MUJ/Q&C/FoMC/SHTM/2022/MoU Activities -HTMi

MANIPAL UNIVERSITY
JAIPUR

FACULTY OF MANAGEMENT & COMMERCE

SCHOOL OF HOSPITALITY AND TOURISM MANAGEMENT

DEPARTMENT OF HOTEL MANAGEMENT

&

HTMi
Hotel and Tourism Management Institute Switzerland

Cross Cultural Hospitality Scopes

Faculty Interaction

14th Oct 2022

03:00 pm onwards

Content of Report

1. Introduction of the Event

School of Hospitality and Tourism Management and HTMi, Hotel and Tourism Management Institute Switzerland had conducted an interactive session to discuss various hospitality scopes.

2. Objective of the Event

The objective of the session was:

- Strengthen the relationship between the two partners and discuss various scopes.

3. Beneficiaries of the Event

- Hospitality & Tourism students
- Faculties

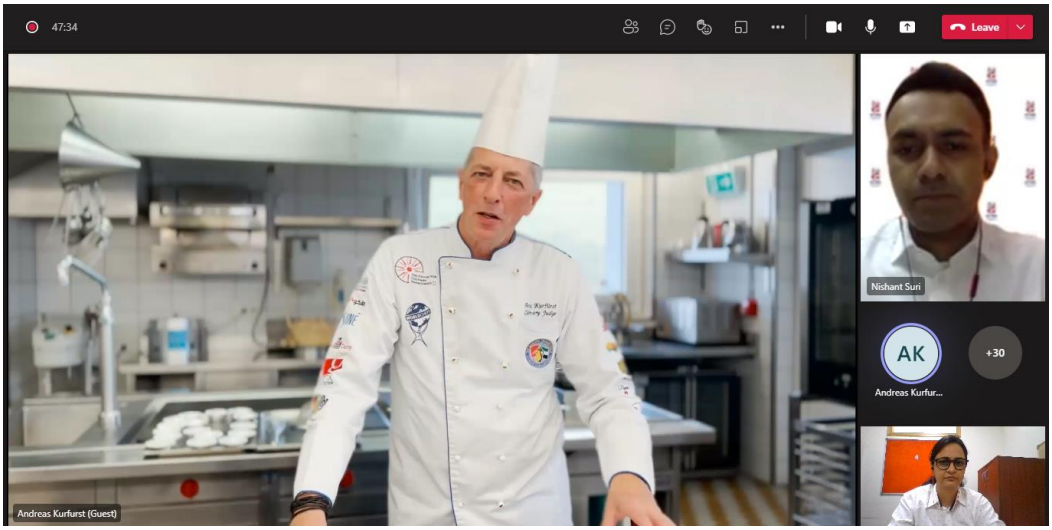
4. Details of the Representatives of HTMI

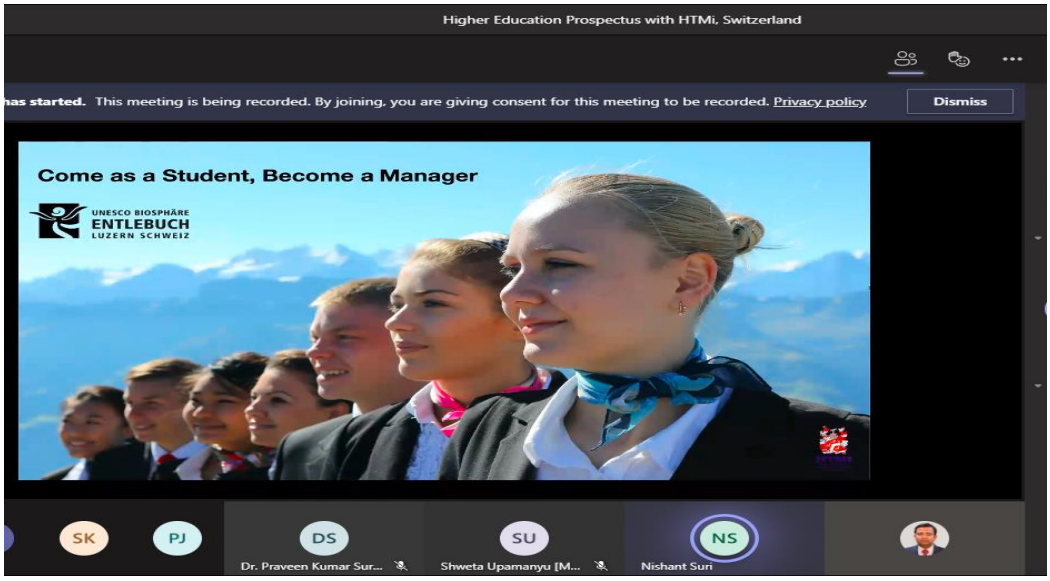
a. Mr. Nishant Suri – Marketing Head, HTMi Switzerland
b. Chef Andreas Kurfrust - Head of HTMi Culinary Education

5. Brief Description of the event

Dr Shweta Upamanyu, Faculty, SHTM opened the session and invited the representatives of HTMi, Switzerland. She also spoke about SHTM, MUJ. Mr. Nishant Suri welcomed the audiences and introduced HTMi Switzerland. Dr Amit Datta spoke about Indian tourism scopes and how the students of the HTMi may be benefitted by exploring the hospitality culture of India. Chef Kurfrust discussed regarding the food culture of Switzerland and how the students of MUJ may be benefitted. The session was very informative for all faculties and how they may explore it further that may be useful for both the MoU partners. Dr. Shweta Upamanyu ended the webinar with a vote of thanks.

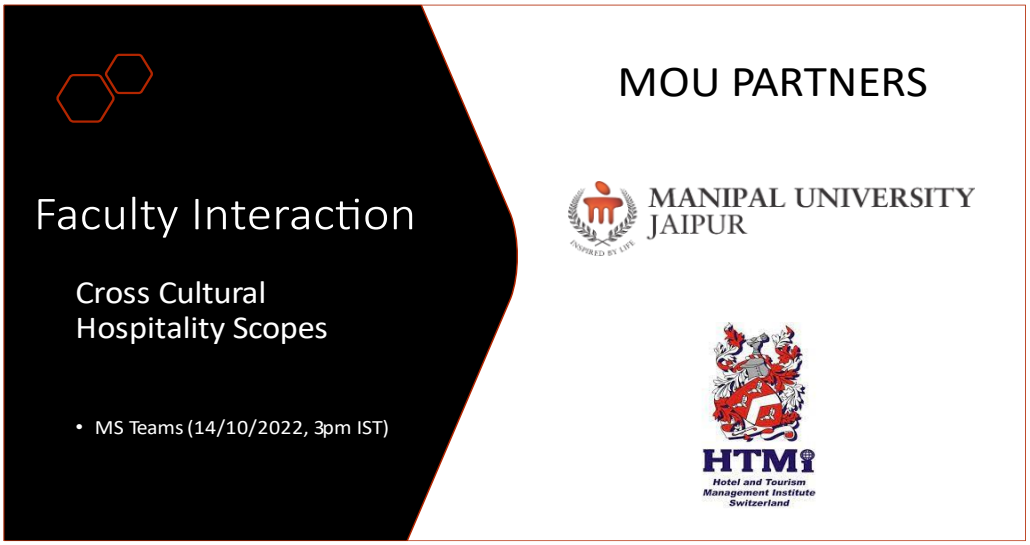
6. Screenshots of the event





Screenshot of the faculty interaction session

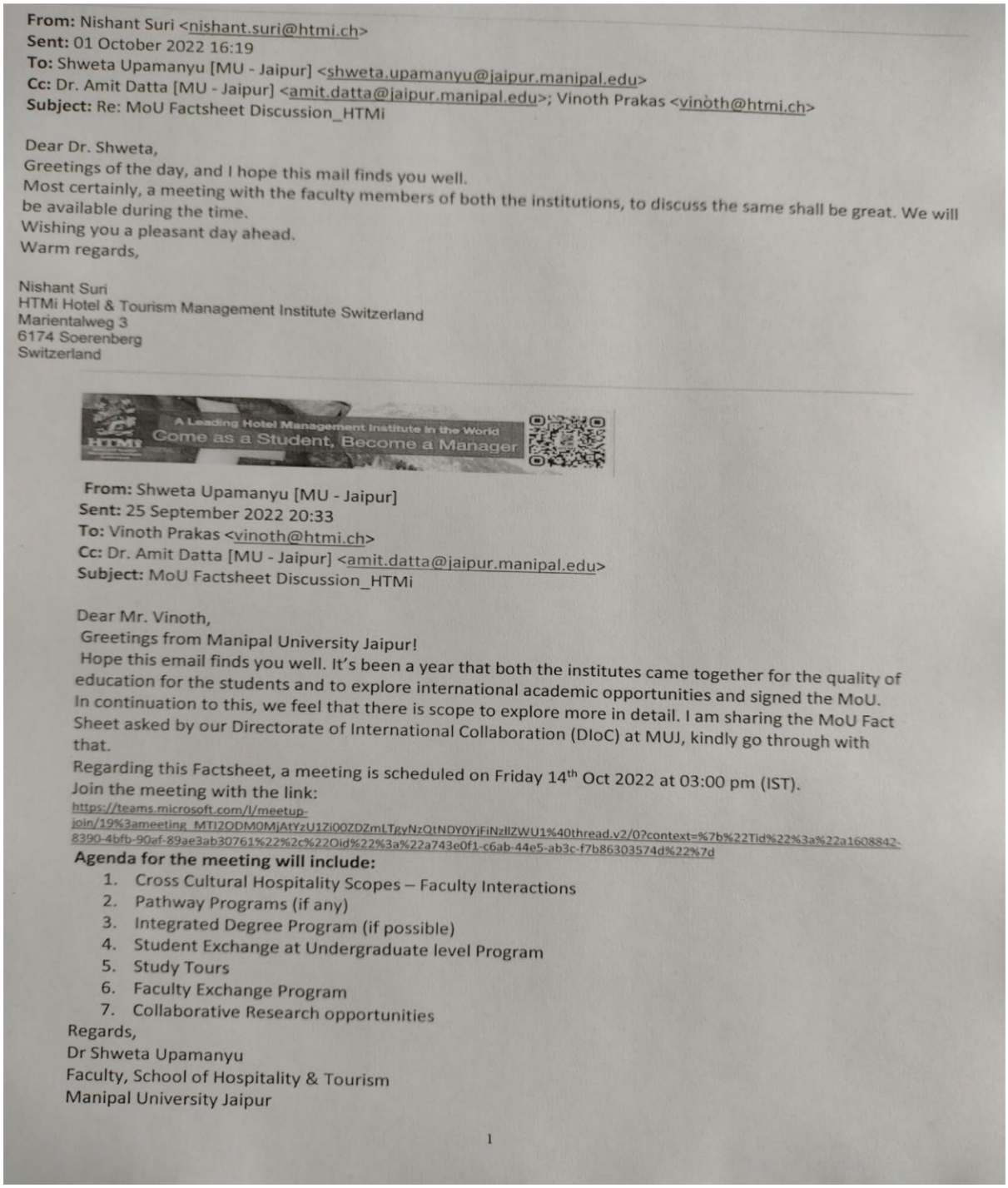
7. Brochure or creative of the event



8. Attendance of the Event: **Total attendee - 07**

Sr. No	Name of Institution	Name of Attendee
1	MUJ	Shweta Upamanyu
2	MUJ	Mukesh Shekhar
3	MUJ	Deepak Pokhriyal
4	MUJ	Amit Datta
5	MUJ	Aravind Kumar Rai
6	HTMi	Nishant Suri
7	HTMi	Andreas Kurfurst

ANNEXURE: Mail



From: Nishant Suri <nishant.suri@htmi.ch>
Sent: 01 October 2022 16:19
To: Shweta Upamanyu [MU - Jaipur] <shweta.upamanyu@jaipur.manipal.edu>
Cc: Dr. Amit Datta [MU - Jaipur] <amit.datta@jaipur.manipal.edu>; Vinoth Prakas <vinoth@htmi.ch>
Subject: Re: MoU Factsheet Discussion_HTMi

Dear Dr. Shweta,
Greetings of the day, and I hope this mail finds you well.
Most certainly, a meeting with the faculty members of both the institutions, to discuss the same shall be great. We will be available during the time.
Wishing you a pleasant day ahead.
Warm regards,

Nishant Suri
HTMi Hotel & Tourism Management Institute Switzerland
Marientalweg 3
6174 Soerenberg
Switzerland

A Leading Hotel Management Institute in the World
Come as a Student, Become a Manager

From: Shweta Upamanyu [MU - Jaipur]
Sent: 25 September 2022 20:33
To: Vinoth Prakas <vinoth@htmi.ch>
Cc: Dr. Amit Datta [MU - Jaipur] <amit.datta@jaipur.manipal.edu>
Subject: MoU Factsheet Discussion_HTMi

Dear Mr. Vinoth,
Greetings from Manipal University Jaipur!
Hope this email finds you well. It's been a year that both the institutes came together for the quality of education for the students and to explore international academic opportunities and signed the MoU.
In continuation to this, we feel that there is scope to explore more in detail. I am sharing the MoU Fact Sheet asked by our Directorate of International Collaboration (DIoC) at MUJ, kindly go through with that.
Regarding this Factsheet, a meeting is scheduled on Friday 14th Oct 2022 at 03:00 pm (IST).
Join the meeting with the link:
https://teams.microsoft.com/l/meetup-join/19%3ameeting_MTI2ODM0MjAtYzU1Zi00ZDZmLTgyNzQtNDY0YjFiNzllZWU1%40thread.v2/0?context=%7b%22Tid%22%3a%22a1608842-8390-4bfb-90af-89ae3ab30761%22%2c%22Oid%22%3a%22a743e0f1-c6ab-44e5-ab3c-f7b86303574d%22%7d

Agenda for the meeting will include:

1. Cross Cultural Hospitality Scopes – Faculty Interactions
2. Pathway Programs (if any)
3. Integrated Degree Program (if possible)
4. Student Exchange at Undergraduate level Program
5. Study Tours
6. Faculty Exchange Program
7. Collaborative Research opportunities

Regards,
Dr Shweta Upamanyu
Faculty, School of Hospitality & Tourism
Manipal University Jaipur

1

MANIPAL UNIVERSITY
JAIPUR
(University under Section 2(f) of the UGC Act)

Post Event Report

FACULTY OF DESIGN

Seminar on

‘Bamboo’

On the occasion of World Bamboo Day

Venue: Smt. Sharda Pai Auditorium

Time: 10:30 AM-12.30 PM

18th September 2022

Index

1. Introduction of the Seminar:

World Bamboo Day is celebrated on 18th September in every year. To celebrate this day The Seminar on Bamboo has been organised in Smt. Sharda Pai Auditorium. The main aim of the seminar was to create awareness of various possibilities of Bamboo construction. It intended to give path to reduce dependency on conventional materials so that we reduce Carbon footprint, and we can further achieve sustainable developments.

2. Objectives of the Seminar:

- Understanding the various application of Bamboo in Exterior and interior spaces.
- To create awareness about bamboo in different regions.
- To understand its production techniques

3. Beneficiaries of the Event:

- UG Students (Architecture, Design and Construction related Fields)
- PG Students (Architecture, Design and Construction related Fields)
- Research Scholars
- Academicians and Industry Professionals
- Farmers

4. Details of the Speakers:

Mr. Pasha Patel is Governing Council Member, Bureau of Indian Standard (BIS) Govt. of India. He is member, Board of trade, Ministry of Commerce & Industry. He is pioneer in promoting Bamboo in various part of India. He has proposed various policy guideline & got implemented in various Government Documents. He is very much passionate to spread message about the possibility of Bamboo. He conducted various seminar and conferences in India as well as Abroad. He has devoted his whole life to facilitate bamboo related studies, its application & preservation strategies to farmers & academicians.

Mr. Sanjeev Shashikant Karpe is a qualified Electrical Engineer has been associated with bamboo Industry for last eighteen years and has pioneered the work in setting up of self- sustainable bamboo-based enterprise in rural India. He is a Founder and Director with Konkan Bamboo & Cane Development Centre (KONBAC), an organization working for sustainable development through use of bamboo as a resource & implementing various bamboo projects successfully for last 17 years. He is also an Expert member on the steering committee of "INBAR

Task Force – Bamboo Construction". International Network for Bamboo & Rattan (INBAR) is an Intergovernmental body having 48 member counties and headquartered at Beijing, China

He is also National governing council member of Bamboo Society of India, Bangalore, a not-for-profit organization (NGO) working for promotion of bamboo in the country for last 40 years.

His specialties include:

- Provide training to use Bamboo with hands on experience.
- Strong communication and presentation skills
- Strong Ability to connect with and relate to students & farmers
- Dynamic team player and an Effective speaker

5. Brief Description of the event:

Faculty of Design conducted a seminar on 'Bamboo' to give awareness to students, researchers, academicians, and Farmers. On the auspicious occasion of world Bamboo Day. Farmers as well as people's representatives of surrounding villages were invited to participate the event. The poster has been circulated in the university portal so that MUJ user & students can get benefited. The event has been covered by various newspaper of Rajasthan and Maharashtra.

The Seminar was held in Smt Sharda pai Auditorium from 10.30 AM to 12.30 on 18th Sept. 2022 PM. The event began with an inaugural session at 10:30 am. Ms Manya Sharma President FOD Student Council, FOD welcomed the speakers, dignitaries, farmers, and Students which was followed by Prof. (Dr) Madhura Yadav, Dean, Faculty of Design addressing the session. Thereafter, the Eminent speaker, Mr. Pasha Patel & Mr. Sanjeev Shashikant Karpe gave their expert lecture/Presentation related to different aspects of Bamboo. Hon arable Prof G.K. Prabhu, President MUJ shared his experience and gave very insightful thought related to Bamboo as sustainable material. People's representatives also shared their views, farmers as well as students have actively participated in question answer sessions. Bamboo plantation ceremony was organised at the end of the event. Keynote speakers, Prof. G. K. Prabhu and dignitaries have participated with commitment that this type of event should be organised in regular interval. The event was concluded with vote of thanks by Ar. Sanjeev Pareek Assistant professor, SA&D to all speakers and Participants.

6. Media Coverage-

Punjab Kesari, 24 September 2022

PUNJAB KESARI
पंजाब केसरी
जयपुर • शनिवार • 24 सितम्बर, 2022

बांस की खेती अपनाने से होगी आर्थिक क्रांति : पाशा पटेल

जयपुर, 23 सितम्बर(ब्यूरो) : ब्यूरो आफ इंडियन स्टैंडर्ड्स के गवर्निंग काउंसिल मेंबर पाशा पटेल ने कहा कि बांस की खेती अपनाने से किसानों की माली हालत सुधरेगी और कृषि क्षेत्र में आर्थिक क्रांति आएगी।

बांस कम पानी और कम उपजाऊ जमीन में भी आसानी से उग सकते हैं। वे मणिपाल यूनिवर्सिटी जयपुर में विश्व बांस दिवस के उपलक्ष्य में फैकल्टी आफ डिजाइन में हुए कार्यक्रम में बतौर वक्ता बोल रहे थी। कार्यक्रम में स्थानीय किसानों और जनप्रतिनिधियों ने भी भाग लिया। उन्होंने कहा कि हमें अपनी आने वाली पीढ़ी को बचाने के लिए हवा और पानी को बचाना होगा। पर्यावरण को संतुलित रखने में बांस की खेती बहुत उपयोगी साबित हो सकती है। कोनवैक के संस्थापक डायरेक्टर इजी. संजीव कारपे ने बांस की खेती करने के लिए किसानों को जागरूक किया। आयोजक और फैकल्टी आफ डिजाइन की डीन प्रो.मथुरा यादव ने कहा बांस की खेती के महत्व पर प्रकाश डाला और विश्व स्तर पर हो रहे प्रयासों की जानकारी दी।

Samachar Jagat, 24 September 2022

विश्व बांस दिवस पर मणिपाल यूनिवर्सिटी जयपुर में कार्यक्रम

बांस की खेती अपनाने से खेती में होगी आर्थिक क्रांति : पाशा पटेल

समाज की सेवा करना और विकास में मदद करना विश्वविद्यालय का कर्तव्य : प्रो. प्रभु

जयपुर,समाचार जगत न्यूज। बांस की खेती अपनाने से किसानों की माली हालत सुधरेगी और कृषि क्षेत्र में आर्थिक क्रांति आएगी। बांस कम पानी और कम उपजाऊ जमीन में भी आसानी से उग सकते हैं। यह बात ब्यूरो आफ इंडियन स्टैंडर्ड्स के गवर्निंग काउंसिल मेंबर पाशा पटेल ने कही। हाल ही में विश्व बांस दिवस के उपलक्ष्य में फैकल्टी आफ डिजाइन में हुए कार्यक्रम में बतौर वक्ता बोल रहे थी। कार्यक्रम में स्थानीय किसानों और जनप्रतिनिधियों ने भी भाग लिया। उन्होंने कहा कि हमें अपनी आने वाली पीढ़ी को बचाने के लिए हवा और पानी को बचाना होगा। अनावश्यक रूप से मशीनों का प्रयोग हमारे पर्यावरण के लिए बड़ा खतरा है। उन्होंने कहा कि पर्यावरण को संतुलित रखने में बांस की खेती बहुत उपयोगी साबित हो सकती है।

कोनवैक के संस्थापक डायरेक्टर इजी. संजीव कारपे ने बांस की खेती करने के लिए किसानों को जागरूक किया। उन्होंने बताया कि बांस से जुड़े उद्योगों की एक बहुत बड़ी मार्केट है जहां पर किसान कम मेहनत में अधिक कमाई कर सकते हैं। बांस से सजावटी सामान के साथ अन्य उपयोगी वस्तुएं बनाई जा सकती हैं। अब तो बांस उद्योग बिजिंक हो गया है और विदेशों में भी इन वस्तुओं की डिमांड है। नए संसद भवन की फर्नीशिंग बांस से बन रही है। मणिपाल यूनिवर्सिटी के प्रेसिडेंट प्रो. (डॉ.) जोके प्रभु ने कहा कि मणिपाल यूनिवर्सिटी जयपुर बांस की खेती के लिए किसानों को मदद करेगी। समाज की सेवा करना और उसके विकास में मदद करना विश्वविद्यालय का कर्तव्य है। मणिपाल यूनिवर्सिटी जयपुर एफपीओ के माध्यम से किसानों को हर संभव सहयोग करेगी। पीयी जयंती पर एपपूरे की ओर से बांस की खेती के प्रति जागरूक करने के लिए अभियान चलाया जाएगा।

कार्यक्रम की आयोजक और फैकल्टी आफ डिजाइन की डीन प्रो. (डॉ.) मधुरा यादव ने कहा कि विश्व बांस दिवस पर हुए कार्यक्रम में पधारे आगंतुकों और किसानों का स्वागत किया। उन्होंने बांस की खेती के महत्व पर प्रकाश डाला और विश्वस्तर पर हो रहे प्रयासों की जानकारी दी। बांस ग्लोबल वार्मिंग को रोकने में सकारात्मक भूमिका निभाता है और यहां रोजगार के अवसर भी खूब हैं। इस दौरान किसान नेता शैलाराम सहल और रामपाल जाट ने भी अपने विचार रखे। कार्यक्रम में रजिस्ट्रार डा. नीतू भटनागर और डिरेक्टिवर रिसुमूदन मुख्य रूप से मौजूद रहे। निदेशक प्रो. संजय कुमार जिन्का, विभाग प्रमुख प्रो. अनंत कुमार ओझरकर, सुरेश कारू, स्नेह सिंह और अन्य शिक्षकों ने भी प्रमुख भूमिका निभाई। विभाग के शिक्षक आर्किटेक्ट संजीव पारिक ने धन्यवाद ज्ञापित किया। छात्रा मान्या शर्मा ने सत्र संचालन किया।

Ujjwal India, 24 September 2022

विश्व बांस दिवस के उपलक्ष्य में मणिपाल यूनिवर्सिटी जयपुर में कार्यक्रम

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जयपुर, उन्मवल इण्डिया संवाददाता। बांस की खेती अपनाने से किसानों की माली हालत सुधरेगी और कृषि क्षेत्र में आर्थिक क्रांति आएगी। बांस कम पानी और कम उपजाऊ जमीन में भी आसानी से उग सकते हैं। यह बात ब्यूरो आफ इंडियन स्टैंडर्ड्स के गवर्निंग काउंसिल मेंबर पाशा पटेल ने कही। हाल ही में विश्व बांस दिवस के उपलक्ष्य में फैकल्टी आफ डिजाइन में हुए कार्यक्रम में बतौर वक्ता उन्होंने यह विचार व्यक्त किए। कार्यक्रम में स्थानीय किसानों और जनप्रतिनिधियों ने भी भाग लिया। उन्होंने कहा कि हमें अपनी आने वाली पीढ़ी को बचाने के लिए हवा और पानी को बचाना होगा। अनावश्यक रूप से मशीनों का प्रयोग हमारे पर्यावरण के लिए बड़ा खतरा है। उन्होंने कहा कि पर्यावरण को संतुलित रखने में बांस की खेती बहुत उपयोगी साबित हो सकती है।

कोनवैक के संस्थापक डायरेक्टर इजी. संजीव कारपे ने बांस की खेती करने के लिए किसानों को जागरूक किया। उन्होंने बताया कि बांस से जुड़े उद्योगों की एक बहुत बड़ी मार्केट है जहां पर किसान कम मेहनत में अधिक कमाई कर सकते हैं। बांस से सजावटी सामान के साथ अन्य उपयोगी वस्तुएं बनाई जा सकती हैं। अब तो बांस उद्योग बिजिंक हो गया है और विदेशों में भी इन वस्तुओं की डिमांड है। नए संसद भवन की फर्नीशिंग बांस से बन रही है। मणिपाल यूनिवर्सिटी के प्रेसिडेंट प्रो. (डॉ.) जोके प्रभु ने कहा कि मणिपाल यूनिवर्सिटी जयपुर बांस की खेती के लिए किसानों को मदद करेगी। समाज की सेवा करना और उसके विकास में मदद करना विश्वविद्यालय का कर्तव्य है। मणिपाल यूनिवर्सिटी जयपुर एफपीओ के माध्यम से किसानों को हर संभव सहयोग करेगी। पीयी जयंती पर एपपूरे की ओर से बांस की खेती के प्रति जागरूक करने के लिए अभियान चलाया जाएगा।

कार्यक्रम की आयोजक और फैकल्टी आफ डिजाइन की डीन प्रो. (डॉ.) मधुरा यादव ने कहा कि विश्व बांस दिवस पर हुए कार्यक्रम में पधारे आगंतुकों और किसानों का स्वागत किया। उन्होंने बांस की खेती के महत्व पर प्रकाश डाला और विश्वस्तर पर हो रहे प्रयासों की जानकारी दी। बांस ग्लोबल वार्मिंग को रोकने में सकारात्मक भूमिका निभाता है और यहां रोजगार के अवसर भी खूब हैं। इस दौरान किसान नेता शैलाराम सहल और रामपाल जाट ने भी अपने विचार रखे। कार्यक्रम में रजिस्ट्रार डा. नीतू भटनागर और डिरेक्टिवर रिसुमूदन मुख्य रूप से मौजूद रहे। निदेशक प्रो. संजय कुमार जिन्का, विभाग प्रमुख प्रो. अनंत कुमार ओझरकर, सुरेश कारू, स्नेह सिंह और अन्य शिक्षकों ने भी प्रमुख भूमिका निभाई। विभाग के शिक्षक आर्किटेक्ट संजीव पारिक ने धन्यवाद ज्ञापित किया। छात्रा मान्या शर्मा ने सत्र संचालन किया।

मराठवाडा नेता

राजस्थान देश व मानव जातीच्या रक्षणासाठी बांबू लागवड करा

मणीपाल युनिव्हर्सिटीमध्ये माजी आमदार पाशा पटेल यांचे आवाहन

बांबू लागवड चळवळ गावागावापर्यंत पोहंचवणार

मणीपाल युनिव्हर्सिटीचे व्हाईस चान्सलर प्रभू यांचे प्रतिपादन

Hindustan Express, 24 September 2022

समाज की सेवा करना और विकास में मदद करना विश्वविद्यालय का कर्तव्य : प्रो. प्रभु

विश्व बांस दिवस के उपलक्ष्य में मणिपाल यूनिवर्सिटी जयपुर में कार्यक्रम

बांस की खेती अपनाने से खेती में होगी आर्थिक क्रांति - पाशा पटेल

हिन्दुस्तान एक्सप्रेस

जयपुर। बांस की खेती अपनाने से किसानों की माली हालत सुधरेगी और कृषि क्षेत्र में आर्थिक क्रांति आएगी। बांस कम पानी और कम उपजाऊ जमीन में भी आसानी से उग सकते हैं। यह बात ब्यूरो आफ इंडियन स्टैंडर्ड्स के गवर्निंग काउंसिल मेंबर पाशा पटेल ने कही। हाल ही में विश्व बांस दिवस के उपलक्ष्य में फैकल्टी आफ डिजाइन में हुए कार्यक्रम में बतौर वक्ता उन्होंने यह विचार व्यक्त किए। कार्यक्रम में स्थानीय किसानों और जनप्रतिनिधियों ने भी भाग लिया। उन्होंने कहा कि मणिपाल यूनिवर्सिटी जयपुर में बांस की खेती के लिए फार्मर प्रोड्यूसर्स ऑफिस बनेगा। जहां किसानों को बांस की खेती के लिए प्रेरित किया जाएगा।

उन्होंने कहा कि हमें अपनी आने वाली पीढ़ी को बचाने के लिए हवा और पानी को बचाना होगा। अनावश्यक रूप से मशीनों का प्रयोग हमारे पर्यावरण के लिए बड़ा खतरा है। उन्होंने कहा कि पर्यावरण को संतुलित रखने में बांस की खेती बहुत उपयोगी साबित हो सकती है। कोनवैक के संस्थापक डायरेक्टर इजी. संजीव कारपे ने बांस की खेती करने के लिए किसानों को जागरूक किया। उन्होंने बताया कि बांस से जुड़े उद्योगों की एक बहुत बड़ी मार्केट है जहां पर किसान कम मेहनत में अधिक कमाई कर सकते हैं। बांस से सजावटी सामान के साथ अन्य उपयोगी वस्तुएं बनाई जा सकती हैं। अब तो बांस उद्योग बिजिंक हो गया है और विदेशों में भी इन वस्तुओं की डिमांड है। नए संसद भवन की फर्नीशिंग बांस से बन रही है। मणिपाल यूनिवर्सिटी के प्रेसिडेंट प्रो. (डॉ.) जोके प्रभु ने कहा कि मणिपाल यूनिवर्सिटी जयपुर बांस की खेती के लिए किसानों को मदद करेगी। समाज की सेवा करना और उसके विकास में मदद करना विश्वविद्यालय का कर्तव्य है। मणिपाल यूनिवर्सिटी जयपुर एफपीओ के माध्यम से किसानों को हर संभव सहयोग करेगी।

7. Images

1.Inaugural Address by Prof. (Dr.) Madhura Yadav, Dean, FoD

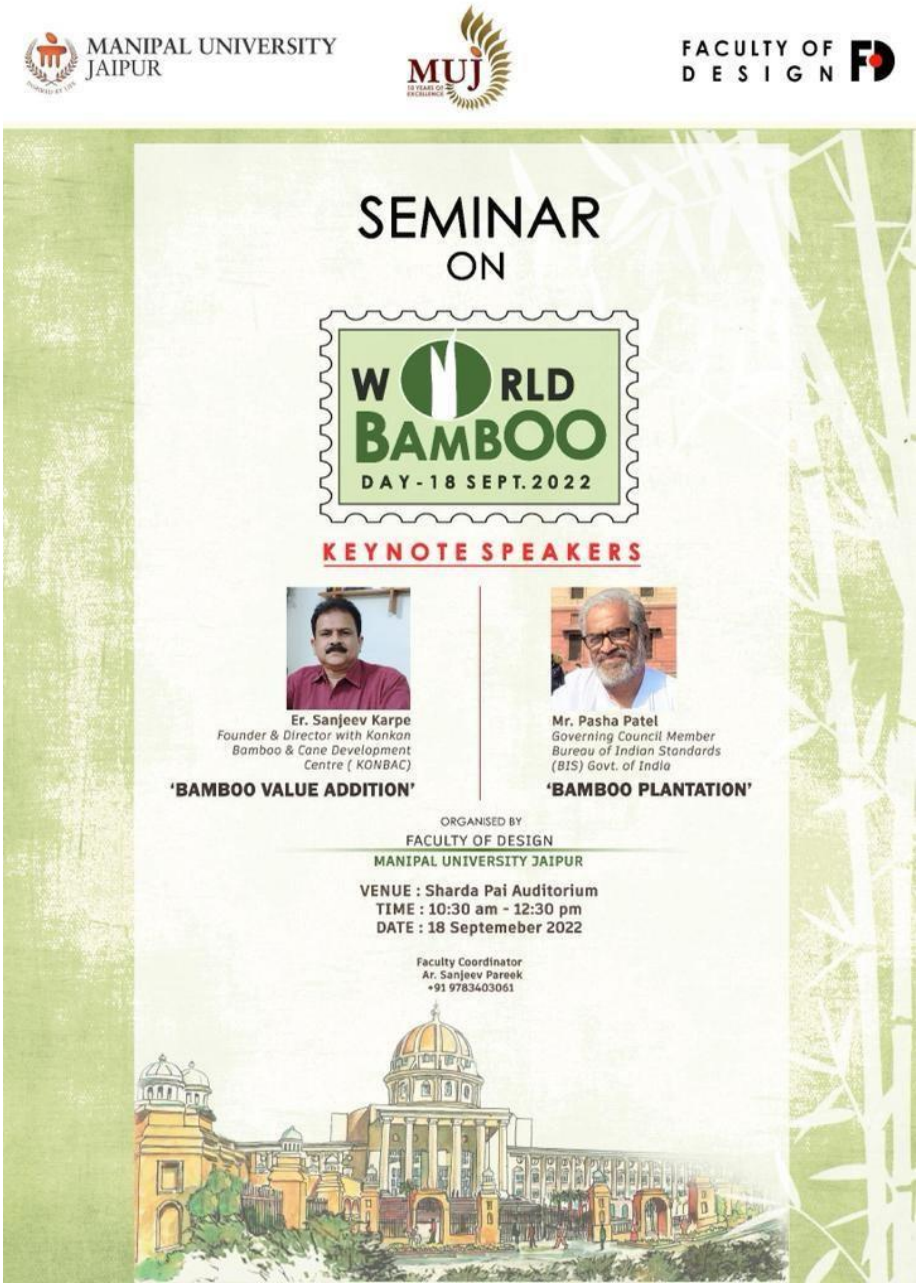
2. Introductory Lecture by Key note speaker-Mr. Pasha Patel and Mr. Sanjeev Shashikant Karpe

3. Sharing experience by Honarable Prof G.K.Prabhu 4.. Discussion session with Farmers & Peoples representatives President MUJ

5. Greetings to key note speakers

6. Bamboo plantation ceremony near S.T.P. at MUJ Campus

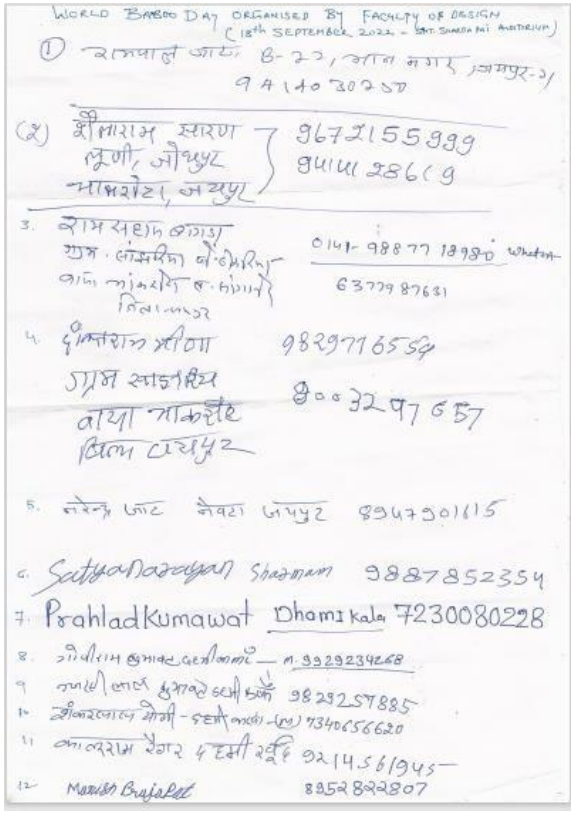
8. Brochure of the event:

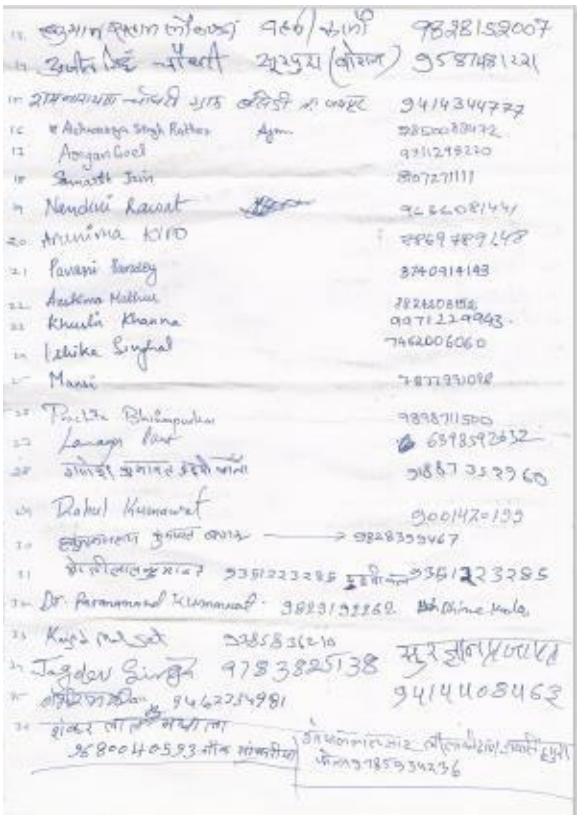


9. Schedule of the event

S.No.	Description	Time
1	Introduction & welcome note by Manya Sharma Student council president	10.30 AM
2	Presentation of Bouquet to Mr. Sanjeev Shashikant Karpe and Mr. Pasha Patel byProf (Dr.) Madhura Yadav and Prof (Dr.) Sampath kumar Padmanabha Jinka	10.35 AM
3	Welcome Address by -Prof (Dr.) Madhura Yadav, Dean Faculty of Design	10.40 AM
4	Presentation on Bamboo Plantation by Mr Pasha Patel.	10.45-11.30
5	Presentation on Bamboo Value Addition by Mr . Sanjeev Shashikant Karpe.	11.30-12.15
6	Vote of Thanks By Ar. Sanjeev Pareek	12.15 PM
7.	Bamboo Plantation Ceremony	12.30 PM

10. Attendance of the Event:





11. Weblink:

https://www.youtube.com/watch?v=wi4edh5WyOo

12. Event Coordinators:

- Ar. Sanjeev Pareek (Assistant Professor, SA&D)

Ar. Sanjeev Pareek (Assistant Professor, SA&D)

Prof. Sunanda Kapoor
Head

MANIPAL UNIVERSITY
JAIPUR
(University under Section 2(f) of the UGC Act)

Event Report

By

Abhigya Club

ECE, SEEC, FoE,

Manipal University Jaipur

SaveSoil Event

at Jaipur Exhibition & Convention Centre (JECC)

3rd June, 2022

Physical / Off-line Event

1. Introduction of the Event:

Save Soil is a global movement launched by Sadhguru, founder of Isha Foundation, to addressthe soil crisis by bringing together people from around the world to stand up for Soil Health, and supporting leaders of all nations to institute national policies and actions toward increasingthe organic content in cultivable Soil.

Students of Abhigya club, MUJ organized 'STAND FOR SOIL' event to show support for thisinitiative and welcome Sadhguru to Jaipur, Rajasthan.

2. Objective of the Event

The objective of the events:

a) To show support for 'Save Soil' initiative of ISHA Foundation

b) To attend the SaveSoil event at Jaipur Exhibition & Convention Centre (JECC)

c) To learn about the significance of this movement from Sadhguru himself.

3. Beneficiaries of the Event

d) MUJ students

e) Society / community / Humanity

4. Details of the Guests

1) Sadhguru from ISHA Foundation

2) Mr. Rajesh Chand Meena, the Minister of Panchayati Raj & Rural Development, Raj,

3) Mr. Lalchand Kataria, the Agriculture Minister of Rajasthan

4) Ila Arun, famous folk singerOther dignitaries

5. Brief Description of the event

Conscious Planet is an effort to raise human consciousness and bring a sense of inclusivenesssuch that multifarious activities of our societies move into a conscious mode.

An effort to align human activity to be supportive of nature and all life on our planet.

In this inclusive undertaking of Save Soil Movement, governments, UN agencies, global leaders, organizations, eminent members of the environmental and scientific community, corporate and individual citizens are uniting behind a common purpose to address the alarming crisis of Soil Extinction. For our children and future generations, it is critical to leave behind a planet capable of producing nutritious food and sustaining all life.

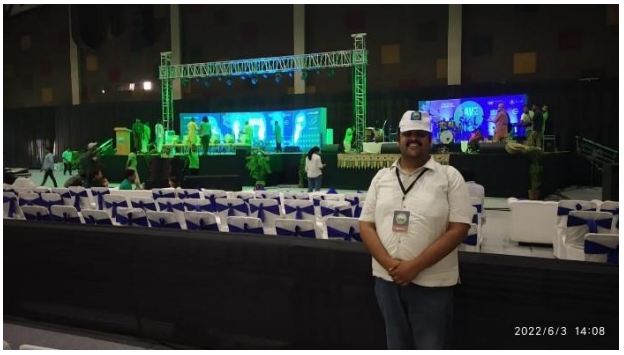
Sadhguru was on a 100-day Motorcycle Journey, from the United Kingdom to India movingacross 27 Countries, covering more than 30,000 kms of distance. The objective of the event was to learn from Sadhguru & understand the opinions of different ministers of Rajasthan government.

Abhigya club students attended this event with other MUJ students. The event was in physicalmode with guests coming from various walks of life at JECC, Jaipur.

6. Photographs

1) Abhigya Club & MUJ Students going to JECC by
MUJ bus

2) Abhigya Club & MUJ Students attending the
Save Soil event at JECC Jaipur



3) Abhigya Club's faculty coordinator volunteering at the Save Soil event, JECC Jaipur

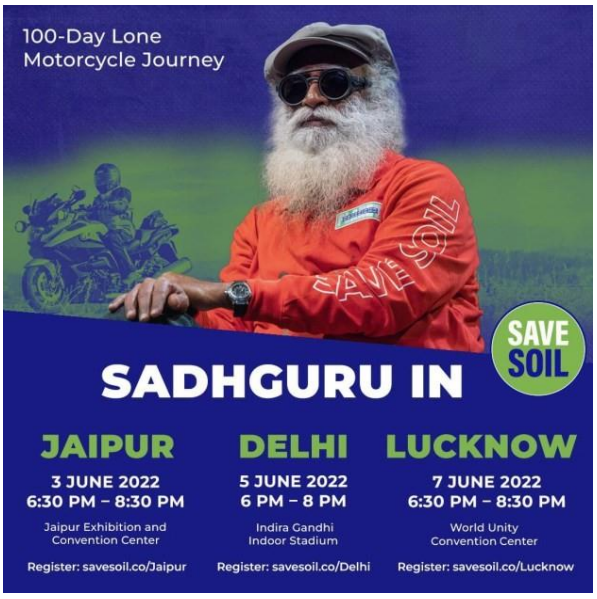
4) Shri Kutle Khan ji performing on stage

5) Smt. Ila Arun ji attending the Save Soil event

6) Isha Samskriti group performing on stage

7) Sadhguru handing over the Save Soil policy document for Rajasthan to Honourable Minister

7. Brochure or creative of the event

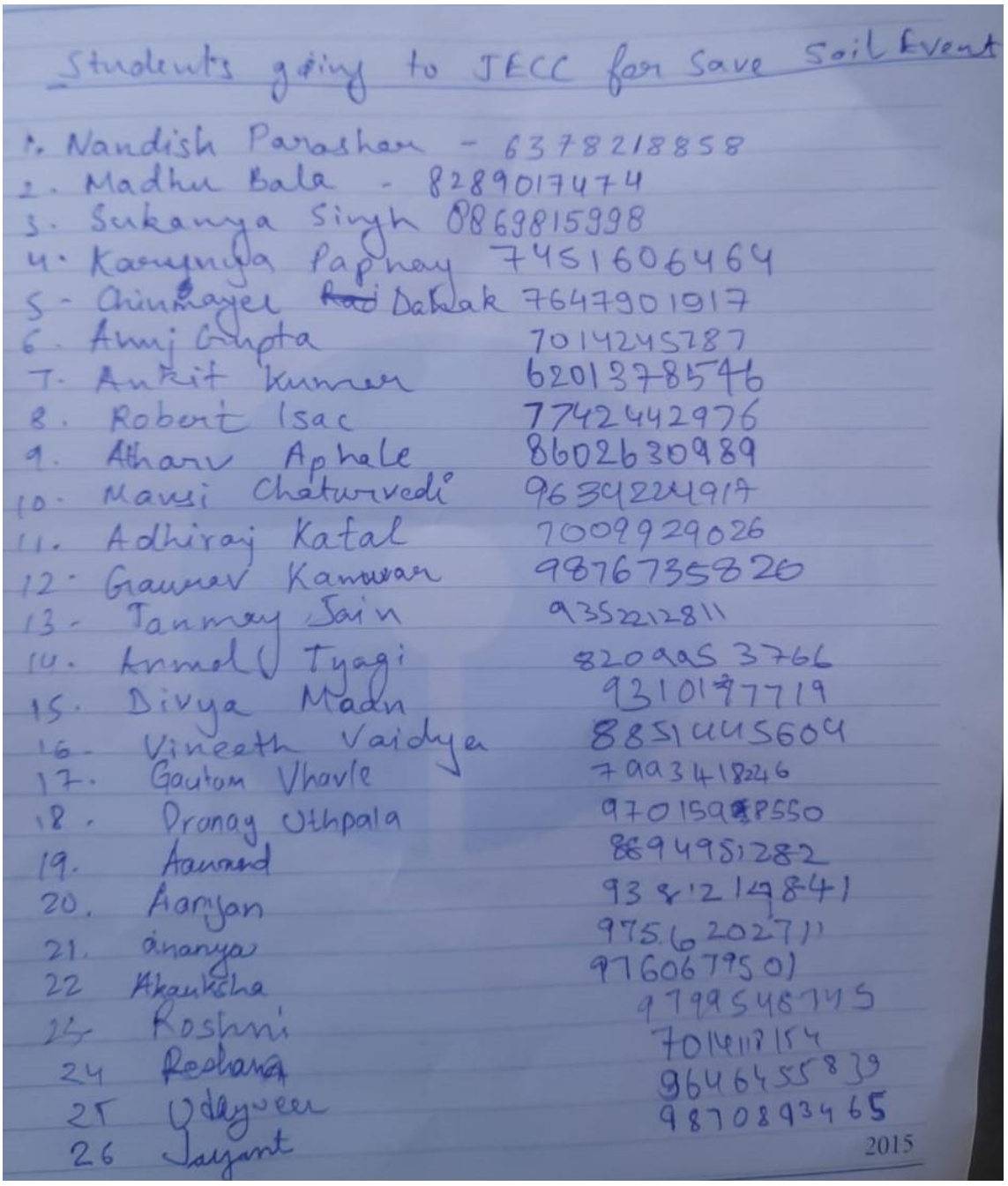


8. Schedule of the Event

Date: 3rd June, 2022 Time: 6:30 PM – 8:30 PM

Venue: Jaipur Exhibition & Convention Centre (JECC)

9. Attendance of the Event



Students going to JECC for Save Soil Event

1. Nandish Parashar - 6378218858
2. Madhu Bala - 8289017474
3. Sukanya Singh 08693815998
4. Kanyuga Paphay 7451606464
5. Chinmayee ~~Rai~~ Dahlak 7647901917
6. Anuj Gupta 7014245787
7. Ankit Kumar 6201378546
8. Robert Isac 7742442976
9. Atharv Aphale 8602630989
10. Mansi Chaturvedi 9634224917
11. Adhiraj Katal 7009929026
12. Gaurav Kanwar 9876735820
13. Tanmay Jain 9352212811
14. Anmol Tyagi 8209953766
15. Divya Madn 9310177719
16. Vineath Vaidya 8851445604
17. Gautam Vhavle 7993418246
18. Pranay Uthpala 9701598550
19. Aanand 8694951282
20. Aanjan 9381214841
21. Ananya 9756202711
22 Akanksha 9760679501
23 Roshni 7994548745
24 Reshma 7014117154
25 Udayveer 9646455839
26 Jayant 9870893465

2015

10. Faculty Coordinator:

-s/d-

Dr. Rohit Mathur - Department of ECE, SEEC, MUJ

MANIPAL UNIVERSITY
JAIPUR
(University under Section 2(f) of the UGC Act)

Event Report

By

Abhigya Club

ECE, SEEC, FoE,

Manipal University Jaipur

Stand for Soil

2nd June, 2022

Physical / Off-line Event

1. Introduction of the Event:

Save Soil is a global movement launched by Sadhguru, founder of Isha Foundation, to address the soil crisis by bringing together people from around the world to stand up for Soil Health, and supporting leaders of all nations to institute national policies and actions toward increasingthe organic content in cultivable Soil.

Students of Abhigya club, MUJ organized 'STAND FOR SOIL' event to show support for thisinitiative and welcome Sadhguru to Jaipur, Rajasthan.

2. Objective of the Event

The objective of the events:

a) To show support for 'Save Soil' initiative and

b) To welcome Sadhguru to Jaipur, Rajasthan.

3. Beneficiaries of the Event

c) MUJ students

d) Society / community / Humanity

4. Details of the Guests

Shri Sadhguru ji, founder of ISHA Foundation

5. Brief Description of the event

MUJ ABHIGYA CLUB took an initiative to bring awareness about soil conservation by organizing a 'Stand for Soil' event near Jaipur-Ajmer Expressway outside Hotel Highway King, a popular hotel on the expressway. Wherein students stood for two and a half hours and conveyed about soil conservation to local people and passerby's.

Sadhguru was on a 100-day Motorcycle Journey, from the United Kingdom to India movingacross 27 Countries, covering more than 30,000 kms of distance. The objective of the event was to bring awareness about soil conservation and to welcome Sadhguru to Jaipur as he moves towards his next destination on his Save Soil journey.

Abhigya club students conducted the event in physical mode with many students of ManipalUniversity and guests present at Hotel Highway King.

6. Photographs

1. Sadhguru acknowledges MUJ students & staff for their support

2. Students, ISHA volenteers and MUJ staff participating in 'STAND for SOIL' event

3. Guests at Hotel Highway King participating in 'STAND for SOIL' event

7. Brochure or creative of the event

8. Schedule of the Event

Date: 2nd
June 2022
Time: 5:30
PM – 7:70 PM
Venue: Hotel HighwayKing, Jaipur-Ajmer Expressway

MANIPAL UNIVERSITY
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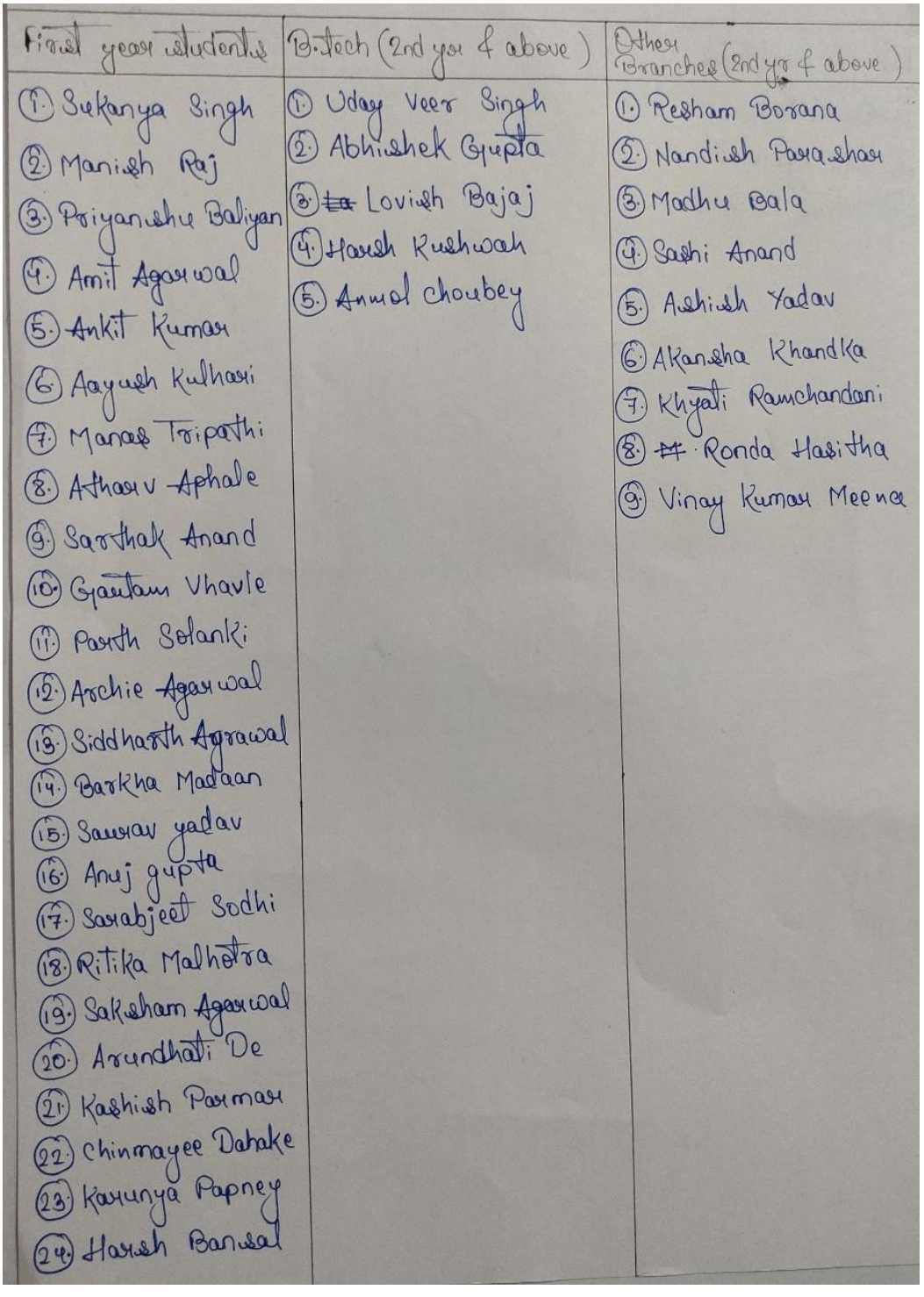
9. Attendance of the Event



First year students	B.Tech (2nd yr & above)	Other Branches (2nd yr & above)
1. Sukanya Singh	1. Uday Veer Singh	1. Resham Borana
2. Manish Raj	2. Abhishek Gupta	2. Nandish Parashar
3. Priyanshu Baliyan	3. Lovish Bajaj	3. Madhu Bala
4. Amit Agarwal	4. Harsh Kushwah	4. Sashi Anand
5. Ankit Kumar	5. Anmol Choubey	5. Ashish Yadav
6. Aayush Kulhari		6. Akansha Khandka
7. Manas Tripathi		7. Khyati Ramchandani
8. Atharv Aphale		8. Ronda Hasitha
9. Sarthak Anand		9. Vinay Kumar Meena
10. Gautam Vhavle		
11. Parth Solanki		
12. Archie Agarwal		
13. Siddharth Agrawal		
14. Barkha Madaan		
15. Saurav yadav		
16. Anuj gupta		
17. Sarabjeet Sodhi		
18. Ritika Malhotra		
19. Sakesham Agarwal		
20. Arundhati De		
21. Kashish Parmar		
22. Chinmayee Dahake		
23. Karunya Papney		
24. Harsh Bansal		

10. Faculty Coordinator:

-s/d-

Dr. Rohit Mathur - Department of ECE, SEEC, MUJ

FACULTY OF MANAGEMENT AND COMMERCE

SCHOOL OF BUSINESS AND COMMERCE

BUSINESS ADMINISTRATION

Visit to Akshaypatra

23/12/2022

1. Introduction of the Event

School of Business and Commerce organized a visit for its students to Akshaypatra, Jaipur on 23rd December 2022.

2. Objective of the Event

The visit to Akshaypatra was organised to provide a chance to the students to interact withthe experts and teachers at Akshaypatra about self-discipline and understanding our roots. Also, during the visit, the students learnt about the functioning of Akshaypatra Foundationand its social contribution.

3. Beneficiaries of the Event

- Students
- Faculties

4. Details of the Guests

During the visit, Shri Pran Vallabh from Akshaypatra was the resource person. Students and faculties from SBC were also present.

5. Brief Description of the event

School of Business and Commerce organised a visit to Akshaypatra, Jaipur on 23rd Dec 2022 for its students. The visit started with an interaction between the teachers of Akshayapatra and students of SBC. During the interaction, various issues like discipline, self-control and yoga were discussed. The teachers explained the students the importance of understanding our culture and roots. After the interaction, the students took a guided tour to the Akshaypatra Kitchen. Experts from the foundation narrated the various systems and processes followed in the kitchen for food preparation. The students also learnt about the social contribution of Akshaypatra, which is working towards providing meals to underprivileged children.

6. Photographs of the event

Figure 1 MUJ students understanding the operation of Akshaypatra

Figure 2 Students are getting inputs : how to work for society"

Figure 3 official of Akshaya Patra introducing the working of organization

Figure 4 Valuable Inputs received by MUJ students

7. Attendance of the Event Total attendee- 35

List of participants

S.NO	NAME	CLASS
1	Kashish Jain	BBA III-C
2	Jaismin Tansukhani	BBA III-B
3	Chirag Saraf	BBA III-C
4	Bhavya Khandelwal	BBA III-B
5	Mayank Tyagi	BBA III-B
6	Manav Sankhla	BBA III-E
7	Jhanvi Agarwal	BBA III-B
8	Jay Sharma	BBA III-A
9	Pragya Jain	B.com Honours III-B
10	Harsh Kumar Singh	B.com Honours III-B
11	Palak Agarwal	B.com Honours III-B
12	Varsha Agarwal	B.com Honours III-B
13	Divyansh Gaur	B.com Honours III-A
14	Ashish Saini	B.com Honours III-A
15	Deepak Sahu	B.com Honours III-A
16	Ambudhi Choudhary	B.com Honours III-A
17	Nishtha Sethia	B.com Honours III-A
18	Purav Bhayana	B.com Honours III-A
19	Yash Dangi	B.com Honours III-A
20	Tanisha Doshi	B.com Honours III-A
21	Hemant Sharma	B.com Honours III-A
22	Manan Sachdeva	B.com Honours III-B
23	Pratibha Keshwani	B.com Honours III-B
24	Jay Sharma	BBA III-A
25	Siddhant Garg	B.com Honours III-A
26	Priyanka Kumari	BBA III-C
27	Yajat tak	BBA III-C
28	Devansh Garg	B.com Honours III-B
29	Ramay Mehta	BBA III-C
30	Tanishq haldiya	BBA III-C
31	Palak Chouhan	BBA III-C
32	Rishik saraf	B.com Honours III-B
33	Varsha Agarwal	B.com Honours III-B
34	Prem Raj	B.com Honours III-B
35	Bharti Vyas	B.com Honours III-B

Head
Department of Business Administration
Manipal University Jaipur

Research Paper

Universal Review

Scientific Information and Technological Board of Sadhana

www.universalreview.in

Index in Cosmos

Impact Factor: 5.525

Volume 10 Number 07 July 2019

ISSN 22772723 (P) 22772726 (O)

Climate Change & Emerging Health Care Issues in India

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Received: 14 June **Revised:** 22 June **Published:** 02 July

Abstract

The impact of climate change has started being felt from long time now and future projections represent an unacceptably high and potentially calamitous risk to human health all over the world. Studies have revealed that India is getting severely effected due to global warming. Climate change affects the health of people due to change in disease patterns as well. This article explores the increasing need of health care expenditure due to increasing impact of climate change on human health. In India, per capita health expenditure is low and moreover, approximately 72% of it is out of pocket. This article explores the impact of climate change on human health, specifically change in disease patterns and number of death caused due to it. It studies increasing episodes of diseases due to climate change and an urgent need to allocate more resources to health expenditure in view of these changing conditions.India being a developing nation, requires health infrastructure for prevention of chronic diseases as well as new diseases patterns arising due to change in global temperature and lifestyle.

Keywords: *Climate change, health expenditure, morbidity, diseases.*

JEL Classification: C33, H51, I12, H75

Health Care & Climate Change

Climate change may be considered to be one of the main challenges of Sustainable Development in present scenario. The Sustainable Development Goal 13 speaks largely of taking correcting steps to limit rising temperatures as well as improving the changes already brought about. In the 2030 Agenda for Sustainable Development, Member States express their commitment to protect the planet from degradation and take urgent action on climate change as well as the Agenda also identifies, in its paragraph 14, climate change as 'one of the greatest challenges of our time' (United Nations, 2015). This is being observed at the global level as with countries are experiencing changes in rainfall, more floods, droughts, intense rain, more frequent heat waves & shifting climate patterns.

Impact on human health can be seen as one of the most important impacts of climate changes happening all over the globe. It is pertinent to mention here that SDG 3 focuses on Universal Health Care and SDG Target 3.8 commits all countries to work towards the achieving of UHC by ensuring access by all to quality essential health-care services, and to safe, effective and affordable medicines and vaccines. (Organization, 2018). The effect on health can be better understood by understanding determinants of climate change. The ambition of development and increasing industrialization & urbanization has led to increasing emission of GHGs and at the same time there is rising deforestation due to which the impact of GHGs increases even more. Industrialized countries owe their current prosperity to years of 'historical' emissions, which have accumulated in the atmosphere since the start of the industrial revolution (Narain, Ghosh, Saxena, Parikh, & Soni, 2009). Though Kyoto protocol in 1997, tried to limit the emission of Green House Gases, there has been a constant war between the developed and developing countries as both the sides are blaming each other for increase environmental concerns &

ISSN 22772723 (P) 22772726 (O)

MANIPAL UNIVERSITY JAIPUR

International Winter School- Manipal University Jaipur [IWSMUJ]-2022

[Hybrid Mode]

Course/Project Overview

Name of Course- Climate change and sun studies for field work

Name of instructor: Mr Sagar Gupta & Dr
Tejbahadur Session: Jan.-Feb. 2022
Language of instruction: English
Number of contact hours: 36
Credit awarded: 03

Objective of Course/Project- The student will be able to

1. Interpretate emission at household and industry level
2. Design and develop tailored sun study data for miscellaneous work.
3. To develop carbon emission inventory for field studies.

Syllabus: introduction to solar radiation studies, climate change. introduction to IPCC, Types of GHG gasses, measurements of carbon emission.

Organization of Course

Total contact hrs 36		
1st week:	10 hrs (classes)	2 hrs (self-study/project)
2nd week:	10 hrs (classes)	2 hrs (Mid-term exam/assessment/discussion)
3rd week:	10 hrs (classes)	2 hrs
4th week:	6 hrs (Classes)	2hrs (End term exam)

Mode of lectures: Hybrid mode lecture/videos/case study/ discussion/ workshop/ hands-on

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Course/Project Plan

Lecture no.	Topic	Lecture mode	Instructor
L: 1-3	Basic concepts of sun studies	power point presentation	Dr Tej Bahadur
L: 4-5	Solar observatory studies at Jaipur city	field visit	Dr Tej Bahadur & Mr Sagar Gupta
L: 6-7	Climate change and the environment	power point presentation	Mr sagar Gupta
L: 8-9	Introduction to IPCC	powerpoint presentation	Dr Tej Bahadur & Mr sagar
L: 10-11	field visit to stp for understanding emissions	field visit	Dr Tej Bahadur & Mr sagar
L:12-13	Household carbon emission calculation	power point	Mr sagar Gupta
L:14-15	Household carbon emission calculation	power point	Mr sagar Gupta
L: 15-19	field based assignment for data collection of industry carbon emission	field	Dr Tej & Mr Sagar Gupta
L:20-21	Assessment of the GHG emission	Group exe	Dr Tej & Mr Sagar Gupta
L: 22-25	Assessment of the GHG emission	Group exe	Dr Tej & Mr Sagar Gupta
L: 26-30	Preparation of report on the field assignment	PowerPoint	Dr Tej & Mr Sagar Gupta
L: 31-34	Preparation of report on the field assignment	PowerPoint	Dr Tej & Mr Sagar Gupta
L: 35-36	Wrap up	Chalk & board	Dr Tej & Mr Sagar Gupta

Brief profile of the instructor

Mr Sagar Gupta is Assistant Professor (Sr.Scale) in the department of Civil Engineering. He has 8 years of experience in teaching and research. His qualification involves B.Tech and M.Tech Degree in civil engineering, Graduate Professional Diploma in sanitation (IHE Delft), Post-graduation certification in leadership in Public Health (MAHE), University Teaching Qualification, Netherlands, He is also pursuing his PhD from MUJ. He has been recipient of BMGF fellowship in sanitation(2018-19) in MSc sanitation Training of Trainers(ToT) Programme at IHE Delft. He is core committee member of IWA India chapter and a young water professional. Also, reviewer for SCOPUS index journals.

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Dr. Tej Bahadur is working as Associate Professor in the Department of Civil Engineering, Faculty of Engineering, Manipal University Jaipur. He completed his Ph.D. in Sedimentary Geology from the University of Rajasthan, Jaipur in 2004. He is the subject expert of Sedimentology, Carbonate Petrography, Stratigraphy, Palaeoecology ,Underground Coal Gasification, and Sun Dial Studies.

International Journal of Biological Macromolecules 216 (2022) 179–192

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

A comparative *in silico* study to detect the effect of food-additives on metabolic protein and its perturbations compensated by osmolytes

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ARTICLE INFO

Keywords:
Molecular docking
Dynamics
Food additives
Osmolytes
Metabolic proteins

ABSTRACT

Since its inception, food additive has been an integral part of the food processing industry with various commercial roles. Besides its advantages, various studies have already highlighted its long-term adverse effects on human health. However, in terms of protein structures and functions, the innate mechanism that triggers these effects has not been elucidated in previously reported studies. Our work takes an *in silico* approach to delve into structural implications resulting from these additives with three well studied metabolic proteins-lysozyme, bovine serum albumin (BSA) and ribonuclease A. Three classes of food additives- synthetic color, preservatives, and phosphate-containing, are taken here to understand their effects on the aforementioned metabolic proteins. Conventional molecular docking and dynamics (MD) studies reveal that these additives induce significant structural perturbations. Among them, carmoisine brings about the most secondary structural changes for lysozyme and ribonuclease A, whereas sodium tripolyphosphate affects BSA the most. To restore the secondary structural loss, we further examine the roles of osmolytes through cross-docking and higher timescale MD simulations. These studies unravel that application of osmolytes like raffinose and trehalose triggers structural restoration for BSA, lysozyme and ribonuclease A, and highlight their roles as co-formulants to alleviate the adverse effects of food additives.

1. Introduction

For the last two decades, food additives have been used on daily basis in the form of synthetic colors, taste enhancers, and preservatives. They are the most common in fast and processed food. For the pace of consumer's point of view, to make it more appealing, the concentration of food additives used in the preparation and processing of food is getting beyond the limit of acceptable daily intake (ADI) [1].

Calcium phosphate and aluminium phosphate are used as enhancers, preservatives, acidifying agents, acidity buffers, chemical leavening of baked goods and emulsifying agents of various foodstuffs. The maximum acceptable daily intake (ADI) value of calcium phosphate and aluminium phosphate proposed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is 70 mg/kg BW (body weight) and 7 mg/kg BW/week to 1 mg/kg BW/week, respectively [1]. The protein-rich foods mainly contain organic phosphate esters that slowly break down in the gastrointestinal tract and then resorbed from the intestine. However, the industrially processed foods have contained much higher phosphates than natural foods. Excessive intake of calcium phosphate in foodstuffs leads to muscle and skin atrophy, the progression of chronic renal failure, and cardiovascular calcifications [2]. In the Chronic Renal Insufficiency Cohort Study, it was studied in the USA patients; the renal failure rate was increasing over time with increasing serum phosphate concentration above 1.45 mmol/L and increasing the risk of hyperphosphatemia [2]. On the other hand, excess aluminium phosphate impairs the calcium and phosphorous uptake by the body and leads to osteoporosis, Parkinson's and Alzheimer's disease [3]. Sodium metaphosphate, a high-molecular-weight sodium polyphosphate, is composed of cyclic sodium metaphosphate with rings of alternating phosphorus and oxygen atoms. Furthermore, sodium hexametaphosphate, which is used in foods as a curing agent, dough strengthener, emulsifier and flavour enhancer has been found to be acting as severe irritant in rabbits. Nevertheless, adverse effects of dietary phosphates showed the incidences of hypocalcemia, hyperparathyroidism, and bone

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https://doi.org/10.1016/j.ijbiomac.2022.06.152
Received 6 April 2022; Received in revised form 15 June 2022; Accepted 23 June 2022
Available online 30 June 2022

resorption in male Sprague-Dawley rats, B7D2F1 Bar Harbour mice, and adult dogs as compared to animals of the control groups [4,5].

Another group of food additives is synthetic food colors that are frequently used in the food industry. Sunset yellow (SY) is an azo dye, used as synthetic food color at high concentrations in beverages, chocolates, colored rice, saffron and fruit juice, ice cream, sauces, seasonings, *etc*. The maximum acceptable daily intake (ADI) value of sunset yellow by the JECFA is 0–4 mg/kg BW [6]. Other food colors like carmoisine, acid red, quinoline, *etc.* have been linked with severe liver damage, gastric upsets, diarrhea, vomiting, urticaria, asthma, and anaphylaxis of abnormal immune responses [6–9]. Various studies have also suggested that food colors at near or higher ADI limits establish a favorable interaction with the residues of essential proteins that lead to misfolding and adversely affect biochemical and physiological processes of vital organs, such as the liver and kidney [10,11]. Persistent use of food additives has been contributing to bioaccumulation that is causing deleterious effects on biological macromolecules such as blood proteins (BSA, lysozyme) and other proteins such as RNase-A (ribonuclease-A) found in the kidney and liver [10–12]. Interaction of food additives actually might lead to perturbed structural and functional aspects of proteins resulting in various diseases [12–14]. So far, very limited numbers of studies have been carried out with respect to the effect of food additives on biological proteins. No systematic work has been carried out on the effect of food additives (within or beyond the ADI limit) on the physiologically important model proteins like RNase-A, BSA, and lysozyme.

In this study, lysozyme, BSA, and RNase-A have been selected as these proteins are well studied and have key enzymatic physiological and pharmaceutical functions. Lysozyme, occasionally called as muramidase, is found in both the animal and plant kingdoms [15]. Among mammals, it is abundant in blood, liver, kidney, phagocytes including macrophages, neutrophils and dendritic cells [16]. In the kidney tubules, enzymes like lysozyme are involved in the biotransformation of varied compounds [16,17]. Laterally, the ligands like food additives undergo proteolytic or hydrolytic effects through the endocytosis process and transformed chemicals may be excreted through the urine or it may recede into the renal venous loop [17]. On the other hand, bovine serum albumin (BSA) is the main circulating blood protein. It is also known to be a transporter protein and it binds many essential ligands such as cations, fatty acids, hormones, vitamin D, *etc*. It maintains the oncotic pressure of blood and regulates blood coagulation [18]. Furthermore, RNase-A also known as pancreatic ribonuclease A is an enzyme that catalyzes the maturation of RNA molecules such as messenger RNAs and non-coding RNAs which have varied roles in cellular processes. It acts as the first defense against RNA viruses for host defense mechanism and plays an important role in angiogenesis and digestion processes [19]. Thus, in our *in silico* studies, these proteins have been subjected to the aforementioned classes of food additives, which provide molecular insights into their effects on the structural and functional aspects of lysozyme, BSA, and RNase-A.

Moreover, these additives are more likely to affect the structural and functional properties of the proteins that will impede the crucial functions of essential biological processes [20,21]. Some strategies have been employed to stabilize the proteins against unfavorable binding interactions with the food additives. Uses of osmolytes, organic solvents, and enzyme immobilization on suitable carriers have shown an enhancement in proteins and enzymes' activity as well as stability [22,23]. Osmolytes are normally found in the living organism in higher concentrations to combat various environmental stresses such as temperature, pH, and other osmotic imbalances [22,23]. These osmolytes, also known as compatible osmolytes and are classified into four groups such as polyols, sugars, methylamines, and their derivatives [24,25]. The mechanism of stabilization of osmolytes is driven by the preferential hydration mechanism where osmolytes are excluded from the surface of the proteins and in turn increase the hydration layer of the proteins. As a result, the protein is folded into a more compact tertiary and secondary structure with the formation of more intramolecular hydrogen bonding within the polypeptides chain and in turn, it helps the protein in a more reduced surface area in which the free energy is minimized [22,26,27].

The present study employs the roles of various classes of osmolytes on the highest affinity of food additives on the protein to understand the molecular interactions among food additives, osmolytes and the protein.

2. Materials and methods

2.1. Protein preparation

Three-dimensional (3D) structures of bovine serum albumin (BSA) (PDB ID: 3V03), lysozyme (PDB ID: 1DPX) and ribonuclease A (PDB ID: 1FS3) were retrieved from protein data bank (PDB). The extracted structures had few missing information on connectivity, bond orders and formal charges, as well as lacked hydrogen atoms. Therefore, to prepare the retrieved structures for further computational studies, protein preparation wizard of Schrodinger suite was used, wherein the bond orders were assigned to the protein, along with hydrogen addition, metal treatment and removal of water molecules hetero-groups beyond 5 Å. Hydrogens were then optimized using exhaustive sampling option and the protein was minimized to RMSD limit from the starting structure of 0.3 Å using the Impref module of Impact with OPLS force field (Schrodinger, LLC, New York, 2020).

2.2. SiteMap analysis of the metabolic proteins

Prior to the molecular docking analysis, the generic metabolic proteins were subjected to SiteMap (Sitemap, Schrödinger, LLC, New York, NY, 2020) analysis to identify their putative binding sites. The top ranked binding pocket for each protein was finalized on the basis of SiteMap score, which was calculated using the criteria like volume, ratio between hydrophobic and hydrophilic residues, exposure to solvent, and degree to which ligand might donate or accept hydrogen bonds. Subsequent docking studies were performed by targeting these top-ranked selected pockets with the food additive molecules [28].

2.3. Ligand-directed conventional molecular docking

Binding affinity of the different classes of food additives with metabolic proteins, were assessed using two independent molecular docking studies involving GLIDE and AutoDock Vina [29,30]. Molecular structures of a total of 15 food additives were retrieved from Pubchem [31]. Sodium aluminium phosphate (Pubchem ID: 72941495), dicalcium phosphate (Pubchem ID: 21862903), sodium hexametaphosphate (Pubchem ID: 56846408), disodium phosphate (Pubchem ID: 24203), sodium tripolyphosphate (Pubchem ID: 24455), carmoisine (Pubchem ID: 19118), sunset yellow (Pubchem ID: 17730), tartrazine (Pubchem ID: 164825), quinoline yellow (Pubchem ID: 88640483), acid red (Pubchem ID: 21520), direct blue (Pubchem ID: 131752638), bisphenol A (Pubchem ID: 6623), MSG (Pubchem ID: 23672308), gluconate (Pubchem ID: 10690), and gluconolactone (Pubchem ID: 7027) were used for targeted docking studies.

The pre-docking preparation of the retrieved molecules was carried out using the LigPrep module of Schrodinger suite [32]. The structures were energy minimized, expanded to protonation and tautomer and conformations were generated by the Monte Carlo method as implemented in Macro Model, using OPLS-2005 force field. The generated conformers were subsequently minimized using truncated Newton conjugate gradient (TNCG) minimization up to 500 iterations. The conformers with an energy difference of 30 kcal/mol as compared to the global energy minimum conformer were retained. The conformational searches were carried out for aqueous solution using the generalized born/solvent accessible surface (GB/SA) continuum solvation model, and the resultant structures were accumulated in a library prior to the docking processes.

For both GLIDE and Autodock Vina, the Emodel scoring function was used to select among the best protein-ligand complexes for a given molecule and the score was used to rank-order compounds to separate compounds that bind strongly (actives) from those that don't (inactives). This scoring function primarily took account for the physics of the binding process including a lipophilic-lipophilic term, hydrogen bond terms, a rotatable bond penalty, contributions from protein-ligand coulomb-vdW energies and hydrophobic enclosure terms. The docked complex was accordingly ranked on the basis of the score and the top-ranked were subjected to molecular dynamics (MD) simulation [33].

2.4. Cross-docking studies using osmolytes

Cross-docking analysis was executed for those protein-ligand complexes, which had shown the highest structural perturbations after the MD simulation studies (protocols described in the next section). Using X-GLIDE tool in Schrodinger, osmolytes like trimethylamine oxide (Pubchem ID: 1145), betaine (Pubchem ID: 247), glucose (Pubchem ID: 5793), trehalose (Pubchem ID: 7427), raffinose (Pubchem ID: 439242), D-sorbitol (Pubchem ID: 5780), and proline were forcefully docked into the binding pockets occupied by the food additives in the complexes and then given for MD run to analyse the extent of water-bridge interactions between the additives and protein [34].

2.5. MD simulation analysis of the top-ranked complexes

The best ranked complexes shortlisted from the conventional and cross-docking analyses were given for MD simulation run using Desmond (Desmond, Schrödinger, LLC, New York, NY, 2020) where OPLS4e (Optimized Potentials for Liquid Simulations Version 4e) force field was used to generate topology and parameter files [35,36]. Each complex was surrounded by a cubic box of TIP3P water molecules with the nearest distance from the complex to the box boundary being no more than 10 Å [37]. The generated systems were subsequently neutralized (net charge was brought to zero) by adding adequate number of positive (Na^+) and negative (Cl^-) ions. Each system underwent one round of steepest- descent minimization, followed by one round of conjugated gradient for 5000 picoseconds (ps) [38]. The systems were then equilibrated in NVT (constant number of particles, volume, and temperature) and NPT (constant number of particles, pressure, and temperature) ensembles with two sets of restrained NVT (for 24 ps and 2000 ps respectively) and one set of restrained (for 24 ps) and unrestrained (for 5000 ps) NPT each [39]. During equilibration, LINCS (LINear Constraint Solver) constraint algorithm was used to apply position restraining force on all the atomic bonds present in the systems [40]. The conventional docking systems were subjected to final MD production for 100 ns, where three replica runs for each conventional docking system were executed. On the other hand, the systems for cross-docking complexes were simulated for 1000 nanoseconds (ns) under no-restrained NPT ensemble and repeated twice more for each system. For all the systems,

Table 1
List of autodock and glide scores of different food dyes with BSA, lysozyme and ribonuclease A.

Protein	Autodock									Glide			
	Additives	Binding energy (kcal/mol)	Ligand efficiency	Inhibition constant	Inter-mol. energy (kcal/mol)	Vander Waals energy (kcal/mol)	Electrostatic energy (kcal/mol)	Total energy (kcal/mol)	H-bond forming residues	XP G score	ΔG bind (kcal/mol)	Glide Vander Waals energy (kcal/mol)	XP H-bond
BSA (3V03)	Carmoisine	−8.9	−0.29	301.67 (nM)	−10.39	−8.73	−1.66	−0.02	Lys431	−6.31	2.68	−21.50	−1.58
	Sunset yellow	−8.82	−0.33	345.11 (nM)	−10.31	−8.27	−2.03	−0.01	Arg435, Tyr147, Lys431, Glu424	−5.30	−37.77	−37.65	0
	Tartarizine	−8.0	−0.26	1.38 (μM)	−9.79	−8.22	−1.56	0.7	Tyr147	−4.28	−12.78	−36.28	−0.03
	Quinoline yellow WS	−7.28	−0.35	4.62 (μM)	−7.58	−7.53	−0.04	−0.03	Ser192	−5.41	−27.79	−33.47	−0.01
	Acid red	No interactions with BSA											
	Direct blue	No interactions with BSA											
Lysozyme (1dpx)	Carmoisine	−8.33	−0.27	786.72 (nM)	−9.82	−8.56	−1.26	−0.31	Arg114, Glu35	−3.25	−27.06	−33.98	−1.078
	Sunset yellow	−8.35	−0.31	755.06 (nM)	−9.84	−8.23	−1.62	−0.04	Asn59, Arg114	1.73	−22.04	−32.71	−0.9
	Tartarizine	−6.6	−0.21	14.42 (μM)	−8.39	−6.84	−1.55	0.17	Arg114	−2.57	−21.02	−33.43	−0.952
	Quinoline yellow WS	−6.62	−0.32	14.13 (μM)	−6.91	−7.14	0.23	−0.27	Asp52	−1.46	−18.31	−27.76	−0.54
	Acid red	−7.87	−0.25	1.71 (μM)	−9.66	−8.34	−1.32	−0.12	Arg 114	−2.87	−24.54	−2.41	−0.7
	Direct blue	No interactions with lysozyme											
RNaseA (1fs3)	Carmoisine	−10.39	−0.34	24.2 nM	−11.88	−8.08	−3.8	−0.02	Lys 41, Lys 66	−3.72	−40.71	−27.70	−1.6
	Sunset yellow	−9.75	−0.36	71.18 (nM)	−11.24	−8.24	−3.0	−0.27	Arg39(2) Arg85, Phe120 (2)	−5.91	−32.77	−27.18	−3.275
	Tartrazine	−10.05	−0.32	43.18 (nM)	−11.84	−7.31	−4.52	0.17	Ser123, Lys41, Lys7, Lys66	−3.01	−34.39	−29.97	−2.233
	Quinoline yellow WS	−7.04	−0.34	6.86 (μM)	−7.34	−7.32	−0.03	−0.15	His12, Phe120	−3.48	−13.72	−24.34	−0.402
	Acid red	−9.63	−0.31	86.5 (nM)	−11.43	−9.22	−2.21	0.11	Thr45, Lys7	−3.13	−41.02	−2.07	−1.6
	Direct blue	No interactions with RNaseA											

the final temperature was kept at 300 K. Post-simulation all the MD simulation data analysis were carried using MD simulation analysis tools available in Desmond and Maestro platform (Desmond and Maestro, Schrödinger, LLC, New York, NY, 2020).

3. Results

3.1. Docking analysis of food dyes and additive molecules with generic proteins

BSA, lysozyme and ribonuclease A were subjected to docking using autodock and glide in presence of three groups of molecules that include food dyes, phosphate containing additives, and other food additives. Among the group of food dyes, carmoisine generated the highest docking scores with all the three proteins (Table 1 and Fig. 1A). Ligand docking of carmoisine with BSA, lysozyme, and ribonuclease A generated top scores of −8.9 kcal/mol, −8.33 kcal/mol, and −10.39 kcal/mol, respectively (Table 1). In case of phosphate-containing additives, sodium-tripolyphosphate showed the best docking scores with BSA (−5.6 kcal/mol), lysozyme (−5.48 kcal/mol) and ribonuclease A (−8.63 kcal/mol) (Table 2 and Fig. 1B). Among the other additives, bisphenol generated highest scores with BSA (−6.21 kcal/mol), lysozyme (−6.56 kcal/mol), and ribonuclease A (−5.14 kcal/mol) proteins (Table 3 and Fig. 1C). The high glide score indicated a high binding affinity towards the target. We checked for the following interactions, hydrogen bonds, salt bridges, halogen bonds, aromatic bonds, pi-cation, and also pi-pi interactions all of which contribute towards the stability of the protein-ligand complexes. These compounds interacted with the target proteins by forming hydrogen bonds, and hydrophobic interactions with the active site residues shown in Figs. S1, S2 and S3.

3.2. Effect of carmoisine on BSA, lysozyme and ribonuclease A

The top ranked carmoisine-BSA complex was then given for molecular dynamics (MD) simulation studies using Desmond (Schrodinger 2020, NY, LLC) for 100 ns, where the interaction fractions were generated to identify the critical amino acids binding with the carmoisine molecule. Interaction fraction analysis revealed that apart from forming intermolecular H-bonds with adjacent water molecules (eight H_2O molecules), carmoisine also forms intramolecular H-bonds with Arg208, Lys211 and Lys350 (Fig. S4). Moreover, it also forms water-mediated interactions with Phe205, Ala209, Thr235 and Glu353 residues as well as hydrophobic interactions with Ala212, Val215, Val234, Lys322, Leu346 and Ala349 residues (Fig. S4). The effect of these interactions was further analysed using RMSD study that showed slight deviation of 0.3 Å for the bound complex (average RMSD: 3.4 Å) when compared with the unbound (average RMSD: 3.1 Å) one (Figs. 2A, and S5–S8). Secondary structural analysis did not show any significant loss or gain of structure, however at amino acid number 500, there has been a slight gain of the alpha helical structure for the carmoisine complex (Fig. 2B).

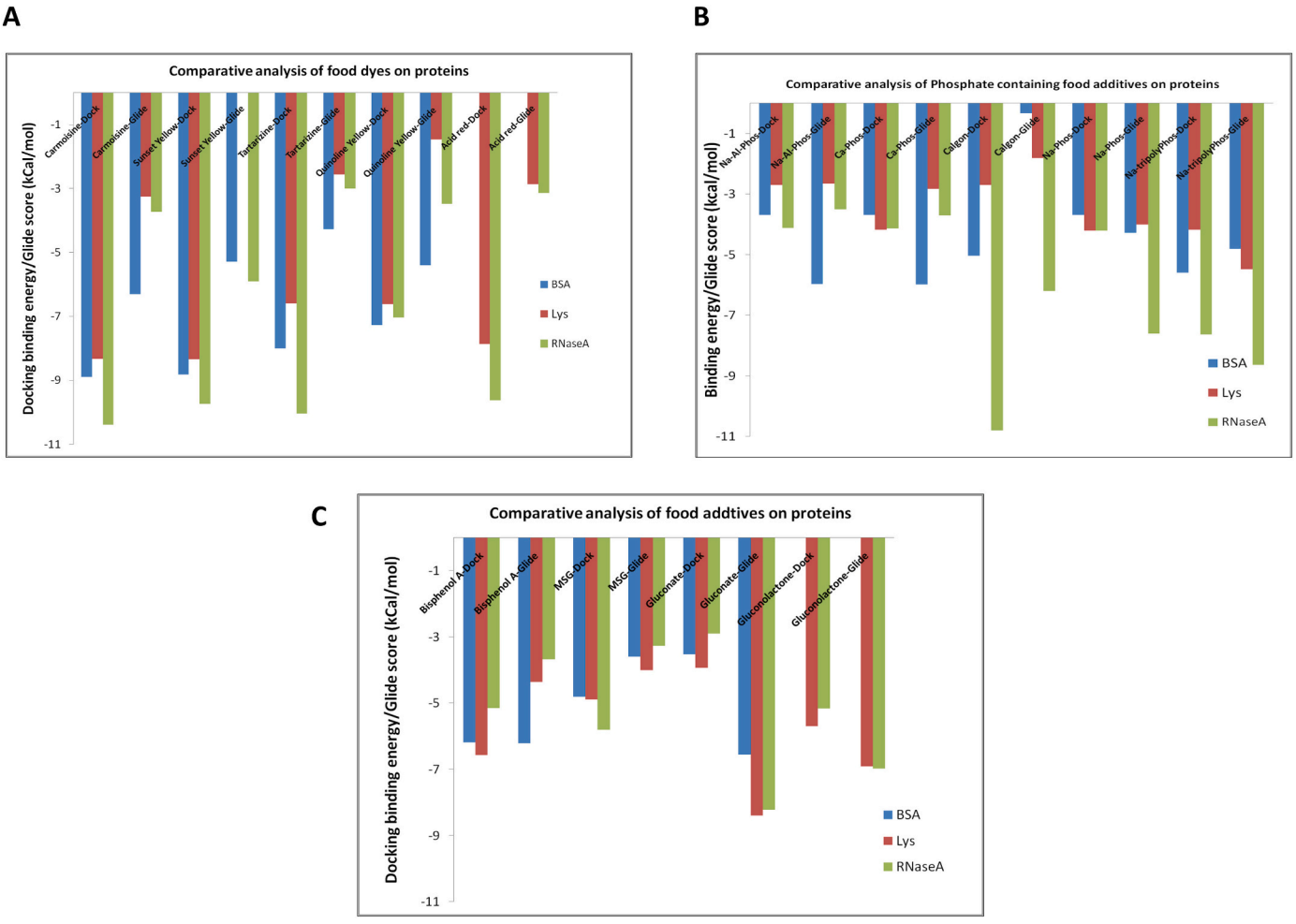

Fig. 1. Comparative analysis of molecular docking scores of the food additives with BSA, lysozyme and ribonuclease A proteins. Glide and AutoDock scores of A) food dyes B) phosphate-containing C) other preservatives are represented through bar graphs where, blue, maroon and green indicates BSA, lysozyme and ribonuclease A, respectively. Each triplet group of bars shows the scores (dock represents AutoDock scores and Glide represents glide scores) of individual additive bound to all the three proteins.

Table 2
List of autodock and glide scores of different phosphate-containing additives with BSA, lysozyme and ribonuclease A.

Protein	Autodock									Glide			
	Additives	Binding energy (kcal/mol)	Ligand efficiency	Inhibition constant	Inter-mol. energy (kcal/mol)	Van der Waals energy (kcal/mol)	Electrostatic energy (kcal/mol)	Total energy (kcal/mol)	H-bond forming residues	XP G score	ΔG bind (kcal/mol)	Glide Van der Waals energy (kcal/mol)	XP H-bond
BSA (3V03)	Sodium aluminium phosphate	−3.69	−0.74	1.97 (mM)	−4.29	−2.94	−1.35	0.06	Ser191, Arg198	−5.97	4.62	−3.48	−3.66
	Dicalcium phosphate	−3.69	−0.74	1.98 (mM)	−4.28	−2.95	−1.33	0.06	Ser191, Arg198	−5.98	4.64	−3.47	−3.67
	Sodium hexametaphosphate (calgon)	−5.04	−0.21	201.52 (μM)	−5.04	−3.11	−1.93	0.0	Lys294, Val342, Arg217	−0.32	32.8	−20.35	−1.39
	Disodium phosphate	−3.69	−0.74	1.99 (mM)	−4.28	−2.98	−1.31	0.06	Ser191, Arg198, Arg194	−4.27	4.61	−7.84	−2.92
	Sodium tripolyphosphate	−5.6	−0.43	78.26 (μM)	−7.39	−4.15	−3.24	2.36	Glu291, Trp213, Arg198, Asp450, Arg217	−4.81	8.66	−9.24	−3.31
Lysozyme (1dpx)	Sodium aluminium phosphate	−2.96	−0.59	6.78 (mM)	−3.56	−2.17	−1.39	0.09	Ile88, Ser86	−2.66	−1.82	−3.90	−0.83
	Dicalcium phosphate	−4.17	−0.83	878.36 (μM)	−4.77	−3.64	−1.12	0.09	Gly102, Gly104, Arg21, Val99	−2.82	−0.83	−2.70	−0.83
	Sodium hexametaphosphate (calgon)	−2.7	−0.11	10.42 (mM)	−2.7	−2.06	−0.65	0.0	Trp62	−1.83	−9.78	−5.76	−0.73
	Disodium phosphate	−4.17	−0.83	878.44 (μM)	−4.77	−3.66	−1.11	0.09	Gly104, Arg21, Gly102, Val99	−3.96	2.74	−5.80	−1.07
	Sodium tripolyphosphate	−4.17	−0.32	873.98 (μM)	−5.96	−2.9	−3.06	2.23	Cys76, Asn93	−5.48	−13.26	−3.78	−2.25
RNase A (1fs3)	Sodium aluminium phosphate	−4.12	−0.82	962.03 (μM)	−4.71	−3.15	−1.15	0.05	Phe120, His12, His119	−3.50	−7.93	−1.76	−1.02
	Dicalcium phosphate	−4.13	−0.83	942.01 (μM)	−4.72	−3.14	−1.59	0.06	His12, His119, Phe120	−3.72	−7.92	−1.39	−1.19
	Sodium hexametaphosphate (calgon)	−10.83	−0.45	11.48 (nM)	−10.83	−3.26	−7.57	0.0	Lys7, Arg39, Gln11, His12	−6.23	−27.3	−8.91	−2.53
	Disodium phosphate	−4.17	−0.83	870.43 (μM)	−4.77	−3.41	−1.36	0.1	Arg39, Asp38	−7.56	−14.9	−1.61	−3.09
	Sodium tripolyphosphate	−7.63	−0.59	2.55 (μM)	−9.42	−4.48	−4.94	1.68	Lys41, His119, Gln11, His12, Arg39	−8.63	−9.73	−2.21	−2.35

Table 3
List of autodock and glide scores of food additives with BSA, Lysozyme and Ribonuclease A.

Protein	Autodock									Glide score			
	Additives	Binding energy (kcal/mol)	Ligand efficiency	Inhibition const	Inter-mol. energy (kcal/mol)	Van der Waals energy (kcal/mol)	Electrostatic energy (kcal/mol)	Total energy (kcal/mol)	H-bond forming residues	XP G score	ΔG bind (kcal/mol)	Glide Van der Waals energy (kcal/mol)	XP H-bond
BSA (3V03)	Bisphenol A	−6.18	−0.36	29.4 (μM)	−7.08	−6.99	−0.99	–0.47	Glu424	−6.21	−33.49	−28.69	−0.64
	MSG	−4.8	−0.48	304.3 (μM)	−6.29	−3.43	−2.86	−1.86	Trp213, Arg217, Arg198	−3.59	−16.09	−8.12	−1.94
	Gluconate	−3.52	−0.27	2.62 (mM)	−6.5	−5.17	−1.34	−2.76	Arg435, Lys431, Ser428	−6.55	−18.03	−11.31	−4.55
	Gluconolactone	No interaction with BSA											
Lysozyme (1dpx)	Bisphenol A	−6.56	−0.39	15.6 (μM)	−7.45	−7.34	−0.11	−0.18	Asn59, Asn103, Ile98	−4.35	−28.24	−22.05	−1.33
	MSG	−4.88	−0.49	262.97 (μM)	−6.38	−4.64	−1.74	−1.67	Asn59, Val109, Asp52, Glu35, Gln57	−3.95	−11.20	−12.47	−1.54
	Gluconate	−3.93	−0.3	1.31 (mM)	−6.92	−6.25	−0.67	−2.24	Trp63, Val109, Gln57, Glu35, Ala107	−8.39	−14.76	−9.91	−5.00
	Gluconolactone	−5.69	−0.47	67.91 (μM)	−5.69	−5.67	−0.02	0.0	Asn59	−6.91	−35.49	−1.35	−3.33
RNaseA (1fs3)	Bisphenol A	−5.14	−0.3	170.43 (μM)	−6.04	−5.7	−0.34	−0.43	Phe120	−3.68	−33.56	−21.28	−1.24
	MSG	−5.8	−0.58	56.19 (μM)	−7.29	−3.67	−3.62	−1.63	Lys7, Lys41, Gln11	−3.26	−12.37	−8.92	−0.62
	Gluconate	−2.89	−0.22	7.66 (mM)	−5.87	−4.26	−1.61	−4.11	Val118, Lys41, Lys7, Phe120	−8.21	−19.16	−11.46	−6.24
	Gluconolactone	−5.16	−0.43	165.87 (μM)	−5.69	−4.92	−0.23	0.0	Lys7, His119	−6.98	−35.28	−0.89	−3.88

Similar docking and MD simulation studies with lysozyme showed that carmoisine molecule forms intermolecular H-bond interactions with a number of water molecules (twelve H_2O molecules) and predominantly interacts with Arg112 (water-bridge) and Lys116 (H-bonds) (Fig. S9). Additionally, it forms H-bond interactions with Tyr23, Gly102, Asn103, Asn106, Asn113 and Arg114 (Fig. S9). Formation of these interactions resulted a significant RMSD deviation of the carmoisine-bound complex where the average RMSD of the bound complex (average RMSD: 2.5 Å) increased by 1.1 Å as compared to the unbound (average RMSD: 1.4 Å) one (Figs. 2C, and S10–S13). Secondary structural analysis also showed significant loss of alpha helical characteristics in the lysozyme-carmoisine complex after 120th residue of the lysozyme (Fig. 2D).

In case of ribonuclease A, the ligand fraction analysis identified Val43, Thr45, Lys66, Asp83, Cys84 and Phe120 residues as important ones that form various H-bonds, water-bridges and hydrophobic interactions (Fig. S14). Though the last frame of the ligand interaction showed strong H-bond interactions with Lys41, cumulative fractional studies over 100 nanoseconds (ns), showed aforementioned residues to be playing more important role in forming strong interaction between carmoisine and ribonuclease A (Fig. S14). Apart from that, the final frame also showed that carmoisine forms intermolecular H-bonds with eleven water molecules (Fig. S14). Although, RMSD analysis did not show any significant deviation of the bound complex from the unbound ribonuclease A (0.08 Å) (Figs. S15–S18), the secondary structural analysis revealed loss of beta-strand characteristics in the carmoisine bound complex (Figs. 2E and F). Total Secondary Structure Estimation (SSE) percentage reduced from 44.4 % to 43.38 % where the significant beta-strand loss was observed at 100th amino acid residue (Fig. 2F).

3.3. Effect of sodium tripolyphosphate on BSA, lysozyme and ribonuclease A

For understanding the extent of structural interference exhibited by the phosphate-containing additives, sodium tripolyphosphate bound BSA, lysozyme and ribonuclease A complexes were subjected to MD simulation analysis for 100 ns. Interaction analysis with BSA suggested that tripolyphosphate forms H-bonds with eighteen water molecules in its vicinity during the MD run (Fig. S19). Moreover, majority of these water molecules are also responsible for formation of critical water-mediated salt bridges with BSA residues like Arg198, Trp213, Arg217, Lys221, His241, Arg256, Ser286, Ala290, Glu291, and Tyr451. Besides forming water-bridges it forms H-bonds predominantly with Arg194, Arg198, Arg217, and Lys221 (Fig. S19). Though, a large number of intermolecular interactions was observed between BSA, and tripolyphosphate, overall RMSD mapping with the unbound BSA trajectories did not confer any significant deviation in the bound complex (Figs. 3A, and S20–S21). However, a reduction in the secondary structure characteristic was observed in BSA where the overall SSE percentage reduced by 0.49 % due to the significant loss of alpha-helical characteristics between 265 and 275 residues (Fig. 3B).

In case of lysozyme, the tripolyphosphate molecule interacts with 24 water molecules where it forms major water-bridges with Arg112, and Lys116 (Fig. S22). The same residues are also involved in the formation

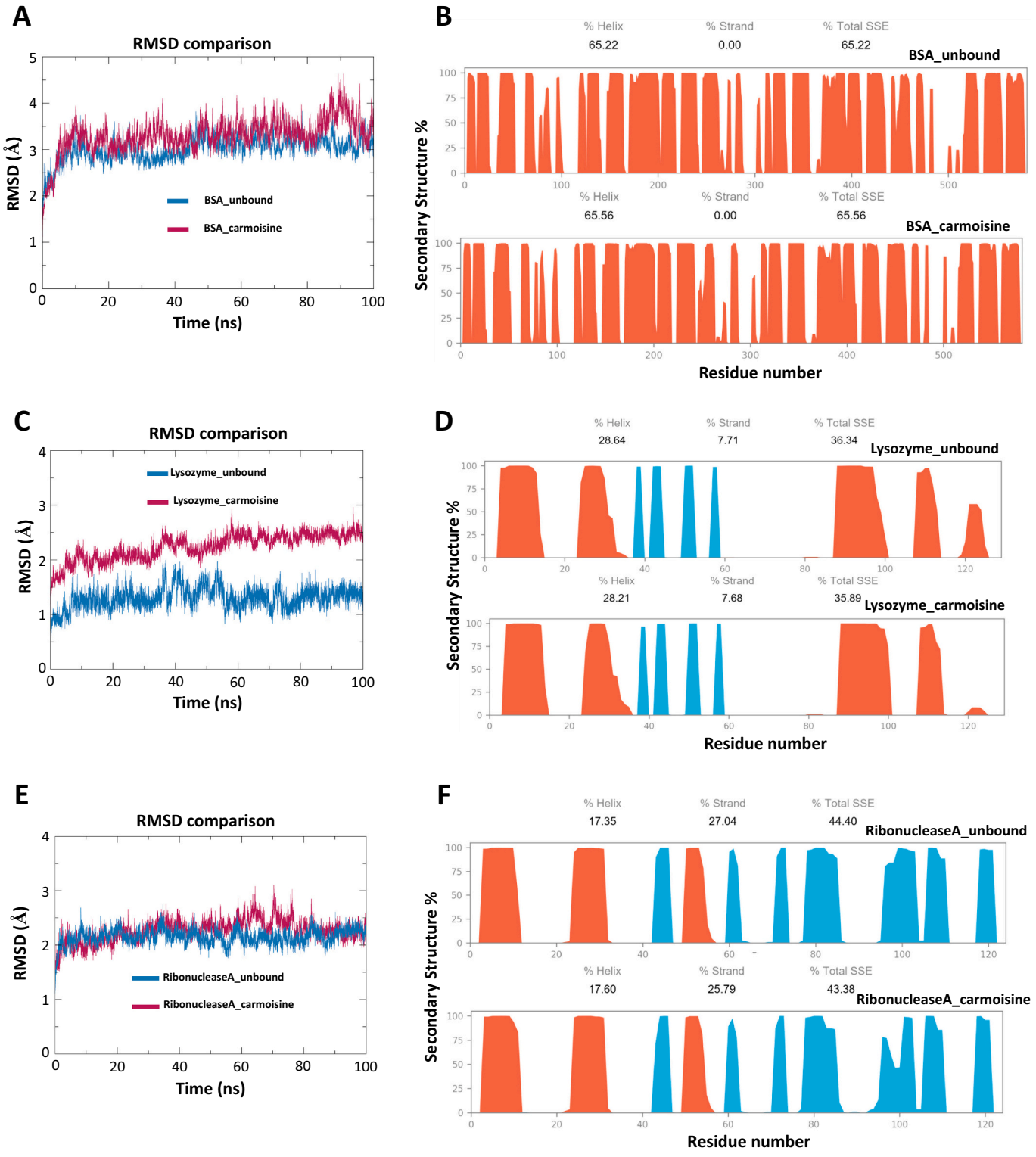

Fig. 2. Effect of carmoisine on BSA, lysozyme and ribonuclease A secondary structures. Comparative root mean squared deviation (RMSD) and secondary structural elements plots are represented for unbound and carmoisine-bound BSA (A and B), lysozyme (C and D) and ribonuclease A (E and F). In RMSD plots (unbound - blue and bound - red) X-axis denotes MD simulation time in nanoseconds and Y-axis denotes RMSD values in Å. In secondary structure elements plot, X-axis denotes residue numbers and Y-axis denotes secondary structure percentage, where the helices and stands are represented in orange and blue, respectively.

of direct H-bonds with the ligand molecule (Fig. S22). These interactions brought significant RMSD disparity in the bound complex in comparison to the unbound lysozyme that revealed almost two-fold deviation in the MD trajectories. The average RMSD of the unbound lysozyme was 1.4 Å, which rose to 2.6 Å in case of the tripolyphosphate-bound lysozyme (Figs. 3C, and S23–S24). In terms of total SSE percentage, the huge RMSD deviation did not result significant secondary characteristics loss, however, loss of alpha-helical characteristics after 120th residue was evident that was also observed in case of carmoisine binding discussed earlier (Fig. 3D).

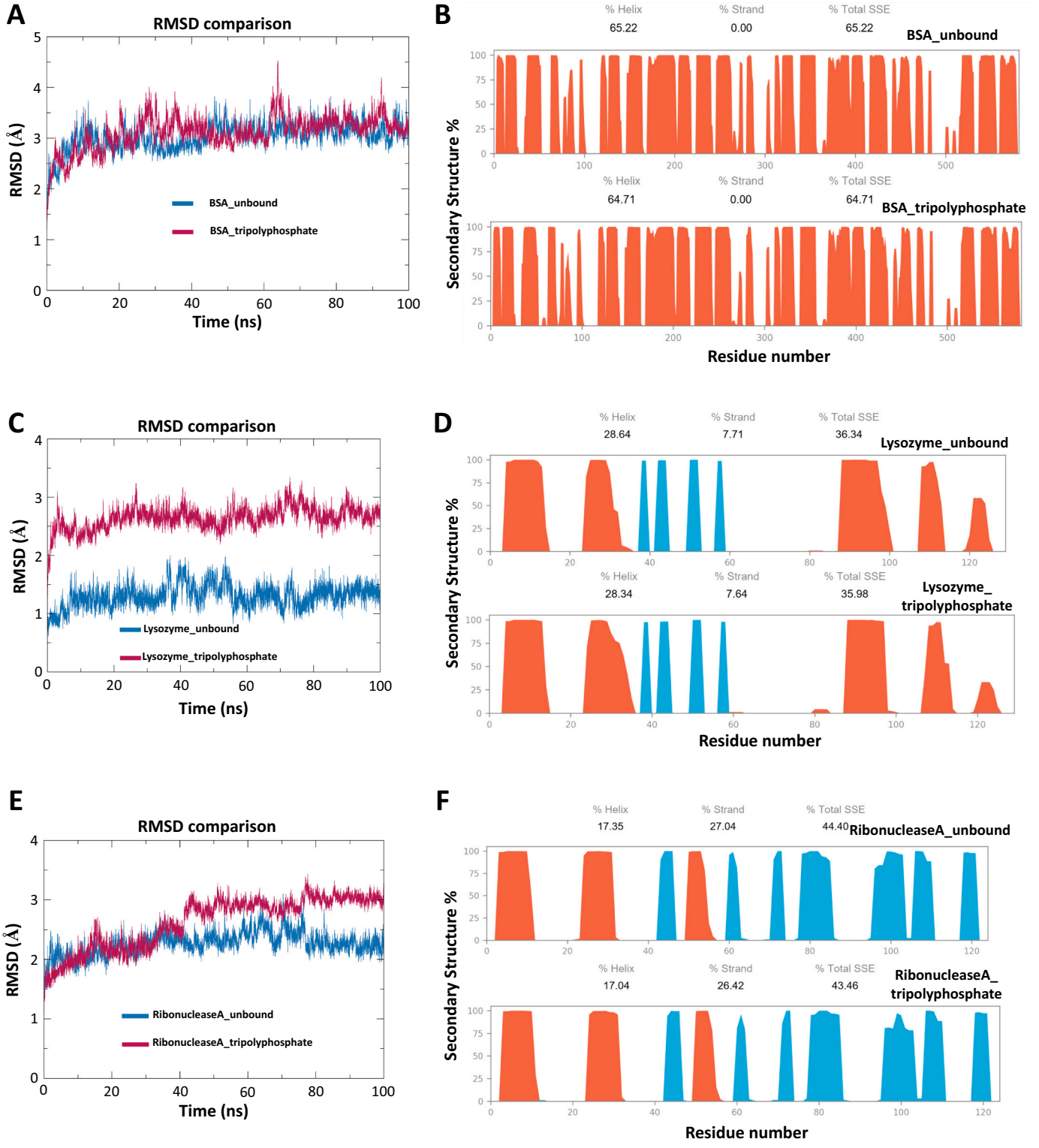

Fig. 3. Effect of sodium tripolyphosphate on BSA, lysozyme and ribonuclease A secondary structures. Comparative root mean squared deviation (RMSD) and secondary structural elements plots are represented for unbound and sodium tripolyphosphate-bound BSA (A and B), lysozyme (C and D) and ribonuclease A (E and F). In RMSD plots (unbound - blue and bound - red) X-axis denotes MD simulation time in nanoseconds and Y-axis denotes RMSD values in Å. In secondary structure elements plot, X-axis denotes residue numbers and Y-axis denotes secondary structure percentage, where the helices and stands are represented in orange and blue, respectively.

Interaction analysis of the tripolyphosphate ligand with ribonuclease A showed intermolecular H-bond formation with sixteen H_2O molecules that facilitated water-bridges with Arg10, Gln11, and Leu35 (Fig. S25). Moreover, direct intramolecular H-bond contacts were also observed with Lys1, Lys7, Arg39, and Lys41 (Fig. S25). Comparative RMSD analysis with the unbound ribonuclease A showed an RMSD deviation of 0.7 Å, where the bound and unbound forms clocked average RMSDs of 2.9 Å, and 2.2 Å, respectively (Figs. 3E, and S26–S27). The total SSE

analysis further demonstrated secondary structure loss in the bound form where the SSE percentage reduced from 44.40 % to 43.46 % (Fig. 3F). Unsurprisingly, similar SSE percentage reduction was also seen in case of carmoisine-bound ribonuclease A.

3.4. Effect of bisphenol on BSA, lysozyme and ribonuclease A

Among the rest of the food additives, bisphenol generated the best docking scores, hence, the bisphenol bound complexes were given for

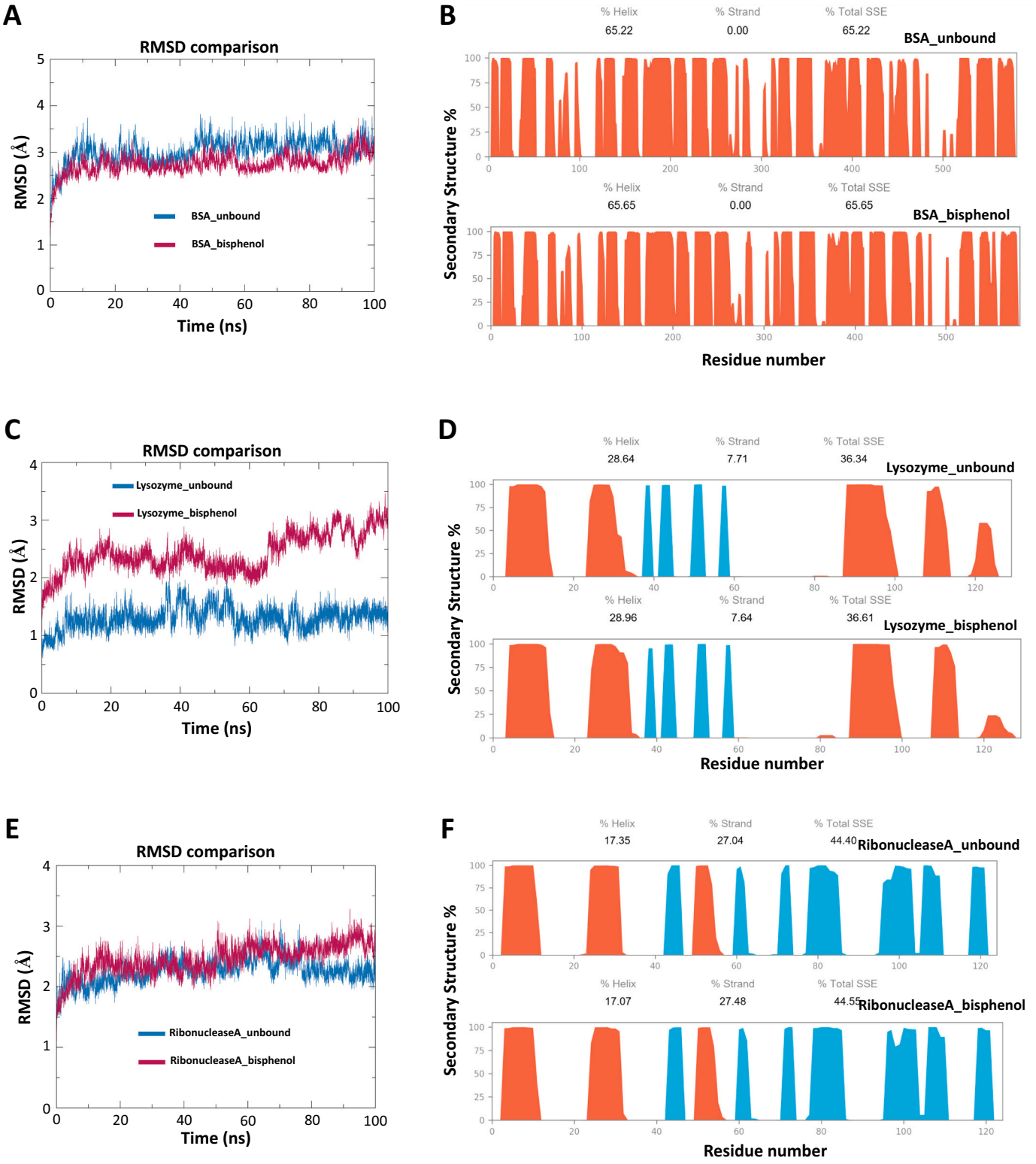

Fig. 4. Effect of bisphenol A on BSA, lysozyme and ribonuclease A secondary structures. Comparative root mean squared deviation (RMSD) and secondary structural elements plots are represented for unbound and bisphenol A-bound BSA (A and B), lysozyme (C and D) and ribonuclease A (E and F). In RMSD plots (unbound - blue and bound - red) X-axis denotes MD simulation time in nanoseconds and Y-axis denotes RMSD values in Å. In secondary structure elements plot, X-axis denotes residue numbers and Y-axis denotes secondary structure percentage, where the helices and stands are represented in orange and blue, respectively.

MD analysis. Interaction studies with BSA showed that bisphenol binds with two neighbouring water molecules, resulting water-bridge formation with Glu424 (Fig. S28). Moreover, it also formed strong H-bond with Glu186, and a moderately strong bond with Glu424. Apart from that, it showed several weak hydrophobic interactions Leu189, Ala193, Val432, Tyr451, Leu454, and Ile455 (Fig. S28). Comparative RMSD analysis, and SSE percentage study between unbound BSA and bisphenol-bound BSA did not result to any significant deviation or loss of secondary structures (Figs. 4A–B, and S29–S30).

Bisphenol binding with lysozyme showed involvement of two water molecules in formation of water-bridges with Arg73 and Asp101 (Fig. S31). A number of weak H-bond interactions were also observed with Trp63, Arg73, Leu75, Lys97, Ser100, Asp101, Gly102, and Asn103. In addition to that few hydrophobic interactions were also observed with Arg61, Trp62, and Leu75 (Fig. S31). These interactions might have resulted RMSD deviation for the bound complex (average RMSD 2.4 Å) when compared to the unbound lysozyme (average RMSD 1.4 Å) (Figs. 4C, and S32–S33). However, this RMSD deviation was not reflected in the SSE analysis unlike the carmoisine-bound or tripolyphosphate-bound lysozyme (Fig. 4D).

Similarly, in case of ribonuclease A, bisphenol binding did not bring about any significant RMSD disparity or SSE percentage changes (Figs. 4E, F, S34–S35). However, intermolecular H-bond formation with water molecules rose to five molecules from two as observed in the other two proteins. Furthermore, one strong H-bond contact with residue Thr45 was also seen along with several hydrophobic interaction formation with Val43, Lys66, Arg85, and Ala122 (Fig. S36).

3.5. Osmolytes restoring the secondary structures of the metabolic proteins

In presence of carmoisine, loss of secondary structures was evident in lysozyme, and ribonuclease A. On the other hand, BSA showed the highest structural perturbations in presence of sodium tripolyphosphate as evident from the aforementioned MD analyses. A number of studies had previously established the potential roles of osmolytes in stabilizing secondary structural elements in various proteins. At first, carmoisine bound lysozyme, and ribonuclease A complexes were subjected to cross-docking in presence of protective or compatible osmolytes [41] like sorbitol (polyol), glucose, trehalose, raffinose (sugars), proline (amino acid), betaine, and trimethylamine oxide (TMAO) (amino acid derivative). Among these osmolytes, raffinose generated the highest docking score of −9.505 kcal/mol, followed by trehalose (−8.505 kcal/mol), betaine (−7.23 kcal/mol), sorbitol (−6.5 kcal/mol), glucose (−6.447 kcal/mol), TMAO (−6.12 kcal/mol), and proline (−5.423 kcal/mol) when bound to lysozyme. In case of ribonuclease A, raffinose exhibited the highest docking score of 9.049 kcal/mol, followed by trehalose (−7.892 kcal/mol), sorbitol (−6.032 kcal/mol), betaine (−5.222 kcal/mol), glucose (−4.849 kcal/mol), proline (−4.273 kcal/mol), and TMAO (−2.019 kcal/mol). Since tripolyphosphate binding with BSA resulted higher structural anomaly in comparison to carmoisine, and bisphenol A, BSA-tripolyphosphate complex was taken into consideration for cross-docking with the osmolytes. Cross-docking analyses revealed that raffinose demonstrated highest binding affinity of −10.243 kcal/mol, whereas trehalose (−7.919 kcal/mol), sorbitol (−5.362 kcal/mol), glucose (−5.038 kcal/mol), betaine (−3.924 kcal/mol), proline (−3.362 kcal/mol), and TMAO (−3.199 kcal/mol) followed. For all the three metabolic proteins, raffinose, and trehalose showed the best docking scores, hence the bound complexes comprising these two osmolytes were chosen for further MD simulation of 1000 ns. The generated results from the MD simulation were analysed to dissect the potential roles of the osmolytes- raffinose, and trehalose in restoring the secondary structural integrity of the metabolic proteins, which were lost due to the presence of the additives. Secondary structural analysis showed increase in the total SSE percentage in lysozyme from 35.89 % (carmoisine bound) to 38.26 %, and 37.15 % in presence of raffinose, and trehalose, respectively (Fig. 5A). Similarly, ribonuclease A also indicated elevated SSE% from 43.38 (carmoisine bound) to 45.26, and 44.41, when bound to raffinose, and trehalose, respectively (Fig. 5B). In case of BSA, the trend was similar as it experienced secondary structural restoration in presence of raffinose and trehalose (SSE% increasing to 66.28, and 65.88, respectively) in comparison to sodium tripolyphosphate bound one (SSE% of 64.71) (Fig. 5C).

Furthermore, in lysozyme, where residues 112–116 (away from the active site) were forming interactions with carmoisine (as shown in Fig. S5) did not show similar interactions in presence of osmolytes like trehalose. When bound to trehalose, one of the lysozyme's active site residues, namely Asp52 [42] showed the highest percentage of interactions and also found to be conserved across species (Figs. S37–S38). Similarly in case of ribonuclease A, the active site residues (His12 His119 and Lys41) [43] were not involved in forming interactions (refer to Fig. S6) with carmoisine, whereas in bound form with raffinose (one of the osmolytes), the interaction percentages for His119 and Lys41 increased considerably (Figs. S39–S40). On the other hand, BSA, which contains two binding sites – Site I (Ala209, Trp213, and Asp450) [44] and Site II (Asn390) [45], did not observe any of its binding site residues to form interaction when bound to sodium tripolyphosphate (as observed in Fig. S7). However, raffinose exhibited interactions with the site I residues, where Asp450 had very high percentage of interactions (Figs. S41–S42).

To further understand the role of these osmolytes in providing structural stability to the metabolic proteins, radial distribution function (RDF) analyses of the osmolyte molecules were performed using 1000 ns MD simulation trajectories. Osmolytes are generally known for aggregating solvent molecules around the protein's structures, inducing improved surrounding solution and effectuating protein folding and structural re-integration [41,46]. In this case, RDF analyses were carried out to calculate probability or number of water molecules at the vicinity of the particular osmolyte or additive. The sodium tripolyphosphate bound BSA, which had maximum solvent molecules probability of 0.48 at 12 Å distance from the binding pocket, showed an increase in probability of 0.55 and 0.73 when bound to trehalose and raffinose (Fig. 6A). Along with probability, absolute number of solvent molecules also increased around the ligand binding pocket of BSA (249 ± 3 in case of tripolyphosphate binding) amounting to 324 ± 4 and 489 ± 2 in presence of raffinose and trehalose, respectively (Figs. 6B and S43). Lysozyme structure also exhibited a similar aggregation of solvents in presence of raffinose and trehalose as the absolute water molecule numbers and probability reached as high as 580 ± 2 and 0.9 (in case of raffinose), while with carmoisine the number and probability were 253 ± 7 and 0.45 respectively (Fig. 6C, D and S44). In addition, raffinose and trehalose binding with ribonuclease A, further demonstrated accumulation of solvent molecules (540 ± 2 and 395 ± 4), in respect to the carmoisine bound complex (344 ± 2 solvent molecules); nonetheless the probability also increased considerably in presence of osmolytes (Fig. 6E, F and S45). Thus, secondary structure and RDF analyses further indicates that osmolytes, especially higher-order sugars like raffinose and trehalose can potentially stabilize metabolic proteins' structures and subsequently restore their functioning if used as co-formulant with existing food additives.

4. Discussion

At native folded state, proteins tend to maintain their structural integrity and in turn physiological functioning. However, structural denaturation effect rendered by external molecules such as food additives, dyes and preservatives might results in structural perturbation of these proteins [47–49]. With prolonged exposure, the accumulated protein dysfunction can hamper crucial cellular networks and pathways leading to serious health problems and diseases [47–49]. Although, a number of studies have previously delineated the adverse effects of these synthetic compounds on human health, structural elaboration of how

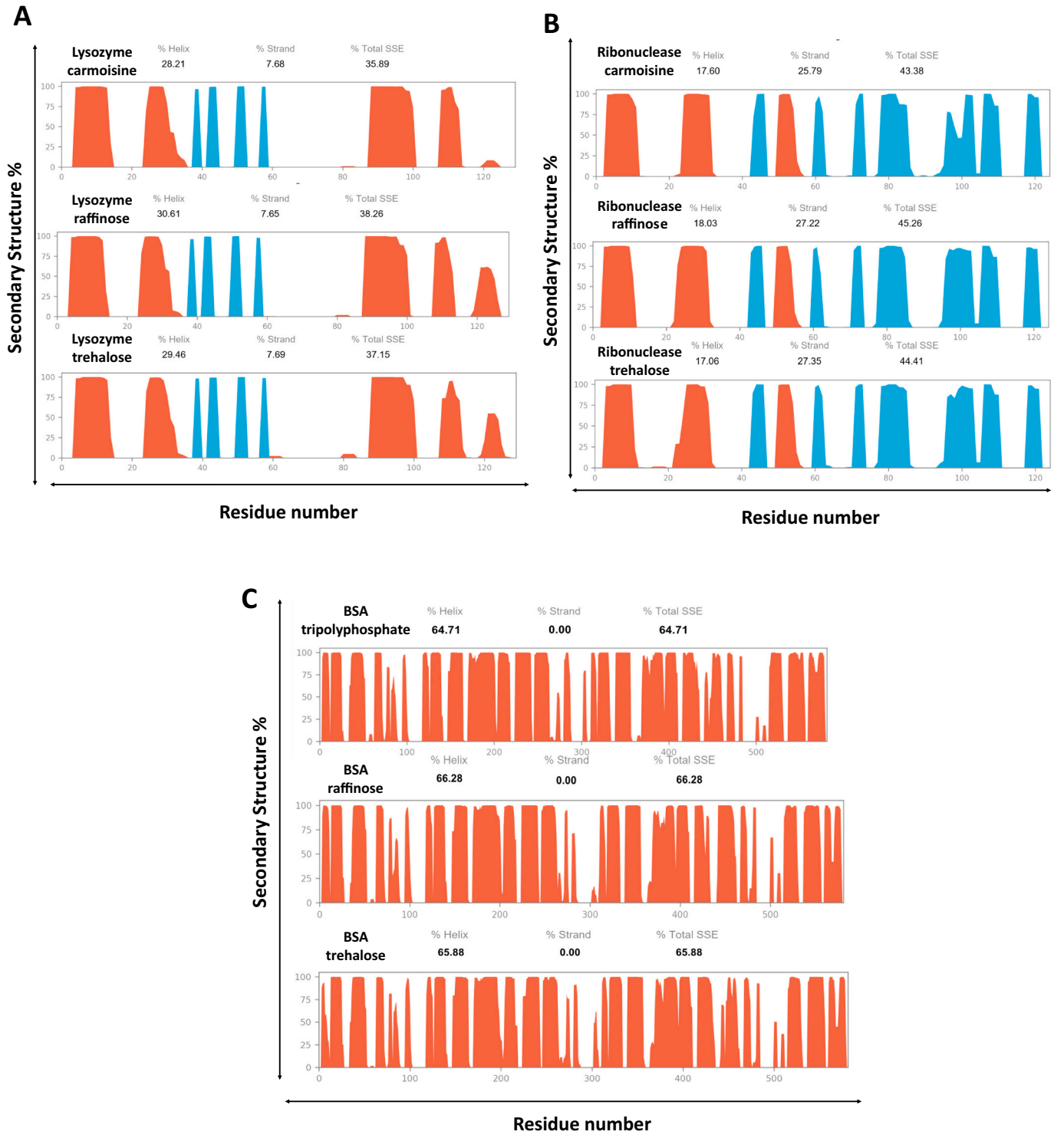

Fig. 5. Secondary structural restoration analysis of BSA, lysozyme and ribonuclease A in presence raffinose and trehalose. Secondary structure percentage (Y-axis) is plotted against residue numbers (X-axis) for A) lysozyme, B) ribonuclease A and C) BSA showing the helices in orange and strands in blue.

these molecules affect protein stability has still remained elusive. To understand the structural implications of these compounds, our study first segregated them into three categories: food dyes, phosphate containing additives and rest of the additives. Extensive series of comparative docking studies with three well studied proteins, *viz.*, BSA, lysozyme, and ribonuclease A, recognised three additives, namely, carmoisine, bisphenol and sodium tripolyphosphate, which showed the highest affinities with all the proteins. Further MD simulation analysis of these compound-bound complexes unraveled that these additives concurred significant structural perturbations in BSA, lysozyme and ribonuclease A. More importantly, the effect of carmoisine on the structure of lysozyme was found to be the highest in terms of loss of secondary structure in lysozyme, followed by ribonuclease A. On the other hand, although, carmoisine did not trigger much structural abnormality, it was sodium tripolyphosphate that demonstrated significant structural perturbations in BSA. To compensate this secondary structure

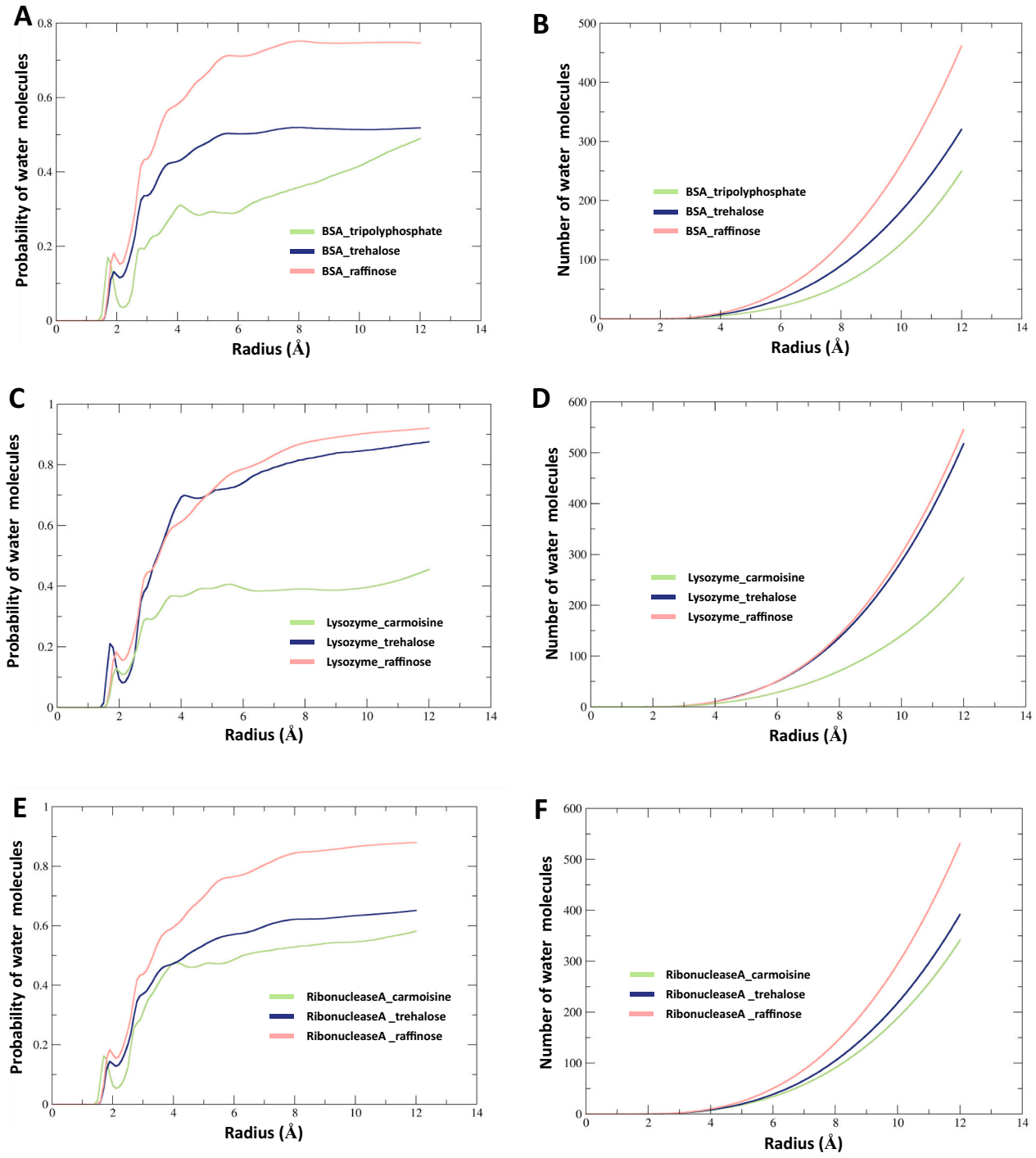

Fig. 6. Radial distribution function (RDF) analysis of the solvent molecules. Graphical representation of the RDF analysis for BSA (A and B), lysozyme (C and D) and ribonuclease A (E and F) in presence of raffinose (light pink), trehalose (deep blue) and sodium tripolyphosphate/carmoisine (light green). The X-axis denotes radius in Å to calculate the distance between the solvent molecules and the ligand (whether carmoisine, sodium tripolyphosphate, trehalose or raffinose), whereas the Y-axis denotes probability of solvent molecules near the ligands in A, C and E; as well as number of solvent molecules near the ligands in B, D and F.

loss, our next course of action was to introduce compatible or protective osmolytes to the additives-bound metabolic protein complexes. Compatible osmolytes are a diverse class of small solute accumulated by organisms to protect the cell macromolecules under different stress conditions [22,27]. Osmolytes like sorbitol, betaine, raffinose, glucose, trehalose, proline and trimethylamine oxide (TMAO) were selected from the four classes of osmolytes, namely polyols, sugars, amino acids and their derivatives [50]. Higher timescale MD simulation of the additives-bound metabolic protein complexes in presence of raffinose and trehalose (two of the osmolytes used in our study) further demonstrated recovery of the secondary structural characteristics for these proteins. Subsequently, the observations from the RDF analyses also confirmed that among the class of sugars, raffinose (trisaccharide) demonstrated highest impact on the refolding of the secondary elements for lysozyme, followed by trehalose (disaccharide) and glucose (monosaccharide). These results corroborate well with previous reports, which demonstrated that higher oligosaccharides with respect to sizes, follow the similar stabilizing effects on the proteins like RNase-A, Cytochrome C

and many more [51,52].

On the other hand, the accumulation of water molecules, as shown in the RDF analysis further substantiate previous experimental reports [53,54], which hypothesized that stabilizing osmolytes with respect to their increasing sizes are more excluded, and increase the hydration layer near the protein domain conducive to proper folding of the protein. Apart from the oligosaccharides, betaine and TMAO from amino acid derivatives class, sorbitol from polyols class and proline from amino acid class reflect different degree of exclusion from the protein domain, resulting in the differential solvation behavior with the peptide backbone that might induce effect on the stability of the protein, similar to the oligosaccharides [41]. In the case of applying these osmolytes in practice, cues can be taken from number of the reports about plant protection products (PPP), which utilizes an approach called co-formulants to increase the bioavailability of active substances/proteins in the plant body [55,56]. Through cross-docking analysis of these osmolytes with food additives such as carmoisine and sodium tripolyphosphate, our study further shed light on possibilities of using them as co-formulants for enhancing protein structure stability and reducing the adverse effect of the food-additives.

Although, the future aspect of this work will be exhaustive *in vitro* biophysical and biochemical analysis that can validate the role of the aforesaid compounds on metabolic protein structures and subsequent role of osmolytes to compensate for the structural loss, this study pinpoint towards the differential role of various classes of osmolytes on protein structural properties. Nevertheless, knowledge regarding the utility of these osmolytes both as co-formulants or solitary compounds can act as an interesting starting point for the food additive industries to ensure the application of these compounds with higher efficacy and reduced harmful side-effects. Thus, along with underlying the severe side-effects of the different food additives on structure and function of stable proteins, this study is the first of its kind to also delineate co-formulation of additives with osmolytes to circumvent the adverse effects.

CRediT authorship contribution statement

NKP conceived and conceptualised the study. SD, NSK and NKP designed the relevant experiments. SD and NSK performed the experiments. SD, NSK and NKP analysed the data. SD, NSK, KB and NKP wrote the manuscript. All of the authors read, revised and approved the manuscript submitted for publication.

Declaration of competing interest

The authors declare that no competing interests exist.

Acknowledgements

Support by Enhanced Seed grant EF/2019-20/QE04-02 (to NKP) from Manipal University Jaipur, Rajasthan, India is gratefully acknowledged. The authors also thank ACTREC (Advanced Centre for Treatment, Research and Education in Cancer) for providing necessary infrastructures to conduct the experiments.

Funding information

The work was funded by ACTREC intramural fund and DBT National Women Bioscientist Award (BT/HRD/NWBA/37/01/2015). The work was also funded by Enhanced Seed grant EF/2019-20/QE04-02 (to NKP) of Manipal University Jaipur, Rajasthan.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2022.06.152.

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Review

Mycorrhiza: An Ecofriendly Bio-Tool for Better Survival of Plants in Nature

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Abstract: Modern agriculture is currently enduring rapid changes in defiance of the continuing increase of the global population and the various consequent environmental challenges. Crop quality is becoming as important as crop yield and can be characterized by several parameters. Extensive use of chemical fertilizers leads to food safety concerns globally; hence, the use of mycorrhizal symbionts have proven to be beneficial for the sustainable growth of the agricultural cropping system. Microflora inhabiting the soil entails various ecological interactions which are associated with agricultural performances. Amongst these microflora, mycorrhizal fungi are the critical suppliers of nutrients, with restricted diffusion capacities of minerals such as phosphorus, nitrate, zinc, sulfur etc. Mycorrhizae are the obligatory biotrophs that depend upon their host plant for the nutritional requirements. They act as the key contributors to sustainable agro-ecological enforcement and impact globally on the eco-systemic processes. These soil inhabitants devote themselves to the continuous nutrient flow and extemporize resistance against various environmental stresses like drought, flood, metal toxicity, salinity, etc. This review briefly highlights the taxonomic co-evolution, factors affecting mycorrhizal behaviors (phytohormonal regulation), and the concise mechanistic approach (improved water status, photosystems, stomatal conductance, ionic uptake, C & N fixation) to combat various environmental stresses (biotic/abiotic). Plant growth regulators play a crucial role in this symbiotic establishment with the plant roots. Auxins, brassinosteroids, and strigolactones are responsible for the establishment of mycorrhizal association. On the other hand, ethylene, abscisic acid, and jasmonic acids can promote or downregulate this process in the plants. Whereas, gibberellic acids and salicylic acids negatively impact on mycorrhizal association. The hormonal homeostasis (in response to fungal associations) leads to the activation of transcriptional and signaling cascades which ensues various physio-morphological changes for the benefit of the plant. The role of phytohormones in the regulation of plant-fungus mutualism, and the impact of mycorrhization on the activation of molecular and transcriptional cascades, have been described along with the potential applications of agricultural produce and soil rehabilitation.

Keywords: Mycorrhizae; phytohormones; biotic/abiotic stress; agricultural produce; soil rehabilitation

Citation: Dhiman, M.; Sharma, L.; Kaushik, P.; Singh, A.; Sharma, M.M. Mycorrhiza: An Ecofriendly Bio-Tool for Better Survival of Plants in Nature. *Sustainability* **2022**, *14*, 10220. https://doi.org/10.3390/su141610220

Academic Editor: Stefania De Pascale

Received: 27 June 2022
Accepted: 10 August 2022
Published: 17 August 2022

1. Introduction

Microbial interactions in the rhizosphere are crucial for nutrient recycling, plant growth, and biotic/abiotic stress reduction in forest and agricultural ecosystems. These interactions vary from one plant species to other, at both the inter and intra-specific scales [1]. Among the various microorganisms (bacteria & fungi) involved in the rhizospheric activities, mycorrhizal fungi exhibit the exceptional feature of dwelling partly inside as well as outside the plant roots. The term mycorrhizae comes from the Greek word 'mykes' and 'rhiza', meaning 'fungus' and 'root' respectively, which was first applied to the association of trees with fungal symbionts [2]. Mycorrhizal fungi, which are members of Glomeromycota, are common on the landscape and associate with over 80% of plants in a diversity of

managed (agricultural) and unmanaged (natural) ecosystems [3]. These fungal symbionts solely rely on the host system to fulfill their carbon requirements, and in exchange provide numerous benefits to the plant system in terms of sustainable nutritional flow, improved plant development, productivity, yield, stress tolerance, water uptake, enzymatic antioxidants accumulation, and soil fertility, etc. [3–5]. These microbial communities are enticed towards their symbiotic partners in response to some signaling factors in the form of root exudates released by the plants [6]. Moreover, in response to the mycorrhizal symbiosis, physicochemical as well as molecular alterations in plants leads to improved plant growth, where phytohormones impart considerable impact in regulating the overall process [7]. Mycorrhization benefits plants by up-regulating the catalytic activities of soil enzymes (such as phosphatases, dehydrogenase, nitrogenase, etc.), assisting in the breakdown of complex organic compounds of soil, and positively influencing other microbes present in the rhizosphere for improved nutrients uptake. Activation of these mechanisms, in turn, provides the ability to withstand drought stress, alleviate salinity, helps with micronutrient absorption and better water absorption, and defense systems in the plants [7]. Owing to these benefits, mycorrhizae have gained a lot of consideration towards multidisciplinary research and have huge applications in agriculture as bio-fertilizers, in fuel production due to the increased plant biomass, and in soil rehabilitation, phytoextraction, and phytoremediation, etc. The impact of mycorrhiza on plant survival in extreme environmental conditions, certain factors (phytohormones) that lead in the successful colonization and their mechanistic approach, and other potential applications are discussed in this paper.

This review aims to provide a better understanding about the plant-mycorrhizal interaction which supports sustained plant growth in the agricultural and forest biomes. These associations have been specified as crucial for regenerating over-exploited or lost forest covers. Due to these characteristics, they can be used as bio-tools for the conservation of many overlooked plant species that are significant to various commercial industries.

2. Methodology

A systematic review of relevant literature was conducted to find articles of relevance to the objective of the study. Keywords such as mycorrhizal association, rhizospheric soil microflora, plant–fungal symbiosis, bioremediation, abiotic stress, soil rehabilitation etc. were used to search the data. More than 200 review and research articles published in peer-reviewed journals were studied, and the most suitable were considered to represent the data in the form of a review article. Literature reviews for this report was searched using different databases such as Research gate, Google Scholar, Scopus, Web of Science and reference book records.

3. History

Arbuscule-like structures evolved during the Ordovician Period in the land plant fossils about 430 million years ago, which specifies mycorrhizal growth and successful colonization of land plants [8,9]. Based on the Rhynie chert evaluation, structural resemblances of subterranean organ fossils, and further molecular verifications in available literature concluded that the origin of AMF was in between the Ordovician and Devonian periods. In addition, co-evolution of these mycorrhizal fungi with plants suggests there are advantages of these symbiotic associations in providing increased strength to each other against diverse environmental conditions [6,8]. AMF are relatively older than nitrogen-fixing symbionts and have likely developed by co-opting mycorrhizal signaling components [10]. Originally, arbuscular mycorrhizae were placed under the zygomycetes classification, considering their morphological features of spores as taxonomic indicators. Lately, these taxonomic markers have been replaced by analyzing the molecular variations through rRNA (small subunit) sequencing. A taxonomic assessment using these molecular markers has led to the re-classification of all the AM fungi into a new phylum i.e., Glomeromycota (the sister clade to Ascomycota and Basidiomycota) [11]. *Rhizophagus irregularis* is the first mycorrhizal fungi with a completely sequenced genome and is the most studied strain in the research

field [12]. The classification of AM fungi has been well described from the very beginning up until recent times. It was divided into different timeline periods as: (a) 1845–1974 (the discovery period), (b) 1975–1989 (alpha taxonomy), (c) 1990–2000 (the cladistics period), and (d) 2001–2012 (the phylogenetic period) by [13]. Mycorrhizal evolution is considered to be one of the major revolutions in the development of global land flora. Endomycorrhizae are the most ancient and abundant of the symbiotic associations and are documented based upon fossil records [8,14]. Endomycorrhizae classified under the phylum Glomeromycota are also designated as 'vesicular-arbuscular mycorrhiza' (VAM) because of the presence of intracellular structures such as vesicles (storage structures) and arbuscules (branched tree-like structures within roots) (Figure 1). However, due to the ephemeral nature of arbuscular structures, they might often be missing or difficult to observe in the roots collected from soil (owing to the age and color of the roots) [15]. On the other hand, vesicles are present in most of the subsections of mycorrhizal symbionts (except some members of Endogonaceae), therefore, the more appropriate terminology given to this group is 'arbuscular mycorrhizae' (AM) [16]. Depending on the morphological and colonization patterns, AM are categorized into "Arum" and "Paris" types (Figure 1). The Arum type describes the linear intercellular spread of hyphal structures within the host roots that form a ramified tree-like arrangement—arbuscules (inside the infected cell) and infection spreads through the side branches penetrating the cortex. The Paris type, on the other hand, describes thick and coiled hyphal growth intracellularly, and infection proceeds from cell to cell through the cortex [15,17]. Another type of AM spread is the "Intermediate" type, where characteristics of both Arum and Paris are present in the infection [6].

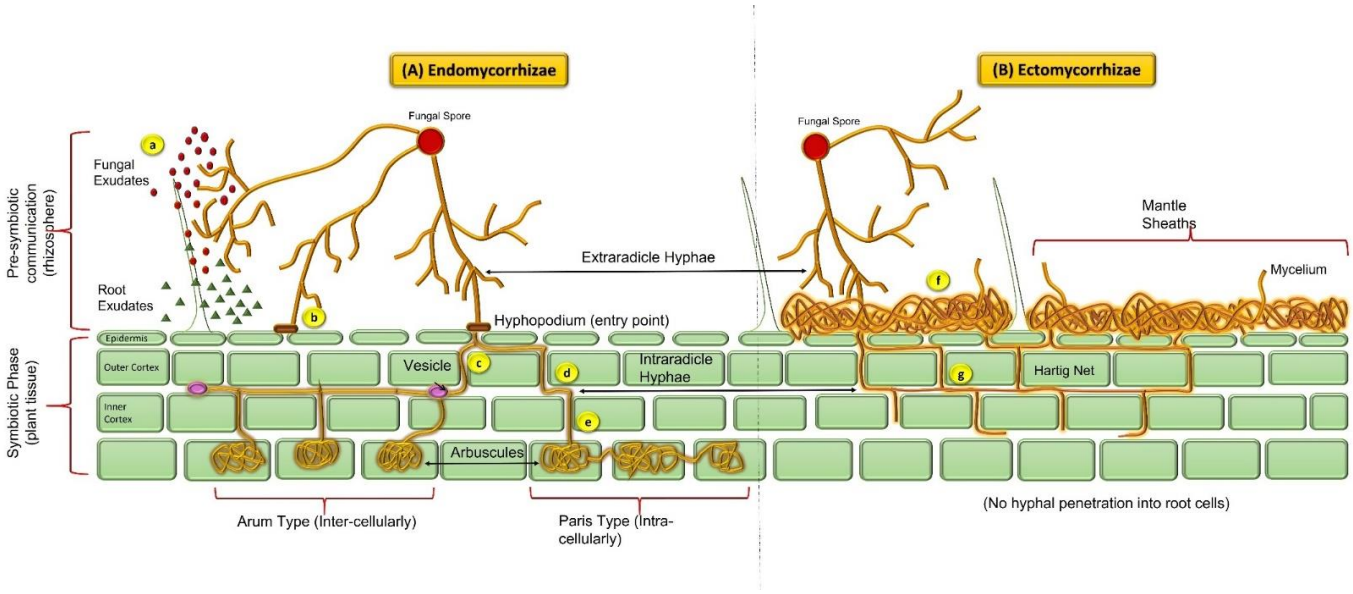


Figure 1. Schematic diagram for mycorrhizal colonization stages of (**A**) Endomycorrhizae; (**a**) pre-symbiotic communication between fungal and root exudates; (**b**) hyphopodium development for fungal entry; (**c**) hyphal penetration into the cortex; (**d**) elongation of intraradical hyphae; (**e**) hyphae branching & arbuscule formation; and (**B**) Ectomycorrhizae; (**f**) mycelium or mantle sheath covering epidermis and cortical cells; (**g**) intraradicle elongation into cortical layer without penetrating cells (Hartig net).

After AM infection spread, cortical cells endure structural modifications and develop a periarbuscular membrane (PAM) outside the fungal hyphae. PAM supports trading of nutrients and photosynthetic material between both the symbiotic partners through an "inter-facial apoplastic compartment" (IAC; the gap between arbuscules and PAM) [6]. The majority of mycorrhizae are arbuscular mycorrhizae, which involves the monophyletic Glomales and a broad range of herbaceous and woody plants [14]. Despite their abundance

and wide range of relationship with plant species (>80% of terrestrial flora), AMF has shown low species diversity. AMF have high functional diversity because different combinations of host plants and AMF have distinct effects on the numerous aspects of symbiosis [17]. AM are obligatory symbionts and rely on the respective partner for the fulfilment of their carbon requirements, thus the establishment of AM cultures are not possible without any host plant [18]. AM fungi belong to nine genera: *Gigaspora*, *Scutellospora*, *Glomus*, *Acaulospora*, *Entrophospora*, *Archaeospora*, *Gerdemannia*, *Paraglomus* and *Geosiphon*, the only known fungal endosymbiosis with cyanobacteria [19].

These associations are true cosmopolitans and are apparent in all of the ecosystems from tropical forests, Arabic deserts, Arctic regions to elevated Himalayan regions, with few exceptions [20]. Moreover, AM fungi are the key contributors to the defense against various biotic and biotic stresses (Table 1).

Table 1. Effect of mycorrhizal symbiosis on stress tolerance.

Sr. No.	Stress Condition	Mycorrhizal sp.	Host Plant	Possible Mechanism	Reference
1	Salinity, 200 mM NaCl	*Rhizophagus irregularis (Formerly* Glomus intraradices)	*R. pseudoacacia*	Improved photosynthetic rate, PS-II photochemistry, water status, K^+, Chloroplast (RppsbA, RppsbD, RprbcL)& transporter genes (RpSOS1, RpHKT1, RpSKOR) up-regulation, lower shoot:root Na^+ content	[21]
2	80 mM NaCl	*Glomus intraradices*	*Lactuca sativa*	Alleviates salt stress through improved stomata performance, photosystem (PS-II), Carotenoid deoxygenase gene (LsNCED2) induction, normalized ABA level and by altering the hormonal profiles (SLs induction)	[22]
3	100 mM NaCl	*Rhizophagus irregularis*	*Solanum lycopersicum*	Elevated K+ and K+/Na+ ratio (prevention of metabolic processes disruption), regulated hormone synthesis & cross talk	[23]
4	200 mM NaCl	*Glomuse tunicatum, Glomus intraradices, Glomus mosseae*	*Cucumis sativus* L.	Photosynthetic pigments regulation, enhanced antioxidant activities, osmolyte (proline& phenols) regulation, improved water status; regulated mineral uptake; reduced uptake of Na^+.	[24]
5	200 mM NaCl	*Claroideoglomus etunicatum*	*Aeluropus littoralis*	Overcome free radical formation by elevated antioxidant activity, high CO_2 synthesis and nitrate assimilation	[25]
6	120 mM NaCl	*Funnelliformis mosseae, Acaulospora laevis, Gigaspora margarita*	*Oryza sativa* L.	Rise in chlorophyll content, K^+/Na^+ ratio, photosynthesis, and dropped shoot/root Na^+ ratio by limiting Na^+ uptake and translocation.	[26]
7	200 mM NaCl	*Funneliformis mosseae*	*Malus domestica Borkh.*	AMF in combination with dopamine help to maintain host cell membrane integrity, improves photosynthesis	[27]
8	35 and 70 mM NaCl.	*Glomus sp. mix (G. mosseae, G. intraradices, G. hoi)*	*Citrus aurantium* L.	Elevation in plant growth, chlorophyll levels, improved water status, gas exchange capacities (increased photosynthetic rate, stomatal conductance and transpiration rate), enhanced oxidative stress defense system	[28]
9	160 mM NaCl	*R. intraradices* and *F. mosseae.*	*Prunusdulcis* × *Prunuspersica* hybrid	Improved physiological parameters (chlorophyll, osmolytes that are soluble sugars and proline content to combat salt toxicity) and increased antioxidant enzymes activity compared to non-inoculated. F. mosseae elevated chlorophyll content more efficiently, whereas R. intraradices prevailed total sugars and proline content.	[29]
10	150 mM NaCl	*Glomus etunicatum, Glomusgeo sporum*, and *Glomus mosseae*	*Oryza sativa* L.	Improved physiological parameters (chlorophyll, osmolytes that are soluble sugars and proline content to combat salt toxicity) and increased antioxidant enzymes activity	[30]

Table 1. *Cont.*

Sr. No.	Stress Condition	Mycorrhizal sp.	Host Plant	Possible Mechanism	Reference
11	-	*Funneliformis mosseae* and *Claroideoglomus etunicatum*	*Puccinellia tenuiflora*	Increased P uptake, high antioxidant capacities, enhanced biomass to dilute the salt concentration, elevated K^+/Na^+ ratio, restricted Na^+ translocation towards aerial parts.	[31]
12	200 mM NaCl	*Glomus monosporum*, *G. clarum*, *Gigasporanigra*, and *Acaulospora laevis*	*Vigna unguiculata* L.	Elevated photosynthetic pigments, soluble sugar contents, ions accumulation and compartmentalization (maintained membrane integrity) and high enzymatic activities.	[5]
13	Drought Stress	*Funneliformis mosseae (formerly Glomus mosseae)* and *Rhizophagus intraradices*	*Solanum lycopersicum*	Stress tolerance varies depending upon the myc-species. F. mossaeae promoted volatile emission (VOC), high arbuscule formation in colonization regions R. intraradices is more efficient towards P uptake (upregulated P transporters; LePT4,5), high plant performance to lower water dispersal by adopting a compact structure (high internode/height ratio), high water utilization efficiency	[32]
14	Drought Stress	*AMF*	*Glycine max* L.	Increased water holding capacity, photosynthesics, osmoregulation	[33]
15	Drought Stress	*Rhizophagus irregularis (formerly Glomus intraradices)* and *Funneliformis mosseae (formerly G. mosseae)*	*Trifolium alexandrinum* L.	Enhanced nutrient uptake, increase in phosphorus acquisition, defense against oxidative stress, increased N_2 fixation, sufficient availability of the photosynthates	[34]
16	Drought Stress	*Funneliformis mosseae* and *Rhizophagus intraradices*	*Solanum lycopersicum*	Aquaporin genes regulation; LeNIP3;1 (overexpressed), LeNIP3;1 & LeTIP2;3 (suppressed) by F. mosseae, and RiAQPF1 & 2 (overexpressed) by R. intraradices, elevated stomatal density, activation of LOX (lipoxygenase) genes, increased antioxidant activity, proline content (osmoregulation)	[35]
17	Drought Stress	*Funneliformis mosseae*	*Triticum durum Desf.*, *Triticum aestivum* L.	Positive impact on root metabolome, high C fixation, high P sugar accumulation, osmoregulatory effects, anti-oxidative behavior, regulated phytohormone profile	[3]
18	Drought Stress	*Rhizophagus irregularis*	*Zea mays*	Efficiency of photosystem II, membrane stability, osmotic regulation via accumulation of soluble sugars and plant biomass production. Root hydraulic conduction via down-regulating aquaporin genes (ZmPIP1;6, ZmPIP2;2, and ZmTIP4;1)	[36]
19	Drought Stress	*Myc-mix. (Rhizophagus intraradices + Funneliformis mosseae + F. geosporum)*	*Triticum aestivum*	Elevation in photosynthetic pigments, high Mg uptake, C fixation (photosynthate) and biomass; improved water status; enhanced PSI & PSII photochemistry	[37]
20	-	*Rhizophagus irregularis*	*Solanum lycopersicum*	Promoted photosynthesis, improved C fixation, osmoregulation and root hydraulic conductivity via enhanced aquaporin.	[38]
21	**Temperature stress** (43–44 °C)	*Funneliformis sp. AMF*	*Zea mays*	Up-regulated water transport and transpiration, regulated PSII heterogeneity, stomatal conductance	[39]
22	(44 °C)	*Rhizophagus intraradices*, *Funneliformis mosseae*, *F. geosporum*	*Zea mays*	Enhanced PSI & PSII photochemistry, high Mg^{2+} uptake.	[40]
23	(35 °C)	*Rhizophagus irregularis*, *Funneliformis mosseae*, *Funneliformis geosporum*, *Claroideoglomus claroideum*	*Triticum aestivum* L.	Increased photosynthetic yield, nutrient distribution and nutrient composition in roots, lowered the K/Ca ratio	[41]
24	(3–5 °C)	*Glomus versiforme and Rhizophagus irregularis*	*Hordeum vulgare* L.	Enhanced membrane stability, antioxidative capacity & phenolics metabolism Glomus sp. imparted more alleviation against cold stress. Rhizophagus found more efficient towards survival rate.	[42]

Table 1. *Cont.*

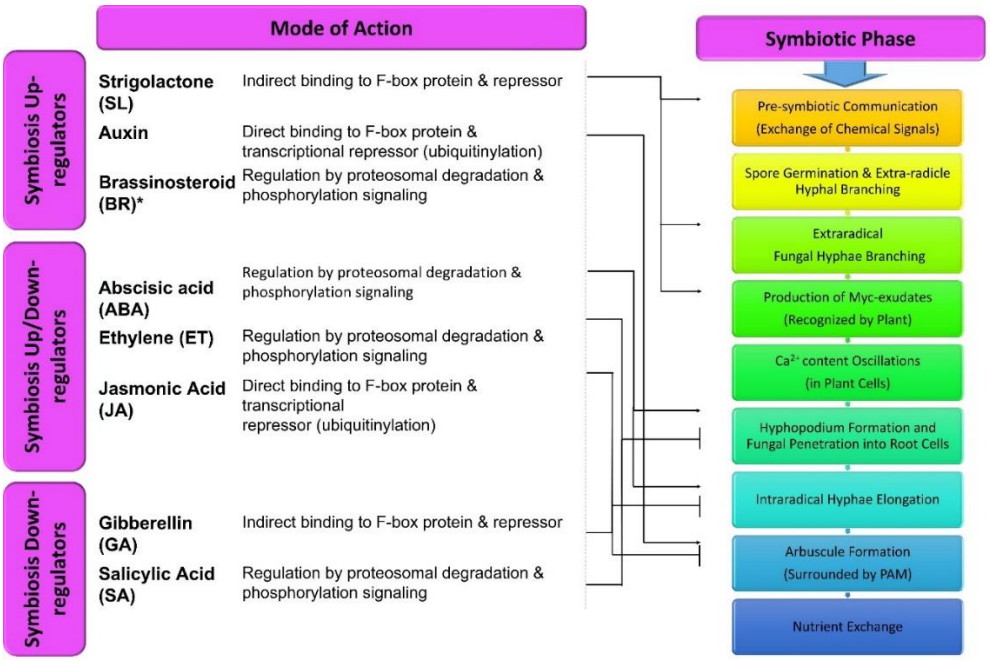
Sr. No.	Stress Condition	Mycorrhizal sp.	Host Plant	Possible Mechanism	Reference
25	(15 °C)	*Rhizophagus irregularis*	*Zea mays* L.	Down-regulated PS-I & PS-II genes and decreased oxidative stress, enhanced C assimilation by metabolic upregulation, high ATP production by increased P concentration	[43]
26	(5–25 °C)	*Funneliformis mosseae, Claroideoglomus etunicatum, Rhizophagus irregularis,* and *Diversispora versiformis*)	*Solanum melongena* L.	Promoted photochemical, antioxidant activities, and maintained membrane integrity, proline and phenolics accumulation (protection against stress)	[44]
27	(4 ± 0.5 °C)	*Glomus intraradices*	*Citrullus lanatus*	Improved photosynthesis, induced peroxidase (POX) activity, restoring photosynthesis efficiency, released oxidative stress	[45]
	Biotic stress				
28	Aphids (*M. euphorbiae*)	*Rhizophagus intraradices*	*Solanum lycopersicum* L.	Indirect defense via enzymatic release of methyl salicylate to attract parasitoid A.ervi	[32]
29	Spodoptera littoralis	*Rhizophagus irregularis*	*Solanum lycopersicum* L.	Enhanced nutrient acquisition, N_2 fixation, defense activation	[46]
30	Caterpillar, Helicover-paarimigera	*Glomus mosseae*	*Solanum lycopersicum Mill.*	Activation of stress responsive genes (LOXD, AOC, PI-I & II) in leaves, regulated JA cascade	[47]
31	Meloidogyne incognita (severe yield losses in tomato)	*Rhizophagus intraradices*	*Solanum lycopersicum*	Improved plant peroxidases for ROS scavenging, Upregulated flavonoid enzymes, modulation of pathogen related genes (LTP), phytohormonal regulation, increased glutathione transferases	[48]
32	Fusarium virguliforme	*Rhizophagus irregularis*	*Glycine max*	Peroxidase genes regulation, decreased. Down-regulation of several genes coding for glutathione-S-transferase (GST)	[49]
33	Xiphynema index	*Rhizophagus intraradices*	*Grapevine rootstock SO_4 (Vitis berlandieri × V.riparia)*	Decreased down-regulation of several genes coding for glutathione-S-transferase (GST)	[50]

The establishment of ectomycorrhiza (ECM) symbiosis occurs through higher fungi mycelia, taxonomically placed under Basidiomycota predominantly, and a few under Ascomycota [51,52]. The most important characteristic of these associations is the non-septate basidium of spore-producing symbionts. These associations usually establish in rootlets of woody flora (bushes or trees) of temperate and some tropic regions. They are mostly categorized under the myrtle family (Myrtaceae), beech family (Fagales), birch family (Betulaceae), rock rose family (Cistaceae), pine family (Pinaceae), willow family (Salicaceae), Dipterocarpaceae and to a lesser extent they are also found in Nyctaginaceae & Polygonaceae [53]. Ectomycorrhizae represent less than 5% of the mycorrhizal associations known in vascular plants, but are ubiquitous in the Pinaceae. Plants under this family dominantly inhabit the diverse climatic conditions and nutrient-deficient soil which are the key influencers of ectomycorrhizal associations. These ECM symbionts are the key players maintaining the nutritional flow through the forests by extending their mycelial network through both the host system and surrounding soils [51]. Ectomycorrhiza form 'mycelium mantle' surrounding the host root system (short lateral roots) which further occupies the epidermal and cortical cells without penetrating through the cortex. This results in highly branched mycelial structure to form 'Hartig net' [53]. Hartig net plays a critically important role in mutualism by forming the plant-fungus interface through which exchange of the nutrient material between both partners takes place. Besides, mycelia emerging through the root mantle are absolute in hunting the nutrient substances from inundating soil. The ECM colonizing plant range is relatively smaller than AM, despite the fact that owing to the larger forest canopy conquered by these associations, their economic value is steadily growing [19]. The most suitable biological habitat for these associations is parched, non-calcareous, sandy soil regions, swamps, and the lowlands of the Northern Hemisphere [54]. The first impression of such associations is not clear however, because of the obscure preservation in the form of fossil remains attributable to their ephemeral nature, minuscular dimensions, and delicate tissues. Acknowledging that the molecular

clock indicates that their first existence is supposed to be from the early Cretaceous period, almost 130 mya [55]. They have evolved as derivatives of saprotrophs and the pattern of their association with the host has evolved autonomously many times [14]. Moreover, the permineralized flora of Princeton chert is apparent for the existence of ectomycorrhizal associations unveiling the ECM structures that are the Hartig net, mantle, and extra-matrical hyphae networks (Figure 1). These findings verify the rise of ECM at least 50 mya [56].

4. Role of Phytohormones in Regulating the Development of Plant-Fungus Symbiotic Association

Phytohormones are the key regulators of all the physicochemical, molecular, and phenotypic expressions of plants. These factors, in very small fractions, facilitate the signal transduction in response to different environmental stimuli. This in turn regulates plant growth, stress resistance, pathogenic resistance, and nutrient flow, etc. [57]. Based on the cellular environment, these molecules can act both in down-regulating or in a synergistic manner. They have been categorized as developmental {auxins (Aux)/cytokinin (Cyt)/gibberellins (GA)/brassinosteroids (BR)/strigolactones (SLs)} and stress resistant {(salicylates (SA)/ethylene (ET) /abscisic acid (ABA)/jasmonates (JA)} hormones. Although, stress-relieving hormones are not limited to this characteristic, and their interaction with growth-regulating hormones control various growth responses and vice-versa [57]. These hormonal interactions flow in a sequential fashion mainly includes: signal input (SI; differential buildup of phytohormones after receiving environmental stimuli), signal processing (SP; a triggered cascade of repressors/stimulators/transcription factors post ligand- receptor binding), and signal output (SO; in the form of phenotypic response post transcriptional changes) [57,58]. Various behavioral responses by different plant hormones have been addressed further with their effects on mycorrhizal colonization (Figure 2). Additionally, significant effects of protein-protein interaction has been evidenced in these regulatory responses [58]. DELLA & JAZ are the key proteins that regulate the GA & Jas pathways, respectively and provide defense and growth benefits to the plants. These interactive regulations (by DELLA & JAZ) are not limited to defense hormones but with other factors also (Aux, ethylene, light, etc.) [59,60].



* Evidences for particular stage affected by BR still require further investigation.

Figure 2. Impact of various hormones on symbiotic phase regulation and suggested mode of action.

4.1. Auxin

Several reports are available describing the contribution of the plant hormone to the formation of mycorrhizal associations since the first evidence of auxin involvement in ectomycorrhizal symbiosis was published [61]. Although, auxins are well known for their ability to regulate the plant root system, their role and action mechanism involved in symbiotic association still needs to be explored [62]. It has been reported that the auxin molecules released by the ECM variety enable the successful establishment of plant-mycorrhiza association that results in an improved root system [63]. Also, in *Terfezia boudieri* and *Cistus incanus,* auxins and P (phosphorus) govern the type of symbiotic associations (AM or ECM) [64]. Various studies have indicated certain impacts of auxins on inoculation and the colonization abilities of fungal symbiosis. The exogenous use of auxins in mycorrhizae associating plants viz. *Papaver croceum* and *Quercus robur* influences greater ECM establishment. Contrary to this, Aux transport inhibitors (TIBA; 2,3,5-triiodobenzoic acid & NPA; 1-N-naphthylphtalamic acid) hamper the mycorrhization process [65,66]. Auxins play a vital role in regulating the structural parameters of roots, which is subjective to the exudation and colonization by the fungal partners [67]. Higher auxin concentrations results in various structural variations in the roots such as attenuated gravitropic progression of taproot, generation of lateral roots to facilitate more colonization [63,68]. Fungal Aux stimulating lateral root proliferation in plants provides more infection sites for the fungal colonization, since the infection occurs closer to the root cap area with a higher probability of mycorrhization. Besides, these small molecules are evident for the increased arbuscular development by activation of various transcriptional mechanisms [6,39,40].

4.2. Strigolactone

Strigolactones (SLs) are plant hormones exuded by plants in the rhizosphere as signaling molecules [69]. They are carotenoid-derived molecules, like the other phytohormones in their action mechanism [10]. Originally, they were discovered as germination stimulating factors of parasitic weed Striga lutea and Orobanche exudates. A few decades later, their role in hyphal branching and the symbiotic association between plant mycorrhiza were witnessed [70,71]. These molecules travel upwardly in plants and down-regulate branching in plants. Their basic action mechanism in symbiosis is identical to the other plant growth regulators, that is, hormone-derived proteolysis [70]. SLs are specifically reported to trigger hyphae formation and branching in mycorrhizal species of Gigasporaceae & Glomeraceae families [10]. Mycorrhizae exhibit extremely sensitive behavior for SLs (GR24; synthetic analog of SL) and hence very low concentrations (approx. 10 nM) are sufficient to establish a symbiotic connection. These molecules recruit the nuclear division, mitochondrial expansion and promote the catalytic performance of NADH dehydrogenases with high ATP production, which are the essential parameters for hyphal growth in *Gigaspora rosea* [72]. For these activities, the fundamental structure of the intact tricyclic (ABC) lactones and a butanolide (D) ring (attached through an ether linkage) is critical and consistent [10,69]. However, both methylation and demethylation processes are imperative in the varying fungal growth response. Also, the study suggested that the developmental parameters such as hyphal branching may not always signify the symbiotic association between both the partners [73]. Additionally, SLs elicit the production of fungal exudates (myc factors i.e., lipochito- oligosaccharides & chito-oligosaccharides) for the enhanced fungal activity in the symbiotic association process [74]. Due to negligible stability in soil, strigolactones establish a gradient surrounding the roots which provide direction to the mycorrhiza [75]. It has also been reported that the extent of colonization in Petunia mutant plants (SL exporter muted) gets abridged due to the blocked exudation of SLs (orobanchol). Before the colonization process, the growing hyphal tip attaches to the root surface after differentiation into the hyphopodium or appressorium. Formation of a pre-penetration apparatus by the plant cells underneath the hyphopodia takes place for the fungal permeation into the plant. After penetration, fungal hyphae grow inter or intracellularly through the cortical cell layer and form arbuscules which supports nutrient exchange between both partners [76].

4.3. Gibberellin

Gibberellins (GA) are associated with different plant growth stages such as seed germination (breaking seed dormancy), pollen growth, root-shoot elongation, and flower induction, etc. These molecules trigger the signaling cascade which is responsible for the degradation of DELLA TFs (transcription factors) because of poly-ubiquitination by another TF-E3 ubiquitin ligases [77]. Hence, loss of function of the DELLA-TFs is key to express the GA induced response [78]. Based on the available reports, notable evidences have suggested negative impacts of the GA application on AM symbiosis in plant species like (*Pisum sativum*, *Lotus japonicas*, *Solanum lycopersicum*, *Oryza sativa*, *Medicago truncatula*, and *Triticum* etc. [40,50,51]. Although, the rise in GA concentration in AM associated plant *Lotus japonicus* roots have been reported and increased, GA levels may regulate the hyphal density in the roots by maintaining the arbuscule formation [79]. The pea variety (na-1), which are GA deficient due to the inactive ent-kauremoic enzyme, exhibit high mycorrhizal colonization than the wild type *P. sativum* plants which authenticates the previous findings [80]. Moreover, stable expression of the DELLA protein (della1-Δ18; non-degradable/ resistant to GA due to the absence of the DELLA domain) is significant in successfully developing mycorrhizae in *L. japonicas* and *M. truncatula* [78,79]. Further, GA signaling initiates the formation of arbuscular structures and not the hyphae branching. This is evident in the DELLA mutant forms of rice plant (slr1), *P. sativum* & *M. truncatula* (della1 & della2) showing a drastic decline in the arbuscular count (than in other fungal structures) [81,82]. On the other hand, overexpressing Della factors SLR-YFP (*O. sativa*), Rht1 and Rht2 (*Triticum* sp.) resulted in a rise in these structures [83].

4.4. Abscisic Acid (ABA)

ABA plays a crucial role in plants with regards to stress management [84,85]. In stress (drought) conditions, ABA levels increase to induce stomatal closure in leaves of mycorrhizae treated plants like *Glycine max* and *Solanum lycopersicum* roots [86,87]. ABA also influences mycorrhization in a dose-dependent manner and supports this perception in *S. lycopersicum* & *M. truncatula*. Lower concentrations of ABA in plants like *S. lycopersicum* ABA mutant (sitens) and *M. truncatula* ABA mutant (PP2A) have shown declined arbuscular branching and fungal penetration into roots, respectively [35]. Besides, in sitens mutant variety, a reduction in AM can be rescued by the application of exogenous ABA. ABA cross-interaction with others suppresses GA (DELLA) cascade, thereby increasing arbuscular formation [75].

There are only a few studies about ABA's role in AM development and apart from the discussed assumptions, further clarification about the molecular mechanism is still needed [75].

4.5. Jasmonate (JA)

Jasmonic acid, a key defense phytohormone active against both biotic and abiotic stress, has significant role in mycorrhizal symbiosis [75]. JA has been reported to exhibit both positive as well as negative to neutral responses for mycorrhizal symbiosis, where JA cascade is triggered in response to a myc-fungus infection which resulted in enhanced synthesis of JA precursor and genes [88]. Negative consequences on mycorrhizal colonization are due to increased JA in *S. lycopersicum*, *T. majus*, *O. sativa*, and *C. papaya*, by activating the plant's defense system and rise in Ca^{2+} spiking. Similarly, mutant rice plants (cpm2; JA deficient) were reported to have increased mycorrhizal colonization in roots [75,88]. In tomato mutant (def-1) plants (JA deficient), mycorrhizal colonization exhibited positive impacts such as improved resistance to biotic stress (via *Spodoptera littoralis*) compared to non-inoculated plants (wild type & constitutively JA producing). With JA-accumulating plants, alleviated colonization has been reported [46]. Contrary to this, tomato plants conferring progressive effect of JA on mycorrhizal colonization have also been reported where suppressed JA levels resulted in a delayed mycorrhization process, and vice-versa [89,90]. However, further clarification about such contrasting effect is still needed. One of the

possible mechanisms reported for the regulated JA functioning has been the environmental factors (i.e., light conditions). Thus, JA homeostasis plays a crucial role in optimal mycorrhizal colonization [75].

4.6. Brassinosteroid

Brassinosteroids (BRs) are steroidal hormones which regulate different aspects of plant growth such as the development of flower parts (stamen and pollen) through cellular expansion and elongation, vascular system development, fruit ripening, shoot elongation, and pathogenic defense systems, etc. [40,65,66]. Few reports are available which state the role of BRs in mycorrhizal symbiosis in plants such as rice, wheat, tomato, pea etc. In recent findings, it has been concluded that deficiencies of BRs (caused by a mutation in BR synthesizing genes) resulted decline in mycorrhization in mutant rice (brd2-1), pea (lk) and tomato (dx) plants [91–93]. Whereas, foliar nourishment of synthetic BRs leads to the improved mycorrhization in wheat [94]. Some studies have suggested the elevated sucrose transport (through a SlSUT2 transporter) and its increased availability to the fungus as a significant factor for improved mycorrhization [91,92]. Recently, it has been reported that the deficient BRs responses and the silencing of the SlSUT2 transporter also decreases mycorrhization in tomatoes, along with reduced pollen development [95]. Further work to demonstrate a detailed understanding behind BRs activity is still required [78].

4.7. Ethylene

Ethylene (ET), a stress hormone, plays an important role in many physiological activities in plants [96]. This volatile phytohormone imparts both positive as well as negative controls on plant growth such as promoted seed germination, fruit ripening, and cell senescence [75]. Very few reports describing the role of ET in mycorrhizal development are available, revealing the regulation of plant immunity and crucial in interactions of the plant with symbiotic or pathogenic microbes. According to the literature surveyed, the elevated concentration of ET is evident in downregulating the mycorrhization process [93]. In a report, this effect was found consistent in the *M. truncatula* mutant (ein2, ET insensitive) where increased mycorrhizal development in response to ET insensitivity was reported [97]. On the other hand, the ET-insensitive pea plant did not favor mycorrhizal development, neither did it suppress AM development when supplemented with ethylene [93]. In the tomato mutant (ET overproducing), the reduction in the roots colonized with mycorrhizal fungi has also been reported [93]. Moreover, different morphological effects in response to the ethylene have been observed, where ET-ABA interactions down-regulate the intraradical colonization without affecting hyphopodia on the root [98]. In other plant species like *P. sativum*, *O. sativa*, and *L. japonicas*, unusual hyphopodial structures were observed as an effect of ethylene with restricted root entry by mycorrhizal species [99,100]. The most probable reason suggested for these ethylene-induced effects has been targeting factors involved in Ca^{2+} spiking cascade and the activation of transcriptional factors and enzymes (amino cyclopropane carboxylate oxidase) involved in the defense system in response to colonization by *Glomus fasciculatum* [75,98]. It has also been reported that ET signaling is associated with phosphate starvation [101]. Although further research is needed to clarify whether ET reduction occurs to promote intra-radical mycorrhization or because of increased P nutrition by symbiosis [75].

4.8. Salicylic Acid (SA)

SA has been regarded as a stress phytohormone which stimulates endogenous signaling cascade to acquire systemic resistance against pathogens. Signaling also gets induced during mycorrhizal symbiosis, which results in an undesirable impact on mycorrhizal colonization with the host root [6,75]. In tobacco plants (transgenic; exhibit constitutive SA synthesis) due to continuous synthesis of SA, alleviation in colonization has been observed. On the other hand, decreased SA concentrations due to SA hydroxylase activity promoted the colonization [102]. Like ABA, SA reduces mycorrhizal colonization in rice, although no

observable effects on hyphopodium development have been reported [103]. Additionally, the SA introduction into rice effects the efficiency of fungal association by lowering the colonization in roots without disturbing the development of appressorium, which indicates indirect influence of SA on fungal growth [6]. Besides, the rise in SA concentration in defective *P. sativa* (for myc-symbiosis) has been reported. Contrary to this, transgenic tobacco (down-regulated SA production) maintained an increased colonization efficiency [102]. In this context, the suppressing effects of SA on fungal penetration into the host via roots has been suggested [104]. Conclusively, based on the literature available, the application of SA in higher concentrations may reduce or delay the process of successful mycorrhizal colonization in plants. There are only a few reports even now and therefore, the role of SA in mycorrhizal symbiosis still requires further investigation.

5. Applications of Mycorrhizal Symbiosis to the Ecosystem

5.1. Positive Impacts on Plant Growth and Nutritional Requirements

The most prominent assistance provided by symbiotic association of plant-mycorrhizae is to improve growth through the sustainable and enhanced supply of micronutrients. The most evident nutrients involved in this phenomenon is Phosphorous (P) which has additional benefits such as carbon assimilation, regulated enzymatic activities, water retention, and improved soil quality which leads to a positive impact on plant growth [105,106]. AMF are associated with the regulated flow of water and nutrients in exchange of carbohydrates from the host [106]. The mycorrhizal association modifies the morphology of the host roots and improves water-mineral uptake from the rhizosphere [107–109]. These associations show varying colonization patterns and capacities depending upon the plant species [110]. AM symbiosis also regulate rhizospheric enzymes such as urease, glucosidases, dehydrogenase, nitrogenase, phosphatase, catalase, peroxidase and soil polyphenol oxidases to provide better soil antioxidant activities [111–114].

Rhizospheric enzymes improve soil aggregation by hydrolysis and the activation of non-available organic matter in soil, the transfer of nutrients within or between the plants, stabilizing mycorrhizal products like hydrophobins, polysaccharides, glomalin related soil proteins and other extracellular composites, and chelating toxic substances in the rhizosphere [114]. Increased phosphatase activities by mycorrhizal association amplifies levels of phosphorus release from the soil organic matter, hence enhanced translocation of nutrients from the soil to the host plant. In addition, the pattern of intra-radical and extra-radical hyphal structures influence the phosphorus metabolisms among AM species [115]. Conclusively, most of the plants in the natural environment depend on mycorrhizal associations for their nourishment, and these associations have been reported for the transport of about 50% of fixed N and 90% of P into the plant [116,117].

5.2. AMF and Mineral Nutrition

Mycorrhizal symbiosis has gained significant attention with regards to agricultural sustainability due to its characteristic properties of mineral nutrients uptake, utilization, translocation, and how it acts as a biocontrol instrument to the plants. As mentioned in previous sections, they exhibit a critical mediator between the roots and soil, where the soil nutrients acquired by fungal partners get moved to the plant partner in exchange for the photosynthetic carbon produce. Mycelial extensions on the roots' surface help plants to capture nutrients more efficiently by increasing the surface area, and hence maximum the absorption of soil minerals [118]. The mycorrhizal association triggers the transfer of minerals such as phosphate, ammonium, nitrate, zinc, copper, potassium, sulfur, etc. with the help of various transporters (Table 2) [21,32]. Phosphate transporters (PTs) present in the mycorrhizal fungi due to their high affinity have been extensively studied for their functional and molecular characteristics imparting nutritional benefits towards plant development [4,117]. AM associations have also been reported to promote P uptake cascade in plants, by triggering expression of some phosphate transporters in many plant species such as in *M. truncatula* (MtPT4), *A. sinicus* (AsPT1) and *O. sativa* (OsPT11) [119–121]. In this

way, phosphate accumulated via mycelial absorption (an active process) is accessible to the plants. These transporter proteins are considered to be indicators of mycorrhizal symbiosis embedded on periarbuscular membranes (PAM) (Figure 3) [122]. Other plant transporter genes for micro and macronutrients like ammonium (AMs), sulfur (SULTF), zinc (ZIPs), nitrate (NPF), potassium (KTs) etc. have also been identified in mycorrhized plants. These transport systems are coupled with a positive impact on arbuscular development as well as a regulatory response to the plant homeostasis [119,123]. In addition, potassium (K^+) plays a significant role in plant physiological processes and a symbiotic association with fungus not only increases the potassium supply, but also provide resistance against drought stress to the plant. Potassium accessibility in soil, however, is of concern due to their high mineral adsorption characteristics. Although, these (K^+) transporters are associated with myc-symbiosis, their significant physiological involvements have been less explored [123]. Moreover, myc-inoculation into the agricultural sites could soon possibly be an effective method for improved crop productivity, nutritional flow, and regulation of symbiotic associations [116].

Table 2. Regulation of mineral nutrition via transporters in mycorrhizae associated plants.

Sr. No.	Mineral	Mycorrhizal sp.	Plant sp.	Host Plant Transporters	Effect of Mycorrhizal Symbiosis	Reference
1.	Phosphate	*Claroideoglomus etunicatum*	*Camellia sinensis*	CsPT1 & CsPT4	AMF up-regulated root CsPT1 expression, while down-regulated the CsPT4 expression. AMF inoculation significantly promoted P acquisition capacity of tea plants, especially in roots through improving root growth and enhancing soil acid phosphatase activity and root CsPT1 expression.	[124]
		Rhizophagus irregularis	*Zea mays*	ZmPht1;6 & ZmPht1;11	AMF improved plant growth and Pi assimilation, AMF colonization strongly improved the nutritional status of the plants and increased the internal P concentration. ZmPht1;6 over expression at a high level in AMF-colonized roots. While less expressed ZmPht1;11 also stimulated by AMF colonization.	[125]
2.		*Gigaspora margarita or Funnelliformis mosseae*	*Lotus japonicus*	LjPT4	LjPT4 affects proper arbuscule formation on the fungal side and for improved Pi uptake on the plant side.	[126]
3.	Sulfur	*Rhizophagus irregularis*	*Zea mays*	ZmSULTR1.2a, ZmSULTR2.1, ZmSULTR3.5	Upregulation of ZmSULTR1.2a & ZmSULTR2.1 in sulfur deprived conditions while downregulation of ZmSULTR3.5 in mycorrhized plants.	[127]
4.	Copper	*Rhizophagus irregularis*	*Medicago truncatula*	MtCOPT2	Preferential expression of MtCOPT2 during mycorrhizal symbiosis.	[128]
	Nitrate	*Rhizophagus irregularis*	*Oryza sativa, Zea mays, Sorghum bicolor, Medicago truncatula*	OsNPF4.5, ZmNPF4.5, SbNPF4.5, MtNPF4.5	Myc-symbiosis resulted in efficient up-regulation of OsNPF4.5, ZmNPF4.5 and SbNPF4.5, while slight induction of MtNPF4.5.	[129]
		Rhizophagus irregularis	*Oryza sativa*	OsNPF genes: NPF2.2/ PTR2, NPF1.3, NPF6.4 and NPF4.12	Enhanced expression of nitrate transporter genes in mycorrhizal roots in nutrient dependent manner.	[130]
5.	Ammonium	*Rhizophagus irregularis*	*Oryza sativa*	OsAM1, OsAM10, OsAM20, OsAM25	Significant upregulation in roots via AMF symbiosis.	[130]
		Rhizophagus irregularis	*Oryza sativa*	OsAMT3.1	Up-regulation of OsAMT3.1 in rice mycorrhizal roots	[129]

Table 2. *Cont.*

Sr. No.	Mineral	Mycorrhizal sp.	Plant sp.	Host Plant Transporters	Effect of Mycorrhizal Symbiosis	Reference
6.	Zinc	*Rhizophagus irregularis*	*Medicago truncatula*	MtZIP5, MtZIP2	AMF symbiosis caused higher expression of MtZIP5 in poor rhizospheric Zn condition and reduction in MtZIP2 at elevated soil Zn concentration.	[131]
		Rhizophagus irregularis/mock-inoculated	*Hordeum vulgare*	HvZIP3, HvZIP7, HvZIP8, HvZIP10, HvZI13	Out of five transporters, HvZI13 found most significantly upregulated, HvZI3 & 8 upregulated also in Zn deficient conditions, while HvZI7 & 10 downregulated.	[132]
7.	Potassium	*Rhizophagus irregularis*	*Lycium barbarum Solanum lycopersicum*	LbKT1, LbSKOR SlHAK10	Regulated expression of LbKT1 and LbSKOR for varying water & potassium availability	[133,134]

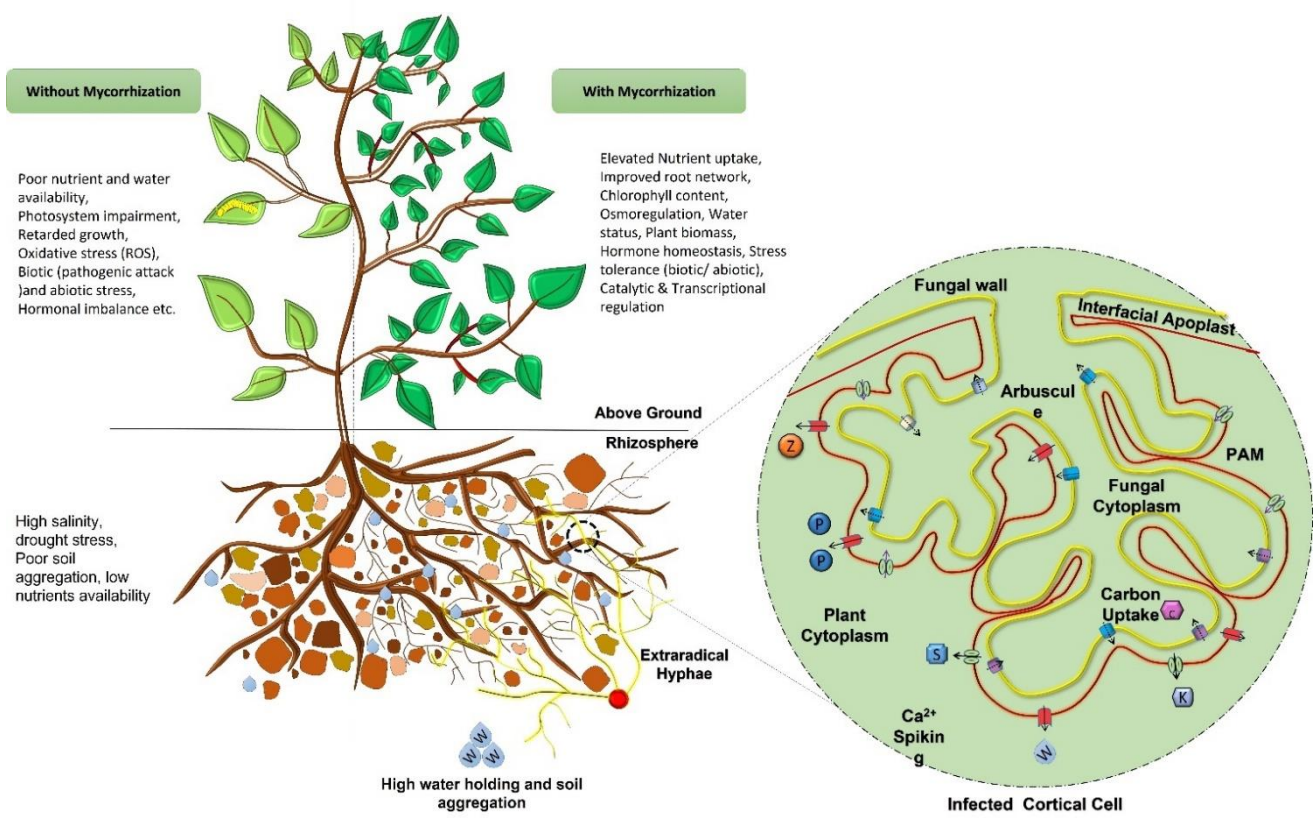


Figure 3. The effect of mycorrhizal associations on plant growth and restoration of soil: alleviated nutrient supply, poor root network and impaired plant growth without mycorrhizal exposure (**left**), and rhizospheric extraradical hyphae extension deep into inaccessible soils (soil aggregation), elevation in nutrient uptake (ionic exchange by arbuscule formation through IFA) and improve plant growth (**right**).

5.3. AMF as Bio-Fertilizer

Generally, bio-fertilizers are substances which include microbial population and when applied to the soil, result in improved plant growth by promoting mineral nutrition uptake, water supply, protection against biotic/abiotic stresses, and soil quality. In particular, the fungal microorganisms (due to thin hyphal structures) have emerged as extremely proficient networks with the capabilities of nutrient acquisition from soil inaccessible to the plant roots [135]. Hence, mycorrhizal symbiosis is promising in alleviating limitations related to nutrient uptake [116]. It is also a very interesting fact that the plants invest almost a hundred times of the energy (in C form) required to produce a root than a single hypha which further travels beyond the exhausted nutritional regions of the soil for sustainable

nutrient supply. These inferences support the cost-effective nature of the mycorrhizal symbiosis [136]. Mycorrhizal symbiosis is propitious for improved soil texture and other physicochemical properties that result in aggregate formation (in dry or wet conditions), improved soil catalytic performances, proper aeration because of hyphal entanglement, balanced soil pH, etc. Fungal hyphae penetrating deep into the soil form a mesh-like hold upon soil particles and result in micro and macroaggregates formation [137]. Glomalin, the fungal exudate is associated with the formation of these aggregates and helps to hold the soil matrix [136]. These aggregates ultimately provide: (a) protection against soil erosion through heavy wind and water flow, (b) porous texture to the soil, (c) carbon fixation by protecting the organic matter decay by other microbial populations and (d) soil moisture regulation [137,138]. In a recent study, it has been indicated that Glomalin related proteins (product of AMF) help in the restoration of eroded lands by increased soil aggregation and organic carbon sequestration [139]. A variety of mycorrhizal biofertilizers are available on the market (such as Rootplus, Vamstar, Myko-win, Rutmy, Farrata, VAM, Mycoxol, etc.) and have been used widely in agriculture for higher crop yield, production, and soil fertility.

5.4. Mitigation of Biotic & Abiotic Stress

Harsh environmental conditions (abiotic stress) and pathogenic attack (biotic stress) are the major intimidations to global agricultural produce. Negative consequences of these stresses can impede plant growth, nutritional inequities, physiological ailments, ionic toxicity, and cause hormonal imbalance. To overcome the negative consequences, plant adopt several physiological, morphological, structural, and biochemical modifications to alleviate stress [39,47]. A mutualistic association with soil microorganisms promises a stress-tolerant approach towards improved plant defense [26]. From previous reports, it appears that myc-plants exhibit more efficiency in growing under stress conditions [23,28,48]. Reports are available describing stress resistance via: (a) regulated ionic uptake for improved osmoregulation (P↑, N↑, Mn, K↑, Na↓, etc.), (b) up-regulated photosynthetic performance, (c) alleviated oxidative stress, (d) enhanced soil catalytic (mainly phosphatases) activities (for improved availability of mineral elements), (e) the dilution effect on harmful salts/minerals, (f) hormonal balancing, (g) regulation of plant-fungus aquaporin and mineral transporter genes, and (h) elevated water status (Figure. 3) [31,36,45,47]. Different mitigation responses for various biotic/abiotic stresses have been listed (Table 3). However, the mitigation mechanisms for various stress conditions have been debatable and, depending upon the associated myc-plant species, mitigation responses may vary.

Table 3. Influence of different mycorrhizal sp. on soil restoration by phytoremediation of toxic metals.

Pollutant	Mycorrhizal Species	Plant Species	Possible Mechanism	Literature Cited
Chromium (Cr)	*Rhizophagus irregularis*	*Daucuscarota*	Reduced translocation, and immobilization of Cr^{6+} through EPS (extracellular polymers) production. distribution of Cr in roots	[140]
	Rhizophagus irregularis	*bermudagrass [Cynodondactylon (Linn.)*	Cr absorption and immobilization by AM roots, Reduction of Cr^{6+} to Cr^{3+} within fungal structures, inhibited Cr flow from roots to shoots,	[141]
	Rhizophagus irregularis	*Taraxacum platypecidum*	Cr absorption and immobilization by AM roots, inhibit Cr translocation from roots to shoots, promoted plant growth	[141]
	Glomus deserticola	*Prosopisjuli flora-velutina*	Accumulation of Cr in vascular tissue and decreased the translocation of Cr into shoots	[142]

Table 3. *Cont.*

Pollutant	Mycorrhizal Species	Plant Species	Possible Mechanism	Literature Cited
Zinc (Zn)	*Glomus mosseae & G. intraradices*	*Vetiver grass*	Increased P uptake by the plant and improved overall growth (*G. intraradices* showed more rehabilitation capacity)	[143]
	Glomu smosseae	*Trifolium pratense*	Zn accumulation in roots which decreases in shoots as the Zn conc. rises to its maximum, improved P sustenance	[144]
	Glomus deserticola	*Eucalyptus globulus*	Increased root to shoot metal accumulation, high metabolic activity, symbiotic effect of saprophytic fungal sp. on mycoremediation process	[145]
Lead (Pb)	*Glomus mosseae& G. intraradices*	*Vetiver grass*	Increased P uptake by the plant and improved overall growth (*G. mosseae* showed more rehabilitation capacity)	[143]
	Glomus mosseae and G. deserticola	*Eucalyptus globulus*	Promoted overall growth, mineral nutrition, chlorophyll production and enzymatic performances (which further increased due to synergistic effect of *G. deserticola* and *T. koningii*), enhanced Pb accumulation	[146]
Aluminium	*Pisolithus* sp.	*Schinusmolle*	Phytoextraction or phytostbilization, Glomalin production supported chelation, rise in photochemical efficacy	[147]
	R. irregularis	*Zea mays*	Increased accumulation of total phytochelating content in shoots	[148]
Copper (Cu)	*Funneliformis mosseae; R. intraradices*	*Capsicum annuum*	Cu Higher total dry weight and the leaf	[149]
	Arbascular Mycorrhizal Fungi (AMF)	*Elsholtzia splendens*	Increase in germination rate and the germination index of the seeds as well as the fresh weights of hypocotyl and radicle	[150]
	Claroideoglomus claroideum	*Oenothera picensis*	Protect plant from metal toxicity, enhance both plant establishment and nutrition	[151]
	R. irregularis	*Phragmites australis*	Stress tolerance via up-regulating photo systems membrane complexes, improved plant growth.	[152]
	Rhizoglomus clarum	*Canavalia ensiformis*	Alleviated amounts of Cu due to phytoextraction in addition to earthworms	[153]
	Rhizophagus clarus	*Canavalia ensiformis*	Alleviated amounts of Cu due to phytoextraction & phytostabilization in addition to bovine	[154]
	Claroideo glomu sclaroideum and	*Oenothera picensis*	Cu chelation with AM-secreted glomalin protein	[155]
Mercury (Hg)	*Glomussp.,Gigaspora* sp. *&Skutelespora* sp.	*Cyperus kyllingia, Lindernia crustacea, Paspalum conjugatum*	P. conjugatum resulted maximum phytoextraction, while C.kyllingia exhibited maximum (Hg) tolerance	[156]
	Native AM fungal morphotypes	*Axonopus compressus, and Erato polymnioides*	A. compressus ensued phythoextracting; Eratopolymnioides–Hg phytostabilization	[157]
	AMF	*Lolium perenne*	Decreased shoot:root (St:Rt) (Hg conc.), increased metal assimilation in roots	[158]
Nickel (Ni)	*Funneliformis mosseae (also named as Glomus mosseae)*	*Festuca arundinacea*	Enhance expression of ABC transporters and metallothione induced metal intoxication, decreased metal translocation	[159]
	Acaulospora sp. *(indigenous)*	*Canavalia ensiformis*		[160]
	AMF mix	*Lens culinaris*	Alleviated uptake by roots and shoots as an effect of mycorrhizal association	[161]
Arsenic (As)	*Rhizophagus intraradices (formerly named G. intraradices)*	*Plantago lanceolata*	Down-regulating phosphate/arsenate transporters could assist plants to enhance the As tolerance	[162]
	Rhizoglomus intraradices & Glomus etunicatum	*Triticum aestivum*	Regulated P/As ratio, enhanced antioxidant production, holding As into non-toxic forms via increased production of biopolymers	[108]
	Rhizoglomus intraradices	*Robiniapseudoacacia*	Induced changes in root morphology, increased shoot-root dry weights, controlled phyto-hormone concentration etc.	[108]
	Acaulospora scrobiculata	*Anadenantheraperegrina*	Promoted P uptake lead to higher growth rates, As concentrations in the roots and shoots.	[109]

Table 3. *Cont.*

Pollutant	Mycorrhizal Species	Plant Species	Possible Mechanism	Literature Cited
Cadmium (Cd)	*Funelliformis mosseae and Piriformos poraindica*	*T. aestivum*	Biomass uplift, imposed catalytic activities for G-SH transferase, catalase, peroxidase etc., and antioxidant genes upregulation	[163]
	Glomus intraradices	*Zea mays*	Mycorrhizae in association with biochar resulted alleviation in Cd accumulation in plant and restricted mobilization, soil rehabiliation	[164]
	Glomus monosporum, G. clarum, Gigaspora nigra, and Acaulospora laevis	*Trigonella foenum-graecum*	Decreased St: Rt Cd ratio, enhanced antioxidant activities	[165]
	Rhizophagus irregularis	*Phragmites australis*	Immobilization of Cd in roots, increased mineral uptake (Mn& P mainly) to survive Cd-toxicity	[166]
	Glomus intraradices, Glomus mosseae, Glomus claroideum, and Glomus geosporum	*Nicotiana tabacum*	Phyto stabilization of lead via immobilization in extraradical mycelial network	[167]
	Glomusmosseae	*Cajanus ajan*	Diminished oxidative disturbances (free radicle formation), high non-protein thiols (-SH) production and high antioxidant activities	[168]
	Claroideoglomus etunicatum	*Sorghum bicolor*	Increased the glomalin content for improved soil, Cd stabilization in mycorrhizal roots &phytoextraction (by shoots), high nutrient uptake	[169]

5.5. Potential Applications in Phytoremediation

Heavy metal accumulation (Pb, Cd, Hg, Al, Cu, Zn, Cr, etc.) in soil due to natural or human activities has been a serious and persistent environmental threat [143,145,147,158,170]. Owing to their non-degradable nature, these toxic substances pollute the natural resources and the food chains which ultimately reflects adverse effects on the atmospheric, aquatic as well as terrestrial ecosystems. Various chemical and physical remediation techniques are there to overcome the negative effects of heavy metals, while bio-remediation techniques have proved to be more promising in terms of cost-effectiveness and maintaining the soil fertility (by preventing serious soil degradation) [72,122,123]. Some of these heavy metals can be excreted from the body, while others accumulate successively depending upon the exposure, dosage, route, etc. and exhibit chronic behavior [170]. Ubiquitous distribution and abundance of Pb has been one of the hazardous effects on the environment, imposing serious harm to plant growth [146]. Some plant species survive in the presence of heavy metals in the soil, which specifies the presence of some expanded mechanistic approach to adapt in such polluted environments, also called phytoremediation. These plants are designated as hyper-accumulators due to this promising characteristic of accumulating high amounts of heavy metals within the tissue [171,172]. Plants involved in detoxification of soil pollutants usually show sluggish growth, taking a longer time in soil cleaning. While, in combination of mycorrhizal symbiosis plants exhibit a high growth rate, increased biomass, phytochemical activities, and therefore, eradication of soil pollutants at a high rate [173]. However, heavy metals as soil contaminants also hamper colonization and spore formation of mycorrhizal fungi due to increment in root exudate production that limits the supply of carbon sources to the fungal symbionts [174,175]. Mycorrhizal association improves phytoremediation efficiencies. The plant species undergo biological modifications such as increased upward translocation of essential minerals (Zn/Cu) and holding harmful metals (Pb/Cd) in the roots to protect the plant which allows them to survive in extreme abiotic stress [9]. There have been some mechanistic behaviors signifying the removal of toxic substances: metal ion immobilization within mycorrhizal structures; blocking the metal uptake by conversion into non-toxic or ineffective complexes in rhizosphere via chelation, bonding with other biomolecules and precipitation; segregation inside the mycorrhizal

vacuole or arbuscules; cytosolic accumulation using biopolymers; stimulating or enhancing antioxidant activities to prevent cellular damage; use of membrane transporters towards or against the concentration gradient for metal translocation; metal ions diffusion to an alleviated response; enhancing nutrient flow to the host; increasing enzymatic efficiencies of soil; stimulating root exudation and up-regulating rhizospheric activities [147,167,169,176]. Further, the impact of mycorrhizal association with hyper-accumulating plant species on phytoremediation efficiencies is depicted (Table 3).

5.6. Enhanced Biological Produce and Agricultural Profitability

Myc-association plays a vital role in managing sustainable plant growth, in addition to improved responses to changing and stressful environmental conditions. As evidenced, the applications of different mycorrhizal species such as *G. coronatum*, *G. mosseae*, *G. decipiens* have been reported for their increased biological yield (cobs per plant, grains, in maize) [110]. Also, these associations have shown enhanced nutritive values through the production of organic (sugars, amino acids) and secondary metabolites (flavonoids, carotenoids, phytochemicals, and volatile organic complexes) [95,111,116,133]. They are responsible for the enhanced C and N fixation, soil fertility, and texture, high food storage, thus a cost-effective approach for the farmers [3]. Another significant factor is the quality yield production, which has been also reported to be enhanced and accompanied by myc-fungi inoculation [81,116,134]. Based on the reported facts, soil microbiota directly influences agricultural profitability [105].

6. Future Prospects

The majority of world flora is associated with the mycorrhizal interactions which contribute to the nutritional or non-nutritional benefits to the host. Based on a systematic literature survey, it can be concluded that mycorrhiza are the key regulators of sustainable ecological performance and contribute to the global flora conservation. To overcome the negative effects of stressful environments, a wide range of mycorrhizal community such as *Funneliformis mosseae*, *Rhizophagus irregularis*, *Glomuse tunicatum*, *Glomus intraradices*, *Glomus mosseae*, *Acaulospora scrobiculata*, and *Claroideoglomus etunicatum* etc. have been observed to successfully mitigate detrimental effects by stimulating the plant's defense system. Mycorrhizae, in response to biotic or abiotic stresses, persuade various plant mechanisms such as the activation of defensive proteins (glomalin), toxins (phenolics and alkaloids), hormonal homeostasis, antioxidants (glutathione, carotenoid), and volatile compounds production (prevents from pathogenic attack) etc. The mechanistic approach behind these effects has been described as (a) the elevated uptake of mineral salts (due to activation of plant and fungal transporter proteins present in the epidermis, root hairs, PAM and extraradical hyphae, respectively; (b) up-regulation of phytohormones; (c) induction of aquaporin genes for increased water uptake; (d) dilution of heavy metal toxicity by increased plant biomass; (e) osmoregulation by producing sugars and amino acids; (f) photosystem (PS) improvisation, etc. Additionally, considering their role in the rehabilitation of contaminated soil, increased soil fertility, stress tolerance or mitigation, improved biological produce, activation of beneficial soil microflora, etc., a huge commercial revenue can be obtained in agricultural terms. While phytohormones are well known to regulate the plant root system and increase stress resistance, their role and action mechanism involved in controlled symbiotic association still needs to be explored. In addition, species' richness of these symbionts and specificity with the host is lesser known due to studies carried out with a limited number of myc-species. Therefore, further studies towards complete understanding of diverse mechanisms underlying mycorrhizal symbiosis are yet to be conducted. In addition, investigation towards the synergistic effects with other microbial moieties can have a great impact. Moreover, the inclination towards ecological habitats is quite diverse, which is of greater interest to the ecological investigations. Transcription and functional analysis have, up to a certain extent, improved the basic understanding of the association between both partners and the taxonomic classification of the mycorrhizal moiety.

Although the molecular profiling of mycorrhizae has elevated in the last decade, further investigations are required for a better understanding of developmental and functional molecular strategies associated with mycorrhizal association in plants.

Author Contributions: Conceptualization, M.M.S. and M.D.; methodology, M.D., L.S. and M.M.S.; validation, M.M.S., P.K. and A.S.; formal analysis, M.M.S., P.K. and A.S.; investigation, M.D.; data curation, M.D.; writing—original draft preparation, M.D.; writing—review and editing, M.D. and M.M.S.; supervision, M.M.S.; project administration, M.M.S. and A.S.; funding acquisition, M.M.S. and A.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded jointly by NMPB (Govt. of India) and Manipal University Jaipur (research project vide Pr. No. R&D/RAJ03/201-17); UGC, India (SRF fellowship).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: The authors express gratitude to University Grants Commission, New Delhi, India, for financial support (in the form of SRF) sanctioned to Mamta Dhiman; National Medicinal Plants Board, Ministry of AYUSH, New Delhi, Government of India, and Manipal University Jaipur for financial support in the form of research project (vide Pr. No. R&D/RAJ03/201-17); and infrastructural support by MUJ.

Conflicts of Interest: The authors declare no conflict to interest.

Abbreviations:

VAM, vascular arbuscular mycorrhiza; rRNA, ribosomal ribonucleic acid; PAM, periarbuscular membrane; AMF, arbuscular mycorrhizal fungi; ECM, ectomycorrhiza; Myc, mycorrhizal ABA, Abscisic acid; Et, Ethylene; GA, Gibberellin; JA, jasmonic acid; SL, strigolactone; SA, salicylic acid.

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Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci.
https://doi.org/10.1007/s40011-022-01359-x

REVIEW

Anogeissus Species in Rajasthan (India): A Comprehensive Review on an Unexplored Plant

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Received: 16 July 2021 / Revised: 12 October 2021 / Accepted: 18 February 2022

Abstract Genus *Anogeissus* of the family Combretaceae is widely distributed in Asia and Africa. In India, this genus includes five species namely *A. acuminata (*Guill. and Perr., *A. latifolia* Wall ex Guill and Perr., *A. pendula* Edgew., *A. sericea var. sericea* and *A. sericea var. nummularia* King ex Duthie. In Rajasthan, all five species are present out of which three (*A. latifolia*, *A. pendula*, and *A. sericea* var. *nummularia)* are of immense importance. These three species are economically advantageous due to their fuel, fodder and timber value. *A. pendula* regarded as the third toughest timber in the world. *A. latifolia* is known for its Ghatti gum, as a substitute for gum Arabic and uses for treatment of fever; wound healing, snake and scorpion bite, etc. *A. sericea* included in red data book of Indian plants is a rare and endemic species of Indian desert which has been extensively overexploited, the narrow extent of occurrence, and very low natural regeneration due to lesser viability causes the species to face severe conservation threat. The present review focuses on distribution, botanical description, economic importance, natural growth constraints, conservation status, and scientific efforts for developing regeneration protocols and most importantly the government initiatives for their conservation on site and off site.

Keywords Propagation · *Anogeissus* · Conservation · Rajasthan · Seed-viability

Significance Statement The review comprised the details of genus Anogeissus and discusses their distribution, botanical description, economic importance, natural growth constraints and conservation status. Also present the scientific efforts made for developing regeneration protocols and government initiatives for their conservation in situ and ex situ.

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Introduction

Anogeissus (Combretaceae) genus comprises species of arid and semi-arid areas producing timber, fuelwood, fodder, and gum [1]. The present review included three species (*A. pendula, A. latifolia and A. sericea*) of genus *Anogeissus which* are economically, ecologically, and ethnomedicinally important in different regions of Rajasthan [2]. According to published report Udaipur, Nagaur, Chittorgarh, and Pratapgarh are four districts of Rajasthan where these species are available for sampling [1]. Among these three species, *A. pendula* and *A. latifolia* found in eastern slopes of the Aravalli ranges, present in four districts (Alwar, Bharatpur, Udaipur, and Dholpur). *A. sericea var. nummularia* King ex Duthie measured as endemic species of Rajasthan and Gujarat included in red data book of Indian plant [3]. Various sites in southern Rajasthan for *A. sericea* var. *nummularia* like Banswara, Bhilwara, Chittor Garh, Dungarpur, Partapgarh, Rajsamand, Sirohi, Tonk, and Udaipur were identified [4]. These mentioned species of *Anogeissus* are widely exploited multipurpose tree species of the Indian desert used for fodder, charcoal, gum production, and furniture manufacture [5]. The regeneration and exploitation rate are not balanced; therefore, two out of three (*A. sericea* and *A. latifolia*) are rare in their natural stands and *A. pendula* may face same problem in the upcoming time. Forest tree species that are

Published online: 03 June 2022

threatened, endemic, and rare are conserved by mass propagation or unconventional methods to prevent these economically viable species. This review describes rapid and efficient methods to increase propagation of these rare taxa of Rajasthan and also discusses about various factors affecting them. Species of *Anogeissus* are commonly used as ethnomedicines in Rajasthan by the Garasia tribes and communities. Gum produced from *A. latifolia* is used in the form of laddu to get relief from back pain after the delivery and to use as a remedy for the damaged tissue [1]. Other medicinal uses are in gastric disorders, skin diseases, wound healing, diabetes, diarrhea, and dysentery [1, 2]. Despite medicinal uses, these forest tree species are also important in ecological aspects (Tables 1 or 2). The wood of these species are immensely important and used in agricultural equipment formation, hut formation, as fodder, and as source of fuel.

Based on the available literature on these species, *A. sericea* has been overexploited for different commercial purposes (for production of agricultural implements and furniture manufacturing) [2, 5]. *A. sericea* has been assigned the status of 'endangered (EN)' and *A. latifolia* 'threatened (*T*)' due to biological uses of resources and excessive use for agriculture equipment's formation [2]. Therefore, the objective of this review is to status of these important species of Aravallis range of arid and semi-arid region.

Material and methods

A thorough literature review was carried out using a variety of peer-reviewed research publications gathered from a variety of internet sources such as Google, ResearchGate, Scopus, Google Scholar, Springer Link, Elsevier, Taylor & Francis imprints, and others. Other resources, including book chapters and web pages, were also evaluated in order to gather as much information as possible about the efficient utilization of Anogeissus species. Several published investigations like- "A critical review on Anogeissus pendula: an important species of arid zone" looked into the significant use of this species in fuel fodder and timber. Online database searching involved the following keywords: Anogeissus sericia var nummalaria, Anogeissus latifolia, Anogeissus pendula, economical importance, ethnobotanical importance, endangered, threatened, critically endangered species, natural regeneration, protocols. Government initiative and conservation steps data collected from different plant conservation board and agencies database system.

Table 1 Comparison of characteristics of major species of genus *Anogeissus* occurs in Rajasthan [6, 8]

Sr. no.	Character	*A. sericea* var. *nummularia* King ex Duthei	*A. pendula*	*A. latifolia*
1	Habit	Moderate size to large trees	Shrub or small trees (9–15 mts)	Small to medium-sized (20 m)
2	Young branch and bark	Tarnished glossy in appearance, non-drooping	Smooth and silvery grey bark, pendulous	The bark is smooth white grey, drooping
3	Leaves	Alternate and many	Opposite or near opposite	Sub opposite
	a)Apex	Blunt and obtuse	Narrow at both ends, acute	Obtuse
	b)Shape	Orbiculate or sub orbiculate Less than 2 cm long	Elliptic	Ovate to elliptic
	c)Size		1–2 cm long Soft and silky with silvery hairs	5–6 cm long Upper surface smooth lower pubescent
4	Flowers	Axillary or terminal	Axillary or terminal	Axillary or terminal
	a)Shape	Globose	Tiny flowers aggregates to form a spherical head (8–14 mm diameter)	Dense sessile flowers form a globose head
	b)Colour	Yellow to brownish-yellow	Greenish-yellow	Yellow
	Calyx	Companulate calyx, pubescent form a cup-like structure, semi-persistent, calyx tube compressed 3–4 mm long	Cup-shaped and prominent	5 sepals form a connate tube
5	Stamen	Exserted, projecting beyond the calyx tube	Present in all-round directions	10 stamens arranged in 2 rows
6	Fruits	Spiky wings on fruit, glabrous	Flat, circular, small, one-seeded, 2 wings present	1 seeded 2 winged, calyx tube persistent packed in a dense head

Table 2 Economical and medicinal uses of different parts of *A. latifolia, A. pendula,* and *A. sericea* var. *nummalaria*

Sr. no.	*Anogeissus* species	Parts used	Uses	References
1	*A. latifolia*	Wood (Timber)	•House construction •Making fuel and agricultural implements	[12–14]
2	*A. pendula*	Wood (Timber)	•Used in agricultural implements such as axles and shafts	[14–16]
3	*A. sericea* var. *nummalaria*	Wood (Timber)	•Wood is useful in making agricultural equipment	[11]
4	*A. latifolia*	Leaves	•Juice is given in purulent discharges from the ear. •Leaf juice is useful in otopyorrhea	[10, 12]
5	*A. pendula*	Leaves	•Produce dark green dye. •Contain tannin, used in the tanning industry. •The paste is used in swelling externally.	[17]
6	*A. sericea var. nummalaria*	Leaves	•Leaves paste is applied for wounds for 3–5 days. •Use in typhoid.	[3]
7	*A. latifolia*	Bark	•Useful in diarrhea, dysuria, cough, colic, liver complaints, snakebite, pain inflammation and skin diseases •The bark is remedy for chronic cough called 'Dangya Khokala'	[1, 18, 19]
8	*A. pendula*	Seeds	•Haemagglutinatig properties against the human A, B, and O red cells	[14, 16]
9	*A. latifolia*	Roots	•Useful in abdominal disorders •Stomachic and thermogenic.	[12]
10	*A. pendula*	Aerial parts	•Used for diabetes •Diuretic and cardiovascular stimulant potential.	[20, 21]
11	*A. latifolia*	Gum	•Consumed generally as a tonic and after delivery	[1]

Distribution in Rajasthan

Total 11 species under the genus *Anogeissus* are reported, and they are majorly distributed in the region of Arabia, South East Asia, and Africa. This genus is a group of three important multipurpose species, namely *A. latifolia, A. pendula, and A. sericea var nummalaria* [6]. *A. pendula* Edgew., locally known as *Kardhai*, is distributed throughout tropical Asia and Africa. Further, it is distributed in many parts of Rajasthan, particularly in Ajmer-Marwar forests and at Abu in the southwest end of Aravalli hills. This species is also a dominant tree of the Aravalli hills of Rajasthan where it forms a pure forest. The main species found in this kind of forest are *A. pendula, A. latifolia.* These forests are mostly distributed in small patches in different parts of the northern and eastern slopes of the Aravalli ranges, accessible in Alwar, Bharatpur, Udaipur, and Dholpur districts (Fig. 1). Patchy growth of these species of dry deciduous forests is found along the dry riverbeds of Jalore, Nagaur, Pratapgarh, Chittorgarh Ganganagar, and Bikaner districts [4]. *A. sericea* var. *nummularia* King ex Duthie is endemic species. It is also popular with the name Indrokiya. Indrok is considered as the threatened plant species of arid and semi-arid regions of India. It is majorly found in the Gujarat, Rajasthan, and Punjab regions of India [7]. In Rajasthan, during the botanical explorations, the species *A. sericea* var. *nummularia* King ex Duthie was observed in specific districts of southern like Banswara, Bhilwara, Chittor Garh, Dungarpur, Partapgarh, Rajsamand, Sirowhi, Tonk, and Udaipur (Fig. 1) [2]. Dry Tropical deciduous type of vegetation is the characteristic feature of the region which includes some important plant species like *A. latifolia* (Roxb. ex DC.) Wall. Ex Guill and Perr., *A. pendula* Edgew, *Balanites aegyptiaca* (L.) Delile., *Boswellia serrata* Roxb., *Diospyros melanoxylon* Roxb., *Madhuca indica* J.F. Gmelin, *Tectona grandis* Linn. f., *Terminalia arjuna* (Roxb. ex DC.) Wight and Arn. etc. [1].

Botanical Description

Genus *Anogeissus* involves shrubs, small-to-medium height trees having pendulous or non-pendulous branches that bear alternately or oppositely arranged petiolate leaves. Flowers, solitary, or racemose born on axillary or terminal peduncle in such a manner that inflorescence appears as the globose head [6]. This dense structure has numerous, small, winged fruits packed in it. These characters are similar in the species which are prominent in

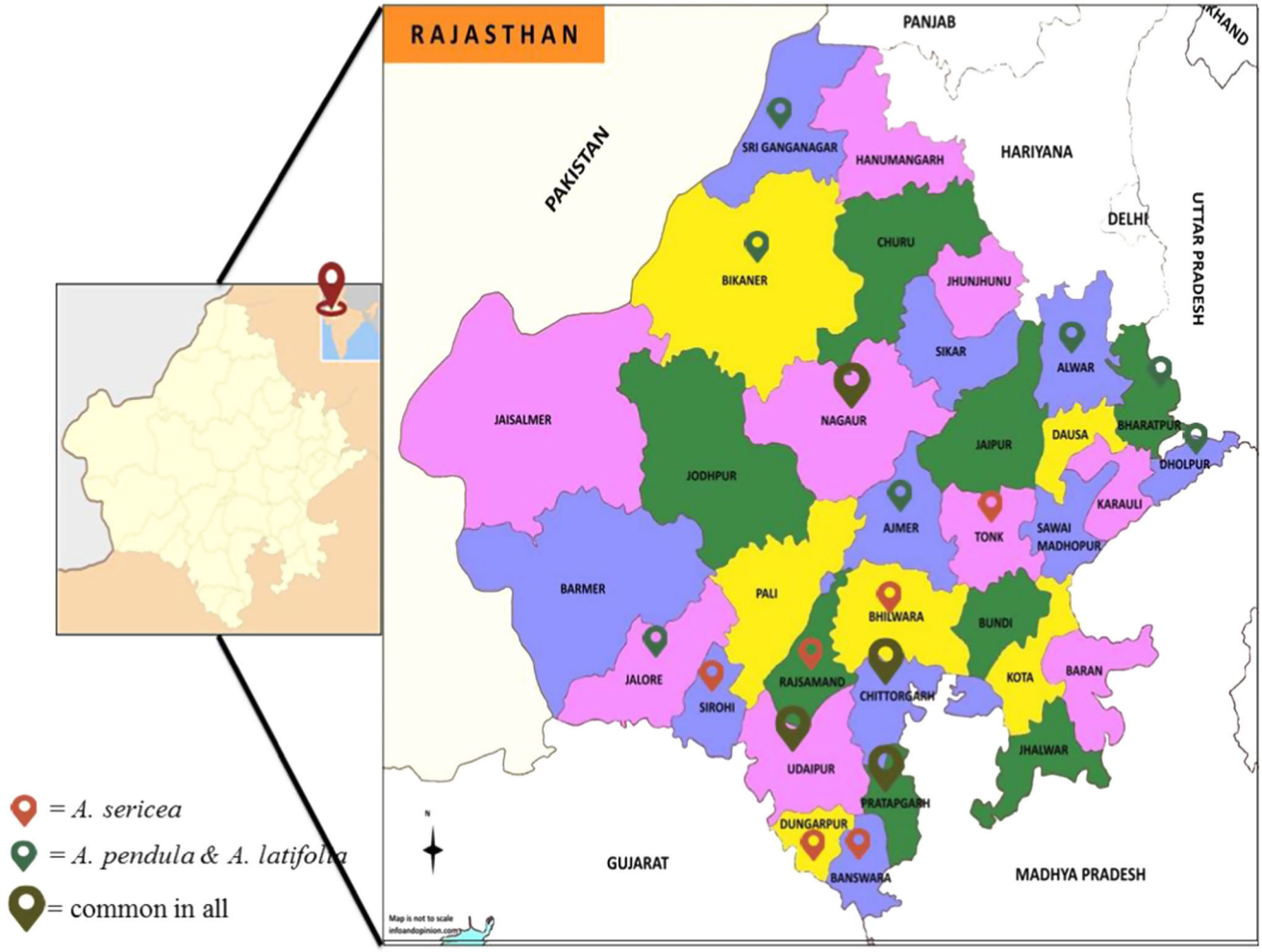

Fig. 1 The distribution of the various species of *Anogeissus* such as *A. sericea, A. penduda,* and *A. latifolia* in different districts of Rajasthan

Rajasthan, i.e., *A. latifolia*, *A. pendula*, and *A. sericea* var. *nummularia* [8]. However, these species can be distinctly identified from each other by the characters explicated in Table 2.

Economic Importance of *Anogeissus* Species

Leaves and bark of *A. latifolia* are used for tanning. *A. latifolia* is also called as the "Ghatti tree" due to the production of "Indian Gum" or "Gum ghatti" which is a non-starch polysaccharide economically viable as an emulsifier [9]. It has been observed that ethanolic extract of *A. latifolia's* bark help in hastening of wound healing. Its bark is used for the treatment of fever by natives in Udaipur and its paste is applied on scorpion sting by the tribal communities residing in the wildlife sanctuary of Gundlabranhmeswaram. Bark also has medicinal value as a sedative and helps in the treatment of hypertension [10].

The foliage of *A. pendula* can be used as fodder for livestock. Also used as fuel and yields timber, which is considered as the third toughest timber of the world and thus valuable [7]. *A. sericea* var. *nummularia* wood is useful in making agricultural equipment and huts by tribal communities. Report shows that leaves of *A. sericea* used in typhoid and also applied on wounds [11].

Growth Constraints

Three species of genus *Anogeissus* (*A. sericea* var. *nummularia*, *A. latifolia,* and *A. pendula*) are widely exploited in the arid and semi-arid regions of Rajasthan due to their immense uses as fodder, charcoal, agriculture equipment's formation, and furniture manufacture [9, 10, 18]. These tree species are beset germination frequency is (0.1–0.2%). Seed viability of *Anogeissus* species declines with time period results in low seed germination. The main reason of low seed germination frequency in *A. pendula* is

production of infertile seeds, in *A. latifolia* seeds are about 95% empty at the time of collection. This emptiness /infertility are responsible for 1–2% seed germination and in *A. sericea* var. *nummularia* seed viability is very low (0.1–0.2%) [22, 23]. Therefore, natural regeneration through seeds is not suitable or reliable. Vegetative propagation through rooting approaches is not available yet for these species due to their inherent regeneration difficulties. Regeneration and exploitation rate are not balanced, lead to these species rare in their natural stands due to low seed viability for these species (*A. pendula*—0.2–0.4%, *A. sericea*—0.1–0.2 %, *A. latifolia*—0.1%) [24]. Taking into the consideration economic importance of these forest tree species and the absence of essential conventional methods of propagation, it would be extremely important to develop *in vitro* procedures or tissue culture-based methods for mass multiplication of important forest tree species.

Conservation Status

Anogeissus latifolia

A. latifolia (Roxb. Ex. DC) Wall. Ex Guill and Perr. is one of the threatened species that need to be conserved. The tree yields good fuelwood and charcoal, its foliage is used to feed silkworms and cattle's and it's the main source of Indian gum, Ghatti gum which is used as an alternative for Arabic gum used in dye production [9]. *A. latifolia* is the survivor of the eroded land which helps in controlling soil erosion and sustains the soil nutrient cycling. The litter decomposition rate is high thus helping in the enrichment of the soil.

Possible Reasons for Becoming Threatened

a) **Seed Viability**

Generally, seed viability is low (0.1%) but it can be increased after a very dry season. Only full mature seeds are capable of germination [23].

b) **Insects and Pest**

The tree is prone to pest attack like stem borers such as *Olenecamptus anogeissus* and *O. indianus.* Sapwood gets infected due to ectoparasitic fungi as *Sarcinella combratcearum.*

iii) **Anthropogenic Activities**

Due to intensified anthropogenic activities, and low seed viability, the rate of regeneration is low in comparison to the pace of the utilization [25]. If the exploitation of *A. latifolia* continues at current rate then this species may be extinct in near future.

Anogeissus pendula

A. pendula is a multipurpose plant. The quality of the fuel, timber, fodder, and value of its foliage make this species important in every aspect of livelihood. Timber is used for production of agricultural implements. The study reveals that *A. pendula* help in maintaining the balance of species in its community as it suppresses the shrubs like *Grewia flavescens* and *Adhatoda zeylancia*. The litter fall of the plant is immense and thus helps in organic matter formation and maintaining the fertility of the soil [21].

Possible Reasons for its Depletion

a) **Low Seed Germination**

The plant is used extensively but the major problem with replenishment is its low germination frequency which makes its propagation difficult in addition to the fact that's only 5% of seeds are viable out of the whole percent which supports that infertile seeds are a drawback for the successful depression [23].

b) **Invasion of Other Species**

Its growth is directly affected by the insects and pathogenic fungi known as *Corynespora* compete, defoliator like *Eutectona machaeralis* are responsible for extensive defoliation of the species [9].

The extensive use and slow rate of germination may create its scarcity shortly may lead the plant to be involved in the threatened categories as the other two species mentioned in this review.

Anogeissus sericea var. nummularia

A. sericea var. nummularia King Ex. Duthie falls under the category of threatened and rare species and also endemic to the region of its occurrence. Leaves are used to feed livestock [26].

Possible Reasons for its Decreasing Population

a) **Anthropogenic Pressure and Low Regeneration**

Continuously increasing Anthropogenic pressure (use of stem for preparation of huts and increasing industrialization), resulting in extinction risk of the species and its very low regeneration rate is the reason species facing severe conservation threat [24].

b) **Grazing**

Fodder present in this region is insufficient to feed livestock, grazing is also a seemingly unavoidable concern that leads to a decrease in the plant populations [9].

iii) **Invasion of Other Species**

The invasion of other species impacts the distribution of the *A. sericea* species. And the pressure for other such species (*Prosopis juliflora*, *Lantana camara*, and *Parthenium hysterophorus*) allelopathy effects leads to the smallest distribution of *A. sericea* in Rajasthan [2]. These all threats in conjunction put excessive pressure on the species pushing it to the belt of endangered.

These need to be raised and planted further to ensure a good population in their ecosystem either by or in-situ conservation strategies.

Scientific Efforts for Replenishment

Due to lacking natural propagation methods, the role of the scientific community is to develop the commercially viable propagation protocol and forest departments should ensure the good population of these plants by raising the seedling and planting them in forest. A protocol has been successfully developed for the large-scale production of *A. pendula* and *A. latifolia* by axillary branching method at Tata Energy Research Institute, Delhi by Sanjay Saxena and Vibha Dhawan [23]. This protocol produced almost 56,0000 tissue cultured plantlets of both species which were successfully planted by state forest department. Similarly, S.K Tiwari et al. tried to use stem branch cutting and standardized the technique by using different concentration of IBA [23, 27]. A very well documented protocol for *in vitro* propagation of *A. sericea* var. *nummularia* has been provided by A. Yusuf for mass propagation of this rare tree at plant Biotechnology laboratory, J.N Vyas University. Jodhpur [24].

Government initiatives for ex-situ or in-situ conservation of species of *Anogeissus.*

Besides scientific efforts, awareness and involvement of native people is mandatory for the depleting *Anogeissus* species. Establishment of more biosphere reserves, National parks, and Wildlife sanctuaries is the key for the protection of state biodiversity. Based on convention on Biological diversity, steps are already been taken like in Southern Rajasthan *Anogeissus* species conserving sanctuaries are established [4].

A. latifolia present in all nine sanctuaries, whereas *A. pendula* present in eight except Phulwari sanctuary, which is second largest of the state. *A. sericea* var. *sericea* is restricted to three sanctuaries in Udaipur and Sirohi district. Sajjangarh, Jaisamand, Kumbalgarh, and Raoli are richest sanctuaries having four *Anogeissus* species out of five, whereas Sitamata, Bassi, Bhensroadgarh, and Mount Abu have only three. Foundation of ecological security (FES) has also been set up which enables the eco-restoration in association with village communities and collect ecological data for conserving the biodiversity of the protected areas. Other initiatives required are mapping of biodiversity, mapping of status of threatened species richness, forming conservation actions plans, and monitoring for their execution. For ex-situ conservation germplasm can be preserved in seeds banks or establishment of Botanical gardens may help. All these measures need support from government to be implemented.

Conclusion

In this review, we have made an effort to explore some important species of genus *Anogiessus* and provide information on their distribution in Rajasthan, botanical description, and their economic importance. Even though *Anogeissus* genus represents 11 species in Asia and Africa, but as mentioned earlier we conclude only three important species, which are distributed in different districts of Rajasthan. Studies were done on these species due to the various wide ranges of reported ethno medicinal and economical uses in different districts of Rajasthan. These species are as *A. pendula*, *A. latifolia* and *A. sericea* are under threat due to over-exploitation for different commercial purposes. These species are used for their fodder, timber, gum, and tanning along with their ethno medicinal uses in tribal communities of Rajasthan. The present review revealed that the most commonly used plant parts, leaves, and stem followed by the gum, seed, root, and its bark. The published report depicts that the traditional mode of use is decoction followed by juice and paste of leaves. Dependency on the products of these species for their daily needs is most prominent which is directly disturbed by the loss of trees. Some studies also show the biotic disturbances, mainly from the overexploitation of tree resources for their fuel and fodder. After reviewing these plants in natural environment, it can be concluded that due to over utilization, these plants are decreasing at fast pace in contrast to the reproduction and it's unavoidable to support their existence by equal efforts of scientific community and government of Rajasthan in collaboration with native people.

Acknowledgements The authors wish to thank Dr. Devendar Bhardwaj RFS, Chief manager technical (retd.) (Rajasthan forest training institute) for his advice and assistance during the course of review. Authors also gratefully acknowledge the facility provided by the Department of Biosciences, Manipal University Jaipur.

Declarations

Conflict of interest All authors declare that they have no conflict of interest.

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