IJOEAR

RESEARCH
TODAY
FOR A GREENER
TOMORROW

NAAS
Rating
4.23
★★★★★

AGRICULTURE JOURNAL

IJOEAR

International Journal of Environmental & Agriculture Research

VOLUME-12
ISSUE-9
SEPTEMBER 2026

Cultivating Knowledge for a Sustainable World

Sustainable Agriculture Brighter Tomorrows

- PEER REVIEWED JOURNAL
- REFEREED JOURNAL
- INDEXED JOURNAL
- WORLDWIDE VISIBILITY

+91-7665235235 | www.ijoear.com | info@ijoear.com

SUBMIT | PUBLISH | SHARE
YOUR RESEARCH, OUR COMMITMENT

Preface

We would like to present, with great pleasure, the volume-12, Issue-9, September 2026 of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas.

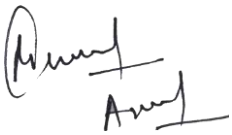
Environmental Research:

Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

Agriculture Research:

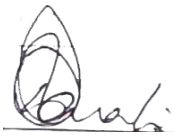
Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.



Mukesh Arora

(Managing Editor)



Dr. Bhagawan Bharali

(Chief Editor)

Fields of Interests

Agricultural Sciences	
Soil Science	Plant Science
Animal Science	Agricultural Economics
Agricultural Chemistry	Basic biology concepts
Sustainable Natural Resource Utilisation	Management of the Environment
Agricultural Management Practices	Agricultural Technology
Natural Resources	Basic Horticulture
Food System	Irrigation and water management
Crop Production	
Cereals or Basic Grains: Oats, Wheat, Barley, Rye, Triticale, Corn, Sorghum, Millet, Quinoa and Amaranth	Oilseeds: Canola, Rapeseed, Flax, Sunflowers, Corn and Hempseed
Pulse Crops: Peas (all types), field beans, faba beans, lentils, soybeans, peanuts and chickpeas.	Hay and Silage (Forage crop) Production
Vegetable crops or Olericulture: Crops utilized fresh or whole (wholefood crop, no or limited processing, i.e., fresh cut salad); (Lettuce, Cabbage, Carrots, Potatoes, Tomatoes, Herbs, etc.)	Tree Fruit crops: apples, oranges, stone fruit (i.e., peaches, plums, cherries)
Tree Nut crops: Hazlenuts. walnuts, almonds, cashews, pecans	Berry crops: strawberries, blueberries, raspberries
Sugar crops: sugarcane. sugar beets, sorghum	Potatoes varieties and production.
Livestock Production	
Animal husbandry	Ranch
Camel	Yak
Pigs	Sheep
Goats	Poultry
Bees	Dogs
Exotic species	Chicken Growth
Aquaculture	
Fish farm	Shrimp farm
Freshwater prawn farm	Integrated Multi-Trophic Aquaculture

Milk Production (Dairy)	
Dairy goat	Dairy cow
Dairy Sheep	Water Buffalo
Moose milk	Dairy product
Forest Products and Forest management	
Forestry/Silviculture	Agroforestry
Silvopasture	Christmas tree cultivation
Maple syrup	Forestry Growth
Mechanical	
General Farm Machinery	Tillage equipment
Harvesting equipment	Processing equipment
Hay & Silage/Forage equipment	Milking equipment
Hand tools & activities	Stock handling & control equipment
Agricultural buildings	Storage
Agricultural Input Products	
Crop Protection Chemicals	Feed supplements
Chemical based (inorganic) fertilizers	Organic fertilizers
Environmental Science	
Environmental science and regulation	Ecotoxicology
Environmental health issues	Atmosphere and climate
Terrestric ecosystems	Aquatic ecosystems
Energy and environment	Marine research
Biodiversity	Pharmaceuticals in the environment
Genetically modified organisms	Biotechnology
Risk assessment	Environment society
Theoretical production ecology	horticulture
Breeding	plant fertilization

Board Members

Dr. Bhagawan Bharali (Chief Editor)

Professor & Head, Department of Crop Physiology, Faculty of Agriculture, Assam Agricultural University, Jorhat-785013 (Assam).

Mr. Mukesh Arora (Managing Editor)

M.Tech (Digital Communication), BE (Electronics & Communication), currently serving as Associate Professor in the Department of EE, BIET, Sikar.

Dr. Kusum Gaur (Associate Editor)

Dr. Kusum Gaur working as professor Community Medicine and member of Research Review Board of Sawai Man Singh Medical College, Jaipur (Raj) India.

She has awarded with WHO Fellowship for IEC at Bangkok. She has done management course from NIHFW. She has published and present many research paper in India as well as abroad in the field of community medicine and medical education. She has developed Socio-economic Status Scale (Gaur's SES) and Spiritual Health Assessment Scale (SHAS). She is 1st author of a book entitled " Community Medicine: Practical Guide and Logbook.

Research Area: Community Medicine, Biostatics, Epidemiology, Health and Hospital Management and Spiritual Health

Dr. Darwin H. Pangaribuan

Associate Professor in Department of Agronomy and Horticulture, Faculty of Agriculture, University of Lampung, Indonesia.

Educational background: (Ir.) from Faculty of Agriculture, IPB University, Bogor, Indonesia; (Dipl. Eng) in Land Evaluation from the University of Tweente (UT-ITC), Enschede, The Netherlands; (M.Sc) in Crop Production from Wageningen University (WU), The Netherlands. (Ph.D) in Horticulture from University of Queensland (UQ), Brisbane, Australia.

Research Interest: Vegetable Production & Physiology; Biostimulant & Biofertilizers; Organic Farming, Multiple Cropping, Crop Nutrition, Horticulture.

Dr Peni Kistijani Samsuria Mutalib

Working as Research coordinator and HOD in the department of Medical Physics in University of Indonesia.

Dr. Govindaraj Kamalam Dinesh, PhD

Department of Biochemistry, Physiology, Microbiology and Environmental Science College of Agriculture, Iroisemba

Central Agricultural University, Imphal, Manipur – 795004, India

Specialization: Ecosystem services, Conservation agriculture, Climate change, Agroecology, Greenhouse gas emissions from agriculture, Agricultural ornithology, Carbon sequestration

Academic Qualifications: B.Sc. (Agriculture), Annamalai University, M.Sc. (Environmental Science), Tamil Nadu Agricultural University (TNAU), Ph.D. (Environmental Sciences), ICAR-IARI, New Delhi (UGC-JRF, IARI PhD SRF, All India Rank – 5)

Professor Jacinta A.Opara

Working full-time and full-ranked Professor and Director, Centre for Health and Environmental Studies at one of the top 10 leading public Universities in Nigeria, the University of Maiduguri-Nigeria founded in 1975.

Dr. Samir B. Salman AL-Badri

Samir Albadri currently works at the University of Baghdad / Department of Agricultural Machines and Equipment. After graduation from the Department of Plant, Soils, and Agricultural Systems, Southern Illinois University Carbondale. The project was 'Hybrid cooling to extend the saleable shelf life of some fruits and vegetables. I worked in many other subject such as Evaporative pad cooling.

Orchid ID: https://orcid.org/0000-0001-9784-7424

Publon Profile: https://publons.com/researcher/1857228/samir-b-albadri

Dr. Goswami Tridib Kumar

Presently working as a Professor in IIT Kharagpur from year 2007, He Received PhD degree from IIT Kharagpur in the year of 1987.

Prof. Khalil Cherifi

Professor in Department of Biology at Faculty of Sciences, Agadir, Morocco.

Dr. Josiah Chidiebere Okonkwo

PhD Animal Science/ Biotech (DELSU), PGD Biotechnology (Hebrew University of Jerusalem Senior Lecturer, Department of Animal Science and Technology, Faculty of Agriculture, Nau, AWKA.

Dr. Jadala Shankaraswamy

Specialization: Horticulture, Post-Harvest Technology, Phytochemistry, Nanobiotechnology, 3D Food Printing

Academic Qualifications: B.Sc. (Horticulture), M.Sc. (Post-Harvest Technology of Horticultural Crops), Ph.D. (Horticulture)

Assistant Professor (Senior Scale - IX), College of Horticulture, Mojerla, Sri Konda Laxman Telangana State Horticultural University, Telangana, India

Dr. Jadala Shankaraswamy is an Assistant Professor (Senior Scale – IX) at Sri Konda Laxman Telangana State Horticultural University, Telangana, India. His research focuses on post-harvest technology, phytochemistry, nanobiotechnology, and value addition in horticultural crops. He has contributed to innovative product development, holds patents in horticultural science, and has received several national and international awards for his research excellence.

Dr. Tarun Kumar Maheshwari

Dr. Tarun Kumar Maheshwari, Head of Agricultural Engineering at Dr. BRA College of Agricultural Engineering and Technology, Etawah, specializes in farm machinery and power engineering. He holds a Ph.D. from Sam Higginbottom University and an M.Tech. from IIT Kharagpur.

Prof. Özhan ŞİMŞEK

Agriculture Faculty, Department of Horticulture, Çukurova University, Adana, 01330 Turkey.

Dr. Anka Ozana Čavlović

Working as Professor in the department of Faculty of Forestry, University of Zagreb, Svetošimunska 25, Zagreb.

Dr. Rakesh Singh

Professor in Department of Agricultural Economics, Institute of Agricultural Sciences, Banaras Hindu University, Also Vice President of Indian Society of Agricultural Economics, Mumbai.

Dr. Sunil Wimalawansa

MD, PhD, MBA, DSc, is a former university professor, Professor of Medicine, Chief of Endocrinology, Metabolism & Nutrition, expert in endocrinology; osteoporosis and metabolic bone disease, vitamin D, and nutrition.

Dr. Smruti Sohani

Dr. Smruti Sohani, has Fellowship in Pharmacy & Life Science (FPLS) and Life member of International Journal of Biological science indexed by UGC and e IRC Scientific and Technical Committee. Achieved young women scientist award by MPCOST. Published many Indian & UK patents, copyrights, many research and review papers, books and book chapters. She Invited as plenary talks at conferences and seminars national level, and as a Session chair on many International Conference organize by Kryvyi Rih National University, Ukraine Europe. Designated as state Madhya Pradesh Coordinator in International conference collaborated by RCS. Coordinator of two Professional Student Chapter in collaboration with Agriculture Development society and research Culture Society. her enthusiastic participation in research and academia. She is participating on several advisory panels, scientific societies, and governmental committees. Participant in several worldwide professional research associations; member of esteemed, peer-reviewed publications' editorial boards and review panels. Many Ph.D., PG, and UG students have benefited from her guidance, and these supervisions continue.

Dr. Ajeet singh Nain

Working as Professor in GBPUA&T, Pantnagar-263145, US Nagar, UK, India.

Dr. Salvinder Singh

Presently working as Associate Professor in the Department of Agricultural Biotechnology in Assam Agricultural University, Jorhat, Assam.

Dr. Salvinder received MacKnight Foundation Fellowship for pre-doc training at WSU, USA – January 2000-March 2002 and DBT overseas Associateship for Post-Doc at WSU, USA – April, 2012 to October, 2012.

Dr. V K Joshi

Professor V.K.Joshi is M.Sc., Ph.D. (Microbiology) from Punjab Agricultural University, Ludhiana and Guru Nanak Dev University, Amritsar, respectively with more than 35 years experience in Fruit Fermentation Technology, Indigenous fermented foods, patulin ,biocolour ,Quality Control and Waste Utilization. Presently, heading the dept. of Food Science and Technology in University of Horticulture and Forestry, Nauni-Solan (HP), India.

Dr. Mahendra Singh Pal

Presently working as Professor in the dept. of Agronomy in G. B. Pant University o Agriculture & Technology, Pantnagar-263145 (Uttarakhand).

Dr. Sanjoy Kumar Bordolui

M.Sc. (Ag.), PhD, FSTA, FSIESRP, Assistant Professor, Department of Seed Science and Technology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia. W.B., India. He received CWSS Young Scientist Award-2016, conferred by Crop and Weed Science Society, received Best Young Faculty Award 2019 conferred by Novel Research Academy, received Innovative Research & Dedicated Teaching Professional Award 2020 conferred by Society of Innovative Educationalist & Scientific Research Professional, Chennai.

Dr.Chiti Agarwal

Dr. Chiti Agarwal works as a postdoctoral associate at the University of Maryland in College Park, Maryland, USA. Her research focuses on fungicide resistance to fungal diseases that affect small fruits such as strawberries. She graduated from North Dakota State University in Fargo, North Dakota, with a B.S. in biotechnology and an M.S. in plant sciences. Dr. Agarwal completed her doctorate in Plant Pathology while working as a research and teaching assistant. During her time as a graduate research assistant, she learned about plant breeding, molecular genetics, quantitative trait locus mapping, genome-wide association analysis, and marker-assisted selection. She wants to engage with researchers from many fields and have a beneficial impact on a larger audience.

DR. Owais Yousuf

Presently working as Assistant professor in the Department of Bioengineering, Integral University-Lucknow, Uttar Pradesh, India.

Dr. Vijay A. Patil

Working as Assistant Research Scientist in Main Rice Research Centre, Navsari Agricultural University, Navsari. Gujarat- 396 450 (India).

Dr. Amit Kumar Maurya

Working asJunior Research Assistantin the Department of Plant Pathology at Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, U.P. India.

Prof. Salil Kumar Tewari

Presently working as Professor in College of Agriculture and Joint Director, Agroforestry Research Centre (AFRC) / Program Coordinator in G.B. Pant University of Agric. & Tech., Pantnagar - 263 145, Uttarakhand (INDIA).

Dr. S. K. Jain

Presently working as Officer Incharge of All India Coordinated Sorghum Improvement Project, S. D. Agricultural University, Deesa, Gujarat.

Dr. Deshmukh Amol Jagannath

Presently working as Assistant Professor in Dept. of Plant Pathology, College of Agriculture polytechnic, NAU, Waghai.

Mr. Anil Kumar

Working as Junior Research Officer/Asstt. Prof. in the dept. of Food Science & Technology in Agriculture & Technology, Pantnagar.

Mr. Jiban Shrestha

Scientist (Plant Breeding & Genetics)

Presently working as Scientist (Plant Breeding and Genetics) at National Maize Research Programme (NMRP), Rampur, Chitwan under Nepal Agricultural Research Council (NARC), Singhdarbar Plaza, Kathmandu, Nepal.

Mr. Aklilu Bajigo Madalcho

Working at Jigjiga University, Ethiopia, as lecturer and researcher at the College of Dry land Agriculture, department of Natural Resources Management.

Mr. Isaac Newton ATIVOR

MPhil. in Entomology, from University of Ghana.

He has extensive knowledge in tree fruit orchard pest management to evaluate insecticides and other control strategies such as use of pheromone traps and biological control to manage insect pests of horticultural crops. He has knowledge in agronomy, plant pathology and other areas in Agriculture which I can use to support any research from production to marketing.

Mr. Bimal Bahadur Kunwar

He received his Master Degree in Botany from Central Department of Botany, T.U., Kirtipur, Nepal. Currently working as consultant to prepare CCA-DRR Plan for Hariyo Ban Program/CARE in Nepal/GONESA.

Table of Contents
Volume-12, Issue-9, September 2026

International Journal of Environmental & Agriculture Research (IJOEAR)
ISSN: [2454-1850]
[Vol-12, Issue-9, September- 2026]
Website: www.ijoear.com
Journal DOI: 10.25125/agriculture-journal

An Updated Review on Tannery Waste Treatment with Emphasis on Fungal Mediated Applications

BK Manjunatha[1*]; Asha Kadamane[2]; Santhosh D.B[3]; Kiran Kumar H.B.[4]

Department of Biotechnology, The Oxford College of Engineering, Bengaluru-560068, Karnataka, India
Department of Microbiology, Jain University, Benguluru.
Department of Biotechnology, Tumkuru university, Tumkuru
Government Science College, Bengaluru
*Corresponding Author

Received:- 08 August 2026/ Revised:- 24 August 2026/ Accepted:- 03 September 2026/ Published: 15-09-2026

Abstract— *Current estimates state that the global leather industry produces 600 million m³ of effluent annually. Since tannery effluents are toxic with high salinity, and contain several recalcitrant organics, they cannot be broken down by conventional biological treatment methods. Biological treatment options are more sustainable, cost-effective, and environmentally friendly, as they rely on microorganisms. Recently, fungi have garnered attention from the research community for their potential use in the remediation of wastes and wastewaters. A number of filamentous fungi are found naturally in wastewater treatment systems as spores or vegetative cells which metabolize organic substances, e.g., Phanerochaete chrysosporium, Trametes versicolor, etc., which produce key enzymes like lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase. These enzymes break down persistent organic pollutants, including polyaromatic hydrocarbons (PAHs), dyes, and pesticides. Other fungi like Aspergillus spp., Irpex lacteus, and Fusarium spp. bioaccumulate heavy metals and degrade complex organic compounds. Tannins represent one of the least biodegradable substances in tannery wastewaters, with high recalcitrant compounds. Further, several biophysical, enzymatic, cellular, and metabolic advantages of fungi have advantages in tannery effluent treatment. The current review with an introduction on tannery treatment delves into the fungal bioremediation in detail. The recent developments in this area are also covered to update the readers on the developments in genomics and proteomics. Additional methods, viz., enzymatic treatment, strain improvement, scale-up biological processes, and microalgae applications, can enhance the quality and efficacy of the treatment.*

Keywords— Chemical oxygen demand (COD), tannery wastewater effluent (TWE), white-rot fungi (WRF), Reverse osmosis, Tannase.

I. INTRODUCTION

Tannery effluent contains heavy loads of tannins, chromium, and sulfides, alongside over 175 other chemicals used in leather processing. This highly toxic, dark-brown wastewater requires complex physical, chemical, and biological treatments to prevent severe soil and aquatic pollution. Because tannery effluents are high in salinity, toxicity, and recalcitrant organics, conventional biological treatment methods (like activated sludge) struggle to break them down. Advanced methods like electrocoagulation, membrane filtration, and advanced oxidation processes (AOPs) are required to target recalcitrant compounds like tannins and toxic metals. Biological treatment options are generally considered more sustainable, cost-effective, and environmentally friendly, as they rely on microorganisms to break down pollutants. Recently, fungi have garnered attention from the research community for their potential use in the remediation of wastes and wastewaters. A number of filamentous fungi are found naturally in wastewater treatment systems as spores or vegetative cells which metabolize organic substances. Using fungi in tannery effluent treatment provides an eco-friendly, highly efficient biological alternative to conventional chemical methods. Fungi excel at breaking down complex organic pollutants and capturing toxic heavy metals, making them a sustainable solution for leather industry wastewater. The present review is an effort to collate literature with

this back with emphasis on fungi. Advancements and current trends are also included to give a comprehensive view on the topic.

The objectives of this review are: (i) to summarize the characteristics and environmental impact of tannery wastewater; (ii) to review conventional and advanced treatment methods; (iii) to provide a comprehensive overview of fungal-mediated bioremediation of tannery wastewater; (iv) to highlight recent developments in genomics, metagenomics, enzyme immobilization, and microbial consortia; and (v) to identify research gaps and future directions for sustainable tannery wastewater management.

II. REVIEW METHODOLOGY

This narrative review was prepared by synthesizing peer-reviewed literature on tannery wastewater treatment with a focus on fungal-mediated applications. Literature was identified through targeted searches of major scientific databases including Web of Science, Scopus, PubMed Central, Google Scholar, and ScienceDirect using search terms such as "tannery wastewater," "tannin degradation," "fungal bioremediation," "white-rot fungi," "tannase," "laccase," "COD removal," and "heavy metal biosorption." The search covered the period from 2000 to 2026, with emphasis on recent developments from 2020 onwards. Foundational papers necessary to define the field were also included. Studies were included if they addressed tannery wastewater treatment, fungal bioremediation, or related enzymatic processes. The retrieved literature was critically evaluated for scientific quality, relevance, and contribution to the understanding of fungal applications in tannery wastewater treatment.

III. TANNINS: CHEMISTRY, TYPES, AND AS SOURCE OF WATER POLLUTION

Tannins are complex, naturally occurring polyphenolic compounds widely distributed in nature. They are produced by plants (pteridophytes, gymnosperms, and angiosperms) and are accumulated in leaves, fruit, bark, roots, seeds, and wood. Tannins differ from other natural phenols in their ability to precipitate proteins (Lorenz et al., 2014), and they are used in the tanning process to bind to the collagen matrix of animal skin and make the leather more durable and resistant to decay. Tanning is an essential phase in one of civilization's oldest processes, the transformation of skins into leather, and vegetable tannins were probably the earliest used reagents. Tannins are applied in the leather tanning industry to transform the animal skin, making leather more durable and resistant to decay.

Tannins used in the tanning processes are polyphenolic compounds with elevated molecular weight (from 500 to 4000 Da). They are considered "recalcitrant" because their complex structures resist standard biological degradation, causing severe aquatic toxicity, high chemical oxygen demand (COD), and dark discoloration in effluents. Because standard biological treatments (such as activated sludge) are largely ineffective against them, specialized tertiary treatment methods are required to break down or remove tannins from industrial wastewater. Technologies like Fenton's oxidation, ozonation, and heterogeneous photocatalysis break down the complex tannin structures into smaller, biodegradable byproducts. Highly effective filter media, such as activated carbon or specialized polymer resins, trap and remove tannin molecules from aqueous solutions. Processes like reverse osmosis and nanofiltration physically filter out the recalcitrant compounds, producing highly purified, reusable water. Figure 1 describes the various steps of tannery treatment.

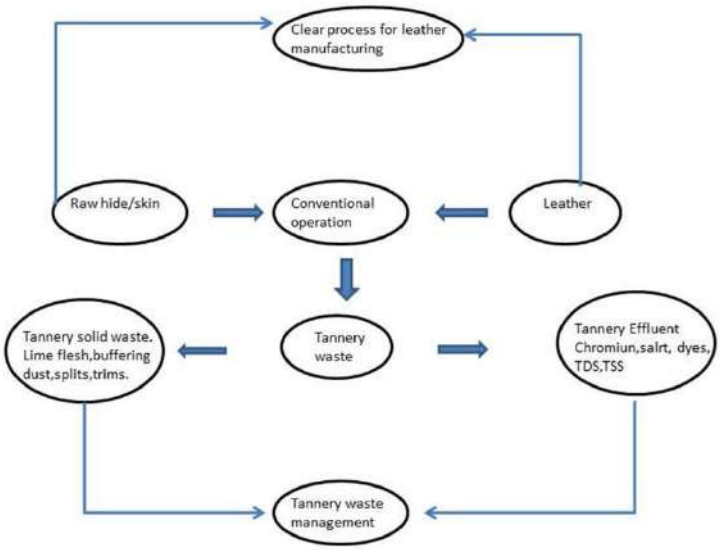


FIGURE 1: Schematic representation of tannery wastewater treatment steps

IV. TANNERY EFFLUENT AS A POTENTIAL POLLUTANT AND ITS TREATMENT

Each year, the global leather industry produces 600 million m^3 of effluent. According to a report on tannery operations in Asia, an estimated 350 million cubic meters of wastewater containing chemicals and other contaminants are produced annually from processing around 10 million tonnes of hides and skins (Rajamani, 2018). This represents a significant environmental challenge, as the effluent can negatively impact local ecosystems and human health (Zaheer et al., 2022; Selvan et al., 2023). Tannery operations require excess water which contains a large quantity of biological oxygen demand (BOD), total dissolved solids (TDS), chemical oxygen demand (COD), sulfides, chlorides, and a variety of other chemicals which contribute 60–70% of the total contaminant load generated by tanneries (Wu et al., 2018). Tannery wastewater is mainly characterized by a variety of organic waste, hazardous substances, and metallic and non-metallic pollutants that arise in tannery wastewater effluent (TWE). These components lead to dark brown coloring and a foul smell (Tamersit & Bouhidel, 2020). Metallic and non-metallic contaminants like T Cr, Cr(III), Cr(VI), Cl, total Kjeldahl nitrogen (TKN), SO_4^{2-}, S^{2-}, NH_4-N, iron, and calcium are present in tannery effluents (Kassim et al., 2022). The number of total solids (TS) increases as a result of either or both organic and inorganic matter in tannery effluent. In addition, high BOD and COD, which decrease dissolved oxygen concentration in the aquatic environment and toxic chemicals, make the effluent extremely acidic (Fouda et al., 2021; Ahmed et al., 2022). Further, the activities involved in tannery operations generate wastewater that can negatively impact the environment and the health of living organisms, including humans and animals. Specifically, the untreated effluent from tanneries can contain high levels of organic matter that consume dissolved oxygen and deprive aquatic life of the oxygen they need to survive. Also, the wastewater may contain nutrients that can promote the growth of aquatic plants and algae, leading to the eutrophication of lakes and streams. Finally, untreated effluent can also harbor pathogenic microorganisms and toxic substances that can cause disease and pose a risk to human health if ingested (Chiampo et al., 2023).

TABLE 1
PHYSICO-CHEMICAL PROPERTIES OF EFFLUENTS: A COMPARATIVE SUMMARY

Parameters	Apaydin et al. 2009	Yadav et al., 2016	Abba et al., 2019	Joël Akea et al., 2022	Bharadwaj et al., 2023
pH	7.4	8.7	8.4	2.69	4
TDS (mg L^{-1})	-	2,219	-	22,566	11,030
Total solids (mg L^{-1})	-	2500	-	28,966	27,300
Suspended solids (mg L^{-1})	2690	281	1600	6,400	912
Volatile Suspended solids (mg L^{-1})	1260	-	-	-	-
COD (mg L^{-1})	3700	449	10670	939.33	8,160
BOD (mg L^{-1})	1470	250	4168	2400	2,200
TKN (mg L^{-1})	-	-	-	-	-
Nitrogen (mg L^{-1})	180	234	85	-	-
Chromium (mg L^{-1})	-	19	-	200	-
Sulfide (mg L^{-1})	440	2,490	560	1,021	-

4.1 Treatment Methods for Tannery Wastewater

The techniques used to manage post-treated effluents in the tannery industry (PTEs) are contingent on the effectiveness of removal, the kind of effluent, operation parameters, and the specifics of the study. Numerous treatment methods currently in use are coagulation/flocculation, advanced oxidation processes (AOPs), biological treatment, membrane separation processes (MSPs), adsorption, and hybrid methods. The treatment of tannery wastewater is categorized into specific, sequential steps to ensure all pollutants are managed effectively.

4.1.1 Pre-treatment (Beamhouse Operations)

This step prevents damage to downstream equipment by removing coarse materials, large solids, and oils. It consists of screening and grit removal: bar screens and settling tanks physically remove hides, fleshings, and heavy debris. Because tanneries discharge waste intermittently, equalization basins mix streams to create a uniform flow with consistent pollutant loads.

4.1.2 Physical-Chemical Treatment

This stage focuses on removing specific chemical pollutants and suspended solids. Sulfides from the unhairing process are oxidized using aeration or chemical catalysts (like manganese sulfate) to prevent toxic hydrogen sulfide gas from forming. Chrome-tanning waste is segregated and precipitated using alkaline chemicals (such as magnesium oxide or lime), forming chromium hydroxide, which can be separated and recycled back into the tanning process.

- **Coagulation/Flocculation:** This is a common method used to treat wastewater due to its ability to effectively remove both organic and inorganic pollutants. Coagulation and flocculation help in the removal of suspended particles in the effluent with a size range from 0.1 to 100 μm (Davis, 2010). The treatment of TWE has already been investigated using different coagulants and flocculants, viz., poly-aluminum chloride (PAC), $Al(OH)_3$, $Fe(OH)_3$, and $FeCl_3$. Biological treatment with chitosan, tannins, and extract from the flowers of *Musa* sp. plants are also reported. Drawbacks of the method include not being suitable as a primary process for treating wastewater due to the harsh quality of the wastewater. The method requires a significant amount of flocculants or coagulants to achieve acceptable decontamination. Another drawback is the formation of large quantities of sludge.
- **Advanced Oxidation Processes (AOPs):** AOPs utilize in situ generated hydroxyl (OH) radicals as the primary oxidant. This approach is environmentally benign and has demonstrated remarkable flexibility in the disintegration of biorefractory contaminants. Several AOP techniques have been shown to have the highest removal efficiency for COD, including zinc oxide-assisted photocatalysis and photo-assisted electrochemical oxidation. The oxidation of substances such as Cr(III) is crucial in establishing effective effluent treatment after tanning using AOPs (Lofrano et al., 2013). High operating cost, time-consumption, and higher energy demands are the drawbacks of this method. Fenton processes can produce a significant amount of sludge.

4.1.3 Biological Treatment Methods

Biological procedures of effluent treatment include the application of microalgae, fungi, activated sludge, bacterial strains, and enzymes. Biological treatment options are generally considered more sustainable, cost-effective, and environmentally friendly, as they rely on microorganisms to break down pollutants. Biological treatment methods, especially activated sludge, play a critical role in environmental protection by removing pollutants from wastewater (Xiao et al., 2015). Recently, some methods have enabled the remediation of wastewater that contains colors or heavy metals (Huang et al., 2015). Due to the complicated aromatic structure of azo dyes and their byproducts, traditional biological treatment approaches are frequently inadequate for degradation (Kertèsz et al., 2014). The azo linkage in the dye molecule might be degraded by the biological treatment processes investigated utilizing aerobic decomposition or fungal treatment, turning them into dinitrogen (N_2) or ammonia (NH_3) or being integrated into biomass (Senthilvelan et al., 2014). A study using fungal treatment at 100 mg/l, pH 5.5, 168 h of treatment and aerobic decomposition indicated that the PTEs had significant removal of COD (above 80%) and color (above 90%) at the effective pH range of 5–7, and the temperature range of 25–35 °C. Chromium (VI) reduction and azo dye removal were accomplished by *Lactobacillus paracase*, an isolate from deep-sea sediment (Huang et al., 2015). Effluent can be a source of organic or inorganic nutrients, such as phosphates and ammoniacal nitrogen, that can be assimilated by various algae species, including *Chlorella*, *Scenedesmus*, *Chlamydomonas*, and *Nanochloropsis* (Saranya & Shanthakumar, 2020). A microalgae consortium containing mainly *Tetraselmis* sp. revealed removals for COD, TOC, and BOD that were consistently below 60%. The main drawbacks of biological treatment methods are time consumption, unstable effluent quality, excessive salinity inhibition, and large-scale sludge formation. Additionally, they can treat a wide range of pollutants and effectively reduce the wastewater's toxicity. However, it is necessary to undertake the primary treatment procedure through the physico-chemical process (screening, equalization, pH adjustment, and chemical treatment). Furthermore, the efficiency of biological treatment processes can be affected by toxic substances or high levels of organic matter.

4.1.4 Membrane Separation Processes

These processes have gained popularity due to their ability to produce high-quality treated water. Membrane separation processes (MSPs) may remove dissolved compounds from tannery wastewater. These methods can be characterized by the pressure difference (reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF), microfiltration (MF)), potential difference, and osmosis pressure (forward osmosis) (Venzke et al., 2018; Li et al., 2021). Based on studies, it has been observed that the maximum level of pollution can be effectively reduced through the use of NF membranes, followed by RO membranes (Nicolini et al., 2016; Licona et al., 2018). UF membranes can eliminate solutes with a molecular weight greater than 1,000 Da. One of the major disadvantages of membrane separation processes is their high energy consumption, which can lead to

high operational costs. Fouling of the membrane is another common issue, resulting in decreased efficiency and increased costs, and they may not be suitable for treating wastewater with high salinity, as salt deposits can damage the membranes and require frequent replacement.

4.1.5 Adsorption

Tannery effluent is challenging to treat due to the presence of phenols in tanning amalgamation, which forms polyphenolic structures that make the effluent refractory (Benvenuti et al., 2017). Adsorption effectively treats wastewater contaminated with heavy metals, aromatic chemicals, and dye by reducing their concentration (Hashem et al., 2022; Shaibur, 2023). However, various other adsorbent materials such as zeolite, green macro-algae, modified kaolin, and commercial activated carbon have also been employed. Results from previous studies indicate that acid dyes (anionic) were successfully removed from chrome-tanned leather shavings by 58–87% using adsorption (Piccin et al., 2016). Commercial activated carbon in powder form produced the highest levels of pollution removal, followed by modified zeolite (Aljerf, 2018) and kaolin with discoloration greater than 90%. Several factors affect adsorption efficiencies, such as pH, dye concentration, temperature, and adsorbent material (Gomes et al., 2016). The high cost of using adsorbents, especially for large-scale operations, is a drawback. Additionally, the adsorption capacity of the adsorbents is influenced by several factors which may require optimization to achieve effective removal of pollutants. Another disadvantage of adsorption processes is the need for frequent replacement of adsorbents and their disposal.

4.1.6 Hybrid Treatment Methods

Studies suggest that combining different treatment methods can achieve a more effective and cost-efficient approach. By carefully considering the pros and cons of each method, a suitable combination of techniques can be chosen that yields multi-effective performance and reduces costs. This is an increasingly popular trend among researchers working on tannery effluent treatment, who have been experimenting with diverse techniques to achieve better results (Grandclément et al., 2017). Combining RO, NF, gravity settling, and coagulation/flocculation among hybrid treatments produced the maximum COD removal. Adsorption was employed after coagulation or flocculation as a pre-treatment in other hybrid procedures (Puchana-Rosero et al., 2018; Mella et al., 2019). In some cases, integrating AOPs with biological treatment can improve their economic viability, as higher oxidant dosages are typically required when used as a standalone final treatment (Ganiyu et al., 2015).

V. FUNGI AND THEIR ROLES IN TANNERY WASTE TREATMENTS

Fungi are abundant worldwide, because they can exist and survive in a wide range of habitats (Madigan et al., 2018). Within their ecosystems, fungi might be symbionts, parasites, or saprophytes of plants, animals, and other fungi (Prescott et al., 2016). Various biochemical activities in the fungi can be exploited for the production of compounds useful in many fields, such as antibiotics, steroids, and cyclosporins. Recently, fungi have garnered attention from the research community for their potential use in the remediation of wastes and wastewaters (Madigan et al., 2018). A number of filamentous fungi are found naturally in wastewater treatment systems as spores or vegetative cells which metabolize organic substances (Subasri et al., 2020). Using fungi in tannery effluent treatment provides an eco-friendly, highly efficient biological alternative to conventional chemical methods (Ameen, 2023). Fungi excel at breaking down complex organic pollutants and capturing toxic heavy metals, making them a sustainable solution for leather industry wastewater. Among the most effective are white-rot fungi (WRF) e.g., *Phanerochaete chrysosporium*, *Trametes versicolor*, and *Pleurotus ostreatus*, which produce key enzymes like lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Deepa et al., 2011). These enzymes break down persistent organic pollutants, including polyaromatic hydrocarbons (PAHs), dyes, and pesticides. Other fungi like *Aspergillus* spp., *Irpex lacteus*, *Penicillium* spp., and *Fusarium* spp. contribute through their bioaccumulation of heavy metals and ability to degrade complex organic compounds (Ghosh et al., 2023).

5.1 Advantages of Fungi in Tannery Effluent Treatment

Fungi have been shown to be capable candidates to remove azo dye compounds during the wastewater treatment process (Rajhans et al., 2021). Fungal biomass can absorb dyes from wastewater regardless of whether the fungus is dry or wet. Further, fungi are a rich source of degrading enzymes (Ugbede et al., 2023) and possess the ability to withstand extremely harsh conditions such as low pH, fluctuating pollution load, and low nutrient concentrations (Yadav et al., 2024). Fungi also have a greater resistance to inhibitory compounds in comparison to bacterial species (Spennati et al., 2021). The hyphal growth of fungi provides greater protection for their sensitive organelles. The cell walls of fungi, a layer of extra-polysaccharide matrix, protect them from inhibitory compounds through adsorption (Gow et al., 2017). The higher number of genes in fungi imparts

greater reproductive selectivity, which might result in better adaptations to the environment (Priest et al., 2020). Fungal enzymes are highly non-specific with regard to their substrate, which gives them the capability to degrade a wide range of highly recalcitrant organopollutants (Ningombam et al., 2025). A number of fungi species, such as *Aspergillus*, *Penicillium*, and a host of others, have been implicated in the removal of carbon and nutrient sources in wastewater (Dalecka et al., 2020). Additionally, some fungi use their fungal hyphae for trapping and adsorbing suspended solids to accomplish their energy and nutrient requirements (Vaksmaa et al., 2023). Unlike chemical precipitation, which produces massive volumes of hazardous chemical sludge, fungal biomass significantly reduces the overall volume of secondary sludge and can often be composted safely after treatment (Civzele et al., 2025). Fungal cell walls contain functional groups that act as an excellent natural sponge for toxic metals like chromium, lead, and cadmium through a process called biosorption (Staszak & Regel-Rosocka, 2024). Species like *Aspergillus niger* have been shown to drastically reduce metal concentration. Although fungi represent a promising biological resource in environmental biotechnology, their application is limited due to a lack of knowledge regarding the optimal process conditions and due to their lack of stability in non-sterile conditions (Palli, 2016; Svobodová & Novotný, 2017).

5.2 Tannin-Degrading Fungal Strains

Despite the antimicrobial properties of tannins, many fungi, bacteria, and yeast are quite resistant to tannins and can use tannins as carbon and energy sources (Cherian & Madhan, 2021). The biodegradation of natural tannins in the environment is mainly associated with fungi rather than bacteria. Fungi express a large number of degradative enzymes including peroxidases, laccases, and tannases (Viswanath et al., 2014). Tannase catalyses the hydrolysis of ester bonds, releasing glucose, gallic acid, and galloyl esters (Tang et al., 2024). Some authors suggest a correlation between tannase activity from fungi in phenol-rich wastewater and the reduction of COD, Total Organic Carbon (TOC), and phenol content (Salgado et al., 2016). In particular, *Aspergillus* spp. and *Penicillium* spp. have been isolated from tannery wastewaters and were reportedly able to biodegrade tannins (Ben et al., 2021).

The following paragraphs summarize various studies spanning source of substrate, carbon source, process parameters, and fungal strains in tannin degradation. Spennati et al. (2020) report four novel rotating submerged packed bed reactors with *Aspergillus tubingensis* MUT 990a immobilized in polyurethane foam cubes carriers and inserted inside a submerged rotating cage reactor for biodegradation of tannins in non-sterile conditions. The results implicate that after two months, 80% of COD and 90% dissolved organic carbon removal was achieved. Further, important tannase activity was also detected. Quebracho tannin and Tara tannin are intensively used by the tanning industry and are two of the most recalcitrant compounds that can be found in tannery wastewaters. Spennati et al. (2019) used two reactors inoculated with *Aspergillus tubingensis* MUT 990. Soluble chemical oxygen demand removals up to 53% and 90% and maximum elimination capacities of 9.1 g sCOD m^{-3} h^{-1} and 37.9 g sCOD m^{-3} h^{-1} of Quebracho and Tara tannins were achieved.

Studies have reported the roles of culture and process parameters as variables influencing fungal degradation. The study by Buenrostro-Figueroa et al. (2021) was aimed at obtaining ellagitannase enzymatic extracts from *Aspergillus niger* GH1 in solid-state fermentation, using dextrose and ellagitannins as inducers. The influence of culture conditions on ellagitannase expression and fungal ellagitannin degradation was studied. Three groups of proteins were identified, viz., β-glucosidase, phospholipase C, and triacylglycerol lipase. However, only phospholipase C was overexpressed with ellagitannins as inducers, showing the most spontaneous reaction with punicalagin (ΔG -8.56). These results suggest that phospholipase could be involved in ellagitannin biosynthesis. Prendecka et al. (2016) studied the dynamic changes in tannase activity depending on the carbon source in the culture medium using fungi *Phellinus pini*, *Fomes fomentarius*, and *Tyromyces pubescens*, respectively. The inducible potential of TA and rapeseed meal on the activity of tannin acyl hydrolase was confirmed during 14 days of culturing. The highest concentrations of GA were observed after stimulation by the TA in the cultures of *F. fomentarius* and *T. pubescens*.

Bhoite et al. (2013) report gallic acid production optimization using response surface methodology (RSM). Process parameters such as pH, moisture, and fermentation period were considered for optimization. Among the various fungi isolated from coffee by-products, *Penicillium verrucosum* produced 35.23 µg/g of gallic acid using a carbon source in solid-state fermentation. The optimum values of the parameters obtained were pH 3.32, moisture 58.40%, and fermentation period of 96 hr. Further, gallic acid production with an increase of 4.6-fold was achieved. Several researchers report the application of fungi in degradation of vegetable and fruit-based tannin substrates. Hydrolysis of pomegranate ellagitannins by *Aspergillus niger* GH1 by solid-state culture using polyurethane foam as support and pomegranate ellagitannins as substrate is reported by Ascacio-Valdés et al. (2016). The results show that ellagitannin reached the maximal biodegradation value at 18 h. The activity of ellagitannase

was 390.15 U/L. Finally, HPLC/MS analysis identified gallagic acid as an intermediate molecule and immediate precursor of ellagic acid. De León-Medina et al. (2023) report rambutan peel as a source of ellagitannins through SSF. Ellagitannase was the main enzyme responsible for biodegradation of ellagitannins using ultrasound/microwave hybrid technology. Fungi have demonstrated degradation of synthetic sources of tannin. Bardi et al. (2019) studied the efficiency of the white-rot fungus, *Bjerkandera adusta* MUT 2295 in the treatment of a synthetic solution prepared with tannic acid. At the end of the process, an increase of fungal dry weight and the presence of ergosterol were detected. COD and TOC removal of 81% and 73% was achieved which correlated with the detection of tannase and Lignin Peroxidase (LiP) activities. Based on these studies, it can be summarized that fungi are the best fit microorganism for treatment of the recalcitrant fraction of wastewater containing a high concentration of tannins, such as tannery wastewater.

VI. RECENT DEVELOPMENTS

Several genetic and genomic studies have been carried out in this realm of bioremediation to study gene expression, identify key genes in pathways, and conduct metagenomics surveys. *Coriolopsis gallica* is a ligninolytic basidiomycete that produces high levels of this extracellular enzyme. The role of tannic acid was shown to be an inductor of laccase activity in this fungus, due to the enhancement of expression of the laccase gene at the transcriptional level (Carbajo et al., 2002). *Aureobasidium melanogenum* T9 isolated from red wine starter showing ability for tannin degradation (Zhang et al., 2019). Highest biomass was achieved when the initial tannic acid concentration was 20 g/L. Genes *tan A*, *tan B*, and *tan C* encoding three different tannases, respectively, were identified. Among these genes, *tan A* and *tan B* can be induced by tannin acid simultaneously at both gene transcription and protein expression levels. Deletion assays showed that the deletion of *tanA* and *tanB* resulted in tannase activity decline (51.3 ± 4.1 and 64.1 ± 1.9 U/mL), respectively.

A study analyzed the tannin biodegradation efficiency and the transcriptomic responses of *A. tubingensis* in liquid and solid cultures with tannin added. A total of 54.6% of the 11,248 differentially expressed genes were upregulated in liquid cultures, including AtWU_03490 (encoding ABC multidrug transporter), AtWU_03807 (ribonuclease III), AtWU_10270 (peptidyl-tRNA hydrolase), and AtWU_00075 (arabinogalactan endo-1,4-beta-galactosidase). Functional and gene ontology enrichment analyses indicated upregulation in processes including oxidation reduction, drug metabolism, and monocarboxylic acid metabolism. Overall, this study provides insight into the transcriptomic response to tannin biodegradation by *A. tubingensis* in different cultures and reveals that liquid cultures induce greater transcriptomic variability compared to solid cultures.

The metagenomics technique is a potent tool for examining the biodiversity of soil microbial communities. Sinduja et al. (2025) report ten most abundant dominant bacteria in metal-contaminated soil samples, viz., Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Cyanobacteria, Acidobacteria, and Archaea. Meanwhile, Nitrososphaerales exhibited the highest abundance in the control samples accounting for 48% of microbial diversity. This study provides an in-depth analysis of microbial composition and metagenomics for bioremediation of metal-contaminated soil. In another study, Quintana et al. (2026) metagenomics revealed microbiome profiles dominated by Pseudomonadota (77.2%), primarily *Thiocapsa* (27.8%) and *Francisella* (23.0%). The phototrophic and sulfur-oxidizing metabolism characteristic of *Thiocapsa* may explain the distinctive coloration of the effluent, while the predominance of *Francisella* is consistent with tolerance to hostile environmental conditions. DNA sequences revealed species including *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Klebsiella pneumoniae*. These findings demonstrate that treated tannery effluent can retain clinically relevant genetic material and ARGs, underscoring the need to integrate metagenomic surveillance into environmental monitoring frameworks to better understand and mitigate emerging resistance determinants in aquatic systems.

An alternative source of tannase from human oral mucosa is reported. Tomás-Cortázar identified and expressed in *E. coli* a tannase from the oral microbiota member *Fusobacterium nucleatum* subs. *polymorphum* (TanBFnp). Sequence analyses revealed close relation to bacterial tannases. The enzyme exhibits biochemical properties that make it an interesting target for industrial use. The enzyme properties were 100% activity at different temperatures (incubations up to 45 °C) and activity against esters of gallic and protocatechuic acid, including tannic acid, gallocatechin gallate, and epigallocatechin gallate.

Condensed tannins (proanthocyanidins) are naturally occurring polyphenols formed by the polymerization of flavonoid units, such as catechin and epicatechin. They also possess antioxidant and astringent properties. Lin et al. (2016) studied polyphenol extraction recovery and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of the extracted polyphenol after litchi pericarp was treated with *Aspergillus awamori*, *Aspergillus sojae*, or *Aspergillus oryzae*. The treatment of *A. awamori* produced the highest antioxidant activity of polyphenol from litchi pericarp. Further studies suggested that the treatment of *A. awamori* releases the non-extractable condensed tannin from cell walls of litchi pericarp. The total extractable tannin in the

litchi pericarp residue after a six-time extraction with 60% ethanol increased from 199.92 ± 14.47 to 318.38 ± 7.59 μg/g dry weight (DW). The ESI-TOF-MS and HPLC-MS^2 analyses further revealed degradation of B-type condensed tannin (condensed flavan-3-ol via C4-C8 linkage).

An engineered ecosystem, based on fungal tannin biodegradation, has been successfully tested in a laboratory-scale bioreactor under non-sterile conditions. Several steps are critical in scaling-up a novel fungal-based technology for tannins biodegradation. A mathematical model was developed by Spennati et al. (2020) and applied to describe both the respirometric profiles and the experimental data collected from the laboratory-scale tests performed in the bioreactor. The microbial growth was described through a Monod-type kinetic equation as a first approach. Substrate inhibition, decay rate, and tannin hydrolysis process were included to better describe the behaviour of immobilised biomass selected in the tannin-degrading bioreactor. The model was implemented in AQUASIM using the specific tool Biofilm Compartment to simulate the attached fungal biofilm.

Maintaining optimal conditions for fungal growth in fluctuating industrial effluents is challenging. AI can bridge this gap through several core applications. Machine Learning (ML) algorithms (like Artificial Neural Networks and Random Forest models) accurately predict pollutant degradation rates and forecast effluent quality in real-time. To this end, Manoj et al. (2025) report a machine learning-assisted prediction of heavy metal adsorption from tannery effluent wastewater using *Delonix regia* with random forest, isotherms, and kinetics modelling.

The outburst of green biotechnology has facilitated a substantial upsurge in the usage of enzymes in a plethora of industrial bioconversion processes. The tremendous biocatalytic potential of industrial enzymes provides an upper edge over chemical technologies in terms of safety, reusability, and better process control. Tannase is one such enzyme loaded with huge potential for bioconversion of hydrolysable tannins to gallic acid (Dhiman et al., 2018). Furthermore, toxic tannery effluents from various tanneries are loaded with significant levels of tannins in the form of tannic acid. Tannase can be principally employed for debasing the tannins that predominately occur in the toxic tannery effluents, thus providing a relatively much cheaper measure for their biodegradation. Over the years, microbial tannase-catalyzed tannin degradation has gained momentum. The plentious availability of tannin-containing agro- and industrial waste paves a way for efficient utilization of microbial tannase for tannin degradation eventually resulting in gallic acid production.

Saad et al. (2024) report bioremoval of tannins and heavy metals using immobilized tannase and biomass of *Aspergillus glaucus*. The results demonstrate that tannase showed high stability with 50% of its activity at 60 °C and pH range 5.0-6.0. Immobilization of tannase on Na-alginate (3%) and covalent binding to chitosan and effects of concentrations on the bead formation and enzyme immobilization revealed that maximum immobilization efficiency (75%). A potential reusability of the immobilized enzyme was shown through keeping 70% of its relative activity up to the fourth cycle. Bioconversion efficiency of tannic acid to gallic acid by immobilized tannase was at 40 °C with tannic acid concentration up to 50 g/L. Bioremediation of heavy metal (Cr^{3+}, Pb^{2+}, Cu^{2+}, Fe^{3+}, and Mn^{2+}) was achieved with uptake percentages of (37.20, 60.30, 55.27, 79.03, and 21.13), respectively. The biomass was successfully used repeatedly for removing Cr^{3+} after using a desorbing agent (0.1 N HCl) for three cycles.

In another study by Prigione et al. (2018), using four different tannins (the hydrolysable chestnut ellagitannin and tara gallotannin and the condensed quebracho and wattle tannins) with an aim of selecting fungal strains capable of growing in the presence of high tannin concentration and thus their potential usefulness in biotransformations of tannery effluent was studied. A total of 125 isolates of filamentous fungi belonging to 10 species and four genera (*Aspergillus*, *Paecilomyces*, *Penicillium*, and *Talaromyces*) were isolated from the tannin industrial preparations. Miniaturized biotransformation tests with 10 fungal strains and the HPLC analysis pointed out a strong activity of all the tested fungi on both chestnut and tara tannins. Two strains (*Aspergillus tubingensis* MUT 990 and *Paecilomyces variotii* MUT 1125), tested against real tannery wastewater, were particularly efficient in COD and tannin removal (>60%), with a detoxification above 74%.

Using bacterial and fungal consortia is a highly effective, eco-friendly approach to bioremediating toxic tannery effluents. These mixed-microbe systems degrade complex organic matter, reduce high heavy metal concentrations (like toxic Chromium), and decolorize the wastewater, performing far better than single-strain treatments (Nagabalaji et al., 2023). Microbial communities are more robust, maintaining their metabolic efficiency under varying pH levels, high salinity, and fluctuating temperatures common in tanning wastewaters (Sharma & Malaviya, 2016). Ameen et al. (2023) report a consortium of microorganisms (five single bacterial and five fungal species from different genera) is more efficient than the single microorganisms in the consortium. The results indicate *Corynebacterium glutamicum* and the fungus *Acremonium* sp. were the most efficient in the single-microbe treatment. In the consortium treatment, the consortium of these two was the most efficient

at treating the effluent. The TDS was reduced to 80 mg/L and BOD to 20 mg/L and the total heavy-metal concentration (Cd, Cr, Cu, Mn, and Pb) was reduced to 0.2 μg/L by the consortium.

VII. DISCUSSION

Tannins are polyphenolic compounds produced by plants that have been used for centuries in the vegetable tanning of leather (Morrison Boyd & Bhattacharjee, 2010). Tannins represent one of the least biodegradable substances in tannery wastewaters, with high recalcitrant soluble COD. A high concentration of tannins can additionally inhibit biological treatment (Maisuria et al., 2022). Moreover, tannins represent a complex class of compounds, and several studies are currently underway to improve our understanding of their physical and chemical properties, their effect on biological activity, their role in microbial ecosystems, and finally, their potential applications in other fields (production of glues, pharmaceuticals, surfactants, etc.).

Tannins in tannery wastewater are treated inefficiently by the activated sludge process and at high cost by physico-chemical treatment. It is worth mentioning that although these treatment methods have been proven effective, they require further development and improvement to meet more stringent regulatory standards (Ramisha et al., 2026). The use of additional techniques such as enzymatic treatment, fungal processes, isolated bacteria, conventional biological processes, and microalgae should be explored to enhance the quality of the effluent further (Venugopal & Khambhaty, 2024). The application of the membrane separation process has been identified as an appropriate technique for reusing treated wastewater, making it a viable option for sustainable tanning processes (Monnot et al., 2020). Some tanneries have implemented closed-loop systems that minimize water usage and treat wastewater onsite for reuse in the tanning process. Although using treated leather tanning effluent as a raw material is not yet a common practice, there are opportunities for innovation and advanced solutions in the leather industry to reduce waste and promote sustainability.

The advantages of fungal treatment over conventional methods are significant. Fungi offer a sustainable, cost-effective alternative to chemical treatments, with the ability to degrade a wide range of recalcitrant compounds and bioaccumulate heavy metals. The non-specific nature of fungal enzymes allows them to break down diverse pollutants, and the fungal biomass itself can be composted safely after treatment, reducing secondary waste. However, challenges remain, including the need for optimized process conditions, stability in non-sterile conditions, and scalability for industrial applications.

TABLE 2
COMPARATIVE ANALYSIS OF TREATMENT METHODS:

Method	Advantages	Disadvantages	Efficiency
Coagulation/Flocculation	Simple, effective for suspended solids	High sludge production, chemical costs	Moderate
AOPs	High degradation of recalcitrant compounds	High energy costs, chemical consumption	High
Biological (Activated Sludge)	Cost-effective, sustainable	Ineffective for recalcitrant compounds, slow	Moderate
Membrane Filtration	High-quality effluent, reusable water	High energy costs, membrane fouling	High
Adsorption	Effective for heavy metals and dyes	High adsorbent cost, disposal issues	Moderate-High
Fungal Treatment	Degrades recalcitrant compounds, biosorbs metals	Slow, requires optimization, non-sterile instability	High

Several avenues for future applications include full-scale use of a segregated flux of vegetable tannin bath with an optimized process based on fungal bioreactors (Bardi et al., 2019). Treatment in a side-stream reactor could reduce the overall cost of treatment, since the tertiary treatment applied for removing soluble recalcitrant COD from the tannery wastewater could be minimised. Also, in-depth investigation is needed into the different degradation pathways of tannins and the chemical compositions and structures of tannins. This research could be carried out, for example, using specially-developed methods and procedures such as LC-MS and MALDI-TOF (Mott et al., 2010). The population monitoring involved in each degradation step, coupled with the enzymatic activity and mechanisms of action, could also improve the production of those enzymes at industrial scale, given that tannase is one of the most expensive enzymes to produce. Additional investigation related to the

different inhibitory mechanisms of QT and TT (and tannins in general) is required. This would require an investigation of the different mechanisms of action of bacteria versus fungi, with an additional consideration of the possible interference of quorum sensing (Zeng et al., 2023). A third potential avenue of investigation would be the study of biofilm dynamics for modelling (Ananta et al., 2025). The diffusion of tannins and oxygen inside the fungal biofilm and the associated biological reactions could be described with microsensors.

VIII. CONCLUSION AND FUTURE PERSPECTIVES

This review has comprehensively examined the current state of tannery wastewater treatment, with a particular focus on fungal-mediated bioremediation. Tannery effluents represent a significant environmental challenge due to their high toxicity, salinity, and recalcitrant organic content. While conventional physical-chemical and biological treatment methods have been employed, they often fall short of achieving complete remediation. Fungi, particularly white-rot fungi and tannin-degrading species, offer a promising, sustainable, and cost-effective alternative for the treatment of tannery wastewater. Their ability to produce non-specific extracellular enzymes (LiP, MnP, laccase, tannase) enables the degradation of a wide range of recalcitrant compounds, while their cell walls facilitate the biosorption of heavy metals.

Recent developments in genomics, metagenomics, enzyme immobilization, and microbial consortia have further enhanced the potential of fungal bioremediation. The integration of AI and machine learning for process optimization, coupled with the development of hybrid treatment systems combining fungal treatment with other methods, represents a promising direction for future research. However, challenges remain, including the need for optimized process conditions, stability in non-sterile environments, and scalability for industrial applications.

Key Recommendations:

1) **Process Optimization**: Further research is needed to optimize culture conditions (pH, temperature, nutrient availability) for fungal growth and enzyme production in non-sterile tannery wastewater.
2) **Strain Improvement**: Genetic engineering and strain improvement techniques should be employed to enhance the efficiency and stability of fungal strains under industrial conditions.
3) **Enzyme Immobilization**: Development of cost-effective immobilization techniques for fungal enzymes to improve reusability and stability.
4) **Consortia Development**: Optimization of fungal-bacterial consortia for enhanced degradation of complex tannery effluents.
5) **Scale-up Studies**: Pilot-scale and full-scale studies are needed to demonstrate the feasibility and cost-effectiveness of fungal treatment at industrial scale.
6) **Integration with Other Methods**: Hybrid treatment systems combining fungal treatment with membrane filtration, AOPs, or adsorption should be explored for complete remediation.
7) **Genomic and Metagenomic Studies**: Further genomic and metagenomic studies are needed to understand the mechanisms of tannin degradation and heavy metal resistance in fungi.
8) **Regulatory Frameworks**: Development of regulatory frameworks and guidelines for the safe and effective use of fungal bioremediation in tannery wastewater treatment.

Limitations of the Review: This review is based on a narrative synthesis of published literature rather than a formal systematic review. The examples are selected to illustrate the diversity of approaches and should not be considered exhaustive. The focus is primarily on fungal bioremediation, with less attention to other biological treatment methods. The review prioritizes recent developments from 2020 onwards, but may not capture all relevant studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Zaheer, M., Zafar, Z. U., Athar, H. u. R., Bano, H., Amir, M., Khalid, A., Manzoor, H., Javed, M., Iqbal, M., Ogbaga, C. C., & Qureshi, M. K. (2022). Mixing tannery effluent had fertilizing effect on growth, nutrient accumulation, and photosynthetic capacity of some

cucurbitaceous vegetables: A little help from foe. *Environmental Science and Pollution Research*, *30*, 28947-28960. https://doi.org/10.1007/S11356-022-24247-3

[2] Selvan, S. T., Chandrasekaran, R., Muthusamy, S., & Ramamurthy, D. (2023). Eco-friendly approach for tannery effluent treatment and CO2 sequestration using unicellular green oleaginous microalga *Tetradesmus obliquus* TS03. *Environmental Science and Pollution Research*, *1*, 1-19. https://doi.org/10.1007/S11356-023-25703-4

[3] Wu, J., Gao, Y., Zhang, J., Wang, Y., & Chen, W. (2018). Chrome complexes in rewetting and neutralizing effluents and hints for recycling posttanning wet-process effluent. *Polish Journal of Environmental Studies*, *27*, 1315-1321.
https://doi.org/10.15244/pjoes/77032

[4] Tamersit, S., & Bouhidel, K. E. (2020). Treatment of tannery unhairing wastewater using carbon dioxide and zinc cations for greenhouse gas capture, pollution removal and water recycling. *Journal of Water Process Engineering*, *34*, 101120.
https://doi.org/10.1016/J.JWPE.2019.101120

[5] Kassim, N. A., Ghazali, S. A. I. S. M., Bohari, F. L., & Abidin, N. A. Z. (2022). Assessment of heavy metals in wastewater plant effluent and lake water by using atomic absorption spectrophotometry. *Materials Today: Proceedings*.
https://doi.org/10.1016/j.matpr.2022.04.671

[6] Fouda, A., Hassan, S. E. D., Saied, E., & Azab, M. S. (2021). An eco-friendly approach to textile and tannery wastewater treatment using maghemite nanoparticles (γ-Fe2O3-NPs) fabricated by *Penicillium expansum* strain (K-w). *Journal of Environmental Chemical Engineering*, *9*, 104693. https://doi.org/10.1016/J.JECE.2020.104693

[7] Ahmed, S., Fatema-Tuj-Zohra, Mahdi, M. M., Nurnabi, M., Alam, M. Z., & Choudhury, T. R. (2022). Health risk assessment for heavy metal accumulation in leafy vegetables grown on tannery effluent contaminated soil. *Toxicology Reports*, *9*, 346-355.
https://doi.org/10.1016/j.toxrep.2022.03.009

[8] Chiampo, F., Shanthakumar, S., Ricky, R., & Pattukandan Ganapathy, G. (2023). Tannery: Environmental impacts and sustainable technologies. *Materials Today: Proceedings*. https://doi.org/10.1016/J.MATPR.2023.02.025

[9] Xiao, Y., De Araujo, C., Sze, C. C., & Stuckey, D. C. (2015). Toxicity measurement in biological wastewater treatment processes: A review. *Journal of Hazardous Materials*, *286*, 15-29. https://doi.org/10.1016/J.JHAZMAT.2014.12.033

[10] Huang, G., Wang, W., & Liu, G. (2015). Simultaneous chromate reduction and azo dye decolourization by *Lactobacillus paracase* CL1107 isolated from deep sea sediment. *Journal of Environmental Management*, *157*, 297-302.
https://doi.org/10.1016/J.JENVMAN.2015.04.031

[11] Kertèsz, S., Cakl, J., & Jiránková, H. (2014). Submerged hollow fiber microfiltration as a part of hybrid photocatalytic process for dye wastewater treatment. *Desalination*, *343*, 106-112. https://doi.org/10.1016/J.D

[12] Senthilvelan, T., Kanagaraj, J., & Panda, R. C. (2014). Enzyme-mediated bacterial biodegradation of an azo dye (C.I. acid blue 113): Reuse of treated dye wastewater in post-tanning operations. *Applied Biochemistry and Biotechnology*, *174*, 2131-2152. https://doi.org/10.1007/s12010-014-1158-x

[13] Saranya, D., & Shanthakumar, S. (2020). An integrated approach for tannery effluent treatment with ozonation and phycoremediation: A feasibility study. *Environmental Research*, *183*, 109163. https://doi.org/10.1016/J.ENVRES.2020.109163

[14] Venzke, C. D., Giacobbo, A., Klauck, C. R., Viegas, C., Hansen, E., De Aquim, P. M., Rodrigues, M. A. S., & Bernardes, A. M. (2018). Integrated membrane processes (EDR-RO) for water reuse in the petrochemical industry. *Journal of Membrane Science and Research*, *4*, 218-226. https://doi.org/10.22079/JMSR.2018.82055.1180

[15] Li, X., Shen, S., Xu, Y., Guo, T., Dai, H., & Lu, X. (2021). Application of membrane separation processes in phosphorus recovery: A review. *Science of the Total Environment*, *767*, 144346. https://doi.org/10.1016/J.SCITOTENV.2020.144346

[16] Nicolini, J. V., Borges, C. P., & Ferraz, H. C. (2016). Selective rejection of ions and correlation with surface properties of nanofiltration membranes. *Separation and Purification Technology*, *171*, 238-247. https://doi.org/10.1016/J.SEPPUR.2016.07.042

[17] Licona, K. P. M., Geaquinto, L. R. d. O., Nicolini, J. V., Figueiredo, N. G., Chiapetta, S. C., Habert, A. C., & Yokoyama, L. (2018). Assessing potential of nanofiltration and reverse osmosis for removal of toxic pharmaceuticals from water. *Journal of Water Process Engineering*, *25*, 195-204. https://doi.org/10.1016/J.JWPE.2018.08.002

[18] Benvenuti, J., Capeletti, L. B., Gutterres, M., & dos Santos, J. H. Z. (2017). Hybrid sol–gel silica adsorbent materials synthesized by molecular imprinting for tannin removal. *Journal of Sol-Gel Science and Technology*, *85*, 446-457. https://doi.org/10.1007/S10971-017-4564-Z

[19] Hashem, M. A., Payel, S., Mim, S., Hasan, M. A., Nur-A-Tomal, M. S., Rahman, M. A., & Sarker, M. I. (2022). Chromium adsorption on surface activated biochar made from tannery liming sludge: A waste-to-wealth approach. *Water Science and Engineering*, *15*, 328-336. https://doi.org/10.1016/J.WSE.2022.09.001

[20] Shaibur, M. R. (2023). Heavy metals in chrome-tanned shaving of the tannery industry are a potential hazard to the environment of Bangladesh. *Case Studies in Chemical and Environmental Engineering*, *7*, 100281. https://doi.org/10.1016/J.CSCEE.2022.100281

[21] Piccin, J. S., Gomes, C. S., Mella, B., & Gutterres, M. (2016). Color removal from real leather dyeing effluent using tannery waste as an adsorbent. *Journal of Environmental Chemical Engineering*, *4*, 1061-1067. https://doi.org/10.1016/J.JECE.2016.01.010

[22] Aljerf, L. (2018). High-efficiency extraction of bromocresol purple dye and heavy metals as chromium from industrial effluent by adsorption onto a modified surface of zeolite: Kinetics and equilibrium study. *Journal of Environmental Management*, *225*, 120-132. https://doi.org/10.1016/J.JENVMAN.2018.07.048

[23] Gomes, C. S., Piccin, J. S., & Gutterres, M. (2016). Optimizing adsorption parameters in tannery-dye-containing effluent treatment with leather shaving waste. *Process Safety and Environmental Protection*, *99*, 98-106. https://doi.org/10.1016/J.PSEP.2015.10.013

[24] Grandclément, C., Seyssiecq, I., Piram, A., Wong-Wah-Chung, P., Vanot, G., Tiliacos, N., Roche, N., & Doumenq, P. (2017). From the conventional biological wastewater treatment to hybrid processes, the evaluation of organic micropollutant removal: A review. *Water Research*, *111*, 297-317. https://doi.org/10.1016/J.WATRES.2017.01.005

[25] Puchana-Rosero, M. J., Lima, E. C., Mella, B., Costa, D. d., Poll, E., Gutterres, M., Puchana-Rosero, M. J., Lima, E. C., Mella, B., Costa, D. d., Poll, E., & Gutterres, M. (2018). A coagulation-flocculation process combined with adsorption using activated carbon obtained from sludge for dye removal from tannery wastewater. *Journal of the Chilean Chemical Society*, *63*, 3867-3874. https://doi.org/10.4067/S0717-97072018000103867

[26] Mella, B., Benvenuti, J., Oliveira, R. F., & Gutterres, M. (2019). Preparation and characterization of activated carbon produced from tannery solid waste applied for tannery wastewater treatment. *Environmental Science and Pollution Research*, *26*, 6811-6817. https://doi.org/10.1007/S11356-019-04161-X

[27] Ganiyu, S. O., van Hullebusch, E. D., Cretin, M., Esposito, G., & Oturan, M. A. (2015). Coupling of membrane filtration and advanced oxidation processes for removal of pharmaceutical residues: A critical review. *Separation and Purification Technology*, *156*, 891-914. https://doi.org/10.1016/j.seppur.2015.09.059

[28] Madigan, M., Bender, K., Buckley, D., Sattley, W., & Stahl, D. (2018). *Brock biology of microorganisms* (Global ed.). Pearson.

[29] Willey, J., Sherwood, L., & Woolverton, C. J. (2016). *Prescott's microbiology* (10th ed.). McGraw Hill Education.

[30] Subasri, M., Gomathi, V., Kavitha, J., & Mary, P. (2020). Fungi: An approach to treat wastewater. *International Journal of Chemical Studies*, *SP-8*(6), 163-166.

[31] Ameen, F. (2023). Improving tannery wastewater treatments using an additional microbial treatment with a bacterial–fungal consortium. *Biology*, *12*(12), 1507. https://doi.org/10.3390/biology12121507

[32] Deepa, S., Valivittan, K., Indira Vincent, I., & Tharadevi, C. S. (2011). Bioremediation of tannery effluent by selected fungal species. *Biosciences, Biotechnology Research Asia*, *8*(1), 143-150.

[33] Ghosh, S., Rusyn, I., Dmytruk, O. V., Dmytruk, K. V., Onyeaka, H., Gryzenhout, M., & Gafforov, Y. (2023). Filamentous fungi for sustainable remediation of pharmaceutical compounds, heavy metal and oil hydrocarbons. *Frontiers in Bioengineering and Biotechnology*, *11*, 1106973. https://doi.org/10.3389/fbioe.2023.1106973

[34] Rajhans, G., Barik, A., Sen, S. K., & Raut, S. (2021). Degradation of dyes by fungi: An insight into mycoremediation. *BioTechnologia*, *102*(4), 445-455. https://doi.org/10.5114/bta.2021.111109

[35] Ugbede, A. S., Abioye, O. P., Aransiola, S. A., Oyewole, O. A., Maddela, N. R., & Prasad, R. (2023). Production, optimization and partial purification of bacterial and fungal proteases for animal skin dehairing: A sustainable development in leather-making process. *Bioresource Technology Reports*, *24*, 101632. https://doi.org/10.1016/j.biteb.2023.101632

[36] Yadav, P., Mishra, V., Kumar, T., Singh, U. K., Vamanu, E., & Singh, M. P. (2024). Cleansing tannery effluent with *Pleurotus opuntiae*: A green solution for environmental restoration and toxicity evaluation. *Water*, *16*(9), 1313. https://doi.org/10.3390/w16091313

[37] Spennati, F., La China, S., Siracusa, G., Di Gregorio, S., Bardi, A., Tigini, V., Mori, G., Gabriel, D., & Munz, G. (2021). Tannery wastewater recalcitrant compounds foster the selection of fungi in non-sterile conditions: A pilot scale long-term test. *International Journal of Environmental Research and Public Health*, *18*(12), 6348. https://doi.org/10.3390/ijerph18126348

[38] Gow, N. A. R., Latge, J. P., & Munro, C. A. (2017). The fungal cell wall: Structure, biosynthesis, and function. *Microbiology Spectrum*, *5*(3). https://doi.org/10.1128/microbiolspec.FUNK-0035-2016

[39] Priest, S. J., Yadav, V., & Heitman, J. (2020). Advances in understanding the evolution of fungal genome architecture. *F1000Research*, *9*, 776. https://doi.org/10.12688/f1000research.25424.1

[40] Ningombam, L., Mana, T., Pradhan, S., et al. (2025). Fungal bioremediation in environmental pollution and recent strategies. *Discover Environment*, *3*, 100. https://doi.org/10.1007/s44274-025-00267-x

[41] Dalecka, B., Strods, M., Juhna, T., & Rajarao, G. K. (2020). Removal of total phosphorus, ammonia nitrogen and organic carbon from non-sterile municipal wastewater with *Trametes versicolor* and *Aspergillus luchuensis*. *Microbiological Research*, *241*, 126586. https://doi.org/10.1016/j.micres.2020.126586

[42] Annika, V., Cruz, G., Ghosh, S., Zeghal, P., Hernando-Morales, E., & Niemann, H. V. (2023). Role of fungi in bioremediation of emerging pollutants. *Frontiers in Marine Science*, *10*, 1070905. https://doi.org/10.3389/fmars.2023.1070905

[43] Civzele, A., Stipniece-Jekimova, A. A., & Mezule, L. (2025). Biodegradation of screenings from sewage treatment by white rot fungi. *Fungal Biology and Biotechnology*, *12*(1), 7. https://doi.org/10.1186/s40694-025-00198-5

[44] Staszak, K., & Regel-Rosocka, M. (2024). Removing heavy metals: Cutting-edge strategies and advancements in biosorption technology. *Materials*, *17*(5), 1155. https://doi.org/10.3390/ma17051155

[45] Cherian, S., & Madhan, R. (2021). Bacterial degradation of bacteriostatic polyphenols, tannins. *Journal of Natural Products and Resources*, *7*, 272-275. https://doi.org/10.30799/jnpr.100.21070201

[46] Viswanath, B., Rajesh, B., Janardhan, A., Kumar, A. P., & Narasimha, G. (2014). Fungal laccases and their applications in bioremediation. *Enzyme Research*, *2014*, 163242. https://doi.org/10.1155/2014/163242

[47] Tang, Z., Shi, L., Liang, S., Yin, J., Dong, W., Zou, C., & Xu, Y. (2024). Recent advances of tannase: Production, characterization, purification, and application in the tea industry. *Foods*, *14*(1), 79. https://doi.org/10.3390/foods14010079

[48] Salgado, J. M., Abrunhosa, L., Venâncio, A., Domínguez, J. M., & Belo, I. (2016). Combined bioremediation and enzyme production by *Aspergillus* sp. in olive mill and winery wastewaters. *International Biodeterioration & Biodegradation*, *110*, 16-23. https://doi.org/10.1016/j.ibiod.2015.12.011

[49] Ashley, B. E. G., Pushpalaksmi, E., Jenson Samraj, J., & Annadurai, G. (2021). Biodegradation of tannery effluent dyes by using *Aspergillus niger* isolated from the tannery effluent and reuse of biotreated water in agricultural field. *Journal of Biodiversity and Environmental Sciences*, *18*(1), 74-80.

[50] De León-Medina, J., Buenrostro-Figueroa, J., Sepúlveda, L., Cristóbal, N., Aguilar, J. A., & Ascacio-Valdés, A. (2023). Fungal biodegradation of ellagitannins extracted from rambutan peel. *Food and Bioproducts Processing*, *141*, 81-90. https://doi.org/10.1016/j.fbp.2023.07.002

[51] Spennati, F., Mora, M., Tigini, V., La China, S., Di Gregorio, S., Gabriel, D., & Munz, G. (2019). Removal of Quebracho and Tara tannins in fungal bioreactors: Performance and biofilm stability analysis. *Journal of Environmental Management*, *231*, 137-145. https://doi.org/10.1016/j.jenvman.2018.10.001

[52] Carbajo, J. M., Junca, H., Terrón, M. C., González, T., Yagüe, S., Zapico, E., & González, A. E. (2002). Tannic acid induces transcription of laccase gene cglcc1 in the white-rot fungus *Coriolopsis gallica*. *Canadian Journal of Microbiology*, *48*(12), 1041-1047. https://doi.org/10.1139/w02-107

[53] Dhiman, S., Mukherjee, G., & Singh, A. K. (2018). Recent trends and advancements in microbial tannase-catalyzed biotransformation of tannins: A review. *International Microbiology*, *21*(4), 175-195. https://doi.org/10.1007/s10123-018-0027-9

[54] Murugaiyan, S., Maheswari, M., Sathya, V., Shobana, N., Dinesh, G. K., Periasamy, D., Shiv, P., & Kalpana, P. (2025). Metagenomic profiling of soil microbial communities in chromium-contaminated tannery sludge. *Geomicrobiology Journal*, *42*, 1-9. https://doi.org/10.1080/01490451.2025.2467404

[55] Quintana, S. A., Sarmiento, M. M., Arrúa Alvarenga, A., Morel, R., Ries, A., & Benitez Rodas, G. (2026). Metagenomic snapshot of treated tannery effluent in Paraguay reveals microbiome diversity and antibiotic resistance genes. *Applied Microbiology*, *6*(2), 28. https://doi.org/10.3390/applmicrobiol6020028

[56] Manoj, K. M., Priya, A. K., & Senthil Rajan, M. (2025). Machine learning-assisted prediction of heavy metal adsorption from tannery effluent wastewater using *Delonix-regia* with random forest, isotherms, and kinetics modelling. *Desalination and Water Treatment*, *324*, 101417. https://doi.org/10.1016/j.dwt.2025.101417

[57] Ameen, F. (2023). Improving tannery wastewater treatments using an additional microbial treatment with a bacterial-fungal consortium. *Biology*, *12*(12), 1507. https://doi.org/10.3390/biology12121507

[58] Nagabalaji, V., Maharaja, P., Nishanthi, R., Sathish, G., Suthanthararajan, R., Shanmugham, & Srinivasan, V. (2023). Effect of co-culturing bacteria and microalgae and influence of inoculum ratio during the biological treatment of tannery wastewater. *Journal of Environmental Management*, *341*, 118008. https://doi.org/10.1016/j.jenvman.2023.118008

[59] Sharma, S., & Malaviya, P. (2016). Bioremediation of tannery wastewater by chromium resistant novel fungal consortium. *Ecological Engineering*, *91*, 419-425. https://doi.org/10.1016/j.ecoleng.2016.03.005.

International Journal of Environmental & Agriculture Research (IJOEAR)
ISSN: [2454-1850]
[Vol-12, Issue-9, September- 2026]
Website: www.ijoear.com
Journal DOI: 10.25125/agriculture-journal

In vitro Activity of *Pseudomonas fluorescens* Strains against *Fusarium verticillioides*, *Fusarium subglutinans* and *Schizophyllum commune*, which Cause Diseases in Sugarcane in Côte d'Ivoire

OKOUE Djédji[1*]; TEHUA Amoa Armist[2]; YEO Navigué Abou[3]; KOFFI Yao Fulgence[4]; KEBE Ibrahima[5]; ALLOUE-BORAUD Waze Aimée Mireille[6]; KONE Daouda[7]

[1,5,6]Laboratoire de Biotechnologie et Microbiologie des Aliments, Unité de Formation et de Recherche en Sciences et Technologie des Aliments (UFR-STA), Université Nangui Abrogoua, 02 BP 801 Abidjan 02, Abidjan, Côte d'Ivoire.
[2,3]Département de Biologie Végetale, UFR Sciences Biologiques, Université Péléforo GON COULIBALY de Korhogo, BP 1328 Korhogo
[4]Département de Biochimie-Génétique, UFR Sciences Biologiques, Université Péléforo GON COULIBALY de Korhogo, BP 1328 Korhogo.
[7]West African Science Service Center on Climate Change and Adapted Land Use (WASCAL0 Capacity Building department, Headquarters Accra)
*Corresponding Author

Received:- 07 July 2026/ Revised:- 10 August 2026/ Accepted:- 07 September 2026/ Published: 15-09-2026

Abstract— *Sugar cane (Saccharum officinarum L.) is a key crop for the economy of Côte d'Ivoire. However, domestic production remains insufficient to meet domestic demand, resulting in a sugar deficit for the country. This low productivity is largely due to fungal diseases, which cause considerable economic losses. Whilst several control methods have been used, some pose risks to human health. It is against this backdrop that this study was conducted, the objective of which was to evaluate the in vitro effect of Pseudomonas fluorescens strains against Fusarium verticillioides, Fusarium subglutinans and Schizophyllum commune, which cause diseases in sugarcane in Côte d'Ivoire. To this end, 150 soil samples were collected from the rhizosphere of healthy and diseased sugarcane plants for the isolation and phenotypic and molecular identification of Pseudomonas fluorescens strains. In vitro antifungal tests were then carried out. The results showed that strains of Pseudomonas fluorescens were isolated and identified from the rhizosphere of both healthy and diseased sugarcane plants. In vitro tests showed that 95 per cent of the bacterial strains inhibited the mycelial growth of the three fungal pathogens, with inhibition rates ranging from 40.16 ± 6.9 per cent to 80.65 ± 6.5 per cent. These results suggest that Pseudomonas fluorescens, when tested under field conditions, could be used effectively as a biocontrol agent against fungal diseases in sugarcane crops in Côte d'Ivoire.*

Keywords— Sugar cane, Pathogenic fungus, Pseudomonas fluorescens, Côte d'Ivoire.

I. INTRODUCTION

Sugar cane (*Saccharum officinarum* L.) is a perennial plant of the Poaceae family. It is native to New Guinea and is cultivated for its stems, which contain a sweet sap (Meslien, 2009). In 2022, global sugarcane production reached approximately 1.92 billion tonnes, accounting for 79% of total global sugar production (ChenhanTech, 2022). This sector constitutes a strategic agro-industrial sector, both for the production of sugar and for that of ethanol or energy co-products. In 2023–2024, the main producing countries were Brazil and India, with annual production ranging from 753 to 783 million tonnes and from 405 to 490 million tonnes respectively (Financial Times, 2023). Sugar cane cultivation generates economic value amounting to tens of billions of US dollars (USD). For example, the Brazilian group Raízen recorded a turnover of 47 billion USD in 2023

(Financial Times, 2023). The Illovo Sugar group (South Africa) is the leading player in Africa, exporting to the European Union, the United States and West Africa. Côte d'Ivoire is a medium-sized producer in Africa. In 2018, the country's annual sugarcane production was estimated at 1.9 million tonnes, ranking it 53rd globally and 16th in Africa (REPERCI, 2024). Within the country, sugarcane is mainly used for direct consumption or in the sugar industry. It is also used in the cosmetics and pharmaceutical sectors. Furthermore, it represents the main source of economic activity for the population in the northern part of the country (Kouamé et al., 2010). Despite the importance of sugarcane, Côte d'Ivoire continues to face a sugar deficit. This sugar deficit is generally caused by diseases affecting sugarcane cultivation, including bacterial diseases such as leaf blight caused by *Xanthomonas albineans*, and fungal diseases such as smut, red mould and pokkah boeng. These fungal diseases result in losses estimated at between 10 and 30 per cent of the national yield (Yao et al., 2020). Although there are no comprehensive official national data, fungal diseases cause economic losses ranging from 20 to 40 per cent of the national yield. Thus, based on an estimated national sugarcane production of around 330,000 tonnes, a 20 per cent loss equates to 66,000 tonnes, representing nearly 13 million USD in annual economic losses (Kouamé et al., 2010). These fungal diseases are characterised by symptoms such as leaf chlorosis and green mottling of varying severity. The most susceptible varieties can be literally stunted to the point of resembling tufts of grass. Sometimes the stems are covered in cankers and the internal tissues are necrotic. This deterioration of the stems leads to a reduction in their sugar content and losses in yield. These diseases have caused crop losses of up to 40 per cent in the Borotou-Koro and Zuénoula sugar complexes (Yao et al., 2020). In order to combat these pathogens effectively, pesticides – particularly synthetic fungicides from the benzimidazole, triazole and strobilurin families – are primarily used. However, the overuse of these synthetic fungicides in sugarcane plantations could lead to the emergence and rapid development of resistant strains, which would result in a resurgence of diseases (Cawoy et al., 2011). In view of these adverse consequences, it is important to find alternative solutions that will enable the continued control of plant pathogens whilst reducing the use of chemicals and also protecting the environment. These may involve the rationalisation of agricultural practices, the use of resistant plant varieties and/or the use of biopesticides (Thakore, 2006). It is against this backdrop that this study is set, the overall objective of which is to evaluate the in vitro effect of *Pseudomonas fluorescens* strains against *Fusarium verticillioides*, *Fusarium subglutinans* and *Schizophyllum commune*, which cause diseases in sugarcane in Côte d'Ivoire.

II. MATERIALS AND METHODS

2.1 Material

The materials used in this study consisted, on the one hand, of samples collected from two major sugarcane-producing areas in Côte d'Ivoire, namely Zuenoula and Borotoukoro. These soils, taken from the rhizosphere of sugarcane, were used to isolate bacterial biocontrol agents. In addition, samples of apparently healthy sugarcane cuttings were used to carry out biocontrol tests on pathogens in a greenhouse. Furthermore, pathogenic fungal strains of *Fusarium verticillioides*, *Fusarium subglutinans* and *Schizophyllum commune*, which cause diseases in sugarcane in Côte d'Ivoire, were used. These fungal strains were obtained from the Laboratory of Food Biotechnology and Microbiology at the Food Science and Technology Training and Research Unit (UFR-STA) of Nangui Abrogoua University (Abidjan, Côte d'Ivoire). These strains were identified from symptomatic parts of sugarcane in Côte d'Ivoire, in accordance with the work of Okoue et al. (2025).

2.2 Methods

2.2.1 Sampling area

Soil samples, as well as apparently healthy sugarcane cuttings, were selected from plots in the two sugarcane production areas, namely the Zuenoula Integrated Agricultural Unit (UAI), situated between 7°30' and 7°40' north latitude, and between 6°50' and 6°15' west longitude, and the Integrated Agricultural Unit (UAI) at Borotou-Koro, situated between 8°31' north latitude and 7°17' west longitude.

2.2.2 Identification of bacterial biocontrol agents

2.2.2.1 Sampling

Soil samples were taken from the rhizosphere of apparently healthy sugarcane plants and those showing symptoms of disease, located at least 50 m apart, at a depth of 0 to 30 cm, with a view to identifying biocontrol agents. A total of 150 soil samples were collected from the Borotou-Koro and Zuénoula sugar complexes, of which 75 were taken from the rhizosphere of apparently healthy sugarcane plants and 75 from those showing symptoms of disease. These samples were placed in sterile

Stomacher bags, labelled, sealed and then stored in a cool box containing dry ice at 4°C, before being transported to the laboratory for various analyses.

2.2.2.2 Isolation of *Pseudomonas fluorescens* isolates

The isolation of presumptive *Pseudomonas fluorescens* was carried out using the suspension-dilution method and by plating dilutions of soil suspensions onto King B agar (Vidhyasekaran et al., 1997). To this end, 1 g of soil was sampled using a sterile spatula and suspended in 9 ml of sterile distilled water. After vortexing the suspension for 10 minutes, decimal dilutions ranging from 10^{-1} to 10^{-7} were prepared. Next, 0.1 ml of each suspension was spread onto Petri dishes, each containing 20 ml of King B agar medium. The Petri dishes were incubated at 30 °C in the dark. After 48 hours of incubation, the characteristic colonies of putative *Pseudomonas fluorescens* were counted and the bacterial load per soil sampling site was determined using equation (2) in accordance with ISO 7218 (2007). After counting, the putative *Pseudomonas fluorescens* isolates were subcultured onto King B agar until pure colonies were obtained. The pure colonies were preserved in 50% glycerol in Eppendorf tubes at -20°C and in tubes containing King B agar poured into slants at 4°C (Bossis et al., 2000).

$$N\left(\frac{ufc}{g}\right) = \frac{\sum C}{V \times (n_1 + 0{,}1\ n_2) \times d} \quad (1)$$

Where

N (ufc/g): Microbial load

C: Number of colonies across all Petri dishes;

V (ml): Volume of the soil solution inoculum;

n1: Number of Petri dishes inoculated with the first dilution under consideration;

n2: Numbers of Petri dishes inoculated with the second dilution under consideration;

d: Dilution selected.

2.2.2.3 Phenotypic identification of putative *Pseudomonas fluorescens* isolates

The phenotypic identification of putative *P. fluorescens* isolates was carried out using various methods based on morphological and physiological criteria. Following purification, the colonies obtained on King B agar were examined with the naked eye. The macroscopic examination was carried out by assessing the size, shape and lustre of the colonies (Benzina et al., 2016). The physiological examination, meanwhile, focused on the production of yellow-green pigment on King B agar at 4 °C. The microscopic examination was based on Gram staining, which involved preparing a bacterial smear on a heat-fixed microscope slide. The smear was then examined under the microscope at magnifications of ×40 and ×100 (Bossis et al., 2000). Presumed *Pseudomonas fluorescens* species appear as pink-stained bacilli, i.e. Gram-negative bacilli (Bossis et al., 2000).

2.2.3 Molecular identification of putative *Pseudomonas fluorescens* isolates

2.2.3.1 DNA extraction

To extract DNA, pre-cultures were prepared by inoculating the strains into a nutrient broth and incubated at 30 °C for 24 hours. A 1.5 ml volume of bacterial suspension was transferred to sterile Eppendorf tubes. The bacterial biomass was recovered by centrifugation at 4000 rpm for 10 minutes. The biomass was then mixed with 500 µl of H_2O. The thermal shock DNA extraction technique, based on the disruption of bacterial membranes by treating the pre-cultures at 100 °C followed by -20 °C, was used to recover genomic DNA (Queipo et al., 2007).

2.2.3.2 16S rRNA gene amplification reaction

The gene encoding the 16S rRNA subunit is a fundamental tool for the genetic identification and phylogenetic tracing of bacteria (Emmanuel et al., 2009). In order to amplify the genes encoding the 16S rRNA subunit, universal primers specific to the bacterial kingdom will be used. The template strand primer is 27F and the complementary strand primer is 1492R (reverse primer). A PCR master mix with a final volume of 25 µl was prepared, consisting of nuclease, Taq buffer, dimethyl sulphoxide (DMSO), $MgCl_2$, deoxyribonucleotides, primer F, primer R, Taq polymerase and the DNA to be analysed (Table I). Amplification was carried out in a thermal cycler (Techne® Prime Thermal Cycler Range, USA) according to the programme described by Arzu et al. (2011). The first stage was the initial denaturation. The second stage comprised 35 cycles, each

consisting of a denaturation phase, a hybridisation phase and an elongation phase. Finally, the third stage was the final elongation (Table II).

TABLE 1
REAGENT USED IN THE PCR REACTION MIXTURE FOR THE MOLECULAR IDENTIFICATION OF BACTERIAL ISOLATES

Reactive	Volume per reaction (µl)
Nuclease Free Water	11.825
Tampon Taq	5
Diméthylsulfoxyde (DMSO)	0.125
$MgCl_2$	4
DNTP	0.6
ADN	2
Amorce F	0.625
Amorce R	0.625
Taq polymérase	0.2
Total	**25**

TABLE 2
PCR PROTOCOL FOR THE MOLECULAR IDENTIFICATION OF BACTERIAL ISOLATES

Stage	Temperature (°C)	Time	Number of cycles
Initial denaturation	94	7 min	
Amplification	94	1 min	35
	53	43 s	
	72	45 s	
Final elongation	72	7 min	

2.2.3.3 Agarose gel electrophoresis

Agarose gel electrophoresis is a widely used method in molecular biology that enables the separation of DNA fragments according to their molecular weight. It is based on the migration of negatively charged nucleic acids in the presence of an electric field. The migration of DNA fragments takes place within the agarose gel matrix, with smaller molecules moving more rapidly than larger ones. Electrophoresis provides essential information, notably the molecular weight of the amplified gene, by comparison with size markers. Following amplification, the PCR products were analysed by electrophoresis on a 1.5% agarose gel. The gel was immersed in an electrophoresis tank containing 0.5× TBE. Aliquots of 10 µl of amplified fragments were mixed with 2 µl of loading buffer (50% TE, pH 8; 50% glycerol; bromophenol blue) before being loaded into the gel wells. The gel was visualised using a transilluminator under UV light following electrophoresis at 90 volts for 90 minutes. The size of the DNA fragments was determined using the Reddy Run Super Ladder-Low 1000 bp molecular weight marker as a reference. The PCR products were subsequently stored at –20 °C for further use.

2.2.4 Evaluation of the in vitro antifungal activity of bacterial biocontrol agents against sugarcane pathogenic fungi

2.2.4.1 Preparation of culture media and direct comparison between pathogens and antagonist

The *P. fluorescens* bacteria were first re-inoculated 48 hours prior to the preparation of the YPGA culture media used. These purified bacteria were then streaked onto the pre-prepared YPGA agar in a straight line dividing the Petri dish into two equal parts. Next, two mycelial discs, each 1 mm in diameter, from seven (7)-day-old pathogenic fungi were placed on either side of the streak, 1 cm from the edge of the Petri dish (Korsten and Jager, 1995). Control plates containing no bacterial biocontrol agent were prepared by inoculating a mycelial fragment of the pathogenic fungus in the centre of the Petri dish. Antifungal activity was assessed by measuring the diameter of the mycelial growth of the pathogenic fungi using a ruler graduated in centimetres (cm) in each of the Petri dishes comprising the treatments. This measurement was taken daily until the pathogenic fungus had filled the control dishes. At the end of these measurements, the rate of inhibition of the pathogen's mycelial growth was calculated using the following formula (2) by Korsten and Jager (1995):

$$TI(\%) = (\frac{R-r}{R}) \times 100 \quad (2)$$

Where

TI (%) = Inhibition rate

r: Radial growth of the fungus in the presence of the bacterial strain

R: Radial growth of the fungus in the absence of the bacterial strain

All in vitro antifungal tests were performed with three replicates per treatment, and each experiment was conducted twice to ensure reproducibility.

2.3 Statistical Analyses

The data collected were analysed using analysis of variance (ANOVA I and II) with Statistica software, version 7.1. One-way analysis of variance (ANOVA I) was used to investigate the variability of bacterial strains across different sugarcane-growing regions. Two-way analysis of variance (ANOVA II) was used to screen for antagonistic bacteria and the concentrations at which they significantly reduce or completely inhibit the in vitro growth of pathogens. Where a significant difference was found, the means were compared using the Newman-Keul test at a 5 per cent significance level. The significance of the test is determined by comparing the probability (P) associated with the test statistic to the value $\alpha = 0.05$. Thus, when $P \geq 0.05$, it is concluded that there is no difference between the means. However, when $P < 0.05$, there is a significant difference between the means.

III. RESULTS

3.1 Potential of Bacteria with Biopesticidal Activity Isolated from the Sugarcane Rhizosphere

3.1.1 Abundance and morphological characteristics of the putative *Pseudomonas fluorescens* isolates

The average concentration of putative *Pseudomonas fluorescens* ranged from 2.5×10^6 to 1.4×10^6 CFU/g of soil for the Zuenoula and Borotou-koro complexes, respectively, in soil samples taken from the rhizosphere of apparently healthy sugarcane plants. Those from symptomatic sugarcane plants ranged from 9.1×10^4 to 3.5×10^4 CFU/g of soil. Regardless of the sampling area, the levels of putative *Pseudomonas fluorescens* in soils from symptomatic sugarcane plants were lower than those in soils from apparently healthy plants. Statistical analyses revealed a significant difference in the load of presumptive *Pseudomonas fluorescens* isolated from the rhizosphere of symptomatic and healthy sugarcane plants (Figure 1). Following purification, a total of 407 bacterial isolates presumed to belong to the genus *Pseudomonas fluorescens* were isolated from soil samples taken from the rhizosphere of apparently healthy and symptomatic sugarcane plants in both study areas,

namely Borotou-koro and Zuénoula. The characterisation of presumptive *Pseudomonas fluorescens* isolates using macroscopic characteristics following purification on King B agar revealed the formation of distinct colonies visible to the naked eye, exhibiting the specific morphological criteria of presumptive *Pseudomonas fluorescens*. On King B agar, presumptive *Pseudomonas fluorescens* isolates are characterised by rapid growth, producing small, creamy, overlapping colonies that are circular, smooth, regular in appearance and invasive, with yellow-green pigment production in the agar (Figure 2A). Identification by microscopic examination reveals straight or slightly curved bacilli with rounded ends. The cells occur singly or in pairs. They are asporangiate. Following Gram staining, the presumptive *Pseudomonas fluorescens* isolates appear as pink rods, indicating that they are Gram-negative bacilli. Indeed, bacteria belonging to the genus *Pseudomonas* are small rods with polar cilia and are Gram-negative (Figure 2B). Following phenotypic identification, a total of 103 bacterial isolates presumed to be *Pseudomonas fluorescens* were selected and coded using letters of the alphabet, followed by an Arabic numeral and a letter, or not. The first letter, 'P', stands for *Pseudomonas*, and the other letters and numbers are arranged at random.

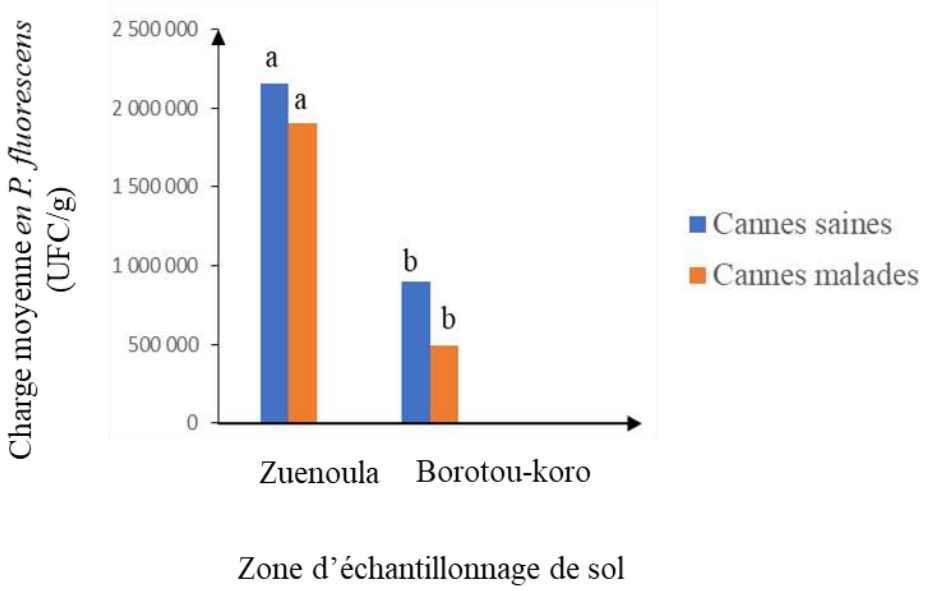


FIGURE 1: Bacterial load of putative *P. fluorescens* isolates from soil samples taken from the two sugar complexes. Bands with the same letters are statistically identical at the 5 per cent significance level, and those with different letters are statistically different at the 5 per cent significance level.

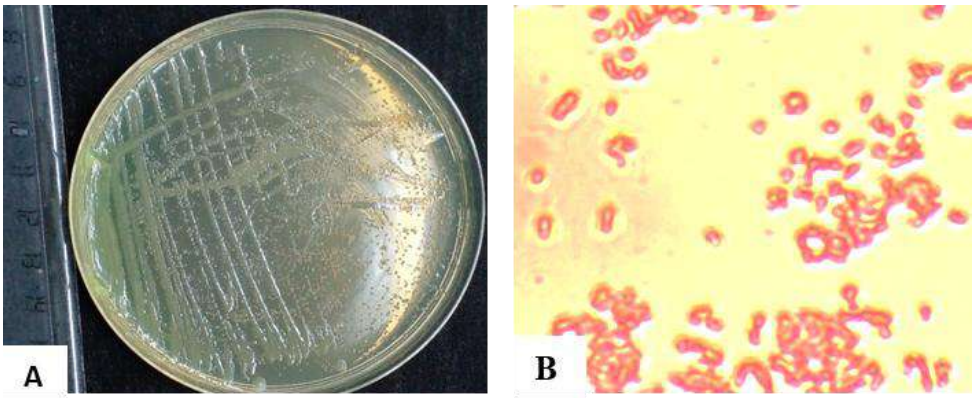


FIGURE 2: Macroscopic and microscopic appearance of putative *P. fluorescens* isolates from the rhizosphere soil of the Zuenoula and Borotou-Koro sugar complexes. A - Macroscopic appearance of putative *Pseudomonas fluorescens*; B - Microscopic appearance of putative *Pseudomonas fluorescens*.

3.1.2 *Pseudomonas fluorescens* strain identified using molecular tests

The search for *Pseudomonas fluorescens* isolates through the analysis of soil samples resulted in the isolation of rhizobacteria distributed across the various wells formed in the agarose gel. The molecular characterisation method, involving amplification of the 16S rDNA gene, enabled the identification of *Pseudomonas fluorescens*. The size of the amplicons was approximately 1,500 bp (Figure 3).

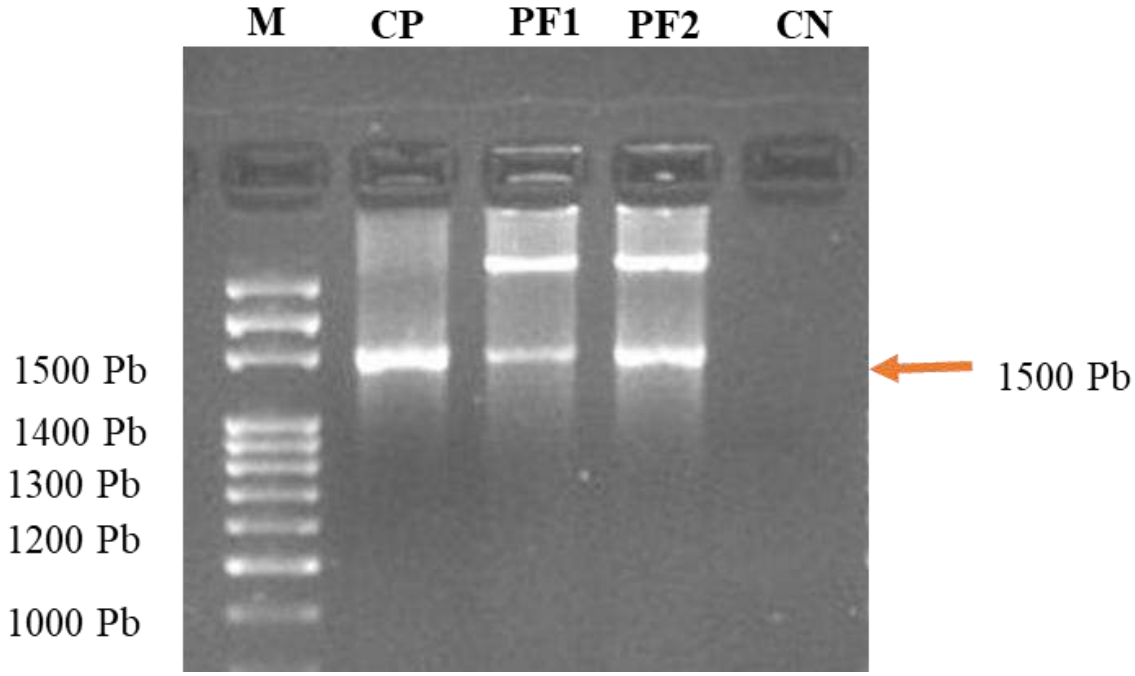


FIGURE 3: Electrophoretic profile of *Pseudomonas* sp. isolates from the sugarcane rhizosphere. M: Molecular weight marker; PF1: *Pseudomonas fluorescens* 1; PF2: *Pseudomonas fluorescens*, CP: *Pseudomonas fluorescens* CI and CN: H_2O.

3.2 In vitro Antagonistic Activity of *Pseudomonas fluorescens* Strains against the Mycelial Growth of Sugarcane Pathogenic Fungi

The results of the antagonism tests carried out showed that the isolates of *Bacillus* sp. and presumptive *Pseudomonas fluorescens* were capable of inhibiting the mycelial growth of sugarcane pathogenic fungi (Figures 4, 5 and 6). The results showing the average rates of inhibition of mycelial growth of pathogenic fungi by the genus *Bacillus* are presented in Table III. Average inhibition rates exceeding 50 per cent were observed for the majority of the identified isolates. These average inhibition rates ranged from 44.12 to 80.47 per cent for the *Pseudomonas* isolates PCI and PZ13, respectively, on the growth of *Schizophyllum commune*, and from 46 to 79.24 per cent respectively for the *Pseudomonas* PE91 and PZ13 isolates on the mycelial growth of *Fusarium verticillioides*, and from 48.68 to 66.34 per cent respectively for the *Pseudomonas* PF26 and PEZ83 isolates on the mycelial growth of *Fusarium subglutinans*. Statistical analyses revealed a significant difference at the 5 per cent significance level between the inhibition rates. The best inhibitory effect was observed for the *Pseudomonas* isolates PZ13 and PEZ83, with mean inhibition rates of 80.47 per cent, 79.24 per cent and 79.52 per cent respectively. These mean inhibition rates are statistically equivalent at the 5 per cent significance level (Table III).

TABLE 3
RATES OF INHIBITION OF MYCELIAL GROWTH IN SUGARCANE PATHOGENIC FUNGI BY STRAINS OF *PSEUDOMONAS FLUORESCENS*

Strains	*Schizophyllum commune*	*Fusarium subglutinans*	*Fusarium verticillioides*
PE75	(53.45±4.40)^{cdef}	(55.30±2.26)^{cd}	(53.71±7.30)^{bc}
PCE	(50.60±3.79)^{def}	(51.12±1.02)^{de}	(54.81±4.34)^{bc}
PE6Z51	(65.07±1.37)^{bc}	(57.09±2.43)^{bcd}	(60.13±2.04)^{ab}
PEZ1	(58.56±5.15)^{cde}	(44.65±1.91)^{e}	(54.70±1.46)^{bc}
PQ94	(72.97±1.62)^{ab}	(61.32±1.43)^{bc}	(59.45±1.90)^{ab}
PEZ2	(61.36±3.74)^{bcd}	(58.11±2.00)^{bcd}	(52.94±3.46)^{bc}
PEZ4	(44.60±3.99)^{f}	(51.12±1.02)^{de}	(57.65±4.60)^{abc}
PCI	(44.12±7.47)^{f}	(54.87±2.00)^{cd}	(61.17±1.25)^{ab}
PEZ83	(78.09±2.18)^{a}	(75.63±2.67)^{a}	(66.34±2.57)^{a}
P65Z9	(53.49±3.57)^{cdef}	(54.87±2.00)^{cd}	(57.65±4.60)^{abc}
PE5	(59.99±1.42)^{cde}	(58.50±1.53)^{bc}	(56.80±4.04)^{abc}
PZ20	(54.76±5.01)^{cdef}	(62.70±1.48)^{b}	(54.27±1.71)^{bc}
PEZ13	(80.47±2.17)^{a}	(79.24±0.71)^{a}	(61.64±2.87)^{ab}
PEZ181	(57.52±6.29)^{cde}	(61.33±2.55)^{bc}	(55.09±4.30)^{bc}
PE91	(48.31±3.50)^{ef}	(46.00±3.24)^{e}	(55.12±2.14)^{bc}
PO12	(59.99±4.28)^{cde}	(59.89±1.27)^{bc}	(60.31±1.43)^{ab}
PE1	(79.52±0.82)^{a}	(54.87±2.00)^{cd}	(58.96±3.97)^{abc}
PZ26	(59.87±4.72)^{cde}	(55.34±1.91)^{cd}	(57.65±4.60)^{abc}
PE60	(55.23±5.01)^{cdef}	(54.31±3.76)^{cd}	(56.04±1.40)^{abc}
PF26	(49.68±3.82)^{def}	(54.25±5.16)^{cd}	(48.68±1.14)^{c}

On the same row, values with the same letters are statistically identical at the 5 per cent significance level.

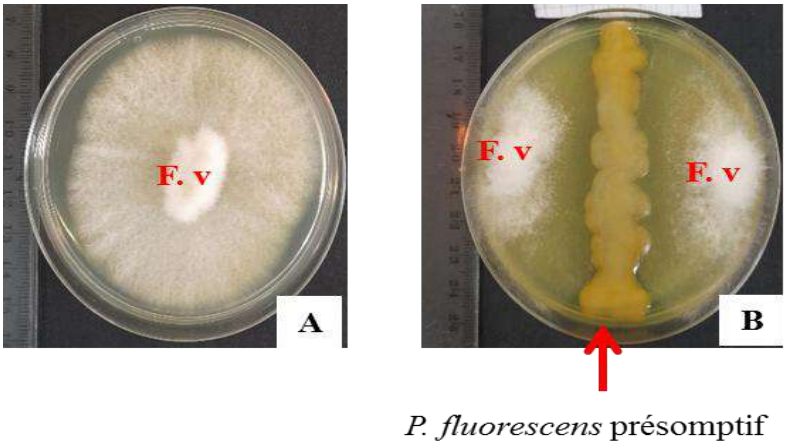


FIGURE 4: Inhibition of *Fusarium verticillioides* mycelial growth by putative *Pseudomonas fluorescens* isolates from the sugarcane rhizosphere. A – *Fusarium verticillioides* (F. v) on YPGA medium without bacterial challenge; B – Co-culture of *Fusarium verticillioides* (F. v) and putative *P. fluorescens* on YPGA medium.

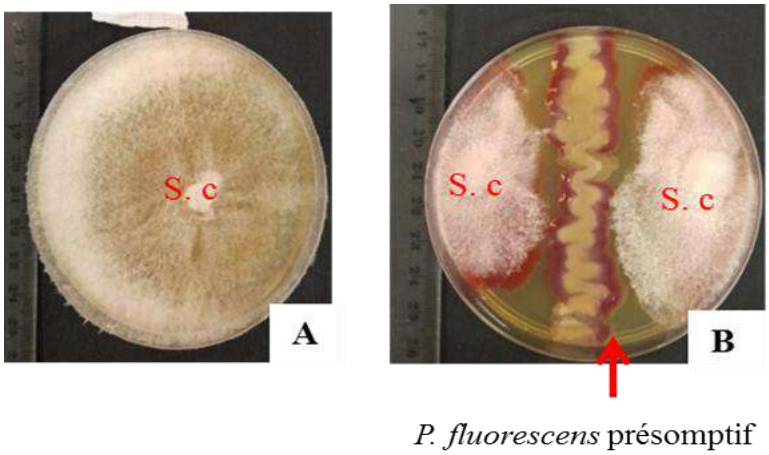


FIGURE 5: Inhibition of mycelial growth of *Schizophyllum commune* by isolates of putative *Pseudomonas fluorescens* isolated from the rhizosphere of sugarcane. A – *Schizophyllum commune* (S. c) on YPGA medium without bacterial challenge; B – Co-culture of *Schizophyllum commune* (S. c) and presumptive *P. fluorescens* on YPGA medium.

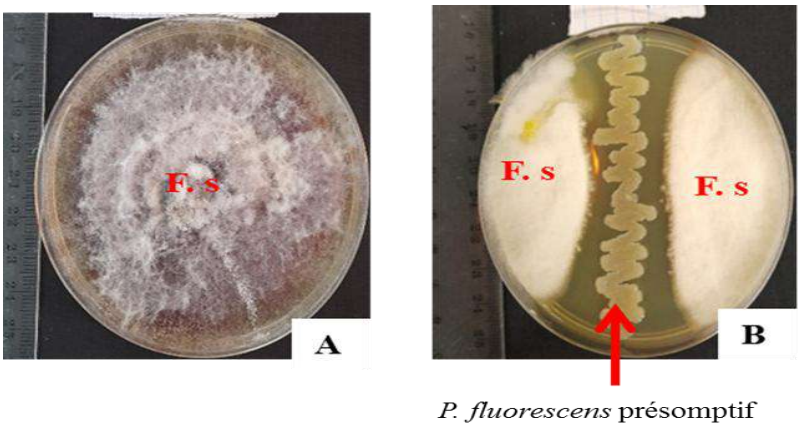


FIGURE 6: Inhibition of *Fusarium subglutinans* mycelial growth by putative *Pseudomonas fluorescens* strains isolated from the sugarcane rhizosphere. A – *Fusarium subglutinans* (F. s) on YPGA medium without bacterial challenge; B – Co-culture of *Fusarium subglutinans* (F. s) and putative *P. fluorescens* on YPGA medium.

IV. DISCUSSION

The aim of this chapter was to identify the bacterium *Pseudomonas fluorescens*, which has biopesticidal potential, from the rhizosphere of sugarcane. The results of the isolation and identification of putative *Pseudomonas fluorescens* from the sugarcane rhizosphere demonstrated the presence of *Pseudomonas fluorescens* in the rhizosphere of sugarcane in the localities of Zuenoula and Borotou-Koro. The *Pseudomonas fluorescens* strains isolated from the rhizosphere of asymptomatic sugarcane plants constituted a significant proportion of the soil microbial community. The isolation of these strains suggests that the sugarcane plantations in the localities of Borotou-Koro and Zuenoula are naturally rich in bacteria of the genera *Pseudomonas*. The low abundance of *Pseudomonas fluorescens* observed in soil samples from the rhizosphere of diseased sugarcane plants was statistically different from that found in soil samples from the rhizosphere of healthy sugarcane plants. This difference could indicate that the microbial population influences the development or absence of sugarcane diseases. Indeed, according to Ongena (2014), better plant protection would require a microbial population of at least 10^6 cfu/g of soil. Recent studies have also shown that strains of *Pseudomonas putida* and *Stenotrophomonas* sp. have been isolated from the rhizosphere of carrots and coffee beans, respectively (Van et al., 2004). The rhizosphere of plants is a zone containing a diverse range of rhizobacteria. Species such as *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Arthrobacter*, *Bacillus*, *Serratia*, *Xanthomonas* and *Erwinia* are abundant there (Suman et al., 2010). Frietas and Germida (1990) isolated strains of *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, *Pseudomonas fluorescens* and *Pseudomonas putida* from the rhizosphere

of millet. Watanabe and Hayano (1993), for their part, isolated strains of *Bacillus licheniformis*, *Bacillus mycoides* and *Bacillus megaterium* from the rhizosphere of rice.

The results showed that all isolates produced inhibition percentages ranging from 50 to 81 per cent. Indeed, the selected isolates were capable of inhibiting the growth of all the fungi they were tested against. These results could be explained by the fact that the selected strains were capable of producing a variety of antimicrobial and antifungal compounds, namely lytic enzymes, siderophores and lipopeptides, thereby enabling them to reduce or completely inhibit the growth of pathogenic strains. Indeed, most microorganisms possessing antagonistic activity are isolated either from fruit, from plants or from the rhizosphere (Janisiewicz and Korsten, 2002). Silva et al. (2015) isolated a variety of bacterial isolates (*Enterobacter aerogenes*, *Lysinibacillus fusiformis*, *Klebsiella oxytoca* and *Hafnia alvei*) from a fruit native to Brazil (*Psychotria hoffmannseggiana*), all of which exhibited antagonistic activity against the pathogenic strain *Aspergillus carbonarius*. These authors stated that the antagonistic activity observed in these different strains was due to their production of cellulase and pectinase. In their research, Oztopuz et al. (2018) demonstrated that the *Bacillus subtilis* strain Ege-B-1.19, isolated from soil, was capable of secreting a variety of hydrolytic enzymes. These enzymes comprised chitinase, chitosanase, N-acetyl-β-hexosaminidase and protease. These enzymes thus enable it to inhibit the growth of a range of phytopathogenic fungi, including *Aspergillus niger* EGE-K-213, *Aspergillus foetidus* EGE-K-211, *Aspergillus ochraceus* EGE-K-217, *Fusarium solani* KCTC6328, *Rhizoctonia solani* KACC 40111 and *Colletotrichum gloeosporioides* KACC 40689. Previous studies have also demonstrated the biocontrol potential of certain strains of *Bacillus subtilis* against various plant-pathogenic fungi. *Bacillus subtilis* was able to inhibit *Aspergillus niger*, *Fusarium verticillioides*, *Aspergillus flavus* and *Penicillium digitatum* (Waewthongrak et al., 2015). These results suggest that *Bacillus subtilis* is effective as a biological control agent against pathogenic fungi. Indeed, strains of *P. fluorescens* possess broad-spectrum antagonistic activity against plant pathogens. These activities include, amongst others, direct antibiosis, spatial competition or nutritional status and the production of siderophores (Showkat et al., 2012). Thus, during the stationary phase of growth, *P. fluorescens* species produce several metabolites with potent antifungal properties, such as phenazines, pyrrolnitrin, pyoluteorin and 2,4-diacetylphloroglucinol (2,4-DAPG), which are reported to inhibit, in vitro, the growth of pathogenic fungi (Haass and Defago, 2005). These metabolites act by impairing the pathogen's germination, growth and/or sporulation, causing distortion of the pathogen's hyphae, altering the appearance of colonies and inducing the production of specific structures such as pseudoparenchymata (Campbell, 1989). These results may also stem from the secretion of lytic enzymes such as β-1,3-glucanase and chitinase, whose mechanism of action involves parasitism through the degradation of the pathogenic fungus's cell walls (Ban Koffi et al., 2015). Showkat et al. (2012) demonstrated that *P. fluorescens* isolates collected from the wheat rhizosphere can inhibit, in vitro, the growth of *Fusarium oxysporum* and species of the genus *Aspergillus* that infect wheat crops in Kashmir.

The mechanisms by which *P. fluorescens* exerts its antagonistic activity can be categorized into several modes of action. First, direct antibiosis involves the production of diffusible antibiotics such as phenazines, pyrrolnitrin, and 2,4-DAPG, which directly inhibit fungal growth by interfering with cellular processes. Second, siderophore production sequesters iron from the environment, making it unavailable to pathogens and thereby limiting their growth. Third, competition for nutrients and space on the root surface prevents pathogen colonization. Fourth, the production of lytic enzymes such as chitinase and β-1,3-glucanase degrades fungal cell walls, leading to lysis of hyphae. Fifth, induced systemic resistance (ISR) can be triggered in the host plant, priming its defense responses against subsequent pathogen attack. The relative contribution of each mechanism may vary depending on the specific strain, the pathogen, and environmental conditions. Future studies should investigate the specific mechanisms operating in the most effective strains identified in this study (PZ13 and PEZ83) to better understand their biocontrol potential.

The results of this study are particularly promising for the development of sustainable disease management strategies in Côte d'Ivoire's sugarcane sector. The high inhibition rates observed (up to 80.47%) demonstrate the potential of these *Pseudomonas fluorescens* strains as effective biocontrol agents. However, several challenges must be addressed before field application. These include: formulation and delivery methods to ensure bacterial survival and establishment in the field; compatibility with existing agricultural practices; potential effects on non-target organisms; and regulatory approval for commercial use. Future research should include greenhouse and field trials to validate the efficacy of these strains under realistic conditions, as well as studies on formulation optimization and application timing.

V. CONCLUSION

This study enabled the isolation and identification of a variety of bacterial isolates from the sugarcane rhizosphere. The strains were selected on the basis of their potential antagonistic activity. The identification of bacterial isolates from the sugarcane

rhizosphere revealed the species *Pseudomonas fluorescens*. All these bacterial strains were capable of inhibiting the three fungi they were tested against, with high inhibition rates. In view of the various results obtained, these bacterial strains could be effective biocontrol agents against fungal diseases of sugarcane in Côte d'Ivoire.

Limitations of the Study: The study was conducted under in vitro conditions, which may not fully reflect field conditions where environmental factors, microbial competition, and plant-pathogen interactions can influence biocontrol efficacy. The specific mechanisms of antagonism were not investigated directly. Field validation of the most promising strains is needed to confirm their efficacy under realistic conditions. Future research should include greenhouse and field trials, formulation development, and investigation of the mechanisms of action.

Future Research Directions: Future studies should focus on: (1) evaluating the most effective strains (PZ13 and PEZ83) under greenhouse and field conditions; (2) investigating the specific mechanisms of antagonism (antibiosis, siderophore production, lytic enzyme activity, induced systemic resistance); (3) developing effective formulation and delivery methods for field application; (4) assessing the compatibility of these strains with other disease management practices; and (5) evaluating the effects of these strains on non-target organisms and soil health.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Ban Koffi, L., Alloue-Boraud, W. A., Assamoi, J., Koussemon, M., & Ongenas, M. (2015). Study of protective effect of *Pseudomonas fluorescens* F19 against microorganisms responsible of tomato fruits (*Lycopersicon esculentum* Mill) spoilage in Côte d'Ivoire. *International Journal of Applied Science and Research*, *9*, 9-16.

[2] Benzina, F., Sahir-Halouane, F., & Hamed, K. (2016). Algerian isolates of fluorescent *Pseudomonas* and *Bacillus* sp. as potential biological control against wilt pathogen (*Verticillium dahliae*). *Plant Omics Journal*, *9*(1), 48-60.

[3] Bossis, E., Lemanceau, P., Latour, X., & Gardan, L. (2000). The taxonomy of *Pseudomonas fluorescens* and *Pseudomonas putida*: Current status and need for revision. *Agronomie*, *20*, 51-63.

[4] Cawoy, H., Debois, D., Franzil, L., De Pauw, E., Thonart, P., & Ongena, M. (2015). Lipopeptides as main ingredients for inhibition of fungal phytopathogens by *Bacillus subtilis*/amyloliquefaciens. *Microbial Biotechnology*, *8*, 281-295.

[5] Haas, D., & Defago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology*, *3*(4), 307-319.

[6] ISO 7218. (2007). *Microbiologie des aliments -- Exigences générales et recommandations* (3rd ed.).

[7] Korsten, L., & Jager, E. S. D. (1995). Mode of action of *Bacillus subtilis* for control of avocado postharvest pathogens. *South African Avocado Grower's Association Yearbook*, *18*, 124-130.

[8] Kouamé, D. K., Péné, C. B., & Zouzou, M. (2010). Évaluation de la résistance variétale de la canne à sucre au foreur de tiges tropical africain (*Eldana saccharina* Walker) en Côte d'Ivoire. *Journal of Applied Biosciences*, *26*, 1614-1622.

[9] Okoue, D., Koffi, F. Y., Tehua, A. A., Kebe, I., & Boraud, M. W. A. A. (2025). Fungal profile of sugar cane (*Saccharum officinarum* L.) pathogens produced in Côte d'Ivoire. *Biotech Research Asia*, *22*(1).

[10] Ongena, M. (2014). *Biopesticides: Une protection plus naturelle pour les cultures*. Université de Liège. http://reflexions.ulg.ac.be

[11] Öztopuz, Ö., Pekin, G., Dong, R. P., & Eltem, R. (2018). Isolation and evaluation of new antagonist *Bacillus* strains for the control of pathogenic and mycotoxigenic fungi of fig orchards. *Applied Biochemistry and Biotechnology*, *8*, 2764-2769.

[12] Showkat, S., Murtaza, I., Laila, O., & Ali, A. (2012). Biological control of *Fusarium oxysporum* and *Aspergillus* sp. by *Pseudomonas fluorescens* isolated from wheat rhizosphere soil of Kashmir. *IOSR Journal of Pharmacy and Biological Sciences*, *1*, 24-32.

[13] Silva, M. S., Broglio, S. M. F., Trindade, R. C. P., Ferreira, E. S., Gomes, I. B., & Micheletti, L. B. (2015). Toxicity and application of neem in fall armyworm. *Comunicata Scientiae*, *6*(3), 359-364.

[14] Suman, G., & Dikshit, A. K. (2010). Biopesticides: An eco-friendly approach for pest control. *Journal of Biopesticides*, 186-188.

[15] Thakore, Y. (2006). The biopesticide market for global agriculture use. *Industrial Biotechnology*, *2*, 194-208.

[16] Van Emden, H. F., & Services, M. W. (2004). *Pest and vector control*. Cambridge University Press.

[17] Vidhyasekaran, P., Sethuraman, K., Rajappan, K., & Vasumathi, K. (1997). Powder formulations of *Pseudomonas fluorescens* to control pigeonpea wilt. *Biological Control*, *8*, 166-171.

[18] Yao, K. J.-E., Kouamé, K. D., Kouamé, K. G., Kassi, K. F. J.-M., N'guessan, A. C., Eponon, E. C. G., & Koné, D. (2020). Structure of *Sporisorium scitamineum* isolates, the causative agent of sugarcane smut in Côte d'Ivoire. *International Journal of Science and Research*, *9*(3), 1376-1381.

International Journal of Environmental & Agriculture Research (IJOEAR)
ISSN: [2454-1850]
[Vol-12, Issue-9, September- 2026]
Website: www.ijoear.com
Journal DOI: 10.25125/agriculture-journal

Effect of different generation cutting on growth, sex expression, yield and quality attributes of cucumber (*Cucumis sativus* L.) in Godawari munacipility, Kailali, Nepal

Basant Raj Bhattarai[1*]; Binod Bohara[2]; Devraj Rajbanshi[3]

Faculty of Agriculture, Far-western University, Tikapur, Kailali, Nepal
*Corresponding Author

Received:- 10 August 2026/ Revised:- 18 August 2026/ Accepted:- 01 September 2026/ Published: 15-09-2026

Abstract— *Cucumber (Cucumis sativus L.) is the seventh most important crop in terms of area in Nepal, with the capability of providing abundant minerals and vitamins. The productivity of this crop is less than half the world's average productivity, which is associated with imbalance in male-to-female flower ratio. To address this issue, a field experiment was carried out at the farmers' field of Godawari municipality, Fakalpur, Kailali, Nepal during the summer season of 2023/24 to evaluate the effect of different generation cutting on growth, sex expression, and yield of cucumber cv. Malini. The experiment was laid out in Completely Randomized Block Design (RCBD) with three treatments viz; T1 (No cutting: Control), T2 (First generation cutting: 2G), T3 (Second generation cutting: 3G), and six replications. Each plot consisted of 2 rows and each row consisting of 5 plants with spacing 100 cm × 100 cm. Each replication size was 40 m² so total field size for research was 240 m² consisting of 18 plots each of size 5 m × 2 m. The results indicated that treatment T3 (Second generation cutting: 3G) had a significant effect on phenological parameters, mainly male and female flowers of primary and secondary branches, female flowers on tertiary branches, and male-to-female flower ratio of primary, secondary and tertiary branches. Similarly, yield parameters, mainly number of fruits per plant (6.05) and yield per hectare (21.48 mt/ha) were found to be significantly superior under treatment T3 (Second generation cutting: 3G). Among the different generation cuttings, treatment T3 (Second generation cutting: 3G) was found to be significant over control. It can be concluded that the second generation cutting (3G) can be judiciously used for increasing the sex expression and yield attributes of cucumber.*

Keywords— 3G cuttings, Male flower, Female flower, Sex ratio.

I. INTRODUCTION

Cucumber (*Cucumis sativus* L.), an important vegetable crop, is mainly used as salad and pickles in Nepal. Among the cucurbits, cucumber is a precious vegetable crop grown for consumption of its immature fruits. It is believed to have originated in the hilly areas of Nepal. It has been cultivated in Nepal since ancient times. Currently, it has taken a commercial form as a cash-generating business. Its commercial cultivation is done from the plains to the mountains or in areas with communication and market facilities. In Nepal, its cultivation is done as a seasonal crop in March/April and fruits are harvested till August/September. Cucumber is useful for people suffering from jaundice, kidney problems, constipation, indigestion, and its oil is considered good for the brain and body (Gautam et al., 2021). In Nepal, it is the seventh most important vegetable crop in terms of area. The area, production and productivity of cucumber in Nepal is 9,978 ha, 1,52,862 mt and 15.32 mt/ha respectively (MoALD, 2023). The productivity of cucumber is very low in Nepal as compared to Asia (49.61 mt/ha) and the world average productivity (39.34 mt/ha) (FAO Stat, 2021).

Cucumber is a vine crop. Depending on the variety, its length of vine extends from 2-3 meters to 8-10 meters, and the height of the branches also varies according to the variety. Cucumber vine can be divided into three parts according to gender characteristics, according to which, male flowers grow in the lower part of the vine, male and female flowers grow in the middle or one after the other, and female flowers grow in the upper part of the vine. When cucumbers are planted in winter months, the length of the day is short and the temperature is low, the production of male flowers is low and the production of female flowers is high, while the production of male flowers is high and the production of female flowers is low in cucumbers planted in summer. The main vine of cucumber planted in normal season produces many female flowers. If cucumbers are planted in summer season, after 4-6 nodes of flowering, male flowers appear for 5-6 nodes and then female flowers appear, while in winter season cucumbers, female flowers appear after 2-3 nodes of flowering, but this process can vary depending on the variety. The ratio of male and female flowers should be low for maximum production and quality of cucumbers (Gautam et al., 2006). There is an increasing problem of low fruit set and if there is fruit set, very small fruits are developed that are deteriorated on the mother plant. Imbalance of sex ratio is the main problem of declining production in Nepal and influences the male and female ratio by various biotic and abiotic factors, environmental issues, disease and pest, and many other factors.

According to research studies, the 1st and 2nd generation branches comprise a majority of male flowers rather than female, leading to a very small ratio of 14:1 (Male: Female) flowers in the branch that creates a false illusion of heavy flowering but with very low fruiting (Chaurasiya et al., 2020). Thus, 3rd generation branches flush with a majority of female flowers. The main aim should be focused on keeping 3rd generation branches rather than others. Normally, there is an unequal male-to-female flower ratio in cucumber, with a greater number of male flowers than female flowers. If we use these techniques, the number of female flowers increases, which triggers increased productivity.

The physiological basis for the observed effects of generation cutting on sex expression lies in the manipulation of plant hormone balance and carbohydrate allocation. Pruning alters the source-sink relationships within the plant, redirecting assimilates from vegetative growth to reproductive development. This redirection influences the levels of endogenous plant hormones, particularly gibberellins, auxins, and cytokinins, which play a critical role in sex determination in cucurbits. Gibberellins are known to promote male flower formation, while cytokinins and ethylene promote female flower development. By removing apical dominance through cutting, the distribution of these hormones is altered, favouring female flower production on the tertiary branches. Additionally, pruning improves light penetration and air circulation within the canopy, enhancing photosynthetic efficiency and carbohydrate availability, which supports the development of female flowers and fruits. This mechanistic understanding provides the theoretical foundation for the observed agronomic benefits of 3G cutting.

The present study was therefore undertaken with the following objectives:

1) To evaluate the effect of different generation cuttings on growth, sex expression, and yield of cucumber.
2) To identify the optimal generation cutting practice for maximizing cucumber productivity in the Godawari municipality area.

II. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was conducted at farmers' fields of Godawari municipality, Fakalpur, Kailali, Nepal during the summer season of 2023/24 to evaluate the effect of different generation cutting on growth, sex expression and yield of cucumber. The experiment field is located in the tropical region at an elevation of 200 m, having 28°46'45.4"N (28.7792800°) latitude and 80°36'00.5"E (80.6001400°) longitude. Phakalpur is located in the region of Sudurpashchim Pradesh. The distance from Phakalpur to Nepal's capital Kathmandu is approximately 478 km.

2.2 Soil Properties of Study Area

Soil samples were collected randomly at a depth of 0-20 cm by using a sampling auger. The collected soil samples were analysed at the soil and fertilizer testing laboratory, Sundarpur, Kanchanpur. The soil properties of the study area are shown in Table 1.

TABLE 1
SOIL PROPERTIES OF STUDY AREA

Parameter	Value
Soil pH	7.32
Bulk Density	1.37
Particle Density	2.14
Water Holding Capacity	53.84
Porosity	35.5
Organic Matter (%)	0.71
Available Nitrogen (%)	0.04
Available Phosphorus (%)	12.58
Available Potassium (%)	88.15
Soil Texture	Sandy clay loam soil

2.3 Experimental Material

Different generations of vine i.e., first and second generation cuttings were used to evaluate the vegetative, flowering and yield attributes. We used the Malini variety of cucumber, which is a hot temperature tolerant variety and good in taste compared to other varieties. Seeds were sown in polybags in February by filling vermicompost and soil in a 2:1 ratio. Seedlings were transplanted when 3-4 true leaves emerged, one month after seed sowing.

2.4 Experimental Design and Treatment Details

The experiment was laid out in Completely Randomized Block Design (RCBD) with 3 treatments viz; T1 (control), T2 (First branch cutting: 2G), T3 (Second branch cutting: 3G), and 6 replications. Each plot consisted of 2 rows and each row consisting of 5 plants, so the total number of plants per plot was 10, with spacing 100 cm × 100 cm. Each replication size was 40 m^2 so the total field size for research was 240 m^2 consisting of 18 plots each of size 5 m × 2 m.

2.5 Treatment Application Method

The treatment application method was as follows:

T1 (Control - No Cutting): The main stem (primary branch) was allowed to grow freely without any cutting or pruning intervention throughout the growing period.

T2 (First Generation Cutting - 2G): The main stem (primary branch) was allowed to grow freely until it reached approximately 6-8 feet in height. No side branches were allowed to develop until 5 leaves from the main stem; if any developed, they were pinched out. The tip of the secondary branch was removed when it reached 2-3 feet in height to promote tertiary branches, which were then allowed to grow freely.

T3 (Second Generation Cutting - 3G): The main stem (primary branch) was allowed to grow freely until it reached approximately 6-8 feet in height. No side branches were allowed to develop until 5 leaves from the main stem; if any developed, they were pinched out. The tip of the secondary branch was also removed when it reached 2-3 feet in height to promote tertiary branches. Then, the tertiary branch was allowed to grow without any further cutting.

2.6 Sampling Techniques

A random sampling method was used where all units had the same chance of selection for the sample. Eighty plants were selected from two hundred and forty plants for sampling from the whole research area. Sampling was done on two plants from every row, that is, four plants from a single plot.

2.7 Data Collection

Vegetative characteristics, flowering and yield behaviours were recorded. The data on internodal length of primary branch, internodal length of secondary branch, stem diameter of primary and secondary branches, male and female flowers of primary, secondary and tertiary branches, number of fruits, fruit length, fruit diameter, fruit weight, and yield per hectare were recorded. Quality parameters including TSS (°Brix), titratable acidity (TA), TSS/TA ratio, colour, size, appearance, and taste were also assessed using the 1-3 scale method (Subedi et al., 2024). Plant protection measures were applied as needed following

recommended practices, including the application of fungicides (mancozeb) and insecticides (cypermethrin) at recommended doses to manage pests and diseases. Harvesting was done when fruits reached marketable size (approximately 15-20 cm length) and was carried out at 3-4 day intervals throughout the harvesting period.

2.8 Statistical Analysis

The data were subjected to one-way Analysis of Variance (ANOVA), and significant means were separated using the Least Significant Difference (LSD) test at 1% or 5% level of significance (Gomez & Gomez, 1984). All statistical analyses were done in R Studio version 4.0.2 using the DoE bioresearch package.

III. RESULTS AND DISCUSSION

3.1 Effect of Different Generation Cutting on Growth Parameters of Cucumber

All the growth attributes of cucumber taken at the maturity stage in the study, viz. internodal length of primary and secondary branches, stem diameter of primary and secondary branches, are shown in Table 2. There is no significant difference in internodal length and stem diameter of primary and secondary branches among the different generation cuttings of cucumber. Internodal length determines the height and number of nodes per plant, but 3G cuttings showed no significant differences in internodal length of cucumber. Similar findings were also reported by Kumar et al. (2008) and Dogra (2012). By limiting the growth of unproductive plant parts, pruning operations promote regulated growth by enhancing photosynthetic efficiency, which in turn promotes cell expansion in other plant parts. This is in close agreement with the findings of Yu et al. (2013), Jat et al. (2011), Coggins et al. (2014), and Singh et al. (2016).

TABLE 2
INTERNODE LENGTH AND STEM DIAMETER OF DIFFERENT GENERATION CUTTINGS OF CUCUMBER

Treatment	Internode Length (cm)		Stem Diameter (cm)	
	Primary Branch	Secondary Branch	Primary Branch	Secondary Branch
T1 (Control)	6.10a	8.67a	8.98a	4.31b
T2 (2G)	5.70a	8.50a	8.84ab	4.94a
T3 (3G)	5.82a	8.91a	8.45b	4.78ab
$LSD_{0.05}$	0.55	0.6002	0.44	0.54
SEm(±)	0.17	0.18	0.13	0.16
F test	NS	NS	NS	NS
CV %	6.49	4.73	3.45	8.014
Grand Mean	5.87	8.69	8.76	4.68

Note: Means followed by same letter(s) in a column are not significantly different at $p \leq 0.05$.

3.2 Effect of Different Generation Cutting on Flowering Parameters of Cucumber

The study assessed the impact of different generation cuttings on the growth, sex expression and yield attributes of cucumber. The results showed that T3 (second generation cutting: 3G cutting) had the most favourable outcomes across the various parameters. The effect of different generation cutting was found to be significant on the number of male and female flowers, as shown in Table 3. The results revealed that T1 (Control) showed statistically maximum number of male and female flowers in the primary branch of the cucumber plants (23.40), whereas treatments T2 (2G: first generation cutting) and T3 (3G: second generation cutting) observed minimum number of male and female flowers, with T2 being statistically at par with T3. In contrast to this, 2G and 3G cutting recorded the lowest number of male and female flowers in primary branches. Similar results of male flowers in primary branches were also reported by Verma et al. (2023). The first-generation branches induced more male flowers than female flowers in a high ratio of 14:1, giving a fallacious conception of heavy flowering with very few numbers of fruiting.

Analysis of secondary branches indicated that treatment T1 (Control) and T2 (2G) had the highest number of male flowers (11.13 and 9.49, respectively), while T3 (3G) had the lowest (4.45) male flowers. Whereas the highest number of female flowers were observed in treatment T3 (1.78) and T2 (1.75), while the lowest number of female flowers were recorded in the control treatment. Similar results were also reported by Singh et al. (2021). Second-generation branches of the Cucurbitaceae family tend to produce more male flowers than female flowers.

In tertiary branches, there were no significant differences in the number of male flowers among the treatments, but the highest number of female flowers (1.99) was recorded in 3G cuttings and the lowest number of female flowers (1.06) was recorded in no-cutting branches. Similar results were also reported by Chapagain et al. (2022). Due to the modulation of hormonal levels in plants, 3G cutting induces tertiary branches which are responsible for the production of female flowers by dominating male flowers.

TABLE 3
MALE AND FEMALE FLOWERS OF DIFFERENT GENERATION CUTTINGS OF CUCUMBER

Treatment	Primary Branch		Secondary Branch		Tertiary Branch	
	Male Flower	Female Flower	Male Flower	Female Flower	Male Flower	Female Flower
T1 (Control)	23.40a	3.15a	11.13a	1.32b	4.92	1.06c
T2 (2G)	12.90b	1.15b	9.49a	1.75a	5.15	1.42b
T3 (3G)	12.95b	1.45b	4.45b	1.78a	5.03	1.99a
$LSD_{0.05}$	1.83	0.41	1.69	0.31	0.39	0.19
SEm(±)	0.56	0.12	0.51	0.09	0.12	0.05
F test	***	***	***	*	NS	***
CV %	7.66	14.96	13.903	13.18	5.41	8.87
Grand Mean	16.41	1.91	8.35	1.62	5.03	1.49

Note: Means followed by same letter(s) in a column are not significantly different at p ≤ 0.05. NS = Non-significant; * = Significant at p ≤ 0.05; *** = Significant at p ≤ 0.001.

Data presented in Table 4 show that different generation cuttings had a highly significant effect on the male-to-female flower ratio of cucumber. The results revealed that the maximum ratio (11.72) was recorded in treatment T3 (second generation cuttings: 3G). No cutting and first generation cutting had no significant differences in flower ratio of cucumber, whereas the minimum ratio (7.46) was recorded in treatment T1 (no cutting: control) in primary branches. The findings of the present study are in accordance with the studies of Rajalingam et al. (2017) and Hirama et al. (2011).

In the case of secondary branches, the higher ratio (6.23) and (5.51) was recorded in no cutting and first generation cutting, respectively, whereas the minimum ratio (3.41) was recorded in second generation cutting. No cutting of branches has been shown to increase the incidence of various diseases and pests that lead to decreasing yield by production of non-productive flowers (Premalatha et al., 2006; Ekwu et al., 2012; Khoshkam, 2016).

Analysis of tertiary branches indicated that the maximum ratio (4.75) was recorded in the control treatment (no cutting) followed by first generation cutting (3.69), whereas the minimum ratio (2.52) was recorded in second generation cuttings. Our results are in accordance with Thakur et al. (2018) and Chapagain et al. (2022). They studied that the ratio of male and female flowers should be low for maximum production and quality of cucumbers (Gautam et al., 2006).

TABLE 4
MALE-TO-FEMALE FLOWER RATIO OF DIFFERENT GENERATION CUTTINGS OF CUCUMBER

Treatment	Ratio of Male and Female Flowers		
	Primary Branch	Secondary Branch	Tertiary Branch
T1 (Control)	7.46b	6.23a	4.75a
T2 (2G)	9.36ab	5.51a	3.69b
T3 (3G)	11.72a	3.41b	2.52c
$LSD_{0.05}$	2.45	1.07	0.91
SEm(±)	0.75	0.32	0.28
F test	*	***	**
CV %	17.64	14.56	17.18
Grand Mean	9.52	5.05	3.65

Note: Means followed by same letter(s) in a column are not significantly different at p ≤ 0.05. * = Significant at p ≤ 0.05; ** = Significant at p ≤ 0.01; *** = Significant at p ≤ 0.001.

3.3 Effect of Different Generation Cutting on Yield Attributing Parameters of Cucumber

Based on the analysis of variance, there is an effect of different generation cuttings on yield attributing parameters of cucumber. Different generation cutting highly affected the fruit number, fruit weight per plant, fruit length, fruit diameter and yield, presented in Table 5. Results indicated that there are no significant differences in weight, length, and diameter of fruit among different generation cuttings of cucumber. Duong (1999) recorded that 3G cutting had no significant impact on the length and fruit weight of cucumber. According to Mir et al. (2019), different generation cutting stimulated the process of photosynthesis that leads to improvement of fruit characters by enlargement of cells. In our findings, there are no significant differences in fruit characters, especially fruit length, diameter and weight of cucumber.

But results observed that the fruit number per plant was positively influenced by different generation cuttings. The greater number of fruits (6.05) per plant was recorded in 3G cutting followed by 2G cutting (5.60), whereas the minimum number of fruits per plant (5.10) was recorded in no cutting treatment. Similar results were obtained by Shah et al. (2020). The findings are in accordance with the findings of Mir et al. (2019) who observed that an increase in the number of female flowers due to generation cuttings might have increased the number of fruits in each plant. Eifediyi and Remison (2009) observed that 3G cutting reduces the diseases and insect pests by uniform circulation of air around the plant canopy that enhances the marketable yield of cucumber.

3G (second generation cuttings) in cucumber produced significantly higher production (21.48 ton/ha) followed by 2G (20.03 ton/ha), while the lowest production (18.18 ton/ha) was recorded in no cutting (control) treatment. This is because different generation cuttings maintain a balance of vegetative growth and reproductive growth, especially the number of fruits on branches, which triggers increased production of cucumber (Humphries and Vermillion, 1994).

The highest production recorded under the 3G cutting plants may have been caused by a larger number of fruits. This is consistent with the research studied on cucumber by Shivaraj et al. (2018). By allowing plants adequate light exposure, pruning boosted photosynthesis, which in turn increased the source-to-sink ratio and raised the yield. Due to the effect of different generation cutting, it helps in cell enlargement, separation, synthesis of protein with maintained auxin concentration. So that contributes to good vegetative growth of side branches and increased number of female flowers which ultimately increased yields. Devi et al. (2016); Chaurasiya et al. (2020); Mardhiana et al. (2017); and Mardhiana et al. (2017) studied that different generation cutting facilitates CO_2 level with optimum use of sunlight, better photosynthesis, and air circulation within the canopy by preventing unnecessary vegetative growth of cucumber which ultimately increases yield. Whereas no cutting branches increases male flowers by slow and dense vegetative growth that decreases yield due to high incidence of various diseases and insect pests (Khoshkam, 2016; Ekwu et al., 2012; Premalatha et al., 2006).

TABLE 5
YIELD AND YIELD ATTRIBUTE TRAITS OF DIFFERENT GENERATION CUTTING OF CUCUMBER

Treatments	Fruit Number	Fruit Weight per Plant (g)	Fruit Length (cm)	Fruit Diameter (cm)	Yield (ton/ha)
T1 (Control)	5.10c	356.63	20.95	5.27	18.18c
T2 (2G)	5.60b	358.03	20.55	5.29	20.03b
T3 (3G)	6.05a	355.23	20.24	5.24	21.48a
$LSD_{0.05}$	0.36	17.63	0.89	0.21	1.29
SEm(±)	0.11	5.4	0.27	0.06	0.39
F test	**	NS	NS	NS	**
CV %	4.44	3.39	2.98	2.74	4.47
Grand Mean	5.58	356.63	20.58	5.27	19.9

Note: Means followed by same letter(s) in a column are not significantly different at $p \leq 0.05$. NS = Non-significant; ** = Significant at $p \leq 0.01$.

3.4 Effect of Different Generation Cutting on Quality of Cucumber

The effect of different generation cutting on the quality of cucumber is shown in Table 6 and Table 7. The results revealed that there are no significant differences in TSS, TA and TSS/TA ratio, colour, size, appearance and taste among different generation cuttings of cucumber. Das et al. (2018) and Kumari & Topno (2023) also reported that Malini had similar TSS, TA, TSS/TA, colour, size, appearance and taste even in different generation cuttings. These findings are in agreement with the results reported

by Kumar et al. (2014). The redirection of nutrients and carbohydrates could theoretically increase sugar accumulation (TSS) in fruits, though no direct TSS data is reported in studies on 3G cutting (Verma et al., 2023; Kumar et al., 2025). Different generation cutting induces mild stress, potentially activating defense mechanisms and ROS metabolism that could stabilize TSS (Madhumala et al., 2024). A study on fresh-cut cucumbers (mechanical stress) noted ROS-related metabolic shifts but did not report significant TSS changes (Guan et al., 2023).

The absence of significant reports on titratable acidity in these studies, despite explicit measurement of quality attributes in some cases, supports the conclusion that different generation cutting does not significantly influence cucumber TA. The emphasis across the 3G cutting literature is on flowering and yield impacts rather than biochemical quality parameters like TA. Kumar, Mishra & Sher (2025) investigated the influence of 3G cutting on cucumber titratable acidity, but it was not highlighted as significantly affected by different cutting levels, indicating that generation cutting did not materially alter acidity levels in cucumber fruit compared to yield and other attributes.

TABLE 6
EFFECT OF DIFFERENT GENERATION CUTTING ON QUALITY OF CUCUMBER

Treatments	TSS (°Brix)	TA (% Malic acid)	TSS/TA
T1 (Control)	2.5	0.165	15.118
T2 (2G)	2.5	0.169	15.794
T3 (3G)	2.683	0.171	15.638
$LSD_{0.05}$	0.29	0.21	0.17
SEm(±)	0.19	0.35	0.48
F test	NS	NS	NS
CV %	7.3	3.93	6.33
Grand Mean	2.64	0.17	15.41

Note: NS = Non-significant.

TABLE 7
EFFECT OF DIFFERENT GENERATION CUTTING ON QUALITY OF CUCUMBER

Treatments	Colour (1-3 scale)	Size (1-3 scale)	Appearance (1-3 scale)	Taste (1-3 scale)
T1 (Control)	2	2.63	2.33	2.25
T2 (2G)	2	2.6	2.3	2.3
T3 (3G)	2	2.65	2.28	2.33
$LSD_{0.05}$	0.27	0.12	0.11	0.24
SEm(±)	0.92	0.44	0.93	0.28
F test	NS	NS	NS	NS
CV %	9.58	4	4.27	7.84
Grand Mean	1.93	2.02	1.86	2.06

NOTE: 1-3 scale, where 1 = less acceptance value and 3 = high acceptance value (Subedi et al., 2024). NS = Non-significant.

Different generation cutting in cucumber generally does not produce significant changes in external fruit colour characteristics, as the existing agronomic studies predominantly report effects on flowering and yield traits rather than on fruit colour, and available data do not show consistent effects of different generation cutting treatments on colour attributes. Independent of cutting, cucumber fruit skin colour is strongly determined by pigment genetics, mainly chlorophyll and flavonoid content, regulated by specific genes rather than horticultural practices like generation cutting (Peng et al., 2010). Fruit colour formation is controlled largely by chlorophyll and pigment gene expression, indicating that colour is mainly genetically determined and less responsive to pruning practices (Wang et al., 2019).

Forss et al. (1962) reported that effects of generation cutting on morphological and yield traits and some basic quality parameters do not identify significant or consistent changes in sensory quality traits (such as taste) attributable to different cutting treatments. Cucumber flavour and taste are complex traits influenced by genetic background and volatile and soluble metabolite profiles rather than pruning alone; available research emphasizes that pruning/cutting effects on biochemical quality like soluble solids or acidity do not consistently translate into noticeable taste differences (Zhang et al., 2004).

IV. DISCUSSION

The results of this study demonstrate that different generation cutting practices significantly influence the sex expression and yield of cucumber, with the second generation cutting (3G) showing superior performance across most parameters. The improved sex expression under 3G cutting can be attributed to the manipulation of plant hormone balance and carbohydrate allocation. Pruning removes apical dominance, altering the distribution of endogenous plant hormones. Gibberellins, which promote male flower formation, are reduced, while cytokinins and ethylene, which promote female flower development, are relatively increased. This hormonal shift favours the production of female flowers on the tertiary branches, leading to a more favourable male-to-female flower ratio.

The significant reduction in the male-to-female flower ratio under 3G cutting (from 7.46 to 11.72 on primary branches, and from 6.23 to 3.41 on secondary branches) is particularly noteworthy. This indicates that 3G cutting effectively promotes female flower production while suppressing male flower production. The higher number of female flowers translates directly into higher fruit set and ultimately higher yield. The 18.15% increase in yield under 3G cutting compared to the control demonstrates the practical significance of this finding.

The lack of significant effects on fruit quality parameters (TSS, TA, colour, size, appearance, taste) suggests that generation cutting primarily affects yield-related traits rather than fruit quality. This is consistent with the findings of previous studies (Kumar et al., 2014; Das et al., 2018; Kumari & Topno, 2023). The stable quality parameters across treatments indicate that farmers can adopt 3G cutting without compromising fruit quality, making it an attractive option for improving productivity.

The findings of this study have important practical implications for cucumber growers in Nepal. The adoption of 3G cutting can significantly increase cucumber yield by improving sex expression and fruit set. The technique is relatively simple and can be easily adopted by farmers with minimal training. However, the additional labour required for pruning should be considered in the economic analysis. Future research should focus on the economics of different cutting practices, the integration of cutting with other management practices, and the validation of these findings across different locations, seasons, and cultivars.

V. CONCLUSION

The study demonstrates that different generation cutting practices have varying impacts on cucumber growth, flowering, yield, and quality. Specifically, the second-generation branch cutting (3G) showed superior performance in terms of phenological and yield parameters. The ratio of male and female flowers was significantly lower in second-generation branch cutting by 3G cutting with 78.50% and 26.10% over primary and secondary branches, respectively. Similarly, the fruit yield was significantly higher by 3G cuttings with 7.24% and 18.15% increase in yield over 2G and no cutting. In quality parameters, there was similar taste among the different treatments. Thus, for optimizing cucumber production and profitability in similar agro-meteorological conditions, adopting the second-generation branch cutting practice is recommended.

Limitations of the Study: The study was conducted at a single location (Godawari municipality, Kailali) over a single season (summer 2023/24) using a single cultivar (Malini). The results may vary under different agro-ecological conditions, seasons, or with other cultivars. The additional labour requirements for 3G cutting were not quantified, and an economic analysis was not performed. Future research should include multi-location trials across different seasons, evaluation of different cultivars, economic analysis of the cutting practices, and investigation of the physiological mechanisms underlying the observed effects.

ACKNOWLEDGEMENTS

This manuscript has been taken from the project "International Centre for Integrated Mountain Development (ICIMOD) for improved yield and quality of cucumber in ICIMOD command areas of Godawari municipality, Fakalpur, Kailali." The farmers of Fakalpur, Kailali are thanked very much for providing their support in making the project accomplished. The Far Western University, FoA faculty members and students were the source of inspiration to accomplish the on-farm research work with grant success.

AUTHORS' CONTRIBUTIONS

- B.R. Bhattarai - Accomplished research on cucumber as a principal researcher.
- *Bohara - Worked as a research assistant.*
- *Rajbanshi - Worked as a research assistant.*

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest pertaining to the publication of this paper.

REFERENCES

[1] Chapagain, U., Bhandari, S., Shrestha, Y. M., Bista, S., & Dahal, J. (2022). Effect of pruning and fertilizers on growth, flowering and yield of cucumber (*Cucumis sativus* L.) under protected structure in Panchthar, Nepal. *International Journal of Applied Sciences and Biotechnology*, *10*(3), 171-181.

[2] Chaurasiya, D. K., Kumar, M., Sahni, S., & Singh, S. (2020). 3G cutting: A wonderful technique to redouble the production of cucurbits. *Biotica Research Today*, *2*(12), 1308-1310.

[3] Coggins, C. W., & Lovatt, C. J. (2014). Plant growth regulators. In L. Ferguson & E. E. Grafton-Cardwell (Eds.), *Citrus production manual*. UC ANR.

[4] Devi, J., Bakshi, P., Wali, V. K., Kour, K., & Sharma, N. (2016). Role of auxin and dates of planting on growth of cutting raised plantlets of Phalsa (*Grewia asiatica* L.). *The Bioscan*, *11*(1), 535-537.

[5] Dogra, L. K. (2012). *Genetic evaluation of some hybrids of cucumber under modified naturally ventilated greenhouse in mid hills of Western Himalayas* [M.Sc. thesis]. CSKHPKV.

[6] Duong, H. X. (1999). *Effect of pruning on yield and quality of cucumber* [Training report]. AVRDC, Kasetsart University.

[7] Eifediyi, E. K., & Remison, S. U. (2009). Effect of time of planting on the growth and yield of five varieties of cucumber (*Cucumis sativus* L.). *Report and Opinion*, *1*(5).

[8] Ekwu, L. G., Nwokwu, G. N., & Utobo, E. B. (2012). Effect of mulching materials and pruning on growth and yield of cucumber (*Cucumis sativus* L.). *International Journal of Agriculture and Rural Development*, *15*(2), 1014-1021.

[9] FAO STAT. (2021). *Food and Agriculture Organization*. Retrieved January 16, 2021.

[10] Forss, D., Dunstone, E., Ramshaw, E., & Stark, W. (1962). The flavor of cucumbers. *Journal of Food Science*, *27*, 90-93.

[11] Gautam, I. P., & Gauli, R. (2006). *Year-round production technology of cucumber*. Nepal Agriculture Research Council, Regional Agriculture Research Centre.

[12] Gautam, I. P., Pradhan, N. G., Subedi, S., & Thakur, M. K. (2021). Evaluation of cucumber hybrids for yield and quality under plastic house and open field conditions. *Nepalese Horticulture*, *15*, 52-63.

[13] Guan, Y., Hu, W., Wang, L., & Yang, B. (2023). Different cutting methods affect the quality of fresh cut cucumbers by regulating ROS metabolism. *Horticulturae*, *9*(4), 514.

[14] Hirama, N., Miusawa, H., & Azuhata, F. (2011). Effects of different levels of greenhouse ventilation and training methods on cucumber growth and yield under forcing culture. *Horticultural Research (Japan)*, *10*, 499-505.

[15] Humphries, E. G., & Vermillion, D. L. (1994). Pickling cucumber vine pruning treatments and their implications for mechanical harvesting. *Transactions of the ASAE*, *37*(1), 71-75.

[16] Jat, G. S. (2011). *Studies on hybrid seed production in bitter gourd under insect-proof net house and open-field conditions* [M.Sc. thesis]. PG School, ICAR-IARI.

[17] Khoshkam, S. (2016). The effect of pruning and planting density on yield of greenhouse cucumber in Jiroft. *International Journal of Scientific Engineering and Applied Science*, *2*(8), 212-227.

[18] Kumar, A., Kumar, S., & Pal, A. K. (2008). Genetic variability and characters association for fruit yield and yield traits in cucumber. *Indian Journal of Horticulture*, *65*(4), 423-428.

[19] Kumar, A., Mishra, S., & Sher, A. (2025). Influence of 3G cutting on the performance of cucumber (*Cucumis sativus* L.) for growth, yield and quality attributes. *International Journal of Research in Agronomy*, *8*(8), 174-178.

[20] Kumar, N. B., & Saravaiya, S. N. (2014). Response of parthenocarpic cucumber to fertilizers and training systems under naturally ventilated polyhouse in sub-tropical conditions. *International Journal of Current Research*, *6*(8), 8051-8057.

[21] Madhumala, K., Kumar, V., & Kumar, K. (2024). Physiology and mechanism of pruning in fruit crops. *International Journal of Advanced Biochemistry Research*, *8*(12), 443-445.

[22] Mardhiana, A. P., Adiwena, M., Kartina, S. D., Wijaya, R., & Maliki, A. (2017). Effects of pruning on growth and yield of cucumber (*Cucumis sativus* L.) mercy variety in the acid soil of North Kalimantan, Indonesia. *Cell Biology and Development*, *1*(1), 13-17.

[23] Mir, A. A., Sadat, M. A., Amin, M. R., & Islam, M. N. (2019). Pruning: A mechanical stress inducing method to improve growth and yield of Bangladeshi local cucumber variety "Baromashi". *International Journal of Business, Social and Scientific Research*, *7*(4), 31-35.

[24] MoALD. (2023). *Statistical information on Nepalese agriculture*. Ministry of Agriculture and Livestock Development, Government of Nepal.

[25] Peng, X., Chen, Z., Shi, F., Wu, X., Wang, Y., & Gao, L. (2010). Effects of different grafting methods on growth and fruit quality of cucumber in solar greenhouse. *Northern Horticulture*, *15*, 122-124.

[26] Premalatha, M. G. S., Wahundeniya, K. B., Weerakkody, W. A. P., & Wicramathunga, C. K. (2006). Plant training and spatial arrangement for yield improvement in greenhouse cucumber (*Cucumis sativus* L.) varieties. *Tropical Agricultural Research*, *18*, 346-357.

[27] Rajalingam, G. V., Rajasree, V., Arumugam, T., & Saraswathi, T. (2017). Influence of different training systems in cucumber under naturally ventilated poly house. *International Journal of Chemical Studies*, *5*(6), 1453-1455.

[28] Shah, P. S., Nabi, G., Khan, M., Hussain, S., & Jan, J. A. (2020). Performance of sponge gourd cultivars under organic and inorganic fertilizer regimes. *Sarhad Journal of Agriculture*, *36*(2), 478-488.

[29] Shivaraj, D., Lakshminarayana, D., Prasanth, P., & Ramesh, T. (2018). Studies on the effect of pruning on cucumber cv. Malini grown under protected conditions. *International Journal of Current Microbiology and Applied Sciences*, *7*, 2019-2023.

[30] Singh, A. K., Munsi, A. D., Veerpal, S., Mukul, K., & Sellam, P. (2016). Influence of gynoecious cucumber varieties and spacing on yield and economics during off-season production under protected condition in North Indian plains. *International Journal of Basic and Applied Agricultural Research*, *14*, 54-58.

[31] Verma, A. K., Goutam, E., Gangwar, V., Singh, P., Prajapati, J., Singh, D., Mishra, A. K., Patel, V., & Singh, R. (2023). 3G cutting: An innovative tool in cucurbitaceous crops to boost the production and doubling the income of small farmers in a per unit area. *International Journal of Plant & Soil Science*, *9*(35), 71-76.

[32] Wang, W., Miao, H., Dong, S., & Zhang, S. (2019). QTL mapping and genome-wide association study reveal two novel loci associated with green flesh color in cucumber. *BMC Plant Biology*, *19*, 243.

[33] Yu, K., Fan, Q., Wang, Y., Wei, J., Ma, Q., Yu, D., & Li, J. (2013). Function of leafy sepals in *Paris polyphylla*: Photosynthate allocation and partitioning to the fruit and rhizome. *Functional Plant Biology*, *40*, 393-399.

[34] Zhang, S., Guo, Y., & Xu, C. (2004). Inheritance of bitterness in cucumber. *Acta Horticulturae Sinica*, *31*, 613-616.

International Journal of Environmental & Agriculture Research (IJOEAR)
ISSN: [2454-1850]
[Vol-12, Issue-9, September- 2026]
Website: www.ijoear.com
Journal DOI: 10.25125/agriculture-journal

Physiological and Photosynthetic Variability among Tomato (*Solanum lycopersicum* L.) Genotypes under Pot Culture in Temperate Kashmir

Hilal A. Bhat[1]; Farooq A Khan[2*]; Zaffar Mahdi Dar[3]; Insha Mushtaq[4]; Urfia Jan[5]; Mohammad Amir[6]; Sumati Narayan[7]; Faheemullah Khan[8]

Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, J&K 190 025, India; #Lovely Professional University, Phagwara (Punjab), India
*Corresponding Author

Received:- 11 August 2026/ Revised:- 24 August 2026/ Accepted:- 06 September 2026/ Published: 15-09-2026

Abstract**—***The present study was conducted to assess genotypic variability in growth, biomass allocation, photosynthetic pigments and leaf gas-exchange characteristics of twelve tomato genotypes under pot culture conditions in the temperate climate of Kashmir. The experiment was conducted during 2018 and 2019 at Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, using a completely randomized design with four replications. Significant differences were observed among genotypes for plant height, shoot and root dry weight, shoot/root ratio, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, net photosynthetic rate, transpiration rate and stomatal conductance. Among the genotypes, 2016/Todvar-9 exhibited the highest plant height (82.13 cm), root dry weight (3.03 g plant^{-1}), chlorophyll a (1.28 mg g^{-1} FW), chlorophyll b (1.04 mg g^{-1} FW), total chlorophyll (2.32 mg g^{-1} FW) and carotenoids (0.32 mg g^{-1} FW). It also recorded the highest net photosynthetic rate (18.57 µmol CO_2 m^{-2} s^{-1}), transpiration rate (2.08 mmol H_2O m^{-2} s^{-1}) and stomatal conductance (237.10 µmol H_2O m^{-2} s^{-1}). Genotypes 2016/Todvar-7 and 2016/Todvar-10 also exhibited relatively higher physiological activity, whereas 2016/Todvar-2 and 2016/Todvar-3 generally recorded lower values. The results demonstrate substantial genotypic variability in physiological and photosynthetic attributes, with 2016/Todvar-9 showing superior overall physiological performance under the experimental conditions. This genotype warrants further evaluation for yield and adaptation under temperate conditions of Kashmir and for potential use in tomato improvement programmes.*

Keywords— Tomato; Genotypic variability; Photosynthesis; Chlorophyll; Gas exchange; Temperate Kashmir.

I. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated vegetable crops and is valued for its nutritional, economic and dietary importance. Its fruits are important sources of vitamins, minerals, carotenoids and other bioactive compounds, while the crop has considerable significance for fresh consumption and processing industries. However, tomato growth, development and productivity are strongly influenced by genotype and environmental conditions, making the identification of genotypes with superior adaptation and physiological performance an important component of crop improvement programmes. Recent studies have demonstrated substantial genetic variation among tomato germplasm for plant growth, biomass production, root characteristics and other agronomic traits, highlighting the value of physiological and phenotypic characterization for genotype selection (Roohanitaziani et al., 2020; Tripodi et al., 2022).

Photosynthesis is a major determinant of plant carbon gain and biomass accumulation and is influenced by the coordinated functioning of photosynthetic pigments, stomatal regulation, carbon assimilation and the photosynthetic apparatus. Chlorophylls are central to light harvesting and energy conversion, whereas carotenoids contribute to light harvesting and

photoprotection. Leaf gas-exchange attributes, particularly net photosynthetic rate, stomatal conductance and transpiration rate, provide important indicators of carbon assimilation and plant water relations. Recent advances in tomato research have emphasized the genetic basis of photosynthetic variation and the potential for exploiting photosynthetic traits in crop improvement (Dong et al., 2025). Differences among genotypes in gas exchange and photosynthetic responses may also become particularly important under environmental constraints, because tomato genotypes can employ contrasting physiological strategies for maintaining growth and photosynthetic function (Francesca et al., 2024; Bhattarai et al., 2024).

Genotypic variation in physiological traits is closely associated with differences in biomass accumulation and resource acquisition. Biomass partitioning between shoots and roots reflects the allocation of assimilates towards above- and below-ground organs and may influence plant establishment, nutrient acquisition and overall growth. Recent evaluation of diverse tomato accessions under contrasting nitrogen regimes demonstrated substantial variation in growth, biomass traits and pigment-related characteristics, with root biomass identified as one of the useful traits associated with nutrient-use efficiency (Flores-Saavedra et al., 2024). Similarly, comparative studies involving diverse tomato genotypes have shown that genotype, environment and their interaction can substantially influence morpho-physiological performance (Tripodi et al., 2022). Such variation provides an opportunity to identify genotypes with favourable physiological attributes for subsequent breeding and adaptation studies.

The importance of physiological characterization is further increased under changing climatic conditions. Temperature extremes can markedly affect photosynthesis, stomatal behaviour, growth and reproductive performance in tomato, with genotypes differing considerably in their physiological responses to environmental stress (Francesca et al., 2024; Bhattarai et al., 2024). Recent studies have shown that physiological and photosynthetic traits can contribute to the identification of contrasting tomato genotypes under temperature stress and other environmental constraints, although individual physiological traits may not always provide sufficient information for genotype selection when considered independently (Francesca et al., 2024; Bhattarai et al., 2024). Therefore, an integrated assessment of growth, biomass allocation, photosynthetic pigments and gas-exchange characteristics can provide a more comprehensive understanding of genotypic physiological performance.

Under the temperate agro-climatic conditions of Kashmir, evaluation of tomato genotypes is particularly relevant because environmental conditions differ from those of the major tomato-growing regions of India. Earlier work under Kashmir conditions reported significant variability among tomato genotypes for plant growth, fruit yield and quality attributes (Bhat et al., 2022). However, the physiological basis underlying such genotypic differences, particularly with respect to biomass partitioning, photosynthetic pigments and leaf gas exchange, remains insufficiently characterized. In particular, there is limited information on the simultaneous evaluation of these physiological attributes among the tomato genotypes evaluated under Kashmir conditions. Such information can complement conventional yield and quality evaluation and help identify genotypes possessing favourable physiological traits for further adaptation and breeding studies.

Therefore, the present study was undertaken to evaluate the physiological and photosynthetic variability among twelve tomato genotypes under pot culture conditions in the temperate climate of Kashmir, with particular emphasis on plant growth, shoot and root biomass, biomass partitioning, photosynthetic pigments and leaf gas-exchange characteristics. It was hypothesized that the tomato genotypes would differ significantly in biomass allocation, photosynthetic pigment concentration and gas-exchange activity, and that genotypes exhibiting superior physiological performance could be identified for further evaluation under temperate conditions of Kashmir.

II. MATERIALS AND METHODS

2.1 Experimental Site and Conditions

The experiment was conducted during 2018 and 2019 at the Division of Basic Sciences and Humanities, Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shalimar, Srinagar, Jammu and Kashmir, India. The experimental site represents a temperate agro-climatic environment. During May - August, temperature ranged from 8.19–30.46 °C and relative humidity from 63.77–71.38%. Twelve tomato (*Solanum lycopersicum* L.) genotypes were evaluated, viz., 2016/Todvar-1 (G1), 2016/Todvar-2 (G2), 2016/Todvar-3 (G3), 2016/Todvar-4 (G4), 2016/Todvar-5 (G5), 2016/Todvar-7 (G6), 2016/Todvar-8 (G7), 2016/Todvar-9 (G8), 2016/Todvar-10 (G9), 2016/Todvar-11 (G10), 2016/Todvar-12 (G11) and 2017/Todvar-3 (G12).

2.2 Experimental Design and Plant Culture

The experiment was laid out in a completely randomized design with four replications. Forty-day-old healthy and uniform tomato seedlings were transplanted on 3rd May into 12-inch pots containing 8 kg soil. Each pot considered one experimental unit with two plant maintained per pot. The soil was collected from the experimental field of the Division of Basic Sciences and Humanities, SKUAST-K, Shalimar, and was classified as silty clay loam. The soil had a pH of 6.98, organic carbon content of 0.86%, and available N, P and K contents of 228.50, 19.25 and 203.20 kg ha^{-1}, respectively. A uniform basal nutrient application comprising 0.96 g N, 0.80 g P and 0.48 g K per pot was applied along with 250 g vermicompost. All genotypes received identical nutrient and crop-management practices throughout the experimental period. Irrigation was provided as and when required to maintain sufficient soil moisture and avoid visible water stress. Pots were maintained under open environmental conditions.

2.3 Growth and Biomass Measurements

Plant height was recorded at final harvest by measuring the distance from the soil surface to the apical growing point and expressed in centimetres. Fruit harvesting commenced after 15th July and continued at regular intervals until the fruits reached physiological maturity. At the end of the harvesting period, plants were uprooted, and roots were washed thoroughly to remove adhering soil. Shoot and root samples were oven-dried at 80 °C for 2 h and subsequently at 60°C until constant weight.

2.4 Photosynthetic Pigment Estimation

Chlorophyll a, chlorophyll b and total chlorophyll contents were estimated from fresh leaf samples using dimethyl sulfoxide (DMSO) extraction as described by Hiscox and Israelstam (1979), and calculated using the equations of Arnon (1949). Carotenoid content was estimated spectrophotometrically following the method of Wellburn (1994). For all pigment measurements, the third fully expanded leaf from the apex was used to ensure consistency across genotypes and replications.

2.5 Leaf Gas-Exchange Measurements

Leaf gas-exchange characteristics were measured using a TPS-2 portable photosynthesis system (PP Systems, USA). The TPS-2 operates as an open gas-exchange system in which CO_2 and H_2O concentrations of air entering and leaving the leaf cuvette are measured to determine photosynthetic and transpiration rates. Measurements were made between 11:00 and 12:00 h on fully expanded, physiologically active leaves (third leaf from the apex) under ambient light conditions (photosynthetically active radiation (PAR) ranging from 800–1200 μmol m^{-2} s^{-1}), with leaf temperature maintained at 25–28 °C. Net photosynthetic rate (Pn), transpiration rate (E) and stomatal conductance (gs) were recorded. Three measurements were taken per plant and the average was used for statistical analysis.

2.6 Statistical Analysis

Data recorded were subjected to analysis of variance using standard statistical procedures for agricultural experiments (Gomez and Gomez, 1984). Treatment means were compared using the critical difference (CD) at the 5% level of significance. Pearson's correlation coefficients were calculated to examine the relationships among the measured physiological traits.

III. RESULTS AND DISCUSSION

3.1 Growth and Biomass Partitioning

Significant differences were observed among the tomato genotypes for all growth and biomass-related attributes (Table 1). Plant height ranged from 64.19 cm to 82.13 cm, with 2016/Todvar-9 recording the greatest plant height, which was significantly higher than most of the other genotypes. Similarly, shoot dry weight varied from 33.10 g $plant^{-1}$ in 2017/Todvar-3 to 41.69 g $plant^{-1}$ in 2016/Todvar-11, closely followed by 2016/Todvar-9 (41.66 g $plant^{-1}$). Root dry weight also differed significantly, ranging from 1.71 g $plant^{-1}$ in 2016/Todvar-3 to 3.03 g $plant^{-1}$ in 2016/Todvar-9. Consequently, the shoot/root ratio varied considerably from 13.74 in 2016/Todvar-9 to 20.92 in 2016/Todvar-5, indicating substantial differences among genotypes in biomass partitioning between above- and below-ground organs.

The superior plant height and root dry weight of 2016/Todvar-9, together with its high shoot dry weight, indicate a comparatively vigorous growth habit and greater overall biomass accumulation under the prevailing pot-culture conditions. In contrast, 2016/Todvar-11 combined the highest shoot dry weight with relatively low plant height and root biomass, suggesting that genotypes may achieve similar shoot biomass through different patterns of biomass allocation. The relatively low shoot/root ratio of 2016/Todvar-9 indicates a greater proportional allocation of biomass towards roots compared with genotypes

such as 2016/Todvar-5 and 2016/Todvar-8. Root biomass is important for water and nutrient acquisition and has been identified as a useful trait associated with physiological performance and nutrient-use efficiency in diverse tomato germplasm (Flores-Saavedra et al., 2024). Genotypic differences in shoot and root biomass and their partitioning have also been reported in tomato under different nutritional and environmental conditions (Abduelghader et al., 2011; Bristow et al., 2021). Recent studies further demonstrate substantial genetic variation for plant growth and biomass traits among tomato accessions, supporting the interpretation that the differences observed in the present study are indicative of genotypic variation in growth strategy rather than uniform responses across genotypes (Flores-Saavedra et al., 2024; Basavaraj and Naik, 2024).

The observed variation in shoot/root ratio is physiologically relevant because biomass partitioning represents the balance between investment in photosynthetic organs and the below-ground system. In tomato, changes in environmental conditions can modify this allocation pattern, with altered shoot/root ratios reflecting adjustments in carbon allocation between shoots and roots (Albacete et al., 2008; Ji et al., 2021). Thus, the relatively greater root biomass of 2016/Todvar-9 may have contributed to its vigorous growth under the temperate conditions of Kashmir, although this relationship should be interpreted cautiously because root physiological activity, nutrient uptake and water-use efficiency were not directly measured in the present experiment.

TABLE 1
GROWTH AND BIOMASS PARTITIONING OF DIFFERENT TOMATO (*SOLANUM LYCOPERSICUM* L.) GENOTYPES UNDER POT CULTURE CONDITIONS (POOLED DATA OF 2018–2019)

Genotype	Plant Height (cm)	Shoot Dry Weight (g plant^{-1})	Root Dry Weight (g plant^{-1})	Shoot:Root Ratio
2016/Todvar-1	70.9	37.8	2.01	18.8
2016/Todvar-2	73	40.16	1.98	20.28
2016/Todvar-3	71.53	34.33	1.71	20.07
2016/Todvar-4	67.19	38.24	2.15	17.78
2016/Todvar-5	72.28	40.18	1.92	20.92
2016/Todvar-7	67	38.38	2.47	14.27
2016/Todvar-8	76.83	39.98	2.73	14.64
2016/Todvar-9	82.13	41.66	3.03	13.74
2016/Todvar-	65.61	37.9	2.23	16.99
2016/Todvar-	64.19	41.69	2.1	19.75
2016/Todvar-	72.45	37.33	1.97	18.94
2017/Todvar-3	64.68	33.1	2.07	15.99
CD (P<0.05)	**2.67**	**2.23**	**0.12**	**0.61**

3.2 Photosynthetic Pigments and Carotenoids

Significant genotypic differences were observed for chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content (Table 2). Chlorophyll a varied from 0.70 mg g^{-1} FW in 2016/Todvar-5 to 1.28 mg g^{-1} FW in 2016/Todvar-9, while chlorophyll b ranged from 0.43 mg g^{-1} FW in 2016/Todvar-2 to 1.04 mg g^{-1} FW in 2016/Todvar-9. Consequently, total chlorophyll ranged from 1.22 to 2.32 mg g^{-1} FW, with 2016/Todvar-9 recording the highest value. Carotenoid concentration also showed considerable variation, ranging from 0.16 mg g^{-1} FW in 2016/Todvar-2 to 0.32 mg g^{-1} FW in 2016/Todvar-9. The differences exceeded the respective critical differences, confirming significant genotypic variation in leaf pigment accumulation.

The consistently higher concentrations of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids in 2016/Todvar-9 indicate a comparatively greater investment in the leaf photosynthetic pigment system. Chlorophyll a is the principal photochemical pigment, whereas chlorophyll b is an important accessory pigment that broadens the light-harvesting capacity of the photosynthetic apparatus. Carotenoids contribute to light harvesting and, importantly, provide photoprotection by dissipating excess excitation energy and limiting photooxidative damage. Therefore, variation in these pigments can contribute to differences in the capacity of genotypes to intercept and utilize incident radiation, although pigment concentration alone does not establish photosynthetic efficiency.

The comparatively high pigment concentration of 2016/Todvar-9 is also consistent with its superior growth performance observed in Table 1. This genotype recorded the highest plant height (82.13 cm), highest root dry weight (3.03 g plant^{-1}) and near-highest shoot dry weight (41.66 g plant^{-1}). Such concurrence suggests that enhanced development of the photosynthetic

apparatus may have contributed to greater carbon assimilation and biomass accumulation. However, this relationship should be considered associative rather than causal because photosynthetic rate is also regulated by stomatal conductance, CO_2 diffusion, biochemical capacity and environmental conditions.

The genotypic variation observed here agrees with recent studies demonstrating substantial differences among tomato genotypes in chlorophyll accumulation and photosynthetic characteristics. For example, genotypic differences in chlorophyll a, chlorophyll b and related physiological traits have been reported among tomato cultivars, with some genotypes maintaining higher pigment concentrations together with superior physiological performance (Abdelkader et al., 2024; Francesca et al., 2024). Similarly, comparative evaluation of tomato cultivars has demonstrated significant variation in chlorophyll and carotenoid concentrations among genotypes (Flores-Saavedra et al., 2024). Genotypic differences in photosynthetic activity and chlorophyll-related traits have also been reported under different environmental and nutritional conditions (Bhattarai et al., 2024; Flores-Saavedra et al., 2024).

The variation in carotenoid concentration is particularly relevant because carotenoids have functions beyond light harvesting, including protection of the photosynthetic apparatus against excess excitation and oxidative stress. Thus, the relatively high carotenoid concentration in 2016/Todvar-9 may indicate a greater capacity for photoprotection under the prevailing environmental conditions. This interpretation is consistent with studies showing coordinated changes in chlorophyll and carotenoids in tomato in response to environmental stress and genetic differences (Ben Sedrine et al., 2024). Recent work has also demonstrated that tomato genotypes can differ considerably in their photosynthetic and physiological responses to environmental stress, reinforcing the importance of considering pigment characteristics together with gas-exchange traits rather than as isolated indicators (Francesca et al., 2024; Dong et al., 2025).

TABLE 2
PHOTOSYNTHETIC PIGMENTS AND CAROTENOID CONTENT IN LEAVES OF DIFFERENT TOMATO (*SOLANUM LYCOPERSICUM* L.) GENOTYPES UNDER POT CULTURE CONDITIONS (POOLED DATA OF 2018–2019)

Genotype	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total Chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
2016/Todvar-1	0.84	0.48	1.32	0.18
2016/Todvar-2	0.79	0.43	1.22	0.16
2016/Todvar-3	0.92	0.66	1.58	0.23
2016/Todvar-4	0.81	0.52	1.33	0.19
2016/Todvar-5	0.7	0.66	1.36	0.22
2016/Todvar-7	1.01	0.94	1.95	0.3
2016/Todvar-8	1	0.72	1.72	0.26
2016/Todvar-9	1.28	1.04	2.32	0.32
2016/Todvar-10	1.08	0.75	1.83	0.31
2016/Todvar-11	0.8	0.55	1.35	0.2
2016/Todvar-12	0.86	0.53	1.39	0.23
2017/Todvar-3	1.06	0.67	1.73	0.28
CD (P<0.05)	**0.15**	**0.12**	**0.16**	**0.08**

3.3 Leaf Gas-Exchange Characteristics

Significant genotypic differences were observed for net photosynthetic rate (Pn), transpiration rate (E) and stomatal conductance (gs) (Table 3). Net photosynthetic rate ranged from 12.45 µmol CO_2 m^{-2} s^{-1} in 2016/Todvar-3 to 18.57 µmol CO_2 m^{-2} s^{-1} in 2016/Todvar-9. The highest Pn was recorded in 2016/Todvar-9, followed by 2016/Todvar-10 (17.67) and 2016/Todvar-7 (16.27 µmol CO_2 m^{-2} s^{-1}), whereas 2016/Todvar-3 showed the lowest value. Transpiration rate varied from 0.91 mmol H_2O m^{-2} s^{-1} in 2016/Todvar-3 to 2.08 mmol H_2O m^{-2} s^{-1} in 2016/Todvar-9, while stomatal conductance ranged from 124.69 to 237.10 µmol H_2O m^{-2} s^{-1}, with the minimum and maximum values recorded in 2016/Todvar-3 and 2016/Todvar-9, respectively. The observed differences exceeded the respective CD values, confirming significant genotypic variation in leaf gas-exchange behaviour.

The superior Pn of 2016/Todvar-9 was accompanied by the highest stomatal conductance and transpiration rate. This coordinated response suggests greater stomatal opening and consequently greater diffusion of atmospheric CO_2 into the leaf, supporting higher carbon assimilation. Stomatal conductance regulates the diffusion of CO_2 into leaves while simultaneously

influencing water vapour loss through transpiration; therefore, differences in gs can contribute substantially to genotypic variation in photosynthetic rate. Recent research on tomato has emphasized the importance of genetic variation in photosynthetic traits and the complex regulation of photosynthesis by stomatal, biochemical and molecular processes (Dong et al., 2025).

The results are also consistent with the pigment data presented in Table 2. 2016/Todvar-9, which recorded the highest chlorophyll a, chlorophyll b, total chlorophyll and carotenoid concentrations, also recorded the highest Pn, suggesting that development of the photosynthetic pigment system was associated with greater photosynthetic activity in this genotype. Chlorophyll concentration can influence the light-harvesting capacity of leaves, but the relationship between pigment concentration and carbon assimilation is not necessarily linear because photosynthesis is also controlled by stomatal conductance, CO_2 diffusion, electron transport and biochemical capacity. Thus, the simultaneous superiority of Todvar-9 in pigment concentration and gas exchange provides stronger evidence of its favourable physiological performance than either trait considered independently. Recent work has similarly emphasized the integrated genetic control of photosynthesis in tomato rather than reliance on a single physiological indicator (Dong et al., 2025).

The relatively high Pn values of 2016/Todvar-10 and 2016/Todvar-7 were also associated with comparatively high stomatal conductance (233.35 and 224.56 μmol H_2O m^{-2} s^{-1}, respectively), indicating that these genotypes maintained relatively active stomatal regulation and CO_2 exchange. In contrast, 2016/Todvar-2 and 2016/Todvar-3 exhibited relatively low stomatal conductance and transpiration, accompanied by lower photosynthetic rates. This pattern supports the contribution of stomatal behaviour to the observed differences in carbon assimilation, although non-stomatal limitations cannot be excluded because biochemical parameters such as Rubisco activity, intercellular CO_2 concentration and chlorophyll fluorescence were not measured.

The observed genotypic variability is physiologically relevant under the temperate conditions of Kashmir, as tomato genotypes can differ substantially in their gas-exchange responses to temperature stress (Singh et al., 2005; Zhou et al., 2017). However, physiological traits alone may not always provide a sufficient basis for identifying superior or stress-tolerant genotypes, highlighting the importance of integrating gas-exchange measurements with pigment, biochemical and other stress-response traits (Bhattarai et al., 2024; Arena et al., 2020).

TABLE 3
LEAF GAS-EXCHANGE CHARACTERISTICS OF DIFFERENT TOMATO (*SOLANUM LYCOPERSICUM* L.) GENOTYPES UNDER POT CULTURE CONDITIONS (POOLED DATA OF 2018–2019)

Genotype	Net Photosynthetic Rate (μmol CO_2 m^{-2} s^{-1})	Transpiration Rate (mmol H_2O m^{-2} s^{-1})	Stomatal Conductance (μmol H_2O m^{-2} s^{-1})
2016/Todvar-1	13.45	1.28	188.29
2016/Todvar-2	12.67	0.96	128.93
2016/Todvar-3	12.45	0.91	124.69
2016/Todvar-4	12.87	1.18	143.22
2016/Todvar-5	13.15	1.3	183.74
2016/Todvar-7	16.27	1.46	224.56
2016/Todvar-8	12.67	1.17	131.87
2016/Todvar-9	18.57	2.08	237.1
2016/Todvar-10	17.67	1.72	233.35
2016/Todvar-11	13.3	1.29	186.25
2016/Todvar-12	13.15	1.31	179.69
2017/Todvar-3	14.42	1.43	214.69
CD (P<0.05)	**1.33**	**0.42**	**5.02**

3.4 Correlation Analysis

To examine the relationships among the measured physiological traits, Pearson's correlation coefficients were calculated (Table 4). Total chlorophyll content showed a strong positive correlation with net photosynthetic rate ($r = 0.892$, $p < 0.01$), indicating that genotypes with higher pigment content tended to have higher photosynthetic rates. Similarly, stomatal conductance was positively correlated with net photosynthetic rate ($r = 0.856$, $p < 0.01$), confirming the role of stomatal regulation in determining photosynthetic capacity. Plant height was positively correlated with root dry weight ($r = 0.743$, $p < 0.05$), suggesting that vigorous above-ground growth was associated with better root development. The shoot/root ratio was negatively correlated with root dry weight ($r = -0.781$, $p < 0.01$), indicating that genotypes with greater root biomass allocation had lower shoot/root ratios. These correlations support the integrated interpretation of the physiological data and suggest that multiple traits contribute to the superior performance of 2016/Todvar-9.

TABLE 4
CORRELATION COEFFICIENTS AMONG PHYSIOLOGICAL TRAITS OF TOMATO GENOTYPES

Trait	Plant Height	Shoot DW	Root DW	S/R Ratio	Chl a	Chl b	Total Chl	Carotenoids	Pn	E	gs
Plant Height	1										
Shoot DW	0.432	1									
Root DW	0.743*	0.512	1								
S/R Ratio	-0.456	0.289	-0.781**	1							
Chl a	0.634*	0.478	0.698*	-0.512	1						
Chl b	0.567	0.423	0.645*	-0.489	0.934**	1					
Total Chl	0.612*	0.456	0.678*	-0.501	0.978**	0.967**	1				
Carotenoids	0.589	0.467	0.634*	-0.478	0.912**	0.889**	0.923**	1			
Pn	0.678*	0.523	0.723*	-0.534	0.867**	0.845**	0.892**	0.856**	1		
E	0.612*	0.489	0.678*	-0.512	0.823**	0.789**	0.834**	0.812**	0.923**	1	
gs	0.645*	0.501	0.689*	-0.523	0.845**	0.812**	0.856**	0.834**	0.856**	0.912**	1

Note: *Significant at p < 0.05; ***Significant at p < 0.01; DW = Dry Weight; S/R = Shoot/Root; Chl = Chlorophyll; Pn = Net Photosynthetic Rate; E = Transpiration Rate; gs = Stomatal Conductance.***

IV. DISCUSSION

The study revealed substantial genotypic variation in growth, biomass partitioning, photosynthetic pigments and leaf gas-exchange characteristics among tomato genotypes under pot culture conditions in the temperate environment of Kashmir. The superior performance of 2016/Todvar-9 across multiple physiological parameters indicates that this genotype possesses a well-coordinated physiological system that supports vigorous growth and carbon assimilation.

The positive correlation between total chlorophyll content and net photosynthetic rate ($r = 0.892$, $p < 0.01$) supports the association between photosynthetic pigments abundance and photosynthetic performance. Genotypes with higher pigment concentrations, such as 2016/Todvar-9, had greater light-harvesting capacity and consequently higher carbon assimilation rates. The strong correlation between stomatal conductance and photosynthesis ($r = 0.856$, $p < 0.01$) further emphasizes the role of stomatal regulation in determining photosynthetic rates. The coordinated increase in pigment content, stomatal conductance, and photosynthetic rate in 2016/Todvar-9 suggests that this genotype has a superior photosynthetic apparatus and efficient stomatal regulation.

The relatively low shoot/root ratio of 2016/Todvar-9 (13.74) compared to other genotypes (up to 20.92) indicates greater investment in root biomass. This is physiologically significant because better root development can enhance water and nutrient

acquisition, supporting vigorous shoot growth. The positive correlation between root dry weight and plant height ($r = 0.743$, $p < 0.05$) supports this interpretation. The greater root biomass of 2016/Todvar-9 may have contributed to its superior overall growth and physiological performance under the pot culture conditions, where root growth is confined.

The genotypic differences observed in this study are consistent with the growing body of literature demonstrating substantial genetic variation in photosynthetic and physiological traits in tomato (Roohanitaziani et al., 2020; Tripodi et al., 2022; Dong et al., 2025). The identification of 2016/Todvar-9 as a superior genotype for physiological performance under temperate conditions provides a valuable genetic resource for tomato improvement programmes in similar agro-climatic regions.

The physiological basis for the superior performance of 2016/Todvar-9 likely involves multiple interacting factors. Higher chlorophyll content enhances light absorption and energy conversion, while higher stomatal conductance facilitates CO_2 diffusion into the leaf. The combination of traits was associated with higher photosynthetic rates and greater biomass accumulation. The relatively greater root biomass allocation provides better access to water and nutrients, sustaining the vigorous shoot growth. This integrated physiological system enables 2016/Todvar-9 to perform well under the temperate conditions of Kashmir.

V. CONCLUSION

The study revealed substantial genotypic variation in growth, biomass partitioning, photosynthetic pigments and leaf gas-exchange characteristics among tomato genotypes under pot culture conditions in the temperate environment of Kashmir. 2016/Todvar-9 consistently exhibited superior physiological performance, recording the highest plant height, root dry weight, chlorophyll a, chlorophyll b, total chlorophyll, carotenoid content, net photosynthetic rate, transpiration rate and stomatal conductance, together with high shoot biomass. The concurrent higher pigment concentrations and gas-exchange activity in 2016/Todvar-9 indicate superior physiological and photosynthetic performance under the experimental conditions. The considerable variation in shoot:root ratio further indicated distinct genotype-specific patterns of biomass allocation. Correlation analysis confirmed strong positive relationships between chlorophyll content and photosynthetic rate ($r = 0.892$), and between stomatal conductance and photosynthesis ($r = 0.856$), supporting the integrated interpretation of the physiological data. Overall, the findings demonstrate that growth, pigment and gas-exchange traits can serve as useful physiological indicators for differentiating tomato genotypes and identifying promising material under temperate conditions. Accordingly, 2016/Todvar-9 is recommended for further multi-location and field evaluation under the temperate conditions of Kashmir for its superior growth and photosynthetic performance.

Limitations of the Study: The study was conducted under pot culture conditions, which may not fully reflect field conditions where roots can explore a larger soil volume. The confined root growth in pots may influence plant development and physiological responses. The study did not include yield data, which would strengthen the practical relevance of the findings. The study was conducted at a single location over two years. Future research should include field validation, yield evaluation, multi-location trials, and investigation of the biochemical basis of the observed physiological differences (e.g., Rubisco activity, electron transport rate).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Abdelkader, M., Elkhawaga, F. A., Suliman, A. A., Puchkov, M., Kuranova, K. N., Mahmoud, M. H., & Abdelkader, M. F. M. (2024). Understanding the regular biological mechanism of susceptibility of tomato plants to low incidences of blossom-end rot. *Horticulturae*, *10*(6), 648. https://doi.org/10.3390/horticulturae10060648

[2] Abduelghader, A. A., Sanders, F. E., & Pilbeam, D. J. (2011). Growth and biomass partitioning in tomato in relation to ratio of nitrogen:Phosphorus supply. *Journal of Plant Nutrition*, *34*(13), 2018-2038. https://doi.org/10.1080/01904167.2011.610488

[3] Albacete, A., Ghanem, M. E., Martínez-Andújar, C., Acosta, M., Sánchez-Bravo, J., Martínez, V., Lutts, S., Dodd, I. C., & Pérez-Alfocea, F. (2008). Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. *Journal of Experimental Botany*, *59*(15), 4119-4131. https://doi.org/10.1093/jxb/ern251

[4] Arena, C., Conti, S., Francesca, S., Melchionna, G., Hájek, J., Barták, M., Barone, A., & Rigano, M. M. (2020). Eco-physiological screening of different tomato genotypes in response to high temperatures: A combined field-to-laboratory approach. *Plants*, *9*(4), 508. https://doi.org/10.3390/plants9040508

[5] Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts: Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, *24*(1), 1-15. https://doi.org/10.1104/pp.24.1.1

[6] Basavaraj, D., & Naik, P. V. (2024). Genetic variability studies for growth, yield and quality traits in tomato (*Solanum lycopersicum* L.) germplasm under North Eastern Dry Zone of Karnataka, India. *Journal of Advances in Biology & Biotechnology*, *27*(8), 1459-1465. https://doi.org/10.9734/jabb/2024/v27i81289

[7] Ben Sedrine, I., Werghi, S., Hachef, A., et al. (2024). Alleviation of drought stress in tomato by foliar application of seafood waste extract. *Scientific Reports*, *14*, 30572. https://doi.org/10.1038/s41598-024-80798-0

[8] Bhat, H. A., Khan, F. A., Bhat, S. A., Narayan, S., Mir, S. A., Masoodi, K. Z., Malik, A. A., Lone, S. A., Sajad, S., & Jan, U. (2022). Evaluation of tomato (*Solanum lycopersicum*) genotypes for plant growth, fruit yield and quality. *Indian Journal of Agricultural Sciences*, *92*(4), 495-499. https://doi.org/10.56093/ijas.v92i4.123978

[9] Bhattarai, S., Harvey, J. T., Lee, C., Joshi, V., & Leskovar, D. I. (2024). Assessment of physiological and biochemical thermotolerance traits in tomato genotypes. *Scientia Horticulturae*, *324*, 112561. https://doi.org/10.1016/j.scienta.2023.112561

[10] Bristow, S. T., Hernandez-Espinoza, L. H., Bonarota, M.-S., & Barrios-Masias, F. H. (2021). Tomato rootstocks mediate plant-water relations and leaf nutrient profiles of a common scion under suboptimal soil temperatures. *Frontiers in Plant Science*, *11*, 618488. https://doi.org/10.3389/fpls.2020.618488

[11] Dong, H., Li, F., Xuan, X., Ahiakpa, J. K., Tao, J., Zhang, X., Ge, P., Wang, Y., Gai, W., & Zhang, Y. (2025). The genetic basis and improvement of photosynthesis in tomato. *Horticultural Plant Journal*, *11*(1), 69-84. https://doi.org/10.1016/j.hpj.2023.06.007

[12] Flores-Saavedra, M., Villanueva, G., Gramazio, P., Vilanova, S., Mauceri, A., Abenavoli, M. R., Sunseri, F., Prohens, J., & Plazas, M. (2024). Nitrogen use efficiency, growth and physiological parameters in different tomato genotypes under high and low N fertilisation conditions. *Plant Physiology and Biochemistry*, *208*, 108447. https://doi.org/10.1016/j.plaphy.2024.108447

[13] Francesca, S., Vitale, L., Graci, S., Addonizio, M., Barone, A., & Rigano, M. M. (2024). Integrated physiological and genetic data reveal key-traits for heat tolerance in tomato. *Plant Stress*, *13*, 100555. https://doi.org/10.1016/j.stress.2024.100555

[14] Gomez, K. A., & Gomez, A. A. (1984). *Statistical procedures for agricultural research* (2nd ed.). John Wiley & Sons.

[15] Hiscox, J. D., & Israelstam, G. F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany*, *57*(12), 1332-1334. https://doi.org/10.1139/b79-163

[16] Ji, Y., Ouzounis, T., Schouten, H. J., Visser, R. G. F., Marcelis, L. F. M., & Heuvelink, E. (2021). Dissecting the genotypic variation of growth responses to far-red radiation in tomato. *Frontiers in Plant Science*, *11*, 614714. https://doi.org/10.3389/fpls.2020.614714

[17] Roohanitaziani, R., de Maagd, R. A., Lammers, M., Molthoff, J., Meijer-Dekens, F., van Kaauwen, M. P. W., Finkers, R., Tikunov, Y., Visser, R. G. F., & Bovy, A. G. (2020). Exploration of a resequenced tomato core collection for phenotypic and genotypic variation in plant growth and fruit quality traits. *Genes*, *11*(11), 1278. https://doi.org/10.3390/genes11111278

[18] Singh, I., Mariko, S., Fukamachi, H., & Suzuki, K. (2005). Effect of heat stress on gas exchange characteristics in tomato. *Indian Journal of Plant Physiology*, *10*(3), 283-286.

[19] Tripodi, P., Figàs, M. R., Leteo, F., Soler, S., Díez, M. J., Campanelli, G., Cardi, T., & Prohens, J. (2022). Genotypic and environmental effects on morpho-physiological and agronomic performances of a tomato diversity panel in relation to nitrogen and water stress under organic farming. *Frontiers in Plant Science*, *13*, 936596. https://doi.org/10.3389/fpls.2022.936596

[20] Wellburn, A. R. (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology*, *144*(3), 307-313. https://doi.org/10.1016/S0176-1617(11)81192-2

[21] Zhou, R., Kjær, K. H., Rosenqvist, E., Yu, X., Wu, Z., & Ottosen, C.-O. (2017). Physiological response to heat stress during seedling and anthesis stage in tomato genotypes differing in heat tolerance. *Journal of Agronomy and Crop Science*, *203*(1), 68-80. https://doi.org/10.1111/jac.12166.

International Journal of Environmental & Agriculture Research (IJOEAR)
ISSN: [2454-1850]
[Vol-12, Issue-9, September- 2026]
Website: www.ijoear.com
Journal DOI: 10.25125/agriculture-journal

Development and Quality Evaluation of Paneer Thokku Prepared Using Oil, Vinegar and Lemon Juice

M. Anna Anandh[1*]; G. Gawdaman[2]

Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Department of Livestock Products Technology, Veterinary College and Research Institute, Orathanadu – 614 625, Thanjavur District, Tamil Nadu. India
*Corresponding Author

Received:- 11 August 2026/ Revised:- 25 August 2026/ Accepted:- 04 September 2026/ Published: 15-09-2026

Abstract— *The present study was undertaken to develop paneer thokku using oil-, vinegar- and lemon juice-based formulations and to evaluate their physicochemical, microbiological and sensory characteristics. Three formulations were evaluated: oil-based paneer thokku (C), vinegar-based paneer thokku (T1) and lemon juice-based paneer thokku (T2). Significant differences (P<0.05) were observed among the formulations for physicochemical, microbiological and sensory characteristics. Vinegar-based paneer thokku (T1) recorded the lowest pH (4.42), highest titratable acidity (0.89%) and lowest total plate count (1.21 log_{10} cfu/g), whereas oil-based paneer thokku (C) recorded the highest product yield (120.85%). T1 also exhibited the lowest moisture content (45.82%). Coliforms and yeasts and moulds were not detected in any of the formulations. Lemon juice-based paneer thokku (T2) recorded the highest sensory scores for appearance and colour (8.08), flavour (8.56), juiciness (8.02), texture (8.06), saltiness (8.01) and overall acceptability (8.12). Among the formulations evaluated, lemon juice-based paneer thokku (T2) was the most preferred based on overall sensory acceptability and showed potential for further product development.*

Keywords— Paneer, paneer thokku, acidulants, vinegar, lemon juice, value addition, sensory quality, microbial quality.

I. INTRODUCTION

Paneer is an important indigenous Indian dairy product prepared by acid-heat coagulation of milk, followed by separation and pressing of the coagulum. It is a fresh, non-ripened and non-melting dairy product characterized by a firm and cohesive body and valued for its protein, fat and mineral contents. The quality characteristics of paneer are influenced by several processing factors, including milk composition, type and concentration of coagulant, coagulation temperature, pressing conditions and other processing parameters (Alam et al., 2024; Ahmed and Bajwa, 2019). Because of its relatively high moisture and nutrient content, paneer is susceptible to microbial deterioration and has a comparatively short shelf life under conventional storage conditions. Several approaches, including thermal processing, packaging, low-temperature storage and incorporation of antimicrobial or preservative agents, have been investigated to improve its storage stability (Goyal and Goyal, 2016). The incorporation of spices has also been reported to contribute to the storage stability of paneer, with individual spices exhibiting varying degrees of antimicrobial and preservative effects (Eresam et al., 2015).

Pickling is a traditional preservation technique in which multiple preservation factors, including acidity, salt, oil, heat treatment and spices, act together to improve product stability. Earlier studies have demonstrated the feasibility of pickling for the preservation of paneer. Rani et al. (2014) reported the application of brine and spice-vinegar pickling for maintaining acceptable microbiological quality of paneer products during storage. Similarly, Shukla and Vaid (2004) reported the storage stability of oil-based paneer pickle, although progressive changes in chemical characteristics were observed during storage.

Organic acids are important components of acidified foods because they reduce product pH and can inhibit the growth of microorganisms. Acetic acid and citric acid are widely used as food acidulants and antimicrobial agents, with their effectiveness being influenced by acid concentration, pH and food-matrix characteristics (Ben Braiek and Smaoui, 2021).

Vinegar, which contains acetic acid, and lemon juice, which naturally contains citric acid, can therefore contribute to both the technological and sensory characteristics of traditional food products. Anna Anandh and Sobana (2021) developed a traditional-style ready-to-eat paneer pickle using vegetable oil, vinegar and lemon juice and reported lower pH and higher titratable acidity in the vinegar-based product, whereas the lemon juice-based product received higher sensory scores. These findings indicate the potential of different acidulant systems for developing acceptable value-added paneer products.

Paneer thokku is a traditional-style preparation in which paneer is combined with oil, spices and condiments to obtain a thick and flavourful product. However, limited information is available regarding the influence of different acidulant systems, particularly vinegar and lemon juice, on the physicochemical, microbiological and sensory characteristics of paneer thokku. Therefore, the present study was undertaken to develop paneer thokku using oil-, vinegar- and lemon juice-based formulations and to evaluate their physicochemical, microbiological and sensory characteristics.

II. MATERIALS AND METHODS

2.1 Procurement of Raw Materials

Fresh cow milk, spices, condiments, gingelly oil, vinegar, lemon fruits, garlic, ginger and other food-grade ingredients required for the preparation of paneer thokku were procured from the local market. All raw materials were examined for freshness, quality and suitability before use.

2.2 Preparation of Paneer

Fresh cow milk was standardized to 4.5% fat and heated to 85°C. Coagulation was carried out using a 1% citric acid solution. The coagulum was allowed to settle, followed by drainage of whey. The curd was subsequently pressed, cooled and cut into approximately 1 cm × 1 cm × 1 cm cubes. Freshly prepared paneer cubes were used for the preparation of the experimental products. The procedure followed the approach reported for traditional-style paneer pickle preparation by Anna Anandh and Sobana (2021), while the use of citric acid for coagulation was consistent with earlier paneer-processing procedures (Bhattacharya et al., 1971).

2.3 Experimental Treatments

Three formulations of paneer thokku were prepared, namely oil-based paneer thokku (C), vinegar-based paneer thokku (T1) and lemon juice-based paneer thokku (T2). The formulations used for the preparation of the experimental products are presented in Table 1. In C, gingelly oil was incorporated at 75%, whereas in T1 and T2, gingelly oil was incorporated at 50% along with 25% vinegar and 25% lemon juice, respectively. The remaining ingredients were incorporated at identical levels in the respective formulations.

TABLE 1
FORMULATION OF TRADITIONAL-STYLE PANEER THOKKU

Ingredients	C: Oil-based	T1: Vinegar-based	T2: Lemon juice-based
Paneer (%)	100	100	100
Gingelly oil (%)	75	50	50
Vinegar (%)	–	25	–
Lemon juice (%)	–	–	25
Turmeric powder (%)	1	1	1
Mustard seeds (%)	1	1	1
Ginger and garlic paste (%)	10	10	10
Roasted cumin seed powder (%)	1	1	1
Roasted fenugreek seeds (%)	1	1	1
Asafoetida powder (%)	1	1	1
Red chilli powder (%)	2.5	2.5	2.5
Salt (%)	3	3	3
Spice mixture (%)	2	2	2

2.4 Preparation of Spice and Condiment Mixture

The dry spice mixture consisted of aniseed (10%), black pepper (10%), capsicum (8%), caraway seed (10%), cardamom (5%), cinnamon (4%), cloves (1%), coriander (20%), cumin seed (22%) and turmeric powder (10%). The spices were cleaned, dried and ground before blending in the specified proportions. Fresh ginger and garlic were peeled, cut into small pieces and ground to a fine paste. The spice and condiment formulation was based on the traditional-style paneer pickle formulation reported by Anandh and Sobana (2021).

2.5 Preparation of Paneer Thokku

Paneer cubes were mixed with turmeric powder and salt and allowed to marinate for one hour. The marinated paneer cubes were fried in gingelly oil until golden brown and kept aside. Mustard seeds were tempered in oil, followed by the addition of ginger-garlic paste, red chilli powder, roasted cumin seed powder, roasted fenugreek seed powder, asafoetida and the prepared spice mixture. The fried paneer cubes were then incorporated and mixed thoroughly.

Based on the respective treatment, gingelly oil, vinegar or lemon juice was incorporated as specified in Table 1. Control (C) contained 75% gingelly oil, whereas T1 and T2 contained 50% gingelly oil along with 25% vinegar and 25% lemon juice, respectively. The mixture was cooked with continuous stirring until a thick thokku consistency was obtained. The prepared products were cooled to room temperature and subjected to physicochemical, microbiological and sensory evaluation.

2.6 Physicochemical Analysis

The pH of the products was determined using a calibrated digital pH meter. Product yield was calculated as the weight of the final product relative to the initial product weight and expressed as a percentage. Titratable acidity was determined and expressed as percentage acidity. Moisture content was determined by the standard hot-air oven method following AOAC procedures (AOAC, 2005). The analytical procedures were based on standard methods and procedures reported for paneer pickle evaluation (Anna Anandh and Sobana, 2021).

2.7 Microbiological Analysis

The microbiological quality of paneer thokku was evaluated by determining total plate count, coliform count, and yeast and mould count. Samples were processed aseptically and analysed using standard microbiological procedures. Microbial counts were expressed as $\log_{10}$ cfu/g. Samples showing no detectable growth under the test conditions were recorded as not detected (ND). The methodology was based on standard procedures described in the Compendium of Methods for the Microbiological Examination of Foods (Salfinger and Tortorello, 2015).

2.8 Sensory Evaluation

Sensory evaluation was carried out using a 9-point hedonic scale by a panel of semi-trained judges. The sensory attributes evaluated were appearance and colour, flavour, juiciness, texture, saltiness, sourness and overall acceptability. Scores were assigned from 1 to 9, with 1 representing extremely undesirable and 9 representing extremely desirable.

2.9 Experimental Replication and Statistical Analysis

The experiment was conducted in six independent replications. The data obtained for physicochemical, microbiological and sensory parameters were subjected to analysis of variance (ANOVA). Treatment means were compared at a significance level of $P<0.05$.

III. RESULTS AND DISCUSSION

3.1 Physicochemical Characteristics

The physicochemical characteristics of paneer thokku prepared using the three formulations are presented in Table 2. Significant differences ($P<0.05$) were observed among the formulations for pH, product yield, titratable acidity and moisture content. The differences were associated with the varying proportions of vinegar and lemon juice incorporated into the formulations. The pH values differed among the formulations, with T1 recording the lowest pH (4.42), followed by T2 (4.71)

and C (5.21). The lower pH observed in T1 and T2 might be attributed to the incorporation of organic acids, predominantly acetic acid from vinegar and citric acid from lemon juice. Organic acids are known to reduce the pH of food systems and thereby contribute to microbial inhibition (Ben Braiek and Smaoui, 2021). The greater reduction in pH observed in T1 indicated stronger acidification under the formulation conditions employed. This observation agrees with Anna Anandh and Sobana (2021), who reported lower pH in vinegar-based paneer pickle, followed by lemon juice- and vegetable oil-based products.

Product yield was highest in C (120.85%), followed by T2 (111.40%) and T1 (99.20%). The higher yield observed in C might be attributed mainly to the greater proportion of gingelly oil incorporated into the formulation and its retention in the final product. The yield values represent final product mass yield and should therefore not be interpreted as recovery of milk solids. Similar treatment-dependent differences in yield were reported by Anna Anandh and Sobana (2021), in which the oil-based paneer pickle showed higher product yield than the acidulant-based formulations.

TABLE 2
PHYSICOCHEMICAL CHARACTERISTICS OF PANEER THOKKU PREPARED USING DIFFERENT TREATMENTS

Parameter	C	T1	T2
pH	5.21 ± 0.08a	4.42 ± 0.06b	4.71 ± 0.05c
Product yield (%)	120.85 ± 0.32a	99.20 ± 0.28b	111.40 ± 0.25c
Titratable acidity (%)	0.12 ± 0.02a	0.89 ± 0.03b	0.18 ± 0.02c
Moisture (%)	49.30 ± 0.24a	45.82 ± 0.22b	47.60 ± 0.20c

Values are Mean ± SE. Means bearing different superscripts within a row differ significantly (P<0.05).

Titratable acidity was highest in T1 (0.89%), followed by T2 (0.18%) and C (0.12%). The markedly higher acidity in T1 corresponded with its lower pH and might be attributed to the incorporation of 25% vinegar. Although T2 contained an equivalent proportion of lemon juice, it exhibited considerably lower titratable acidity, indicating differences in the acid contribution and buffering interactions of the respective formulations. This trend was consistent with the findings of Anna Anandh and Sobana (2021), who reported higher titratable acidity in vinegar-based paneer pickle than in lemon juice-based products.

Moisture content was lowest in T1 (45.82%), followed by T2 (47.60%) and C (49.30%). The differences in moisture content might be associated with cooking-related moisture loss and the varying proportions of oil and acidulants incorporated into the formulations. The relatively lower moisture content of T1 occurred along with its lower pH and might have contributed to its improved microbiological stability. Similar differences in moisture content among oil-, vinegar- and lemon juice-based paneer pickle formulations have previously been reported by Anandh and Sobana (2021).

3.2 Microbiological Characteristics

The microbiological characteristics of paneer thokku are presented in Table 3. Total plate count differed significantly among the formulations. T1 recorded the lowest TPC (1.21 $\log_{10}$ cfu/g), followed by T2 (1.43 $\log_{10}$ cfu/g) and C (1.72 $\log_{10}$ cfu/g). The lower TPC observed in the acidulant-based formulations may be associated with the combined effects of acidification, heat treatment, salt, spices and oil. The vinegar-based formulation showed the lowest TPC, corresponding with its lowest pH and highest titratable acidity. Organic acids such as acetic and citric acids can exert antimicrobial effects by lowering environmental pH and interfering with microbial cellular processes (Ben Braiek and Smaoui, 2021; Presser et al., 1999). The present findings are in agreement with those of Anna Anandh and Sobana (2021), who observed lower microbial counts in vinegar- and lemon juice-based paneer pickle than in the vegetable oil-based product. Rani et al. (2014) also demonstrated the potential of pickling as a preservation method for paneer. In addition, the preservative contribution of spices might have complemented the effects of acidity, as several spices have been reported to influence the microbial and chemical stability of paneer during storage (Eresam et al., 2015).

Coliforms and yeasts and moulds were not detected in any of the formulations. This indicates satisfactory microbiological quality of the products under the processing and analytical conditions employed. However, the absence of detectable

organisms should be interpreted as a result under the specific test conditions and should not be considered evidence of complete microbial sterility.

TABLE 3
MICROBIOLOGICAL CHARACTERISTICS OF PANEER THOKKU PREPARED USING DIFFERENT TREATMENTS

Microbial profile (log_{10} cfu/g)	C	T1	T2
Total plate count	1.72 ± 0.10a	1.21 ± 0.08b	1.43 ± 0.09c
Coliform count	ND	ND	ND
Yeast and mould count	ND	ND	ND

Values are Mean ± SE. ND = Not detected. Means bearing different superscripts within a row differ significantly (P<0.05).

3.3 Sensory Characteristics

The sensory characteristics of paneer thokku are presented in Table 4. Lemon juice-based paneer thokku (T2) recorded the highest scores for appearance and colour (8.08), flavour (8.56), juiciness (8.02), texture (8.06), saltiness (8.01) and overall acceptability (8.12). The consistently higher sensory scores of T2 indicate a favourable overall sensory response to the lemon juice-based formulation. The higher flavour and overall acceptability scores of T2 might be associated with the relatively mild and balanced acidic character of lemon juice, which complemented the flavour of the spice and condiment system. The use of fruit-derived acidulants has previously been associated with acceptable sensory characteristics in paneer products (Ahmed and Bajwa, 2019).

TABLE 4
SENSORY CHARACTERISTICS OF PANEER THOKKU PREPARED USING DIFFERENT TREATMENTS

Sensory Attributes*	C	T1	T2
Appearance and colour	7.05 ± 0.11a	7.52 ± 0.10b	8.08 ± 0.12c
Flavour	7.08 ± 0.10a	7.64 ± 0.12b	8.56 ± 0.10c
Juiciness	7.10 ± 0.10a	7.58 ± 0.09b	8.02 ± 0.11c
Texture	7.12 ± 0.11a	7.60 ± 0.10b	8.06 ± 0.10c
Saltiness	7.02 ± 0.12a	7.55 ± 0.11b	8.01 ± 0.10c
Sourness	6.05 ± 0.09a	7.62 ± 0.10b	7.12 ± 0.12c
Overall acceptability	6.92 ± 0.10a	7.62 ± 0.11b	8.12 ± 0.10c

Sensory attributes were evaluated on a 9-point hedonic scale (wherein 1 = extremely undesirable and 9 = extremely desirable). Values are Mean ± SE. Means bearing different superscripts within a row differ significantly (P<0.05).

T1 recorded the highest sourness score (7.62), reflecting its greater acidification. Although the stronger acidity of T1 was advantageous in terms of pH reduction and microbial control, the greater perceived sourness may have limited its preference relative to T2. The present findings are consistent with those of Anandh and Sobana (2021), who reported higher sensory scores for lemon juice-based paneer pickle compared with vinegar- and vegetable oil-based formulations. This agreement suggests that lemon juice may provide a favourable balance between acidity and sensory acceptability in traditional-style paneer products. Among the three formulations, overall acceptability was highest for T2 (8.12), followed by T1 (7.62) and C (6.92). Thus, the incorporation of oil, acidulant and spice-condiment systems resulted in distinct sensory responses, with T2 demonstrating the highest overall acceptability.

3.4 Overall Interpretation

The three paneer thokku formulations demonstrated distinct advantages with respect to different quality attributes. Vinegar-based paneer thokku (T1) exhibited the greatest acidification, as indicated by the lowest pH (4.42) and highest titratable acidity (0.89%), and also recorded the lowest TPC (1.21 log_{10} cfu/g). Oil-based paneer thokku (C) recorded the highest final product yield (120.85%), which was primarily associated with its greater oil incorporation. In contrast, lemon juice-based paneer thokku (T2) demonstrated the most favourable sensory profile, with the highest overall acceptability score (8.12).

The sensory advantage of T2 might be attributed to a favourable balance between acidity and the characteristic flavour of the spice-condiment system. Similar sensory superiority of lemon juice-based paneer pickle has previously been reported by Anna Anandh and Sobana (2021). Thus, the results indicate that formulation performance varied according to the intended quality attribute: T1 was advantageous for acidification and microbial control, C for final product yield, whereas T2 was superior in sensory acceptability. Under the conditions of the present study, lemon juice-based paneer thokku may therefore be considered the preferred formulation, primarily on the basis of sensory acceptability.

IV. CONCLUSION

Paneer thokku was successfully developed using oil-, vinegar- and lemon juice-based formulations. Vinegar-based paneer thokku exhibited the greatest acidification, as indicated by the lowest pH and highest titratable acidity, while oil-based paneer thokku recorded the highest product yield. Lemon juice-based paneer thokku exhibited the highest sensory acceptability (8.12) and was the most preferred formulation under the conditions of the study. Coliforms and yeasts and moulds were not detected in any of the formulations. Further storage and shelf-life studies are recommended to establish product stability and commercial shelf life.

Limitations of the Study: The study was conducted on freshly prepared products, and storage stability was not evaluated. The sensory evaluation was conducted using semi-trained panelists rather than consumers. Future research should include storage studies to evaluate the shelf life and stability of the products, consumer acceptability testing, and optimization of the formulation for commercial production.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Ahmed, A., & Bajwa, U. (2019). Composition, texture and microstructure appraisal of paneer coagulated with sour fruit juices. *Journal of Food Science and Technology*, *56*(1), 253-261. https://doi.org/10.1007/s13197-018-3484-4

[2] Alam, M., Rawat, M., Das, R., Das, D., Kaur, R., & Sharma, P. (2024). Exploring the impact of processing and compositional factors on the improvement of quality of milk paneer: A comprehensive review. *International Dairy Journal*, *158*, 106038. https://doi.org/10.1016/j.idairyj.2024.106038

[3] Anandh, M. A., & Sobana, A. S. (2021). Development and quality evaluation of traditional styled ready to eat pickle from paneer. *The Pharma Innovation Journal*, *10*(7), 393-396.

[4] AOAC. (2005). *Official methods of analysis of AOAC International*. Association of Official Analytical Chemists.

[5] Ben Braïek, O., & Smaoui, S. (2021). Chemistry, safety, and challenges of the use of organic acids and their derivative salts in meat preservation. *Journal of Food Quality*, *2021*, 6653190. https://doi.org/10.1155/2021/6653190

[6] Bhattacharya, D. C., Mathur, O. N., Srinivasan, M. R., & Samlik, O. (1971). Studies on the method of production and shelf life of paneer (cooking type of acid coagulated cottage cheese). *Journal of Food Science and Technology*, *8*, 117-120.

[7] Eresam, E. K. K., Pinto, S., & Aparnathi, K. D. (2015). Evaluation of selected spices in extending shelf life of paneer. *Journal of Food Science and Technology*, *52*(4), 2043-2052. https://doi.org/10.1007/s13197-013-1226-1

[8] Goyal, S., & Goyal, G. K. (2016). Maximizing shelf life of paneer—A review. *Critical Reviews in Food Science and Nutrition*, *56*(8), 1253-1261. https://doi.org/10.1080/10408398.2012.763157

[9] Presser, K. A., Ratkowsky, D. A., & Ross, T. (1999). Modelling the inhibitory effects of organic acids on bacteria. *International Journal of Food Microbiology*, *47*(3), 189-201. https://doi.org/10.1016/S0168-1605(99)00012-4

[10] Rani, M., Dabur, R. S., Khanna, S., Potliya, S., & Verma, S. (2014). Application of pickling for preservation of paneer. *Asian Journal of Dairy and Food Research*, *33*(3), 183-186. https://doi.org/10.5958/0976-0563.2014.00599.5

[11] Salfinger, Y., & Tortorello, M. L. (Eds.). (2015). *Compendium of methods for the microbiological examination of foods* (5th ed.). American Public Health Association.

[12] Shukla, F. C., & Vaid, J. (2004). Studies on the storage stability of oil-based paneer pickle. *International Journal of Dairy Technology*, *57*(1), 15-18. https://doi.org/10.1111/j.1471-0307.2004.00116.x.

International Journal of Environmental & Agriculture Research (IJOEAR)
ISSN: [2454-1850]
[Vol-12, Issue-9, September- 2026]
Website: www.ijoear.com
Journal DOI: 10.25125/agriculture-journal

Propagating for Survival: Conservation Strategies for the Endangered Himalayan Medicinal Plant *Picrorhiza kurrooa*

G. G. Mohi Ud Din[1*]; Irshad A. Nawchoo[2]; B. A. Wafai[3]

[*1]Department of Botany, Govt. Degree College Bemina, Srinagar J&K, India
[2,3]Department of Botany, University of Kashmir, Srinagar, J&K, India
*Corresponding Author

Received:- 12 August 2026/ Revised:- 01 September 2026/ Accepted:- 11 September 2026/ Published: 15-09-2026

Abstract— *Studies on propagation and conservation of an endangered medicinal herb - Picrorhiza kurrooa reveals that the seed germination did not occur in darker conditions and seem to be photoblastic. Among various treatments the species showed 93.33±2.88% germination when the seeds were germinated in a medium of 0.125mM GA_3 with mean germination time (MGT) of 8.0±0.866 days as against the MGT 18.66±0.57 days of controlled seeds (seeds without any treatment). In field conditions however, the seeds germinate only when placed on the upper layer of soil covered with a thin film of water exposed to light. Although the plants grew nicely on various soils, maximum plant survival and vigorous growth was obtained in loamy and sandy loam textured soils. The plants responded to various nutrient treatments and in loamy textured the maximum dry biomass was shown by the plants treated with nitrogen (500mg/kg soil) produced 9.50±2.50gm dry biomass and the plants treated with NPK (500mg/kg soil) produced 9.16±4.481gm dry biomass as against the control of 4.83±0.473gm in loam textured soils. In sandy loam (1:2) textured soils the plants treated with nitrogen and NPK (500mg/kg soil) produced the dry biomass of 13.66±0.581 and 10.16±0.763gm respectively as against the control of 5.06±1.401gm per individual. The experimental manipulations also reveal that the species has a potential to propagate through underground stem cuttings in sandy loam soils. The cuttings treated with IAA, IBA and GA_3 showed 93.33, 80.0 and 95.0% survival in top segments while as 60.0, 55.0 and 68.0% survival in basal segments respectively as against the control of 75.0% in top and 42.10% in basal segments.*

Keywords— Picrorhiza kurrooa, endangered medicinal plant, propagation, growth regulators, NPK treatments, seed germination, ex situ conservation.

I. INTRODUCTION

P. kurrooa (English: Hellabore; Hindi: Kutki; Punjabi: Karu; Kashmiri: Koad) is the major source of a multipurpose drug valued as a bitter tonic, antiperiodic, laxative and cathartic, when used in small and large doses respectively (Kirtikar and Basu, 1988) and hepatoprotective (Ansari et al., 1991). In the entire range of distribution the species is confined to alpine habitats and when viewed from a global perspective, the distribution range of the species turns out to be negligibly small. Even in this extremely narrow range of distribution, the species constitute small sized populations which are sparsely distributed and contain scant individuals. Justifiably therefore, the herb have been recognised as endangered (EN) (*P. kurrooa*) and accordingly listed in Red Data Book of BSI (Nayar and Shastry, 1998). Despite the poor and dismal populational picture of the herb in its range of distribution, the underground plant parts, which represent the main sources of its survival and multiplication over generations in natural setting, are ruthlessly and indiscriminately extracted from the soil as drugs. This practice has been going on for years together and continues unabated even today. The result is that most of the populations are bereft of this herb and that day is not

far when no single plant will be traceable to the posterity. There is an urgent need to rise to the occasion and come up with proactive strategies to salvage whatever is left and plan long term rescue measures not only to save this high potential herb from loss but also to rejuvenate the species, return to its natural ecosystem and ensure supply of raw material to the drug industry on sustained basis. In this backdrop the present studies have been conducted. The specific objectives of this study were: (i) to develop efficient seed germination protocols for *P. kurrooa*; (ii) to evaluate the species' performance on different soil types and nutrient regimes for ex situ cultivation; and (iii) to develop vegetative propagation techniques using stem cuttings for conservation and commercial multiplication.

II. MATERIALS AND METHODS

2.1 Study Sites and Source Material

Six alpine sites of Kashmir Himalaya ranging in altitude from 3,150 to 3,400m a.s.l. were monitored and used as a source material for seed, soil and propagule collection (Table 1). All populations were located in alpine habitats with soils rich in moisture, mosses and leaf litter. Threatened status of the species necessitated careful collection of material to avoid further depletion of natural populations.

TABLE 1
SALIENT FEATURES OF SOME KASHMIR HIMALAYAN SITES SELECTED FOR STUDIES ON *PICRORHIZA KURROOA*

Habitat character	Populations					
	Thajwas I	**Zaildar Dub**	**Arm Pathri**	**Thajwas II**	**Sangam Top**	**Razaka ka Paja**
Altitude (m)	3150	3150	3300	3350	3400	3400
Latitude/Longitude	34°30'N 75°30'E	34°30'N 75°30'E	34°02'N 75°30'E	34°30'N 75°30'E	34°30'N 75°30'E	34°30'N 75°30'E
Direction with reference to Srinagar	north east	north east	south east	north east	north east	north east
Climatic zone	alpine	alpine	alpine	alpine	alpine	alpine
Habitat	Rocky slope with partial shade	open grassy/ rocky slope	shady moist slope under *Betula utilis* cover	shady slope under *Betula utilis* cover	shady moist slope	moist shady slope
Threat factor	extraction, grazing, landslides	extraction, grazing	extraction, grazing	extraction, grazing	extraction, grazing	extraction, grazing

Note: In all populations soils are rich in moisture, mosses and leaf litter.

2.2 Seed Germination Studies

For seed germination, mature seeds of *Picrorhiza kurrooa* of the same age (seed cohort) were collected from a selected natural population, Thajwas I, 3150m a.s.l (Kashmir Himalaya). The seeds were treated with 0.1% mercuric chloride for 5 minutes and washed with double distilled water. Seeds were germinated in petriplates on Whatman filter paper (in vitro) using different media (Table 2). For each treatment three replicates were used each with a set of control. The mean germination time (MGT) was calculated following Joshi and Dhar (2003) as given below:

$$MGT = (n \times d) / N \quad (1)$$

where 'n' = number of seeds germinated on each day, 'd' = number of days from the beginning of the test, and 'N' = total number of seeds germinated at the termination of the experiment.

In field conditions, seeds were sown on the soil surface with a thin film of water, and at depths of 1 cm and 1.5 cm to assess the effect of burial on germination. The photoblastic nature of seeds was tested by germinating seeds in complete darkness.

TABLE 2
EFFECT OF DIFFERENT PHYSICAL AND CHEMICAL TREATMENTS ON SEED GERMINATION OF *PICRORHIZA KURROOA*

S. No.	Treatment	Mean germination time (MGT) days	% germination
1	**Lab. conditions (LC)**		
	Control	18.66 ± 0.57*	57.5 ± 3.53
	Chilling (days)		
	20	25.0 ± 5.0	6.66 ± 2.88
	40	-	-
	75	-	-
	90	-	-
	Conc. H_2SO_4 dip	16.83 ± 0.57	19.66 ± 0.57
	GA_3 (mM)*		
	0.125	8.0 ± 0.866	**93.33 ± 2.88****
	0.25	17.66 ± 0.288	65.0 ± 5.0
	0.5	16.66 ± 1.607	41.66 ± 17.55
	1	15.83 ± 1.04	57.66 ± 12.50
	1.5	17.16 ± 0.76	16.66 ± 2.88
	2	16.16 ± 1.119	70.00 ± 15.49
	Thiourea (mM)*		
	0.25	15.5 ± 1.322	**73.33 ± 2.88****
	0.5	16.33 ± 1.059	62.33 ± 7.50
	1	16.66 ± 0.763	69.33 ± 4.04
	1.5	16.0 ± 0.50	70.0 ± 5.0
	2	16.66 ± 0.763	40.0 ± 5.0
	Kinetin (mM)*		
	0.25	-	-
	0.5	17.16 ± 0.58	3.33 ± 2.88
	1	-	-
	1.5	-	-
	2	17.33 ± 0.812	15.0 ± 5.0
2	**Field conditions (FC)**		
	Seeds placed on upper surface of soil covered with thin film of water	14.33 ± 0.57	53.33 ± 8.50
	Seeds sown 1cm deep	-	-
	Seeds sown 1.5cm deep	-	-

***Mean ± Standard Deviation; **Maximum seed germination; Seed sample for each replica: LC = 20; FC = 200; Temperature range: LC = 15-20°C; FC = 18-25°C; ****Used as mediums; Seed germination did not occur in dark**

2.3 Development of Plant Production Technique (Ex Situ Conservation)

To make the cultivation of this herb possible in an effective way at lower altitudes, its response was observed in different soil textural classes and fertilizer applications (Table 3). The following soil textural classes were used: (i) Natural soil (collected from natural habitat). (ii) Loam : organic manure (2:1). (iii) Loam soil (garden soil) (iv) Sandy loam (2:1) (v) Sandy loam (1:2) (vi) Sand : silt : clay (1:2:2).

Young seedlings (of the same age) of *P. kurrooa* were collected from natural habitats in May and sown in the above mentioned soil textural classes in pots. Each set of pots (containing equal amount of soil) were irrigated at different intervals for the first year. In the next growing season the stabilized and adapted plants were treated with various inorganic fertilizers (Table 3) in different concentrations each with a set of control (Mohi-Ud-Din et al., 2022). The plants were kept in diffused light and harvested (at full bloom) to determine dry biomass (after oven dried at 80°C for 72 hours; Singh and Purohit, 2003).

TABLE 3
EFFECT OF VARIOUS TREATMENTS ON THE PRODUCTION OF *PICRORHIZA KURROOA* GROWN ON DIFFERENT SOIL TYPES

S. No.	Soil type & fertilizer applied	Dry weight per plant (gm)		Total dry biomass per plant (gm)
		below ground	above ground	
1	**Natural soil**	10.166 ± 2.020*	5.33 ± 0.763	**15.5 ± 2.783****
2	**Loamy soil**			
	N-300mg/kg soil	2.166 ± 1.040	5.0 ± 1.322	7.16 ± 2.084
	N-500mg	3.166 ± 0.763	6.33 ± 1.757	**9.50 ± 2.50****
	K-300mg	1.66 ± 0.766	3.833 ± 0.288	5.06 ± 1.914
	DAP-500mg	3.83 ± 1.755	4.166 ± 1.154	8.0 ± 2.179
	NPK-500mg†	4.33 ± 1.757	5.0 ± 2.783	**9.16 ± 4.481****
	Control	3.16 ± 0.763	1.66 ± 1.044	4.83 ± 0.473
3	**Loam with organic manure (2:1)**	3.0 ± 1.802	3.6 ± 0.60	**6.60 ± 2.260****
4	**Sandy loam (2:1)**			
	N-300mg/kg soil	1.833 ± 0.288	4.066 ± 2.328	5.9 ± 2.424
	N-500mg	3.33 ± 0.763	5.66 ± 1.040	**9.0 ± 0.866****
	K-300mg	1.67 ± 0.970	3.40 ± 1.153	5.06 ± 1.914
	DAP-500mg	2.80 ± 0.764	3.66 ± 0.763	6.5 ± 1.0
	NPK-500mg†	4.0 ± 0.50	5.066 ± 0.404	**9.066 ± 0.901****
	Control	1.66 ± 0.288	1.33 ± 0.577	3.0 ± 0.866
5	**Sandy loam (1:2)**			
	N-300mg/kg soil	5.33 ± 1.258	4.0 ± 1.50	**9.33 ± 1.258****
	N-500mg	8.0 ± 1.50	5.66 ± 2.02	**13.66 ± 0.581****
	K-300mg	3.30 ± 0.764	1.83 ± 0.577	5.0 ± 1.322
	DAP-500mg	1.33 ± 0.290	1.67 ± 0.763	3.0 ± 0.50
	NPK-500mg†	5.33 ± 1.040	4.833 ± 0.288	**10.16 ± 0.763****
	Control	1.83 ± 1.04	3.23 ± 0.461	5.06 ± 1.401
6	**Sand: Silt: Clay (1:2:2)**			
	N-300mg/kg soil	4.0 ± 0.50	5.5 ± 1.0	**9.50 ± 1.50****
	N-500mg	3.83 ± 1.154	2.80 ± 0.764	6.60 ± 1.260
	K-300mg	1.10 ± 0.360	1.0 ± 0.50	2.10 ± 0.692
	DAP-500mg	1.16 ± 0.288	1.0 ± 0.5	2.10 ± 0.583
	NPK-500mg†	3.83 ± 0.577	2.50 ± 1.0	6.16 ± 1.755
	Control	1.30 ± 0.36	1.0 ± 0.50	2.33 ± 0.763

***Mean* ± *Standard Deviation;* *Showed maximum dry biomass; †NPK (N-300mg + P-100mg + K-100mg) = 500mg/kg soil.**

2.4 In Vivo Propagation

The fresh underground as well as above ground stems of the species were collected from natural population Thajwas I, 3150m a.s.l (Kashmir Himalaya) in the first week of May and were cut horizontally into Basal (without shoot apex) and Top cuttings (with shoot apex). Both the types of cuttings were treated with 100ppm IAA, IBA and GA_3 for 48 hours and were sown in sandy loam soils under pots and trays at Herbal Garden (1495m), University of Kashmir, Srinagar (India). The pots and trays were kept in diffused light to monitor the leafy shoot generation, survival and comparison with the control (untreated cuttings) (Table 4).

TABLE 4
IN VIVO PROPAGATION OF *PICRORHIZA KURROOA*

Treatment	No. of cuttings studied		Days required for shoot regeneration		No. of plantlets survived till senescence		% survival	
	top*	basal**	top	basal	top	basal	top	basal
Control	12	19	8 to 12	10 to 12	9	8	75.0	42.10
IAA (100ppm)	15	20	6 to 9	8 to 15	14	12	93.33	60.0
IBA (100ppm)	15	20	7 to 10	8 to 15	12	11	80.0	55.0
GA_3 (100ppm)	20	25	4 to 10	6 to 14	19	17	95.0	68.0

**** cutting with shoot apex; ** cutting without shoot apex; *** Temperature Range = 21 to 27 Degree Centigrade***

2.5 Statistical Analysis

All experiments were conducted with three replications unless otherwise specified. Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by appropriate post-hoc tests where applicable. Differences were considered significant at $P < 0.05$. All statistical analyses were performed using [specify software, e.g., SPSS or GraphPad Prism].

III. RESULTS

3.1 Development of Agrotechniques

The studies on agrotechniques reveal that the total dry biomass per individual works to 15.5 ± 2.783gm in natural soil (soil collected from natural habitat). In loam and organic manure (2:1) the plants produce 6.60 ± 2.260gm dry biomass per individual. In the loam soil, among different fertilizers used nitrogen (N) (500mg/kg soil), DAP (500mg/kg soil) and NPK (500mg/kg soil) showed maximum dry biomass of 9.50 ± 2.50gm, 8.0 ± 2.179gm and 9.16 ± 4.481gm per individual respectively as against the control of 4.83 ± 0.473gm.

In the sandy loam (2:1) soil type among different fertilizers NPK (500mg/kg soil) and N - 500mg/kg soil showed good biomass production, which works to 9.066 ± 0.901gm and 9.0 ± 0.866gm respectively as against the control of 3.0 ± 0.86gm. In the sandy loam (1:2) soil type N - 500mg/kg soil and NPK - 500mg/kg soil showed the dry biomass production of 9.33 ± 1.258gm, 13.66 ± 0.581gm and 10.16 ± 0.763gm respectively as against the control of 5.06 ± 1.401gm.

In sand: silt: clay (1:2:2) soil type only the plants treated with nitrogen (300mg/kg soil) showed production of 9.50 ± 1.50gm of dry biomass per individual as compared to 2.33 ± 0.763gm of control.

The plants grown on the natural soil showed maximum belowground dry biomass (10.166 ± 2.020gm) per individual because of the loose aerated and moist soils full of decomposed litter.

FIGURES 1-6: Development of agrotechnology of *Picrorhiza kurrooa* growing in ex situ conditions under different fertilizer treatments showing differences in vegetative growth. 1. Control Plant; 2. DAP treated; 3. Nitrogen (300mg/kg soil) treated; 4. Nitrogen (500mg/kg soil) treated; 5. NPK (500mg/kg soil) treated; 6. Plant growing in soil (collected from natural habitat) - note the increased number of shoots.

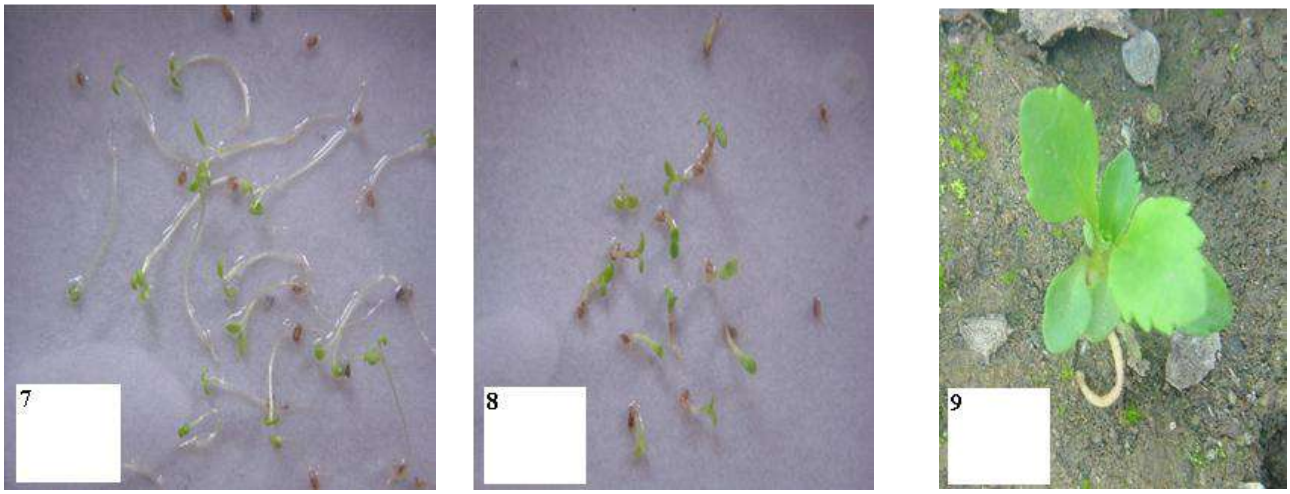
3.2 Seed Germination

The small perforated seeds of *Picrorhiza kurrooa* germinated nicely (57.5 ± 3.53% germination) without any treatment. However, the mean germination time (MGT) is higher which averages 18.66 ± 0.57 days.

The seeds fail to germinate when chilled at 4°C for varying periods (20 - 90 days) while as the seeds treated with GA_3 (0.125mM) and thiourea (0.25mM) germinate in large numbers (93.33 ± 2.88% and 73.33 ± 2.88% respectively) and relatively in lesser time compared to other treatments and control. The seeds kept for germination in different concentrations of kinetin also fail to germinate, however, the 2.0mM concentration showed 15.0 ± 5.0% germination.

In the field conditions, the seeds germinate only when placed on the upper layer of soil covered with thin film of water and exposed to light. The seeds fail to germinate when sown 1 - 1.5cm deep in the soil. The seedlings are too fragile and minute and show very less survival rate.

The seeds also germinate efficiently under laboratory conditions within the soil collected from natural habitats and covered with a thin film of water. In natural habitat the seeds sown after winter germinate in lesser quantity but mostly the seedlings fail to recruit while as the seeds sown before winter do not germinate at all.



FIGURES 7-9: In vitro seed germination of *Picrorhiza kurrooa*. 7 & 8: Small & fragile seedlings generated in Petri plates under laboratory conditions. 9: Transplanted seedling.

3.3 In Vivo Propagation

For the study of in vivo propagation the underground stem was transversely cut into top and basal segments. The study reveals that the cuttings without any treatment showed 75.0% and 42.10% survival in top and basal segments respectively in sandy loam soils. The number of days required for shoot regeneration is 8 - 12 days in case of top segments and 10 - 20 days in case of basal segments.

The cuttings treated with IAA (100ppm) showed 93.33% and 60.0% survival for top and basal segments respectively while as the cuttings treated with IBA (100ppm) showed 80.0% and 55.0% survival for the top and basal segments respectively. The number of days required for shoot generation in IAA treated cuttings is 6 - 9 days for top and 8 - 15 days for basal segments while as IBA treated cuttings require 7 - 10 days for top and 8 - 15 days for basal segments.

The GA_3 (100ppm) treated cuttings show best results and the number of days required for shoot generation was only 4 - 10 for top and 6 - 14 for basal segments and survival was 95.0% for top and 68.0% for basal segments.

FIGURES 10-12: In vitro propagation of *Picrorhiza kurrooa* through stem cuttings. 10: Regenerated cuttings treated with IAA. 11 & 12: Regenerated cuttings treated with GA_3, note increased number of shoots as compared to IAA treated cuttings.

IV. DISCUSSION

The alpine and sub-alpine medicinal plant species constitute an important natural bioresource. This wealth has been exploited extensively as a source of traditional herbal drugs and also in the manufacture of many allopathic drugs. The overexploitation of important species is the basic reason of their extinction. This has necessitated the development and standardization of agrotechniques for their cultivation and consequent conservation (Joshi et al., 1990). The major conservation strategies

recommended are development of germplasm centers, in situ and ex situ conservation of critically endangered species, establishment of high altitude nurseries, systematic collection and domestication etc. (Joshi and Rawat, 1997; Dwivedi, 1999).

Considering the increasing demand of herbal drugs in general and Himalayan medicinal plants in particular and consequent depletion of several species, it is important to urgently initiate proper steps for conservation (Nautiyal et al., 2001). In view of large-scale exploitation, biotic interferences and increasing demand for *Picrorhiza kurrooa* as herbal drug, its assessment as threatened species in nature, and consequently the need for its cultivation to salvage it from loss and also raise the germplasm and provide economic avenues for the poor, it becomes obligatory to initiate steps for its large scale cultivation and development of elementary agrotechniques at lower altitudes under ex situ conditions.

The plants performed well under shady conditions. Being a moisture loving plant, it requires good amount of moisture. Under shady conditions irrigation after 4 - 5 day interval resulted in highest survival rates and biomass production. Similar kind of responses has been reported by Nautiyal et al. (2001) while assessing the germinability of *Picrorhiza kurrooa*. *P. kurrooa* in its natural habitat grows on sandy, loamy silt and moisture rich humus soils while at the low altitude of Botanical Garden Kashmir University, 1495m, the plants got domesticated nicely on different textural classes. Thus, the plant enjoys and tolerates a wide range of soil texture and reaction.

Among different soil textural classes, the plants grown in natural soil (collected from its natural habitat) showed the best survival and performance with dry biomass of 15.5 ± 2.783gm per individual. On loamy soil among different nutrient trials nitrogen (500mg/kg soil) and NPK (500mg/kg soil) produced dry biomass of 9.50 ± 2.50gm and 9.16 ± 4.481gm per individual respectively as against the control of 4.83 ± 0.473gm.

The plants also showed good survival under sandy loam (2:1) textured soil and produced dry biomass of 9.066 ± 0.901gm on application of NPK (500mg/kg soil) as against the control of 3.0 ± 0.866gm and in sandy loam (1:2) textured soil, the NPK treated plants resulted in 10.16 ± 0.763gm as against the control of 5.06 ± 1.401gm per individual possibly because of the fact that the plant prefers sandy loam with organic matter in natural habitat. Such soils facilitate the underground rooting as the plants do have underground horizontal stem. In the loose aerated soil they grow better as compared to other textural soil classes. Similar kind of rooting and underground stem production was observed by Nautiyal et al. (2001) in *P. kurrooa* under sandy soils with litter treatments.

The species however, shows a poor performance in sand: silt: clay (1:2:2) soil. Possibly the rooting is difficult in clayey soils as the plant mostly prefers higher moisture content and loose soils. The plants also show best performance in loam soils grown together with many other grasses as compared to the plants growing in isolated populations. This perhaps is due to the fact that the combination of grasses taller than the species under discussion provides shade and protects the soil against exposure to direct sunlight due to which the moisture content remains higher than in the open beds where the species grows lonely. Under such conditions the plants of the *P. kurrooa* showed maximum number of vegetative shoots and thus propagate more efficiently as compared to the plants grown lonely.

The increasing demand for herbal drugs in general and Himalayan medicinal plants in particular and the depletion of important species from natural resources make it imperative to develop proper methods and strategies for their conservation and commercial production (Raina et al., 2003).

4.1 Seed Germination

Maximum viability and vigour of the seed coincide with the attainment of maximum dry weight or physiological maturity by it (Rasyad et al., 1990; Hay and Probert, 1995). However, some recent studies on a range of diverse crops have consistently shown that seed longevity, as well as other seed quality traits, continue to increase after the end of seed maturity phase (Kameswara et al., 1991; Zanakis et al., 1994).

Seeds of many plants fail to germinate immediately and pass through a phase of dormancy that may be caused by several factors thus delaying the life cycle of these plants. Some plants display a wide range of dormancy breaking and germination requirements (Leon, 1985). Besides, germination at the right time and in the right place largely determines the probability of seedling survival (Thompson, 1974). The dormancy characteristics and germination responses are considered to be the key elements in plant life history strategies (Meyer et al., 1990).

The dormancy state of seeds is determined by incubating populations of them on a moist substrate over a range of environmental conditions. Nondormant seeds germinate over the widest range of conditions possible for the species, while dormant ones fail

to germinate at any condition. Conditionally dormant seeds germinate over only a portion of the range of conditions possible for the species (Vegis, 1964; Baskin and Baskin, 1985). Buried seeds of many species change from one dormancy state to another and back again during the course of a year in response to seasonal cycles of environmental conditions (Baskin and Baskin, 1998).

The physiological and chemical treatments have been found to improve the seed germination, seedling growth and nitrogen metabolism in plants (Bose et al., 1982; Hay and Probert, 1995; Prassad, 1999; Bose and Sarma, 2000; Joshi and Dhar, 2003; Mohi-Ud-Din et al., 2005). Once the seed germinates successfully, establishment of the young seedlings as new recruits is a crucial demographic event and depends on the nutritional reserves within the seed (Thomas, 1966; Willson, 1983) and the ability of the seed to emerge from greater soil depths (Harper et al., 1970; Willson, 1983).

To understand the occurrence and distribution of a species in a certain habitat and its life history strategy, the timing of germination and the nature of dormancy breaking mechanisms assume great significance. The development of seed germination protocols is therefore, an important step (especially for threatened medicinal plants) to offset the increasing demand of the species in pharmaceutical industry (Joshi and Dhar, 2003). Such studies will also help in developing conservation strategies for the proper management and the maintenance of the bioresources especially economically important plants in general and medicinal plants in particular at lower and easy-to-approach habitats (Mohi-Ud-Din et al., 2005).

The species is endangered in status. Therefore availability of material for multiplication from the natural habitats is an arduous task (Nautiyal et al., 2001). The species produces a large quantity of small sized seeds, which mostly fail to recruit, with the result that the seedlings are rarely seen in natural habitat. The seed viability was tested by subjecting the seeds to different physical and chemical treatments (Table 2). The results reveal that under controlled conditions with a temperature range of 15°C to 20°C, the seeds germinate with ease without any treatment (control) and register 57.5 ± 3.53% germination.

GA_3 concentrations (0.125mM, 0.25mM and 0.5mM) showed 93.33 ± 2.88, 65.0 ± 5.0 and 41.66 ± 17.55% germination as against the control of 57.5 ± 3.53%. However, higher concentrations of GA_3 (1.5mM) resulted into only 16.66 ± 2.88% germination. Thiourea when given in 0.25mM, 0.5mM, 1.0mM, 1.5mM and 2.0mM concentrations resulted into 73.33 ± 2.88%, 62.33 ± 7.50%, 69.33 ± 4.04%, 70.0 ± 5.0% and 40.0 ± 5.0% germination.

Kinetin and conc. H_2SO_4 dip resulted into low germination, while the seeds chilled at 20 days resulted into 6.66 ± 2.88% germination and those at 40, 75 and 90 days suffered negative effects. In field conditions, however, the seeds germinate only when sown on the soil surface covered with thin film of water under exposed conditions. The seeds thus seem to be photoblastic in nature. The photoblastic nature of seeds has also been reported in a cofamilial taxon *Agalinis fasciculata* by Baskin et al. (1998).

The seedlings raised are too fragile and minute to survive after transplantation. If the species produces viable seeds, then why it fails to recruit in its natural home? To answer this question, the following manipulations were made:

1) Seeds were kept for germination within the soil (collected from natural habitat) and monitored under lab. conditions.
2) Seeds were sown in natural home (basic habitat) on pre-identified and selected spots before winter and after winter.
3) Seeds were sown in natural soil (in Lab. conditions) with a thin film of water.

These experiments reveal that in lab. conditions the seeds germinate in good quantity (60%) but only when kept on soil surface under a thin film of water. In natural habitat the seeds sown before winter fail to produce seedlings in the next growing season, while the seeds sown after winter (chilling) germinate but the seedlings fail to recruit because of seedling mortality.

From the above information, it is evident that the possible reasons for seed failure of the species in natural populations are:

1) Prolonged exposure to extreme chilly conditions in alpine sub-alpine habitats may lead to loss of viability in natural habitats.
2) The seeds are very small and photoblastic, thus non-availability of light due to burial of seeds below the leaf litter of the co-existing species may also inhibit germination.
3) Water seems to be essential for the germination both under lab. as well as field conditions. Thus due to non-availability of appropriate moisture in the natural habitat may lead to loss of seed's viability hence failure of germination.

4) The seedlings are too fragile and minute hence, may hardly survive in natural conditions as the smaller seedlings cannot compete with the larger seedlings and plants growing in the vicinity of the species.

Seeds of *P. kurrooa* are small in size. Such seeds are known to store less food material and thus have weaker ability to recover from competition and herbivory (Willson, 1983). Further, smaller seeds in general produce fragile seedlings with less survival potential (Edwards et al., 1934). The effects of seed size on seedling growth and survival have been studied for a variety of angiospermic taxa. The studies of Schaal (1980) reveal that the large sized seeds have a distinct advantage over the smaller seeds. Such seeds give the seedlings an attendant advantage in developing leaves earlier so as to meet the competition from co-occurring plants as compared to smaller seeds (Willson, 1983). Seeds also affects the time of seedling emergence which in turn is known to register a significant impact on seedling success (Ross and Harper, 1972). It is also important to know the extent to which seed size exerts an effect on seedling emergence.

From the above discussion, it becomes evident that the *Picrorhiza kurrooa* seed requires light and water to germinate. The seedlings being smaller and weak hardly survive in the harsh conditions of the alpine habitat. Thus in order to thwart this bottleneck, the species has established an efficient method of vegetative propagation.

4.2 Vegetative Propagation

Earlier, the micropropagation studies of the species have been carried out in vitro under tissue culture (Lal et al., 1988; Upadhyay et al., 1989). The present studies were undertaken to develop a simple and cost effective technique for the conservation and commercial production of the species. The studies reveal that the underground stem segments are most successful not only for multiplication but also for higher commercial production in a short period of time. Since multiplication through seeds is difficult (discussed earlier), this method can be used as an alternative for quick multiplication of the species under ex situ conditions.

The studies reveal that the top segments are efficient explants as compared to the basal segments. The segments treated with IAA, IBA and GA_3 (100ppm) show better growth, best survival and require lesser time to regenerate as compared to the control. Thus, the species demonstrates better regeneration and multiplication from the stems. Similar kind of trend has earlier been reported by Nautiyal et al. (2001) in the same species.

For the cultivation and conservation of *Picrorhiza kurrooa*, the methods developed during the present study can provide not only an alternate income generating resource but can also provide the opportunity for their sustainable management and long term supply of the drug.

V. CONCLUSION

The present study has successfully developed comprehensive propagation and conservation protocols for the endangered Himalayan medicinal plant *Picrorhiza kurrooa*. The key findings reveal that:

1) **Seed Germination**: The species is photoblastic, requiring light for germination. Maximum germination (93.33 ± 2.88%) was achieved with 0.125mM GA_3 treatment, with significantly reduced mean germination time (8.0 ± 0.866 days) compared to control (18.66 ± 0.57 days). The failure of natural recruitment is attributed to the combination of photoblastic nature, small seed size, fragile seedlings, and harsh alpine conditions.

2) **Ex Situ Cultivation**: The species performs best in loamy and sandy loam soils, with maximum dry biomass production (13.66 ± 0.581g) achieved in sandy loam (1:2) soil with nitrogen supplementation (500mg/kg soil). NPK supplementation also significantly improved biomass production.

3) **Vegetative Propagation**: Underground stem cuttings provide an efficient alternative for multiplication, with GA_3 (100ppm) treated top segments showing 95.0% survival and rapid shoot regeneration (4-10 days).

Recommendations for Conservation and Cultivation:

1) **Seed-Based Propagation**: For large-scale multiplication, seeds should be germinated in vitro with 0.125mM GA_3 treatment under light conditions at 15-20°C, followed by careful transplantation of seedlings.

2) **Vegetative Propagation**: For quick multiplication, underground stem cuttings (top segments) treated with GA_3 (100ppm) should be used, ensuring high survival rates and rapid regeneration.

3) **Cultivation**: Plants should be grown in sandy loam or loamy soils with adequate organic matter, under diffused light conditions, with nitrogen or NPK supplementation (500mg/kg soil).

4) **Conservation**: In situ conservation should focus on protecting natural populations from overexploitation, while ex situ cultivation should be promoted to meet the demand for raw material and reduce pressure on wild populations.

Limitations of the Study: The study was conducted at a limited number of sites in the Kashmir Himalaya, and the findings may not be directly applicable to other regions. The long-term survival and reproductive success of propagated plants in natural habitats were not assessed. Future research should include reintroduction trials, genetic diversity studies of propagated populations, and assessment of the economic viability of large-scale cultivation.

ACKNOWLEDGEMENTS

The authors thank the University of Kashmir for providing facilities and the Department of Forests, Jammu and Kashmir, for permission to collect plant material from the study sites.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Ayensu, E. S. (1981). Assessment of threatened plant species in the United States. In H. Singe (Ed.), *The biological aspects of rare plant conservation* (pp. 19-58). John Wiley & Sons.

[2] Ansari, R. A., Tripathi, S. C., Patnaik, G. K., & Dhawan, B. N. (1991). Antihepatotoxic properties of Picroliv, an active fraction from the rhizomes of *Picrorhiza kurroa*. *Journal of Ethnopharmacology*, *34*, 61-68.

[3] Anwar, S., Bashir Ahmad Subhan, M., Gul, W., & Nazar-Ul-Islam. (2003). Biological and pharmacological properties of *Aconitum chasmanthum*. *Journal of Biological Sciences*, *3*, 989-993.

[4] Anonymous. (1997). *Threatened medicinal plants of Himalayas—A checklist*. CAMP Workshop.

[5] Baskin, J. M., & Baskin, C. C. (1985). The light requirement for germination of *Aster pilosus* seeds: Temporal aspects and ecological consequences. *Journal of Ecology*, *73*(3), 765-773.

[6] Baskin, C. C., & Baskin, J. M. (1998). *Seeds: Ecology, biogeography, and evolution of dormancy and germination*. Academic Press.

[7] Bose, B., & Sarma, M. C. S. (2000). Influence of maize seed treatment with nitrates on nitrogen economy and nitrogen pollution. *Indian Journal of Plant Physiology*, *5*, 93-95.

[8] Bose, B., Srivastava, H. S., & Mathur, S. N. (1982). Effect of some nitrogenous salts on nitrogen transfer and protease activity in germinating *Zea mays* L. seeds. *Biologia Plantarum*, *24*(2), 89.

[9] Dhar, U., & Kachroo, P. (1983). *Flora of Kashmir Himalaya*. Scientific Publishers.

[10] Dwivedi, S. W. (1999). Traditional health care among tribals of Rewa district of Madhya Pradesh with special reference to conservation of endangered and vulnerable species. *Journal of Economic and Taxonomic Botany*, *23*, 315-320.

[11] Edwards, T. I., Pearl, R., & Gould, S. A. (1934). Influence of temperature and nutrition on the growth and duration of life of *Cucumis melo* seedlings. *Botanical Gazette*, *96*, 118-135.

[12] Giri, A., Ahuja, P. S., & Kumar, P. V. A. (1993). Somatic embryogenesis and plant regeneration from callus cultures of *Aconitum heterophyllum* Wall. *Plant Cell, Tissue and Organ Culture*, *32*, 213-218.

[13] Gurumurthi, K., Gupta, B. B., & Kumar, A. (1984). Hormonal regulation of root formulation. In S. S. Purohit (Ed.), *Hormonal regulation of plant growth and development* (pp. 387-400). Agrobotanical Publishers.

[14] Harper, J. L., Lovell, P. H., & Moore, K. G. (1970). The shapes and sizes of seeds. *Annual Review of Ecology and Systematics*, *1*, 327-356.

[15] Hay, F. R., & Probert, R. J. (1995). Seed maturity and the effects of different drying conditions on desiccation tolerance and seed longevity in foxglove (*Digitalis purpurea* L.). *Annals of Botany*, *76*(6), 639-647.

[16] Joshi, D. N., & Rawat, G. S. (1997). Need for conservation and propagation of alpine and subalpine medicinal plants of North West Himalaya. *Indian Forester*, *123*, 811-814.

[17] Joshi, G. C., Pandey, N. K., Tiwari, K. C., & Tiwari, R. N. (1990). A preliminary synecological approach for the study of herbal wealth of high altitude Himalaya and their conservation strategy. *Bulletin of Medico-Ethnobotanical Research*, *11*, 96-102.

[18] Joshi, M., & Dhar, U. (2003). Effect of various presowing treatments on seed germination of *Heracleum candicans* Wall. ex DC.: A high value medicinal plant. *Seed Science and Technology*, *31*, 737-743.

[19] Lal, N., Ahuja, P. S., Kukreja, A. K., & Pandey, B. (1988). Clonal propagation of *Picrorhiza kurroa* Royle ex Benth. by shoot tip culture. *Plant Cell Reports*, *7*, 202-205.

[20] Leon, J. A. (1985). Germination strategies. In P. J. Greenwood, P. H. Harvey, & M. Slatkin (Eds.), *Evolution: Essays in honour of John Maynard Smith* (pp. 129-142). Cambridge University Press.

[21] Kameswara Rao, N., Appa Rao, S., Mengesha, M. H., & Ellis, R. H. (1991). Longevity of pearl millet (*Pennisetum glaucum* R. Br.) seeds harvested at different stages of maturity. *Annals of Applied Biology*, *119*(1), 97-103.

[22] Khan, A. A., & Zaidi, S. H. (1991). Cultivation prospects of *Mentha arvensis* Linn. at Peshawar, Pakistan. *Pakistan Journal of Forestry*, *41*, 170-173.

[23] Kirtikar, K. R., & Basu, B. D. (1988). *Indian medicinal plants* (2nd ed., Vol. I, pp. 195-196). Lalit Mohan Basu.

[24] León, J. A. (1985). Germination strategies. In P. J. Greenwood, P. H. Harvey, & M. Slatkin (Eds.), *Evolution: Essays in honour of John Maynard Smith* (pp. 129-142). Cambridge University Press.

[25] Manjikola, S., Dhar, U., & Rawal, R. S. (2005). Phenology and biology of *Arnebia benthamii*: A critically endangered medicinal plant of the Himalaya. *Proceedings of the National Academy of Sciences, India*, *75B*(4), 283-287.

[26] Meyer, S. E., Monsen, S. B., & McArthur, E. D. (1990). Germination response of *Artemisia tridentata* (Asteraceae) to light and chill: Patterns of between-population variation. *Botanical Gazette*, *151*, 176-183.

[27] Mohi-Ud-Din, G. G., Nawchoo, I. A., & Wafai, B. A. (2005). Seed germination studies on *Saussurea costus* (Falc.) Lipsch.—A threatened medicinal plant of N.W. Himalaya. *Indian Journal of Plant Physiology*, *10*(4), 394-396.

[28] Mohi-Ud-Din, G. G., Nawchoo, I. A., & Wafai, B. A. (2007). Reproductive biology of *Picrorhiza kurrooa* Royle ex Benth.—An endangered medicinal plant of the north west Himalaya. *Phytomorphology*, *57*, 109-116.

[29] Mohi-Ud-Din, G. G., Nawchoo, I. A., & Wafai, B. A. (2009). Meiotic system and pollination mechanisms of the critically endangered *Aconitum chasmanthum* Stapf ex Holmes—A novel species endemic to Kashmir Himalayan region. *International Journal of Plant Reproductive Biology*, *1*(2), 153-162.

[30] Mohi-Ud-Din, G. G., Nawchoo, I. A., & Wafai, B. A. (2022). Efficient propagation methods and management implication of *Aconitum chasmanthum* Stapf ex Holmes: A critically endangered and endemic medicinal herb of Kashmir Himalaya. *Indian Journal of Agricultural Research*, *58*(4), 657-663. https://doi.org/10.18805/IJARe.A-5990

[31] Nadeem, M. A., Kumar, A., Nand, S. K., & Palni, L. M. S. (2001). Tissue culture of medicinal plants with particular reference to Kumaun Himalaya. In S. S. Samant, U. Dhar, & L. M. S. Palni (Eds.), *Himalayan medicinal plants: Potentials and prospects* (pp. 231-268). Gyanodaya Prakashan.

[32] Naidu, C. V., Josthna, P., Swamy, P. M., & Bhupathi Naidu, M. (2006). Effect of auxins on the vegetative propagation of VI mulberry variety (*Morus alba* L.) stem cuttings. *Journal of the Indian Botanical Society*, *85*, 33-34.

[33] Nautiyal, B. P., Prakash, V., Chauhan, R. S., Purohit, H., & Nautiyal, M. C. (2001). Assessment of germinability, productivity and cost benefit analysis of *Picrorhiza kurrooa* cultivated at lower altitudes. *Current Science*, *81*, 579-585.

[34] Nayar, M. P., & Sastry, A. R. K. (Eds.). (1988). *Red data book of Indian plants* (Vol. 2). Botanical Survey of India.

[35] Prakash, V., Bisht, H., & Nautiyal, M. C. (2011). Seed germination enhancement in high altitude medicinal plants of Garhwal Himalaya by some pre-sowing treatments. *Research Journal of Seed Science*, *4*(4), 199-205.

[36] Prasad, P. (1999). Enhancement of seed germination of *Podophyllum hexandrum* Royle and *Aconitum heterophyllum* Wall. by different treatments. *Journal of Hill Research*, *12*(2), 102-106.

[37] Primack, R. B. (1980). Variation in the phenology of natural populations of montane shrubs in New Zealand. *Journal of Ecology*, *68*, 849-862.

[38] Raina, R., Behera, M. C., Chand, R., & Sharma, Y. (2003). Reproductive biology of *Gentiana kurroo* Royle. *Current Science*, *85*(5), 667-670.

[39] Ramesh, M. N., Farooqui, A. A., & Tilak, S. (1989). Influence of sowing date and nutrients on growth and yield on Isbagol (*Plantago ovata*). *Crop Research*, *2*, 169-174.

[40] Rasool, G. (1998). *Medicinal plants of the Northern Areas of Pakistan* (pp. 13-15). SAAD Printo Pack.

[41] Rasyad, A., VanSanford, D. S., & TeKrony, D. M. (1990). Changes in seed viability and vigor during wheat seed maturation. *Seed Science and Technology*, *18*, 259-267.

[42] Ross, M. A., & Harper, J. L. (1972). Occupation of biological space during seedling establishment. *Journal of Ecology*, *60*(1), 77-88.

[43] Schaal, B. A. (1980). Reproductive capacity and seed size in *Lupinus texensis*. *American Journal of Botany*, *67*(5), 703-709.

[44] Singh, A. K., & Neoparly, B. (1993). Effect of NPK nutrition and spacing on yield attributes in ginger. *Haryana Journal of Horticultural Sciences*, *22*, 143-148.

[45] Singh, A., Kuniyal, C. P., Lata, H., Rajasekaran, C., Prasad, P., Bhadula, S. K., & Purohit, A. N. (1998). In vitro propagation of *Aconitum atrox* (Bruhl) Muk.: A threatened medicinal herb from Garhwal Himalaya. *Physiology and Molecular Biology of Plants*, *4*, 171-174.

[46] Singh, V. P., & Purohit, S. (2003). *Research methodology in plant sciences* (p. 1). Scientific Publishers (India).

[47] Thomas, R. L. (1966). The influence of seed weight on seedling vigor in *Lolium perenne* L. *Annals of Botany*, *30*, 111-121.

[48] Thompson, P. A. (1974). Effects of fluctuating temperatures on germination. *Journal of Experimental Botany*, *25*(1), 164-175.

[49] Vegis, A. (1964). Dormancy in higher plants. *Annual Review of Plant Physiology*, *15*, 185-224.

[50] Upadhyay, S., Kukreja, G. S., & Ahuja, P. S. (1989). In vitro propagation of *Picrorhiza kurroa* Royle ex Benth. through shoot tip culture. *Plant Cell Reports*, *8*, 215-218.

[51] Willson, M. F. (1983). *Plant reproductive ecology*. John Wiley & Sons.

[52] Yousef, E., & Mahmood, M. (2014). An efficient method in breaking of dormancy from *Bunium persicum* (Boiss.) Fedtsch seeds: A valuable herb of Middle East and Central Asia. *Asian Pacific Journal of Tropical Biomedicine*, *4*(8), 642-649.

[53] Lodhi, Y., Sangeeta, S., Chakravorty, S., & Prasad, B. V. G. (2019). Enhanced effect of nitrogen and phosphorus on growth and yield of *Capsicum*: A review. *International Journal of Current Microbiology and Applied Sciences*, *8*(11), 2425-2433.

[54] Zanakis, G. N., Ellis, R. H., & Summerfield, R. J. (1994). Seed quality in relation to seed development and maturation in three genotypes of soybean (*Glycine max*). *Experimental Agriculture*, *30*(2), 139-156.

International Journal of Environmental & Agriculture Research (IJOEAR)
ISSN: [2454-1850]
[Vol-12, Issue-9, September- 2026]
Website: www.ijoear.com
Journal DOI: 10.25125/agriculture-journal

Income Distribution and Inequalities among Rural Farmers of Nagaland, India

Sungjeminla Longkumer

Assistant Professor, Department of Economics, Tetso College, Dimapur Nagaland

Received:- 12 August 2026/ Revised:- 18 August 2026/ Accepted:- 31 August 2026/ Published: 15-09-2026

Abstract— *The study attempts to analyze sources and distribution of rural households' income from farm and non-farm activities and examines the extent of income inequalities across farm sizes. The study is based on primary data collected from 200 farm households of Nagaland through a multi-stage simple random sampling technique. The results indicate that nearly half of the income is from non-farm sources (48.57 percent). Contrary to the popular belief that farming is the principal source of income for rural farm households, the study shows that households derive nearly half of their income from non-farm sources. Another significant finding from the analysis is that across farm size, farm size and farm income show a positive relationship, whereas farm size and non-farm income show an inverse relationship. The non-farm income is most unequally distributed among sources of income, followed by other farm income. The estimation of Gini ratio also reveals that income inequality is higher among marginal and small farmers.*

Keywords— Farm income, Non-farm income, Participation, Gini coefficient.

I. INTRODUCTION

The rural economy in India is predominantly dependent on agriculture and allied activities, on which roughly 54 percent of the rural households depend, while 46 percent depend on non-agricultural activities. However, for the past few years, the structure of Indian agriculture has been experiencing a decline in farm size, which has led to a rise in the marginalization of holdings (Meena et al., 2017). The distribution of area in percentage for marginal farmers increased from 29.8 percent to 34.5 percent between 2012–13 and 2018–19. In contrast, the percentage area for medium farms decreased from 23.1 percent in 2002–03 to 14.7 percent in 2018–19, and for large farmers, it decreased from 11.6 percent in 2002–03 to 5.8 percent in 2012–13 and then further declined to 3.9 percent (National Statistical Survey Report, 2019). As one of the developing economies, India still relies heavily on agriculture to generate income and employment in its rural areas, while it is widely acknowledged that this sector alone cannot keep up with the country's growing population.

It has long been assumed that rural households in developing countries are almost entirely focused on farming and neglect other non-farm activities in the countryside, according to the World Bank (2008). In order to improve rural well-being and lower poverty in the world's growing nations, a comprehensive approach that goes beyond agricultural development is therefore required. According to Chand et al. (2011), small landowners who relied solely on agriculture for their income were likely to have continued to live in poverty. Given the issue of income variability, rural farmers turned to non-farm income streams as an alternate source of income. Identifying the most effective ways to promote non-farm activities is vital, since non-farm income is essential for rural households to sustain and improve their standard of living and may even be a means of escaping poverty (FAO, 2002).

Since the beginning of the green revolution in the early 1960s, Indian agriculture has evolved towards modern agriculture, resulting in the adoption of improved technology and the development of infrastructure within the agricultural sector. While

productivity grew, the experience of its benefits was uneven across regions and farm sizes, which has created economic inequality among agricultural households (Dhanagare, 1988; Freebairn, 1995). The green revolution promoted expensive technology investments for the richer farmers in those resource-rich areas, keeping away the small farmers in resource-scarce areas (Shiva, 1993). Because of scarce resources, farmers were more competitive, which increased the gaps between social classes and geographic areas and, ultimately, led to an uneven distribution of income. The adoption of economic reforms and liberalization in India, which has predominantly benefited the top 1 percent of the population, who held more than one-fifth of the entire national income in 2021, is another example of growing inequality. The bottom half, however, only has 13 percent of the income (World Inequality Lab, 2022). India is one of the most unequal countries as a result. Land ownership and agricultural revenue are strongly related. The very unequal distribution of land in India has resulted in significant economic inequalities within rural communities, as disclosed by Ranganathan et al. (2016).

Formulating policy measures that target welfare efforts at the most disadvantaged farmers becomes easier with information on various income sources and the degree of inequality across farm-size households. Numerous studies have been conducted on the distribution of income among rural populations at the national level (Birthal et al., 2014; Ranganathan et al., 2016; Chakravorty et al., 2016; Priscilla et al., 2021). However, the research on income inequality that specifically deals with farm households and focuses on various farm-size categories is still few (Kaur & Singh, 2014). While national-level studies provide valuable insights, there is a need for region-specific research, particularly in less-explored areas like Nagaland, where the agrarian structure, cultural practices, and livelihood strategies differ significantly from other parts of India. The present study addresses this gap by examining income sources and inequalities among rural farm households in Nagaland, a state with a unique socio-economic and agricultural context.

The objectives of the study are:

1) To evaluate the distribution of rural household income from both farm and non-farm activities across farm sizes.
2) To examine the extent to which income inequality exists among various farm size categories.

II. CONCEPTUAL FRAMEWORK

The study is guided by the conceptual framework linking farm size, income diversification, and inequality. In rural economies of developing countries, farm households are not homogeneous; they differ in terms of land holdings, access to resources, and livelihood strategies. The farm household model suggests that households make decisions about resource allocation (labor, capital, land) across farm and non-farm activities to maximize income and minimize risk (Ellis, 1998). Income diversification theory posits that households with smaller land holdings are more likely to diversify into non-farm activities due to land constraints, while larger farmers tend to specialize in agriculture (De Janvry et al., 2005).

The relationship between farm size and income inequality operates through multiple channels. First, land is the primary productive asset in rural areas, and its unequal distribution leads to unequal income from agriculture. Second, access to non-farm opportunities is often influenced by factors such as education, social networks, and capital, which are correlated with farm size. Third, the green revolution and technological change have favored larger farmers who can afford expensive inputs and machinery, exacerbating inequalities (Shiva, 1993). The present study examines these relationships in the context of Nagaland, where small and marginal farmers constitute the majority of the agricultural population.

The conceptual framework posits that:

- Farm income increases with farm size due to economies of scale and better access to resources.
- Non-farm income decreases with farm size as smaller farmers are pushed into non-farm activities due to land constraints.
- Income inequality is higher among smaller farmers due to greater variability in non-farm income sources.
- Participation in different income sources varies systematically across farm sizes.

III. METHODOLOGY

3.1 Study Area

Nagaland, one of the smallest states in the country, has an agrarian economy, with 60 percent of its working population engaged in the agricultural sector. As per the Advance Estimate (AE) of the Nagaland Economic Survey (2025–26), the sector's percentage share in the Gross State Domestic Product (GSDP) has decreased from 31.41 percent in 2011–12 to 23 percent in 2025–26. As per the 2011 census, the state has a total population of 19,78,502, with 71.14 percent living in rural areas and the remaining 28.86 percent in urban areas. The estimated number of rural households in the state is 2,40,200. Out of which, 1,91,800 were agricultural households, accounting for 79.9 percent of total rural households (Agriculture Statistics at a Glance, 2024-05).

An agricultural household earned an average income of ₹9950 per month, whereas a non-agricultural household earned an average of ₹10043 in Nagaland. The average monthly income of agricultural households in the state is somewhat higher than the ₹8951 of the national average (NABARD, 2021). With an estimated 1.287 ha of operating area per household, Nagaland has a higher average than other states. As per the National Statistical Survey report (2019), the state's medium and large farmers accounted for only 23.5 percent of farm households during the agricultural year 2018–19, while 76.5 percent of farm households were small and marginal farmers.

3.2 Data Base

A set of well-structured questionnaires was used in a multi-stage simple random sample procedure to gather primary data. Initially, the Peren district was selected to serve as the sample district. In the following stage, out of the four blocks in the district, two blocks—Peren and Jalukie—were chosen for the study. Four villages were then chosen: Punglwa and Gaili from the Peren block, and Jalukie B and Samziuram from the Jalukie block. Finally, 200 rural farm households were chosen at random to serve as sample units, consisting of 50 households from each village under study. Based on the size of the farm, the sample households were categorized into four groups: Marginal farmers (operating up to 2.5 ac), small farmers (between 2.51 to 5.0 ac), Medium farmers (between 5.0 to 10 ac), Large farmers (more than 10.01 ac). The survey was conducted during the agricultural year 2020-21, covering both pre-monsoon and post-monsoon seasons to capture seasonal variations in income.

3.3 Data Analysis

For the data analysis, two income sources are considered, i.e., farm and non-farm income.

- **Farm income** includes crop production such as income from paddy, oilseeds, vegetables, spices, etc., and other farm income, i.e., income from livestock output, diary, etc., sale of seeds and plants, agricultural wages, renting or hiring out implements and machinery, plantations, etc.
- **Non-farm income** includes income from business, self-employment, salary, casual labour, and land rent received.

The following are the statistical tools used for the study:

To find out the level and distribution of various income sources, averages and percentages were used to identify the share of contributions from various farm and non-farm incomes across the farm size categories.

The Gini coefficient was used to calculate the disparity of income (farm and non-farm) among the farmers by farm size. This provides a statistical measure of distribution. The value of the coefficient is a number that ranges from 0 to 1, where 1 represents perfect inequality and 0 represents perfect equality.

$$G = \frac{\sum_{i=1}^{n}\sum_{j=1}^{n}|x_i - x_j|}{2n^2\bar{x}} \quad (1)$$

Where x is an observed value, n is the number of values observed, and $\bar{x}$ is the mean value. Adequate equality is shown by a coefficient in the range of 0.3 to 0.4. This indicates that while wealth or income is allocated suitably, it may still be disbursed more equitably. A significant income disparity is indicated by a coefficient larger than 0.4 (Hasell, 2023).

IV. RESULTS AND DISCUSSION

4.1 Distribution of Income and Participation of Rural Households in Farm Activities

The distribution of income by sources and participation rate of rural farm households in a range of farm activities are depicted in Table 1.

TABLE 1
HOUSEHOLDS' FARM INCOME (AVERAGE IN ₹) AND PARTICIPATION (IN %) IN FARM ACTIVITIES

Sources of Farm Income	Marginal (N=85)		Small (N=75)		Medium (N=29)		Large (N=11)		All size (N=200)	
	Annual Income	Participation (%)	Annual Income	Participation (%)	Annual Income	Participation (%)	Annual Income	Participation (%)	Annual Income	Participation (%)
Crop production	54164.71 (35.2)	100	111449.33 (41.79)	100	172368.97 (41.1)	100	209818.18 (38.95)	100	101347 (39.61)	100
Livestock	56402.94 (36.65)	97.65	84694.54 (31.76)	100	152054.82 (36.26)	96.26	273500 (50.77)	100	92822.15 (36.28)	98.5
Sale of seeds and plants	688.24 (0.45)	12.94	1533.33 (0.57)	20	4851.72 (1.16)	48.28	4000 (0.74)	36.36	1791 (0.69)	22
Agri. Wage	20361.18 (13.23)	83.53	16664 (6.25)	66.67	10689.66 (2.55)	41.38	6363.64 (1.18)	27.27	16802.5 (6.57)	68
Hiring-out implements & machinery	8775.29 (5.7)	27.06	21000 (7.87)	48	41862.07 (9.98)	62.07	20909.09 (3.88)	63.64	18824.5 (7.36)	42
Plantation	10564.71 (6.86)	30.59	28282.67 (10.6)	29.33	35344.83 (8.43)	55.17	22272.73 (4.13)	36.36	21446 (8.38)	34
Others	2941.18 (1.91)	16.47	3093.33 (1.16)	14.67	2241.38 (0.53)	17.24	1818.18 (0.34)	9.09	2835 (1.11)	15.5
Total	**153898.25 (100)**		**266717.2 (100)**		**419413.45 (100)**		**538681.82 (100)**		**255868.15 (100)**	

Source: Computed from field survey data, 2020-21
Note: Figures in the bracket represent percentage of income in the total

A rural household by and large earns about ₹2,55,868.15 from farming activities annually. The primary source of income for the household on the farm is crop production, which accounted for 39.53 percent of total farm income. Livestock came in second with 36.32 percent. Therefore, 75.85 percent of the total farm income has come from the production of crops and livestock combined. Additional secondary sources include earnings from agriculture wages (6.58 percent), plantations (8.39 percent), and the hiring out of machinery and other assets (7.37 percent).

The average annual household farm income for each farm size category is estimated to be ₹153,898.25, ₹266,717.2, ₹419,413.45, and ₹538,681.82 for marginal, small, medium, and large farmers, respectively. For marginal farmers, livestock accounts for the largest proportion of farm income (36.65 percent), followed by crop production (35.2 percent) and agricultural wages (13.23 percent). For small and medium-sized farmers, the production of crops (41.79 percent and 41.1 percent, respectively) and livestock (31.76 percent and 36.36 percent, respectively) constitute the main sources of revenue. For medium farms, the secondary sources were renting out machinery and implements (9.98 percent), while for small farmers, plantations accounted for 10.6 percent of the farm income. On the other hand, livestock accounts for 50.77 percent of the income earned by large farms, with crop production coming in second at 38.95 percent.

Approximately two-thirds of the household's total farm revenue across all farm sizes is contributed by crop and livestock productions.

4.2 Farmers' Participation in Farm Activities

It was found that 100 percent of the sample households are involved in agricultural production. Livestock production is the second major farm activity, with 98.5 percent of households involved. The study confirms that raising livestock is a crucial component of rural livelihood, supplementing income earned from growing crops for all farm size categories. There is 100 percent involvement for both large and small farmers in livestock production. For marginal and medium farmers, it is 97.65 percent and 96.26 percent, respectively. By tradition in Nagaland, livestock is considered an important asset that determines household wealth and ensures security, especially in rural areas, which can be readily converted into cash when required. Primary livestock are piggery, poultry, cattle, etc.

Agricultural wage activity is another important source of income for marginal and small farmers, with participation rates of 83.53 percent and 66.67 percent, respectively. Both the income from agricultural wages and its participation rate decreases as

farm sizes grow; that is, as farms grow in size, the portion of the income and participation rate in wages declines. Birthal et al. (2014) shared similar conclusions from their research conducted in India. For medium and large farmers, the participation rate in hiring out machinery and implements was estimated at 62.07 percent and 63.64 percent, respectively. It is followed by plantations with 55.17 percent and 36.36 percent, respectively.

Across all farm size categories, the income from crops and livestock contributes substantially more than that from all other activities. Furthermore, compared to larger farmers, small and marginal farmers are more dependent on agricultural wage income. Larger farm households, on the other hand, diversify their sources of income by leasing out equipment and machinery, selling seeds and plants, and operating plantations, all of which supplement income from the output of livestock and crops. Similar conclusions were made by Kaur (2017), who stated that farm revenue accounts for a sizable portion of the total income in large and medium-sized farm households. However, compared to larger farmers, the participation of small and marginal farmers in high-yield varieties was lower due to their smaller farmland and lower income levels.

4.3 Distribution of Income and Participation of Rural Households in Non-Farm Activities

TABLE 2
HOUSEHOLDS' INCOME (AVERAGE IN ₹) AND PARTICIPATION (IN %) FROM NON-FARM ACTIVITIES

Sources of Non-Farm Income	Marginal (N=85)		Small (N=75)		Medium (N=29)		Large (N=11)		All size (N=200)	
	Annual Income	Participation %	Annual Income	Participation %	Annual Income	Participation %	Annual Income	Participation %	Annual Income	Participation %
Business/self employed	69254.12 (31.68)	80	52496 (24.57)	45.33	42068.97 (11.86)	31.03	20363.64 (6.53)	36.36	56339 (23.32)	57.5
Regular/salaried employees	138436.5 (63.33)	49.41	150260 (70.34)	54.67	306000 (86.25)	75.86	287090.91 (92.02)	63.64	175343 (72.57)	56
Land rent received	3058.82 (1.4)	4.71	1813.33 (0.85)	4	0 (0)	0	2727.27 (0.87)	9.09	2130 (0.88)	4
Casual labour	7847.06 (3.59)	35.29	9053.33 (4.24)	40	6724.14 (1.9)	31.03	1818.18 (0.58)	9.09	7805 (3.23)	35
Total	**218596.5 (100)**		**213622.66 (100)**		**354793.11 (100)**		**312000 (100)**		**241617 (100)**	

Source: Computed from field survey data, 2020-21
Note: Figures in the bracket represent percentage of the total income

4.4 Distribution of Non-Farm Income

The average household's annual income from non-farm sources is ₹2,41,617. The primary source of non-farm income is salary income (72.57 percent), with a participation rate of 56 percent. It shows that a substantial amount of the non-farm income is generated by salaried activities, which are carried out by more than half of the farm households. This is true for all farm size categories; for marginal, small, medium, and large farmers, this percentage is 63.33 percent, 70.34 percent, 86.25 percent, and 92.02 percent, respectively. In comparison to smaller farm sizes, larger farms have a higher percentage.

Three other sources of non-farm income include rent (0.88 percent), casual labour (3.23 percent), and small businesses (23.32 percent).

4.5 Farmers' Participation in Non-Farm Activities

Different farm sizes have different situations when it comes to participation rates. Only 31.68 percent of marginal farmers' total income came from non-farm small business operations, despite the fact that 80 per cent of them had participated in such activities. It shows that marginal farmers are mostly engaged in small businesses, which have low returns compared to salaries. Although the participation rate in salaries is only 49.41 percent, they account for almost 60 percent of all non-farm income.

Small and marginal farmers have greater participation rates (35.29 percent and 40 percent, respectively) for casual wages than large farmers (9.09 percent only). Less than 5 percent of the average income of farm households across all categories came from casual wages, which is more common among small and marginal farmers. This is in accordance with the findings of Ohno et al. (2021). The current study reveals an interesting finding that there is a negative correlation between farm size and participation in business and casual wages, which tend to decline with farm size.

On the other hand, salary income tends to rise, suggesting a positive correlation with farm size. Similarly, Lanjouw and Shariff (2002) noted that wages are skewed toward the marginal farmers and the salary incomes are distributed more favorably to the

affluent farmers in the country. Land rent provides a minimum income of less than 2 percent, with marginal farmers receiving the largest percentage of this income at 1.4 percent of their individual average non-farm income. Therefore, it can be concluded that small farmers have more diverse income sources than large farmers, which is also supported by past research (Subramanian, 2018; Saini & Kaur, 2022).

4.6 Distribution of Income by Farm and Non-Farm Sources

In addition to farming, rural farmers also engage in non-farm activities as a means of generating income. The percentages of income from different sources have been organized by farm size categories in Table 3.

TABLE 3
AVERAGE INCOME FROM FARM AND NON-FARM INCOME AMONG RURAL FARMERS IN PEREN DISTRICT

Farm Size	Farm Income			Non-farm Income	Total Income (Farm & Non-farm)
	Crop Production	Other Farm Income	Total Farm Income		
Marginal	54164.71 (14.54)	99733.53 (26.78)	153898.2 (41.32)	218596.47 (58.68)	372494.71 (100)
Small	111449.33 (23.20)	155267.87 (32.32)	266717.2 (55.52)	213622.67 (44.47)	480339.87 (100)
Medium	172368.97 (22.26)	247044.48 (31.91)	419413.5 (54.17)	354793.10 (45.83)	774206.55 (100)
Large	209818.18 (24.66)	328863.64 (38.66)	538681.8 (63.32)	312000 (36.68)	850681.82 (100)
All size	**101347 (20.37)**	**154521.15 (31.06)**	**255868.2 (51.43)**	**241617 (48.57)**	**497485.15 (100)**

Source: Computed from field survey data, 2020-21
Note: Figure in the bracket represents percentage of the total. Total Farm income is the sum of the crop production and other farm income.

In Nagaland, the average total household income differs considerably depending on the size of the farm; for marginal and small farmers, it was only ₹372,494.71 and ₹480,339.87, but for medium and large farmers, it was ₹774,206.55 and ₹850,681.82, respectively. The study shows a positive relationship between rural farmers' average income and farm sizes. Singh (2019) found a similar pattern in India, stating that the average revenue declined as farm size decreased. In comparison to marginal farmers, large farmers earn more than twice as much on average annually. It is also observed that a substantial portion of income comes from other farm activities (31.06 percent), which is more significant than crop production (20.37 percent), hence, the total farm income accounted for 51.43 percent of the average income of a farmer's household in Nagaland. Additionally, 48.57 percent of the income comes from non-farm sources. The survey results reveal that households obtain almost half of their income from non-farm sources, which is in contrast to the widely held belief that farming is the primary source of income for rural farm households. Birthal et al. (2014) also share a similar perspective.

The impoverished households with smaller land holdings are more likely to engage in low-paying, non-agricultural activities. The small and marginal farm households diversify their sources of income away from farming due to their tiny land holdings, low agricultural yield, and excess labour from farming. For marginal farmers, the percentage of total income from crop production was only 14.54 percent, while the percentage from other farm activities was 26.78 percent. As a result, total farm income accounted for only 41.32 percent, while the percentage from non-farm income was 58.68 percent. According to De Janvry et al. (2005), large farmers concentrate more on agriculture, whereas small farmers are rapidly diversifying into non-farm pursuits in the country. A similar scenario is observed among the large farmers in rural Nagaland.

4.7 Income Inequality

Each rural farm household has a portfolio of income streams depending on their willingness to take risks, family size, educational attainment, and resource availability. As a result, the distribution of income levels varies both within and between farm size categories. In order to determine the pattern of income inequality within each farm size category, income inequality is examined by farm size.

TABLE 4
DECOMPOSITION OF INCOME INEQUALITY (GINI COEFFICIENT)

Farm Size Categories	Crop Production	Other Farm Income	Total Farm Income	Non-farm Income	Total Income
Marginal	0.22	0.335	0.241	0.446	0.311
Small	0.097	0.353	0.216	0.455	0.253
Medium	0.096	0.435	0.265	0.435	0.285
Large	0.061	0.467	0.285	0.433	0.251
All size	**0.293**	**0.432**	**0.33**	**0.466**	**0.326**

Source: Calculated from the field survey data, 2020-21

The overall rural household income has a 0.326 Gini coefficient. With an estimated Gini coefficient value of 0.466, non-farm income is the most unequally distributed source of income, followed by other farm income (0.432). With a 0.293 Gini coefficient, crop production income is distributed less unequally. This result is consistent with that of Thakur et al. (2000), whose research found that revenue from agricultural activities is distributed less unequally than income from non-farm sources.

Across farm size categories, the Gini coefficients of total income distribution are 0.311, 0.253, 0.285, and 0.251 for marginal, small, medium, and large farm sizes, respectively. The income inequality estimates for marginal farmers are higher than large farm households.

Income inequality from crop production is higher for marginal farmers (0.220) and non-farm sources is most unequally distributed within small farm families (0.455), while other farm income is most unequally distributed among large farm households (0.467). Compared to medium-sized and large farms, marginal and small farmers experience higher levels of disparity in the distribution of income from crop production and non-farm sources. It shows that there is a negative correlation between income disparity and farm size; that is, as farm size grows, income inequality begins to decline. Meanwhile, income from other farm activities is less inequitably distributed among marginal and small farmers than that of medium and large farms, suggesting a positive association with farm sizes.

The higher inequality among marginal farmers can be attributed to several factors. First, marginal farmers have limited land resources, which restricts their ability to generate income from agriculture. Second, their participation in non-farm activities is often in low-paying sectors such as casual labour and small businesses, which have high income variability. Third, access to salaried employment, which provides more stable and higher income, is limited among marginal farmers compared to larger farmers. These factors collectively contribute to the higher income inequality observed among marginal and small farmers.

V. DISCUSSION

The findings of this study reveal several important patterns regarding income sources and inequality among rural farm households in Nagaland. The significant contribution of non-farm income (48.57 percent) to total household income challenges the traditional assumption that farming is the primary source of livelihood for rural households. This finding is consistent with national-level studies (Birthal et al., 2014) and reflects the broader trend of income diversification in rural India. The high dependence on non-farm income, particularly salaried employment, suggests that rural households in Nagaland are increasingly integrating into the non-agricultural economy.

The positive relationship between farm size and total income is consistent with the agrarian structure literature (Singh, 2019; Chand et al., 2011). Larger farmers benefit from economies of scale, better access to resources, and higher productivity, resulting in higher incomes. However, the inverse relationship between farm size and non-farm income indicates that smaller farmers are compelled to diversify into non-farm activities due to land constraints and limited agricultural income. This pattern is consistent with the findings of De Janvry et al. (2005) and Subramanian (2018), who observed that small farmers in India are rapidly diversifying into non-farm pursuits.

The higher income inequality among marginal and small farmers is a matter of concern. The Gini coefficients for marginal (0.311) and small (0.253) farmers are higher than for medium (0.285) and large (0.251) farmers, indicating greater disparity within these groups. This may be attributed to the heterogeneity in non-farm income sources, with some households having access to salaried employment while others rely on casual labour or small businesses with lower and more variable returns. The finding that non-farm income is the most unequally distributed source of income (Gini = 0.466) underscores the importance of addressing disparities in access to non-farm opportunities.

The high participation in livestock production (98.5 percent) reflects the cultural and economic importance of livestock in Nagaland. As noted by Priscilla et al. (2021), livestock serves as a crucial asset for rural households, providing both income and security. The reliance on agricultural wages among marginal and small farmers (83.53 percent and 66.67 percent participation) indicates the prevalence of wage labour as a coping strategy for land-constrained households, consistent with the findings of Ohno et al. (2021).

The study has several policy implications. First, the significant contribution of non-farm income suggests that rural development policies should go beyond agricultural development and support non-farm employment opportunities. Second, the higher inequality among marginal and small farmers calls for targeted interventions to improve their access to productive assets, education, and non-farm employment. Third, the importance of livestock in rural livelihoods suggests that livestock development programmes could be an effective way to enhance income and reduce inequality.

VI. CONCLUSION

In Nagaland, the study shows a positive relationship between rural farmers' average total income and farm sizes. The estimation of Gini ratio varies from 0.293 to 0.466 across farm sizes. The marginal farmers exhibited the highest inequality in terms of overall income (0.311) and crop production income (0.220) and for non-farm income the highest inequality is among small farmers (0.455). Hence, there is a need to reduce inequality particularly among marginal and small farmers.

The study suggests that it is imperative to intensify crop production process by prioritizing high-value crops with better infrastructure base and to maximize benefits from non-farm income, specific steps must be taken to educate impoverished households on how to create opportunities for earning income from non-farm sources. To address disparities in income distribution and promote more inclusive and equitable growth in the State, the government, agricultural organizations, and local administration must collaborate to ensure their effectiveness at the regional level and regularly evaluates their impact across farm size. This will be helpful in reducing inequalities between marginal and large farm households.

Limitations of the Study: The study is based on a single district (Peren) in Nagaland, which limits the generalizability of the findings to other districts with different socio-economic and agro-climatic conditions. The cross-sectional design captures income at a single point in time and does not capture seasonal or inter-annual variations. The study did not examine the determinants of income diversification and inequality, which could provide insights for policy interventions. Future research should extend the analysis to multiple districts, employ panel data to capture income dynamics, and examine the factors influencing income diversification and inequality.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Birthal, P. S., Negi, D. S., Jha, A. K., & Singh, D. (2014). Income sources of farm households in India: Determinants, distributional consequences and policy implications. *Agricultural Economics Research Review*, *27*(1), 37-48. https://doi.org/10.5958/j.0974-0279.27.1.003

[2] Chand, R., Prasanna, P. A. L., & Singh, A. (2011). Farm size and productivity: Understanding the strengths of smallholders and their livelihoods. *Economic and Political Weekly*, *54*(26/27), 5-11.

[3] Chakravorty, S., Chandrasekhar, S., & Naraparaju, K. (2016). *Income generation and inequality in India's agricultural sector: The consequences of land fragmentation* (Working Paper No. 2016-028). Indira Gandhi Institute of Development Research. https://www.igidr.ac.in/pdf/publication/WP-2016-028.pdf

[4] De Janvry, A., Sadoulet, E., & Zhu, N. (2005). *The role of non-farm incomes in reducing rural poverty and inequality in China* (Working Paper No. 1001). Department of Agricultural and Resource Economics, University of California, Berkeley.
https://repositories.cdlib.org/are-ucb

[5] Dhanagare, D. N. (1988). The green revolution and social inequalities in rural India. *Bulletin of Concerned Asian Scholars*, *20*(2), 2-13. https://doi.org/10.1080/14672715.1988.10404444

[6] FAO. (2002). *The state of food and agriculture: Agriculture and global public goods ten years after the Earth Summit*. Food and Agriculture Organization.

[7] Freebairn, D. K. (1995). Did green revolution concentrate incomes? A quantitative study of research reports. *World Development*, *23*(2), 265-279. https://doi.org/10.1016/0305-750X(94)00116-G

[8] Government of India. (2024-25). *Agriculture statistics at a glance*. Ministry of Agriculture and Farmers Welfare, Department of Agriculture and Farmers Welfare, Economics and Statistics Division.

[9] Government of India. (2019). *Situation assessment of agricultural households and land and holdings of household in rural India* (Report No. 587 (77/33.1/1)). Ministry of Statistics & Programme Implementation, National Statistical Office.

[10] Government of Nagaland. (2025-26). *Nagaland Economic Survey 2025-26*. Directorate of Economics and Statistics, Government of Nagaland.

[11] Hasell, J. (2023). Measuring inequality: What is the Gini coefficient? OurWorldInData.org. https://ourworldindata.org/what-is-the-gini-coefficient

[12] Kaur, K. (2017). *Disparities in ownership of assets and income among rural households in Punjab: A case study of Sri Muktsar Sahib District* [Unpublished M.Phil. dissertation]. Punjabi University.

[13] Kaur, S., & Singh, G. (2014). Analysis of income distribution among marginal and small farmers in rural Punjab. *International Journal of Scientific Research*, *33*, 755-759. https://www.ijsr.net/archive/v3i3/MDIwMTMxMTk5.pdf

[14] Lanjouw, P., & Shariff, A. (2002). *Rural non-farm employment in India: Access, income and poverty impact* (Working Paper No. 81). National Council of Applied Economic Research.

[15] Meena, M. S., Singh, K. M., Singh, R. K. P., Kumar, A., Kumar, A., & Chahal, V. P. (2017). Inequality and determinants of income among rural households in tribal dominated areas of Jharkhand. *Indian Journal of Agricultural Sciences*, *87*(1), 92-96.

[16] Meher, S., & Sharma, R. K. (2010). *Pattern of farm investment among cultivating households: A comparative study of Haryana and Orissa*. Jawaharlal Nehru University. http://handle.net/10603/31428

[17] Micevska, M., & Rahut, D. B. (2008). Rural non-farm employment in the Himalayas. *Economic Development and Cultural Change*, *57*(1), 163-193.

[18] NABARD. (2021). *Farmers welfare in India: A state-wise analysis*. Department of Economic Analysis and Research (DEAR), National Bank for Agriculture and Rural Development.

[19] Ohno, A., Fujita, K., & Vatta, K. (2021). Agrarian structure of Punjab in the post-green revolution era: Household strategies for distress coping. *Economic and Political Weekly*, *56*(40), 56-64. http://www.epw.in/journal/2021/40/special-articles/agrariam-structure-punjab-post-green-revolution.html

[20] Priscilla, L., Singh, O. K., & Vatta, K. (2021). Effects of income sources on inequality among agricultural households in North-east India. *Indian Journal of Agricultural Economics*, *76*(4), 659-671.

[21] Ranganathan, T., Tripathi, A., & Rajoriya, B. (2016). *Changing sources of income and income inequality among Indian rural households*. https://www.researchgate.net/publication/301355952_changing_sources_of_Income_and_income_inequality_among_IndianRural_Households

[22] Saini, R., & Kaur, M. (2022). Income inequality and its decomposition among farm households in Punjab. *Cogent Food & Agriculture*, *8*(1), 2069314. https://doi.org/10.1080/23311932.2022.2069314

[23] Shiva, V. (1993). *The violence of the green revolution: Third world agriculture, ecology and politics* (2nd ed.). Zed Books.

[24] Singh, G. (2019). Income inequalities among farm households in Hoshiarpur District of Punjab. *Journal of Krishi Vigyan*, *7*(2), 153-157. https://doi.org/10.5958/2349-4433.2019.00026.6

[25] Subramanian, S. (2018). *Participation of rural households in farm, non-farm and pluri-activity: Evidence from India* (Working Paper No. 412). The Institute for Social and Economic Change.
https://www.isec.ac.in/WP%20412%20-%20S%20subramanian%20-%20final.pdf

[26] Thakur, J., Bose, M. L., Hossain, M., & Janaiah, A. (2000). Rural income distribution and poverty in Bihar: Insights from village studies. *Economic and Political Weekly*, *35*(30), 4657-4663.

[27] The World Bank. (2008). *Agriculture for development* (World Development Report 2008). https://doi.org/10.1596/978-0-8213-7233-3

[28] World Inequality Lab. (2022). *World Inequality Report 2022*. https://wir2022.wid.world.

International Journal of Environmental & Agriculture Research (IJOEAR)
ISSN: [2454-1850]
[Vol-12, Issue-9, September- 2026]
Website: www.ijoear.com
Journal DOI: 10.25125/agriculture-journal

Horticulture-Voltaic: The Earth's Solar Horticulture Turns Every Ray into Food and Power - A Comprehensive Review

Jadala Shankaraswamy

Assistant Professor, Department of Fruit Science, College of Horticulture, Mojerla, Sri Konda Laxman Telangana Horticultural University (SKLTGHU), Telangana 509382, India
Orcid : 0000-0002-5623-6384

Received:- 14 August 2026/ Revised:- 27 August 2026/ Accepted:- 08 September 2026/ Published: 15-09-2026

Abstract— *Horticulture-voltaic systems - the co-location of photovoltaic (PV) electricity generation with horticultural crop production on the same land - are emerging as a central strategy for climate-resilient horticulture. This in-depth review examines the technology, engineering, intercultural operations and empirical evidence for horticulture-voltaics applied to fruit, vegetable and flower crops. We cover solar panel technologies, system architectures, the full design-component and engineering stack (mounting heights of 2.5-5 m, panel spacing and tilt, deep-buried armoured cables, structural safety under ASCE-7-16 wind loads, smart irrigation integration), the electrical architecture (module/string voltage, inverter, battery, transformer, grid connection), and intercultural operations adapted for sub-array horticulture. Empirical evidence shows that shade relieves heat and drought stress, with grape +277% (Mediterranean, LER 3.54), tomato and chiltepin doubled or tripled (Arizona), and partial shade increasing floral abundance in flowers. Shading must stay below ~30% for fruit crops and 20-40% for most vegetables and flower crops. With continued research and engineering, horticulture-voltaics can deliver climate-adaptive, resource-efficient and energy-positive horticultural systems.*

Keywords— agrivoltaics; horticulture-voltaic; climate-resilient horticulture; photovoltaic; solar energy; fruit crops; vegetable crops; flower crops; shading; ground coverage ratio; wind load; intercultural operations.

I. INTRODUCTION

Horticulture-voltaic systems - the co-location of photovoltaic (PV) electricity generation with horticultural crop production on the same land - are emerging as a central strategy for climate-resilient horticulture. The concept of agrivoltaics (agri-PV, or AV) was first proposed by Goetzberger and Zastrow (1982) and has since grown from a niche idea into a rapidly expanding global field of research and commercial deployment [1,2]. The core proposition is dual land use: solar panels generate electricity while the shade they cast moderates the microclimate beneath, reducing heat and drought stress, lowering evaporative demand and, in hot dry regions, often improving crop performance and water-use efficiency [3,4].

The rationale is compelling. Global food demand is projected to rise by 35-56% by 2050 while agricultural land is finite, and the transition to renewable energy requires large land areas; co-locating the two uses avoids the land-use conflict between food and energy [4,5]. Empirical studies report that agri-voltaic arrays can raise land-use efficiency above 180% - producing electricity and crops on the same hectare - while cooling panels (improving their electrical efficiency) and cooling the crops and soil beneath [4,6].

For horticulture the stakes are especially high. Perennial fruit crops (apple, grape, citrus, fig, olive, pomegranate), high-value vegetables (tomato, chilli, brinjal, leafy greens) and flower crops (rose, gerbera, marigold, chrysanthemum, tuberose) are increasingly exposed to heat waves, drought, salinity and erratic weather. The shade, cooler soil and conserved soil moisture under PV arrays offer a measurable resilience benefit [7,8]. At the same time the same shade can reduce yield and quality if it exceeds crop-specific thresholds: for fruit crops, shading above roughly 30% risks substantial declines [9,10].

This review provides an in-depth, engineering-oriented account of horticulture-voltaic systems, restricted to horticultural crops - fruits, vegetables and flower crops. It covers the solar technology models, system architectures, mounting designs, electrical architecture, infrastructure and installation components, engineering design parameters, intercultural operations adapted for sub-array cropping, and empirical evidence and ongoing research supporting the technology, with four labelled diagrams for systems engineering, design components, intercultural operations, and electrical schematics.

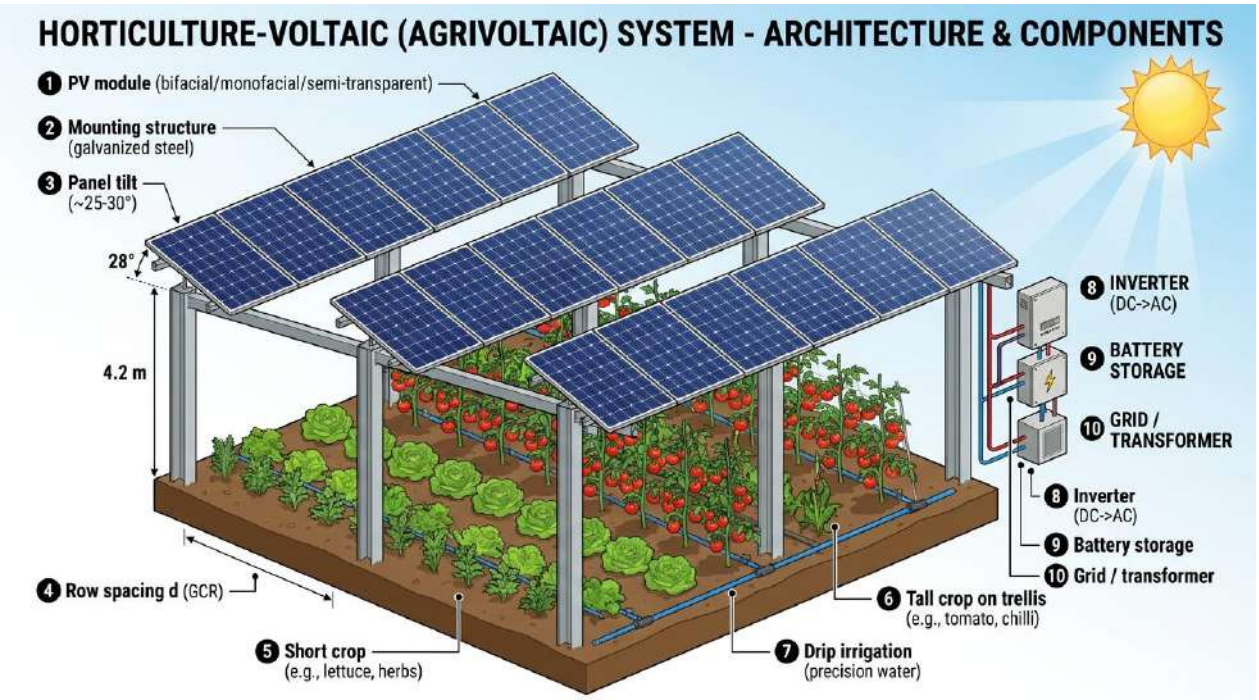


FIGURE 1: Three-dimensional architecture of a horticultural-voltaic system showing elevated PV modules on mounting structures, crops beneath, drip irrigation, and electrical components (inverter, battery, transformer). Numbered labels 1-10 identify all design elements.

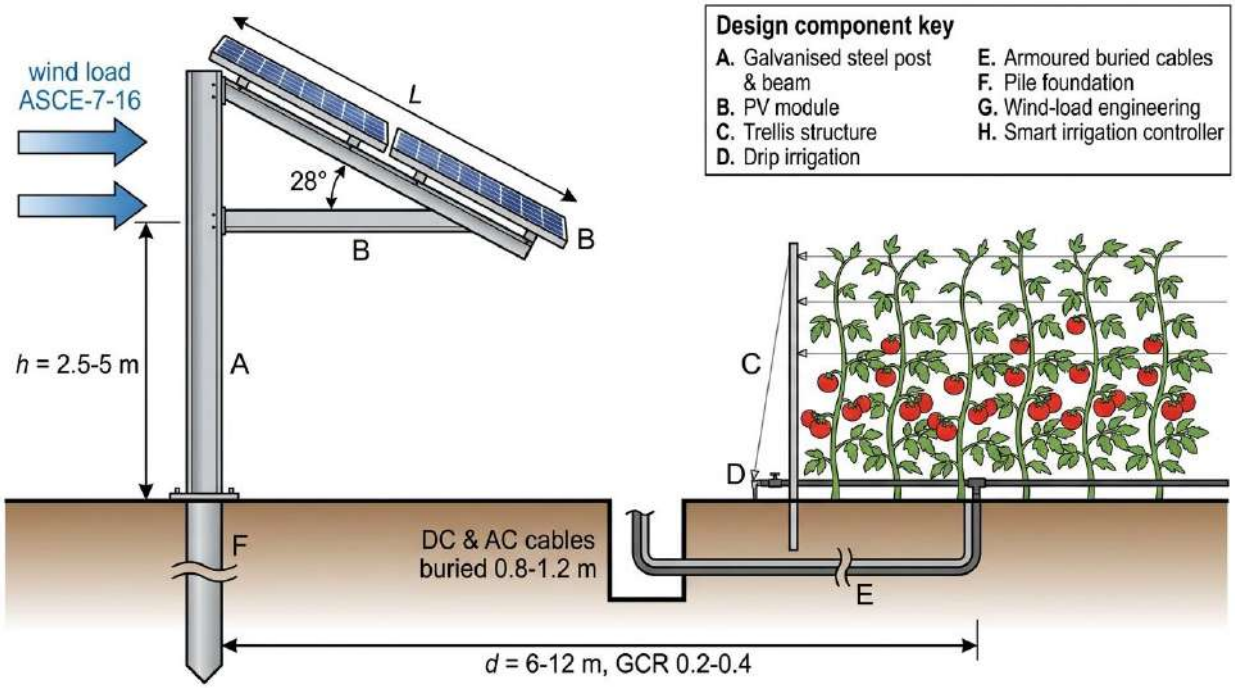


FIGURE 2: Engineering design components and dimensional layout: mounting height (h = 2.5-5 m), panel tilt (28 degrees), row spacing (d = 6-12 m, GCR 0.2-0.4), trellis structure, drip irrigation, armoured buried cables and wind-load annotations (ASCE-7-16); design component key A-H.

II. REVIEW METHODOLOGY

This narrative review was prepared by synthesizing peer-reviewed literature on horticulture-voltaic systems with a focus on fruit, vegetable, and flower crops. Literature was identified through targeted searches of major scientific databases including Web of Science, Scopus, ScienceDirect, Google Scholar, and PubMed Central using search terms such as "agrivoltaics," "horticulture-voltaic," "agri-PV," "photovoltaic," "shading," "fruit crops," "vegetable crops," "flower crops," and "climate-resilient horticulture." The search covered the period from 1982 (the foundational Goetzberger and Zastrow paper) to 2026, with emphasis on recent developments from 2019 onwards. Foundational papers necessary to define the field were also included. Studies were included if they addressed agrivoltaic systems, horticultural crop production under PV arrays, or related engineering and microclimate aspects. The retrieved literature was critically evaluated for scientific quality, relevance, and contribution to the understanding of horticulture-voltaic systems.

III. SOLAR PHOTOVOLTAIC TECHNOLOGY MODELS

The PV module is the fundamental building block; its technology determines efficiency, light transmission, cost and suitability for a given crop and architecture. Table 1 summarises the principal panel types relevant to horticulture-voltaic systems.

TABLE 1
PRINCIPAL PHOTOVOLTAIC PANEL TYPES AND THEIR ROLE IN HORTICULTURE-VOLTAIC SYSTEMS

Panel type	Typical efficiency	Light transmission	Agrivoltaic role	Source / Notes
Monofacial crystalline silicon (c-Si)	18-22%	Opaque	Standard elevated arrays	Mature, low cost; common baseline
Bifacial c-Si	18-23%	Opaque (rear captures albedo)	Elevated + vertical arrays	+5-30% energy vs monofacial [11]
Thin-film (CdTe, a-Si, CIGS)	12-18%	Semi-transparent options	Diffuse-light arrays; Laterza vineyard system [9]	Uniform shading; low temperature coeff.
Semi-transparent / spectral-splitting	8-15% (transmission-adjusted)	20-60%	Greenhouses, high-value crops	Tunable PAR vs NIR; crop-friendly light [12]
Organic / dye-sensitised (OPV/DSSC)	5-12%	Tunable, coloured	Emerging, niche	Flexible, low cost, early stage
Perovskite (emerging)	25%+ (lab)	Tunable	Research stage	High potential; stability/toxicity to resolve

Bifacial modules are particularly attractive for agri-voltaics because the rear face harvests light reflected from soil and crops, adding 5-30% energy output, and because they enable vertical and elevated geometries that leave more light for crops [11,13]. Semi-transparent and spectral-splitting modules are the key to greenhouse-integrated and high-value-crop systems: they can pass photosynthetically active radiation (PAR) while converting near-infrared into electricity, or simply trade a controlled fraction of light for power [12].

IV. SYSTEM ARCHITECTURES AND MOUNTING MODELS

Agri-voltaic systems are classified by their geometry and mounting. The main architectures relevant to horticulture are:

4.1 Elevated fixed-tilt (pergola / canopy) arrays:

Panels are mounted on elevated structures several metres above the ground, tilted typically 20-35 degrees. This is the most common architecture for field vegetables and berries. The clearance height (panel underside to soil) is the key crop-compatibility parameter: 2.5-4 m for field vegetables, 4-6 m for orchards and vineyards to allow machinery and tall crops [14,15]. Examples include the Fraunhofer APV-RESOLA system at Heggelbach (Germany) [6] and the Ferrara/Bari sites in Italy [9].

4.2 Single-axis and dual-axis tracking arrays:

Panels rotate to follow the sun. Trackers raise annual energy yield by 20-35% (single-axis) relative to fixed tilt, but moving structures cast dynamic shade that can be managed to protect crops during heat peaks or to maximise light in cool seasons [16]. The Scalea (Calabria) lemon trial directly compares fixed panels against single-axis trackers [17].

4.3 Vertical bifacial arrays:

Panels are mounted vertically, facing east-west, in rows between crop strips. This geometry minimises shading of the crop (shade is brief and passes), allows full mechanisation between rows, and suits horticultural rotations. Vertical bifacial systems are well documented in Belgium and Germany [11,18].

4.4 Semi-transparent and diffuse-light arrays:

Panels with reduced or tuned transmission spread light more evenly, reducing hot spots and allowing crops to be grown directly beneath. These are favoured for high-value, shade-tolerant crops and for greenhouse integration [12].

4.5 Greenhouse-integrated (rooftop) PV:

Panels are mounted on or integrated into greenhouse roofs, generating power while the greenhouse continues to produce vegetables and flowers. Semi-transparent modules on the roof balance light for the crop with electricity output - the "electricity above, crop below" model [19].

4.6 Floating agri-voltaics (emerging):

Panels float on water bodies; relevant where land is scarce.

V. ENGINEERING DESIGN COMPONENTS

Engineering design is what makes horticultural integration possible while keeping the array safe and durable. The principal design components (Figure 2) are:

5.1 Elevated and vertical structures:

Panels are typically mounted 2.5 to 5 m (6-12 feet) high to allow standard farm machinery and tractors to pass underneath, or configured as vertical bifacial arrays to accommodate large commodity equipment like combines [14,20]. Mounting structures are typically hot-dip galvanised steel or aluminium; posts are sized for wind, snow and crop loads with the larger heights raising the wind-load design values per ASCE-7-16 [21,22]. The first row of panels is generally taller and stronger than subsequent rows to carry asymmetric loading; field data show meaningful differences between upstream and sheltered downstream rows in vertical arrangements [22].

5.2 Panel spacing and tilt:

Rows and individual modules are spaced further apart than in traditional solar farms to control the shading ratio and ensure sufficient sunlight filters down for crop photosynthesis [14,15,20]. Tilt is typically 20-35 degrees (28 degrees is widely used); the structure can be designed for adjustable tilt to suit seasonal radiation. The ground coverage ratio (GCR = panel area / land area) directly controls the shading fraction; for fruit crops shading should stay below ~30% (GCR roughly 0.3-0.4) [10,15].

5.3 Electrical and wire management:

Underground DC and AC cables must be installed at a greater depth than typical PV installations - armored and buried 0.8-1.2 m deep - to prevent damage during tilling, plowing and heavy agricultural groundwork [20,23]. Cable routes must avoid cultivation zones or be armoured; combiner boxes and isolators should be at serviceable heights. Partial shading from crops/structures is mitigated with module-level optimisers or micro-inverters [23].

5.4 Structural safety and wind loads:

Engineering frameworks must account for increased wind loads on taller columns; builders design customisable adjustments based on local disaster resistance (cyclone, tornado, hail) and terrain [14,20,22]. Considerations include ASCE-7-16 wind-pressure coefficients, exposure category and panel height; a 3D CFD study showed meaningful pressure differences between panels in different positions of a vertical array [21,22]. Bolted base plates, pile-driven foundations, or concrete piers are used depending on soil and water table.

5.5 Smart irrigation integration:

Irrigation lines (overhead or buried drip) are mapped into the initial PV layout to optimise water delivery and avoid operational conflicts with the structure and cabling [20,24]. Smart controllers integrate soil-moisture sensors, weather data and module-level ET models to schedule variable-rate irrigation; the agri-voltaic array's shade reduces crop water demand by up to 70% in arid or high-heat environments, so the irrigation controller can save the corresponding energy and water.

5.6 Re-tillage reversibility:

Because land is farmland, foundations should minimise soil disturbance and be reversible at the end of the array's life. Pile-driven or screw foundations are preferred over concrete slabs for this reason [20].

VI. ELECTRICAL SYSTEM ARCHITECTURE

A horticultural-voltaic system is a complete PV power plant plus storage and monitoring. The electrical architecture (Figure 4) is summarised in Table 2.

TABLE 2
ELECTRICAL SYSTEM ARCHITECTURE OF A HORTICULTURAL-VOLTAIC ARRAY

Stage	Component	Typical specifications	Notes
Module	PV module	400-600 Wp, Voc 40-50 V	c-Si or bifacial
String	Series-parallel of modules	20-35 modules, 600-1500 Vdc	String voltage stays within inverter MPP window
DC side	DC cabling + combiner box + SPD	4-6 mm² PV cable, 0.8-1.2 m burial	Armoured for tillage
Conversion	String inverter	1000/1500 Vdc to 230/400 Vac	Micro-inverters for partial-shade cases
Storage	Battery rack	5-50 kWh (Li-ion / LFP)	Self-consumption + farm loads
Distribution	AC distribution + transformer	11-33 kV MV for export	230/400 V three-phase for on-farm loads
Grid	Meter + protection	Net metering / feed-in tariff	Compliance with DISCOM regulations

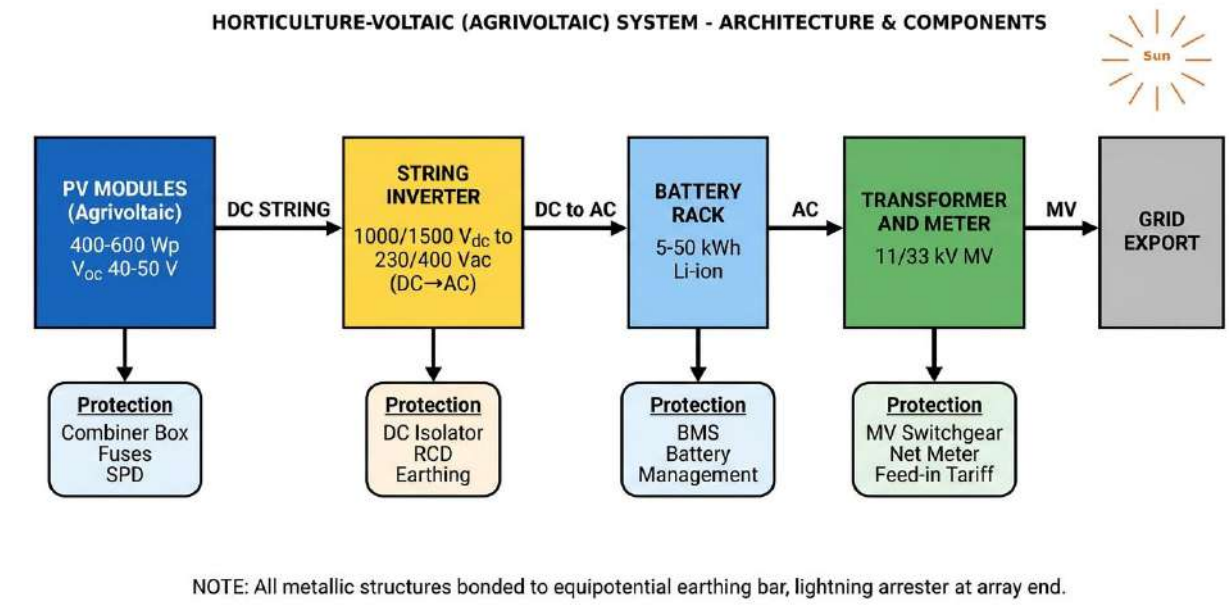


FIGURE 3: Electrical schematic of an AV system from PV modules (Voc 40-50 V, 400-600 Wp) through string inverter, battery rack, transformer and grid connection, with protection and monitoring stages.

A single c-Si module typically delivers an open-circuit voltage (Voc) of ~40-50 V and a maximum-power voltage (Vmp) of ~30-40 V at a power of 400-600 Wp. Modern inverters accept a maximum system voltage of 1000 Vdc or 1500 Vdc, which sets the maximum string length (typically 20-35 modules) [23]. The string must stay within the inverter's MPP (maximum power point) voltage window and below the maximum voltage at cold temperatures (when Voc rises) [23,25].

VII. INFRASTRUCTURE AND INSTALLATION

The physical infrastructure comprises mounting structures (galvanised steel or aluminium), foundations (pile-driven posts, ground screws or concrete ballast), cabling and cable management (armoured, deep-buried), water infrastructure (drip lines, sensors, optional water treatment such as reverse osmosis) and balance-of-system (BOS) cabinets housing inverters, batteries, transformers, metering, monitoring and protection [14,20,23].

Access and machinery clearance are critical: row spacing must accommodate tractors, sprayers and harvest machinery; orchard structures span between tree rows so equipment can pass beneath [14,20]. For elevated systems, increasing the structural height raises material costs and wind-load design values, so mounting structure is sized to the minimum clearance required for the chosen crop and machinery [21,22,26].

VIII. INTERCULTURAL OPERATIONS UNDER AN AGRI-VOLTAIC ARRAY

Intercultural operations are the routine field practices carried out after planting/sowing but before harvesting - weeding, hoeing, mulching, training, pruning, thinning, staking, plant protection and so on. Under an agri-voltaic array these operations must be adapted to the geometry, shaded microclimate, machinery clearance and cabling layout. The main classes (Figure 3) are:

8.1 Soil and weed management:

Under partial shade the soil is cooler and wetter than in full sun, which slows weed germination but also favours certain moisture-loving weeds. Regular weeding (manual, mechanical or chemical) is therefore still required; shallow hoeing breaks the soil crust and aerates around the root zone without damaging shallow-buried drip lines and armoured cables [27,28]. Mulching - with organic materials (straw, leaves) or plastic sheets - further suppresses weeds, conserves soil moisture and regulates soil temperature; it is one of the most effective intercultural operations in shaded systems. Earthing-up - piling soil around the stem base - is essential for tuber crops (potato, ginger) and cabbages, and is generally compatible with array structure provided post spacing allows [27].

8.2 Canopy and growth regulation:

Training the plant on a trellis (grape, tomato, kiwi, passion fruit, certain flower crops) directs growth, optimises light interception and reduces the risk of branches breaking under their own weight. Pruning systematically removes dead, diseased or overcrowded branches to balance vegetative growth with reproduction and improve air circulation; in fruit trees and grapevines, pruning interacts directly with the shading pattern imposed by the array and must be timed to keep the canopy alert to photosynthetically beneficial periods without excessive shading [27,29]. Thinning - removing excess flowers, buds or immature fruits - prevents branch breakage, increases the size and quality of remaining fruits, and is a standard practice in grape, apple, peach, mango and citrus [27,29]. Pinching (removing terminal growing tips to encourage lateral branching) is standard in flower crops like marigold and carnation; desuckering (removing non-productive vegetative shoots) is essential in banana and tomato [27].

8.3 Crop protection and support:

Staking (bamboo sticks, wooden stakes, strings) prevents tall or weak-stemmed crops (tomato, pea, carnation) from lodging under their own weight; the array's posts can serve as a structural backbone for vertical-string systems. Blanching (tying outer leaves over cauliflower heads) keeps them white; wrapping individual pomegranate fruits, fig fruits or grape bunches protects them from sun-scald, birds and hail - particularly valuable because the array's shade reduces but does not eliminate sun-scald risk [27,28]. Plant protection (targeted bio-pesticides, fungicides, insecticides) follows integrated pest management (IPM) principles; the agri-voltaic microclimate's altered humidity may shift pest pressure and disease cycles and requires adapted spray timing [27,28].

8.4 Operations adapted to the AV microclimate:

The agri-voltaic microclimate (lower air temperature at midday, higher night temperature, lower vapour-pressure deficit, less wind speed, more uniform light) calls for specific cultivation adjustments [9,29]: selecting shade-tolerant cultivars; adjusting

row orientation along the array's east-west axis to maximise morning light; choosing machinery sized to the array's clearance; and modifying planting schedules so flowering and fruiting windows coincide with the array's most beneficial shading periods (e.g., shade during afternoon heat peaks in summer for leafy vegetables and flowers). Mulching and drip irrigation scheduling must coordinate with the array's drip line plan and with the increased soil-moisture retention that shading provides [24,27].

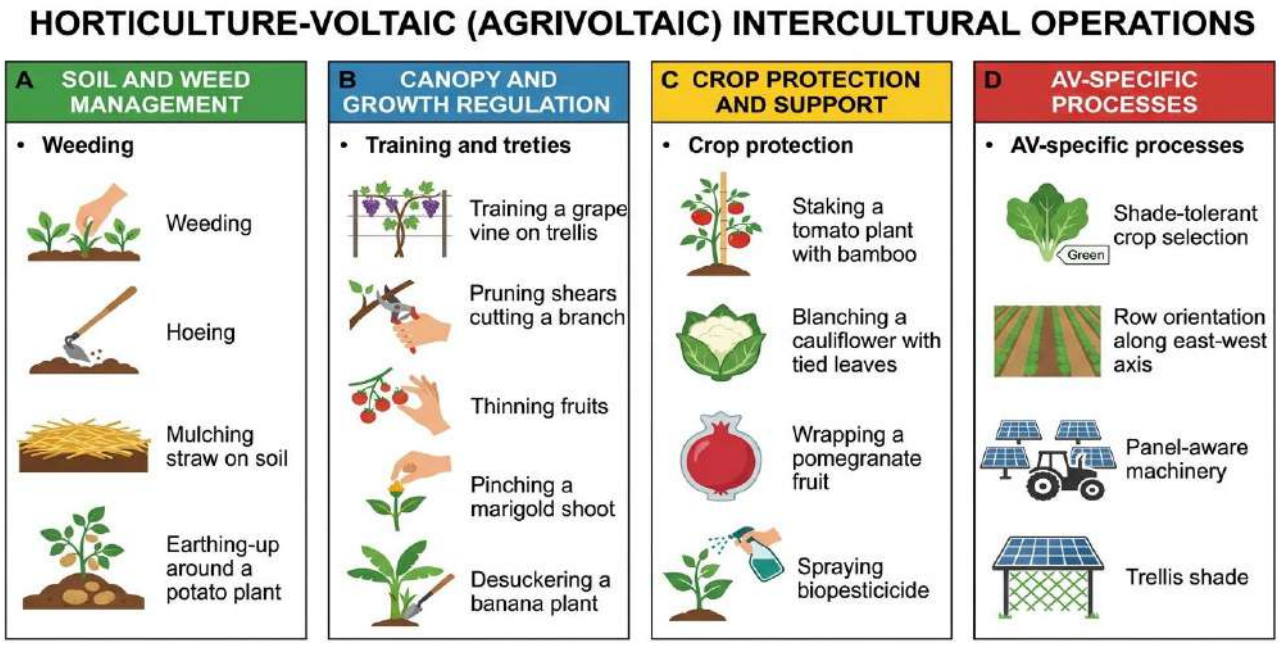


FIGURE 4: Intercultural operations for horticultural crops under an agrivoltaic array: soil and weed management, canopy and growth regulation, crop protection and support, and AV-specific processes.

IX. SUITABILITY OF HORTICULTURAL CROPS

Engineering design determines whether a crop thrives or suffers under the array. The principal parameters are GCR, panel height vs crop height plus machinery envelope, tilt, row spacing and PAR transmission [10,14,15]. Table 3 summarises the suitability of horticultural crops for agri-voltaic installation.

TABLE 3
SUITABILITY OF HORTICULTURAL CROPS (FRUITS, VEGETABLES, FLOWER CROPS) FOR AGRI-VOLTAIC INSTALLATION

Crop group	Examples	Height class	Shade tolerance	Recommended shading
Leafy vegetables & herbs	Lettuce, spinach, chard, kale, basil, mint	Low (<0.5 m)	High	30-50%
Fruiting vegetables	Tomato, chilli, brinjal, sweet pepper	Tall (trellised)	Medium	20-35% (heat-stress relief)
Root & other vegetables	Onion, garlic, carrot, radish	Low-medium	Medium	20-30%
Berry fruits	Strawberry, raspberry, blueberry	Low-medium	Medium-high	20-40%
Vine fruits	Grape (table/wine)	Tall (trellis)	Medium	<30% (quality-sensitive)
Pome & stone fruits	Apple, peach, plum	Tree (2-4 m)	Medium	<30%
Citrus & subtropical fruits	Lemon, orange, fig, olive, pomegranate	Tree	Medium-high	<30-40%
Flower crops (cut & loose)	Rose, gerbera, marigold, chrysanthemum, tuberose	Low-medium	Medium-high	20-40% (partial shade often improves floral abundance) [30]

X. EMPIRICAL EVIDENCE: FRUIT CROPS

Fruit crops are the most extensively studied horticultural group under agri-voltaics. Shade relieves heat and drought stress but can reduce yield and quality beyond a threshold.

10.1 Grapevine:

Magarelli, Mazzeo and Ferrara (2025) at Laterza (Puglia, Italy) report that Primitivo grapevines beneath a fixed thin-film (a-Si) array covering 43% of the ground yielded +277% more grapes than vines in full sun, with better bud fruitfulness; the array reduced wind speed and vapour-pressure deficit and the land equivalent ratio (LER) reached 3.54, demonstrating high dual-use efficiency in Mediterranean conditions [9]. Earlier work by Juillion et al. (2022) in southern France showed tracking photovoltaic panels reduced temperature by ~3.8 degrees C and increased humidity by 14% in an apple orchard at 50-55% shading, reducing irrigation requirements by 6-31% and alternate bearing, but also reduced photosynthetic capacity with negative effects on flowering and yield [29].

10.2 Apple:

Juillion et al. (2022) reported fruit dry-matter content under shading was reduced by an average of 24%, and concluded that agrivoltaic systems may reduce alternate bearing while requiring careful management of shading below ~40% to preserve yield [29,31]. The Bari group's 2024 review with Ferrara recommended holding shading below a 30% threshold to avoid substantial declines in fruit yield and quality [10].

10.3 Citrus and subtropical fruits:

At Scalea (Calabria), ENEA and EF Solare Italia built a lemon trial running lemons under fixed panels against lemons under single-axis trackers, with bifacial modules, a lithium battery rack (5.12-20.48 kWh) and a reverse-osmosis unit converting brackish water to up to 240 L/h [17]. Ferrara's group is tracking lemon and lavender there; the two Italian sites (Laterza and Scalea) are designed to complement each other by testing different crops, environments and PV configurations [17].

10.4 Fig and olive:

At the Vigna Agrivoltaica di Comunita at Laterza (Taranto), a 970 kW array of ~7,770 thin-film panels (135 W, 125 W and 100 W) tilted at 28 degrees on reinforced concrete pergola frames is being used to study fig and olive under the glass [17]. Ferrara reported soil under the array more than 20 degrees Celsius cooler than soil in open sun at ~11 a.m. on a hot day, though he flagged this as a peak and noted it moves with latitude, time of day, ambient temperature and soil characteristics [17].

XI. EMPIRICAL EVIDENCE: VEGETABLE CROPS

Vegetables show the clearest yield benefits under agri-voltaics in hot, dry conditions, because heat and water stress relief outweigh light reduction.

11.1 Tomato:

Barron-Gafford et al. (2019) at Biosphere 2 (Arizona) found cherry tomato production was twice as great under PV panels than in the open field, with chiltepin pepper fruit production three times greater; jalapeno produced similar fruit with 65% less transpirational water loss, and soil moisture remained ~15% higher in the agri-voltaic system [4]. A Fraunhofer ISE pilot for Maharashtra (India) projected up to 40% higher yields for tomato under agri-voltaic shading [6]. A Princeton modelling study (Hosseini and Bou-Zeid, 2024) coupled air-heat-moisture simulation over a New Jersey tomato field; simulated leaves were 1.84-7.56 degrees C cooler under panels, with 47% less direct sunlight, 31% lower photosynthesis, 22.4% lower daily leaf water loss (42.6% for crops and soil) and 4.46 degrees C lower perceived temperature for farm labour; panels themselves ran 5.6 degrees C cooler, trimming heat-related efficiency losses by 15% [32].

11.2 Chilli and pepper:

The Arizona results for chiltepin (3x fruit) and jalapeno (similar yield, -65% transpirational water loss) are the strongest pepper evidence [4]. Many chilli/pepper types tolerate a 35-50% reduction in PAR compared to open sunlight, making them well suited to agri-voltaic installation [33].

11.3 Brinjal (eggplant):

Brinjal is heat- and drought-sensitive; agri-voltaic shade relieves heat stress and conserves soil moisture, though shading should be kept moderate (20-35%) to avoid yield loss [7,33].

11.4 Leafy vegetables and herbs:

Leafy greens (lettuce, spinach, chard, kale) and herbs (basil, mint) are shade-tolerant and among the most suitable crops for agri-voltaics, with stable or improved yields at 30-50% shading under hot and water-limited conditions [7,33].

XII. EMPIRICAL EVIDENCE: FLOWER CROPS

Flower crops (ornamentals and cut flowers) are an emerging but promising horticultural category for agri-voltaics, because many flower species are adapted to partial shade and benefit from reduced heat stress and moderated microclimate.

12.1 Partial shade and floral abundance:

Oregon State University research found floral abundance was greatest in partial-shade plots, with 4% more blooms than full-sun or full-shade plots, with benefits most pronounced in late summer when heat stress is highest [30].

12.2 Rose and gerbera:

Rose and gerbera are high-value cut flowers grown in greenhouses and shade houses; semi-transparent and greenhouse-integrated PV can supply electricity while preserving light quality for flower development [12,19]. Gerbera is routinely produced under shade nets in tropical regions, making it well suited to diffuse-light agri-voltaic systems.

12.3 Marigold, chrysanthemum and tuberose:

Marigold and chrysanthemum are photoperiodic loose-flower crops that respond to light quality and day length; partial shading can be managed to modulate flowering while protecting blooms from heat damage. Tuberose, a shade-tolerant bulbous flower, performs well under partial shade [27]. These crops are candidates for agri-voltaic installation in tropical and subtropical floriculture belts.

12.4 Design considerations for flower crops:

Because flower value depends on visual quality (colour, stem length, vase life), light management must be precise: semi-transparent panels that transmit PAR while converting NIR are preferable, and shading should be kept in the 20-40% range [12,30].

XIII. ONGOING RESEARCH AND EMERGING SYSTEMS

13.1 Multi-site field networks:

The University of Bari, ENEA, Agridatalog and partner companies run complementary trials at Laterza (fig, olive, grape under fixed pergola arrays) and Scalea (lemon, lavender under fixed vs tracking bifacial arrays), generating paired microclimate and yield data across different crops and PV configurations [9,17].

13.2 Modelling and digital twins:

Coupled physics models (air-heat-moisture) such as the Princeton tomato model [32], irradiance models for orchard integration and digital-twin approaches are being used to simulate shading, microclimate and crop response before field deployment.

13.3 Crop-specific shading thresholds:

Research is converging on crop-specific shading ceilings: ~30% for fruit crops [10], 20-40% for most vegetables and flower crops [7,30] - and on dynamic tracking strategies that protect crops during heat peaks while maximising light in cool seasons [16,29].

13.4 Greenhouse and protected-cultivation integration:

Semi-transparent rooftop PV for greenhouses producing vegetables and flowers is an active area, balancing electricity output with PAR transmission [12,19].

13.5 Wind-load engineering:

Field studies and CFD simulations of wind loads on elevated and vertical arrays are informing revised design codes for taller agri-voltaic columns [21,22].

13.6 Water-energy-food integration:

Systems pairing PV with drip irrigation, battery storage and water treatment (e.g., reverse osmosis at Scalea) are demonstrating the water-energy-food nexus in practice [17,24].

XIV. CHALLENGES AND FUTURE DIRECTIONS

Despite rapid progress, several challenges limit adoption:

14.1 Shading and yield trade-off:

Shade relieves heat stress but can reduce yield and quality beyond thresholds; for fruit crops shading must stay below ~30%, and for quality-sensitive crops (grape for wine, apple) the light environment must be managed carefully [9,10,29].

14.2 Capital cost and structure:

Elevated, orchard-spanning structures are costlier than ground-mounted arrays; foundations, galvanised steel and cable management must be engineered for 25+ year service [14,21]. The higher initial investment requires careful economic analysis, though the dual revenue streams (crop + electricity) can improve returns over time.

14.3 Crop-specificity and generalization:

Yield responses are crop- and site-specific; the same array can raise one crop and reduce another, so design must be tailored to crop height, shade tolerance and local climate [7,9].

14.4 Electrical and safety complexity:

String design, MPP voltage windows, inverter selection, battery sizing and safety clearances require specialist engineering; partial shading of modules by crops/structures must be managed with optimisers or micro-inverters [23].

14.5 Intercultural and operational complexity:

Agricultural operations - weeding, pruning, spraying, harvesting - must be adapted to the array's geometry and clearance; training, mulching and pinch management interact with the altered microclimate in counter-intuitive ways [27,28].

14.6 Skills and regulation:

Growers and extension staff need training in PV engineering, data systems and microclimate-aware cultivation; grid-connection, land-use and subsidy regulations vary by region.

14.7 Economic viability:

The economic viability of horticulture-voltaic systems depends on several factors: capital costs (which can be 2-3 times higher than ground-mounted PV due to elevated structures), crop revenue, electricity revenue, and operational costs. Payback periods typically range from 8-15 years depending on location, crop value, and electricity tariffs. Government subsidies and feed-in tariffs can significantly improve economic returns. For smallholder farmers, the high upfront costs remain a barrier, though leasing models and community-owned systems are emerging as alternatives.

14.8 Policy and regulatory considerations:

Regulatory frameworks for agrivoltaics vary widely across regions. Key considerations include: land-use classification (whether the land remains classified as agricultural), grid connection regulations, feed-in tariffs and net metering policies, building permits for elevated structures, and agricultural subsidies. Some countries (e.g., Germany, France, Japan) have established specific regulatory frameworks for agrivoltaics, while others are still developing them. Clear policy frameworks are essential for scaling up horticulture-voltaic deployment.

Future directions include: dynamic tracking programmed for crop protection; semi-transparent and spectral-splitting modules tuned to crop PAR needs; digital twins and AI for design optimisation; integrated water-energy-food systems; and standardised, open data ecosystems linking microclimate, yield, intercultural practice and energy data across sites [11,16,24,27,32].

XV. CONCLUSION

Horticulture-voltaic systems offer a compelling route to climate-resilient horticulture by producing renewable electricity and protecting fruit, vegetable and flower crops on the same land. The shade, cooler soil and conserved moisture under PV arrays relieve heat and drought stress: grape yield rose by 277% under a Mediterranean array, tomato and chiltepin production doubled or tripled in Arizona, and partial shade increased floral abundance in flower crops. The engineering - panel type, mounting height and tilt, structural safety under wind loads, deep-buried armoured cables, smart irrigation and inverter-battery grid architecture - must be tailored to each crop's height and shade tolerance, with shading kept below ~30% for fruit crops and 20-40% for most vegetables and flower crops. Intercultural operations (weeding, mulching, training, pruning, thinning, staking, plant protection) integrate with the array's geometry and microclimate: the AV site is at once an energy plant, a horticultural field and a managed agro-ecosystem.

Limitations of the Review: This review is based on a narrative synthesis of published literature rather than a formal systematic review. The examples are selected to illustrate the diversity of approaches and should not be considered exhaustive. The focus is primarily on peer-reviewed studies from 2019 onwards, but may not capture all relevant research, particularly from non-English sources or grey literature. The economic and policy analyses are based on general observations rather than comprehensive regional assessments. Future work should include systematic reviews, meta-analyses of crop responses, and region-specific economic assessments.

Key Recommendations:

1) **For Researchers**: Conduct systematic meta-analyses of crop responses to shading; develop crop-specific shading threshold models; investigate cultivar variability in shade tolerance; and establish standardised data collection protocols across sites.
2) **For Engineers**: Develop cost-effective elevated mounting structures; optimise panel configurations for crop light requirements; and improve wind-load design codes for tall agri-voltaic structures.
3) **For Policymakers**: Establish clear regulatory frameworks for agrivoltaics; provide subsidies and incentives for dual-use systems; and support research and demonstration projects.
4) **For Farmers**: Start with pilot projects on small areas; select shade-tolerant crops; and seek technical advice on system design and operation.

With continued research, standardised data and capacity building, horticulture-voltaics can deliver climate-adaptive, resource-efficient and energy-positive horticultural systems worldwide.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Goetzberger, A., & Zastrow, A. (1982). On the coexistence of solar-energy conversion and plant cultivation. *International Journal of Solar Energy*, *1*(1), 55-69.

[2] Dupraz, C., Marrou, H., Talbot, G., et al. (2011). Combining solar photovoltaic panels and food crops for optimising land use: Towards new agrivoltaic schemes. *Renewable Energy*, *36*(10), 2725-2732.

[3] Pacheco-Ruiz, P., et al. (2025). From data to decisions: A paradigm shift in fruit agriculture through the integration of multi-omics, modern phenotyping and bioinformatic tools. *Frontiers in Plant Science*, *16*, 1707289.

[4] Barron-Gafford, G. A., Pavao-Zuckerman, M. A., Minor, R. L., et al. (2019). Agrivoltaics provide mutual benefits across the food-energy-water nexus in drylands. *Nature Sustainability*, *2*(9), 848-855.

[5] van Dijk, M., Morley, T., Rau, M. L., & Saghai, Y. (2021). A meta-analysis of projected global food demand and population at risk of hunger for the period 2010-2050. *Nature Food*, *1*(7), 494-501.

[6] Fraunhofer ISE. (2019). *Agrophotovoltaics: High-harvesting yield in hot summer of 2018* [Press release]. Fraunhofer Institute for Solar Energy Systems. https://www.ise.fraunhofer.de/en/press-media/press-releases/2019/agrophotovoltaics-hight-harvesting-yield-in-hot-summer-of-2018.html

[7] Widmer, J., et al. (2024). Agrivoltaics: A review of the current state of research and implementation. *Renewable and Sustainable Energy Reviews*. https://www.sciencedirect.com/science/article/pii/S1364032123011358

[8] International Energy Agency (IEA). (2023). *Agrivoltaics: Solar and farming can co-exist* (PVPS Task 13 report).

[9] Magarelli, A., Mazzeo, A., & Ferrara, G. (2025). Agrivoltaics in a Mediterranean vineyard: Soil moisture, microclimate, yield and land equivalent ratio. *Horticulturae*, *11*(2), 160. https://www.mdpi.com/2311-7524/11/2/160

[10] Ferrara, G., Magarelli, A., & Mazzeo, A. (2024). *Agrivoltaics for fruit crops: Shading thresholds and resilience* [Review]. University of Bari Aldo Moro.

[11] Hussain, S. N., et al. (2024). Bifacial photovoltaic modules in agrivoltaic systems: Performance and benefits. *Solar Energy*. https://www.sciencedirect.com/science/article/pii/S0038092X24007977

[12] The Innovation Energy. (2025). Agrivoltaic greenhouses: Electricity above, crop below. https://www.the-innovation.org/article/doi/10.59717/j.xinn-energy.2025.100124

[13] PVcase. (n.d.). *Agrivoltaics: The technical guide to design and implementation*.

[14] Solar Racks. (2026). *Agrivoltaics mounting: Dual-use design guide*. https://www.solaracks.com/agrivoltaics-mounting-structures-design-guide-for-dual-use-solar-and-agriculture/

[15] Applied Energy. (2025). Design parameters of agrivoltaic systems: Ground coverage ratio, clearance height and tracking. https://www.sciencedirect.com/science/article/pii/S0306261925004805

[16] Energies. (2025). Fixed-tilt versus tracking in agrivoltaic systems. *Energies*, *18*(10), 2553.

[17] pv magazine. (2024-2026). Agrivoltaics in Italy: Laterza and Scalea projects (Vigna Agrivoltaica di Comunita, Le Greenhouse). https://www.pv-magazine.com

[18] Renewable Energy. (2023). Interspersed bifacial agrivoltaic systems: Fixed vertical versus dynamic single-axis tracking in Belgium. https://www.sciencedirect.com/science/article/abs/pii/S0960148123010960

[19] Carrasco-Astudillo, N., et al. (2026). Rooftop agrivoltaic systems integrating urban agriculture and photovoltaic generation. https://www.sciencedirect.com/science/article/pii/S0360132326005718

[20] OpenEI / NREL. (2024). *InSPIRE Design & Configurations Guide* (Agrivoltaics Technical Guide). https://docs.nlr.gov/docs/fy25osti/91672.pdf

[21] Gautam, S., et al. (2024). Impact of solar panel spacing on wind load in an elevated solar panel structure. https://link.springer.com/article/10.1007/s44245-024-00081-4

[22] Sentnet Engineering. (2026). *Vertical agrivoltaic wind loads: 15 months of AgriPV field data*.

[23] Leonics. (n.d.). *Sizing of PV systems: Module, string and inverter specifications*. https://www.leonics.com/support/article2_12j/articles2_12j_en.php

[24] AIP Conference Proceedings. (2024). Smart irrigation integration with PV systems for water-energy-food nexus.

[25] Mayfield Energy. (2023). *How to calculate PV string size: Maximum system voltage (1000/1500 Vdc)*. https://www.mayfield.energy/technical-articles/2023-update-how-to-calculate-pv-string-size/

[26] ASCE 7-16. (2016). *Minimum design loads and associated criteria for buildings and other structures*. American Society of Civil Engineers.

[27] Special Cultural Operations in Vegetable Production for Enhanced Yield and Quality. (2024). ResearchGate. https://www.researchgate.net/publication/381915259

[28] AgriScan. (2025). *Inter-cultural operations in crop production*. https://agriscan.in/inter-cultural-operations-in-crop-production/

[29] Juillion, P., et al. (2022). Shading effect of photovoltaic panels on apple fruit quality and alternate bearing. *Scientia Horticulturae*. https://www.sciencedirect.com/science/article/abs/pii/S0304423822005544

[30] Oregon State University. (2023). *Partial shade from solar panels increases abundance of flowers in late summer*. https://news.oregonstate.edu/news/partial-shade-solar-panels-increase-abundance-flowers-late-summer

[31] OpenEI. (n.d.). *Tracking optimization in agrivoltaic systems: A comparative study for apple orchards*. https://openei.org/wiki/Tracking_Optimization_in_Agrivoltaic_Systems:_A_Comparative_Study_for_Apple_Orchards

[32] Hosseini, E., & Bou-Zeid, E. (2024). Modeling the microclimate and crop response of agrivoltaic systems: A tomato field case study. *Journal of Advances in Modeling Earth Systems*.

[33] NCAT. (n.d.). *Crops uniquely suited to growth in agrivoltaic settings*. https://www.ncat.org/blog/crops-uniquely-suited-to-growth-in-agrivoltaic-settings/.

International Journal of Environmental & Agriculture Research

Published by
AD Publications

Contact us +91-7665235235 www.ijoear.com info@ijoear.com