax back to ground state, releasing their stored energy as an emitted photon. Usually, the emitted light has lower energy than the absorbed radiation. Devices called fluorometers are used to measure fluorescence. A fluorometer is a very sensitive device consisting of Light source, Excitation filter, Sample holder, Dichroic mirror, Emission filter and Detector. It has applications in various fields like bioscience, industrial, chemical, agricultural, pharmaceutical and disease diagnosis. In first case study, external label-free fluorescence spectroscopy was used for the first time to determine spectral profiles of internal fluorophores tryptophan, NADH, and FAD in fresh brain samples of a mouse suffering from Alzheimer's disease (AD). Results depicted that fluorescence intensities of tryptophan, NADH, and FAD were higher in the brain tissues of a AD mouse compared with that of normal brain tissues. In second case study Tryptophan and its three important metabolites (N-formyl-L-kynurenine, kynurenine and kynurenic acid) were quantified with the help of fluorescence spectroscopy. Preliminary results depicted a difference in the optical signatures in metabolites between patients with AD and control. These studies verified that tryptophan and NADHcould be employed as biomarkers for AD diagnosis. Fluorescence spectroscopy is an effective technique to detect differences of fluorophore compositions in AD and normal brain tissues, and to diagnose the progression of AD by examining the spectral profiles of various fluorophores (Gore, 2000; Lakowicz, 2013; Shi *et al*., 2017 and Sordillo *et al*., 2018).

## CRISPR/CAS9 Mediated Genome Editing

Genome editing is a method that enables scientists to change the DNA of organisms using different genome editing tools. In recent years, the CRISPR system has been adapted as a tool that can edit the genome of nearly any organism. However, the application of CRISPR/Cas9 in gene therapy is helpful for gene correction. Gene therapy is the introduction of genes into existing cells to prevent or cure wide range of diseases. Genes are delivered using genetically engineered vectors. And the adeno-associated virus vector act as a safe vehicle for in vivo delivery of gene. And mutating the terminal resolution site sequence on the one side of the inverted terminal repeats, leads to production of self –complementary AAV and is used as vector for CRISPR-Cas9 genome editing components for the correction of Duchenne muscular dystrophy, a severe genetic disorder caused due to loss of function of dystrophin gene. This loss of function is due to mutation caused by deletion of exon 44 which is the most common hotspot for mutation of dystrophin gene. High dosage of adeno – associated virus (AAV) was efficiently for in vivo genome editing but this was challenging for clinical application. While low dosage of self complimentary adeno associated virus scAAV showed the marked restoration of dystrophin expression (Min *et al*., 2019; Min *et al*., 2019(a); Wang *et al*., 2019 and Zhang *et al*., 2020).

## References

Alhazmi, HA., Ahsan, W., Ibrahim, AM., Khubrani, RAY., Haddadi, ZAA., Safhi, AYF., Shubayr, N., Al Bratty, M. and Najmi, A., (2021). Investigation of bovine serum albumin aggregation upon exposure to silver (I) and copper (II) metal ions using Zetasizer. *Open Chemistry*, *19*(1), 987-997.

Aljamali, NM., (2015). Zetasizer technique in biochemistry. *Biochemistry and Analytical Biochemistry*, *4*(2), 1.

Anna Pratima G., Nikalje, (2017), A Handbook of Chromatography,NikaljeScholar's Press Verlag Omniscriptam, Deutschland, Germany.

Anusha, S., and Sirisha, P. (2018). A overview on affinity chromatography: a review. *Pharmaceutical Research*, *8*(07).

basic principles and application. In Protein chromatography (209-223). Humana Press, New York, NY.

Caro, LG. and Van, TRP. (1962). High-resolution autoradiography: Methods. *Int. J. Cell Biol*. 15(2): 173-188.

Carvalho, PM., Felício, MR., Santos, NC., Gonçalves, S. and Domingues, MM., (2018). Application of light scattering techniques to nanoparticle characterization and development. *Frontiers in chemistry*, *6*, p.237.

Chadar DA., Chudasama NA., Vadodariya N., Meena R., Prasad K. and Siddhanta AK., (2019). Protein-Mimicking Functions of Nano-Size Monoamido Amino Acids Derived from Polysaccharides of Marine Origin. *Macromol. Chem. Phys.*, 220(19): 1-13

Chatwal GR.(2010). Instrumental methods of Chemical Analysis fifth edition, 340-351.

Crabbe P., 1972. ORD and CD in Chemistry and Biochemistry. Academic Press, London. 1-21.

Creeth, JM. and Pain, RH.(1967). The determination of molecular weights of biological macromolecules by ultracentrifuge methods. *Prog. Biophys. Mol. Biol.* 17: 217–287 .

Cummins, PM., Rochfort, KD., and O'Connor, B.F. (2017). Ion-exchange chromatography

Darwish, IA. (2006). Immunoassay Method and their Applications in Pharmaceutical Analysis: Basic Methodology and Recent Advances. *Int. J. Biomed.* 2:(3), 217-235.

David, EM., Carol, MM. and David, JS. (2006). Biochemistry, The Chemical Reactions of Living Cells. Harcourt/Academic Press, USA. P106-108.

Gan, SD. and Patel, KR. 2013. Enzyme Immunoassay and Enzyme-Linked Immunosorbent Assay. *J. Invest. Dermatol.* 133(9): 1-3.

Gaonkar, CC., Keni, M., Banavath, B.,Mudaraddi, TY., Hiremath, SV., Biradar, PM., and Veeranagoudar, DK. (2018). Purification and characterization of alkaline Lipase from *P. aeruginosa*strain. *IJASRM*, 3(4): 47-54

Ghosh, P. 2021. Geiger-Muller counter. *J Eng Math*. 3(3): 138-139.

Gola, D., Malik, Namburath, M. and Ahammad, SZ. (2018). Removal of industrial dyes and heavy metals by *Beauveria bassiana*: SEM, TEM and AFM investigations with Pb (II). *Environ. Sci. Pollut. Res.,* 25(21): 20486-20496.

Gore, MG. (Ed.). (2000). *Spectrophotometry and spectrofluorimetry: a practical approach*. New York: Oxford University Press.

Grange, RD., Thompson, JP. and Lambert, DG. (2014). Radioimmunoassay, Enzyme and Non-Enzyme-Based Immunoassays. *Br. J. Anaesth*. 112:(2) 213-217.

Greenfield NJ., (2000). Analysis of Circular Dichroism Data. *Meth. Enzymol.*, 383: 282-289

Griem-Krey, N., Klein, AB., Herth, M. and Wellendorph, P. (2019). Autoradiography as a simple and powerful method for visualization and characterization of pharmacological targets. J. Vis. Exp. (145).

Hall, C.E. (1954). Introduction to Electron Microscopy**.** *LWW.,*77(3): 254-263.

Harding, SE. and Winzor, DJ. (2001) Sedimentation velocity analytical ultracentrifugation, In (Harding, SE., Chowdhry, BZ. eds) *Protein-Ligand Interactions: Hydrodynamics and Calorimetry*, Oxford University Press, pp. 75–103.

Jaibiba, P., Naga Vignesh, S. and Hariharan, S. (2020). Working principle of typical bioreactors. Bioreactors. 145–173. doi:10.1016/b978-0-12-821264-6.00010-3

Junaidi, Y., Partiwiningrum, A., and Erwanto, Y. (2018). Purification by ion exchange chromatography and enzyme characterization of potential de-hairing alkaline protease from *Bacillus cereus* LS2B. *PJBT*, 15(2), 413-421.

Labrou, NE. (2014). *Protein downstream processing* (Vol. 1129). Totowa, NJ: Humana Press.

Lakowicz, JR. (Ed.). (2013). *Principles of fluorescence spectroscopy*. Springer science and business media.

Lequin, RM. (2005). Enzyme Immunoassay (EIA)/ Enzyme- Linked Immunoassay (ELISA). *Clin. Chem*. 51(21): 2415-2418.

Lodish, H., Berk, A., Kaiser, CA., Kaiser, C., Krieger, M., Scott, MP. and Matsudaira, P. (2008). Molecular cell biology.4. W H Freeman, Macmillan. 156-160.

Miles AJ.W. Janes R., Wallace. BA., (2021). Tools and Methods for Circular Dichroism Spectroscopy of Proteins: A Tutorial Review. *Chem. Soc. Rev.,* 50: 8401- 8405

Min, Yi-Li; Duby, Bassel, Eric N. (2019a). CRISPR Correction of Duchenne muscular dystrophy. Annu. Rev. Med. Wang, D; Tai, P.W.L; Gao, G. (2019 ).Adeno-associated virus vector as a platform for gene therapy delivery. *Nat Rev Drug Discov*.

Min, Yi-Li; Li, H, Caycedo, Rodriguez, Alex A; Huang, Shelton, M, McAnally, John R, Amoasii, Mammen, P A; Duby, Rhonda and Olson, N. ( 2019 ). CRISPR-Cas9 corrects Duchenne muscular dystrophy exon 44 deletion mutation in mice and human cells. Department of molecular biology.

Morzunova, TG., (2006). Capillary electrophoresis in pharmaceutical analysis (A review). *Pharmaceutical Chemistry Journal*, *40*(3), 158-170.

Muniraj, IK., Desikan, R. and Subburamu, K. (2019). Perspectives and Prospects of Fermentation Technology. *Advances in Food Bioproducts and Bioprocessing Technologies*. 217–232.

Nejad, JG., Park Kyu-Hyun, Fereidoun, F., Lee, Hong-Gu, Lee, Jae-Sung and Sung, K. (2019). Measuring Hair and Blood Cortisol in Sheep and Dairy Cattle Using RIA and ELISA Assay: A Comparison.*Biol. Rhythm Res*. 51(6): 887-897.

Nelson FA., Weaver HE. (1964). Nuclear magnetic resonance spectroscopy in superconducting magnetic fields. *Science* 146, 223–232.

Phillips-Jones, Mary K.; Channell, Guy; Kelsall, Claire J.; Hughes, Charlotte S.; Ashcroft, Alison E.; Patching, Simon G.; Dinu, Vlad; Gillis, Richard B.; Adams, Gary G.; Harding, Stephen E. (2017). Hydrodynamics of the VanA-type VanS histidine kinase: an extended solution conformation and first evidence for interactions with vancomycin. *Scientific Reports. 7(8): 46-180*.

Polyakova, EV., Shuvaeva, OV. and Borisov, A.S., (2018). Determination of Citrate Ions in Blood Plasma by Capillary Zone Electrophoresis. *Journal of Analytical Chemistry*, *73*(9): 906-909

Silverstein RM. and Bassler GC., (1964) Spectroscopic identification of organic compounds, Wiley, New York

Rathore, K., and Pinky, G. (2012). An overview on ion exchange chromatography. *IJARPB*, 1(1): 55-64.

Rodriguez, EL., Poddar, S., Iftekhar, S., Suh, K., Woolfork, AG., Ovbude, S., and Hage, DS. (2020). Affinity chromatography: A review of trends and developments over the past 50 years. *Journal of Chromatography B*, 122332.

Sawhney, SK. and Singh, R. 2014.Introductory practical biochemistry, Alpha Science Int'l Ltd. 245.

Shi, L., Lu, L., Harvey, G., Harvey, T., Rodríguez-Contreras, A., and Alfano, RR. (2017). Label-free fluorescence spectroscopy for detecting key biomolecules in brain tissue from a mouse model of Alzheimer's disease. *Scientific reports* *7*(1), 1-7.

Singh, J., Kaushik, N. and Biswas, S. (2014). Bioreactors- Technology and Design Analysis. *The Scitech. J.* 1(6): 28-36.

Singhal, G., Verma, V., Bhagyawant, SS. and Srivastava, N. (2018). Fermentation Technology Prospecting on Bioreactors/Fermenters: Design and Types. *In:* Kuila, A. and Sharma, V. (eds.) Principles and Applications of Fermentation Technology. Scrivener Publishing LLC. pp. 65-83.

Sivasailanathan, V., Kumar, P., and Sagadevan, S. (2017). Calibration and estimation of efficiency of Geiger Muller counter using a standard radioactive source. *Int. J. Phys. Sci.* 12(1).

Soleimanifard, M., Sadeghi Mahoonak, A., Sepahvand, A., Heydari, R. and Farhadi, S., (2019). Spanish olive leaf extract-loaded nanostructured lipid carriers: Production

and physicochemical characterization by Zetasizer, FT-IR, DTA/TGA, FE-SEM and XRD. *Journal of Food Processing and Preservation*, *43*(7): 13994.

Sordillo, LA., Zhang, L., Shi, L., Sriramoju, V., Sordillo, PP., and Alfano, RR. (2018). Alzheimer's disease evaluation using label-free, stainless, fluorescence to measure tryptophan metabolism along the kynurenine pathway. *Optical Biopsy XVI: Toward Real-Time Spectroscopic Imaging and Diagnosis* (Vol. 10489, p. 104891E). International Society for Optics and Photonics.

Spier, MR., Vendenberghe, LPS., Medeiros, A.B.P. and Soccol, CR. (2011). Application of different types of bioreactors in bioprocesses. *In:*Antolli, P.G. and Liu, Z. (eds.) Bioreactors: Design, Properties and Applications. Nova Science Publishers, Inc. 55-90.

Suzuki, S. and Honda, S., (1998). A tabulated review of capillary electrophoresis of carbohydrates. *Electrophoresis*, *19*(15): 2539-2560.

Tamizi, E. and Jouyban, A., (2015). The potential of the capillary electrophoresis techniques for quality control of biopharmaceuticals—a review. *Electrophoresis*, *36*(6): 831-858.

Thimmaiah, SR. (2006). Standard Methods of Biochemical Analysis, Kalyani Publishers, Ludhiana. 181-183.

Towbin, H., Staehelin, T. and Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose. Proc. Natl. Acad. Sci. 76(9): 4350-4354.

Ul-Hamid, A. (2018). A beginners' guide to scanning electron microscopy. Springer International Publishing, New York.

Urh, M., Simpson, D., and Zhao, K. (2009). Affinity chromatography: general methods. *Methods in enzymology*, *463*: 417-438.

Wang D., Dong Z., Zhang Y., Guo K., Guo P., Zhao P., Xiao Q. (2017). Proteomics provides insight into the interaction between mulberry and silkworm. *J. Proteome Res*., 16: 2472–2480.

Watanabe M., Maemura K., Kanbara K., Tamayama T., and Hayasaki H. (2002). GABA and GABA Receptors in the Central Nervous System and Other Organs. *Int. Rev. Cytol*., 213, 1–47.

Wilson, K., Hofmann, A., Walker, JM. and Clokie, S. (2018). Principles and Techniques of Biochemistry and Molecular Biology.7. Cambridge University Press, New York. 553-580

Wilson, K., Hofmann, A., Walker, JM. and Clokie, S. (2010). Principles and Techniques of Biochemistry and Molecular Biology Cambridge University Press, New York. 422-424.

Wilson, K., Hofmann, A., Walker, JM. and Clokie, S. (2018). Principles and Techniques of Biochemistry and Molecular Biology, Cambridge University Press, New York. 407-420

Wishart DS., Sykes BD., and Richards FM. (1992). The chemical shift index: a fast and simple method for the assignment of protein secondary structure through NMR spectroscopy. *Biochemistry* 31, 1647–1651.

Wu, J., Ding, X., Zhang, J. and Chen, W., (2019). Online Determination of Colloidal Properties of Tannin Solutions under Microwave Irradiation using a Modified Zetasizer. *Analytical Letters*, *52*(6), 879-891.

Zhang, Y, Li, H., Min, Y., Oritz, Sanchez; H., Jian; Mireault, Alex A; Shelton, John M; Kim Mammen, Pradeep PA; Duby, Rhonda, Olson EN. (2020).Enhanced CRISPR/Cas9 correction of Duchenne muscular dystrophy in mice be self complementary AAV delivery system. Molecular genetics.

Zou, X., Zhang, Q., Lu, H., Lin, D., and Yao, S. (2019). Development of a hybrid biomimetic ligand with high selectivity and mild elution for antibody purification. *Chemical Engineering Journal*, *368*, 678-686.

**Biotech Today (January-June 2023) : 13 (1) : 97 - 103**

DOI : 10.5958/2322-0996.2023.00014.5

**RESEARCH ARTICLE**

# Biosurfactant Production using Mustard cake and *Bacillus subtilis*

**Abhinav Singh[1*], Yashi Rathi[2], Baby Verma[3], Neelesh Kapoor[4] and Rakesh Singh Sengar[5]**

**Received**: 25 July, 2022, **Revised**: 28 September, 2022, **Accepted**: 16 October, 2022, **Published**: 1 January 2023

## Abstract

Bio-surfactants are surface-active molecules which are produced by the wide range of microbes including bacteria, fungi and yeast. A study was conducted to produce biosurfactants by *Bacillus subtilis* using industrial waste (Mustard cake). *Bacillus subtilis*, one of the most potential bio-surfactant producers, was isolated from soil sample of hydrocarbon contaminated site. Isolates were grown in a Minimal Salt Media (MSM) with 10% mustard oil cake. The presence and potential of surfactant was determined by the oil spreading technique, emulsification index and surface tension measurement. The emulsification index value was found to be highest in mobil oil from the bio-surfactant extracted from mustard cake. It was found that the surface tensions of cell free extract was were wer 55.40 from mustard cake as compared to distilled water (72.09) at 25 ºC. In conclusion, strain of *Bacillus subtilis* was found to be the potential surface active agent producer on the mustard oil cake, and can be a useful medium for various environmental, food and industrial processes.

**Key words:** Bio-surfactants; Hydrocarbon; Minimal Salt Media; Surface Tension; Emulsification Index, Mustard cake, *Bacillus subtilis*.

[1,4,5]Division of Plant Biotechnology, College of Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut

[2,3]Department of Biotechnology and Microbiology, Meerut Institute of Engineering and Technology, Meerut

*Corresponding Author: abhinavsinghmiet@gmail.com

## Introduction

Surfactants are amphipathic molecules that lower the surface tension of a liquid or the interfacial tension between two liquids, such as that of oil and water. They are characteristically organic compounds containing both hydrophobic tails and hydrophilic heads. Surfactants can be synthetic or biological in origin. For over several decades, surfactants are chemically synthesized and classified according to their dissociation level in water. There can be anionic, cationic and non-ionic surfactants (Kenichi *et al*., 1977; Jadhav *et al*., 2011; Paulino *et al*., 2017). Good performing synthetic surfactants are called Gemini or dimeric surfactants which are composed of two hydrophobic chains and two hydrophilic moieties linked by a spacer group that can be hydrophobic/hydrophilic and flexible/rigid. Microbe-derived surfactants (or in short, biosurfactants), on the other hand, are those being naturally produced by microorganisms either constitutively or inducibly. A biosurfactant is an amphipathic molecule that can be classified into two different classes, low and high molecular weight biosurfactants. Low molecular weight biosurfactants are generally glycolipids, such as rhamnolipids or lipopeptides (Benincasa, 2007), whereas the high molecular weight biosurfactants encompass amphipathic polysaccharides, lipopolysaccharides, proteins and lipoproteins (Amaral, 2006). More attention has been paid to biosurfactants production in recent times due to the perceived advantage over synthetic

surfactants, chiefly, their ease of biodegradation which significantly reduced the environmental burden in contrast to chemical processing, ecological adaptability which helps in bioremediation processes, low toxicity which made them appealing for uses in the pharmaceutical and food industry and high specificity that allow them to promote detoxification of selected pollutants under extreme conditions.

Biosurfactant-producing microorganisms are able to produce biosurfactants in aqueous media with the addition of carbon sources like glucose, fructose, glycerol, mannitol and olive oil (Desai and Banat, 1997)

Biosurfactant produced in the culture medium acts to ease the movement of insoluble substrates across the cell membrane for the growth of microorganisms. In other instances, it helps to stabilize oil or hydrocarbon in water and vice versa. For example, biosurfactants can greatly reduce the surface tension of water from 72 Mn/m to 22 Mn/m. Owing to these properties, biosurfactants can solve some of the problems involving oil spill in oceans and enhance biodegradation of Polycyclic Aromatic Hydrocarbon (PAH) compounds. Biosurfactants also play important roles in several industries; in agriculture, biosurfactants help to improve plant growth by getting rid of phytopathogens whereas in the pharmaceutical industry, it can ease the introduction of foreign genes to the selected cells during gene therapy. Likewise, in the food industry, they can serve as an emulsifier. In confectionery, production of solubilizer in foods containing fats and oils, such as margarine and dairy foods.

Bio-surfactant occupies an advantageous position both for research and industrial production .The most prevalent bacterial species capable of producing surfactant belong to the genera are *Pseudomonas* (Das and Mukherjee; 2007; Guerra-Santos, 2010; Jadhav *et al.*, 2011), *Micrococcus flavobacterium, , Bacillus, Acinetobacter, Achromobacter, Arthrobacter, Klebsiella, Aeromonas, Alkaligenes, Streptococcus sp., Corynebacterium sp., Moraxella sp.* and *Proteobacteria* (Arima *et al.,* 2013). *Bacillus* is one of such genus of bacteria associated with the production of crucial bio-surfactant. They not only produce good bio-surfactants, but are also capable of growing under facultative or anaerobic conditions and have also been reported to be non-pathogenic, which permits their use in wide range industries, apart from environmental applications (Edwards and Hayashi, 2013).

Hydrocarbons are commonly used as the substrate for the production of bio-surfactants. It has been postulated that the biological function of surface-active compounds is related to hydrocarbon uptake and therefore a spontaneous release occurs with these substrates (Zhao *et al.,* 2010). Reduction in cost of substrate by using lower grade and cheap substrate and using more efficient microbes could significantly reduce the manufacturing cost (Yin *et al.*, 2009).

The present study focused at the production of bio-surfactant by *B. subtilis* on the industrial wastes obtained from mustard oil, which is cheaper and low grade substrate (mustard oil cake) obtained from mustard oil which is in turn obtained from mustard crop that is found locally and in abundance.

## Objectives of the study

1. The study aimed for the production of bio-surfactant by *B. subtilis* on the industrial wastes, cheaper and low grade substrates (mustard oil cake).
2. To explore the presence and potential of surfactant by the oil spreading technique, emulsification index and surface tension measurement.

## Materials and Methods

Six soil samples from mustard oil extraction plant were collected in aseptically dried and clean plastic bags and were transported to laboratory, immediately.

## Bacterial isolation

The test organism (*Bacillus subtilis*) was isolated form soil sample, by heating test samples at 40°C for 10 min by using water bath to destroy all the vegetative cells. Then 1 g of soil was weighed and was suspended in 9 ml sterile distilled water. Serial dilution of soil sample was done up to$10^4$ dilutions. Nutrient agar with 1% soluble starch was prepared onto which an aliquot (0.1ml) of $10^2$ to $10^4$ dilutions were inoculated and spread plate method was followed. The plates were incubated aerobically at 37°C for 48 hours.

Colonies that showed big, creamy, wrinkle and spreading colonies were picked and sub cultured on nutrient agar broth and on nutrient agar slant. Gram staining and endo-spore staining were carried out for the presumptive identification of the isolates.

Different biochemical tests viz; catalase, citrate, urease, indole, starch hydrolysis, methyl red voges-proskauer, sugar fermentation test (lactose, mannitol, sucrose), Triple Sugar Iron (TSI) and Sulfur, Indole and Mobility (SIM) tests were carried out to identify the isolates.

The medium used for the experiment was a Minimal Salt Medium supplemented with 10% substrate (Mustard oil cake). It was prepared by dissolving 1.73 g dipotassium phosphate, 0.68 g potassium dihydrogen phosphate, 0.1 g magnesium sulphate hepta hydrade, 0.33 g ferrous sulphate, 4 g sodium chloride, 1g ammonium nitrate, 0.02 g calcium chloride and 5 g glucose.

## Inoculation and incubation

1 ml of 24 hours broth culture of the isolate was pipetted into each flask. All six flasks were plugged with cotton and allowed to stand in water bath shaker for 5 days. Temperature of the water bath shaker was maintained at 37 °C. After 5 days all flask were taken out from shaker and centrifugation was done to obtain cell free supernatant.

## Oil spreading technique

A Petri-dish (150 mm diameter) was filled with 40 ml of distilled water. 15 ml of weathered mobil-oil was added. The mobil oil formed a thin oil layer on the water surface. Then, 10 ml of free cell culture supernatant was carefully placed on the center of the oil film. If there were microbial surfactants present in the supernatant, the oil was displaced and a clearing zone was formed.

## Determination of emulsification activity

0.5 ml of the extracted supernatant was added to 7.5 ml of 1 M tris-HCl buffer and 0.1 ml of oil (mustard oil and mobil oil). The mixture was vigorously vortexed and allowed to stand for 1 hour. Absorbance was measured at 540 nm, 5 times at an interval of 1 hour. After obtaining the absorbance, emulsification activity was calculated.

## Measurement of Emulsification Index (E24)

The bacterial broth was centrifuged and was studied for its emulsifying ability by a modified method. 2 ml cell-free broth was pippeted into the screw cap test tube and 3 ml of oil (Mustard, mobil oil) was then added. The mixture was vortexed at high speed for 2 min and left at room temperature. The result was observed after 24 h for the stability of emulsion. The total volume of the mixture, volume of emulsified and volume of non-emulsified phase was observed .The emulsification index (E24) was calculated by the equation:

$$E24 = \frac{\text{Height of emulsion layer}}{\text{Total Height}} \times 100$$

The method was followed at the end of each day to obtain reading for 5 day.

## Results

## Bacterial Isolation and identification

The test organism (*Bacillus subtilis*) was isolated form a soil sample of automobile workshop. Presence of *Bacillus subtilis* was

confirmed based on the macroscopic examination of colonies and microscopic examination.

### Emulsification activity (EA)

The emulsification activity was observed highest in mustard oil with the cell free extract from mustard oil cake, whereas mobil oil showed the lowest.

## Discussion

Bio-surfactants, compounds with such wonderful advantages over chemical ones, have not yet been commercialized significantly as a result of high production cost. Since the cost of substrates, chemical ones (used more widely), account for 10-20% of the production cost of biosurfactants, the production cost is higher. The possible measure to minimize the cost is to reduce the cost of substrate i.e. use of cheap substrates preferably natural substrates like mustard oil cake, soya cake and sunflower oil cake can be used as an alternative to mustard oil cake. Present study was performed with the objective of identifying the utility of the substrate mentioned above for production of bio-surfactants using bacterial species isolated from oil contaminated soil.

Many similar studies have identified the bacterial strain as well as the possible substrates of bio-surfactant production. Cameotra and Makkar (2004), have reported the studies on bio-surfactant production by *Bacillus* strains under thermophilic conditions on sucrose and molasses as substrate. Al-Bahry *et al.* (2013) reported production of bio-surfactant by *Bacillus subtilis* B20 using date molasses and its possible application in enhanced oil recovery. Nevertheless, the mustard oil cake, an agro-industrial waste, could be the potential substrate for the commercial bio-surfactant production as suggested by present study. Although the potential of the crude biosurfactant produced, is lower than expected. It cannot be denied that higher potential could have been achieved with better resources. Moreover, the extracted crude surfactant was also able to give satisfying result despite of being crude. However, the study could have been considered more achieving if the purity of the crude extract could have been increased.

## Conclusion

The result of this study showed that the strain of *Bacillus subtilis* isolated from Mustard cake was found to be the potential surface active agent producer and is a useful tools for various environmental, food and industrial processes. The organism isolated from oil contaminated area showed greater ability to produce bio-surfactants. Furthermore, each of the substrates obtained from industrial and household waste were found to show great potential as substrate for the production of bio-surfactant. Among them, the mustard oil cake substrate is cheap and best among the three studied substrate for bio-surfactant production.

## References

Adamczak, M. and Bednarski, W. (2000). Influence of medium composition and aeration on the synthesis of biosurfactants produced by *Candida antartica*. *Biotechnology Letters*. 22: 313-316.

Al-Bahry, SN., Al-Wahaibi, YM., Elshafie, AE., Al-Bemani, AS., Joshi, SJ., Al-Akhmari, HS. (2013). Biosurfactant production by *Bacillus subtilis* B20 using date molasses and its possible application in enhanced oil recovery. International Biodeterioration and Biodegradation. 81: 141–146. doi: 10.1016/j.ibiod.2012.01.006.

Alejandro, CS., Humberto, HS. and Maria, JF. (2011). Production of glycolipids with antimicrobial activity by *Ustilago maydis* FBD12 in submerged culture. *African Journal of. Microbiology Research*. 5: 2512-2523.

Amani, H. and Kariminezhad, H. (2016). Study on emulsification of crude oil in water using emulsan biosurfactant for pipeline transportation. *Petrolium Science and Technology*. 34: 216–222. doi: 10.1080/10916466.2015.1118500.

Amaral, PFF., da Sliva, JM., Lehocky, JM., Barros-Timmons, AMV., Coelho, MAZ., Marrucho IM. and Coutinho, JAP. (2006). Production and characterization of a bioemulsifier from *Yarrowia lipolytica*. Process Biochemistry, 41: 1894-1898.

Aneja, KR. (2012). Experiments in Microbiology, Plant Pathology and Biotechnology. 4th ed. New Delhi: New Age International Limited. 135-138.

Arima, K., Kakinuma, A. and Tamura, G. (2013). Surfactin, a crystalline peptidelipid surfactant produced by *Bacillus subtilis*: Isolation, characterization and its inhibition of fibrin clot formation. *Biochemical and Biophysical Research Communications*. 3: 488-494.

Arutcheli, J. and Doble, M. (2010). Mannosylerythoital Lipids: Microbial production and their Application. *Biosurfactants*. 5(6): 145-177.doi 10.1007/978-3-642-14490-5-6.

Banat, IM., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, MG. and Fracchia, L. (2010). Microbial biosurfactants production, applications and future potential. *Applied Microbiology and Biotechnology*. 87: 427–444. doi: 10.1007/s00253-010-2589-0.5.

Benincasa, M. (2007). Rhamnolipid produced from agroindustrial wastes enhances hydrocarbon biodegradation in contaminated soil. *Current Microbiology*. 54: 445-449.

Cameotra SS. and Makkar RS. (2004). Recent applications of biosurfactants as biological and immunological molecules. *Current Openion in Microbiology*. 7: 262-266.

Casas, JA., De Lara., SG., and Garcia-Ochoa, F. (2017). Optimization of a synthetic medium for *Candida bombicola* growth using factorial design of experiments. *Enzyme and Microbial Technology*. 21: 221-229.

Cavalero, DA. and Cooper, DG. (2003). The effect of medium composition on the structure and physical state of sophorolipids produced by *Candida bombicola* ATCC 22214. *Journal of Biotechnology*. 103: 31-41.

Chandran, P. and Das, N. (2010). Biosurfactant production and diesel oil degradation by yeast species *Trichosporon asahii* isolated from petroleum hydrocarbon contaminated soil. *International Journal of Engineering Science and Technology*. 2: 6942-6953.

Cooper, DG., Macdonald, CR., Duff, SJB. and Kosaric, N. (2011). Enhanced production of surfactin from *Bacillus subtilis* by continuous product removal and metal cation additions. *Applied Environmental Microbiology*. 42: 408-412.

Cunha, CD., Do-Rosario M., Rosado, AS., Leite, SGF. (2004). *Serratia* sp. SVGG 16: A promising bio-surfactant producer isolated from tropical soil during growth with ethanol- blended gasoline. *Process Biochemistry*. 39: 2277-2282.

Das, K. and Mukherjee, AK. (2007). Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from petroleum oil contaminated soil from North-East India. *Bioresource Technology*. 98: 1339- 1345.

Desai, JD. and Banat, IM. (1997). Microbial production of surfactants and their commercial potential. *Microbiology and Molecular Biology Reviews*. 61: 47- 64.

Edwards, JR. and Hayashi, JA. (2013). Structure of a rhamnolipid from *Pseudomonas aeruginosa*. *Archives of Biochemistry and Biophysics*. 111: 415-421.

Fakruddin, M. (2012). Biosurfactant: Production and Application. *Journal of Petroleum and Environmental Biotechnology*. 3(4):124. doi:10.4172/2157-7463.1000124.

Gautam, KK. and Tyagi, VK. (2006). Microbial surfactants: A review. *Journal of Oleo Science*. 55: 155- 166.

Gharaei-Fathabad, E. (2011). Biosurfactants in pharmaceutical industry: A Mini Review. *American Journal of Drug Discovering and Development*. 1: 58-69.

Guerra-Santos, LH., Kappeli, O. and Fiechter, A. (2010). *Pseudomonas aeruginosa* biosurfactant production in continuous culture with glucose as carbon source. *Applied and Environmental Microbiology*. 48(2): 301–305.

Hitzman, DO. (1984). Enhanced Oil Recovery Process Using Microorganisms. U.S. Patent application No. 4,450,908. Patent No 6,090,051. Washington, DC: U.S. Patent and Trademark Office.

Holdom, R. and Turner. AG. (2008). Growth of *Mycobacterium rhodochrous* on Decane: a New Growth Factor and Emulsifying Agent. *Journal of Applied Microbiology*. 32: 448-456.

Hu, Y. and Ju, LK. (2001). Purification of lactonic sophorolipids by crystallization. *Journal of Biotechnology*. 87: 263-272.

Jadhav, M., Kalme, S., Tamboli, D. and Govindwar, S. (2011). Rhamnolipid from *Pseudomonas desmolyticum* NCIM-2112 and its role in the degradation of Brown 3REL. *Journal of Basic Microbiology*. 51: 385-396.

Kachholz, T. and Schlingmann, M. (1987). Possible food and agricultural application of microbial surfactants: an assessment. In: Kosaric N, Cairns WL, Grey NCC, (eds.), *Biosurfactant and Biotechnology*, New York, Marcel Dekker Inc.: 183- 208.

Hisatsuka, K., Nakahara, T., Minoda, Y. and Yamada, K. (1977). Formation of Protein-like Activator for *n*-Alkane Oxidation and its Properties. *Agricultural and Biological Chemistry*. 41(3): 445–450. https://doi.org/10.1080/00021369.1977.10862518

Kim, SY., Oh, DK., Lee, KH. and Kim, JH. (1997). Effect of soybean oil and glucose on sophorose lipid fermentation by *Torulopsis bombicola* in continuous culture. *Applied Microbiology and Biotechnology*. 48: 23-26.

Konishi, M., Fukuoka, T., Morita, T., Imura, T. and Kitamoto, D. (2008). Production of new types of sophorolipids by *Candida batistae*. *Journal of Oleo Science*. 57: 359-369.

Krishnaswamy, M., Subbuchettar, G., Ravi, TK. and Panchaksharam, S. (2008). Biosurfactants properties, commercial production and application. *Current Science*. 94: 736-747.

Kugler, JH., Le Roes-Hill, M., Syaldatk, C. and Hausmann, R. (2015). Surfactants tailored by the class Actinobacteria. *Frontiers of Microbiology*. 6: 212.

Lee, YJ., Choi, JK., Kim, EK., Youn, SH. and Yang, EJ. (2008). Field experiments on mitigation of harmful algal blooms using a Sophorolipid: Yellow clay mixture and effects on marine plankton. *Harmful Algae*. 7: 154-162.

Lotfabad, TB., Shourian, M., Roostaazad, R., Najafabadi, AR. and Adelzadeh, MR. (2009). An efficient biosurfactant-producing bacterium *Pseudomonas aeruginosa* MR01, isolated from oil excavation areas in south of Iran. *Colloids and Surfaces B: Biointerfaces*. 69: 183-193.

Makkar, RS. and Rockne, KJ. (2003). Comparison of synthetic surfactants and biosurfactants in enhancing biodegradation of polycyclic aromatic hydrocarbon. *Environmental Toxicology Chemistry*. 22: 2280-2292.

Mohan, PK., Nakhla, G. and Yanful, EK. (2006). Biokinetics of biodegradation of surfactants under aerobic, anoxic and anaerobic conditions. *Water Research*. 40: 533-540.

Mukherjee, AK. (2007). Potential Application of Cyclic lipopeptide biosurfactants produced by *Bacillus subtilis* strains in laundry detergent formulations. *Letters in Applied Microbiology*. 45: 330-335.

Mukherjee, S., Das, P and Sen, R. (2006). Towards commercial production of microbial surfactants. *Trends in Biotechnology*. 24: 509-515.

Paulino, BN., Pessoa, MG., Molina, G. and Neto, AAK. (2017). Biotechnological production of value-added compounds by Ustilagino mycetous yeasts. 101(6): 253-256. doi:10.1007/s00253-017-8156-x.

Perfumo, A., Rancich, I. and Banat, IM. (2010). Possibility and challenges for biosurfactant uses in petroleum industry. *Advances in Experimental Medicine and Biology*. 672: 135-145.

Rahman, PKSM. and Gakpe, E. (2008). Production, characterization and application of Biosurfactants-Review. *Biotechnology*. 7: 360-370.

Roy, A. (2017). A Review on the Biosurfactants: Properties, Types and its Applications. *Journal of Fundamentals of Renewable Energy and Applications*. 8(1): 248. doi:10.4172/20904541.1000248.

Singh, A., Van Hamme, JD. and Ward OP. (2007). Surfactants in microbiology and biotechnology Part 2. Application aspects. *Journal of Biotechnology Advances*. 25(1): 99-121.

Sobrinho, HBS., Luna JM., Rufino, RD., Porto, ALF. and Sarubbo, AL. (2014). Biosurfactants: Classification, Properties and Environmental Applications. *Recent Developments in Biotechnology*. *Studium Press LLC, USA*. 1.: 303-330.

Yin, H., Qiang, Y., Jia, Y., Ye, J., Peng, H., Qin, H., Zhang, N. and He, B. (2009) Characteristics of biosurfactant produced by *Pseudomonas aeruginosa* S6 isolated from oil –containing wastewater. *Process Biochemistry*. 44(3): 302-308.

Zanotto, AW., Valerio, A. and Andrade, CJD. (2019). New sustainable alternatives to reduce the production costs for surfactin 50 years after the discovery. *Applied Microbiology and Biotechnology*. 103(21-22): 8647-8656..doi 10.1007/s00253-019-10123-7 .

Zhao, Z., Wang, Q., Wang, K., Brain, K. and Liu, C. (2010). Study of the antifungal activity of *Bacillus vallismortis* ZZ185 in vitro and identification of its antifungal components. *Bioresource technology*. 101: 292-297.

Zinjarde, SS. and Pant, A. (2002) Emulsifier from a tropical marine yeast *Yarrowia lipolytica* NCIM 3589. *Journal of Basic Microbiology*. 42(1): 67-73.

**Statement About Onwership of**
**International Journal of Biological Sciences-Biotech Today**

**(Regd. No. 1138)**

**From IV**

**(As per rule 18)**

| | | |
|---|---|---|
| **Place of Publication** | : | Meerut, India |
| **Periodicity of Publication** | : | Half Yearly |
| **Printer's Name** | : | Learning Media Publication |
| **Nationality** | : | Indian |
| **Address** | : | A-16, Aman Vihar, Mawana Road, Meerut-250001 |
| **Publisher's Name** | : | **SOCIETY OF GREEN WORLD FOR SUSTAINABLE ENVIRONMENT** |
| **Nationality** | : | Indian |
| **Address** | : | H.No. 35, Akashardham, Roorkee Road, Modipuram, Meerut-250110 (U.P.) |
| **Chief Editor** | : | R.S. Sengar |
| **Nationality** | : | Indian |
| **Address** | : | H. No. 35, Akshardham, Roorkee Road, Modipuram, Meerut-250110 (U.P.) India |

I Prof. R.S. Sengar, hearby

sd. **R.S. Sengar**

Signature of the Publisher

For & on behalf of SOCIETY OF GREEN WORLD

FOR SUSTAINABLE ENVIRONMENT

Phone : 9412472292, 7906045253

Email: biotechtoday@gmail.com, chiefeditor@bitotechtoday.co.in

# RECENT NEWS

## Climate resilient rice varieties 'Swtarna Unnat Dhan' and 'Swarna Sukha Dhan' released

Swarna Unnat Dhan (IET 27892), a high yielding multiple stresses tolerant rice variety, has been released and notified by Central Sub-Committee on Crop Standards, Notification and Release of Varieties for Agricultural Crops, Govt. of India for cultivation under irrigated transplanted condition in the states of Bihar, Odisha, West Bengal, Madhya Pradesh and Maharashtra of India. Swarna Unnat Dhan is an early duration (115-120 days), semi-dwarf, high yielding (5.0- 5.5 t ha-1), multiple stresses (drought, disease and insect pest) tolerant, lodging resistant with desirable cooking quality traits and having long slender grain type. Swarna Unnat Dhan showed moderately resistant to bacterial leaf blight (BLB), false smut, leaf blast, sheath blight, RTD and brown spot diseases and major pests like stem borer, leaf folder, whorl maggot, and brown plant hopper (BPH) under natural condition.

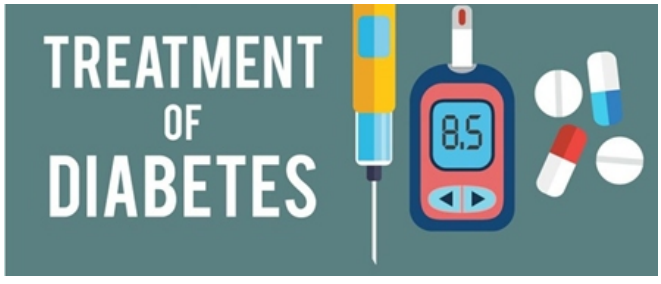
Swarna Sukha Dhan (IET 24692) a high yielding multiple stresses tolerant rice variety. Swarna Sukha Dhan is an early duration (110- 115 days), semi-dwarf, high yielding (3.5-4.0 t ha-1), multiple stresses (drought, disease and insect pest) tolerant, with desirable quality traits and high micronutrient [(Zinc:23.1ppm)] content.It is suitable for cultivation under direct seeded condition in rainfed midland to upland ecosystem of Uttar Pradesh. Quality wise, Swarna Sukha Dhan possesses 78.4% hulling, 70.9% milling, 68.4 % head rice recovery (HRR) with desirable intermediate alkali spreading value (ASV=4.0), amylose content (22.32%) with medium slender grain type.

These varieties have promise for restricted irrigated areas and drought-prone rainfed areas and thus, will contribute to increasing rice productivity and improve the socio-economic status of the farmers.


## IIT Mandi team discovers molecule that can be used for treatment of diabetes

Researchers at the Indian Institute of Technology (IIT) Mandi have discovered a drug molecule that can be used to treat diabetes. The molecule, named PK2, is able to trigger the release of insulin by the pancreas and can potentially be used as an orally administered medicine for diabetes. Dr. Prosenjit Mondal, Associate Professor, School of Basic Sciences, IIT Mandi said "Current drugs such as exenatide and liraglutide used for diabetes, are administered as injections, and they are costly and unstable after administration. We seek to find simpler drugs that are stable, cheap, and effective against both Type 1 and Type 2 diabetes."

Dr. Prosenjit Mondal points to another critical finding in their work, "Beyond increasing insulin release, PK2 was also able to prevent and even reverse beta cell loss, a cell essential for insulin production, making it effective for both Type 1 and Type 2 diabetes."



## A new wearable technology for plants

Researchers have created a wearable sensor for plant leaves to detect water loss. The system wirelessly transmits data to a smartphone app, allowing for remote management of drought stress in gardens and crops.

Similar to the wearable smart watches, plant-wearable devices could help farmers and gardeners remotely monitor their plants' health, including leaf water content, which is a key marker of metabolism and drought stress. Previously, researchers had developed metal electrodes for this purpose, but the electrodes had problems staying attached, which reduced the accuracy of the data. Renato Lima and colleagues wanted to identify an electrode design that was reliable for long-term monitoring of plants' water stress, while also staying attached. The researchers created two types of electrodes: one made of nickel deposited in a narrow, squiggly pattern, and the other cut from partially burnt paper that was coated with a waxy film.

The device wirelessly shared data to a smartphone app and website, and a simple, fast machine learning technique successfully converted these data to the percent of water content lost. The researchers say that monitoring water content on leaves can indirectly provide information on exposure to pests and toxic agents. Because the plant-wearable device provides reliable data indoors, they now plan to test the devices in outdoor gardens and crops to determine when plants need to be watered, potentially saving resources and increasing yields.

## India's first open sea cage farm under the Mariculture Policy


In a first in the country, an open sea cage farm has been launched in Goa under the Mariculture Policy with the technical guidance of the Karwar Regional Station of ICAR-Central Marine Fisheries Research Institute (CMFRI).

Realising the huge potential in mariculture, Government of Goa became the first state in India to notify the Mariculture Policy for open sea cage farming in territorial waters. Following this, ICAR-CMFRI extended technical support to Shri Sheldon Fernandes, a Candolim resident of North Goa belonging to fishermen community to become the first entrepreneur in India with an open sea cage farm under the Policy. The cage culture farm at Candolim, North Goa was inaugurated by Dr Sharmila Monteiro, Director, Department of Fisheries, Govt. of Goa on 28 October 2022.

Shri Sheldon Fernandes was sanctioned 5 cages under centrally sponsored scheme of Department of Fisheries, Govt. of Goa. 2 cages were also provided under All India Network Project on Mariculture of ICAR-CMFRI, Karwar for demonstration of Integrated Multitrophic Aquaculture. Hatchery produced seeds of Asian seabass,

Lates calcarifer and silver pompano (Trachinotus blochii) were stocked in these cages. The technical guidance for the entire period of cage culture will be extended by ICAR-CMFRI, Karwar.

## Ten New Breeds of Indigenous Farm Animals Registered by ICAR-NBAGR

ICAR-NBAGR registered 10 new breeds of indigenous livestock species in the country. These breeds are -Kathani cattle (Maharashtra), Sanchori cattle (Rajasthan) and Masilum cattle (Meghalaya); Purnathadi buffalo (Maharashtra); Sojat goat (Rajasthan), Karauli goat (Rajasthan) and Gujari goat (Rajasthan); Banda pig (Jharkhand), Manipuri Black pig (Manipur) and Wak Chambil pig (Meghalaya). Accession numbers were also assigned to these breeds by the Bureau. Earlier, Breed Registration Committee (BRC) in its 10th meeting held on 31 August 2022, approved the registration of these livestock breeds of different states. After including these breeds, total number of registered indigenous breeds is 212, including 53 cattle, 20 buffalo, 37 goat, 44 sheep, 7 for horses and ponies, 9 or camel, 13 pig, 3 donkey, 3 dog, 1 yak, 19 chicken, 2 duck and 1 of geese.

## Bio-pesticide Thar Jaivik 41EC from *Citrullus colocynthis*- Patented

Dr D K Samadia, the Director Of ICAR-Central Institute for Arid Horticulture, stated that the bio-pesticide prepared by Dr S M Haldhar, Scientist (Entomology) and his team is absolutely safe and effective to control insect-pests in arid zone fruits and vegetables. The Institute has released this product with the name of "Thar Jaivik 41 EC" in 2019 and had sent for patent. The patent office, Government of India has granted and issued a certificate of patent entitled bio-pesticide compositions and formulation from tumba (Citrullus colocynthis) for insect control under Patents Act, 1970. This bio- pesticide is effective against Helicoverpa armigera, Spodoptera litura, white fly and aphid, and safe for natural enemies. The data on phyto-toxicity effect on plant was also recorded and found that no effect was observed on plant when applied 10 times more dose of the recommended dose of bio-pesticide (Thar Jaivik 41 EC). It was also observed that there was no effect on fruits and vegetables after 3 days spraying of bio-pesticide (Thar Jaivik 41 EC) for human consumption.

Indian arid zone is characterized by high and low temperatures and variable precipitation, which limit the scope for high crop productivity. The existing low productivity could be increased by following improved new technologies and inputs. The area and yield potential of arid horticultural crops has increased many-fold because of the development of new varieties, agro-techniques and plant protection measures in arid region. Insect-pests are the major constraint for increasing the production of arid horticulture crops in India. Chemical pesticides have played an important role in managing pests and diseases and increasing arid horticulture crops production in the past, but, their indiscriminate use for over three decades has led to several problems such as development of resistance in pest to pesticides, pesticides residues, destruction of beneficial insects and the outbreak of secondary pests. These negative effects have provided the impetus for the development of alternatives including botanical pesticides. Organic botanical pesticides are an important group of naturally occurring, often slow-acting crop protectants that are usually safer to human and with minimal residual effects. On the basis of above background, we have made bio-pesticide formulation that is Thar Jaivik 41 EC from tumba and desi cow urine.

## Inactivated Low Pathogenic Avian Influenza (H9N2) Vaccine for Chicken

Inactivated low pathogenic avian influenza (H9N2) vaccine for chickens', developed by the scientists of ICAR-NIHSAD, Bhopal was transferred to M/s Globion India Pvt Ltd, Secunderabad, M/s Venkateshwara Hatcheries Pvt Ltd, Pune, M/s Indovax Pvt Ltd, Gurgaon and M/s Hester Biosciences Ltd, Ahmedabad on 27 December 2022, facilitated by M/s Agrinnovate India Ltd. (AgIn) at NASC, New Delhi. The event was graced by Dr Himanshu Pathak, Secretary (DARE) & Director General (ICAR) and Chairman, AgIn, Dr B N Tripathi, DDG (Animal Science), Dr Praveen Malik, CEO, Agrinnovate India Ltd., Dr Aniket Sanyal, Director, ICAR-NIHSAD, representatives of commercial firms, and other officials from ICAR and AgIn.

Dr Himanshu Pathak appreciated the sincere efforts of the ICAR-NIHSAD scientists in development of the first indigenous vaccine for H9N2 virus and commended the Agrinnovate India limited (AgIn) for the efforts in the transfer of the technology to industry. DDG (AS) asserted that the vaccine will meet the standard of the market both in India and abroad. The vaccine will contribute significantly to increase the income of poultry farmers by reducing the economic loss due to the disease.


## Bioengineering breakthrough increases DNA detection sensitivity by 100 times

"DNA detection is in the center of bioengineering," says Jinglei Ping, Assistant Professor of Mechanical and industrial engineering, Center for Personalized Health Monitoring, Institute for Applied Life Sciences, UMass Amherst, England. He said "Everyone wants to detect the DNA at a low concentration with a high sensitivity and we just developed this method to improve the sensitivity by about 100 times with no cost". According to him the challenge is that there are lots of molecules present in a sample that aren't the target DNA that can interfere with the result. That's where this method is different. The test sample is put within an alternating electric field. Then, the DNA is allowed to free-move. Dr. Ping says "when the strands of DNA dance, they have a specific oscillation frequency." Researchers can then read samples to see if there is a molecule moving in a way that matches the movement of the target DNA and easily distinguish it from different movement patterns. This even works when there is a very low concentration of the target DNA.

This new method has huge implications for speeding up disease detection, because it is highly sensitive. With this method, diagnoses can happen at earlier stages of a disease progression, which can greatly impact health outcomes. This method just takes a few minutes because it is all electrically managed. "This makes it suitable for point of care," he says. "Usually, we provide samples to a lab and they can provide the results quickly or slowly, depending on how fast they can process, and it can take 24 hours or longer." For instance, he cites how with a diagnosis, a biopsy sample is frozen and then sent to a lab for processing, which can take up to two

months. The near-instant results with this new method mean treatment does not have to wait for lab processing times.

Another benefit is its portability. Ping describes the device to be similar in size to a blood sugar test tool, which opens the doors to improvements in health on a global scale. "It can be used at places where resources are limited. In remote areas, the doctor usually goes to a village once or twice a year, and now, maybe they can have a base that has this kind of tool and they will have the chance to test for it quickly and easily."

Ping is enthusiastic about the breadth of possible applications for this discovery. He added "The nano-mechanoelectrical approach can be also integrated with other bioengineering technologies, like CRISPR, to elucidate nucleic acid signaling pathways, comprehend disease mechanisms, identify novel drug targets and create personalized treatment strategies, including microRNA-targeted therapies".

**II T researchers creates bio-jet fuel from waste biomass**

IIT Jodhpur's research team has successfully developed an iron-based catalyst (Fe/Silica-Alumina) that is abundantly available. This catalyst has been used in the production of bio-jet fuel, utilizing non-edible oils and waste biomass. Their breakthrough solves a long-standing industry challenge, making the manufacturing process of bio-jet fuel economically viable. The development of this catalyst offers the potential for cheaper and cleaner fuels, leading to transformative changes in the energy sector. The bio-jet fuel has the potential to contribute significantly to the reduction of greenhouse gas emissions.

The research finding holds significant importance in the development of aviation fuel under mild reaction conditions, characterized by low $H_2$ pressure and high reusability of the Fe/SiO2-Al2O3 catalyst. The catalyst demonstrates excellent reusability for up to 10 cycles in the production of bio-jet fuel. These results are particularly promising, considering the catalyst's high acidity and unique textural properties, all achieved under relatively mild process conditions, including low $H_2$ pressure and solvent-free conditions

**Fluorescent nanotubes will now detect presence of bacteria and viruses**

A team of researchers from Bochum, Duisburg and Zurich has developed a new method for creating modular optical sensors capable of detecting viruses and bacteria. The researchers used fluorescent carbon nanotubes with a novel type of DNA anchors that act as molecular handles. These anchors can be used to conjugate biological recognition units, such as antibodies aptamers, to the nanotubes, which can then interact with bacterial or viral molecules. The team's findings were published in the Journal of the American Chemical Society. The key to success was the use of DNA structures with guanine quantum defects, which allowed the detection unit to be adapted to the target molecule for identifying specific viral or bacterial proteins. The researchers demonstrated the sensor concept using the SARS CoV-2 spike protein, using aptamers that selectively bind to proteins. The sensors

demonstrated high reliability and stability in solution, making them suitable for diagnostic applications in complex environments.

## New health-boosting probiotic bacteria

The Institute of Life Sciences (ILS), Bhubaneswar has discovered health-promoting probiotic bacteria from the tribals of Odisha. The isolated probiotic is claimed to be useful in preventing diarrhoea and obesity. The name of the bacteria discovered by the ILS is *Ligilactobacillus salivarious*. The ILS has identified 30 to 40 such probiotic bacteria from the bodies of the tribal people of Odisha and their foods. The bacteria have successfully been tested on rats in laboratories.

"We have discovered some probiotic bacteria. We have even done the whole genome sequencing of some bacteria. Now we are confirmed that these bacteria are of probiotic type and can be used for promoting health of tribal people," said senior scientist of ILS, Dr Shantibhushan Senapati. Another scientist of ILS, Dr Jaylaxmi Dash said, "Since we have done basic research, proof of validation is there to take the finding of the research to the next level. Their evaluation has also been completed, confirming their safety in animal studies. It will be available for all after passing through the regulatory checks."

With the changing lifestyle, these health-promoting bacteria are getting lost. Since there is a possibility of these bacteria may go extinct, the ILS has planned to set up a Probiotic Bank.

## Mitochondrial genome editing technique yields useful traits for hybrid Tobacco seed production

Researchers at North Carolina State University have developed a method to generate Cytoplasmic Male Sterility (CMS) in tobacco plants. The researchers used a unique strategy to move an essential mitochondrial gene called *atp1* to the nucleus, allowing it to be expressed in every cell of the plant except those responsible for pollen production. They then used the genome editing tools to permanently remove the native *atp1* gene from the mitochondria. The researchers found that the plants looked normal until they began to flower, but failed to produce pollen because the transferred atp1 gene was no longer expressed. This trait will be inherited maternally, which is crucial for large-scale hybrid seed production. The researchers are now working to decouple these results so that researchers can achieve either pollen infertility or the seedless trait alone, instead of both at the same time. Their next generation of experiments will include testing the seedless trait in tomato and a grain such as rice to test the efficacy of their system in a crop where hybrid seed production is important for achieving maximal yields. The researchers believe that the technology can be effectively transferred to other plant species.

# NATIONAL AND INTERNATIONAL CONFERENCES

1) 5th International Conference on Biotechnology, Biomedicines and Genetic Engineering "Biotechnology as a Trajectory for Future Lives " Oct 09-11, 2023 Paris, France
2) International conference in Latest Advancements in Science, Management, Commerce and Educational Research (LASMCER-2023)25th - 26th October 2023, Toronto, Canada
3) International conference on advances in Science, Engineering & Technology (ICASET) 27th - 28th October 2023 | Hochi Minh city, Vietnam
4) 2ndWorld Conference on Multidisciplinary Research & Innovation (WCMRI) 28th - 29th October 2023 | Singapore
5) 03rd - 04th November 2023 | Chennai, India | Hybrid Conference
6) 44thWorld Conference on Applied Science, Engineering & Technology 25th - 26th November 2023 | Malaysia
7) 6thInternational Conference on Recent Trends in Multi-Disciplinary Research (ICRTMDR-23)
8) 10th - 11th November 2023 | Istanbul, Turkey
9) 01st International Conference on Contemporary Engineering, Technology and Management (ICCETM-2023) 4th - 5th December 2023 | Dubai
10) 45thWorld Conference on Applied Science, Engineering & Technology 29th - 30th December 2023 | Goa, India
11) 6th International Conference on Applied Sciences, Engineering, Technology And Management (ICASETM-2023) 29th - 30th December 2023 | Prime Plaza Hotel Sanur - Bali
12) 4thInternational Conference on Advancing Knowledge from Multidisciplinary Perspectives in Engineering & Technology 26th - 27th January 2024 | Manila, Philippines
13) International Conference on Computer, Cybernetics and Education (ICCCE-2024) 23rd - 24th February 2024 | Jakarta, Indonesia
14) 4th International Conference on Engineering, Social- Sciences, And Humanities(ICESSU-2024) 29th - 30th March 2024 | Manila, philippines
15) 5th International Conference on Multidisciplinary and Current Educational Research (ICMCER-2024) 26th - 27th April 2024 | Bangkok, Thailand
16) 7th International Conference on Multi-Disciplinary Research Studies and Education (ICMDRSE-2024) 30th - 31st May 2024 | Malaysia

Society of Green World for Sustainable Environment
An International Journal of Biological Sciences
Biotech Today
A Peer Review Journal

# INFORMATION FOR CONTRIBUTORS

## GENERAL

All manuscripts should be addressed to the Editor in-chief, Biotech Today, 35, Akshardham, Roorkee Raod, Modipuram, Meerut – 250110 U.P. India. Submission of an article implies that it has not bee previously published and is not under consideration for publication elsewhere; and further, that if accepted, it will not be published elsewhere. Two copies of anuscripts of all categories are to be submitted with a letter of transmittal, giving names and complete addresses (including telephone numbers, e-mail i.d. etc. if available) of all the authors.

International Journal of Biological Sciences Biotech Today is a multi disciplinary journal and therefore research and review papers of general significance that are written clearly and well organized will be given preference. All papers's solicited and unsolicited, will be first assessed by a Reviewing Editor. Papers found unsuitable in terms of the overall requirements of he journal will be notified of acceptance, revision or rejection of the paper. It may be noted that papers once rejected cannot be resubmitted. Illustrations and other materials to be reproduced from other publications must be properly credited; it is the authors' responsibility to obtain permission for reproduction of figures, tables, etc. from published sources (copies of letters of permission should be sent to the editor-in-chief).

### Declarations to be made regarding ethical issues

Manuscripts that deal with clinical findings should be enclosed with a statement on informed consent of the patients under study. If humans and animals are the subject of a clinical study, it is essential for the study to have been carried out in accordance with the ethical standards of the country/ countries where the research described in the article has been conducted. A declaration to that effect must accompany the manuscripts.

### Author conflict of interest statement

Authors must knowledge the organizations that have provided financial support for their work. Any other conflict of interest

must be declared while submitting the manuscripts.

## CATEGORIES OF MANUSCRIPTS

General articles not exceeding 5000 words, 8 display items (tables and figures)] discuss current trends in research in a field that would be of interest to readers outside the field. These include interdisciplinary topics, science policy and science administration, some aspects of the application of science and technology to human needs or the impact of science and technology on society/ecosystem/life. The articles should include an abstract, introductory paragraph, brief subheads at appropriate places, illustrations that will help a general reader and references .

**Review articles** (not exceeding 7000-8000 words, cited references to be limited to about 100 in number) are expected

to survey and discuss current developments in a field. They should be well focused and organized, and avoid a general ..textbook' style.

**Research articles** (not exceeding 5000 words) report research result of major significance. They should include an abstract,

an introductory paragraph and brief subheads.

**News** (not exceeding 1000 words) can cover important scientific events or any other news of interest to scientist in

general.

## MANUSCRIPT PREPARATION

Manuscripts should be typed/printed double spaced on one side of white paper (21×28 cm). The pages should be numbered consecutively, starting with the title page and through the text, reference list, tables and figures legends. The title should be brief, specific and amenable to indexing. Not more than five keywords should be indicated separately; these should be chosen carefully and must not be phrases of several words. Abstract and summary should be limited to 100 words and convey the main points of the paper, outline the results and conclusions, and explain the significance of the results. The full length paper should have the following headings.

**SHORT TITLE** : A short title, not exceeding 35 letters, should be typed at the top of first page in first letters capitals and underlined.

**TITLE** : The title should be short and informative, typed in first letters capital, Latin names underlined

**AUTHORS** : Names of authors to be typed in first letters capital.

**ADDRESS** : Address of the institution where the work was carried out. Footnote should indicate the authors for correspondence.

**MAIN HEADINGS** : Each full-length research paper should be divided into the following main headings which are to be bold and typed in first letters capitals on the left hand side of the pag: Abstract, Introduction : Materials and Methods, Results, Discussion, References.

**SUB-HEADINGS** : Typed flush left in first letter capital, bold and italicized.

**Abstract** : A brief abstract, not exceeding 200 words should be typed after address.

**Key words** : The Abstract should be followed by about five key words indicating the contents of the manuscript.

**Introduction** : Introduction should be brief and pertinent.

**Materials and Methods** : Materials and Methods should include the source and nature of material, experimental design and the techniques employed.

**Result** : Result should contain data which are essential for drawing main conclusion from the study. Wherever needed the data should be statistically analyzed. Same data should not be presented in tables and figure form.

**Discussion** : The discussion should deal with the interpretation of the results. Wherever possible, Results and Discussion can be combined.

**Tables** : Tables should be typed on separate sheets, each with a heading. Tables should be typed with the first letter capital, table number in Arabic numerals, followed by a period.

**Figures** : For each figure submit a glossy print or original drawing. Photomicrograph should have a scale bar. Line drawings should be roughly twice the final printed single column size of 7.5 cm width. Text figures should be numbered in Arabic numerals. Captions and legends to illustrations should be typed on a separate sheet of paper. Line drawings and photograph should contain figure number and authors' name, on the back with soft lead pencil. Colour photographs will be printed if author(s) pay Rs 600/- (or US $ 20) as processing charges.

**References** : References should be cited in Text Form. In brackets as (Sengar, et al., 2013). Listing of References should be alphabetically. References should not include unpublished source materials. The list of References at the end of the text

should be in the following format.

Rynold, M.P. (1994). The Archaean grey gnesisses and the genesis of continental crust. In: Archean Crustal Evolution (ed. Candie, K.C.) Elsevier, Amsterdam, pp. 205–259.

Sengar,R.S. (2013). Estimation of population growth and extinction parameters from noisy data. Ecol. Appl., 13 : 806–813.

Sirova, D., Adamec, L. and Verba,J. (2013). Enzymatic activities in trops of four aquatic pieces 3 of the Carnivorous genus Vlricularia, New Phytology 159(3) : 669-675.

Sengar, R.S., Sharma, A.K., Chaudhary, R. and Kureel, R.S. (2009). Biodiesel plant Jatrophaneed for future.

Procedings of ..5th World Congress of Cellular & Molecular Biology (WCCMB, 2012).., November 02- 06, School of Biotechnology, Devi Ahilya University Indore, India & World Society of Cellular & Molecular Biology, France. 142-143.

**Abbreviations** : Use standard abbreviations. Some of the common abbreviations are given below: A (absorbance); h (hour); min (minutes); sec (seconds); cpm (counts per minutes); Ci (Curie); molwt (molecular weight); kD (kilo Dalton); kb (kilo base); sp act (specific activity); wt (weight); SD (standard deviation); SE (standard err); DAF (days after flowering)

**Acknowledgment** : It should be as brief as possible.

**Footnotes** : They are not normally allowed except to identify the author for correspondence.

**Jurisdiction** : The jurisdiction for all disputes concerning submitted articles, published material, advertisement, subscription and sale will be at court/tribunals situated in Meerut only.

## Write to Us

You are welcome to send any comments about any article published in Biotech Today, or share some information with our readers. Write to us at:

**Editor**

**Biotech Today**

35, Akshardham, Roorkee Road, Modipuram,
Meerut-250110 (U.P.) India

Or send an email at :

biotechtoday@gmail.com, sengarbiotech7@gmail.com

# Society of Green World for Sustainable Environment

**An International Journal of Biological Sciences**

## Biotech Today

**Corporate & Business Office: 35, Akshardham, Roorkee Road, Modipuram, Meerut-250110 U.P.**

**Editorial Office : Department of Ag. Biotechnology, College of Agriculture, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut 250110 U.P.**

**Email:biotechtoday@gmail.com**

**A Peer Review Journal**


# MEMBERSHIP FORM

Dear Sir,

I wish to enroll myself as Annual Member / Life Member of **"Society of Green World for Sustainable Environment"**. I agree to abide by the Memorandum, Rules and Regulations of the Society as framed from time to time.

I am sending herewith a Demand Draft/ Cheque No. ..... Dated ..... drawn in favour of the -Society of Green World for Sustainable Environment payable at Modipuram.

1. Name of applicant .....
2. Date of birth .....
3. Address for the correspondence .....
   .....
   Designation and Official Address .....
   .....
   Phone (R).....(O).....(Mobile).....
   E-mail.....
5. Educational Qualifications.....
6. Area of Specialization/ current research interest .....
   .....

Enclosed is a demand draft/cheque no. .....date: ..... drawn..... on..... for.....

Date:

Sincerely yours

Signature

**DETAIL OF FEE**

**PERSONAL MEMBERSHIP**

| | India (INR) | Abroad (US$) |
|---|---|---|
| • Annual Membership | 1000/- | 200 |
| • Life Membership | 5,000/- | 1500 |

**INSTITUTIONAL MEMBERSHIP**

| Library/Institutions | Corporate |
|---|---|
| 2000/- | 2000/- |
| 10000/- | 12000/- |

**You can pay all payment through online banking. Details are :**
Name : Society of Green World for Sustainable Environment
A/c n.:-31992603714
Bank name.;- State Bank of India
Branch :- S.V.P.U.A&T., Meerut
Branch Code :- 10653
IFSC:- SBIN00110653

**Note:** Members Shall receive the Journal at gratis.

**Please send your payment to:**
Dr. R.S. Sengar Secretary, SGWSE
35, Akshardhan Colony, Roorkee Road,
Modipuram, Meerut-250110 U.P. (India)