Bio-stimulants and Plant Growth Regulators

Applications and Signaling Mechanism

Editors

Dr. Vijaya Nikam
Dr. Swarupa Agnihotri
Dr. Pratishtha Nagane
Dr. Manasi Patil

NP
Nature Light Publications

An International Edited Book *ISBN-978-81-981142-7-3*

Biostimulant and Plant Growth Regulator: Applications and Signaling Mechanism

Editors

Dr. Vijaya Nikam
Associate Professor
Department of Botany
Rayat Shikshan Sanstha's
Sadguru Gadage Maharaj College, Karad
(Dist. Satara) - 415 124, Maharashtra, India.

Dr. Swarupa Agnihotri
Assistant Professor
Department of Botany
B.N. Bandodkar College of Science (Autonomous), Thane (MH.).

Dr. Pratishtha Nagane
Assistant Professor
Department of Botany
Rayat Shikshan Sanstha's
Balwant College, Vita, Tal- Khanapur, Dist.- Sangali (MH.).

Dr. Manasi Patil
Assistant Professor
Department of Botany
Rayat Shikshan Sanstha's
Sadaguru Gadage Maharaj College Karad
(Dist. Satara) - 415 124, Maharashtra, India.

Published By

Nature Light Publications, Pune

Biostimulant and Plant Growth Regulator: Applications and Signaling Mechanism

Editors

Dr. Vijaya Nikam
Dr. Swarupa Agnihotri
Dr. Pratishtha Nagane
Dr. Manasi Patil

First Edition: 14 January, 2025

An International Edited Book

ISBN- 978-81-981142-7-3

Published by:

Nature Light Publications, Pune
309 West 11, Manjari VSI Road, Manjari Bk.,
Haveli, Pune- 412 307.
Website: www.naturelightpublications.com
Email: naturelightpublications@gmail.com
Contact No: +91 9822489040 / 9922489040

Preface

We are happy to welcome the idea of publishing a book on relevant topic, "Biostimulant and Plant Growth Regulator: Applications and Signaling Mechanism". Further, it is good that the articles from various sub-disciplines are included in the book. The scholars from biological science have attempted to identify the current trend and to provide ideas to doing the recent study.

Agricultural productivity and sustainability are critical to ensuring food security in an era of increasing global challenges such as climate change, soil degradation, and environmental stress. To address these concerns, innovative approaches that enhance plant growth, resilience, and yield without relying excessively on synthetic agrochemicals have gained significant attention. Among these, bio-stimulants and plant growth regulators (PGRs) have emerged as powerful tools in modern agronomy, offering sustainable solutions to improve crop performance through physiological and biochemical modulation.

This edited volume, Biostimulant and Plant Growth Regulator: Applications and Signaling Mechanism, presents a comprehensive exploration of the role of bio-stimulants and PGRs in plant development. It brings together contributions from leading researchers and experts in the field to provide insights into their mechanisms of action, signaling pathways, and practical applications in agriculture. The book covers a broad spectrum of topics, including: Biological Suppression of Parthenium hysterophorus by Some Strong Allelopathic Weeds, Plant Bio Stimulants Implication in Sustainable Agriculture, Biochar and Its Interactions with Soil Microbiota: Mechanisms and Applications, A Probable Bacterial Fungicide for Management of Powdery mildew diseases, Natural Bio-stimulants for Enhancing Plant Tolerance to Salt and Salinity Stress: A Review, A perspective of Allelopathy in Agriculture, Influence of Various Growth Regulators on Morphological Status of Curcuma caesia Roxb, Study of Antioxidant Activity by Various Methods in Averrhoa carambola L.: A Review, Silver Nanoparticles as Bio stimulants, Phytonutrient Studies and Antimicrobial Activity of Sphagnum Moss, Use of Plant Growth Regulators for In-

vitro Culture of Plants, Biosorption And Growth Promoting Efficacy of Seaweed, Liquid Fertilizer from Ulva Fasiciata (L.), Analysis for Pesticide Residues and its impact on the Molecular Profile of Common Leafy Vegetables, The response of plants growing under stress environmental conditions, Physiology Of Mangrove Fern Acrostichum Aureum L. From West Coast of Maharashtra, Recent advances, emerging trends, and regulatory considerations in biostimulant and PGR research.

By bridging fundamental scientific knowledge with real-world agricultural applications, this book serves as an essential resource for researchers, agronomists, students, and industry professionals seeking to harness the potential of bio-stimulants and PGRs for sustainable crop production. The insights presented here aim to foster innovation in plant science and agronomy, paving the way for more resilient and efficient agricultural practices.

We extend our heartfelt gratitude to all contributors whose expertise and dedication have enriched this volume. We also acknowledge the ongoing efforts of scientists and practitioners working toward the development of sustainable solutions for global agriculture. We hope this book serves as a valuable reference and inspires further research in this dynamic field.

Date: *14 January 2025*

Editors

Biostimulant and Plant Growth Regulator: Applications and Signaling Mechanism

Table of Content

BIOSTIMULANT AND PLANT GROWTH REGULATOR: APPLICATIONS AND SIGNALING MECHANISM

ISBN: 978-81-981142-7-3| Year: 2025 | pp: 01 - 08 |

Biological Suppression of *Parthenium hysterophorus* by Some Strong Allelopathic Weeds

Jai Knox

Associate Professor, Department of Botany, Wilson College (Autonomous), Mumbai-400007

Email: jai.knox@wilsoncollege.edu

Article DOI Link: https://zenodo.org/uploads/14785563

DOI: 10.5281/zenodo.14785563

Abstract

In the present investigation Shoot Cut Bioassay, Seedling Bioassay, Chlorophyll, Nitrogen, and Protein Content, was analysed on *Parthenium hysterophorus* by the shoot leachates of *Cassia occidentalis* and *Calotropis procera* at 25%, 50%, 75% and 100% including control. It has been observed that at high concentration of *Cassia* shoot leachates against *Parthenium*; the phytotoxic damage rating scale was maximum and was found to be statistically significant, followed by *Calotropis*. Similar results were also observed in total chlorophyll, nitrogen and protein content of *Parthenium*. Data revealed that *Cassia* can be a promising weed to curb the population of *Parthenium*.

Keywords: Allelochemicals, Biochemical interaction, Coffee weed, Congress grass, Small Crown flower plant.

Introduction:

Parthenium weed is an aggressive annual herb, colonising roadsides, fallow land and overgrazed pastures where grass cover is having less growth. It does not normally establish in vigorous pastures or intact native vegetation. As it can only reproduce and spread by seed, the seed ecology of *Parthenium* weed contributes greatly to its success in semi-arid environment. *Parthenium hysterophorus* L. is a branching, annual herb with pale green lobed leaves, a deep taproot, and an erect stem with several branches. It can grow to more than two metres high. It grows best on alkaline, clay-loam to heavy clay soils in areas where rainfall is greater than 500 mm per year. *Parthenium* weed is a serious problem. In grazing country, it can dominate pastures under continued heavy grazing and has the potential to exclude useful forage plants, thus decreasing pastures productivity,

carrying capacity and land values. *Parthenium* weed has been shown to be related to health problem for some people living or working in close proximity to it. Individuals in contact with *Parthenium* can develop sensitivity to the plant, which may then manifest as an allergy-type response. Many plants release some chemicals called allelochemicals during different growth stages which may affect the growth and development of adjoining plants. The phenomenon of one plant having detrimental effect on another plant through the production or release of toxic chemicals has been termed Allelopathy. The first observation that antagonistic competitor plants could replace *P. hysterophorus* and therefore had potential for biological control appears to have been made by Singh ,1983 who noted that *Cassia uniflora* moved into areas previously occupied by *Parthenium* weed, in the Maharashtra State of India. Aqueous foliar leachates of *Azadirachta indica* and *Prosopis juliflora*, inhibit seed germination of *Parthenium* by more than 95 percent (Dhawan et. al., 1994). It has been observed that *Tephrosea purpurea, Croton bonplandianum, Sida cordifolia* and other species of *Cassia* have been found to suppress *Parthenium*. Based on the field survey it has been observed that *Cassia* occidentalis and *Calotropis procera* is showing strong allelopathic potential against *Parthenium*. *Cassia occidentalis* Linn. commonly called as coffee weed belongs to the family Caesalpiniaceae. It is an erect, annual shrub. The leaves are pubescent and are 3 to 6 cm long. The inflorescence is axillary cyme. The flowers are purplish in colour and its flowering time is July to September. Fruit is a legume. *Calotropis procera* (Willd.) Dryand. ex W. Ait. commonly called as milk weed belongs to the family Asclepiadaceae. It is an annual, erect shrub. Milky latex is present in stem and leaves of plants. The inflorescence is polychasial cyme. The flowers are purplish white in colour and its flowering time is July to November. Fruit is an Etaerio of follicle. Therefore, this study is performed to assess the potential of above selected weeds on the growth and development of *Parthenium*.

Materials and Methods

The upper parts of shoot tips were collected from the selected botanic agents/plants. 100 grams of shoot tips were soaked in 500ml of double distilled water each under aseptic conditions for 9 days and placed in conical flasks in a refrigerator at 8 ± 1°C. The aqueous leachates were filtered through three layers of muslin cloth/cheese cloth to remove debris. The filtrate was then re-filtered through one layer of Whatmann no. 1 filter paper. Leachates of 25%, 50%, 75% and 100% concentration were prepared with sterilized distilled water and used for bioassay.

Shoot Cut Bioassay

Shoots of *Parthenium* (5.0 cm) with one or two inflorescences were taken. The inflorescences are washed in tap water. Then they were dipped in 1% NaOCl solution for 3 minutes. The tips of the shoots were immediately washed in sterilized distilled water to remove any

residual trace of the chemical. An inclined cut was made at the tip and the shoot were placed in test tubes containing 10 ml of shoot leachates of 25%, 50%, 75% and 100% concentrations of botanic agents. The test-tubes were sealed with cotton buds and aluminium foil to make it air tight. Effect of leachates was observed after 72 hours at room temperature. Phytotoxic damage was recorded on the basis of rating scale of 0-5, where 0 = no effect, 1 = slight chlorosis and slight necrosis, 3 = acute chlorosis and marked necrosis/drooping of entire twig, 4 = falling of flowers and leaves/high necrosis and chlorosis, 5 = acute chlorosis and very high necrosis leading to death of the whole shoot.

Seedling Bioassay

Seedling of *Parthenium* were raised in plastic pots containing sterilized soil, sand and peat (1:1:1) and placed at room temperature 25 ± 1°C. These seedlings were sprayed with shoot leachates of 25%, 50%, 75% and 100% concentrations of botanic agents. Observations regarding the toxicity of seedlings were made after 72 hours. Phytotoxic damage was recorded on the basis of rating scale of 0 – 4 where 0 = no effect, 1 = slight chlorosis, 2 = marked chlorosis, 3 = drooping of seedling, 4 = death of seedling.

Chlorophyll Estimation

Chlorophyll content of *Parthenium hysterophorus* was estimated according to Arnon's, 1949. 40mg (0.04gm) of *Parthenium* leaves were treated with 25%, 50%, 75% and 100% concentration of shoot leachates of botanic agents. The treated *Parthenium* leaves were placed in black plastic bottles containing 10ml of 80% acetone and then it was sealed with adhesive tape at its mouth so that acetone may not get evaporated and kept undisturbed in a refrigerator for 5 – 6 days at 8 ± 1°C temperature. After 6 days optical density was recorded by spectrophotometer at different wavelength i.e. 480, 510, 630, 645, 652, and 665 nm. Using following formula amount of Chl 'a', Chl 'b' and total chlorophyll was calculated and tabulated in tables. Chl 'a' = 15.6 (O.D. at 665nm) – 2.0 (O.D. at 645nm) – 0.8 (O.D. at 630nm)

$$\text{Total chlorophyll} = \frac{\text{O.D.at 652nm}}{34.5} \times 1000$$

Chl 'b' = Total chl – Chl 'a'

Estimation of Nitrogen

Nitrogen was estimated by following the method of Snell and Snell, 1955. 100 mg (0.1 gm) of *Parthenium* leaves were treated with 25%, 50%, 75% and 100% concentration of shoot leachates of botanic agents. Then, the treated *Parthenium* leaves were placed in 50 ml conical flask and mixed with 2ml of conc. H2SO4 and then it was heated on hot plate at 40°C. When volume reduces to half of the original volume, 1.5ml of 30% H2O2 was added. Then the solution was heated gently at 10 - 20°C till the clear extract was obtained. The content was then transferred in 100 ml volumetric flask and the volume was made up to the mark with distilled water. After preparation of acid extract of plant

material, the nitrogen was estimated as follows:

1.0 ml of prepared acid extract from plant material was taken in 50 ml volumetric flask. To these 10 drops of 10% NaOH and 10 drops of 10% sodium silicate was added and the solution was diluted up to the mark. 1.0 ml of freshly prepared nesseler's reagent was added to the flask, the colour intensity was measured by colorimeter after 15 minutes at transmittance of 420 nm using a reagent blank as reference. With the help of standard curve prepared with 100 ppm NH_4Cl solution the amount of N_2 in the sample was found out.

Protein content

The protein content in plant sample was calculated by multiplying percentage nitrogen content of plant sample by the factor of 6.25.

Percentage of Protein = % of Nitrogen × 6.25

Statistical analysis

Statistical analysis of the data recorded was done. The design is Factorial Completely Randomized Design (FCRD) and conclusion was drawn from the data on the basis of Twoway Analysis of Variance (ANOVA) technique. The software used is INDOSTAT, version 98. The calculated values were compared with tabulated value at 5% level of significance (Fisher, 1935) for the appropriate degree of freedom.

Results and Discussion

Shoot Cut Bioassay

Table-1 revealed that maximum phytotoxic damage on the rating scale was inflicted in 100% shoot leachates of Cassia occidentalis i.e. 5.00 against shoots of *Parthenium*, followed by P2 in which phytotoxic damage on the rating scale was found to be 4.20. In control, no effect was observed. In 75% and 50% concentration similar results were observed. Minimum inhibition was observed in 25% concentration that is 3.2 and 1.3 in P1 and P2 plant, respectively. It was observed that shoot leachates of 9th day, at 100% concentration after 72 hours shows maximum phytotoxic damage on the rating scale, and they differ significantly.

Seedling Bioassay

It was observed in table-2 that maximum phytotoxic damage on the rating scale was inflicted in 100% shoot leachates of *Cassia occidentalis* i.e. 4.00 against shoots of *Parthenium*, followed by P2 in which phytotoxic damage on the rating scale was found to be 3.20. In control, no effect was observed. In 75% and 50% concentration 2.8 and 1.8 damage rating scale was observed in P1 and P2 plant, respectively. Minimum inhibition was observed in 25% concentration that is 1.0 and 0.3 in P1 and P2 plant, respectively. It was observed that shoot leachates of 9th day, at 100% concentration after 72 hours shows maximum phytotoxic damage on the rating scale, and they differ significantly.

Chlorophyll Estimation

Chlorophyll 'a', 'b' and Total Chlorophyll

Table - 3 revealed that maximum inhibition in Chl 'a' of Parthenium was

by 100% shoot leachate of Cassia (P1) i.e. 8.20, followed by Calotropis (P2) in which 12.56 Chl 'a' was observed. Highest value was observed in control i.e. 21.24. 100% concentration was found to be significant over other concentrations. Table – 3 depicted that maximum inhibition in Chl 'b' of *Parthenium* was by shoot leachate of *Cassia* i.e. 2.34, followed by *Calotropis* in which 3.00 Chl 'b' was observed. In control 3.42 Chl 'b' was observed. 100% concentration was found to be significant. Maximum inhibition in total chlorophyll of *Parthenium* was observed by 100% shoot leachate of Cassia i.e. 10.54, followed by *Calotropis* in which 15.56 total chlorophyll was observed. Highest total chlorophyll was observed in control i.e. 24.66.

Estimation of Nitrogen and Protein content

Table-3 revealed that maximum inhibition in percent nitrogen of *Parthenium* was by 100% shoot leachate of *Cassia* i.e. 3.15, followed by 75%, 50% and 25% in which 3.48, 3.88 and 4.33 nitrogen percentage was observed, respectively. Different data was observed in P2 plant. At 100% shoot leachate of *Calotropis* nitrogen percentage was found to be i.e. 5.81, followed by 75%, 50% and 25% in which 6.00, 6.87 and 7.89 nitrogen percentage was observed, respectively. Highest percent nitrogen was observed in control i.e. 5.85. 100% concentration is significant over other concentrations. Table-3 revealed that maximum inhibition in protein content of *Parthenium* was observed in the 100% shoot leachate of *Cassia* i.e. 19.68, followed by *Calotropis* in which 36.31 protein content was observed. Highest protein content was observed in control i.e. 36.56. The data differs significantly. The allelopathic properties of plants can be explained successfully as a tool for weed reduction. The chemical exudates from allelopathic plants are proposed to play a major role in the allelopathy mode of action. *Parthenium hysterophorus* L., a common problematic weed of wastelands as well as crop fields in India is well known of its lethal allelopathic effect on many agricultural crops including chickpea, kodo mustard, linseed etc. (Oudhia and Tripathi, 1998). It also has the potential to disrupt natural ecosystem and becoming a curse for biodiverity at many places in India. In order to manage this noxious weed in ecologically safe and viable manner, at present there is a trend towards searching for allelopathic plant species which has the potential to replace this weed through allelopathic mechanism. During field surveys it has been observed that at some sites *Cassia occidentalis, Calotropis procera, Chenopodium album, Croton bonplandianum, Abutilon theophrasti, Acacia arabica* have replaced this weed in patches. Mamatha and Mahadevappa (1992) based on their preliminary surveys have reported that *Cassia sericea, C. tora, Tephrosia purpurea* and *Croton bonplandianum* restricted *Parthenium* invasion in many states in India. The allelochemicals present in *Azadirachta indica* have inhibitory effect

on the germination and seedling growth of *Parthenium* and *Amaranthus*. There was a variability in germination percentage, shoot and root growth of seedling due to the treatments. The degree of phytotoxicity of senescent leaves was more as compared to green leaves and bark was more inhibitory to germination of *Parthenium* and *Amaranthus*.

Inhibition of *Parthenium* seed germination was absolute in aqueous foliar extracts of *Cassia occidentalis*, *Andrographis paniculata* and *Abutilon indicum*. There was no stimulatory effect of extracts on seed germination and seedling growth to test *P. hysterophorus*. All the species tested, inhibited the early seedling growth and fresh weight of the seedling. *Cassia sericea* recorded the highest reduction in seedling length i.e. 87.66 and 87.31% by leaf and whole plants extract, respectively at 50% concentration followed by Hyptis suaveolens (83.89 & 84.55%). These plants also caused maximum reduction in the seedling fresh weight of *Parthenium hysterophorus* (Senthil et. al., 2004). The grazing animals dislike Cassia due to this pungent odour and this is considered as desirable traits, as it repelled the cattle and successfully replaced *Parthenium* even in unprotected land. Its rapid rate of growth, wide range of ecological adaptability, sufficient quantity of seed production and its utility are also considered for its choice to suppress this weed. *Cassia* also has the allelopathic action which hampers the growth of *Parthenium*. Further, research at the University of Agricultural Science, Bangalore confirmed that the plant botanical agents (BA's) could exert allelopathic impact and hinder germination and growth of *Parthenium* both in green house and in its natural habitat.

Conclusion

Evidence showed that higher plants release a diversity of allelochemicals into the environment which includes phenolics, alkaloids, long-chain fatty acids terpenoids and flavonoids. Once the most promising phytochemicals are identified along with their preference, they can be recommended for mass scale cultivation in *Parthenium* infested pockets for subsequent replacement of this noxious weed.

Table-1. Herbicidal potential of shoot leachates of *Cassia occidentalis* and *Calotropis procera* on bioactivity of *Parthenium hysterophorus* Shoot-Cut Bioassay

Allelopathic plants	Incubation periods and concentration ± Phytotoxic damage rating			
	9 th day extract after72 hours exposure time			
	25%	50%	75%	100%
P_1	3.2±0.2	4.3±0.3	4.3±0.7	5.0±0.0
P_2	1.3±0.4	3.6±0.3	3.8±0.2	4.2±0.0
Control	0.0±0.0			

n = 10; Mean± Standard Error; Shoot cut bioassay; 0 = No phytotoxicity, 5 = Highest phytotoxicity

P_1= *Cassia occidentalis*
P_2= *Calotropis procera*

Table-2. Herbicidal potential of shoot leachates of *Cassia occidentalis* and *Calotropis procera* on bioactivity *of Parthenium hysterophorus*
Seedling Bioassay

Allelopathic plants	Incubation periods and concentration ± Phytotoxic damage rating			
	9 th day extract after72 hours exposure time			
	25%	50%	75%	100%
P_1	1.0±0.0	2.0±0.4	2.8±1.4	4.0±0.0
P_2	0.3±0.0	1.6±0.1	1.8±0.2	3.2±0.0
Control	0.0±0.0			

n = 10; Mean± Standard Error; Shoot cut bioassay; 0 = No phytotoxicity, 4 = Highest
phytotoxicity
P_1= *Cassia occidentalis*
P_2= *Calotropis procera*

Table-3. Effect of *Cassia occidentalis* and *Calotropis procera* on chlorophyll, nitrogen and protein content of *Parthenium hysterophorus*

Allelopathic plants	Concentration	Chl 'a'	Chl 'b'	Total Chl	Nitrogen %	Protein Content
P_1	25	11.32	3.01	14.33	4.33	27.06
	50	10.55	2.87	13.42	3.88	24.25
	75	9.78	2.56	12.34	3.48	21.75
	100	8.20	2.34	10.54	3.15	19.68
P_2	25	15.33	3.98	19.31	7.89	49.31
	50	14.59	3.44	18.03	6.87	42.93
	75	13.28	3.21	16.49	6.00	37.50
	100	12.56	3.00	15.56	5.81	36.31
Control		21.24	3.42	24.66	5.85	36.56

P_1= *Cassia occidentalis*
P_2= *Calotropis procera*

References

1. Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts: Polyphenol oxidase in Beta Vulgaris. Plant. Physio. 24:1-15.
2. Dhawan, S.R.; Narwal, S.S. & Dhawan, P. (1994). Allelopathic potential of 10 trees species for biocontrol of congress grass – Parthenium hysterophorus L. Proc. IInd National Symp. On Allelopathy in Sust. Agric. For. and Environ. Indian Soc. of Allelopathy, Jodhpur.

3. Fisher, R.A. (1935). The design of experiments. Edinburgh: Oliver and Boyd.
4. Mamatha, M. & Mahadevappa, M. (1992). Biological survey in relation to Parthenium control. Adv. Plant Sci. 1(2): 238-240.
5. Oudhia, P. & Tripathi, R.S. (1998). Allelopathic effects of Parthenium hysterophorus L. on Kodo mustard and problematic weeds. In: Proceedings of the First International conference on Parthenium Management. University of Agricultural Sciences, Dharwad, India. 6-8 Oct. 1997, 136-139.
6. Senthil, A.; Chinnusamy, C.; Shanmugasundaram, R. and Kandasamy, O.S. (2004). WM 20. Identification of competitive or allelopathic plant species for the management of Parthenium hysterophorus. Abstracts IVth International Conference Allelopathy in Sustainable Terrestrial and Aquatic Ecosystem. Ed., S.S. Narwal, Haryana Agri. University, Hisar, Aug. 23-25 p.24.
7. Singh, N.P. (1983). Potential biological control of Parthenium hysterophorus L. Current Science 52, 644.
8. Snell, H. & Snell, C. (1995). Colorimetric method of analysis of nitrogen Vol. II 813-816

BIOSTIMULANT AND PLANT GROWTH REGULATOR: APPLICATIONS AND SIGNALING MECHANISM

ISBN: 978-81-981142-7-3| Year: 2025 | pp: 09 - 22 |

Plant Bio Stimulants Implication in Sustainable Agriculture

[1]Shantanu Y. Chavan

[2]Rupali R. Taur

[1]Dept of Botany, Sant Dnyaneshwar Mahavidyalaya Soegaon, Dist. Chhatrapati Sambhajinagar 431120 (MS) India.

[2]Dept of Biosciences and Technology, MGM University, Chhatrapati Sambhajinagar 431003 (MS) India.

Email: alysicarpus@gmail.com

Article DOI Link: https://zenodo.org/uploads/14785863

DOI: 10.5281/zenodo.14785863

Abstract

Biostimulants are substances or microorganisms that enhance plant growth, germination, and crop productivity by influencing bioactive molecules, thereby improving various physiological and agronomic traits in crops. This study explores the definition, types, mechanisms of action, application, challenges, and future prospects of biostimulants in sustainable agriculture.

Keywords: Biostimulants, sustainable agriculture, crop productivity, nutrient uptake, stress tolerance.

Introduction

The term biostimulant originated in horticulture to describe substances that promote plant growth without being fertilizers, soil improvers, or pesticides. It was first defined in a 1997 web journal by Zhang and Schmidt, who described biostimulants as materials promoting plant growth in small quantities, distinguishing them from nutrients applied in larger amounts. Early examples included humic acids and seaweed extracts. Later, Kauffman et al. (2007) refined the definition, explicitly excluding fertilizers and classifying biostimulants into humic substances, hormone-containing products, and amino acid-containing products.

Over time, the scope of biostimulants expanded to include microorganisms like plant growth-promoting rhizobacteria (PGPR), with effects such as stress tolerance and development modulation. (Halpern et al 2015). Industry stakeholders, such as the European Biostimulants Industry Council, have

played a significant role in promoting the concept, culminating in global acceptance through events like the 2012 World Congress on Biostimulants. This chapter aims to clarify biostimulant categories and their agricultural applications.

Definition of Biostimulants

A plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content (Jardin 2015)

Importance of biostimulant in sustainable agriculture

Biostimulant improve tolerance to abiotic stress

Climate change induces severe and unpredictable environmental stresses on crops, such as drought, salinity, and nutrient deficiencies, reducing yields and product quality. Biostimulants—natural substances or microorganisms—offer innovative tools to enhance plant resilience and productivity by stimulating adaptive responses. Goud et al 2022)

Key studies highlight various biostimulant applications: (Ruzzi et al2024)

1. **Seed Germination and Early Growth:** Cellular sucrose levels and priming agents like sodium silicate improve germination under stress.
2. **Drought Tolerance:** Products such as melatonin, glycine betaine, and protein hydrolysates stabilize photosynthetic machinery, enhance enzyme activity, and improve water use efficiency.
3. **Salt Stress:** Applications of 2-keto-L-gulonic acid, protein hydrolysates, and microbial inoculants alleviate salinity effects by promoting nutrient uptake and photosynthetic performance.
4. **Nutritional Stresses:** Biostimulants like Pseudomonas spp. and compost improve nutrient availability, soil health, and crop yield under nutrient deficiencies.
5. **Heavy Metal and Oxidative Stresses:** Silicon and iron oxide nanoparticles, as well as flavonoid complexes, mitigate metal toxicity and oxidative damage in contaminated soils.
6. **Microbial Synergies:** Combining microbial biostimulants like mycorrhizal fungi with plant growth-promoting rhizobacteria enhances stress tolerance through symbiotic mechanisms.

These studies underscore the multifaceted benefits of biostimulants in sustainable agriculture, suggesting their integration into farming practices to close the gap between potential and achievable yields under climate stressors.

Types of Biostimulants

Humic and fulvic acids

Humic substances (HS) are natural components of soil organic matter, originating from the decomposition of plant, animal, and microbial residues, as well as the metabolic activities of soil microbes. They are categorized into humins, humic acids, and fulvic acids based on molecular weight and solubility. These substances form complex supra-molecular colloids whose

dynamics are influenced by plant roots and decomposition processes. (Rose et al 2014). HS are essential for soil fertility, improving physical, chemical, and biological properties. They enhance root nutrition by increasing nutrient uptake through mechanisms like boosting the soil's cation exchange capacity and improving phosphorus availability. (Jindo et al 2012). HS also activate plasma membrane H^+-ATPases, facilitating nutrient import, cell wall loosening, and growth. They may enhance respiration and enzymatic activities, providing carbon substrates and potentially exhibiting hormonal-like effects. (Olivares et al 2015) HS contribute to plant stress protection by modulating secondary metabolism, including phenylpropanoid pathways, which are crucial for stress responses. High-molecular-mass HS have shown effects on key metabolic enzymes in plants. Application of HS (e.g., humic and fulvic acids) to crops shows variability in results, with generally positive effects on growth, such as an average increase in dry weight by ~22% for shoots and ~21% for roots. The variability arises from differences in HS sources, environmental conditions, plant species, and application methods. HS are extracted from sources like peat, composts, vermicompost, or mineral deposits, and efforts to use them for crop promotion must optimize interactions between organic matter, microbes, and plant roots. (Schivaon et al 2010).

Protein Hydrolysates and Other Nitrogen-Containing Compounds

Protein hydrolysates, derived from agro-industrial by-products of plants and animals, contain amino acids and peptides obtained through chemical or enzymatic hydrolysis. These compounds, along with other nitrogenous molecules like betaines and polyamines, act as biostimulant, enhancing plant growth and stress tolerance (Calvo et al., 2014). Direct effects include improved nitrogen uptake and assimilation, cross-talk between carbon and nitrogen metabolism, and hormonal activities. They also exhibit antioxidant properties and chelating effects, aiding in micronutrient mobility and stress mitigation. (Colla et al., 2014). Indirectly, protein hydrolysates enhance soil fertility by increasing microbial activity and nutrient availability. Commercial products based on these compounds have shown significant improvements in crop yield and quality. While their safety has been affirmed in bioassays, the EU restricts the use of animal-derived protein hydrolysates on organic crops due to food safety concerns. (Corte et al.2014).

Seaweed and plant based

Seaweed extracts, long used as fertilizers, are now recognized for their biostimulant properties. They contain polysaccharides (laminarin, alginates, carrageenans), micro- and macronutrients, sterols, and nitrogenous compounds like betaines. These compounds enhance soil properties (water retention, aeration, and cation exchange), promote beneficial soil microflora, and provide nutrients. (Khan et al., 2009).

Seaweed extracts also influence plant growth through hormonal effects, impacting seed germination, establishment, and development. Hormonal effects are linked to both the regulation of plant hormone biosynthesis and the compounds in the extracts. Anti-

stress benefits are attributed to antioxidants and stress-responsive gene regulation. (Craigie et al 2011)

Botanicals, plant-derived substances, show potential as biostimulants, although they are better known for pesticidal properties. Allelochemicals, which mediate plant interactions, offer additional prospects for sustainable crop management and new biostimulant development. (Seiber et al., 2014).

Chitosan and Other Biopolymers

Chitosan, a deacetylated derivative of chitin, is a versatile biopolymer produced both naturally and industrially. It is widely utilized in sectors such as food, cosmetics, medicine, and agriculture. Chitosan oligomers, due to their polycationic nature, interact with a variety of cellular components, including DNA, plasma membranes, and cell walls. These interactions also involve binding to specific receptors, activating plant defence mechanisms similar to natural elicitors. (Katiyar et al., 2015). Distinct from chitin, chitosan utilizes unique receptors and signalling pathways. Its binding to cell receptors leads to physiological changes such as hydrogen peroxide production and calcium ion leakage, critical for stress response signalling and developmental regulation. Proteomic and transcriptomic studies confirm its significant impact on plant physiology. (Ferri et al., 2014). In agriculture, chitosan is primarily used for plant protection against fungal pathogens and enhancing tolerance to abiotic stresses like drought, salinity, and cold. It also improves traits related to plant metabolism. For instance, chitosan-induced stomatal closure, mediated by an ABA-dependent mechanism, helps plants manage environmental stress. (Iriti et al., 2009). Beyond chitosan, other biopolymers like seaweed polysaccharides and laminarin (a glucan from brown algae) are increasingly applied as plant defence elicitors. These substances often blur the line between biocontrol and biostimulation, as they can enhance both pathogen resistance and abiotic stress tolence by interconnecting signaling pathways. (Gozzo and Faoro,2013).

Inorganic Compounds

Beneficial elements, such as aluminium (Al), cobalt (Co), sodium (Na), selenium (Se), and silicon (Si), promote plant growth under specific conditions, even though they are not universally essential. They are present in soils as soluble salts or insoluble forms like silica. Their roles include strengthening cell walls, aiding in stress responses (e.g., selenium during pathogen attack, sodium under osmotic stress), and enhancing plant tolerance to abiotic stress. (Pilon-Smits et al., 2009). These elements contribute to plant growth through mechanisms like osmoregulation, enzyme activation, antioxidant protection, and interactions with symbionts. Inorganic salts of beneficial elements, such as phosphates and silicates, serve as biostimulant by improving nutrient efficiency and stress tolerance, separate from their roles as fungicides or fertilizers. Their diverse effects on plant physiology warrant further exploration. (Deliopoulos et al., 2010).

Beneficial Fungi

Fungi form diverse relationships with plants, ranging from mutualistic symbioses to parasitism. (Behie and Bidochka, 2014). Mycorrhizal fungi, especially Arbuscular Mycorrhizal Fungi

(AMF), establish symbiotic relationships with over 90% of plant species, enhancing nutrient uptake (especially phosphorus), water balance, and stress tolerance. AMF also form hyphal networks that facilitate interplant signalling and nutrient transfer. (Johnson and Graham, 2013).

Non-mycorrhizal fungal endophytes like Trichoderma and Piriformospora indica play roles in nutrient transfer, stress tolerance, and growth promotion, with applications as biostimulant and biopesticides. Despite challenges in large-scale AMF propagation and understanding host specificity, fungi are increasingly recognized for their potential in sustainable agriculture to enhance nutrient efficiency, crop yield, and resilience. (Nicolás et al., 2014).

Beneficial Bacteria

Beneficial bacteria interact with plants in various ways, ranging from mutualism to parasitism. (Ahmad et al.,2008) They inhabit various niches, including soil, rhizosphere, rhizoplane, and even the interior of plant cells, and can be transmitted through seeds. Bacteria play crucial roles in nutrient cycling, improving nutrient use efficiency, enhancing disease resistance, increasing abiotic stress tolerance, and modulating plant growth through plant growth regulators. (Berg et al.,2014). Two primary types of beneficial bacteria are used as biostimulants: Rhizobium (biofertilizers) and Plant Growth-Promoting Rhizobacteria (PGPRs). PGPRs influence multiple aspects of plant life, such as growth, development, and stress responses. (Arora et al., 2011) While PGPR applications can be complex due to variable plant responses and environmental conditions, they are gaining popularity as "plant probiotics," improving plant nutrition and immunity. The market for bacterial biostimulants is growing, despite technical challenges in formulation and consistency. (Berendsen et al., 2012).

Plant-Derived Biostimulants

Mechanisms of Action

The mechanisms of action of plant-derived biostimulants involve impacts on general biochemical, molecular, and physiological pathways, distinguishing them from modes of action, which target specific biochemical processes. Biostimulants are not yet characterized at the specific level required to define their modes of action. Research focuses on understanding their composition, bioactive ingredients, and how they alter plant metabolism. Advanced omics tools like transcriptomics, metabolomics, proteomics, and phenomics are crucial for identifying these mechanisms and tailoring formulations for specific crops. (Xu and Geelen 2018). Such determinations are vital for optimizing formulations and are essential for the commercialization of biostimulants, as emphasized by the European Biostimulant Industry Council (EBIC). The mechanisms are explored at both cellular/molecular levels and the whole-plant scale. (Diego and Spíchal 2022).

Plant based Biostimulants, Mode of action

Whole-Plant Level Effects of Plant-Based Biostimulants

Plant-derived biostimulants impact plant physiological processes at the whole-plant level, enhancing germination, root and shoot growth, flowering, and fructification, which are crucial for crop

yield, quality, and economic profitability.

Germination

Plant derived biostimulants promote successful and uniform germination, even under stress. For example, treatments improve germination rates in wheat and mung bean and counteract salinity's negative effects in crops like maize and zucchini. Various plant plant-derived PHs were shown to regulate higher survival rates in Arabidopsis seeds in presence of high salinity, revealing a highly synchronous germination. (Gilroy, and Jones 2000) cannabis plants, treated with a biostimulant componants was produced from aloevera, fish, and kelp extracts inhanced the number of root tips and branch points, and also the total surface area of the root system. (Wise et al 2024)

Root Growth and Morphology

Biostimulants enhance root system development by increasing root length, weight, and surface area in crops such as tomato, maize, and radish. They also improve root growth under stresses like salinity and high temperatures. In Mustard, bioactive peptides observed in hydrolysates produced from soybean byproducts which inhanced root thickness, and generated a higher number of adventitious roots and root hairs of greater length. (Matsumiya, and Kubo 2011) However an inhanced trichoblasts and atrichoblasts was found, in the location pattern did not change, which reveals the biostimulant influanced root growth differently from ethylene (Ashraf et al 2016)

Shoot Growth and Morphology

These treatments boost plant height, stem diameter, and leaf area while increasing biomass in crops like lettuce, spinach, and tomato. They also mitigate stress impacts, ensuring better growth under salinity or water scarcity. (Colla et al 2013). Biostimulants produced from plant byproducts triggered a same response, demonsrated by carob germ-based extracts, that inhanced stem diameter and plant height in tomato (Rady, and Rehman, 2016)

Flowering

Biostimulants advance flowering, increase flower numbers, and improve flower size and post-harvest characteristics in ornamental and food crops like gladiolus and common bean (Elzaawely et al 2018). Spraying of gladiolus with moringa leaf extract developed in rapied spike emergence and an inhanced in the number of spike florets per plant, with its prolonged its inhanced vase life in sucrose (Younis et al 2018) However, PDBs provides a wide application for cultivation of ornamental plants, ranging from accelerated flowering to improvements in flower quantity, size, and post-harvest characteristics

Fructification and Fruit Quality

PDBs enhance fruit yield, size, and quality by increasing nutrient, bioactive compound, and antioxidant content. They also reduce stress-related losses in crops like tomatoes, sweet cherries, and mangoes. (Sharony et al 2015). In tomato plants, biostimulant treatments inhanced fruit production and increased the content of minerals, bioactive compounds like, lycopene, phenols, ascorbic acid, organic acids and soluble solids. The treated tomatoes resulted in increased antioxidant activity and fruit brightness with dark redness colour.

(Caruso et al 2019). Biostimulants demonstrate a broad-spectrum potential to improve plant development and resilience, making them essential tools for sustainable agriculture.

Application of biostimulants for agronomic and physiological traits improvements of crops (Calvo et al 2014)

1. **Hydrolyzed Collagen and Gelatin Hydrolysate**

Gelatin hydrolysate improves cucumber growth by increasing the expression of genes for amino acid transport, acting as a sustained nitrogen source and biostimulant.

2. **Commercial PBs on Lettuce**

Three PBs derived from plants enhanced lettuce shoot fresh weight and stimulated epiphytic bacteria (e.g., Pantoea, Pseudomonas) with plant growth-promoting and pathogen-controlling properties.

3. **Organic Biostimulants**

Formulations containing vermicompost, malt sprouts, and organic herbs improved plant growth by altering microbiota on plant surfaces and surroundings.

4. **Biopolymer-Based PBs on Melons**

Biopolymers with peptides and lignosulfonates promoted biomass and root traits in melon transplants, likely via endogenous phytohormone stimulation.

5. **Humic Acids and Seaweed Extracts**

Humic acids from waste and seaweed extracts (especially Ascophyllum nodosum) positively influenced maize growth by promoting root traits and nutrient accumulation through phytohormonal pathways.

6. **Microbial PBs**

Cladosporium sphaerospermum boosted growth and stress tolerance in peppers and tobacco via mechanisms like photosynthesis, phytohormone balance, and defense responses.

Trichoderma erinaceum enhanced tomato growth under fungal stress by upregulating defense-related genes and antioxidative enzymes.

7. **Animal-Based PBs on Snapdragon**

Foliar or substrate applications improved ornamental traits in a dose- and cultivar-dependent manner.

8. **Stress Tolerance**

Exogenous jasmonic acid alleviated pesticide-induced oxidative stress in Brassica juncea, and sedaxane exhibited hormone-like activity aiding root establishment and stress resilience in maize. Overall, PBs demonstrate versatile applications, from improving growth and productivity to enhancing stress tolerance, with potential benefits for sustainable agriculture.

Application of biostimulants for enhancing produce quality (Rouphael, et al 2015)

Metabolism and Nutritional Quality

Biostimulants can influence plant primary and secondary metabolism, leading to the accumulation of antioxidant molecules, which are beneficial for human health. Key findings include:

1. **Tomato Quality Enhancement**

Application of earthworm-grazed and Trichoderma harzianum-biofortified spent mushroom substrate improved tomato fruit quality by increasing

antioxidant capacity, total sugars, carotenoids (e.g., lycopene, lutein, β-carotene), polyphenols, flavonoids, and mineral content (P, K, Ca, Mg, Fe, Mn, Zn).

2. Stress-Responsive Compounds in Brassica

Under varying conditions of light, phosphate, and phosphite, Brassica campestris and Brassica juncea increased biosynthesis of flavonoids and glucosinolates, likely as a defense mechanism against nutrient stress.

3. Fruit Trees and Grapevines: Apples

Seaweed extract-based PBs improved red coloration intensity in "Jonathan" apples without major changes in size, firmness, or sugars.

Olive Trees

Selenium application enhanced EVOO quality by increasing antioxidant molecules, improving oxidative stability and shelf life.

Grapes

Brassinosteroids improved anthocyanins, berry color, and soluble solids in "Redglobe" table grapes.Abscisic acid applications boosted anthocyanin and flavonoid content in Vitis vinifera × Vitis labrusca grapes by upregulating key biosynthetic genes (e.g., CHI, DFR, F3H, UFGT) and transcription factors (VvMYBA1, VvMYBA2).

Application of Biostimulants for Abiotic Stress Tolerance (Rouphael, et al 2015)

Abiotic stresses like drought, salinity, and extreme temperatures contribute to significant yield losses. Biostimulants (microbial and non-microbial) offer promising solutions for enhancing crop resilience under such conditions

1. Drought Tolerance

Plant-based hydrolysates (PH) and microbial biostimulants, including arbuscular mycorrhizal fungi (AMF), improve water-use efficiency and antioxidant responses in tomatoes.

AMF enhance substrate hydraulic properties, enabling better water extraction and root performance under drought stress.

2. Salinity Tolerance

Polysaccharides derived from brown and red algae mitigate salinity effects in wheat by reducing lipid peroxidation, boosting chlorophyll, and enhancing ion compartmentalization.

PGPR strains like Bacillus spp. improve salinity tolerance in crops like mung bean and durum wheat through growth-promoting traits and stress resilience.

3. Other Abiotic Stresses

Biostimulants like carboxylic acids and 5-aminolevulinic acid enhance photosynthesis and nutrient uptake under salinity and high-conductivity conditions.

Biostimulants show potential in mitigating abiotic stress impacts, safeguarding crop productivity through biochemical, physiological, and molecular mechanisms. Further research could optimize their application across various crops and conditions.

Application of Biostimulants for Improving Nutrient Use Efficiency (Pascale et al 2017)

Biostimulants, including bioactive substances and microbial inoculants, enhance nutrient availability, uptake, and utilization in plants, significantly

improving Nitrogen Use Efficiency (NUE) and Phosphorus Use Efficiency (PUE):

Nitrogen Use Efficiency (NUE):

Legume-derived Protein Hydrolysates (PH)

Boosted leaf growth, SPAD index, and biomass in tomatoes by enhancing root development and N uptake.

Upregulated genes related to N assimilation under suboptimal N conditions.

Trichoderma Strains

T. virens GV41 improved NUE in lettuce and favored soil N uptake.

Effects varied by plant species and N levels, influencing rhizosphere microbial composition.

1. Phosphorus Use Efficiency (PUE)

Microbial inoculants (bacteria and fungi) enhanced nutrient availability and utilization.

Mixed inoculants (e.g., Azotobacter, Bacillus, Trichoderma) were effective in improving wheat production in N- and P-deficient soils in Australia.

2. Zinc Solubilization

Zinc-solubilizing rhizobacteria (Pantoea, Enterobacter cloacae, Pseudomonas fragi) were identified as effective biostimulants to address zinc deficiencies in crops.

Biostimulants, through physiological and molecular mechanisms, offer eco-friendly solutions to improve nutrient use efficiency, benefiting both agricultural productivity and environmental sustainability.

Future Prospects of Biostimulants in Agriculture

Integration with Digital Farming Technologies

Biostimulants can be effectively integrated into precision agriculture systems, utilizing sensors, drones, and data analytics to optimize application rates, timing, and methods. This synergy can improve resource efficiency, reduce waste, and tailor biostimulant use to specific crop needs, maximizing their benefits.

Potential Role in Mitigating Climate Change

Biostimulants contribute to sustainable agricultural practices by enhancing plant resilience to abiotic stresses caused by climate change, such as drought, salinity, and temperature extremes. They can also improve carbon sequestration through enhanced biomass production and healthier root systems, supporting global climate mitigation efforts.

Expanding Use in Organic and Regenerative Agriculture

With growing consumer demand for organic products, biostimulants are increasingly recognized for their compatibility with organic farming practices. Their role in promoting soil health, enhancing nutrient cycling, and reducing reliance on chemical fertilizers aligns with the principles of regenerative agriculture, fostering sustainable food systems.

Innovations in Biostimulant Formulations

Advances in biotechnology and material science may lead to the development of more efficient biostimulant formulations, such as encapsulated or slow-release products, ensuring longer-lasting effects and reduced environmental impact.

Global Regulatory Frameworks and Standardization

As the biostimulant industry matures, harmonized global regulations and standards are anticipated, addressing challenges related to efficacy, safety, and consistency. This will bolster farmer confidence and encourage wider adoption.

Conclusion

Biostimulants are poised to become indispensable tools in sustainable agriculture. By enhancing plant growth, productivity, and resilience to environmental stressors, they contribute to closing the gap between potential and actual crop yields. Their diverse types—ranging from humic acids and protein hydrolysates to beneficial microorganisms—offer tailored solutions for specific agricultural challenges.

Key mechanisms, such as improved nutrient uptake, stress tolerance, and enhanced plant physiology, underscore their multifaceted benefits. Applications in agronomic improvements, produce quality enhancement, and abiotic stress mitigation further demonstrate their potential.

Despite challenges like variability in effectiveness, lack of standardization, and cost barriers, the future of biostimulants looks promising. Integration with digital farming, their role in climate change mitigation, and growing adoption in organic systems highlight their transformative potential.

By fostering collaborations among researchers, policymakers, and industry stakeholders, biostimulants can lead the way toward a more resilient and sustainable agricultural future.

References

1. Ahmad, I., Pichtel, J., & Hayat, S. (2008). Plant-Bacteria Interactions: Strategies and Techniques to Promote Plant Growth. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.
2. Arora, N. K., Khare, E., & Maheshwari, D. K. (2011). Plant growth-promoting rhizobacteria: Constraints in bioformulation, commercialization, and future strategies. In D. K. Maheshwari (Ed.), Plant Growth and Health Promoting Bacteria (pp. 97–116). Springer, Berlin/Heidelberg.
3. Ashraf, R., Sultana, B., Iqbal, M., & Mushtaq, M. (2016). Variation in biochemical and antioxidant attributes of Raphanus sativus in response to foliar application of plant leaf extracts as plant growth regulator. Journal of Genetic Engineering and Biotechnology, 14(1), 1–8.
4. Behie, S. W., & Bidochka, M. J. (2014). Nutrient transfer in plant-fungal symbioses. Trends in Plant Science, 19(10), 734–740.
5. Berendsen, R. L., Pieterse, C. M. J., & Bakker, P. A. H. M. (2012). The rhizosphere microbiome and plant health. Trends in Plant Science, 17(8), 478–486.
6. Berg, G., Grube, M., Schloter, M., & Smalla, K. (2014). Unraveling the plant microbiome: Looking back and future perspectives. Frontiers in Microbiology, 5, Article 148. https://doi.org/10.3389/fmicb.2014.00148
7. Calvo, P., Nelson, L., & Kloepper, J. W. (2014). Agricultural uses of plant biostimulants. Plant and Soil, 383(1–

2), 3–41. https://doi.org/10.1007/s11104-014-2131-8

8. Caruso, G., De Pascale, S., Cozzolino, E., Cuciniello, A., Cenvinzo, V., Bonini, P., Colla, G., & Rouphael, Y. (2019). Yield and nutritional quality of Vesuvian Piennolo Tomato PDO as affected by farming system and biostimulant application. Agronomy, 9(9), 505. https://doi.org/10.3390/agronomy9090505
9. Colla, G., Rouphael, Y., Canaguier, R., Švecová, E., & Cardarelli, M. (2014). Biostimulant action of a plant-derived protein hydrolysate produced through enzymatic hydrolysis. Frontiers in Plant Science, 5, 1–6. https://doi.org/10.3389/fpls.2014.00448
10. Colla, G., Rouphael, Y., Cardarelli, M., Švecová, E., Reynaud, H., Canaguier, R., & Planques, B. (2013). Effectiveness of a plant-derived protein hydrolysate to improve crop performances under different growing conditions. Acta Horticulturae, 1009, 175–179. https://doi.org/10.17660/ActaHortic.2013.1009.20
11. Corte, L., Dell'Abate, M. T., Magini, A., Migliore, M., Felici, B., Roscini, L., Sardella, R., Tancini, B., Emiliani, C., Cardinali, G., & Benedetti, A. (2014). Assessment of safety and efficiency of nitrogen organic fertilizers from animal-based protein hydrolysates—a laboratory multidisciplinary approach. Journal of the Science of Food and Agriculture, 94(2), 235–245. https://doi.org/10.1002/jsfa.6292
12. Craigie, J. S. (2011). Seaweed extract stimuli in plant science and agriculture. Journal of Applied Phycology, 23(3), 371–393. https://doi.org/10.1007/s10811-010-9560-4
13. De Diego, N., & Spíchal, L. (2022). Presence and future of plant phenotyping approaches in biostimulant research and development. Journal of Experimental Botany, 73(16), 5199–5212. https://doi.org/10.1093/jxb/erac311
14. De Pascale, S., Rouphael, Y., & Colla, G. (2017). Plant biostimulants: Innovative tools for enhancing plant nutrition in organic farming. European Journal of Horticultural Science, 82(6), 277–285. https://doi.org/10.17660/eJHS.2017/82.6.2
15. Deliopoulos, T., Kettlewell, P. S., & Hare, M. C. (2010). Fungal disease suppression by inorganic salts: A review. Crop Protection, 29(9), 1059–1075. https://doi.org/10.1016/j.cropro.2010.06.001
16. Du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories, and regulation. Scientia Horticulturae, 196, 3–14. https://doi.org/10.1016/j.scienta.2015.09.021
17. El-Sharony, T. F., El-Gioushy, S., & Oa, A. (2015). Effect of foliar application with algae and plant extracts on growth, yield, and fruit quality of fruitful mango trees cv. Fagri Kalan. Journal of Horticulture, 2(162).

https://doi.org/10.4172/2376-0354.1000162

18. Elzaawely, A. A., Ahmed, M. E., Maswada, H. F., Al-Araby, A. A., & Xuan, T. D. (2018). Growth traits, physiological parameters, and hormonal status of snap bean (Phaseolus vulgaris L.) sprayed with garlic cloves extract. Archives of Agronomy and Soil Science, 64(8), 1068–1082. https://doi.org/10.1080/03650340.2017.1409611
19. Ferri, M., Franceschetti, M., Naldrett, M. J., Saalbach, G., & Tassoni, A. (2014). Effects of chitosan on the protein profile of grape cell culture subcellular fractions. Electrophoresis, 35(12–13), 1685–1692. https://doi.org/10.1002/elps.201300594
20. Gilroy, S., & Jones, D. L. (2000). Through form to function: Root hair development and nutrient uptake. Trends in Plant Science, 5(2), 56–60. https://doi.org/10.1016/S1360-1385(99)01551-4
21. Gozzo, F., & Faoro, F. (2013). Systemic acquired resistance (50 years after discovery): Moving from the lab to the field. Journal of Agricultural and Food Chemistry, 61(50), 12473–12491. https://doi.org/10.1021/jf404156x
22. Halpern, M., Bar-Tal, A., Ofek, M., Minz, D., Muller, T., & Yermiyahu, U. (2015). The use of biostimulants for enhancing nutrient uptake. In D. L. Sparks (Ed.), Advances in Agronomy (Vol. 129, pp. 141–174). Academic Press. https://doi.org/10.1016/bs.agron.2014.10.001
23. Iriti, M., Picchi, V., Rossoni, M., Gomarasca, S., Ludwig, N., Gargano, M., & Faoro, F. (2009). Chitosan antitranspirant activity is due to abscisic acid-dependent stomatal closure. Environmental and Experimental Botany, 66(3), 493–500. https://doi.org/10.1016/j.envexpbot.2009.02.013
24. Jindo, K., Martim, S. A., Navarro, E. C., Aguiar, N. O., & Canellas, L. P. (2012). Root growth promotion by humic acids from composted and non-composted urban organic wastes. Plant and Soil, 353(1–2), 209–220. https://doi.org/10.1007/s11104-011-1024-3
25. Johnson, N. C., & Graham, J. H. (2013). The continuum concept remains a useful framework for studying mycorrhizal functioning. Plant and Soil, 363(1–2), 411–419. https://doi.org/10.1007/s11104-012-1406-8
26. Katiyar, D., Hemantaranjan, A., & Singh, B. (2015). Chitosan as a promising natural compound to enhance potential physiological responses in plants: A review. Indian Journal of Plant Physiology, 20(1), 1–9. https://doi.org/10.1007/s40502-015-0139-6
27. Kauffman, G. L., Kneivel, D. P., & Watschke, T. L. (2007). Effects of a biostimulant on the heat tolerance associated with photosynthetic capacity, membrane thermostability, and polyphenol production of perennial ryegrass. Crop Science, 47(1), 261–267. https://doi.org/10.2135/cropsci2006.07.0485

28. Khan, W., Rayirath, U. P., Subramanian, S., Jithesh, M. N., Rayorath, P., Hodges, D. M., Critchley, A. T., Craigie, J. S., Norrie, J., & Prithiviraj, B. (2009). Seaweed extracts as biostimulants of plant growth and development. Journal of Plant Growth Regulation, 28(4), 386–399. https://doi.org/10.1007/s00344-009-9103-x
29. Matsumiya, Y., & Kubo, M. (2011). Soybean peptide: Novel plant growth promoting peptide from soybean. In Soybean and Nutrition (pp. 55–72). IntechOpen. https://doi.org/10.5772/23755
30. Nicolás, C., Hermosa, R., Rubio, B., Mukherjee, P. K., & Monte, E. (2014). Trichoderma genes in plants for stress tolerance: Status and prospects. Plant Science, 228, 71–78. https://doi.org/10.1016/j.plantsci.2014.04.006
31. Olivares, F. L., Aguiar, N. O., Rosa, R. C. C., & Canellas, L. P. (2015). Substrate biofortification in combination with foliar sprays of plant growth-promoting bacteria and humic substances boosts production of organic tomatoes. Scientia Horticulturae, 183, 100–108. https://doi.org/10.1016/j.scienta.2014.11.012
32. Pilon-Smits, E. A. H., Quinn, C. F., Tapken, W., Malagoli, M., & Schiavon, M. (2009). Physiological functions of beneficial elements. Current Opinion in Plant Biology, 12(3), 267–274. https://doi.org/10.1016/j.pbi.2009.04.009
33. Rady, M. M., & Rehman, H. (2016). Supplementing organic biostimulants into growing media enhances growth and nutrient uptake of tomato transplants. Scientia Horticulturae, 203, 192–198. https://doi.org/10.1016/j.scienta.2016.03.027
34. Rose, M. T., Patti, A. F., Little, K. R., Brown, A. L., Jackson, W. R., & Cavagnaro, T. R. (2014). A meta-analysis and review of plant-growth response to humic substances: Practical implications for agriculture. In D. L. Sparks (Ed.), Advances in Agronomy (Vol. 124, pp. 37–89). Academic Press. https://doi.org/10.1016/B978-0-12-800138-7.00002-4
35. Rouphael, Y., Franken, P., Schneider, C., Schwarz, D., Giovannetti, M., Agnolucci, M., ... Colla, G. (2015). Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Scientia Horticulturae, 196, 91–108. https://doi.org/10.1016/j.scienta.2015.09.002
36. Rouphael, Y., Kyriacou, M. C., & Colla, G. (2018). Vegetable grafting: A toolbox for securing yield stability under multiple stress conditions. Frontiers in Plant Science, 8, 2255. https://doi.org/10.3389/fpls.2017.02255
37. Ruzzi, M., Colla, G., & Rouphael, Y. (2024). Editorial: Biostimulants in agriculture II: Towards a sustainable future. Frontiers in Plant Science, 15, 1427283. https://doi.org/10.3389/fpls.2024.1427283
38. Schiavon, M., Pizzeghello, D., Muscolo, A., Vaccaro, S., Francioso,

O., & Nardi, S. (2010). High molecular size humic substances enhance phenylpropanoid metabolism in maize (Zea mays L.). Journal of Chemical Ecology, 36(6), 662–669. https://doi.org/10.1007/s10886-010-9790-7

39. Seiber, J. N., Coats, J., Duke, S. O., & Gross, A. D. (2014). Biopesticides: State of the art and future opportunities. Journal of Agricultural and Food Chemistry, 62(50), 11613–11619. https://doi.org/10.1021/jf504252n
40. Wise, K., Selby-Pham, J., Chai, X., Simovich, T., Gupta, S., & Gill, H. (2024). Fertiliser supplementation with a biostimulant complex of fish hydrolysate, aloe vera extract, and kelp alters cannabis root architecture to enhance nutrient uptake. Scientia Horticulturae, 323, 112483. https://doi.org/10.1016/j.scienta.2023.112483
41. Xu, L., & Geelen, D. (2018). Developing biostimulants from agro-food and industrial by-products. Frontiers in Plant Science, 9, 1567. https://doi.org/10.3389/fpls.2018.01567
42. Younis, A., Akhtar, M. S., Riaz, A., Zulfiqar, F., Qasim, M., Farooq, A., Tariq, U., Ahsan, M., & Bhatti, Z. M. (2018). Improved cut flower and corm production by exogenous Moringa leaf extract application on Gladiolus cultivars. Acta Scientiarum Polonorum Hortorum Cultus, 17(5), 25–38. https://doi.org/10.24326/asphc.2018.5.3

BIOSTIMULANT AND PLANT GROWTH REGULATOR: APPLICATIONS AND SIGNALING MECHANISM

ISBN: 978-81-981142-7-3| Year: 2025 | pp: 23 - 32 |

Biochar and Its Interactions with Soil Microbiota: Mechanisms and Applications

[1]D. J. Faldu,

[2]M. N. Gajera

[3]A. V. Bhogesara

[3]A. K. Barad

[3]V. U. Bambhaniya

[3]G. J. Ram

[1]Ph.D. scholar, Department of Renewable Energy Engineering, College of Agricultural Engineering & Technology. Junagadh Agricultural University, Junagadh-362001, Gujarat, India.

[2]Master scholar, Department of Farm Machinery and Power Engineering, College of Agricultural Engineering & Technology. Junagadh Agricultural University, Junagadh-362001, Gujarat, India.

[3]Master scholar, Department of Renewable Energy Engineering, College of Agricultural Engineering & Technology. Junagadh Agricultural University, Junagadh-362001, Gujarat, India.

Email: dhruvfaldu2000@gmail.com

Article DOI Link: https://zenodo.org/uploads/14785940

*DOI: **10.5281/zenodo.14785940***

Abstract

Biochar, a carbon-rich material produced through pyrolysis, has gained significant attention for its ability to enhance soil health and sustainability. This chapter, titled "Biochar and Its Interactions with Soil Microbiota: Mechanisms and Applications" focuses on the intricate relationship between biochar and soil microbial communities. Biochar's porous structure, nutrient retention capacity, and ability to modulate soil pH create an optimal environment for microbial colonization and activity. These interactions drive key soil processes such as nutrient cycling, organic matter decomposition, and pathogen suppression, contributing to improved soil fertility and plant health.

The chapter also highlights the mechanisms through which biochar influences microbial diversity and community structure, facilitating processes like nitrogen fixation and phosphate solubilization. Applications in agriculture and environmental remediation, including carbon sequestration and pollutant mitigation, demonstrate biochar's versatility. However, challenges such as variability in properties and economic barriers must be addressed to ensure its effective use.

Future advancements in biochar research, particularly in tailoring its properties and integrating it with other sustainable technologies, are critical to unlocking its full potential. By elucidating the mechanisms and applications of biochar-soil microbiota interactions, this chapter underscores its role as a transformative tool for sustainable agriculture and ecosystem restoration.

Keywords: Biochar, Carbon Sequestration, Microbial Interactions, Nutrient Cycling, Soil Microbiota, Sustainable Agriculture.

Introduction

The interplay between soil health and agricultural productivity is pivotal for sustainable food systems, and biochar emerges as a transformative solution at this intersection. Biochar, a carbon-rich material produced through the pyrolysis of organic biomass in oxygen-limited conditions, has gained prominence for its multifaceted benefits in environmental management and agriculture. Beyond its well-known attributes as a soil amendment, biochar holds unique potential in shaping soil microbiota dynamics—a key factor driving soil fertility and plant health.

Soil microbiota, comprising bacteria, fungi, archaea, and other microorganisms, form the backbone of soil ecosystems. They perform critical functions, including nutrient cycling, organic matter decomposition, and pathogen suppression. However, modern agricultural practices, coupled with climate change-induced stressors, have disrupted the delicate balance of these microbial communities, leading to declines in soil quality and productivity. In this context, biochar offers a promising intervention.

Biochar's highly porous structure, extensive surface area, and chemical stability create a conducive microenvironment for microbial colonization. Its ability to adsorb and retain nutrients further enhances microbial activity, supporting processes like nitrogen fixation and phosphate solubilization. By fostering beneficial microbial interactions and mitigating environmental stressors, biochar not only improves soil health but also contributes to the resilience of agroecosystems.

This chapter explores the intricate mechanisms through which biochar interacts with soil microbiota and examines its applications in enhancing soil fertility, mitigating climate impacts, and promoting sustainable agriculture. By delving into these dynamics, we aim to provide a comprehensive understanding of biochar's role in unlocking the potential of soil microbial communities for agricultural innovation.

Biochar: A Tool for Soil Microbiota Modulation

Biochar serves as more than a soil amendment; it is a versatile medium that profoundly impacts soil microbiota, fostering conditions for their growth and

activity. The interplay between biochar and soil microbes is rooted in biochar's unique physical and chemical properties, which make it an excellent habitat and support system for microbial communities.

1. Physical Properties Enhancing Microbial Habitat

Biochar's highly porous structure offers a protective microenvironment for soil microorganisms. These pores provide shelter from environmental stresses such as desiccation, temperature fluctuations, and predation by other soil organisms. Additionally, the large surface area enhances microbial attachment, enabling microbes to establish biofilms that aid in their survival and functionality.

2. Chemical Interactions and Nutrient Availability

The chemical properties of biochar further modulate soil microbial activity. Its high cation exchange capacity (CEC) helps retain essential nutrients like potassium, calcium, and magnesium, making them more available to microbes and plants. Biochar can also adsorb and immobilize toxins and heavy metals, reducing their harmful effects on soil microbes.

3. Modulating Soil pH for Microbial Functionality

Many soil microorganisms are pH-sensitive. Biochar, often alkaline in nature, can neutralize acidic soils, creating a more favourable pH range for microbial growth. This pH adjustment enhances enzymatic activities and microbial-mediated nutrient cycling processes.

4. Enhanced Microbial Diversity and Community Structure

By altering the soil's physical and chemical environment, biochar influences the diversity and abundance of soil microbiota. Studies have shown that biochar-enriched soils exhibit greater microbial diversity, particularly of beneficial groups such as nitrogen-fixing bacteria and phosphate-solubilizing fungi. This diversity enhances soil resilience and ecosystem services, such as improved nutrient cycling and reduced greenhouse gas emissions.

5. Microbial Processes Enhanced by Biochar

Biochar supports various microbial processes critical to soil health, such as:

Nitrogen Fixation: Biochar provides sites for nitrogen-fixing bacteria to colonize, increasing nitrogen availability for plants.

Decomposition of Organic Matter: By hosting decomposers, biochar accelerates the breakdown of organic residues, enriching soil organic matter.

Pathogen Suppression: Some biochars release antimicrobial compounds or create conditions that suppress soil-borne pathogens, promoting plant health.

Applications Of Biochar in Enhancing Soil Microbiota

Biochar's ability to support soil microbiota translates into a wide range of applications that enhance soil health, agricultural productivity, and environmental sustainability. By fostering microbial activity, biochar contributes to several crucial soil functions, creating a cascade of benefits for plants and ecosystems.

1. Improving Soil Fertility and Nutrient Cycling

Biochar improves soil fertility by enhancing microbial-mediated nutrient cycling. Beneficial microbes, such as nitrogen-fixing bacteria and mycorrhizal fungi, thrive in the nutrient-retentive and protective microenvironment created by biochar. These microbes transform organic and inorganic forms of nutrients into plant-accessible forms, improving the efficiency of fertilizer use and reducing nutrient leaching.

2. Promoting Plant Growth and Health

The interaction between biochar and soil microbiota boosts plant resilience by enhancing nutrient uptake and providing natural disease resistance. Biochar-treated soils often harbor higher populations of plant-growth-promoting rhizobacteria (PGPR), which secrete phytohormones and suppress harmful pathogens. This leads to better root development and increased crop yields.

3. Enhancing Carbon Sequestration

Biochar amplifies the soil's role as a carbon sink by stabilizing organic matter and fostering microbial communities that contribute to long-term carbon storage. Decomposers in biochar-amended soils metabolize organic residues more efficiently, incorporating them into stable soil organic carbon pools. This process simultaneously reduces greenhouse gas emissions.

4. Pollution Mitigation and Remediation

Biochar is a powerful tool for bioremediation, working in tandem with microbes to detoxify contaminated soils and water. Its adsorption capacity immobilizes heavy metals and organic pollutants, reducing their bioavailability. This creates a safer environment for microbes to degrade or transform toxins, enhancing the remediation process.

5. Supporting Drought-Resilient Agriculture

In arid and semi-arid regions, biochar's ability to retain moisture and create microhabitats for drought-tolerant microbes helps maintain soil microbial activity even under water-limited conditions. These microbes aid in soil aggregation and organic matter decomposition, improving soil structure and resilience to climate stressors.

6. Sustaining Soil Microbial Biodiversity

Biochar applications enhance microbial biodiversity, which is critical for maintaining ecosystem functions. Diverse microbial communities ensure redundancy in ecological processes, making soils more resilient to disturbances such as erosion, pollution, or climate change.

Incorporating biochar into agricultural systems not only strengthens microbial ecosystems but also promotes a circular economy by utilizing organic waste as feedstock for biochar production. These applications underscore the potential of biochar as an integral component of sustainable soil management strategies.

Biochar-Enhanced Microbial Processes

The integration of biochar into soil systems significantly enhances microbial processes that drive nutrient cycling, organic matter decomposition, and ecosystem resilience. By creating favorable conditions for microbial activity, biochar facilitates various soil processes critical for maintaining soil

health and boosting agricultural productivity.

1. Nitrogen Fixation

Biochar provides an ideal habitat for nitrogen-fixing bacteria, such as Rhizobium and Azospirillum. Its porous structure protects these microbes and enhances their access to nutrients and moisture. The symbiotic relationship between nitrogen-fixing bacteria and plant roots is strengthened in biochar-enriched soils, resulting in improved nitrogen availability for crops.

2. Phosphate Solubilization

Certain soil microbes, including phosphate-solubilizing bacteria and fungi, can convert insoluble forms of phosphorus into plant-accessible forms. Biochar's ability to retain phosphate and its interaction with these microbes amplifies this solubilization process, improving phosphorus availability and uptake by plants.

3. Organic Matter Decomposition

Decomposer microorganisms, such as saprophytic fungi and bacteria, benefit from biochar's capacity to retain moisture and nutrients, which are essential for enzymatic breakdown of organic matter. Biochar's porous surface acts as a site for colonization and enzymatic activity, accelerating the decomposition of organic residues and enriching the soil with humus.

4. Microbial-Mediated Disease Suppression

Biochar contributes to the suppression of soil-borne plant pathogens through two primary mechanisms:

Direct Antimicrobial Action: Some biochar's release bioactive compounds that inhibit the growth of harmful microbes.

Promotion of Beneficial Microbes: Biochar enhances populations of antagonistic microorganisms that outcompete or suppress pathogens, creating a healthier rhizosphere.

5. Greenhouse Gas Mitigation

Methanogenic and denitrifying bacteria, responsible for methane (CH_4) and nitrous oxide (N_2O) emissions, are influenced by biochar's adsorption properties. Biochar-modulated microbial activity can reduce these emissions by shifting microbial communities toward processes that Favor nitrogen retention and carbon stabilization.

6. Soil Aggregation and Structure Formation

Biochar facilitates soil aggregation by supporting microbial processes that produce polysaccharides and other binding agents. These aggregates improve soil structure, enhance aeration, and prevent erosion, creating a stable environment for microbial ecosystems.

7. Enzymatic Activity Enhancement

Biochar-enriched soils often exhibit higher enzymatic activities due to improved microbial biomass and nutrient availability. Enzymes such as cellulase, phosphatase, and urease play crucial roles in nutrient cycling, organic matter turnover, and overall soil fertility. By enhancing these microbial processes, biochar contributes to a synergistic relationship between soil microorganisms and plants, enabling more sustainable and efficient agricultural practices. These processes exemplify the pivotal role of biochar in

transforming soil microbiota into active agents of soil health and productivity.

Challenges And Limitations

Despite its vast potential, the application of biochar faces several challenges that stem from variability in its properties, economic constraints, and technical limitations. Addressing these barriers is crucial to unlocking the full benefits of biochar for agricultural and environmental sustainability.

1. Variability in Biochar Properties

The properties of biochar can vary significantly depending on the feedstock, pyrolysis temperature, and production methods used. This variability impacts its effectiveness and makes it challenging to standardize biochar for specific applications.

- **Feedstock Influence:**

Different organic materials yield biochar's with varying chemical compositions and nutrient content. For instance, wood-based biochar's are often more carbon-rich, while manure-based biochar's are nutrient-rich but may introduce contaminants.

- **Production Conditions:**

Pyrolysis conditions, such as temperature and residence time, influence biochar's physical and chemical properties, including porosity, pH, and cation exchange capacity. This inconsistency can lead to unpredictable effects on soil and microbial communities.

- **Lack of Standardization:**

The absence of universally accepted quality standards makes it difficult for users to choose the right biochar for their needs, hindering widespread adoption.

2. Economic and Technical Barriers

Biochar production and application often involve high costs and technical complexities that limit its large-scale use.

- **High Production Costs:**

The costs of building and operating pyrolysis facilities, along with feedstock collection and transportation, are significant. These costs can make biochar economically unviable for small-scale farmers and industries without subsidies or incentives.

- **Energy Requirements:**

The energy-intensive nature of the pyrolysis process, particularly at higher temperatures, can offset the environmental benefits of biochar production. Finding energy-efficient methods is essential to reduce costs.

- **Technological Limitations:**

Scaling biochar production is challenging due to a lack of advanced and affordable technologies. Existing systems often require substantial capital investments, and maintaining consistent quality at larger scales remains problematic.

- **Market and Policy Support:**

Limited government incentives and lack of awareness among stakeholders impede the commercial viability of biochar. Policies promoting biochar use, such as subsidies, grants, or carbon credits, are often insufficient.

- **Knowledge Gaps:**

Many users lack technical expertise regarding biochar's production, handling, and application methods, which can lead to suboptimal results and reduced confidence in its benefits.

3. Addressing the Challenges

Efforts to overcome these barriers must focus on:

- **Standardization:**

Developing global guidelines for biochar production and quality assurance.

- **Economic Incentives:**

Introducing subsidies, tax benefits, and carbon credit programs to reduce the financial burden on producers and users.

- **Technological Innovation:**

Investing in research and development to create cost-effective and scalable biochar production methods.

- **Capacity Building:**

Educating stakeholders through training programs and outreach initiatives to enhance their understanding of biochar's applications and benefits.

By tackling these challenges, biochar can achieve its potential as a cornerstone of sustainable agricultural and environmental practices.

Future Perspectives

The future of biochar lies in its ability to evolve as a key player in sustainable agriculture, environmental remediation, and climate change mitigation. Advancements in research and integration with other sustainable technologies can unlock biochar's full potential, driving innovative applications and global adoption.

1. Potential Advancements in Biochar Research

Ongoing and future research efforts aim to enhance the effectiveness, efficiency, and scalability of biochar production and applications.

- **Optimizing Production Techniques:**

Research is focused on developing energy-efficient pyrolysis methods and exploring alternative feedstocks, including invasive plants, algae, and industrial by-products. These innovations could lower costs and improve biochar's environmental footprint.

- **Tailoring Biochar Properties:**

Advances in production techniques can help customize biochar properties to suit specific applications. For example, biochar designed for nutrient retention in agriculture may differ from that engineered for heavy metal adsorption in contaminated soils.

- **Biochar Activation:**

Activation processes, such as steam or chemical treatments, are being refined to increase biochar's porosity and adsorption capacity, enhancing its effectiveness in soil and water remediation.

- **Understanding Biochar-Soil-Microbiota Interactions:**

Deeper exploration of the mechanisms governing biochar's influence on soil microbial communities can lead to optimized formulations for specific crops or ecosystems.

- **Carbon Accounting and Lifecycle Analysis:**

Comprehensive studies on the carbon sequestration potential of biochar and its lifecycle emissions will provide clearer insights into its role in combating climate change.

2. Integrating Biochar with Other Sustainable Technologies

The synergy between biochar and emerging sustainable technologies holds

significant promise for building a circular and low-carbon economy.

- **Biochar in Renewable Energy Systems:**

Combining biochar production with biomass or solar energy systems can create closed-loop systems that utilize waste biomass efficiently. This approach reduces reliance on fossil fuels while producing valuable by-products such as bio-oil and syngas.

- **Biochar in Waste Management:**

Integrating biochar into waste-to-energy technologies can transform organic waste into a high-value resource. This not only reduces landfill use but also mitigates methane emissions from decomposing waste.

- **Soil Microbiota Enhancement Technologies:**

Biochar can be co-applied with microbial inoculants, bio stimulants, or compost to create synergistic effects on soil health and plant growth. These combinations can enhance nutrient cycling, suppress pathogens, and increase agricultural productivity.

- **Advanced Water Filtration Systems:**

Activated biochar can be incorporated into filtration systems to improve the removal of contaminants, including microplastics, pharmaceuticals, and heavy metals, from water sources.

- **Climate Resilience in Agriculture:**

Biochar can play a critical role in climate-smart agriculture by improving water use efficiency, enhancing soil fertility, and reducing greenhouse gas emissions. When integrated with precision farming tools, biochar's applications can be precisely targeted to maximize benefits.

- **Biochar in Construction Materials:**

Research on using biochar in eco-friendly construction materials, such as carbon-negative concrete or insulation, can pave the way for sustainable building practices while sequestering carbon.

The integration of biochar into a broader framework of sustainable technologies and practices can address pressing global challenges, including food security, climate change, and waste management. Collaborative efforts among researchers, policymakers, and industries are essential to scale these innovations and realize biochar's transformative potential.

By aligning research with practical applications, biochar can evolve into a cornerstone of sustainable development, bridging the gap between ecological restoration and industrial innovation.

Conclusions

Biochar, as a versatile and sustainable material, holds immense potential for transforming soil health, boosting agricultural productivity, and addressing critical environmental challenges. Through the chapters outlined, we have explored its multifaceted roles and the mechanisms by which it interacts with soil microbiota, enhances microbial processes, and supports sustainable practices.

1. **Dynamic Interplay with Soil Microbiota:**

Biochar creates a conducive environment for microbial activity, fostering biodiversity and promoting nutrient cycling. Its interactions with soil

microorganisms amplify essential processes such as nitrogen fixation, organic matter decomposition, and pathogen suppression.

2. Broad Applications:

From improving soil fertility and plant health to carbon sequestration and pollution mitigation, biochar's applications showcase its adaptability and effectiveness in diverse ecosystems.

3. Challenges to Overcome:

Variability in biochar properties and economic barriers remain significant challenges. Addressing these issues through standardization, cost-efficient technologies, and policy support will be critical for its widespread adoption.

4. Future Innovations:

Advancements in production methods, biochar activation techniques, and understanding its soil-microbiota interactions will pave the way for more targeted and effective applications. Additionally, integrating biochar with other sustainable technologies—such as renewable energy systems, bio stimulants, and waste management solutions—can create synergistic benefits.

5. Vision for Sustainability:

As a tool for enhancing soil resilience, mitigating climate change, and fostering circular economies, biochar aligns with global goals for sustainability. Its role in supporting climate-smart agriculture and eco-friendly industrial practices underscores its transformative potential.

So, biochar serves as a bridge between traditional agricultural practices and modern sustainability challenges. By leveraging advancements in research and technology, biochar can become a cornerstone of sustainable development, driving progress toward a healthier and more resilient planet.

References

1. Faldu, D. K. J., Lakhani, A. L., Gojiya, M. J., & Chauhan, P. M. (2024). Biochar Activation: A Sustainable Carbon Production from Biomass. Journal of Scientific Research and Reports, 30(5), 385-391.
2. Ferreira, O. E., Silva, H. D. M., Alves, A. B., de Aguilar, M. G., Pimenta, L. P. S., Costa, G. H. G., ... & Machado, A. R. T. (2024). Biochar enhances soil interactions and the initial development of sugarcane. Scientific Reports, 14(1), 27610.
3. Hardy, B., Sleutel, S., Dufey, J. E., & Cornelis, J. T. (2019). The long-term effect of biochar on soil microbial abundance, activity and community structure is overwritten by land management. Frontiers in Environmental Science, 7, 110.
4. Hardy, B., Sleutel, S., Dufey, J. E., & Cornelis, J. T. (2019). The long-term effect of biochar on soil microbial abundance, activity and community structure is overwritten by land management. Frontiers in Environmental Science, 7, 110.
5. Jin, X., Zhang, T., Hou, Y., Bol, R., Zhang, X., Zhang, M., ... & Wang, J. (2024). Review on the effects of biochar amendment on soil microorganisms and enzyme activity. Journal of Soils and Sediments, 1-14.
6. Kerner, P., Struhs, E., Mirkouei, A., Aho, K., Lohse, K. A., Dungan, R. S., & You, Y. (2023). Microbial

responses to biochar soil amendment and influential factors: a three-level meta-analysis. Environmental science & technology, 57(48), 19838-19848.

7. Kerner, P., Struhs, E., Mirkouei, A., Aho, K., Lohse, K. A., Dungan, R. S., & You, Y. (2023). Microbial responses to biochar soil amendment and influential factors: a three-level meta-analysis. Environmental science & technology, 57(48), 19838-19848.
8. Kracmarova-Farren, M., Alexova, E., Kodatova, A., Mercl, F., Szakova, J., Tlustos, P., ... & Stiborova, H. (2024). Biochar-induced changes in soil microbial communities: a comparison of two feedstocks and pyrolysis temperatures. Environmental Microbiome, 19(1), 87.
9. Kracmarova-Farren, M., Alexova, E., Kodatova, A., Mercl, F., Szakova, J., Tlustos, P., ... & Stiborova, H. (2024). Biochar-induced changes in soil microbial communities: a comparison of two feedstocks and pyrolysis temperatures. Environmental Microbiome, 19(1), 87.
10. Li, H., Lin, Y., Qin, X., Song, L., Fan, F., Liu, Y., & Li, S. (2024). An updated review on how biochar may possess potential in soil ARGs control on aspects of source, fate and elimination. Biochar, 6(1), 24.
11. Makavana, J. M., Kalaiya, S. V., Dulawat, M. S., Sarsavadiya, P. N., & Chauhan, P. M. (2020). Development and performance evaluation of batch type biomass pyrolyser for agricultural residue. Biomass Conversion and Biorefinery, 1-8.
12. Wang, H., Zhang, R., Zhao, Y., Shi, H., & Liu, G. (2022). Effect of biochar on rhizosphere soil microbial diversity and metabolism in tobacco-growing

BIOSTIMULANT AND PLANT GROWTH REGULATOR: APPLICATIONS AND SIGNALING MECHANISM

ISBN: 978-81-981142-7-3| Year: 2025 | pp: 33 - 36 |

A Probable Bacterial Fungicide for Management of Powdery mildew diseases

[1]S.N. Bagal

[1]S.V. Thite

[2]M.S. Patil

[1]Karmaveer Bhaurao Patil Mahavidyalaya, Pandharpur (Autonomous) Dist. Solapur (M.S.) India Pin – 413304

[2] Sadguru Gadage Maharaj College, Karad Dist. Satara, 415124 (M.S) India (Autonomous)

Email: manasipatil202@gmail.com

Article DOI Link: https://zenodo.org/uploads/14786212

*DOI: **10.5281/zenodo.14786212***

Abstract

Powdery mildew fungi represent one of the most widely distributed and destructive groups of plant pathogens worldwide. To minimize these losses agrarians have dramatically used pesticides and fungicides which are certainly not ecofriendly. In this context, microbial control through the use of natural antagonistic microorganisms has emerged as a promising alternative. Indeed, these microbial pesticides present many advantages in term of sustainability, mode of action and toxicity compared to chemical pesticides. Here, we focus in detail on the versatile utilization of Bacillus based products as microbial pesticide.

For current article a culture of *Bacillus subtilis*, a gram-positive soil inhabiting bacterium has been used. It is an opportunistic pathogen to humans. The in vitro pure culture of *B. subtilis* in nutrient medium with final density of 1×105 count was used for spray. The effect of this spray was estimated by visual observation for the next 8 days. It was found that the spray of microbial pesticide controls the disease effectively, but needs frequent application.

Keywords: Powdery Mildew, Biopesticides, *Bacillus subtilis*

Introduction

The increasing demand for a steady, healthy food supply requires an efficient control of the major pests and plant diseases. Current management practices are based largely on the application of synthetic pesticides. The excessive use of agrochemicals has caused serious

environmental and health problems. Therefore, there is a growing demand for new and safer methods to replace or at least supplement the existing control strategies. Microbial control, to combat pests or plant diseases has emerged as a promising alternative to chemical pesticides.[2] The *Bacilli* offer a number of advantages for their application in agricultural biotechnology Bacillus-based products represent the most important class of microbial products for phytosanitary use viz microbial pesticide, fungicide & fertilizers are commercially available [3, 4]. *Bacillus*-based biopesticides are widely used in conventional agriculture, by contrast, implementation of *Bacillus*-based biofungicides and biofertilizers is still a pending issue. Plant pathogenic fungi and oomycetes are major threats for crops and plant production. Therefore, the control of fungal diseases by bacilli represents another interesting opportunity for agricultural biotechnology. Indeed, several commercial products based on various *Bacillus* species such as *B. amyloliquefaciens, B. licheniformis, B. pumilus and B. subtilis* have been marketed as biofungicides [3]. Biological control of the plant diseases may be a good alternative to reduce the use of chemical pesticides. The chief objective of this research is to suppress the growth of powdery mildew disease with the help of *Bacillus subtilis*.

Materials and Methods

Isolation of Bacterial Strains

Bacterial strains were isolated from soil samples, plant surfaces, and rhizosphere environments associated with crops suffering from powdery mildew infections. The isolation procedure involved serial dilution plating on nutrient agar and subsequent screening for antifungal activity against Erysiphe hosagoudarii (a model powdery mildew pathogen) using the dual-culture method.

Bacterial Identification and Characterization

Bacterial isolates were identified based on morphological characteristics, gram staining, and biochemical assays. Molecular identification was performed by sequencing the 16S rRNA gene and comparing sequences with known bacterial strains in public databases (e.g., GenBank).

Field Trials

Field trials were conducted on *Nyctanthus arbotristis* L plants which are highly susceptible to powdery mildew. Plants were inoculated with *E. hosagoudarii conidia* and treated with bacterial suspensions (1×105) at various intervals. This culture was diluted and used to spray on plants periodically.

Result and Discussion

Isolation and Identification of Bacterial Strains:

A total of 15 bacterial isolates were obtained, with Bacillus subtilis, Pseudomonas fluorescens and Streptomyces griseus showing the most promising antifungal activity against different Erysiphe sp. The molecular analysis of these isolates confirmed their identities, with high similarity to the respective type strains in GenBank.

Antagonism to pathogens is the main mechanism of biocontrol that has been exploited to combat plant diseases with Bacillus species. Cell wall-degrading enzymes (such as chitinases, glucanases and proteases), peptide antibiotics and other small molecules (such as volatile

organic compounds) are secreted by various species of Bacillus. And many of these have been shown to contribute to pathogen suppression [10]. A high number of reports have described the beneficial effects of several Bacillus species against diseases elicited by fungal pathogens. Some examples are the suppression of root diseases (such as avocado root rot, tomato damping-off), foliar diseases (such as cucurbit and strawberry powdery mildews) and postharvest diseases (such as green, grey and blue moulds) [1, 5, 7-9]. Most of these reports highlight the need to integrate these Bacillus agents mainly with fungicides to optimize disease management.

Unfortunately, very little work has been done on their integration with other management tools such as cultural practices, host resistance, natural products and other biological control agents. The research progress made in the use of bacilli as microbial fungicides during the past two decades has been remarkable; if this pace continues, the use of Bacillus-based products to combat fungal diseases will be greatly expanded in the future. This microbial pesticide is very successful at initial stages of infection.

Although bacterial treatments showed promising results, their efficacy in the field can be influenced by environmental factors such as temperature, humidity, and plant variety. The persistence of bacterial inoculants on plant surfaces and their potential to induce systemic resistance in plants should be further studied. Additionally, combining bacterial agents with other biocontrol strategies or natural products may enhance their effectiveness and provide a more comprehensive disease management approach.

Conclusion

The use of bacterial strains as biocontrol agents for the management of powdery mildew diseases holds great promise. This study demonstrated that B. subtilis, is effective antagonists of Erysiphe hosagoudarii, offering a sustainable and eco-friendly alternative to chemical fungicides. Further research on the formulation and application methods of these bacteria, along with their integration into integrated pest management (IPM) programs, is needed to optimize their use in agricultural systems.

Reference

1. Cazorla FM, Romero D, Pe´ rez-Garcı´a A, Lugtenberg BJJ, de Vicente A, Bloemberg G: Isolation and characterization of antagonistic Bacillus subtilis strains from the avocado rhizoplane displaying biocontrol activity. J Appl Microbiol 2007, 103:1950-1959.
2. Droby S, Wisniewski M, Macarisin D, Wilson C: Twenty years of postharvest biocontrol research: is it time for a new paradigm? Postharvest Biol Technol 2009, 52:137-145.
3. Fravel DR: Commercialization and implementation of biocontrol. Ann Rev Phytopathol 2005, 43:337-359.
4. Jacobsen BJ, Zidack NK, Larson BJ: The role of Bacillus-based biological control agents in integrated pest management systems: plant diseases. Phytopathology 2004, 94:1272-1275.
5. Jayaraj J, Radhakrishnan NV, Kannan R, Sakthivel K, Suganya D,

Venkatesan S, Velazhahan R: Development of new formulations of Bacillus subtilis for management of tomato damping-off caused by Pythium aphanidermatum. Biocontrol Sci Technol 2009, 15:55-65.

6. Leelasuphakul W, Hemmanee P, Chuenchitt S: Growth inhibitory properties of Bacillus subtilis strains and their metabolites against the green mold pathogen (Penicillium digitatum Sacc.) of citrus fruit. Postharvest Biol Technol 2008, 48:113
7. Liu B, Qiao H, Huang L, Buchenauer H, Han Q, Kang Z, Gong Y: Biological control of take-all in wheat by endophytic Bacillus subtilis E1R-j and potential mode of action. Biological Control 2009, 49:277-285
8. Pertot I, Zasso R, Amsalem L, Baldessari M, Angeli G, Elad Y: Integrating biocontrol agents in strawberry powdery mildew control strategies in high tunnel growing system. Crop Protection 2008, 27:622-631.
9. Romero D, de Vicente A, Zeriouh H, Cazorla FM, Ferna´ ndez- Ortun˜ o D, Tore´ s JA, Pe´ rez-Garcı´a A: Evaluation of biological control agents for managing cucurbit powdery mildew on greenhouse-grown melon. Plant Pathol 2007, 56:976-986.
10. Shoda M: Bacterial control of plant diseases. J Biosci Bioeng 2000, 89:515-521.

BIOSTIMULANT AND PLANT GROWTH REGULATOR: APPLICATIONS AND SIGNALING MECHANISM

ISBN: 978-81-981142-7-3| Year: 2025 | pp: 37 - 41 |

Natural Biostimulants for Enhancing Plant Tolerance to Salt and Salinity Stress: A Review

Dr. Varsha Sanjay Khude

Department Of Botany, Devchand College, Arjunnagar

Email: drvarshakhude81@gmail.com

Article DOI Link: https://zenodo.org/uploads/14786243

DOI: 10.5281/zenodo.14786243

Introduction

Soil salinity is a significant problem worldwide, affecting over 800 million of hectares of land and impacting agricultural productivity, food security, and the environment. The causes of soil salinity are multifaceted, including over-irrigation, poor drainage, sea level rise, and geological processes. Over-irrigation, can lead to the accumulation of salts in the soil, while poor drainage can cause water to accumulate in the soil, leading to salt buildup. The effects of soil salinity are far-reaching, resulting in reduced crop yields, water scarcity, soil erosion, and environmental impacts. Saline soils can decrease crop yields and affect plant growth, while also exacerbating water scarcity as plants require more water to survive. Additionally, saline soils can be more prone to erosion, leading to soil degradation and loss of fertile land. Salt stress can significantly impact plant growth and productivity, leading to reduced crop yields and economic losses.

To mitigate the impacts of soil salinity, various management and mitigation strategies can be employed. Implementing effective drainage systems can help reduce soil salinity, optimizing irrigation practices, such as using drip irrigation, can minimize salt accumulation. Planting salt-tolerant crops can also help maintain agricultural productivity, while adding organic amendments, such as compost, can improve soil structure and fertility. Furthermore, Natural biostimulants have emerged as a promising solution to enhance plant tolerance to salt and salinity stress by improving their antioxidant defense systems, nutrient and phytohormone balance, and molecular biology.

Natural Biostimulants

Biostimulants are substances that stimulate plant growth and development, improving crop yields and plant

resilience. These substances can be derived from natural sources, such as plants, microorganisms, and seaweed, or produced synthetically. Biostimulants have gained increasing attention in recent years due to their potential to improve crop productivity, reduce environmental impact, and promote sustainable agriculture. Biostimulants can be classified into several categories based on their origin, composition, and mode of action. Some of the main types of biostimulants include:

1. Plant-Derived Biostimulants

Plant-derived biostimulants are obtained from plants and plant extracts. For Examples, Plant growth-promoting rhizobacteria (PGPR), Plant extracts, such as those from seaweed or algae and Humic and fulvic acids. A study by Abou-Sreea AIB et. al. (2021) in chili pepper state that, Plant biostimulants like honey bee (HB) and silymarin (Sm) are a strategic trend for managing stressed crops by promoting nutritional and hormonal balance, regulating osmotic protectors, antioxidants, and genetic potential, reflecting plant growth and productivity. They applied diluted honey bee (HB) and silymarin-enriched honey bee (HB- Sm) as foliar nourishment to investigate their improving influences on growth, yield, nutritional and hormonal balance, various osmoprotectant levels, different components of antioxidant system, and genetic potential of chili pepper plants grown under NaCl-salinity stress (10 dS m–1).

A study by Ahmad A et al. (2022) stats that, Use of natural plant extracts (PEs) (or "botanicals") could be one of the salinity mitigation ecofriendly strategies. PEs are potential alternatives to chemical fertilizers. PEs fall under the umbrella of plant biostimulants, and are used to enhance plant growth. PEs are the concentrates of plants and could be prepared using any part of the plant, i.e., seeds, roots, stems, leaves, bark, flowers, etc. The application of PEs could be either in liquid form as foliar spray and/or root treatment, or as soil preparations like granules, concentrates, solutions added to soil, or powders. However, the effect of PEs is often concentration dependent. Similarly, plant part and age of plant used as an extract also influences the PEs overall proficiency.

Seaweed extracts are rich in bioactive compounds, including amino acids, vitamins, and minerals. They have been shown to enhance plant tolerance to salt stress by reducing oxidative stress, improving nutrient uptake, and regulating plant hormone production. Theodora Ntanasi et. al (2024) demonstrates that different tomato cultivars, with varying fruit sizes, responded differently to salt stress. The application of 'Algastar' was shown to increase fruit diameter and TSSC (°Brix), while 'Nitrostim' treatment was linked to improved fruit firmness and higher Fe concentration of salinity-stressed plants. These findings highlight the significant role of biostimulants in mitigating saline stress but also indicate that biostimulants should serve as strategic tools to optimize the productivity of the crops under adverse growth conditions.

Humic and fulvic acids:

Humic substances (HS) are natural constituents of the soil organic matter, resulting from the decomposition of plant, animal and microbial residues, but also from the metabolic activity of soil

microbes using these substrates. Humic substances and their complexes in the soil thus result from the interplay between the organic matter, microbes and plant roots. Humic acids can improve soil fertility and plant growth. They have been shown to enhance plant tolerance to salt stress by improving nutrient uptake, reducing oxidative stress, and regulating plant hormone production. The biostimulation activity of HS for stress protection, High-molecular mass HS have been shown to enhance the activity of key enzymes of this metabolism in hydroponically-grown maize seedlings, suggesting stress response modulation by HS (Olivares et al., 2015, Schiavon et al., 2010). El-Sayed M. (2018) in Egypt studied the integrative application of HM (HA+FA) and MLE was effective in alleviating the physiological response of salt stress damages. The integrative HM+MLE-treated sorghum plants under salt stress maintained higher osmoprotectants, phytohormone, nucleic acids and antioxidants, concluding that this integrative treatment plays a very important role in plant growth, development and metabolism and responses to salt stress. It could also be concluded that HM and MLE can be used as plant bio-stimulants/nutritive means in integration under normal or abnormal conditions as an economic and natural source of mineral nutrients, phytohormone, amino acids, osmoprotectants, and antioxidants.

2. Microbial Biostimulants

Microbial biostimulants are derived from microorganisms, such as bacteria and fungi. E.g. Mycorrhizal fungi, Trichoderma spp. and Bacillus spp. Mycorrhizal fungi form symbiotic relationships with plant roots, providing nutrients and water in exchange for carbohydrates. They have been shown to enhance plant tolerance to salt stress by improving nutrient uptake, reducing oxidative stress, and regulating plant hormone production. Plant Growth-Promoting Rhizobacteria (PGPR): PGPR are beneficial bacteria that colonize plant roots and promote plant growth. They have been shown to enhance plant tolerance to salt stress by producing antioxidants, solubilizing minerals, and regulating plant hormone production. A study by El-Sayed M et al., 2020) states that Plant growth- promoting rhizobacteria (PGPRs) can suppress salt stress effects and improve plant productivity. This study elucidated the mechanisms of growth medium-inoculated PGPRs (Bacillus cereus, Serratia marcescens, and Pseudomonas aeruginosa), which contribute to improving salinity tolerance in Triticum aestivum plants grown under two NaCl-salinity levels (150 and 300 mM).

3. Synthetic Biostimulants

Synthetic biostimulants are man-made substances designed to mimic the effects of natural biostimulants. E.g. Amino acids and peptides, Glycine betaine and Polyamines.

Mechanisms of Action

Natural biostimulants can enhance plant tolerance to salt and salinity stress through several mechanisms, including:

1. Antioxidant Production:

Natural biostimulants can produce antioxidants that neutralize reactive oxygen species (ROS) and reduce oxidative stress.

2. Nutrient Uptake and Utilization

Natural biostimulants can improve nutrient uptake and utilization, reducing the impact of salt stress on plant growth.

3. Hormone Regulation:
Natural biostimulants can regulate plant hormone production, reducing the impact of salt stress on plant growth and development.

4. Soil Microbiome Modulation
Natural biostimulants can modulate the soil microbiome, promoting the growth of beneficial microorganisms that can help plants tolerate salt stress.

Applications in Agriculture:
Natural biostimulants have several applications in agriculture, including:
1. Crop Production:
Natural biostimulants can be used to enhance crop yields and improve plant tolerance to salt and salinity stress. Biostimulants assist in combating the effects of environmental stresses. Biostimulants promote enhanced germination and root development, leading to increased vigor and greater stress resistance. An enhanced root system promotes more efficient nutrient and water uptake and translocation throughout the growing season.

2. Promoting growth and improving a plant's metabolism
It can benefit overall plant growth and health. In addition, providing a catalyst at specific developmental stages can lead to increased yield, improved uniformity and overall crop quality. Biostimulants increase grain fill and quality. Biostimulants generally operate through different mechanisms than standard fertilizers and provide essential nutrients for plant metabolism that stimulate plant growth. These metabolic pathways regulate gene expression that can have an effect on cell division and sizing, root and shoot growth, and reproductive development and timing.

3. Soil Remediation:
Natural biostimulants can be used to remediate saline soils, improving soil fertility and plant growth.

4. Irrigation Management:
Natural biostimulants can be used to improve irrigation management, reducing water usage and improving crop yields.

Conclusion:
Natural biostimulants offer a promising solution to enhance plant tolerance to salt and salinity stress. Further research is needed to fully explore the potential of natural biostimulants and to develop effective application strategies.

Future Directions:
Future research should focus on:
1. Identifying New Natural Biostimulants: Identifying new natural biostimulants and understanding their mechanisms of action can provide new opportunities for enhancing plant tolerance to salt and salinity stress.
2. Optimizing Application Strategies: Optimizing application strategies, such as timing, dosage, and method of application, can help maximize the benefits of natural biostimulants.
3. Integrating Natural Biostimulants with Other Sustainable Practices: Integrating natural biostimulants with other sustainable practices, such as conservation agriculture and organic farming.

Referances

1. Abou-Sreea AIB, Azzam CR, Al-Taweel SK, Abdel-Aziz RM, Belal HEE, Rady MM, Abdel-Kader AAS, Majrashi A, Khaled KAM. (2021): Natural Biostimulant Attenuates Salinity Stress Effects in Chili Pepper by Remodeling Antioxidant, Ion, and Phytohormone Balances, and Augments Gene Expression. Plants (Basel).;10(11):2316. doi: 10.3390/plants10112316. PMID: 34834678; PMCID: PMC8617650.
2. Ahmad A, Blasco B and Martos V (2022): Combating Salinity Through Natural Plant Extracts Based Biostimulants: A Review. Front. Plant Sci. 13:862034. doi: 10.3389/fpls.2022.862034
3. El-Sayed M. Desoky, Ahmed M. Saad, Mohamed T. El-Saadony, Abdel-Rahman M. Merwad, Mostafa M. Rady, (2020): Plant growth-promoting rhizobacteria: Potential improvement in antioxidant defense system and suppression of oxidative stress for alleviating salinity stress in Triticum aestivum (L.) plants, Biocatalysis and Agricultural Biotechnology,.30:101878, ISSN 1878-8181, https://doi.org/10.1016/j.bcab.2020.101878.
4. El-Sayed M. Desoky, Abdel-Rahman M. Merwad & Mostafa M. Rady (2018): Natural Biostimulants Improve Saline Soil Characteristics and Salt Stressed-Sorghum Performance, Communications in Soil Science and Plant Analysis, DOI: 10.1080/00103624.2018.1448861
5. Theodora Ntanasi, Ioannis Karavidas, George P. Spyrou, Evangelos Giannothanasis Konstantinos A. Aliferis Costas Saitanis, Vasileios Fotopoulos, Leo Sabatino, Dimitrios Savvas and Georgia Ntatsi (2024): Plant Biostimulants Enhance Tomato Resilience to Salinity Stress: Insights from Two Greek Landraces Plants, 13(10), 1404; https://doi.org/10.3390/plants13101404
6. F.L. Olivares, N.O. Aguiar, R.C.C. Rosa, L.P. Canellas (2015): Substrate biofortification in combination with foliar sprays of plant growth promoting bacteria and humic substances boosts production of organic tomatoes. Sci. Hortic., 183 (2015), pp. 100-108
7. M. Schiavon, D. Pizzeghello, A. Muscolo, S. Vaccaro, O. Francioso, S. Nardi (2010): High molecular size humic substances enhance phenylpropanoid metabolism in maize (Zea mays L.) J. Chem. Ecol., 36, pp. 662-669

BIOSTIMULANT AND PLANT GROWTH REGULATOR: APPLICATIONS AND SIGNALING MECHANISM

ISBN: 978-81-981142-7-3| Year: 2025 | pp: 42 - 52 |

A perspective of Allelopathy in Agriculture

[1]Supriya R. Nikam

[2]Dr. D. D. Namdas

[1]Research Scholar, Department of Botany and Plant Protection, Sadguru Gadage Maharaj College, Karad,Maharashtra 415-110.

[2]Research Guide, Department of Botany and Plant Protection, Yashwantrao Chavan Institute of Science,Satara. Maharashtra 415-110.

Email: supriyanikam2043@gmail.com

Article DOI Link: https://zenodo.org/uploads/14786537

DOI: 10.5281/zenodo.14786537

Abstract

Allelopathy is the process by which one plant produces chemicals that impede the growth and development of another plant. Allelochemicals have been suggested as a promising drug development method for environmentally friendly biological weedicides and frequently promote or inhibit plant growth. As a biological weedicide, the identification of plant materials with abundant sources, reasonable costs, and strong allelopathic effects will surely have a major ecological impact. To ascertain the impact of allelopathic phenomena in agriculture with regard to specific weeds, soybean (Glycine max L.) seeds were treated in a lab setting with aqueous extracts of four chosen weeds at concentrations of 5, 10, and 15% (w/v). A completely randomized design was used to set up the treatments in a three-replication factorial configuration. The date of the test was January, 2024. For the previously listed parameters, the results indicated a considerable variation between plant sections, concentration levels, and their interaction. It was discovered that certain weeds, including *P. hysterophorus L., A. sessilis, C.dactylon, and B.diffusa,* were allelopathic. They contained water-soluble allelochemicals in their plant parts, and their phytotoxic potency was strong enough to inhibit the growth and nutrient accumulation of related agricultural plants. In the current study, four different weeds that are found locally and are related to the soybean crop in the Sangli district are used. The allelopathic state of these weeds will be

investigated, and their extracts will be used to gauge the soybean crops' growth response. The suggested study could aid in comprehending the allelopathic experiment.

Keywords: Aqueous extract, weed, allelopathy, germination, Sangli district.

Introduction

It is a true statement that without plants, life could not exist for a single minute. This is due to the fact that plants have bestowed upon us the invaluable gift of oxygen, a gas necessary for our survival. But in addition to these many benefits, plants also provide us with benefits in different forms. First of all, a wide range of well-known ayurvedic medications are made possible by medicinal herbs. The second plant yields spices, which have long captivated people due to their aroma. Furthermore, there are plants utilized in agriculture, plants that yield fiber, and plants that are aesthetically pleasing. The aforementioned plant varieties already have their own economic value, which is the cause behind the disagreement. On the other side, a class of plants known as "Weed Plants" are those that have consistently been disregarded and have been identified as troublesome plants while having unclear economic significance. Around 3%, or 8000 species, of the approximately 250,000 plant species that exist in the globe act as weeds. The most prominent and well-known characteristic of weeds is their bothersome nature in general.Weeds are unwanted plant grows in a place where some other plants are also growing or no other plant has to grow at all. Weeds are unwanted, harmful, dangerous or economically detrimental. Weeds are found common in both kharip and rabbi season (Chavan et.al2013) Weed flora of Sangli district is very rich with family Asteraceae and Poaceae (Uday Prakash et.al. Weeds are plants which do not have any specific requirement with respect to climatic conditions, nutrients, space. They grow at disturbed and inhabited places. Weeds are useful to human beings as food, erosion control, medicine, aesthetic value, shelter, supply of organic matter and mineral nutrients to the soil (Shreshtha2017).

About allelopathy

The term allelopathy was first used in 1937 by the Austrian professor Hans Molisch in the German-language book Der Einfluss einer Pflanze auf die andere - Allelopathie (The Effect of Plants on Each Other - Allelopathy), which derives from the Greek words allelo- and -pathy (meaning "mutual harm" or "suffering"). He used the phrase to refer to the biochemical interactions that a plant uses to prevent its neighbours' plants from growing. Allelopathy, according to Rice (1984), is any detrimental influence that one plant may have on another by the creation of chemical compounds that escape into the environment. The allelopathic plants, also referred to as Nature's Weed Killers, are particularly powerful in eliminating weeds. Allelopathic compounds limit plant growth by interfering negatively with critical physiological processes like cell

wall modification, cell division inhibition, and enzyme activity.

Selected test crop

Description- Soybean (Glycine max (L.) Merrill) is a superior leguminous crop of exceptional nutritional value, being richer in protein (40-42 per cent) of higher quality than pulses and groundnut and unlike pulse, is a good source of edible oil (20-22 per cent). So, it feeds the man as a pulse and oilseed; his cattle as a quality feed and the soil by enhancing its fertility status.

Origin and Distribution

Although the origins of the soybean plant are uncertain, many botanists believe that it was first domesticated in central China around 7000 BCE. For thousands of years, China, Japan, and Korea have used soybeans as food and a component of medicines. This is a traditional crop. Soybeans were initially brought into the nation in 1804, but their significance only increased in the South and Midwest during the mid-1900s. Brazil and Argentina are significant producers as well.The major Soybean growing states in India are Maharashtra, Madhya Pradesh, Rajasthan, Karnataka, Andhra Pradesh and Chattisgarh, Among these states of Maharashtra ranks second in acreage under Soybean after Madhya Pradesh. In Maharashtra state the area under cultivation of soybean is 3.8 million hectoros with production of 3.07 million tonnes (Jaybhay et al, 2016). The Soybean crop cultivating districts on large scale are mainly, Latur, Nanded, Parabhani, Aurangabad, Jalna, Beed, Osmanabad, Pune, Solapur, Sangli and Satara (I.S.M., Vol.11, 2004). Soybean (Glycine max) crop is ideally suited to areas of moderate rainfall. It requires warm and moist climate, temperature (150C to 320C) and mostly grown in mansoon season from June to October (Kharif). Due to environmental factors, such as temperature, soil, water effects on production of soybean. An increase in plant population per acre can cause competition for water, nutrients and stress. Weeds can also compete with the soybean crop, lengthening time between vegetative stages or shortening time between reproductive stages. In order for a disease to occur, you need a pathogen a susceptible host and a favourable environment. Soybean varieties differ in disease susceptibility however, increased population could create a more favourable environment for some disease. Insecticides constitute large proportion of insect management as they are used to control most insect pests in some cases are the primary method of control. To overcome this problem, the effect of insect, pest, pathogen, the farmers use chemical pesticides and fertilizers such as Asana XL, Larvin 3.2 (thiodicarb). Azaxystrobin, Bascalid, Carbendazim (MBC), Atrazine Fluridone, Metolachlor, Introduction 40 Trifluralin, 2, 4 Dimethyl phenyl formamide (DMPF), Dieldrin. Recently the farmers use plant extract to minimize. insect pest like Neem, garlic, tobacco, petroleum ether extract of periwinkle etc. In addition to these some people use weed extracts Pendimethalin, Fluchloralin, Imaxzithapayar, Weed block etc. In present investigation an

attempt to study these weeds to study morphology, germination, seedling growth and its allelopathic effect in agriculture In present investigation an attempt is made use locally occurring four various weeds in associated with soybean crop from study area (Sangli district).These
weeds will be studied to their phytochemical status and applied in the form of their extracts to measure the growth response in soybean crops.

Information about weed and crop selected for research work
Allelopathic studies on selected weeds

***Alternanthera sessilis* (L.) R.Br.ex DC.**

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Caryophyllales
Family	Amaranthaceae
Genus	*Alternanthera*
Species	*sessilis* (L.) R.Br.ex DC.

Description

A perennial herb, stalkless joyweed is frequently found in and around ponds, canals, and reservoirs. It can be found in marshes, small ditches, and abandoned rice fields since it loves environments with high humidity that is either persistent or intermittent. An herb with many branches that is prostrate and frequently roots at the lower nodes. It has simple, opposite, slightly fleshy leaves that are lanceolate, oblanceolate, or linear-oblong, obtuse or subacute, and glabrous. It also has small, white flowers in axillary clusters, compressed obcordate utricles, and suborbicular seeds. Tender shoots and leaves are consumed in Manipur together with fermented soy beans and rice. At elevations between 200 and 2000 meters, the Himalayas are home to stalkless joyweed.

Impact on Agriculture

A. sessilis is an invasive plant that primarily grows in wetlands and is a weed in both agriculture and the environment. If conditions are right, it can develop pure stands of tightly woven stems that suffocate semi-aquatic and aquatic ecosystems, obstruct irrigation channels, displace native flora, and obstruct pastures and crops in low-lying, poorly drained places.

Parthenium hysterophorous

Kingdom	Plantae
Class	Magnoliopsida
Order	Asterales
Family	Asteraceae
Genus	*Parthenium*
Species	*hysterophorus* L.

Description

The upright, whitish-green *Parthenium hysterophorus* plant has branches and is green in color. It has loosely haired stems. It is between 30 and 40 cm tall, although it can grow as high as 2 m. The leaves are separated into deep, slender segments and are alternating. The inflorescence is arranged in tiny globular capitula in groups of 4 or 5. Flowers in the Centre are ivory white, while those in the peripheral are white. A peduncle carries each capitulum. The entirety creates a big, loose inflorescence with tiny white capitulum. The fruit is a hairy-topped, black, obovoid achene that is 2 mm long and 1.5 mm wide.

Impact on Agriculture

P. hysterophorus allelopathic characteristics have the most of an effect on crops. Caffeic acid, ferulic acid, vanicillic acid, anisic acid, fumaric acid, and sesquiterpene lactones, primarily parthenin and/or hymenin, occur in all parts of the plant and significantly inhibit the germination and subsequent growth of a wide range of crops, including pasture grasses, cereals, vegetables, other weeds, and tree species (Evans, 1997).

Boerhavia diffusa

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Caryophyllales
Family	Nyctaginaceae
Genus	*Boerhaavia.*
Species	*diffusa* (L.)

Description

Herbs with prolonged trailing branches and a tomentose, scarlet stem. Uneven, ovate, obtuse, undulating, truncate to subcordate, tomentose leaves with petioles up to 1 cm long. Flowers 4 mm long, 4–10 in a group, in terminal or axillary peduncled umbels; bracts 5, oval, glandular; pink perianth; 3 stamens; capsule 3 x 1 mm; clavate; 5-ribbed; glandular.

Impact on Agriculture

It is the most common principal weed of date palm orchards in India (Josan et al., 1993) and is one of the most problematic weeds in mustard in India (Rajput et al., 1993), where it is also recorded as a weed in tobacco, pearl millet and

groundnut (Singh and Prasad, 1991; Kennedy et al., 1992

Cynodon dactylon (L.) Pers.

Kingdom	Plantae
Class	Liliopsida
Order	Poales
Family	Poaceae
Genus	*Cynodon*
Species	*dactylon* (L.) Pers.

Description

Creeping perennials that are thin and stoloniferous. Sheaths are keeled; ligules are fimbriate and membranous; leaves are linear-lanceolate, acuminate, and glaucous, measuring 1–10 x 0.1–0.5 cm. spikes that are 1-sided, oblong, and up to 5 cm long; inflorescence of terminal, digitate 3–4 spikes. Spikelets are sessile, 2-3 mm long, oblong-lanceolate, compressed laterally, and unbranched. Lance-shaped, chartaceous, and one-nerved lower glume, 1.5–2 x0.5 mm. Lanceolate, chartaceous, and 1-nerved upper glume, approximately 2× 0.5 mm. Lemma 2-3 x 1.5–2 mm, keeled, and ovate-oblong when spread. Palea 2-2.5 x 0.5-1 mm, distributed into a boat form or an oblong shape, chartaceous. 3-stamen, 1- to 1.5-mm-long anthers. 0.5 mm length, oval ovary; 0.5–1 mm long, pink stigmas. 1 mm or so linear caryopsis.

Impact on Agriculture

According to Holm et al. (1977), C. dactylon is the second most significant weed in the world (after Cyperus rotundus), a designation that is supported by the fact that it is present in almost every tropical and subtropical nation as well as in almost every crop grown there. It is recognized as a "serious" or "principal" weed in no less than 57 nations in their later book (Holm et al., 1979).

Selected Test crop

Glycine max

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Fabales
Family	Fabaceae / Leguminosae
Genus	*Glycine* Willd.

Species *max* (L.) Merr.

Description

Soybean (Glycine max (L.) Merrill) is a superior leguminous crop of exceptional nutritional value, being richer in protein (40-42 per cent) of higher quality than pulses and groundnut and unlike pulse, is a good source of edible oil (20-22 per cent). So, it feeds the man as a pulse and oilseed; his cattle as a quality feed and the soil by enhancing its fertility status.

Result and discussion

Allelopathic study- Petri plate culture

Germination

Soybean seed germination was strongly impacted by the aqueous extract of every plant. With higher doses of various aqueous extracts, it was discovered that the inhibitory effect grew stronger. In comparison Parthenium extract had the strongest allelopathic effect on seed germination. The aqueous extract of all the weeds significantly affected the germination of soybean seeds Germination percentage in control was maximum germination

Allelopathy has gained increased significance in agricultural practices as a result of the primary goals of employing this phenomenon in biological weed management (Rice 1984). This finding should be further examined and used to filter plant species that are allelopathic (Leather, 1982). Two test plant species in the present study validated the growth inhibitory effects on four Nepalese medicinal plants. Due to the production of allelochemicals from many chemical classes, particularly polyphenolic compounds (flavonoids and tannins), cyanogenic glycosides, and alkaloids (Einhelling, 1999), plants demonstrated allelopathic activity. Putative allelochemicals may be the cause of the plant extracts' inhibition of seed germination and seedling growth. In the current study, phenolic chemicals including flavonoids, tannins, and phenols can be used to explain the allelopathic action of specific weed plants Additionally, it was noted that the response threshold of inhibitory compounds affected how well receiver plants responded to allelochemicals (Lovett et al. 1989). These weed plants had various inhibitory effects on the test plant species.

The variation may be explained by variations in the types, quantities, and characteristics of allelochemicals produced by the several species studied in this study. According to Chon et al. (2005), the allelopathic activity of lettuce plant extracts varied depending on cultivar, extract, or fraction. Lower concentrations of plant extracts resulted in higher rates of germination, whereas larger concentrations of extracts resulted in lower rates of germination This could be because concentrated extracts contain more phytochemicals than extracts with lesser concentrations. At increasing concentrations of Parthenium, *A. sessilis*, and *C. dactylon, B. difusa* weed extracts, soybean seeds exhibited a minimum germination rate Phenolic chemicals may alter the growth hormone Giberellic acid's actions in the seed, which are necessary for stimulating seed germination (Olofsdotter 2001). Tefera

(2002) discovered that leaf extract had a stronger inhibitory allelopathic effect than other vegetative portions. However, the outcome in the case of soybean seeds was a little bit different.At greater concentrations of *Parthenium, A.sessilis*, *B.difusa* and *C.dactylon* extracts, the lowest germination rate was seen. These plant extracts were discovered to have an adverse effect on the germination of soybean seeds. This might be because these two plants have stronger allelopathic potential. Interestingly it was observed that *P.hysterophorus,A sessilis, C. dactylon, Boerrhavia diffusa* found growth stimulant effect with increasing concentrations. *P. hysterophorus* varied greatly depending on the extract concentration and grew longer as it did.

Biomass study

Under the application of three different concentrations of extracts, the biomass of the soybean plant shown a declining trend, suggesting that the weeds may be impeding crop growth. The effects of various extract concentration levels on seedling biomass varied significantly. Soybean seedlings' below-ground dry weight was more responsive than their above-ground dry weight. The aboveground dry weight of soybean seedlings, in contrast, was more susceptible to *Parthenium* and *C.dactylon* extracts than the belowground dry weight. The biomass of soybean was examined under various additional amounts of weed extract concentrations in order to determine whether weeds had allelopathic effects on soybean. The findings demonstrated that low concentrations had no overtly inhibitive effects on the crops, but that inhibition was dose-dependent. In other words, the soybean crops are more strongly inhibited by higher concentration levels. Plant biomass is crucial for the research of allelopathy since it is a key indicator of seedling growth. Generally speaking, the aboveground and belowground components of cultivated plants respond differently to changes in temperature, soil moisture, fertilizer application of nitrogen and phosphorus, light, etc. Depending on the species and concentration, the allelopathic effects on radicle length, seedling fresh weight, and other early growth indicators varied. All of the weed extracts under study decreased total seedling dry weight (g), although *P. hysterophorus* and *A. sessilis* showed the greatest reductions; B. diffusa and *C. dactylon* showed the least reductions.

Bhowmik and Doll (1984) also noted that many annual weeds inhibited the growth of soybean seedlings. Compared to the shoot and roots, these plant sections (Kanchan and Jaycharad, 1980). Similar to this, parthenin, which is released to the soil during the breakdown of *Parthenium* leaves, is reported by Belz et al. (2007) to be the main poisonous substance producing phytotoxicity. According to the findings of Tefera (2002) and Wakjira et al. (2005), soybean and haricot bean roots appeared to be more susceptible to the allelopathic effect than shoots. This could be as a result of the root coming

into direct touch with the extracts and then with the inhibitory chemicals (Tefera, 2002). Allelochemicals, which may impede the function of gibberellin and indoleacetic acid, may be responsible for the decreased rate of cell division that is causing the lengthening of seedling roots (Tomaszewski and Thimann, 1966). The results of this study showed that *Parthenium* weed has an allelopathic effect on soybean, as evidenced by its inhibitory effect on seedling germination, germination rate, growth, and dry matter production. Similar findings have been recorded for a variety of crops, including cabbage (Kohli et al., 1985), tomato (Mersie and Singh, 1988), pumpkin (Cucurbita moschata) (Guzman, 1988), multipurpose trees, and arable crops (Evans, 1997).

It can be inferred from the current exploratory experiment conducted under laboratory conditions that Parthenium and *A. sessilis, C.dactylon, B.difusa* has the capacity to prevent soybean seed germination and seedling growth. This is a blatant sign that *Parthenium* and *C.dactylon, A.sessilis, B.difusa* and will pose a threat to crop output. Therefore, it is necessary to seek an integrated management plan for *Parthenium* and *Phyllanthus* which necessitates a cooperative effort from all institutions involved in agricultural production.

Conclusion

Some selected plants such as *Parthenium, A. sessilis, C.dactylon, B.difusa* shows greater inhibition. Higher inhibitory effect on the germination of test seeds by the *Parthenium* extracts must be due to the presence of phytochemicals especially the phenolic compounds in higher concentration. Seedling growth inhibition was maximum by *Parthenium* weed extracts. Overall inhibitory effect in soybean plant was highest for *Parthenium* extracts and lower for *Boerhaavia* and *Cynadon* extract. Presence of phenolic compounds and tannins in higher quantity in *A. conyzoides* and *Parthenium* responsible for the least soybean biomass production. So, the phytochemicals phenolic compounds, alkaloids and saponins present in the selected weed plants under study which show different medicinal properties were responsible for positive and negative allelopathic activities of the plants. So, the presence of allelopathic phytochemicals in the weed plants such as *Parthenium*, were responsible for the inhibitory effect of the plants extracts on seed germination and seedling growth of these plants. This result may be useful to future workers to select a group of plants having similar constituents to isolate biologically active principle or prepare bio-weedicide or biostimulants for particular case.

References

1. Almodovar-Vega, L., Guzman-Perez, C. D., & Semidey-Laracuente, N. (1988). Allelopathic effects of seven weed species on pumpkin (Cucurbita moschata) under greenhouse conditions
2. Bhuvaneswari, S., Aravind, R., Kaviyarasan, V., Kalaivanan, K., Hariram, S. B., & Udayaprakash, N.

(2011). A comparative study on antibacterial activity of common weeds. International Journal of Pharma and Bio Sciences, 2(1).

3. Belz, R. G., Reinhardt, C. F., Foxcroft, L. C., & Hurle, K. (2007). Residue allelopathy in Parthenium hysterophorus L.—Does parthenin play a leading role? Crop Protection, 26(3), 237-245.
4. Bhowmik, P. C., & Doll, J. D. (1984). Allelopathic effects of annual weed residues on growth and nutrient uptake of corn and soybeans 1. Agronomy journal, 76(3), 383-388.
5. Channappagoudar, B. B., Panchal, Y. C., Manjunath, S., & Koti, R. V. (1990). Studies on influence of parthenium on sorghum growth under irrigated conditions.
6. Chavan, Y. R., Thite, S. V., Aparadh, V. T., & Kore, B. A. (2013). Phytochemical analysis of some weeds. The global journal of pharmaceutical research, 2(1).
7. Chiang, M. Y., & Leu, L. S. (1981). Weeds in paddy field and their control in Taiwan. Weeds and weed control in Asia.
8. Chon, S. U., Jang, H. G., Kim, D. K., Kim, Y. M., Boo, H. O., & Kim, Y. J. (2005). Allelopathic potential in lettuce (Lactuca sativa L.) plants. Scientia Horticulturae, 106(3), 309-317.
9. Doll, J., Pinstrupandersen, P., & Díaz, R. (1977). An agro-economic survey of the weeds and weeding practices in cassava (Manihot esculenta Crantz) in Colombia. Weed Research, 17(2), 153-160.
10. Einhellig, F. A. (1999). An integrated view of allelochemicals amid multiple stresses. Principles and practices in plant ecology, 479-494.
11. Ekwealor, K. U., Echereme, C. B., Ofobeze, T. N., & Okereke, C. N. (2019). Economic importance of weeds: A review. Asian J Plant Sci, 3, 1-11.
12. Evans, H. C. (1997). Parthenium hysterophorus. a review of its weed status and the possibilities for biological control. Biocontrol News and information, 18, 89N-98N.
13. Holm, L., Doll, J., Holm, E., Pancho, J. V., & Herberger, J. P. (1997). World weeds: natural histories and distribution. John Wiley & Sons.
14. Holm, L., Pancho, J. V., Herberger, J. P., & Plucknett, D. L. (1979). A geographical atlas of world weeds (pp. xlix+-391).
15. Josan, J. S., Thatai, S. K., & Monga, P. K. (1993). Principal weeds of date palm orchards.
16. Kohli, R. K., Anita Kumari, A. K., & Saxena, D. B. (1985). Auto-and teletoxicity of Parthenium hysterophorus L.
17. Jaybhay, S. A., Taware, S. P., Varghese, P., & Nikam, V. R. (2018). Soybean cultivation by farmers of Maharashtra: Identification and analysis of the problems. Legume Research-An International Journal, 41(3), 474-479.

18. Kanchan, S. D., & Jayachandra. (1980). Allelopathic effects of Parthenium hysterophorus L: Part IV. Identification of inhibitors. Plant and Soil, 55, 67-75.
19. Kennedy, F. J. S., Lourduraj, A. C., & Rajamanickam, K. (1992). Weeds as alternate hosts for groundnut leaf miner.
20. Kohli, R. K., Anita Kumari, A. K., & Saxena, D. B. (1985). Auto-and teletoxicity of Parthenium hysterophorus L.
21. Lovett, J. V., Ryuntyu, M. Y., & Liu, D. L. (1989). Allelopathy, chemical communication, and plant defense. Journal of Chemical Ecology, 15, 1193-1202.
22. Mao, J., Yang, L., Shi, Y., Hu, J., Piao, Z., Mei, L., & Yin, S. (2006). Crude extract of Astragalus mongholicus root inhibits crop seed germination and soil nitrifying activity. Soil Biology and Biochemistry, 38(2), 201-208.
23. Mersie, W., & Singh, M. (1987). Allelopathic effect of parthenium (Parthenium hysterophorus L.) extract and residue on some agronomic crops and weeds. Journal of Chemical Ecology, 13, 1739-1747.
24. Nikam, S. R., & Namdas, D. D.2021. Preliminary Phytochemical Analysis of Alternanthera sessilis Leaves (Linn). R. Br. ex-DC.
25. Olofsdotter, M. (2001). Rice—a step toward use of allelopathy. Agronomy Journal, 93(1), 3-8.
26. Prakash, N. U., Jahnavi, B., Abhinaya, K., Rajalin, A. G., Babu, H. S., Kumar, M. P., ... & Bhuvaneswari, S. (2011). Phytochemical analysis of common weeds of Northern districts in Tamil Nadu. Int. J. Appl. Biol, 2, 25-28.
27. Rice, E. L. (1985). Allelopathy—an overview. Chemically mediated interactions between plants and other organisms, 81-105.
28. Rajput, R. L., Gautam, D. S., & Verma, O. P. (1993). Studies on cultural and chemical weed control in mustard (Brassica campestris).
29. Shreshtha, S., Anushi, J., Joshi, A. N., Joshi, N., & Anupma, H. (2017). Study of total phenol, flavonoid contents and phytochemical screening of methanolic crude extracts of two weed plants. Annals of Plant Sciences, 6(6), 1645-1648.
30. Singh, P. P., & Prasad, R. (1991). Studies on weed control in pearlmillet.
31. Tefera, T. (2002). Allelopathic effects of Parthenium hysterophorus extracts on seed germination and seedling growth of Eragrostis tef. Journal of Agronomy and Crop Science, 188(5), 306-310.
32. Tomaszewski, M., & Thimann, K. V. (1966). Interactions of phenolic acids, metallic ions and chelating agents on auxin-induced growth. Plant physiology, 41(9), 1443-1454.
33. Wakjira, M., Berecha, G., & Bulti, B. (2005). Allelopathic effects of Parthenium hysterophorus extracts on seed germination and seedling growth of lettuce. Tropical Science, 45(4), 159-162.

BIOSTIMULANT AND PLANT GROWTH REGULATOR: APPLICATIONS AND SIGNALING MECHANISM

ISBN: 978-81-981142-7-3| Year: 2025 | pp: 53 - 59 |

Influence of Various Growth Regulators on Morphological Status of *Curcuma caesia* Roxb

[1]Vijaya K. Nikam

[2]Mayuri D. Salunkhe

[1,2]Department of Botany, Sadguru Gadge Maharaj College, Karad

Email: vijunikam18@gmail.com

Article DOI Link: https://zenodo.org/uploads/14786426

DOI: 10.5281/zenodo.14786426

Abstract

Curcuma caesia Roxb. belongs to family Zingiberaceae. The production of bioactive Components and morphological development growth of plants may be affected by the endogenous levels as well as exogenous application of growth regulators. This study conducted to evaluate the effect of various growth regulators like Gibberellic acid, Indole -3-butyric acid and Vipul on morphological development in *Curcuma caesia*. Morphological features were extensively documented after three-month interval of DAP. The present study reported that morphological characters like plant height, number of leaves, leaf area, fresh weight of shoot, dry weight of shoot, fresh weight of rhizome and dry weight of rhizome in *Curcuma caesia* significantly improved after exogenous application of selected growth regulators.

Keywords: *Curcuma caesia*, Growth regulators, Gibberellic acid, Indole -3-butyric acid, Vipul and Morphological development

Introduction

In the family Zingiberaceae, there exist 53 genera and over 1,200 species. It is considered as the powerhouse of active phytochemicals which exhibit a wide range of biological effects including antibacterial, anti-Inflammatory, antiviral, anti- aging, anticancer activities (Nag et al., 2021). Curcuma caesia Roxb belongs to family zingiberaceae. It is an underutilized perennial herb, endemic to the North - Eastern and central India (Paliwal et al., 2011). *Curcuma caesia* is recognized by several common names across different regions of India. These include Kali

Haldi, Nar Kachura, and Krishna Kedar in Hindi; Yaingang Amuba or Yaimu in Manipuri and Kali Halad in Marathi, Nalla pasupu in Telagu, kariarishina and Naru kachora in kannada, Aihang in Mizo, Kaalo Haledo in Nepalese, Kala Haladhi in Assamese (Baghel et. al., 2013). *Curcuma caesia* is usually erect and grows between 0.5 to 1.0 meter in height. The leaves of *C. caesia* grow in bunch of 10-20 leaves which are characterized by deep violet patch that runs throughout leaf lamina. They have parallel venation. Plant bears pale yellow colour, long tubular flowers (Das et.al., 2013). *Curcuma caesia* consist of large Underground ovoid, bluish-black rhizome having camphoraceous sweet odour. The *C. caesia* possesses numerous biological properties and high medicinal potentialities. The plant contains many bioactive compounds like ar-tumerone, borneol, (Z) ocimene, bornyl acetate, ar-curcumene, elemene, curcumin (Mahato and Sharma, 2012). The rhizome extracts are used to treat human health Such as smooth muscle relaxant activity, haemorrhoids, leprosy, asthma, cancer, epilepsy, fever, menustral disorders, anthelmintic, inflammation etc, (sahu et al., 2022).

Plant requires light, water, minerals and other nutrients for their growth and development apart from these requirements plants also need certain organic compounds to signal, regulate and control the growth of plants these compounds are known as plant growth regulators or Phytohormones. They are organic substances produced naturally in plants sometime they may accelerate or inhibit the growth of plants. The growth of plants, production of phytochemicals and other physiological processes may be affected by the endogenous levels as well as exogenous application of growth regulators thus study conducted to evaluate the effect of selected growth regulators Such as Gibberellic acid, Indole-3-butyric acid and vipul on morphological characters and growth in *Curcuma caesia* Roxb.

Methods and Materials

A field investigation was carried out at Borgaon, Satara (Maharashtra). The latitude and longitude coordinators of study site are 17.5577°N and 741680° E respectively. The experiment was carried out in randomized block design with three replication and 4 treatments. Treatments are as follows:

T1 = control (without any treatment)

T2=GA 25 ppm

T3= IBA 25 ppm

T4 =Vipul 25 ppm

Cultivated Plant material of *Curcuma caesia* collected from farmers of Nagthane (Satara). The rhizomes of *Curcuma caesia* were planted on raised beds. Beds are prepared by using dung manure and Trichoderma powder. Pre sowing soaking treatment of 25 ppm concentration of selected growth regulators for 2 hrs was givened to the rhizomes. Soaked rhizomes further allow for shade drying separately after complete drying of rhizome, plantation has been done. The Crop requires high soil moisture conditions for optimum

growth and development. crop was irrigated with the help of drip irrigation system and time to time weeding and other cultural practices were done up to harvest. Two foliar applications of selected growth regulators were givened to the plants after 3-month interval of day after Plantation. Observations of morphological characters like Plant height, Number of leaves, Leaf area, fresh weight of rhizome, Dry weight of rhizome, fresh weight of shoot and Dry weight of shoot were extensively documented on 10 randomly selected clumps in each treatment at three-month interval of day after plantation till harvest.

Statistical analysis

The statistical analysis of data was performed by using analysis of Variance (ANOVA) and using data analysis tool pack of MS Excel. The significance differences in two treatment means were evaluated using Least significance difference (LSD) at 5 % Probability level (Lohar and Hase, 2021).

Table 1: First morphological analysis of *Curcuma caesia* Roxb. after three-month interval of DAP:

Treatment	Height of plant (Cm)	Number of leaves	Leaf area (Cm^2)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of rhizome (g)	Dry weight of rhizome (g)
Control	41.51	3.7	161.4	10.88	1.298	6.38	0.56
GA-25 ppm	49.74	3.9	204.2	12.68	1.591	6.86	0.66
IBA-25 ppm	54.34	7	165	32.88	4.127	32.94	3.726
VIPUL-25 ppm	53.97	4	167.5	20.46	2.413	6.49	0.613
SE±	0.646525	0.361325	5.409996	0.557106	0.083856	0.499758	0.072828757
LSD	1.854336	1.036336	15.51672	1.597867	0.240513	1.433386	0.208884384
CV%	4.097998	24.57224	9.802555	9.163707	11.2494	12.00208	16.57166755
P value	2.08E-16	1.55E-07	4.39E-06	4.6E-26	1.61E-23	6.48E-32	1.03922E-28

Note: LSD- Least significant difference at P=0.05, SE- Standard error of mean, CV%- Coefficient of variation.

Table 2: Second morphological analysis of *Curcuma caesia* Roxb. after three-month interval of DAP:

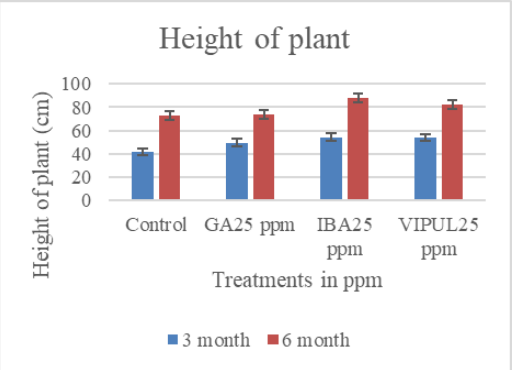
Treatment	Height of plant (Cm)	Number of leaves	Leaf area (Cm^2)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of rhizome (g)	Dry weight of rhizome (g)
Control	73	10.9	234.8	69.3	11	55.5	15.02
GA25 ppm	74	11	335	70.9	17.91	61	18.46
IBA25 ppm	87.9	14.6	236.8	108.8	13.32	100.8	27.82
VIPUL25 ppm	82.4	11.7	322.6	91.3	12.56	50.7	16.47
SE±	0.707342	0.805191	6.165225	1.430035	1.019816	0.985591	0.899007168
LSD	2.02877	2.309416	17.68284	4.101566	2.924994	2.82683	2.57849464
CV%	2.819809	21.13061	6.906183	0.053155	23.54402	4.651808	14.62214368
P value	1.4E-17	0.007542	1.09E-15	2.29E-21	0.000216	5.52E-30	1.03964E-11

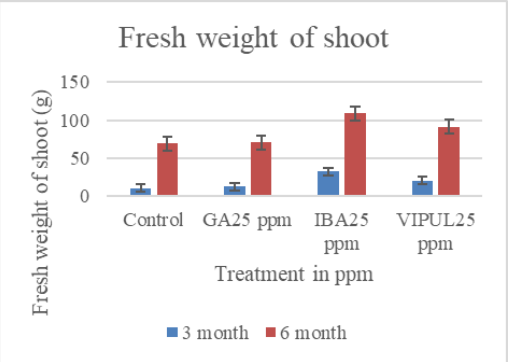
Note: LSD- Least significant difference at P=0.05, SE- Standard error of mean, CV%- Coefficient of variation.

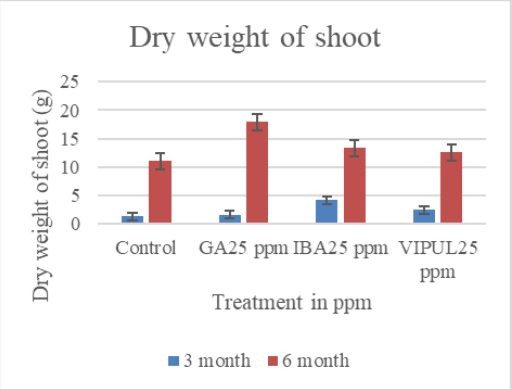
Table 3: Third morphological analysis of *Curcuma caesia* Roxb. after three-month interval of DAP

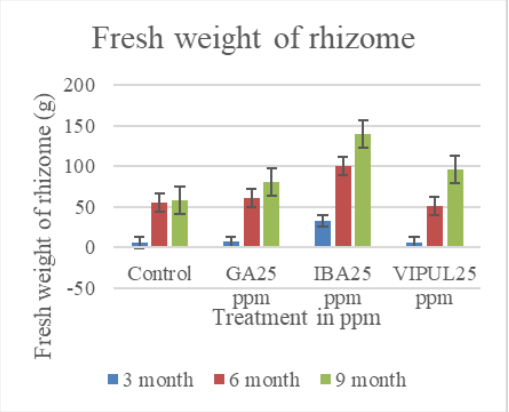
Treatment	Fresh weight of rhizome (g)	Dry weight of rhizome (g)
Control	58.4	7.32
GA25 ppm	80.6	13.114
IBA25 ppm	139.7	15.375
VIPUL25 ppm	96.4	14.748
SE±	7.325242	0.876500063
LSD	17.80114	2.513940706
CV%	26.63941	21.92959685
P value	0.000181	3.45464E-07

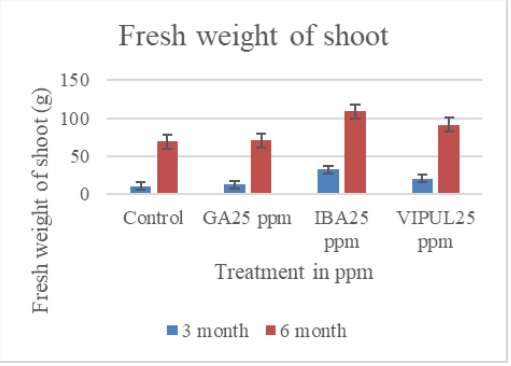
Note: LSD- Least significant difference at P=0.05, SE- Standard error of mean, CV%- Coefficient of variation.

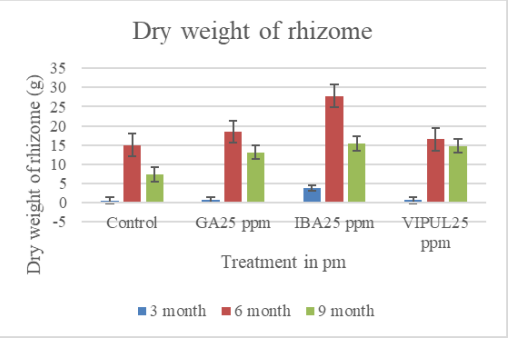


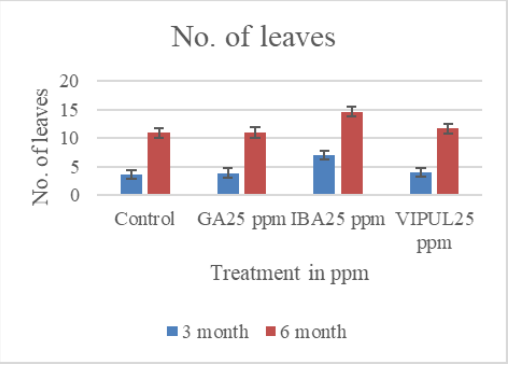












Results and discussion:

Early sprouting and shoot development observed in the T3 as compared to the other treatments. Similar results were recorded by Bhagya and Sreeram (2013). The plant height was significantly influenced by different treatments, Among the treatments maximum plant height was recorded in T3 (54.34 cm and 87.9 cm) followed by T4 (53.97 cm and

82.4 cm), T_2 (49.74 cm and 74 cm) and minimum Plant height was observed in T1 (41.51 cm and 73 cm) at 3rd month and 6th month after day of Plantation. The maximum number of leaves was found in T3 (7 and 14.6) followed by T4 (4 and 11.7) and least number of leaves observed in T_2 (3.9 and 11) and T1 (3.7 and 10.9) at 3rd and 6th month after DAP. From all the treatments highest leaf area was recorded in T_2 (204.2 cm^2 and 335 cm^2) followed by T4 (167.5 cm^2 and 332.6 cm^2), T3 (165 cm^2 and 236.8 cm^2) and least leaf area was recorded in T1 (161.4 and 234.8 cm^2) at 3rd and 6th month after DAP. The maximum fresh weight (32.88 g and 108. 8 g) and dry weight (4.127 g and 13.32 g) of shoot was recorded in T3 at 3rd and 6th of month after day of plantation, it was followed by T4 and T1. The minimum fresh weight (10.88 g and 69.3 g) and dry weight (1.298 g and 11 g) was recorded in T1. In 3rd, 6th and 9th month of morphological analysis the highest fresh weight of rhizome (32.94 g, 100.8 g and 139.7 g) and dry weight of rhizome (3.726 g, 27.82 g and 15.37 g) was recorded in T3 in comparison to the remaining treatments of the growth regulators & control. Plants treated with 25 ppm of GA showing significant increase in fresh weight of rhizome (80.69 g) at 9th month after DAP. Similarly, Venugopal et al., (2017) noted that foliar application of GA3 100 ppm increased rhizome yield in turmeric and Sengupta et al., (2008) recorded that foliar spray of GA3 150 ppm is best for getting higher yield of ginger. Study concluded that all the treatments of selected growth regulators significantly having positive effect on growth and yield in Curcuma caesia.

References

1. Baghel, S.S., Baghel, R.S., Sharma, K., & Sikarwar, I. (2013). Pharmacological activities of Curcuma caesia. International Journal of Green Pharmacy, 7(1),1-5.
2. Bhagya, H.P., & Sreeramu, B.S. (2013). Effect of growth regulators on vegetative propagation of Vitex negundo. Asian Journal of Horticulture, 8(1), 209-212.
3. Das, S., & Zaman, M.K. (2013). Curcuma caesia Roxb. and it's medicinal uses: a review. International Journal of Research in Pharmacy and Chemistry, 3(2):370-375.
4. Lohar, R.R., & Hase, C.P. (2021). Effect of chemical fertilizers and organic sources on growth, yield and quality of turmeric (Curcuma longa L.) and soil fertility. Agricultural Research Journal,58(4):624-631.
5. Mahato, D., & Sharma, H.B. (2018). Kali Haldi, an ethnomedicinal plant of Jharkhand state- A review. Indian Journal of Traditional Knowledge,17(2):322-326.
6. Nag, A., Banerjee, R., Goswami, P., Bandyopadhyay, M., & Mukherjee, A. (2021). Antioxidant and antigenotoxic properties of Alpinia galanga, Curcuma amada, and Curcuma caesia. Asian Pacific Journal of Tropical Biomedicine, 11(8):363 - 374.

7. Paliwal, P., Pancholi, S.S., & Patel, R.K. (2011). Pharmacognostic parameters for evaluation of the rhizomes of Curcuma caesia. Journal of Advanced Pharmaceutical Technology & Research, 2, 56 - 61.
8. Sahu,V., Tantwai,K., Mishra, N., Tiwari, P., Sapre, S., Tiwari, G., & Tiwari, S. (2022) Morphological characterization of Curcuma caesia germplasm collected from different regions of Madhya Pradesh. The Pharma Innovation Journal.11(7S): 4080-4084.
9. Sengupta, D.K., Maity, T.K., & Dasgupta, B. (2008). Effect of growth regulators on growth and rhizome production of ginger (Zingiber officinale Rosc.) in the hilly region of Darjeeling district. Journal of Crop and Weed, 4(2):10-13.
10. Venugopal, S., & Pariari, A. (2017) Effect of Growth Regulators on Growth and Yield of Turmeric (Curcuma longa L.) Varieties in Gangetic Alluvial Plains of West Bengal. International Journal of Agriculture Sciences, 9 (15) :4104-4106.

BIOSTIMULANT AND PLANT GROWTH REGULATOR: APPLICATIONS AND SIGNALING MECHANISM

ISBN: 978-81-981142-7-3| Year: 2025 | pp: 60 - 64 |

Study of Antioxidant Activity by Various Methods in *Averrhoa carambola* L.: A Review

[1]Vijaya K. Nikam

[2]Madhuri A. Pawar

[1,2]Department of Botany, Sadguru Gadge Maharaj College, Karad

Email: vijunikam18@gmail.com

Article DOI Link: https://zenodo.org/uploads/14786577

DOI: 10.5281/zenodo.14786577

Abstract

The fruit of *Averrhoa carambola* L. belongs to the family Oxalidaceae, is probably native to Ceylon and Moluccas, and is commonly known in various places as 'star-fruit' or 'kamrakh'. It is used as traditional medicine in various diseases. Fruits having many phenolic compounds that are beneficial in health. Now a day's there is extensive research on the phenolic compounds and the antioxidant activity of the fruit of *A. carambola* L. In this study antioxidant activity of these fruits content of polyphenols, vitamin C and β-carotene are reviewed. The antioxidant activities were determined by various methods of total polyphenol content y(TP), total flavonoids content (TFC), total antioxidant activity method and 1,1-diphenyl-2-picrylhydrazyl method (DPPH), and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). Whereas HPLC analysis was performed to analyse the content of phenolic acids, flavonoids, vitamin C and β-carotene.

Keywords: *A. carambola* L.; Antioxidant Methods, Total Polyphenol Content, Total Flavonoid Content; HPLC.

Introduction

There are many species in the genus *Averrhoa*. One of these is *A. carambola* L., also referred to as kamrakh or star-fruit. It belongs to the family Oxalidaceae, a perennial tree native to tropical and subtropical regions. It is native to Ceylon and Moluccas (Kurup & Mini, 2017; Manda et al., 2012; Payal et al., 2012). Southeast Asia, Malaysia, southern China, Taiwan, India, the Philippines, Queensland, Australia, and

several Pacific regions are among the places where it is cultivated. The fruit is available from March to August (Dembitsky et al., 2011; Payal et al., 2012). Plant secondary metabolites known as phenolic compounds serve as defences against both biotic and abiotic stress factors (Li et al., 2018). They are classified as phenolic acids, simple phenols, flavonoids, lignans, and tannins based on their chemical structure, which consists of one or more hydroxyl components joined to an aromatic ring (Valduga et al., 2019). In India cultivated the edible fruit of *Averrhoa carambola* L., which is also used to treat a number of illnesses (Thomas et al., 2008). The purpose of the current study was to screening of phytochemicals and examine the antioxidant activity and brine shrimp lethality of the methanolic extract of *Averrhoa carambola* L. Typically fruit is oblong has a tart-sweet taste. The fruit gets its name because it looks like a star in cross section. It is used in traditionally, it has been used to cure stomach, kidney and bladder disorders, fever, and eye difficulties. This fruit has been the subject of numerous scientific investigations to ascertain its hypoglycemic potential, antioxidant activity, anti-inflammatory, antibacterial, and anti-ulcer properties (Manda et al., 2012; Muthu et al., 2016; Payal et al., 2012). The fruit is used to treat piles and hepatic problems and has laxative, antidysenteric, antiphlogistic, febrifuge, anti-inflammatory, and antispasmodic properties (Khare et al., 2007). In addition to their nutritional value, *Averrhoa carambola* L. fruits include phytochemicals that antioxidant activities, such as polyphenols, carotenoids, or vitamin C. Numerous research are currently being conducted on natural bioactive chemicals that may be useful in preventing a number of illnesses. Plant polyphenols in human diet are beneficial to health as their consumption decreases the incidence of cardiovascular diseases, diabetes, and cancers and produce antimicrobial effects (Kranz et al.,2020).

Antioxidant Activity: An antioxidant is a substance that can delay or prevent the oxidation of cell contents such as DNA, proteins, carbohydrates, and lipid in plants.

Methodology

1. DPPH Method

The antioxidant capacity of the fruit juices was determined by a modified Yen and Chen method, using 0.1 mmol/L methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich, St. Louis, MO, USA). An amount of 0.1 mL of a sample was added to 2.9 mL of DPPH solution and mixed. The absorbance was measured on a Rayleigh UV-1800 V/VIS spectrophotometer at 517 nm after 30 min of incubation at room temperature in the dark. For each juice, samples were analyzed in three replicates and the results were used to calculate an average value. The percentage of DPPH scavenging was calculated using the equation:

$$\% \text{ scavenging} = [(ADPPH - A \text{ juice})/ADPPH] \times 100$$

where ADPPH is the absorbance of the DPPH blank solution and A juice is the absorbance of the sample solution.

The obtained value was then substituted into the equation of a previously

prepared 6- hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox-Sigma-Aldrich) calibration curve. The antioxidant capacity of the samples was expressed as milligrams of Trolox equivalents (Sigma-Aldrich) per litre of sample (mg Tx L−1).

2. ABTS Method

The antioxidant capacity was determined using the (Re et al., 1999) method with small modifications. In the ABTS method, 2,20 -azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS, Sigma-Aldrich, St. Louis, MO, USA) and potassium persul-fate solutions were mixed and stored overnight at room temperature in the dark for 12–16 h. ABTS solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. After addition of 1.0 mL of diluted ABTS solution (A734 nm = 0.700 ± 0.020) to 0.01 mL of antioxidant compounds or Trolox standards in methanol, the absorbance was measured on a Rayleigh UV-1800 V/VIS spectrophotometer at 734 nm against methanol after 1 min. Quantification was performed using a Trolox standard curve. The antioxidant capacity of the samples was expressed as milligrams of Trolox equivalents (Sigma-Aldrich, St. Louis, MO, USA) per litre of sample (mg Tx L−1).

Total Flavonoid Content

The total flavonoid content was measured using the colorimetric assay developed by Kapci et al. Briefly, 0.3 mL of 5% sodium nitrite was added to 1 mL of the sample at zero time. After 5 min, 0.3 mL of 10% aluminium chloride was added. At the 6th min, 2 mL of 1M sodium hydroxide was added. The mixture was diluted by addition of 2.4 mL distilled water and mixed. The absorbance was measured on a Rayleigh UV-1800 V/VIS spectrophotometer at 510 nm. The total flavonoids content was determined using a (+)-catechin (Sigma-Aldrich, St. Louis, MO, USA) standard curve and was expressed as milligrams of catechin equivalents per litre of sample (mg CAE L−1).

Total Polyphenol Content

The total polyphenol content (TP) of the samples was determined in the Folin–Ciocalteu method (Sigma-Aldrich). First, 0.3 mL of a sample was placed in a 10-mL capacity tube; next, 0.05 mL 2 mol/L Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) and 0.5 mL 20% sodium carbonate solution were added. The mixture was diluted by addition of 4.15 mL distilled water and mixed. The absorbance was measured on a Rayleigh UV-1800 V/VIS spectrophotometer at 765 nm after 30 min incubation in the dark at room temperature. A calibration curve was performed with gallic acid. The results were expressed as milligrams of gallic acid equivalents per litre of sample (mg GAE L−1).

HPLC Analysis of Ascorbic Acid and β-Carotene

Ascorbic acid was determined using HPLC methods of AOAC with slight modification. The separation was performed on a Thermo Electron Co., column BetaBasic-18 (150 mm × 4.6 mm, 5 µm). The isocratic mobile phase consisted of 0.1% o-phosphoric acid in water.

β-carotene was determined using HPLC normalised methods of EN-12823-2:2000 with slight modification. The separation was performed on a Kinetex

C18 (Phenomenex) column (150 mm × 4.6 mm, 2.6 µm).

Results

Antioxidant capacity of the Averrhoa carambola L. fruit were analyzed by DPPH method (1268+ 80b) and ABTS method (1906+146b) for their antioxidant capacity. Total polyphenol content was determined by Folin-ciocalteu method (Ruiz-Torralba et al.,2018). Results investigated that total polyphenol content (2464+153b) and high level of vitamin C (1345+17a). The total flavonoid content was measured using the colorimetric assay, results stated that juice of Averrhoa carambola had high content of total flavonoids (1345mgCAEL-1) and the composition of these compounds was dominated by flavanols Epicatechin (Vasco et al.,2008).

Conclusions

In this review we concluded that highest antioxidant activity which had a very high vitamin C content as well as total phenolic content, regardless of the analytical method used. Polyphenols were the key compounds in the composition of juices from *Averrhoa carambola* L. affecting their antioxidant activity. Furthermore, the flavonoids determined in the *Averrhoa carambola* L. juice contained mostly flavanols (mainly epicatechin).

References

1. Cebulak, T., Krochmal-Marczak, B., Stryjecka, M., Krzysztofik, B., Sawicka, B., Danilcenko, H., & Jarienè, E. (2023). Phenolic acid content and antioxidant properties of edible potato (Solanum tuberosum L.) with various tuber flesh colours. Foods, 12, 100. https://doi.org/10.3390/foods12010100
2. Dembitsky, V. M., Poovarodom, S., Leontowicz, H., Leontowicz, M., Vearasilp, S., Trakhtenberg, S., & Gorinstein, S. (2011). The multiple Food Sci. Technol, Campinas, v42, e69920, 2022 19nutrition properties of some exotic fruits: biological activity and active metabolites. Food Research International, 44(7), 1671-1701. http://dx.doi.org/10.1016/j.foodres.2011.03.003.
3. Du, H., Wu, J., Li, H., Zhong, P. X., Xu, Y.J., Li, C.H., & Wang, L.S. (2013) Polyphenols and triterpenes from Chaenomeles fruits: Chemical analysis and antioxidant activities assessment. Food Chemistry., 141, 4260–4268.
4. Kapci, B., Neradova, E., Cizkova, H., Voldrich, M., Rajchl, A., & Capanoglu, E. (2013) Investigating the antioxidant capacity of chokeberry (Aronia melanocarpa) products. Journal of Foods and Nutrition Research, 52, 219–229.
5. Khare, C.P. (2007) Indian Medicinal Plants—An Illustrated Dictionary. First Indian Reprint, Springer (India) Pvt. Ltd., New Delhi, 717-718.
6. Kranz, S., Guellmar, A., Olschowsky, P., Tonndorf-Martini, S., Heyder, M., Pfister, W., Reise, M., & Sigusch, B. (2020) Antimicrobial effect of natural berry juices on common oral pathogenic bacteria. Antibiotics, 9, 533.
7. Kurup, S. B., & Mini, S. (2017). Averrhoa bilimbi fruits attenuate hyperglycemia-mediated oxidative stress in streptozotocin-induced

diabetic rats. Journal of Food and Drug Analysis, 25(2), 360-368. http://dx.doi.org/10.1016/j.jfda.2016.06.007. PMid:28911678

8. Li, Z., Shi, W., Cheng, L., Pan, S., & Wang, C. (2018). Screening of the phenolic profile and their antioxidative activities of methanol extracts of Myrica rubra fruits, leaves and bark. Journal of Food Measurement and Characterization,12(1),128-134. http://dx.doi.org/10.1007/s11694-017-9623-1.
9. Manda, H., Vyas, K., Pandya, A., & Singhal, G. (2012). A complete review on: Averrhoa carambola L. World Journal of Pharmacy and Pharmaceutical Sciences, 1(1), 17-33.
10. Muthu, N., Lee, S. Y., Phua, K. K., & Bhore, S. J. (2016). Nutritional, medicinal and toxicological attributes of star-fruits (Averrhoa carambola L.): A review. Bioinformation, 12(12), 420-424. http://dx.doi.org/10.6026/97320630012420. PMid:28405126.
11. Payal, G., Pankti, K., Manodeep, C., & Kamath, J. (2012). Phytochemical and pharmacological profile of Averrhoa Carambola Linn: an overview. International Research Journal of Pharmacy, 3(1), 88-92.
12. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26, 1231–1237.
13. Schimpf, K., Thompson, L., & Baugh, S. (2012) Vitamin C in infant formula and adult/pediatric nutritional formula by HPLC with UV detection: First Action., Journal of AOAC International, 96, 802–807.
14. Singleton, V.L., Orthofer, R., & Lamuela-Raventos, R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin—Ciocalteu reagent. Methods Enzymol, 299, 152–178.
15. Thomas, S., Patil, D., Patil, A., & Naresh, C. (2008). Pharmacognostic evaluation and physicochemical analysis of Averrhoa carambola L. fruit. Journal of Herbal Medicine and Toxicology, 2(2), 51-54. http://www.verypdf.com
16. Valduga, A. T., Gonçalves, I. L., Magri, E., & Delalibera Finzer, J. R. (2019). Chemistry, pharmacology and new trends in traditional functional and medicinal beverages. Food Research International, 120, 478-503. http://dx.doi.org/10.1016/j.foodres.2018.10.091. PMid:31000264.
17. Yen, G., & Chen, H.Y. (1995) Antioxidant activity of various tea extract in relation to their antimutagenicity. J. Agric. Food Chem, 43, 27–32.
18. Zhang, Z., Li, X., Sang, S., McClements, D.J., Chen, L., Long, J., Jiao, A., Jin, Z., & Qiu, C. (2022) Polyphenols as plant-based nutraceuticals: Health effects, encapsulation, nano-delivery, and application. Foods, 11, 2189.

BIOSTIMULANT AND PLANT GROWTH REGULATOR: APPLICATIONS AND SIGNALING MECHANISM

ISBN: 978-81-981142-7-3| Year: 2025 | pp: 65 - 70 |

Silver Nanoparticles as Bio stimulants

[1]Varsha Yadav

[2]Ahilya Waghmode

[3]Manasi Patil

[1,2,3]Department of Botany, Sadguru Gadge Maharaj College, Karad

Email: manasipatil202@gmail.com

Article DOI Link: https://zenodo.org/uploads/14788716

*DOI: **10.5281/zenodo.14788716***

Abstract

Despite their numerous possible applications in agriculture, silver nanoparticles (AgNPs) have shown great potential as bio stimulants. Microorganisms or compounds known as bio stimulants improve plant productivity, growth, and stress tolerance. AgNPs has potential in enhancing crop performance because of their distinct physicochemical characteristics, which include high surface area, increased reactivity, and antibacterial action. According to recent research, AgNPs may have a beneficial effect on seed germination, root shoot elongation, as well as general plant health. Through enzyme activity modulation and signaling pathway activation related to stress tolerance, they improve photosynthetic efficiency and nutrient uptake. Additionally, by shielding plants from harmful microbes, their antimicrobial qualities aid in the reduction of biotic stresses. However, factors such as nanoparticle size, concentration, and application technique impact how efficient AgNPs are as bio stimulants. AgNPs can promote growth at the proper concentration, while high quantities may show phytotoxic effects. In order to maximize agricultural results, research is also examining their synergistic effects with other fertilizers and bio stimulants. Even with the encouraging potential, further research is necessary to address issues with bioaccumulation, environmental impact, and nanoparticle persistence in ecosystems. This chapter puts light on the potential of AgNPs to be groundbreaking techniques for sustainable agriculture, but simultaneously urges the value of careful utilization and complete risk evaluation.

Keywords: Silver nanoparticles, Bio stimulants, growth promoters, sustainable agriculture, chilli.

Introduction

A plant bio stimulant is any material or microbe, regardless of nutrient content, that is ingested to plants to improve crop quality features, abiotic stress tolerance, and/or nutrition efficiency. Thus, plant bio stimulants also refer to commercial products that contain combinations of these compounds and/or microorganisms. (Du Jardin,2015)

Nanoparticles are defined as particulate dispersion or solid particles with a size in the range 10-100nm (Mohanraj and Chen, 2006)

Plants act as a better source for synthesis of nanoparticles as they lack toxic chemical and do contain natural capping agents (Khan et al., 2018). Therefore, green synthesis is a better approach for nanoparticles synthesis considering the safety of environment. Plant extracts, microbial cell biomass or cell-free growing media, and biopolymers have all been used in green synthesis of AgNPs. Wide range of plants consisting of Algae to angiosperms are employed for the synthesis of AgNps; however, there are few data on lower plants, therefore angiosperm plants are the best option (Srikar et. al.,2016).

Agriculture is a main occupation in the developing countries (Sadique et al., 2017). Silver nanoparticles (AgNPs) have gained significant attention in recent years for their potential use as bio stimulants in agriculture and horticulture. AgNPs' potential as bio stimulants in agriculture, increases crop yield and stress tolerance, was shown by Kole et. al. (2013). AgNPs enhanced plant growth, flowering, and fruiting in horticulture crops, according to Rajiv et. al., (2016).

AgNPs are used as bio stimulants in soil remediation, increasing soil microbial activity and plant growth, according to Sharma et. al., (2019).

Benefits of Silver Nanoparticles as Bio stimulants

1. Plant growth promotion

AgNPs have been shown to stimulate plant growth, increase biomass production, and enhance root development. One of the most promising scientific disciplines, nanotechnology offers countless opportunities for the advancement of numerous industries, including horticulture and agriculture. One of the nanomaterials whose effects on plants are now being studied is nano-silver. The potential of nano-silver as a crop plant growth regulator and as a way to increase the post-harvest longevity of cut flowers and ornamental foliage was assessed in a number of scientific investigations. Byczyńska (2017).

2. Stress Tolerance

AgNPs can help plants to tolerate abiotic stresses such as drought, salinity, and temperature extremes. To achieve sustainable agriculture there is a need to exploit the properties of nanoparticles and study its impact on growth and abiotic stress tolerance in crop plants. The great loss in crop yield take place due to different abiotic stress such as drought, salinity, flood, chilling, freezing, UV radiations etc (Wani et. al., 2016; Li et. al., 2017). Because of their impact on stress tolerance, silver nanoparticles (AgNPs) are becoming

more and more popular in agriculture. The potential role of various AgNPs nanoparticle morphologies in abiotic stress protection has been studied. According to Alabdallah and Hasan (2021). By overcoming nutrient shortages, boosting enzymatic processes, and aiding in the adherence of bacteria that promote plant development to plant roots under abiotic stresses, these silver nanoparticles have been demonstrated to improve crop stress tolerance.

3. Improved nutrient uptake

Nanoparticles have a lot of potential to increase nutrient uptake, and some of them can increase the efficiency of plant nutrient use by using mechanisms including controlled release, sustained release, or directional delivery (Solanki et. al., 2015; Dutta et. al., 2022; Taware et. al., 2024). AgNPs can boost the uptake and utilization of nutrients, which will improve the nutrition of plants. AgNPs applied topically as nano fertilizers improve plant growth and nutrient uptake. (Khan et. al., 2023) Among its many advantages, nanoparticles improve soil physiochemical characteristics, facilitate nutrient uptake, and modulate rhizosphere microorganisms to promote plant growth and development. (Zhang et. al., 2024)

4. Antimicrobial properties

AgNPs can help to control plant infections and lower the incidence of illness because of their antibacterial qualities. The quality and productivity of plants can be seriously threatened by plant diseases. Utilizing their remarkable cell penetration capabilities and distinct surface characteristics, some nanoparticles showed promise in suppressing a wide range of harmful microorganisms, ultimately achieving the goal of successfully managing and controlling plant diseases (Gordienko et.al., 2019; Gao et.al., 2023; Rahimizadeh et.al., 2023; Scandolera et. al., 2024).

Mechanisms of Action

Cellular regulation:

Plant cells have the ability to absorb AgNPs, which then interact with other cellular components to affect a number of physiological processes stated (Wang et. al., 2020)

Modulation of reactive oxygen species (ROS):

AgNPs have the ability to alter plant ROS levels, which can affect cellular signaling cascades and stress reactions (Yin et. al., 2012)

Hormone regulation:

According to Tripathi et. al., (2022) Auxins, gibberellins, and cytokinin's are among the hormones that AgNPs can affect in plants.

Silver nanoparticles as Bio stimulant on Chilli Crops

Seed germination in chilli is prolonged and uneven which affect the productivity especially under stress conditions (Demir and Okcu,2004). The reason for low yield of chilli is delayed and unpredictable seed germination (Alam et. al.,2014). AgNPs have been shown to stimulate plant growth, enhance yield, and improve resistance to diseases in various crops, including chilli.

Some Effects on Chilli Crops

1. **Seed germination:** Seed germination rate and vigor is enhanced with the use of AgNPs (Mawale and Giridhar 2024)

2. Plant growth: AgNPs can increases shoot and root length and stimulate plant growth (Aqeel et. al., 2023)

3. Yield enhancement: AgNPs can improve fruit quality and fruit yield. (Li et. al., 2020)

4. Disease resistance: AgNPs can induce diseases resistance in plants (Gowda and Sriram 2023)

Challenges and Future Directions

The potential toxicity and safety concerns associated with AgNP use in agriculture and horticulture need to be addressed. Standardization and regulation of AgNP production and use are necessary to ensure consistency and safety. Further research is needed to understand the mechanisms underlying AgNP bio stimulation and to optimize their use in various applications. AgNP synthesis and application in agriculture require defined techniques and more attention should be given towards environmental safety.

Conclusion

These chapter emphasizes on the potential of AgNPs as bio stimulants in agriculture and horticulture; it also highlights the need for further research on their mechanisms of action, safety, and environmental impact. AgNPs are of great potential on growth performance of chilli crop. Hence, the ultimate goal of any scientific development or research is to increase well-being and studying green nanoparticles which act as bio stimulants can help to alleviate abiotic stress and help in enhancing the germination in crop plants.

Reference

1. Alabdallah, N. M., and Hasan, M. M. (2021). Plant-based green synthesis of silver nanoparticles and its effective role in abiotic stress tolerance in crop plants. Saudi Journal of Biological Sciences, 28(10), 5631-5639.
2. Alam M Z, Hamim I, Ali M A, and Ashrafuzzaman M, (2014) effect of seed treatment on seeding health of chilli,J.Environment science and natural resources7(1);177-181
3. Aqeel, M., Khalid, N., Nazir, A., Irshad, M. K., Hakami, O., Basahi, M. A., and Noman, A. (2023). Foliar application of silver nanoparticles mitigated nutritional and biochemical perturbations in chilli pepper fertigated with domestic wastewater. Plant Physiology and Biochemistry, 194, 470-479.
4. Byczyńska, A. (2017). Nano-silver as a potential biostimulant for plant–A review. World Scientific News, 86(3), 180-192.
5. Demir I, amd Okcu G, (2004) Accelerated hydration treatment for improving germination and seedling growth in aubergine (Solanum melongena) and pepper (Capsicum annum) Ann.Appl.Biol.144;121-123
6. Du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. Scientia horticulturae, 196, 3-14.
7. Dutta, S., Pal, S., Panwar, P., Sharma, R. K., and Bhutia, P. L. (2022). Biopolymeric nanocarriers for nutrient delivery and crop biofortification. ACS Omega 7, 25909– 25920.
8. Gao, Q., Wang, Z., Rao, Y., Zhao, Y., Cao, J., Ho, and K. -F. (2023).

Oxygen vacancy mediated a-MoO3 bactericidal nanocatalyst in the dark: Surface structure dependent superoxide generation and antibacterial mechanisms. J. Hazard. Mater. 443, 130275.

9. Gordienko, M. G., Palchikova, V. V., Kalenov, S. V., Belov, A. A., Lyasnikova, V. N., and Poberezhniy, D. Y. (2019). Antimicrobial activity of silver salt and silver nanoparticles in different forms against microorganisms of different taxonomic groups. J. Hazard. Mater. 378, 120754.
10. Gowda, S., and Sriram, S. (2023). Green synthesis of chitosan silver nanocomposites and their antifungal activity against Colletotrichum truncatum causing anthracnose in chillies. Plant Nano Biology, 5, 100041.
11. Khan M Z H., Tareq F K., Hossen M A., and Roki M N A M. (2018). Green synthesis and characterization of silver nanoparticalas using Coriandrum sativum leaf extract,Journal of Engineering Science and Technology,13(1);158-166.
12. Khan, S., Zahoor, M., Khan, R. S., Ikram, M., and Islam, N. U. (2023). The impact of silver nanoparticles on the growth of plants: The agriculture applications. Heliyon, 9(6).
13. Kole, C., Kole, P., Randunu, K. M., Choudhary, P., Podila, R., Ke, P. C. and Marcus, R. K. (2013). Nanobiotechnology can boost crop production and quality: first evidence from increased plant biomass, fruit yield and phytomedicine content in bitter melon (Momordica charantia). BMC biotechnology, 13, 1-10.
14. Li N, W-j Qian, L Wang, H Cao, X Hao, Y Yang and X Wang. (2017), Isolation and expression features of hexose kinase genes under various abiotic stresses in the tea plant (Camellia sinensis), Journal of Plant Physiology, 209;95-104.
15. Li, M., Liu, H. L., Dang, F., Hintelmann, H., Yin, B., and Zhou, D. (2020). Alteration of crop yield and quality of three vegetables upon exposure to silver nanoparticles in sludge-amended soil. ACS Sustainable Chemistry and Engineering, 8(6), 2472-2480.
16. Mawale, K. S., and Giridhar, P. (2024). Green approaches for the synthesis of silver nanoparticle and its augmentation in Seed germination, growth, and antioxidant level in Capsicum annuum L. Plant Nano Biology, 10, 100107.
17. Mohanraj V J and Chen Y (2006), Nanoparticle-A Review, Tropical Journal of Pharmaceutical Research,5(1);516-573.
18. Rahimizadeh, K., Zahra, Q., Chen, S., Le, B. T., Ullah, I., and Veedu, R. N. (2023). Nanoparticles-assisted aptamer biosensing for the detection of environmental pathogens. Environ. Res. 238, 117123. doi: 10.1016/j.envres.2023.117123
19. Rajiv, S., Jerobin, J., Saranya, V., Nainawat, M., Sharma, A., Makwana, P and Chandrasekaran, N. (2016). Comparative cytotoxicity and genotoxicity of cobalt (II, III) oxide, iron (III) oxide, silicon dioxide, and aluminum oxide nanoparticles on human lymphocytes in vitro. Human and

experimental toxicology, 35(2), 170-183.

20. Sadique S, Nisar S, Dharmadasa R M and Jilani M I. (2017), Effect of nano-fertilizer and growth hormones on different plant, International Scientific Organization,11;113-119.
21. Scandolera, T., Teano, G., Naderpour, M., Geffroy, V., and Pflieger, S. (2024). Insights into the effects of elevated atmospheric carbon dioxide on plant-virus interactions: A literature review. Environ. Exp. Bot. 221, 105737. doi: 10.1016/ j. envexpbot.2024.105737
22. Sharma, V. K., Sayes, C. M., Guo, B., Pillai, S., Parsons, J. G., Wang, C., ... and Ma, X. (2019). Interactions between silver nanoparticles and other metal nanoparticles under environmentally relevant conditions: A review. Science of the Total Environment, 653, 1042-1051.
23. Solanki, P., Bhargava, A., Chhipa, H., Jain, N., and Panwar, J. (2015). NanoFertilizers and their Smart Delivery System, in Nanotechnologies in Food and Agriculture. Eds. M. Rai, C. Ribeiro, L. Mattoso and N. Duran (Springer International Publishing, Cham), 81–101.
24. Srikar, S. K., Giri, D. D., Pal, D. B., Mishra, P. K., and Upadhyay, S. N. (2016). Green synthesis of silver nanoparticles: a review. Green and Sustainable Chemistry, 6(01), 34.
25. Taware, A. S., Rathod, P. B., Katariya, A. P., Tagad, C. K., Wagh, P. S., and Sonar, J. P. (2024). Technological advancement in the development of nano fertilizers for sustainable agriculture. J. Soil Sci. Plant Nutr. 24, 1592–1608.
26. Tripathi, D., Singh, M., and Pandey-Rai, S. (2022). Crosstalk of nanoparticles and phytohormones regulate plant growth and metabolism under abiotic and biotic stress. Plant Stress, 6, 100107.
27. Wang, L., Sun, J., Lin, L., Fu, Y., Alenius, H., Lindsey, K., and Chen, C. (2020). Silver nanoparticles regulate Arabidopsis root growth by concentration-dependent modification of reactive oxygen species accumulation and cell division. Ecotoxicology and Environmental Safety, 190, 110072.
28. Wani S H, Kumar V, Shriram V and Sah S K (2016), Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants, The Crop Journal, 4 (3);162-176
29. Yin, J. J., Liu, J., Ehrenshaft, M., Roberts, J. E., Fu, P. P., Mason, R. P., and Zhao, B. (2012). Phototoxicity of nano titanium dioxides in HaCaT keratinocytes—generation of reactive oxygen species and cell damage. Toxicology and applied pharmacology, 263(1), 81-88.
30. Zhang, H., Zheng, T., Wang, Y., Li, T., and Chi, Q. (2024). Multifaceted impacts of nanoparticles on plant nutrient absorption and soil microbial communities. Frontiers in Plant Science, 15, 1497006.

BIOSTIMULANT AND PLANT GROWTH REGULATOR: APPLICATIONS AND SIGNALING MECHANISM

ISBN: 978-81-981142-7-3| Year: 2025 | pp: 71 - 80 |

Phytonutrient Studies and Antimicrobial Activity of *Sphagnum* Moss

[1]P. V. Mahanwar

[1]S. T. Charapale

[2]C. U. Narayanakar

[1]Department of Botany, Dahiwadi College Dahiwadi (MS) India 415508

[2] Department of Botany, Chikitsak Samuha's S.S & L.S Patkar-Varde College, Goregaon (West), Mumbai – 400104

Email: narayankarchirag7@gmail.com

Article DOI Link: https://zenodo.org/uploads/14788758

*DOI: **10.5281/zenodo.14788758***

Abstract

Mosses are also pioneers that retain moisture and reduce erosion along streams. Mosses are considered a highly developed and dominant group of mosses, occupying a unique position between vascular and non-vascular plants. Along with angiosperms, mosses have the most green plants. They are the simplest and most primitive of land plants. They do not have a well-developed conducting tissue system. *Sphagnum* is a genus of about 380 recognized species of mosses, of which are commonly known as *Sphagnum* moss, also bog moss and Quail moss (although the term is sometimes used for peat). *Sphagnum* is the common name and genus name of mosses (Division Bryophyta) whose leaf appendages are adapted to absorb and store large amounts of water. *Spagnum* moss have diverse medicinal properties it proved by qualitative test of *Sphagnum* for various secondary metabolites, it also exhibits antimicrobial, antifungal activity against various microorganism. *Sphagnum* moss is worthwhile to use in medicinal sector.

Keywords: *Sphagnum*, qualitative tests, antimicrobial, moss.

Introduction

Bryophytes (including mosses, liverworts and hornworms) are important plants that can act as biological indicators because they are usually abundant in the environment. Mosses are also pioneers that retain moisture and reduce erosion along streams. About

17,900 species are known worldwide, consisting of about 12,500 mosses, 4,444 5,250 liverworts, and 100-150 hornworms. In India, moss species represent Eastern and Western taxa, eastern represent biodiversity hotspots and acquire many Endemic mosses. The term Bryophyta is derived from two Greek words; Bryon means moss and phyton means plant. Robert Brown was the first person to use the term in 1864 including algae, fungi, lichens and mosses. (Sahaya Sathish, 2013). Bryophytes play an important role in nutrient cycling, soil creation, they provide a micro habitat for other plants and animals, promote seed germination and fill habitat gaps. (Dandotya, 2011).

Mosses are considered a highly developed and dominant group of mosses, occupying a unique position between vascular and non-vascular plants. Along with angiosperms, mosses have the most green plants. They are the simplest and most primitive of land plants. They do not have a well-developed conducting tissue system. It is abundantly grown in Lonawala and Khandala, Mahabaleshwar in the Western Ghats of India (Dabhade,1964). They play an important role in regulating ecosystems because they act as a buffer system for other plants. Because of their unique ecology and physiology, mosses affect water, energy, and element cycles differently than vascular plants (Turetsky, 2003). Mosses act as very effective filters and absorb nutrients from rain, drafts, dust and decaying debris on their surface (Rieley et al., 1979 and Tamm,1953).

Sphagnum is a genus of about 380 recognized species of mosses, of which are commonly known as Sphagnum moss, also bog moss and Quail moss (although the term is sometimes used for peat). *Sphagnum* is the common name and genus name of mosses (Division Bryophyta) whose leaf appendages are adapted to absorb and store large amounts of water. *Sphagnum* sp are also often called peat mosses because it is one of the most important plant species in peat. Like mosses, they are non-vascular land plants. They are often found in humid tundra, swamp forests, and around ponds and lakes, often in dense clumps or floating mats formed in open water (Michaelis, 2019). Mosses are an important part of ecosystems, especially in the northern hemisphere, where they are the main element of swamp vegetation and the most important peat former (*Sphagnum* mosses), but they are also important in forest ecosystems.

The main structural components of moss are Carbohydrates (Maksimova et al., 2014 and Klavina, 2015), but they also contain other Secondary metabolites with potentially high biological activity. *Sphagnum* mosses produce many metabolites that can be released into the environment (Fudyma et al., 2019 and Hamard et al., 2019). These metabolites promote *Sphagnum* growth, defence and competitiveness by interacting with the environment and/or other organisms in the environment (Erb and Kliebenstein, 2020, Khare et al., 2020). These metabolites are thought to be involved in specific or multiple functions, but are likely to play a role in moss fitness and stress tolerance (Tissier et al., 2014). Specifically, which is a large and important part of primary metabolites (carbohydrates, carotenoids, lipids, proteins) and specialized metabolites

(phenols, proline, flavonoids, tannins, alkaloids), can play a crucial role in growth, photosynthesis, waste resistance to decomposition and tolerance of mosses to abiotic stresses (Xie and Lou, 2009).

Sphagnum plants form unique host plants for microorganisms and are known for their antimicrobial activity. Interestingly, no pathogenic fungi specific to *Sphagnum* are known. Many chemical compounds that are currently largely unknown are responsible for this antimicrobial activity (Asakawa and Heidelberger, 1982, Frahm, 2001). It is also interesting to determine if *Sphagnum* plants contain antagonistic bacteria involved in the antimicrobial activity.

Fungi (including fungi with pathogenic properties) are known to prefer habitats with acidic conditions. Effective defence strategies against pathogenic fungi are therefore important for *Sphagnum* plants that occur in low pH habitats. The potent antifungal bacterium found in this study suggests that the bacteria engage in an antifungal defence strategy, and has been shown for rhizosphere bacteria (Cook et al., 1995 and Weller et al., 2002). The antibacterial activity of mosses has been found to be active against gram-negative bacteria. This changes the benefits of selected mosses as antimicrobial agents. Most studies have shown no antifungal activity of Indian mosses. Although studies in other countries show a wide range of antifungal activity of including Porella, *Sphagnum* etc. (Subhisha S and Subramoniam A, 2005). Many mosses have antimicrobial activity against fungi and bacteria (Sabovljevic et al., 2006). Antimicrobial activity can be due to the presence of flavonoids, steroids, terpenoids and other polyphenolic compounds (Lorimeres et al., 1994).

The peat moss species *Sphagnum* is an economically important moss. The harvesting, processing and sale of *Sphagnum* peat is a multi-million-dollar industry. Peat is used in horticulture, as a source of energy (fuel) and to a limited extent in the mining of organic products, whiskey production and insulation. It has many uses in nutrition, medicine, cosmetics, horticultural and agricultural practices, etc. Decomposed, dried *Sphagnum* moss is called peat or peat moss. It is used as a soil amendment that increases soil and its ability to retain water and nutrients by increasing capillary forces and cation exchange capacity. (Hood and Gerry, 1995) *Sphagnum* moss has been used for centuries as a wound dressing, during World War II (Bold, 1967). Formulations using *Sphagnum*, such as Sphagnol Soap, have been used for a variety of skin conditions, including acne, ringworm and eczema. The soap was used by the British Red Cross to treat facial wounds and ulcers during both world wars. (Facts about peat moss (*Sphagnum*) - Encyclopaedia of Life).

Materials and Methodology

The *Sphagnum* of Bryophyte was collected from Sanjay Nursery Pune (18° 31' 0.2136" N and 73° 51' 22.5180" E.) Maharashtra, India. The collected Sphagnum sp was washed in the water and dried in the shady place for 3 to 4 days. Dried material was then converted into powder form for further studies.

Preparation of Sample

5 g of sample was dissolved 50 ml of solvents such as methanol, acetone and chloroform.

Qualitative Tests

A) Primary Metabolites

1) Carbohydrates

It is tested by Molisch test. The extract is treated with alcoholic α - naphthol and few drops con.H2SO4. The Violet / purple coloration is observed.

2) Proteins

By Ninhydrin: Add 3 drops of 5% ninhydrin solution to the 3ml of ethanolic extract then boiling in a water bath for 10min. Formation of purple or bluish colour is observed.

By Biuret method: An aq. Sample is treated with an equal volume of 1% strong base sodium or potassium hydroxide followed by few drops of aq. Copper II sulphate. Solution turns purple it contains protein.

3) Lipids (By Hong et al., 1988)

Materials Needed are Test tube, Test solutions: Sudan III solution, ethanol, and distilled water and sample containing lipids. Add a small amount of the sample test tube. Add 1-2 ml of Sudan III solution to test tube. Shake the test tube vigorously to mix the contents well. Allow the test tube to stand for a few minutes to allow the Sudan III solution to separate and form a distinct layer at the top. Add 2-3 ml of ethanol to test tube. Shake the test tube again to mix the contents thoroughly. Allow the test tube to stand for a few more minutes to allow the Sudan III solution to dissolve in the ethanol layer. Carefully observe the test tube. A red-coloured layer at the top of the ethanol layer indicates the presence of lipids (triglycerides).

B) Secondary Metabolites

• **Alkaloid test:** 1 ml plant extract add few drops of Mayer's reagent formation of radish brown indicates presence of alkaloids.

• **Flavonoid test:** Few drops of 10% lead acetate solution were added in 1 ml plant extract presence of yellow colour precipitation indicates the presence of flavonoids

• **Test for phenols:** 1 ml plant extract add 0.5 ml of 1% lead acetate formation of precipitation indicates presence of phenols.

• **Saponin test:** Take a 0.5 ml plant extract in attest tube and add few drops of 5% sodium bicarbonate shake the mixture immediately and keep for three minutes, formation of honey comb like structure shows the presence of saponin.

• **Test for Terpenoids:** Take 2 ml plant extract and then add 2 ml of acetic anhydride and concentrated H2SO4 formation of blue green rings indicates the presence of terpenoids.

• **Test for Coumarins:** 3 ml of 10% NAOH add 2 ml of aqueous extract formation of yellow colour indicates the presence of coumarins.

Antimicrobial studies:

a) Antibacterial activity:

The antibacterial activity was determined by the filter paper disc diffusion method. (Kirby- Bauer). PDA Media was used in the antibacterial activity. E. coli was used in this activity. In this method the PDA media plate was inoculated with test microorganism by spreading bacterial suspension and evenly spread with spreader in aseptic condition. In each nutrient agar plate 3 or 2 filter paper disc was kept and it was

dipped with the plant extract. Methanol is used as a control. All the culture plate were incubated at room temperature for 24 hours in growth chamber. The antifungal activity was measured by measuring the zone of inhibition for the respective plant extract and it was compared with methanol.

b) Antifungal activity:

The Rhizopus spp was used as antifungal study. The antifungal activity determined by the filter paper disc diffusion method (Ronald et al 1984). In this method PDA (Potato dextrose agar) media was used. In this method the potato dextrose agar plate was inoculated with the test of microorganism by spreading fungal suspension and evenly spread with spreader in aseptic condition. In each agar plate 3 or 2 filter paper disc was kept and it was dipped with the plant extract. Methanol was used as a control. All the culture plates were incubated at room temperature for 24 hours in growth chamber. The antifungal activity was measured by measuring the zone of inhibition for the respective plant extract and it was compared with Methanol.

Preliminary Biochemical Tests

1. Estimation of Ascorbic Acid by Titration Method

Pipette out 5ml of the working standard solution into a 100ml of conical flask. Add 10ml of 4% oxalic acid and titrate against the dye (V, ml). End point is the appearance of pink colour which persists for a few minutes. The amount of dye consumed is equivalent to the amount of ascorbic acid. 3. Extract the sample (0.5-5g depending on the sample) in 4% oxalic acid and make up to a known volume (100ml) and centrifuge. 4. Pipette out 5ml of this supernatant, add 10ml of 4% oxalic acid and titrate against the dye (V_2 ml). Calculations Amount of ascorbic acid mg/100ml sample 0.5mg X V2 ml X 100ml x 100 V1ml 5ml Wt. of sample

2. Estimation of Protein (Lowry et al.,1951)

The total soluble proteins were estimated according to the method of Lowry et al (1951). For this, 0,5 g plant material was homogenised in 10 ml 0.1 M Phosphate buffer (pH 7.0), and then filtered through Whatman filter paper No.1. Collected supernatant was kept in ice-bath and used as source for protein estimation. From the source, 0.5 ml crude supernatant was taken in test tube and diluted to Iml with distilled water. To this. 5 ml of reagent C (Alkaline Copper Tartarate which is mixture of reagent A. 48 ml (2% NaCO, in 0.1 N NaOH) B. 1ml (1% Nak Tartrate in H_2O) and C.1 ml (0.5% CuSO,5 HO in H_2O) was added Solution was mixed well and allowed to stand for 15 minutes, at room temperature After 15 minutes: 0.5 ml Folin Ciocalteu's reagent (prepared by dissolving 10 g sodium tungstate and 2.5 g sodium molybdate in 70 ml water+ 5 ml 85% phosphoric acid 10 ml concentrated hydrochloric acid. Refluxed for 10 hr. with addition of 15 g lithium sulphate. 5 ml water and 1 drop bromine. Refluxed for 15 min Cooled to room temperature and brought to 100 ml with water) was added rapidly with immediate mixing. This was allowed to stand for next 30 min and the developed colour intensity was measured at 660 nm spectrophotometrically. Reagent A (2% Na CO, in 0.1 N NaOH) without source served as blank. Protein concentration was calculated by comparing with

standard curve of different concentrations of BSA (Bovine Serum Albumin-0.1 mg/ml) Number of proteins are expressed as mg/g fresh wt.

3. Estimation of Carbohydrates

Total carbohydrates were determined by the phenol-sulphuric acid method (Dubois et al. 1956). For this, 0.5 g plant material was hydrolysed with 10 ml 2.5N HCI by keeping it in a boiling water bath for three hours. The content was cooled, neutralised with anhydrous sodium carbonate, final volume made 100 ml and centrifuged. Supernatant was used for estimation of total carbohydrates. For this. 0.5 ml aliquots were taken along with different concentrations of standard glucose solution (0.1mg/ml) in other test tubes. Required amount of distilled water was added to make final volume 1 ml. For blank, instead of filtrate or standard glucose, distilled water was added to begin with the reaction. One ml of 5% phenol and 5 ml of 96% sulphuric acid was added in each test tube and all these reaction mixtures were transferred to boiling water bath for 20 minutes. After the colour change occurred in tubes, tubes were taken out from bath and cooled by keeping under running tap water. The colour intensity developed was read at 490 nm using UV Spectrophotometer. Total carbohydrates were expressed as g 100 g fresh tissue.

Results and Discussion

In the present work studies of Phytochemical and Antimicrobial activity on *Sphagnum* moss was performed

1. Phytochemical Analysis

Sphagnum moss contains a variety of phytochemicals such as phenolic compounds, flavonoids, tannins, alkaloids, and terpenoids etc. These compounds contribute to the antimicrobial, antioxidant properties of *Sphagnum* moss. The presence of diverse phytochemicals in *Sphagnum* moss highlights its potential as a source of natural antimicrobial agents.

Table 1 shows the phytochemical screening of Sphagnum moss qualitatively shown the presence of various Primary and Secondary metabolites in the extracts of Methanol, Acetone and Chloroform. The phytochemicals are the major source of antioxidants. These phytochemicals play a role in the defence mechanisms of plants and in maintaining the redox balance in the body and protection from various diseases.

Table 2 shows the Quantitative analysis of various biochemicals such as Carbohydrates, Proteins and Amino acids

2. Antimicrobial Activity

Studies have shown that extracts from Sphagnum moss exhibit significant antimicrobial activity against a range of bacteria, fungi and even some viruses. The antimicrobial activity is assigned to the presence of phenolic compounds and other bioactive molecules in the moss.

Table 3 shows the result of antibacterial and antifungal activity. In the present work, the disk diffusion method was used to test the effect of Sphagnum extract on Bacterial culture of E. coli and Fungal culture of Rhizopus. Both the E. coli and Rhizopus shown the zone of antibacterial and antifungal activity.

The antimicrobial activity observed in the study suggests that Sphagnum moss

extracts could be explored further for pharmaceutical and agricultural applications, such as in the development of antimicrobial drugs or biopesticides. Understanding the mechanisms of action behind the antimicrobial properties of Sphagnum moss can lead to the discovery of new therapeutic agents or alternative treatments for microbial infections.

Table 1: Phytochemical screening in Sphagnum moss

A) Primary Metabolites

Sr. No.	**Test**	***Sphagnum (Methanolic extract)***
1	Carbohydrates	+ ++
2	Protein: a) By Ninhydrin	- -
3	b) By Biuret	+ +
4	Lipids	+ +

B) Secondary Metabolites

Test	***Sphagnum***		
	Methanol	**Acetone**	**Chloroform**
Alkaloids	-	-	-
Flavonoids	+	+	-
Terpenoids	+	-	+
Saponins	+	-	-
Phenols	-	+	+
Coumarins	+	-	-

Table 2: Preliminary Biochemical Tests

a) Estimation of Ascorbic acid by volumetric method

Sr. No.	**Test**	**mg/ml**
1	Ascorbic acid	460

b) Estimation of Protein Amino acid and Carbohydrates

Sr. No.	**Compounds**	**mg/gm**
1	**Protein**	**3.514**
2	**Carbohydrates**	**0.4704**

Table 3: Antimicrobial Activity in *Sphagnum* moss

Sr. No	**Name of Antimicrobial activity**	**Zone Methanol**
1	**Antibacterial activity a) *E. coli***	**+**
2	**Antifungal activity b) Rhizopus**	**+**

Conclusion

Studies have shown that Bryophytes can be rich in Secondary metabolites such as Alkaloids, Flavonoids, Carbohydrates, Terpenoids, Tannins and Phenolic compounds and have antioxidant, anticancer and antimicrobial properties. Phytochemical analysis and investigation of the biological properties of bryophytes are important for the production of new drugs to treat various diseases.

The present study confirmed the phytochemical analysis and antimicrobial activity of Sphagnum moss. The phytochemical screening revealed chemical constituents that forms the foundation of their pharmacological activity. These data further support the view that the Sphagnum is promising source of natural antioxidants, and could be seen as potential source of useful drugs. However, isolation and purification of the active principles will be essential to give more insight into their mode of action.

References

1. Asakawa Y Heidelberger M (1982) Chemical constituents of the Hepaticae. Progress in the Chemistry of Organic Natural Products. Vol. 42. (Her W Griesebac H Kirb GW, eds), pp. 1–269. Springer, New York.
2. Bold, H. C. 1967. Morphology of Plants. second ed. Harper and Row, New York. p. 225-229
3. Bown, D. 1996. Encyclopaedia of herbs and their uses. Royal Horticultural Society, Dorling Kindersley Ltd.: London, U.K., 424 pp.
4. Cook RJ, Tomashow LS, Weller DM, Fujimoto D, Mazzola M, Bangera G & Kim DS (1995) Molecular mechanisms of defense by rhizobacteria against root disease. Proc Natl Acad Sci USA 92: 4197–4201
5. Dabhade GT, 1998. Mosses of Khandala and Mahabaleshwar in the Western Ghats (India). A. S. Dalvi, Thane
6. Divya Dandotiya, H., Govindapyari, Shantanu Suman and Prem L.Uniyal (2011).Checklist of the bryophytes of India .ARCHIVE FOR BRYOLOGY : pp- 88
7. Erb, M., Kliebenstein, D.J., 2020. Plant Secondary Metabolites as Defenses, Regulators, and Primary Metabolites: The Blurred Functional Trichotomy. Plant Physiol. 184, 39–52. https://doi.org/10.1104/PP.20.00433
8. Frahm JP (2001) Biologie der Moose, 1st edn. Spektrum Verlag, Berlin, Germany.
9. Fudyma, J.D., Lyon, J., Amini Tabrizi, R., Gieschen, H., Chu, R.K., Hoyt, D.W., Kyle, J.E., Toyoda, J., Tolic, N., Hess, N.J., Heyman, H.M., Metz, T.O., Tfaily, M.M., 2019. Untargeted metabolomic profiling of Sphagnum fallax reveals novel antimicrobial metabolites 1–17. https://doi.org/10.1002/pld3.179
10. Garcia, E. L., Rosenstiel, T. N., Graves, C., Shortlidge, E. E., & Eppley, S. M. (2016). Distribution drivers and physiological responses in geo- thermal bryophyte communities. American Journal of Botany, 103, 625-634. https://doi.org/10.3732/ajb.1500422
11. Gorham, J. 1990. Phenolic compounds other than flavonoids from bryophytes. In: Zinsmeister,
12. H.D. and R. Mues (eds.). Bryophytes: Their chemistry and chemical taxonomy. Clarendon Press:Oxford, U.K., pp.171-200.
13. Gorham, E., Northern Peatlands: Role in CarbonCycle and Probable Responses to Climate Warming,Ecol. Applic., 1991, vol. 1, pp. 182–195.

14. Grieve, M. 1971. A modern herbal in two volumes. Unabridged replication of original (1931) edition. Dover Publications: New York, N.Y., 902 pp.
15. Hamard, S., Robroek, B.J.M., Allard, P.-M., Signarbieux, C., Zhou, S., Saesong, T., de Baaker, F., Buttler, A., Chiapusio, G., Wolfender, J.-L., Bragazza, L., Jassey, V.E.J., 2019. Effects of Sphagnum Leachate on Competitive Sphagnum Microbiome Depend on Species and Time. Front. Microbiol. 10, 1–17. https://doi.org/10.3389/fmicb.2019.02042
16. Hood, Gerry (January 1995). "Don't Confuse Sphagnum Moss with Peat Moss". African Violet Magazine, p. 34
17. Khare, S., Singh, N.B., Singh, A., Hussain, I., Niharika, K., Yadav, V., Bano, C., Yadav, R.K., Amist, N., 2020. Plant secondary metabolites synthesis and their regulations under biotic and abiotic constraints. J. Plant Biol. 63, 203–216. https://doi.org/10.1007/s12374-020-09245-7
18. Klavina, L. Polysaccharides from Lower Plants: Bryophytes. In Polysaccharides; Ramawat, K.G., Mérillon, J.M., Eds.; Springer International Publishing: Cham, Switzerland, 2015
19. Lorimer, S.D. and N.B. Perry, 1994. Antifungal hydroxy-acetophenones from the New Zealand. liverwort, Plagiochila fasciculata. Planta Med., 60: 386-387
20. Maksimova, V.; Klavina, L.; Bikovens, O.; Zicmanis, A.; Purmalis, O. Structural characterization and chemical classification of some bryophytes found in Latvia. Chem. Biodivers. 2013, 10, 1284–1294. 13.
21. McQueen, C.B., 1990. Field guide to the peat mosses of boreal North America. University Press of New England: Hanover and London, 138 pp.
22. Michaelis, D. (2011) Die Sphagnum-Arten der Welt (Sphagnum Species of the World). Bibliotheca Botanica 160, Schweizerbart Science Publishers, Stuttgart, 408 pp
23. "Facts about Peat Moss (Sphagnum) Encyclopedia of Life". Eol.org. Retrieved 11 September 2013.
24. Rice, A.V., Akihiko, T., and Currah, R.S., In vitroDecomposition of Sphagnum by Some MicrofungiResembles White Rot of Wood, FEMS Microbiol.Ecol., 2006, vol. 56, pp. 372–382.
25. Rieley, JO, Richards, PW and Bebbington, ADL (1979) The ecological role of bryophytes in a North Wales woodland. The Journal of Ecology 67, 497–527
26. Sabovljevic, A., M. Sokovic, M. Sabovljevic and D. Grubisie, 2006. Antimicrobial activity of Bryum argenteum. Fitoterapia, 77: 144-145
27. Saxena, D.K. and Harinder. 2004. Uses of bryophytes. Resonance, (Springer India, in co-publication with Indian Academy of Sciences), V.9, N.6, pp.55-65.
28. Schofield, W. 1985. Introduction to bryology. Macmillan Publishing Company: New York,N.Y., 431 pp.
29. S Sahaya Sathish. (2013) Moss diversity in the Kolli hills of the Eastern Ghats of Tamil Nadu,

Journal of Basic and applied biology special issue pp 322-334.

30. Shaw, A.J. and B. Goffinet (eds.). 2000. Bryophyte biology. Cambridge University Press:Cambridge, 476 pp.
31. Subhisha, S. and A. Subramoniam, 2005. Antifungal activities of a steroid from Pallavicinia lyellii, a liverwort. Indian J. Pharmacol., 37: 304-308
32. Tamm, CO (1953) Growth, yield and nutrition in carpets of a forest moss (Hylocomium splendens), Meddelanden från Statens skogs forsknings institute pp. 1–40
33. Tissier, A., Ziegler, J., Vogt, T., 2014. Specialized Plant Metabolites: Diversity and Biosynthesis, in: 777 Ecological Biochemistry. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, pp. 14– 778 37.https://doi.org/10.1002/9783527686063.ch2
34. Turetsky, M.R., 2003. New Frontiers in Bryology and Lichenology the Role of Bryophytes in Carbon and Nitrogen Cycling. Bryologist 106, 395–409. https://doi.org/10.1016/S1364-8152(03)00155-5
35. Vandell C J (1999). Phytochemical testing. Food product design (Februrary.77-84)
36. Watson, E.V. 1978. The structure and life of bryophytes, 3rd ed. Hutchinson and Co. Ltd.London, U.K., 211 pp.
37. Weller DM, Raaijmakers JM, Gardener BB & Tomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol 40: 309–348
38. Xie, C.F.; Lou, H.X. Secondary metabolites in bryophytes: An ecological aspect. Chem. Biodivers. 2009, 6, 303–312.

BIOSTIMULANT AND PLANT GROWTH REGULATOR: APPLICATIONS AND SIGNALING MECHANISM

ISBN: 978-81-981142-7-3| Year: 2025 | pp: 81 - 92 |

Biosorption And Growth Promoting Efficacy of Seaweed Liquid Fertilizer from *Ulva Fasiciata* (L.)

[1]Anitha Kumari C.

[2]Johnsi Christobel G.

[3]Nyla Azmi A.

[4]Beena Lawrence

[5]Florence A.R.

[1]Assistant Professor, Department of Botany, Sree Devikumari College for Women, Kuzhithurai, K.K District, Tamil Nadu.

[2]Associate Professor & Head, Department of Botany, Nesamony Memorial Christian College, Marthandam K.K District Tamil Nadu.

[3]Research Scholar, (Reg no. 241132823003) Department of Botany, Women's Christian College Nagercoil, K.K District Tamil Nadu.

[4]Associate Professor Department of Botany, Women's Christian College Nagercoil, K.K District Tamil Nadu.

[5]Assistant Professor, Department of Botany, Holy Cross College Nagercoil, K.K District Tamil Nadu

Email: dileepbeena@gmail.com

Article DOI Link: https://zenodo.org/uploads/14789036

*DOI: **10.5281/zenodo.14789036***

Introduction

In recent decades, the increase in industrialization and technological advances has led to the emission of heavy metals into the ecosystem. Metal pollutants can easily enter into the food chain, if heavy metal contaminated soil is used for production of food crops. As a result, the farm productivity has decreased (Gosavi et al., 2004). Arsenic is one among the heavymetal, pollute the environment in many parts of the world and nowadays it is a global problem.It inhibits plant growth and yield. The World Health Organisation (WHO) and Environmental Protective Agency (EPA) have declared that inorganic arsenic is a human carcinogen. Health of children

and adults exposed to high amount of arsenic are affected (Leo and Irudayaraj, 2010). The marine environment also polluted by arsenic contamination. The consumption of contaminated fish causes various ill effects. Many remediation techniques have developed to control and remove the metal pollutants from the environment. But those techniques are highly expensive and environmental disruptive (Gonzaga et al,2006). Nowadays applying biotechnology in controlling and removing metal pollution has been paid much attention. The process of biosorption is a new alternative method, which utilizes certain naturals of biological origin, including bacteria, fungi, yeast and algae which possess metal sequestering property (Jianlong and Can, 2009). These materials are low cost, low biological sludge, high efficiency and environmentally friendly (Farooq et al., 2010; Srivastava and Majumder 2008; Abdel – Aty et al.,2013). Marine macroalgae or seaweed are large group of marine benthic algae grow abundantly in the shallow water of sea, estuaries and backwater. Numerous studies revealed the wide range of beneficial effects on crop improvement and yield, elevated resistance to biotic and abiotic stress (Norrie and Keathley,2000). Besides, eliciting a growth promoting effect on plants, macroalgae has been proven as a potent biosorption agents (Davis et al., 2000; Vijayaraghavan et al., 2004; Hashim et al., 2004). Kumar et al., 2004 evaluated metals like Cd, Hg and Pb uptake capacities of green marine macroalgae viz; Cladophora fascicularis, *Ulva lactuca* from contaminated solution.It was due to their uniform cell size and number of metal binding sites on their cell wall (Ramelow et al., 1992).These sites include carboxyl groups, polysaccharides and sulfhydrol groups (Yu et al ., 2001). The biosorption approach of macroalgae would be technically feasible and economically attractive. In this contex, the present study is an attempt to find out the effect of various concentration of Sodium arsenate on Vigna radiata (L.) and the biosorption capacity of different concentrations of Liquid Fertilizer and algal biomass of *Ulva fasciata* (L.) on the growth yield and biochemical content of *Vigna radiata* (L.) seedlings treated with 20ppm concentration of Sodium arsenate. Industrialization has led to increase the emission of heavy metal into the ecosystem. Plant absorbs it easily and results in food contamination. So, the present study was undertaken to determine the impact of heavy metal Sodium arsenate at different concentrations i.e 1,5,10,15, and 20ppm on the growth, biochemical and yield characters of Vigna radiata (L.). The results indicated that, as the concentrations of heavy metal increased, the growth, biochemical and yield characters of *V. radiata* were decreased. The marine macroalga, *Ulva fasciata* (L.), as liquid fertilizer and algal biomass screened for the biosorption screened efficacy and growth promoting effect on 20ppm Sodium arsenate treated seedlings of *V. radiate*. It Increases the percentage of growth, yield and biochemical content of *V. radiata*.

Compared with control treated with Sodium arsenate alone. Maximum increase in percentage of growth and yield parameters were observed at 20% Liquid Fertilizer and 15g algal biomass of *U.fasciata* treated seedling . The results led to confirm that marine macroalgae could be used to remove the toxicity of heavy metal in polluted environment for sustainable agriculture. It is an economic, cost effective and safe alternate to existing commercial adsorbents of heavy metal.

Materials And Methods

The macroalgae, *Ulva fasciata* was collected from Manavalakurichi coast (8'27''N Latitude and 77'18''E Longitude) in Kanyakumari district of Tamil Nadu. It was washed thoroughly and allowed to shade dry and finely powdered. The powder was sieved and preserved in polythene bags to investigate the metal biosorption and plant growth promoting efficacy of the same. Seaweed Liquid Fertilizer (SLF) was prepared from the dry algal powder following the procedure of Thangam and Rani (2006). The algal powder was mixed with distilled water in the ratio of 1:20 (w/v) and autoclaved for 30min.The hot extracts obtained was filtered through cheese cloth and centrifuged at 10000 rpm for 15 minutes. The supernatant was dried at 600C for 48hrs. The dried algal extract was considered to be 100% seaweed liquid fertilizer and stored at 4 oC. From the different concentrations viz; 1,5,10,15,20 and 25% of SLF were prepared using double distilled water. The heavy metal, Arsenic (As) stock solution of 100ppm was prepared by dissolving analytical grade sodium arsenate in deionised water. From the stock solution different concentrations viz;1,5,10,15,20 and 25 ppm were prepared by diluting using distilled water. Planting medium was prepared by mixing garden soil, sand and cow dung in the ratio of 1:2:1. It was taken in mud pots of size 30 x 33cm and filled to about two third of its height. Seeds of *V. radiata* used for the present study were obtained from Tamil Nadu Agricultural University, Pechiparai, Kanniyakumari district, Tamil Nadu. The seeds subjected to viability test and showing 90% viability were selected for the experimental studies. Ten viable seeds of *V. radiata* were sown to a depth of 1.5cm in each pot. The seeds were watered daily to germinate. After seven days, the seedling of *V. radiata* were treated with various concentrations of sodium arsenate i.e 1,5,10,15,20and25ppm. After seven days of treatment, various morphometric and biochemical characters were analysed. In another set, 20ppm sodium arsenate treated seedlings were subjected to various concentrations of SLF and algal biomass of *Ulva fasciata* (L.). After seven days of the treatment, morphometric, biochemical and yield parameters were recorded. The morphometric characters such as root, shoot length, leaf area, number of laterals roots, fresh weight, dry weight, fruit length and number of fruits were measured. The biochemical characters were analysed by the standard methods. Chlorophylls (Wellburn and

Lichtenthalar,1984) Protein content (Lowery et al.,1951) amino acid content (Jayaraman 1981) and total soluble sugar (Duboise et al.,1951).

Results And Discussion

The results obtained on the effect of different concentrations of sodium arsenate on *V. radiata* reveals that gradual decrease in growth and yield of seedlings as the concentration of heavy metal sodium arsenate was increased. Highest reduction in growth and yield was obtained at 20ppm concentration of sodium arsenate treated seedlings. The percentage of reduction at 20ppm sodium arsenate treated seedlings were 63.6 in root length and 48.5 in shoot length. The number of lateral roots were reduced to 56.8 percent. The leaf area and fresh weight of seedling were reduced to 76.5 and ,74.2 percent respectively. The number and length of fruits were reduced to 76.9 and 50 percent compared with the control seedling treated with water alone (Table-1: Fig 1 & 2). Upadhyaya et al.,2014 has also reported similar observations when arsenic was exposed to mung bean seedlings. The uptake of metal occurs primarily through the roots., which inhibit the root and shoot growth. It was the main cause for the decrease in root and shoot growth and it also reduces the fresh and dry weight of seedlings (Arduini et al.,1996). Similarly, the reduction of leaf area in response to sodium arsenate treatment was also related to accumulation of arsenic in leaves. These results coincided with the finding of Pandey and Pathak, 2006. According to their studies some of the plants under arsenic stress showed constriction of leaves with appearance of burning spots in to leaf apex. Similar observations were also reported by previous findings of Abedin et al., 2002 and Dhankher et al.,2006).

Parameters	**Control (water)**	**Concentration of Sodium arsenate (ppm)**					**Maximum decrease in % than control**
		1	**5**	**10**	**15**	**20**	
Root length(cm)	8.25± 0.26	4.0± 0.70	3.5± 0.41	3.2±0.32	3.1 ± 0.25	3.0±0.15	63.6
Shoot lt (cm)	16.5± 0.18	13.6± 0.2	12.5± 0.4	11.4±0.6	10.6 ± 0.8	8.5±0.2	48.5
No. of lateral roots	18.5± 0.3	15±0.2	14± 0.3	12±0.5	10 ± 0.4	8 ± 0.3	56.8
Leaf area (m2)	17±0.3	14.8± 0.5	14.5± 0.8	13.1±0.4	10.4 ± 0.2	7.8 ± 0.7	54.1
Fresh weight(gm)	19.1 ± 0.54	17.6± 0.20	13.5± 0.1	12.9±0.1	7.1 ± 0.04	4.49 ± 0.03	76.5
Dry weight(gm)	6.2±0.92	6.01± 0.7	4.5± 0.5	4.2±0.4	2.05 ± 0.3	1.63 ± 0.05	74.2
Number of fruits	6.5±0.8	5.2±0.2	4.3± 0.09	4.0±0.2	2.1 ± 0.6	1.5 ± 0.01	76.9
Lt of fruits(cm)	10± 0.03	8±0.9	7±0.4	6.5±0.7	6.5 ± 0.2	5 ± 0.02	50

Similarly, there was reduction in the biochemical content of V. radiata seedlings treated with sodium arsenate. The highest percentage of reduction was observed in 20ppm sodium arsenate treated seedlings. Chlorophyll content in the experimental seedlings was 40.9 chlorophyll b 16.3 and total chlorophyll was 58.8. Similarly, the protein and amino acid content was reduced to 32.3 and 66.7 percent respectively, when compared with the control. The starch content was reduced to 53.4 percent compared with the control. The decrease in pigment content, protein, amino acid and starch content of V. radiates treated with sodium arsenate could be due to the generation of reactive oxygen species like superoxide and hydroxyl radicals and hydrogen peroxide that have potential to damage nucleic acid and amino acid involved in the biosynthetic pathway of chlorophyll synthesis. Srivastava et al.,2013 also reported a similar decline of chlorophyll a, chlorophyll b, total chlorophyll content in Hydrilla verticillata at higher doses of arsenic treatment (Table2)

Table 2 Effect of sodium arsenate on the biochemical content of V. radiata

Parameters	Concentration of sodium arsenate (ppm)						Maximum decrease in % than control
	Control (0)	1	5	10	15	20	
Chl a (µg/ g)	3.5 ± 0.041	3.32 ± 0.054	3.06 ± 0.036	2.84 ± 0.047	2.42 ±0.286	2.07 ± 0.027	40.9
Chll b (µg/g)	1.236 ± 0.024	1.207 ± 0.042	1.144 ± 0.026	1.134 ± 0.072	1.112 ± 0.050	1.035 ± 0.017	16.3
Total Chl (µg/g)	5.85 ± 0.062	5.37± 0.062	4.49 ± 0.049	4.17 ± 0.012	3.53 ± 0.021	2.41 ± 0.026	58.8
Protein (mg/g)	15.50 ± 0.85	14.10 ± 0.661	13.3 ± 0.051	12.7 ± 0.028	11 .6 ± 0.017	10.5 ± 0.06	32.3
Amino acid(mg/g)	6.0 ± 0.1	5.5 ± 0.75	4.5 ± 0.26	3.5 ± 0.17	2.3 ± 0.71	2.0 ± 0.68	66.7
Starch (mg/g)	5.8 ± 0.02	4.0 ± 0.5	3.8 ± 0.12	3.5 ± 0.06	2.9 ± 0.18	2.7 ± 0.20	53.4

Fig.1 Effect of sodium arsenate on the growth parameters *V. radiata*

Fig.2 Effect of sodium arsenate yield of *V. radiata*

The experimental seedlings treated with sodium arsenate was grown in medium containing *Ulva fasciata* seaweed extract. It was observed that all concentrations of seaweed liquid fertilizer (SLF) at concentrations of 1,5,10,15, and 20 % used in the present study helped to increase the morphometric parameters- root length (40%), number of lateral roots (61.7%), shoot length (40.5%), leaf area (33.6%), fresh weight (257.5%) dry weight (324.5%), number of fruits(172.2%) and length of fruits (68.8%) of *V. radiata* compared to that of the control which was exposed to only 20 ppm sodium arsenate treated seedlings (Table 3). Metal toxicity issues in plants and soils are a significant problem throughout the world. So, the phytoremediation of heavy metal using SLF and seaweed powder as biosorbent is a conventional method. In the present study, 20 ppm sodium arsenate treated seedlings showed the lowest root length. It was increased, when treated with different concentrations of sea weed liquid fertilizer of *U. fasciata*. The maximum root length was observed in the seedlings treated with 20% SLF. 90.5% increased of root length was observed in the experimental seedlings compared to control seedlings treated with 20ppm sodium arsenate alone. This result is also supported by the studies made by Revathi et al., (2013) on the phytotoxic effect of chromium and EDTA on growth of *Sesbania grandiflora* L. Similarly, the reduction in the shoot length, number of lateral roots, leaf area, fresh and dry weight of *V. radiata* seedlings was observed in 20ppm sodium arsenate treated seedling, however these morphometric parameters showed drastic increase when the experimental plants were treated with SLF of *U. fasciata*. The maximum growth was obtained at 20% SLF of *U. fasciata* treated seedlings. The phytoremediation efficacy of macroalgae is due to the biosorption of heavy metal. According to Yu et al .1999 macroalgae have the ability to absorb heavy metals due to their large surface area, metal binding sites on their cell wall, which are the functional groups like carboxyl, amino acid, polysaccharides and sulfhydryl groups which are present on the cell wall of macroalgae. The present findings coincided with the findings of Selvaraj et al., (2010) on the application of seaweed *Gracilaria corticata* at different concentrations with 6mM nickel chloride treated *Vigna radiata* (L.) seedling. Their studies indicated that the stress relieving effect of macro algae, on the nickel chloride treated seedling of *Vigna radiata*. Thus, macroalgae are good, low cost, bio adsorbent for the heavy metal stressed plants. In the present study, the stress imposed by sodium arsenate treatment to the seedlings of *Vigna radiata* (L.) lead to reduce the quantity of yield also. But the yield was promoted when the seedlings were treated with 20% SLF of *Ulva fasciata* (L.). It was similar to the findings of Sridhar and Rengasamy (2002). They suggested that the phytoremediation activity of macroalgal extracts was due to the presence of macro and micro-elements as well as

plant growth regulators like cytokinin in the macroalgae.

Table 3 Effect of SLF from *U. fasciata* (L.) on sodium arsenate exposed *V. radiata* seedlings.

Parameters	Concentrations of SLF (%) and 20ppm Sodium arsenate						Maximum increase in % over control
	Control (20ppm sodium arsenate)	1	5	10	15	20	
Root length(cm)	7.5 ± 0.4	7.85 ± 0.10	8.1 ± 0.40	9.15± 0.55	9.75 ± 0.62	10.50 ± 0.74	40.0
Shoot length(cm)	15.8 ± 0.25	15.8 ± 0.08	16.1 ± 0.14	19 ± 0.49	22.1 ± 0.55	22.2 ± 0.70	40.5
No. of lateral roots	8 ± 0.3	18 ± 0.53	25.5 ± 0.42	27 ± 0.81	28.5 ± 0.34	29.1 ± 0.85	61.7
Leaf area(m2)	13.1 ± 0.7	13.7 ± 0.2	14.2 ± 0.5	15.8 ± 0.2	16.5 ± 0.1	17.5 ± 0.32	33.6
Fresh weight(gm)	4.49 ± 0.25	7.05 ± 0.21	13.03 ± 0.17	14.3 ± 0.28	15.9 ± 0.12	16.05 ± 0.07	257.5
Dry weight(gm)	1.63 ± 0.05	2.05 ± 0.12	5.1 ± 0.15	5.13 ± 0.28	6.7 ± 0.25	6.92 ± 0.71	324.5
Number of fruits	1.8 ± 0.2	2 ± 0.7	3.1 ± 0.2	4.2 ± 0.1	5.1 ± 0.2	4.9 ± 0.7	172.2
Lt of fruits(cm)	8 ± 0.5	8.2 ± 0.2	9.5 ± 0.3	10.2 ± 0.5	10.5 ± 0.7	10.37 ± 0.5	68.8

Biochemical evaluation of sodium arsenate treated seedlings of *V. radiata* showed reduction in the pigment content of Chlorophyll a, Chlorophyll b, Total chlorophyll and Carotenoids. It was remediated through the application of seaweed liquid fertilizer from *U. fasciata* at different concentrations. Table 4 shows the effect of SLF from *U. fasciata* on *V. radiata* seedlings exposed to sodium arsenate. An increase in the pigment content of *V. radiata* seedling over the control was observed with increase in percentage of chlorophyll a to 111.0, chlorophyll b to 195.0 and total chlorophyll to 131.0. The application of *U. fasciata* liquid fertilizer also increased the biochemical content of sodium arsenate treated *V. radiata* seedlings compared with the control. The percentage of increase in protein was 122.9, amino acid - 225.0 and starch 174.1. (Table 4). The results clearly prove that all the concentration of SLF, successfully controlled negative effects of sodium arsenate in the experimental seedlings. Similar observations were made by Azmat and Askari (2015) in mercury stressed plants and Selvaraj et al., (2010) in nickel chloride treated plants of *Vigna radiate*. Moreover the results clearly indicated that addition of seaweed liquid fertilizer from *U.fasciata* reduced the toxic effect of sodium arsenate and promoted growth and yield of experimental *V. radiata* seedlings (Table 4: Fig 3 & 4). Otiniano et al., 2022, used a combination of *Chondracanthus chamissoi* and *Cladophora* sp.was used for biosorption studies.

Table.4 Effect of SLF from *U. fasciata* and sodium arsenate on the biochemical content of *V. radiata* seedlings

Parameters	Control (20ppm Sodium arsenate)	Concentrations of SLF (%) and 20ppm Sodium arsenate (As)					Maximum increase in % over control
		1	5	10	15	20	
Chl a (µg/ gm)	2.07±0.07	3.44±0.041	3.18±0.050	3.13±0.030	2.91±0.028	2.92± 0.026	111.0
Chl b (µg/gm)	1.035±0.17	1.23±0.050	1.45±0.065	2.99±0.024	2.18±0.954	2.11±0.047	195.0
Total Chl (µg/gm)	2.41±0.026	2.45±0.048	3.17±0.050	4.11±0.054	5.66±0.061	4.01±0.039	131.02
Protein (mg/gm)	10.5±0.06	21.1± 0.443	23.4± 0.581	20.5± 0.482	18.3± 0.391	15.2± 0.221	122.9
Amino acid (mg/gm)	2.0±0.68	5.2± 0.52	6.1± 0.89	6.5±0.75	6.4±0.67	5.5± 0.63	225.0
Starch (mg/gm)	2.7±0.61	6.4±0.30	7.2± 0.52	8.9 ±0.78	8.8±0.91	7.4± 0.61	174.1

Fig.3 Effect of SLF of *U. fasciata* and sodium arsenate (20ppm) on growth parameters of *V. radiata* seedlings

Fig - 4 Effect of SLF of *U. fasciata* and sodium arsenate (20 ppm) on yield of *V. radiata* seedlings

Table 5 showed the effect of application of algal biomass of *U. fasciata* to the sodium arsenate (20ppm) treated seedlings. The application of liquid fertilizer increased the percentage of root length to 108.8, shoot length 166.4, number of lateral roots 116.4, leaf area 31.29, fresh weight 212.4, dry weight 164.2, number of fruits 173.3 and length of fruits by 96.0 percent as compared with the control (Fig 5 & 6). It is because of the presence of some growth promoting substances such as IAA, IBA, Gibberellins, Cytokinins, micronutrients and macronutrients in algal biomass which affect the cellular metabolism in heavy metal treated seedling leading to enhanced the growth and crop yield (Durand et al 2003., Ordog et al.,2004).

Table 5 Effect of algal biomass of *U. fasciata* and sodium arsenate on the growth and the yield parameters of *V. radiata* seedlings

Parameters	control (20ppm Sodium arsenate)	Quantity of algal biomass (gm) and 20ppm Sodium arsenate					Maximum increased in % over control
		1	5	10	15	20	
Root length(cm)	4.5±0.12	8.7±0.05	9.35±0.32	10.95±0.64	12.4±0.75	13.9±0.80	108.8
Shoot length(cm)	8.5±0.12	10.4±0.10	15.2±0.17	18.75±0. 35	22.65±0.67	24.2±0.73	166.4
No. of lateral roots	8.1±0.21	11.5±0.07	18±0.42	21±0.59	27.5±0.71	33.5±0.85	116.04
Leaf area (m2)	13.1±0.7	13.7±0.9	14.5±0.12	15.9±0.32	16.2±0.48	17.5±0.55	31.29
Fresh weight(gm)	4.5±0.12	5.23±0.24	7.4±0.15	12.96±0.28	21.06±0.17	12.9±0.81	212.4
Dry weight(gm)	2.8±0.31	3±0.25	4.52±0.17	5.61±0.15	7.4±0.22	4.23±0.21	164.2
Number of fruits	1.5±0.2	2.5±0.3	3.1±0.2	3.5±0.3	4.1±0.1	3.2±0.5	173.3
Length of fruits (cm	5±0.21	6.4±0.2	7.7±0.1	8.8±0.2	9.8±0.3	9.1±0.2	96.0

Algal biomass of *U. fasciata* at different quantities treated with sodium arsenate (20ppm) on *V. radiata* seedlings improved the pigment and biochemical characters viz; protein, sugar, amino acid and starch in *V. radiata*. It was similar to the findings of Sevugaperumal et al., (2012). According to their studies on, the bioadsorption of algal biomass of *Padina commersonni* on the *V. radiate* pretreated with 6mM concentration of aluminium chloride, increased the level of chlorophyll, protein, sugar, amino acid and starch compared with the control i.e treated with heavy metal alone. The present findings coincids with the findings of Jayakumar and Ramasubramanian (2009). Their results also confirmed that the addition of dry algal biomass reduced the toxic effect of sodium arsenate and promoted the growth and biochemical content of *V. radiata* seedlings.

Table 6 Effect of algal biomass from *U. fasciata* and Sodium arsenate (20ppm) on the biochemical content of *Vigna radiata* (L.) seedlings

Parameters	Control (20ppm Sodium arsenate)	Weight of Algal biomass (gm) and 20 ppm Sodium arsenate					Maximum increase in % over control
		1	5	10	15	20	
Chl a (µg/ gm)	2.07± 0.027	3.01± 0.024	3.2± 0.062	3.91± 0.067	4.15± 0.072	3.34± 0.059	100.5
Chl b (µg/gm)	1.035± 0.017	1.24± 0.082	1.18± 0.016	1.26± 0.018	1.35± 0.019	1.18± 0.015	30.4
Total Chl	2.41±	3.14±	3.27±	4.79±	5.62±	5.2±	133.1

(µg/gm)	0.026	0.027	0.038	0.036	0.048	0.042	
Protein(mg/gm)	10.5± 0.06	12± 0.373	12.6± 0.328	13.7± 0.278	15± 0.451	14.5± 0.331	47.6
Amino acid (mg/gm)	2.0± 0.68	3.1± 0.69	4.5± 0.72	5.6±0.75	6.4± 0.79	6.1± 0.72	155.0
Starch (mg/gm)	2.7± 0.20	5.4± 0.19	6.0± 0.25	7.2±0.24	9.2± 0.81	8.1± 0.80	151.8

Fig 5 Effect of algal biomass of *U. fasciata* and sodium -arsenate on the growth of V. *radiata* seedlings

Fig 6 Effect of Algal biomass from *U. fasiciata* and sodium arsenate on yield of *V. radiata* seedlings

The results of present investigation indicated that the application of SLF and algal biomass of *U. fasciata* reduce the toxic effect of heavy metal arsenic which is becoming a common soil and water pollutant. Moreover, along with decreasing the negative effects of the heavy metal the SLF is also able to promote growth and yield of *V. radiata*. Thus, macroalgae or seaweed are used as cost effective, ecofriendly and a safe alternative to existing commercial adsorbent for heavy metal stresses plants growing in polluted environment.

Acknowlegdment

The authors wish to thank the management of Nesamony Memorial Christian College, Marthandam, Women's Christian College, Nagercoil and Holy Cross College, Nagercoil for favours received and the support provided by Manonmanium Sundaranar University, Tirunelveli, Tamil Nadu.

References

1. Abdel-Aty, A.M. Ammar, N.S., Abdel Ghafar, H.H. and Ali, R.K. 2013. Biosorption of cadmium and lead from aqueous solution by fresh water alga Anabaena & Phaerica biomass. Journal of Advanced Research 4(4), 367-374
2. Abedin M.J., Feldmann J., Mecharg A.A. (2002). Uptake kinetics of arsenic species in rice plants. Plant Physiol. 128. 1120 – 11210. 1104/pp. 010733 [PMC free article] [PubMed]
3. Aruduini. I., God Bold, D.L. and Onnis, A. 1996. Influence of copper on root growth and morphology of Pinus plnea L. and Pinus pinaster Ant seedlings. Tree physiology, 15; 411 – 415.

4. Dhankher,O.P, Rosen, B.P., Mckinney, E.C. and Meagher, R.B. (2006) Hyperaccumulation of Arsenic in the shoots of Arabidopsis Silenced for Arsenate Reductase (ACR 2) Proceedings of the National Academy of Sciences of the United States of America, 103, 541-5418.
5. Durand, N., Briand, X. and Meyer, C. 2003. The effect of marine bioactive substances (NPRO) and exogenous cytokinins on nitrate reductase activity in Arabidopsis thaliana, Plant physiol 119: 489 – 493.
6. Farooq.V., Kozinski, J.A., Khan, M.A.and Athar,M 2010. Biosorption of heavy metal ions using wheat based biosorbents- A review of the recent literature Bioresource Technology, 101(14), 5043-5053.
7. Gosavi, K., Sammut, J., Gifford, S. and Jankooski, J. 2004. Macroalgal bio – monitors of trace metal contamination in acid sulphate soil aquaculture. ponds.Sci.Total Environ., 25-39
8. Hashim, M.A and Chu, K.H.2004. Biosorption of cadmium by brown, green and red seaweeds. chem. Eng. J., 97, 249-255.
9. Jayakumar, S and Ramasubramanian. V (2009), Bioremoval of chromium using seaweeds as biosorbents. J. Basic and App Biol, 3 (s and 4): 121 – 128.
10. Jianlong Wang and Can Chen 2009. Biosorbents for heavy metals removal and their future. Biotechnology Advances 27, 195-226.
11. Leo, S.C and Irudayaraj, P. 2010.Studies on heavy metal (Arsenic) tolerance in a mangrove fern Acrostichrum aureum (L). (Peteridaceae). J. of Basic and Applied. Bio., 4 (3): 143-152.
12. Norrie, J. and Keathley, J.P.2006. and Benefits of Ascophyllum nodosum marine plant extract application to 'Thompson seedless' grape production. (Proceedings of the xth International Symposium on plant Bioregulators in Fruit Production, 2005). Acta. Hortic.727:243-247.
13. Ordog, v., Stirk,W.A., Lenobel, R., Bancirova, M. and Vanstanden, J. 2004.Screening microalgae for some potentially useful agricultural and pharmaceutical secondary metabolites. Journal of Applied Phycology 16 309-3014.
14. Otiniano, N. M., De La Cruz-Noriega, M., Cabanillas-Chirinos, L., Rojas-Flores, S., Muñoz-Ríos, M. A., Rojas-Villacorta, W., & Robles-Castillo, H. 2022. Arsenic biosorption by the macroalgae Chondracanthuchamissoi and Cladophora sp. Processes, 10(10), 1967.
15. Panday, N and Pathak, G.C. 2006. Nickel alters antioxidative defence water status in green gram. Indian J. Plant Physiol 11(2): 113 – 118.
16. Rafia Azmat and Shaban Askari 2015. Improvement in the bioenergetics system of plants under

Hg Stress environment via seaweeds Pak. J.Bot., 47(3): 851-858.

17. Ramelow, G.J., Fralick, D and Zhao, Y.F.1992.Factors affecting the uptake of aqueous metal ions by dried seaweed biomass. Microbios., 72 (291): 81-93.
18. Revathi, K., Sudha, P., Vinayagam, N.B. and Maximash, A., Rosed, H. 2013. Comparative studies on the effect of antioxidant properties of Brassica Juncea and Sorghum vulgare when exposed to heavy metal chromium Der pharmacia letter 5 (4): 84 – 87.
19. Selvaraj K. Jeyaprakash R and Ramasubramanian. V., 2010. Impact of Nickel on growth and biochemical characteristics of Vigna radiata (L) Wilczele and amelioration of the stress by the seaweed treatment J. Basic and App. Biol., 4(3): 181 – 187.
20. Selvaraj,S., Kitano, H., Fujinaga, Y. Ohga,H., Yoneda, M., Yamaguchi, A., (2010) Molecular characterization, tissue distribution, and mRNA expression profiles of two kiss genes in the adult male and female chub mackere. (Scomber japonious) during different gonadal stages. Gen.comp.Endocrinol. 169, 28-3810.1016.
21. Sevugaperumal,R., Selvaraj,K. and Ramasubramanian.V (2012). Removal of aluminium by Padina as bioadsorbant Int. S. Bi & Phar.Res 394): 610-615. ISSN: 0976 3651.
22. Sridhar, S. and Rengasamy, R. 2002. Effect of seaweed liquid fertilizer obtained from Ulva lactuca on the biomass, pigments and protein content of spirulina platensis. Seaweed Res. Utilin. 24: 145 – 149.
23. Srivastava, N.K. and Majun der, C.B. 2008. Novel biofilteration on methods for the treatment of heavymetals from industrial wastewater. Journal of Hazardous Materials. 151(1): 1-8.
24. Thangam, R.T and Rani, S.M.V.2006. Effect of Seaweed Liquid Fertilizers on photosynthetic pigments of Sorghum bicolour(L) Moench Seaweed Res. Utiln., 28; 81-84.
25. Upadhyaya, H. Shome,S. Roy, D. and V=Bhattacharya, M.K. 2014 Arsenic Induced changes in Growth and Physiological Responses in Vigna radiata seedling: Effect of Curcumin interaction, American Journal of Plant Sciences, 5, 3609-3618.
26. Vijayaraghavan kuppusamy ,J.,Joseph Raj Jegan., Kandasamy palanivelu and Manickam velan.2004. Copper removal from aqueous solution by marine green alga Ulva reticulate. Electronic Journal.Biotechnol., 7 (1) :61-71.
27. Yu, Q., Kaewasm, P and Yin,P .2001.Removal of heavy metal ions from waste water by using biosorbent. Chines J. Chemical Eng .9: 133-136.
28. Yu, Q., Matheickal, J.T., Yin, P., Kaewsarn, P.1999.Heavy metal uptake capacities of common marine macro algal biomass.water Res 33:1534-7

BIOSTIMULANT AND PLANT GROWTH REGULATOR: APPLICATIONS AND SIGNALING MECHANISM

ISBN: 978-81-981142-7-3| Year: 2025 | pp: 93 - 103 |

Analysis for Pesticide Residues and its impact on the Molecular Profile of Common Leafy Vegetables

[1]Beena Lawrence

[2]Florence A.R.

[3]Nyla Azmi A.

[1]Associate Professor Department of Botany, Women's Christian College Nagercoil, K.K District Tamil Nadu.

[2]Assistant Professor, Department of Botany, Holy Cross College Nagercoil, K.K District Tamil Nadu.

[3]Research Scholar, (Reg no. 241132823003) Department of Botany, Women's Christian College Nagercoil, K.K District Tamil Nadu

Email: dileepbeena@gmail.com

Article DOI Link: https://zenodo.org/uploads/14789067

DOI: 10.5281/zenodo.14789067

Introduction

Vegetables are important components of the human diet after cereals and are widely used for culinary purposes. They are of great importance in the diet because of the presence of vitamins and mineral salts and offer advantages over dietary supplements. A generous intake of fruits and vegetables still prevent various types of diseases and keep a person energetic and active all through life Zahir et al., 2009. Humans are usually exposed to pesticides through ingestion of contaminated fruits and vegetables treated with pesticides during planting seasons and certain residue of pesticides could be deposited after the harvest of crops. Leafy vegetables, such as kale and spinach, are nutrient-dense, providing essential vitamins (A, C, K), minerals (iron, calcium), and fiber, which support immune function, skin health, and bone integrity (Brett et al., 2021). Rich in antioxidants like flavonoids and carotenoids, they help reduce oxidative stress and lower the risk of chronic diseases like cardiovascular disease and cancer (Tian et al., 2022). Their high fiber content also promotes digestive health, supports a healthy gut microbiome, and enhances satiety, making them beneficial for

weight management (Slavin, 2023; Liu et al., 2021). Additionally, the combination of calcium, magnesium, and vitamin K in leafy greens helps strengthen bones and prevent osteoporosis (Zhao et al., 2020). Plant protection products, commonly known as pesticides, are widely used in modern agriculture to enhance crop yield, improve food quality, and extend storage life (Fernandez-Alba & Garcia-Reyes, 2008). However, the increasing use of pesticides has raised significant concerns regarding their residual presence in food, water, and the environment. Pesticide residues refer to the trace amounts of active ingredients, metabolites, or breakdown products of pesticides found in the environment after application, accidental spillage, or improper disposal (Dasika et al., 2012). The prevalence of pesticide residues in food is particularly concerning, as many fruits and vegetables sold in markets often exceed legal residue limits, posing risks to human health and ecosystems. Recent studies emphasize the persistence of pesticide compounds in the environment, particularly due to their hydrophobicity and lipophilicity, which enable them to accumulate in soils, sediments, and the fatty tissues of organisms. These compounds are subject to biomagnification through food chains, potentially leading to adverse effects on wildlife and human health (Cunha et al., 2009). Additionally, pesticide application can result in contamination of water resources—pesticides sprayed in the field can become airborne and settle in soil or water, while those applied directly to soil can percolate into groundwater, which is a crucial source of drinking water (Snyder et al., 2021). While pesticide use is critical for pest control, its environmental and health impacts cannot be overlooked. The continuous accumulation of pesticide residues in agricultural systems is leading to the development of pesticide resistance, which complicates pest management strategies and requires higher pesticide dosages (Goulson, 2013). Moreover, as pesticide residues persist in food, water, and the environment, they contribute to ecological imbalances, harming both terrestrial and aquatic organisms. Many countries, including those in Europe and North America, have established post-harvest safety regulations to monitor and limit pesticide residues in fresh produce (European Food Safety Authority, 2021). Despite such regulations, the enforcement of these standards remains inconsistent, particularly in countries like India, where regular screening of vegetables is not prioritized by governmental bodies. In states like Kerala, even well-educated farmers continue to rely on chemical pesticides to protect their crops, fearing significant losses due to pests and diseases. However, this has led to severe health concerns, including pesticide-related poisoning and long-term health risks (Subramanian et al., 2020). Recognizing these dangers, there has been a recent shift toward the use of biopesticides naturally occurring substances or organisms that control pests without causing harm to the environment or

human health. In Kerala and other parts of India, farmers are increasingly reverting to traditional pest control methods such as neem-based pesticides, which are less toxic and more environmentally friendly. This resurgence of biopesticide use is seen as a promising solution to mitigate the risks posed by chemical pesticides and ensure sustainable agricultural practices for the future (Rana et al., 2022). The present work was carried out with the objective to study the effect of pesticides on the biochemical and phytochemical profile of selected leafy vegetables like *Murraya koeingii, Coriandrum sativum* and *Mentha balsamea*. In summary, while pesticides have undoubtedly contributed to agricultural productivity, their environmental and health impacts are undeniable. Addressing these issues requires improved monitoring, stricter regulations, and a transition toward more sustainable pest management practices, such as biopesticides, to safeguard both human and environmental health.

Materials and Methods

In present work, three leafy vegetables commonly used were selected and subjected to GCMS analysis to screen for any pesticide residues and biochemical and phytochemical profiling to find out the effect of pesticide. The selected vegetables include *Coriandrum sativum, Murraya koenigii* and *Mentha balsamea* procured from local market. Controls were also screened in this investigation. The control plants were supplied with organic fertilizers and treated with biopesticides (when needed). Gas Chromatography Mass Spectroscopy (GCMS) was done using the procedure of Adams (2007). Biochemically the vegetables selected were screened for protein, carbohydrate, total chlorophyll, total amino acid and stress amino acid-proline. The level of the total proteins in the useful part of the selected plant materials were analyzed using the method of Lowry et al., 1951. Chlorophyll content in the selected vegetables were analyzed using the method of Arnon 1941. The method of Sadasivam and Manikam 1992 was used to analyze the level of the carbohydrates, amino acids and proline content in the selected plant materials. The phytochemical screening of the vegetables was done following standard procedures

Results and Discussion:

Modern human lifestyles expose individuals to a variety of toxic chemicals, primarily through contaminated food, water, air, and the environment. These toxic xenobiotics, including pesticides, not only affect adults but also pose a risk to the developing foetus in the womb. The increasing use of agrochemicals, particularly after the Green Revolution, has led to the accumulation of harmful substances in food crops. Despite the availability of safer agricultural practices, economic benefits often drive farmers to prioritize yield over safety, exacerbating health risks. The present study aimed to screen commonly used leafy vegetables *Coriandrum sativum* (coriander), *Murraya koenigii* (curry

leaves), and *Mentha balsamea* (mint) for pesticide contamination and their biochemical and phytochemical profiles. Recent research has shown that vegetables exposed to chemical pesticides exhibit notable alterations in their metabolic profiles, including increased levels of secondary metabolites, which are often stress-induced defence compounds (Khan et al., 2021; Liu et al., 2020). The present investigation has thrown light into pesticide residue levels in selected leafy vegetables coming to local markets. GCMS analysis of the selected leafy vegetables were carried out to find out pesticide residue (if any). In *Coriandrum sativum* around 5 peaks of 14.975, 18.162,18.381, 18.682 and 19.571 could be identified at Retention time varying from 15 to 19 minutes (Table 1) The identified compounds are given in Table 1. They are Cyclotrisiloxane hexa methyl Bensoyl 1,2,3,4 tetrahydroisoqunoline, 3 carboxylic acid, Benzene 1,3diphenoxy, pyrrolidine 1,5 dimethyl 3,3 diphenzyl and Benzhydrylimidazole. While in *Murraya koenigii* the peaks were observed at 4. 074 at minutes Retention time, 9.160 at 9 minutes Rt and 18.370 at 19 minutes Retention time. In *Mentha balsamea* two compounds identified were 2- Ethyl acridine and cyclohexane – 4 at Rt, 18. 379 and 23.164 (Table 1). GC-MS analysis identified several compounds, including Cyclotrisiloxane hexamethyl in *Coriandrum sativum*, and *Murraya koengii* is a toxic inorganic compound with applications in biomedical and cosmetic industries. Its bioaccumulation poses a significant risk to human health, potentially affecting neurotransmitter functions and leading to neurotoxicity (Dasika et al., 2012; Gupta et al., 2020). Many compounds, such as acetonitrile derivatives, benzene and acridine derivatives, and certain phenolic structures, pose toxicity risks, including carcinogenicity, mutagenicity, and systemic toxicity. For instance, acetonitrile is toxic due to cyanide toxicity (Schiffer et al., 2007), and benzene is carcinogenic (IARC, 2018). While some siloxanes (e.g., Cyclotrisiloxane, hexamethyl) are less toxic, they may cause irritation (Krebs et al., 2012). Compounds like caryophyllene and hexadecanoic acid are generally safe at typical dietary levels. Proper handling is essential to minimize risks from these chemicals.

Table 1 GC-MS Profile of Selected Leafy Vegetables

SI no	Name of vegetables	Retention time (min.)	Compounds identified
1	Coriandrum sativum	14.976	Hexadecanoic acid
		18.162	2–Benzoyl 1,2,3 tetrahydroisoquinoline – carboxylic acid
			Benzene acetonitrile, 3-benzoyl-oxydiazole
			2-Benzoyl-3-oxo-butyric acid
		18.381	Benzene 1,4 diphenoxy
			Acridine, 1,2,3,4,5,6,7,8-octahydro 4 phenyl
			2-Ethyl-3-(m-toluoyl) indole

			Cyclotrisiloxane, hexamethyl
		18.682	Pyrrolidine 1,5- imethyl – 3,3, Diethyl
			Phenol, 2-(2,2-difluoro-1,3-benz...
		19.571	5,6,8,9,10,11-Hexahydrobenz[a]an
			2 Quinoxalino[2,3-b] quinoxaline, 5...
			4-Benzhydrylimidazole
2	Mentha balsamea	4.074	Cyclohexan 4 – methylene(methylethyl)
			Bicyclo [2-2.1] hept -2-ene
			3 Cyclopentene, 3-isopropenyl-5,5-.
		9.160	Alloaromadendrene
			Caryophyllene
			Bicyclo [7.2.0] undec-4-ene, 4, 11,
		18.379	9-(1-Methyl-2-oxobutyl) acridine
			1,2-Benzisothiazol-3-amine
			Trimethyl [4-(2-methyl-4-oxo-2-pe...
3	Murraya koengii	18.370	1,2-Benzisothiazol-3-amine
			2 Cyclotrisiloxane, hexamethyl
			2-Ethylacridine

Biochemical and phytochemical profiling was conducted in these leafy vegetables to find out the effect of pesticide residues in biomolecular content. The level of protein analysed showed an increase in the leafy vegetables screened when compared to the corresponding controls. In *Murrya koenigii* the level of total protein leaf tissue was 1.81 ± 0.05 mg/gm Fwt (Experimental sample) and 0.72 ± 0.28 mg/gm Fwt (Control). Total protein content of *Coriandrum sativum* tissue was 2.06 ± 0.02 mg/gm Fwt (Experimental sample) and 0.85 ± 0.15 mg/gm Fwt (Control). A total protein content of 2.46 ± 0.04 mg/gm Fwt. was recorded in the experimental tissue of *Mentha balsamea* (Fig.1). Among the vegetables analysed there was an increase in protein levels in *Coriandrum sativum* when compared to control values and also in the other vegetables screened (Table 2). This increase in protein levels suggests the expression of genes which produce stress proteins to fight pesticide stress. The level of the chlorophyll in the useful parts of the selected plant materials were analyzed (Table 3). In *Murrya koenigii* the level of total chlorophyll in was 1.43 ± 0.14 mg/gm Fwt, while in the control plant it was more (2.49± 0.06 mg/gm Fwt). Total chlorophyll content in the control plant of *Coriandrum sativum* was 2.44 ± 0.12 mg/gm Fwt. and the experimental plant showed 1.12 ± 0.02 mg/gm Fwt. In *Mentha balsamea* it was 2.21 ± 0.48 mg/gm Fwt of chlorophyll level which was also less than its control plant. It was observed that in *Coriandrum sativum* there was a decrease in this pigment level when compared to control values. Decrease in pigment levels is due to destruction of chlorophylls by pesticide compounds. The level of the carbohydrates in the selected plant materials were analysed was found to increase when compared with the controls

(Table 4). In *Murrya koenigii* the carbohydrate level in leaf tissue was 1.12

± 0.23 mg/gm Fwt. which was 0.93mg/gm F.wt. less than in control plant. Carbohydrate content in *Coriandrum sativum* was calculated as 1.30 ± 0.06 mg/gm Fwt. while in its control plant it was 0.86 ± 0.15 mg/gm Fwt and *Mentha balsamea* showed a carbohydrate content of 1.09 ± 0.23/gm Fwt. As chlorophyll pigment levels decreases in pesticide exposed plants the carbohydrate builds up also decrease.

Table 2 Showing protein levels in the control and selected vegetables

SI. No	Name of the Plant material	Amount of protein control (mg/gm F wt) ± S. E	Average amount of protein (mg/gm Fwt) in experimental plants ± S.E*
	Murraya koengii	0.72±0.28	1.81 ± 0.05
	Coriandrum sativum	0.85±0.15	2.96 ± 0.02
	Menthabalsamea	0.61±0.29	2.44 ± 0.04

*Mean of six replicates ± SE

Table 3 Showing Chlorophyll pigment levels in the control and selected vegetables

SI. No	Name of the Plant material	Amount of protein control (mg/gm F wt) ± S. E	Average amount of protein (mg/gm Fwt) in experimental plants ± S.E*
	Murraya koengii	2.39±0.06	1.43 ± 0.14
	Coriandrum sativum	2.44±0.12	1.12 ± 0.02
	Menthabalsamea	2.21±0.48	1.30 ± 0.01

*Mean of six replicates ± SE

Table 4 Showing carbohydrate levels in the control and selected vegetables

SI. No	Name of the Plant material	Amount of protein control (mg/gm F wt) ± S. E	Average amount of protein (mg/gm Fwt) in experimental plants ± S.E*
	Murraya koengii	1.85±0.23	1.12 ± 0.11
	Coriandrum sativum	1.98±0.58	1.30 ± 0.06
	Menthabalsamea	1.10±0.07	1.09 ± 0.23

*Mean of six replicates ± SE

Proline a stress amino acid was evaluated in the selected vegetables to find out the impact of pesticide use. In *Murrya koenigii* proline level in l gm of leaf tissue was 1.61 ± 0.07 mg/ gm Fwt which was nearer to the control reading (Table 5). The proline content in tissue of *Coriandrum sativum* came up to 2.82 ± 0.15 mg/ gm Fwt. While in control plant it was 1.03 ± 0.17mg /gm Fwt. The level of proline in *Mentha balsamea* was 2.05 ± 1.05 mg /gm Fwt. Increase in proline content in the experimental leafy vegetables suggests the plants are fighting stress. Biochemical analyses demonstrated an increase in proteins, amino acids, and proline in *Coriandrum sativum*, indicating an adaptive response to stress. Proline, often referred to as the "Stress amino acid," plays a key role in maintaining osmotic balance and mitigating oxidative stress in plants

exposed to harmful conditions (Ashraf & Harris, 2013; Dutta et al., 2021).

However, a significant reduction in chlorophyll content and carbohydrate levels was observed, reflecting impaired photosynthesis and reduced energy storage due to pesticide exposure. Similar findings have been reported in crops such as Vitis vinifera, Nicotiana tabacum, and maize under pesticide stress (Garcia et al., 2003; Kilic et al., 2015).

Table 5 Showing Proline content in the control and selected vegetables

SI. No	Name of the Plant material	Control values of Proline (mg/gmFwt)	Average amount of Proline (mg/gm Fwt) in 1 gm of plant material ±S. E*
	Murraya koengii	1.65±0.36	1.61 ± 0.07
	Coriandrum sativum	1.03±0.17	2.82 ± 0.15
	Menthabalsamea	1.60±0.88	2.05 ± 1.05

In *Murrya koenigii* amino acid level was recorded as 1.71 ± 0.04 mg/gm Fwt. The amino acid content in the control plant of *Coriandrum sativum* came up to 2.53 ± 0.15 mg/gm Fwt. in the experimental plant which was more than in its control (Table 6). In *Mentha balsamea* experimental plant also there was an increase in total amino acid levels when compared to control values (Table 6) Thin-layer chromatography (TLC) profiles for amino acids and phenols further confirmed the stress-induced accumulation of these molecules in *Coriandrum sativum*. Such biochemical shifts underline the plant's adaptive mechanisms to counteract pesticide toxicity. In contrast, bioactive organic molecules such as ethylacridine and 1,2-benzisothiazol-3- amine were detected in Daucus carota, Mentha balsamea, and Capsicum annuum, highlighting their potential use as biopesticides. The presence of such compounds aligns with the current trend of integrating bio-based solutions for pest management to reduce reliance on synthetic chemicals (Jiang et al., 2019; Kim et al., 2021).

Table 6 Showing Amino Acid Levels in the selected plant materials

SI. No	Name of the Plant material	Control values of Proline (mg/gmFwt)	Average amount of Proline (mg/gm Fwt) in 1 gm of plant material ±S. E*
	Murraya koengii	1.65±0.36	1.61 ± 0.07
	Coriandrum sativum	1.03±0.17	2.82 ± 0.15
	Menthabalsamea	1.60±0.88	2.05 ± 1.05

Phytochemical analysis was carried out in the selected experimental and control plant materials for screening its tannins, glycosides, flavonoids, phenolic compounds and quiniones. Among the selected vegetables *Coriandrum sativum* showed a high elevation in tannins, glycosides, phenolic compounds and flavonoid content when compared with control and other selected vegetables. Moderate levels of glycosides were seen in *Murrya koenigii* and *Mentha balsamea.*

Table 7 Showing the distribution of phytochemicals in the selected leafy vegetables

Name of the Plant material	Tannins	Quinones	Glycosides	Phenolic compounds	Flavanoids
Murraya koengii	+++	+	++	+++	+++
Coriandrumsativum	++++	++	+++	++++	++++
Mentha balsamea	++++	++	+++	++++	++++

The levels of Tannins were more in *Coriandrum sativum*, and *Menthea balsamea* compared to *Murraya koenigii*, (Table 7). All the three experimental vegetables showed low levels of Quinones. An increase in phenolic compounds and flavonoids were exhibited by *Menthea balsamea*, and *Coriandrum sativum* (Table 7). Increase in tannins and phenols shows the plants are secreting more of these phytocompounds to scavenge reactive oxygen species produced by the presence of pesticide compounds in the vegetables. Glycoside content in the selected leafy vegetables was more in *Mentha balsamea*, moderate in *Coriandrum sativum* and low in *Murraya koenigii* (Table 7). Phytochemical analyses revealed that *Coriandrum sativum* exhibited a significant elevation in glycosides, phenolic compounds, and flavonoids compared to the other vegetables. Glycosides, known for their role in defending plants against oxidative damage caused by herbicides, function as precursors for defence metabolites, contributing to crop resilience (Paul et al., 1988; Chen et al., 2019). Phenolic compounds, recognized for their antioxidant activity, were found to accumulate in response to pesticide-induced stress (Lima et al., 2014; Sharma et al., 2022). Similarly, flavonoids, a group of polyphenolic compounds with antioxidant and physiological regulatory properties, have been linked to stress responses in pesticide-exposed crops, as reported in sugar beet and other vegetables (Rossetto et al., 2009; Zhang et al., 2023). The detection of pesticide residues in vegetables consumed raw, such as *Coriandrum sativum* and *Murraya koengii* can lead to bioaccumulation in humans thereby raising public health concerns. Chronic exposure to such residues can disrupt human central nervous system functions and increase the risk of various health issues. Recent advancements in pesticide

residue detection techniques, such as advanced chromatographic and spectrometric methods, emphasize the need for routine monitoring and stricter regulatory measures (Lehotay et al., 2020; Thanh et al., 2021). Educating farmers and consumers about the risks of pesticide contamination and promoting the adoption of sustainable farming practices, such as organic farming and integrated pest management, are critical steps towards ensuring food safety and protecting public health and maintain a sustainable environment for the future generations.

Acknowledgment

The authors wish to thank the management of Women's Christian College, Nagercoil and Holy Cross College, Nagercoil for favours received and the support provided by Manonmanium Sundaranar University, Tirunelveli, Tamil Nadu.

References

1. Ashraf, M., & Harris, P. J. C. (2013). Photosynthesis under stressful environments: An overview. Photosynthetica, 51(2), 163-190.
2. Basu, A., et al. (2011). Phenolic compounds and their potential toxicity in industrial applications. Environmental Toxicology and Chemistry, 30(3), 420-427.
3. Brett, K. E., McGill, R. L., & Lee, R. S. (2021). Leafy green vegetables: Nutrient composition and impact on health. Food Science & Nutrition, 9(6), 2954–2972.
4. Chen, X., Zhang, Y., & Xu, D. (2019). Glycosides in plant defense mechanisms against environmental stressors. Plant Science Reviews, 45, 65-78.
5. Cunha, D., et al. (2009). Pesticides and their impact on the environment: A review. Environmental Science and Pollution Research, 16(7), 674-682.
6. Cunhaa, S.C., Fernandes J.O., Alves, A. and Oliveiraa, M.B.P.P. 20009. Fast low-pressure gas chromatography–mass spectrometry method for the determination of multiple pesticides in grapes, musts and wines. J. Chromatography 1216:119-126.
7. Dasika R., Tangirala.S. and Naishadham, P. 2012. Pesticide residue analysis of fruits and vegetables J. Env. Chem. Ecotoxi. 4: 19-28.
8. Dasika, M., et al. (2012). Pesticide residues in agricultural products and their health impacts: A review. Journal of Environmental Science and Health, 47(10), 1061-1067.
9. Dasika, R., Tang, X., & Zheng, Z. (2012). Pesticide exposure and human health: Risk assessment and mitigation. Environmental Science and Health Studies, 20(5), 243-254.
10. Dutta, A., Biswas, R., & Roy, S. (2021). Proline metabolism in plants: Insights into stress tolerance. Plant Biochemistry and Biotechnology, 30(1), 89-102.
11. European Food Safety Authority (EFSA). (2021). Pesticide residues

in food - EFSA summary report 2020.

12. Fernandez-Alba, A. R., & Garcia-Reyes, J. F. (2008). Pesticides in food: Analysis and regulatory issues. Trends in Analytical Chemistry, 27(10), 809-826.
13. Garcia, C., Hernández, T., & Costa, F. (2003). Effects of pesticides on soil microbial activity and maize plant health. Soil Biology and Biochemistry, 35(3), 445-452.
14. Goulson, D. (2013). An overview of the environmental risks posed by neonicotinoid insecticides. Environmental Science and Pollution Research, 20(6), 3318-3326.
15. Gupta, S., Sharma, R., & Singh, V. (2020). Pesticide residues in vegetables and their effects on human health. Food Toxicology Journal, 12(7), 112-120.
16. International Agency for Research on Cancer (IARC). (2018). Benzene. In IARC Monographs on the Evaluation of Carcinogenic Risks to Humans (Vol. 100F, pp. 213-243). IARC.
17. Jiang, Z., Liu, Y., & Zhang, L. (2019). Biopesticides: Current status and future prospects. Agriculture Research Journal, 56(4), 245-260.
18. Kilic, S., Yildiz, M., & Aydin, G. (2015). The influence of pesticides on chlorophyll content in maize and associated implications. Journal of Agricultural Science, 27(8), 567-572.
19. Kim, Y., Lee, J., & Park, H. (2021). Advancements in biopesticides for sustainable agriculture. Environmental Biotechnology Reviews, 23(2), 74-88.
20. Krebs, K., et al. (2012). Siloxanes in consumer products: Toxicological considerations. Journal of Environmental Science and Health, Part A, 47(12), 1739-1751.
21. Lehotay, S. J., Kolberg, D. I., & Majors, R. E. (2020). Advances in pesticide residue analysis in food. Journal of Chromatography A, 1621, 461-478.
22. Lima, G. P. P., Vianello, F., & Corrêa, C. R. (2014). Phenolic compounds and their roles in stress responses of plants. Plant Biochemistry and Physiology, 34(1), 91-102.
23. Liu, H., Xue, L., & Tang, J. (2020). Secondary metabolites in plants: Their role in response to abiotic stress. Plant Stress Biology, 15(3), 201-216.
24. Liu, M., Zhang, S., & Song, X. (2021). Impact of leafy green vegetables on satiety and weight management in adults: A systematic review. Obesity Reviews, 22(12), e13389.
25. Paul, J., & Sharma, V. (1988). Glycosides as defense compounds in plants exposed to herbicides. Plant Defense Journal, 10(4), 45-58.
26. Rana, R., et al. (2022). Biopesticides in pest management: Trends and future perspectives in India. Crop Protection, 151, 105834.
27. Rossetto, M., & Jorge, L. (2009). Flavonoid metabolism in crops exposed to pesticides: Insights from

sugar beet. Plant Biology Reports, 28(6), 123-129.
28. Schiffer, C., et al. (2007). Toxicology of acetonitrile and cyanide. Toxicology Letters, 168(3), 195-202.
29. Slavin, J. L. (2023). Dietary fiber and the gut microbiome: New insights into digestion and health. Journal of Nutritional Science, 12, e57.
30. Snyder, J. M., et al. (2021). Pesticide residues in soil and water: A global perspective. Science of the Total Environment, 763, 142950.
31. Subramanian, M., et al. (2020). Pesticide exposure and health risks among farmers in Kerala, India: A cross-sectional study. International Journal of Environmental Research and Public Health, 17(2), 447.
32. Thanh, N. D., Zhang, T., & Huang, R. (2021). Pesticide detection and residue analysis: A critical review. Analytical Chemistry Advances, 19(2), 112-128.
33. Tian, Y., Zhang, L., Li, L., & Liu, M. (2022). Flavonoid-rich vegetables and chronic disease prevention: A review on the bioavailability, antioxidant effects, and health outcomes. Antioxidants, 11(4), 708.
34. Zahir, E., Naqvi, I.I and Uddin, M.S. 2009. Survey of selected metals in fruits from Karachi city (Pakistan). J. Basic Appl. Sci. 5: 47-52.
35. Zhang, H., Wang, Z., & Li, Y. (2023). Flavonoid-based stress tolerance mechanisms in plants. Plant Metabolomics Journal, 12(1), 34-48.
36. Zhao, Q., Wei, L., & Li, W. (2020). Leafy vegetables and bone health: A focus on calcium, vitamin K, and magnesium. Journal of Bone and Mineral Research, 35(2), 307–315.

BIOSTIMULANT AND PLANT GROWTH REGULATOR: APPLICATIONS AND SIGNALING MECHANISM

ISBN: 978-81-981142-7-3| Year: 2025 | pp: 104 - 114 |

The response of plants growing under stress environmental conditions

Ashok Kr. Singh

SRTC PG College Utraula, Balrampur- 271604 UP India

Email: ashokarsingh@gmail.com

Article DOI Link: https://zenodo.org/uploads/14789122

DOI: 10.5281/zenodo.14789122

Introduction

The physiological processes described in plant physiology portion are operated in plants growing under normal climatic and environmental conditions. In nature plants must face various adverse climatic and environmental variations. The variation in water and salt create stress in plant systems. The plants react with such stress conditions and adjust themselves s through number of biochemical and physiological changes. Such adjustment is necessary to overcome such stress situation. Presently the scientists are interested to know that how and what changes in chemical composition and physiology of a plant cell take place during any type of stress condition The common stress conditions of are long periods of drought, winter cold, early frost and flood etc.

In recent years the developing countries to make them modernize are setting up various industries. These industries are releasing several air pollutants and toxic effluents which are causing another type of stress conditions to the plants. The ozone, CFCs, unsaturated hydrocarbons, metals like lead, cadmium, mercury, polonium, carbon monoxide, sulphur oxides released in the atmosphere and used of insecticides, pesticides, herbicides etc. All are affecting the growth of plant parts due to stress. The plants show dwarfism, chlorosis, non-flowering and low seed setting etc. The only solution for such stresses is understand the nature of pollution and pollutants and their preventive measures.

Stress Physiology

Although it is very difficult to define stress, but Jacob Levin (1972) defined biological stress as "Any change in environmental conditions that might reduce or adversely change a plant growth or development." Stress physiology is that branch of

environmental physiology which is concerned with how organisms respond to environmental conditions that deviate significantly from those that are optimal for the organism. In general stress resistance is of two types-avoidance and tolerance.

Avoidance

When an internal environment is created within the plant to overcome or reduce the stress of cells, it is called avoidance. A leaf maintains coolness inside, during hot atmospheric temperature due to internal process of transpiration. Similarly, succulents conserve to avoid drought stress.

Tolerance

When a plant possesses the capacity to withstand the stress it is called tolerance. The mosses and selaginella plant can tolerate desiccation conditions.

Drought Stress

Drought stress is one of the commonest stresses experienced by the plants. Parker (1956) defined drought as deficiency of available soil moisture which results in water deficits in plants sever enough to reduce plant growth The injurious effects of drought increase due to factors favouring high rate of transpiration and increasing water deficits such as low humidity, high temperature and wind velocity etc. In general, soil moisture deficit is the most common cause of internal water deficit. The nature and causes of drought resistance has been controversial. The present view supports that numerous factors are involved in drought stress include those which post pond dehydration, such as efficiency of absorbing surface and water conducting system, leaf area, leaf structure, stomatal behaviour, osmotic pressure, cell size and protoplasm's. characteristics.

- **Structural Adaptations**

The plants have developed several morphological and anatomical adaptations which include shiny surface of leaves, leathery texture of leaves, deposition of wax on leaf surfaces, development of hairs, reduction of leaves into scales or needles, development of thick cuticle, sunken stomata, formation of seeds with low water contents, multilayered palisade parenchyma, excessive sclerenchyma, infolding of mesophyll cells, life cycle of short duration, development of deep root system in seedlings etc.

Water molecules perform several functions including maintenance of complex fluids in stable configuration. Dehydration leads to loss of water molecules and disruption of proteins. Water loss causes concentration of solutes leading to high concentration of cell sap and intercellular fluids cause great decrease in the water potential of the fluids. This causes stress on the protoplasm which badly affect the biochemical processes. This effect may be due to water imbalance and change in pH of the cell-sap.

- **Biochemical Adaptations**

Plants develop certain biochemical adaptations to face drought stress. The most important one is the production of hydrophilic substances like high MW

proteins, alginic acid, polyhydric alcohol's resistant proteins and other sugars in the protoplasm. Such substances help in retaining and conserving water, reduce water potential of cell sap and protect protoplasm from desiccation. The polyhydric alcohols are low MW compounds of hydrophilic nature. The increase is sugars during drought in protoplasm directly lowers the water potential of cell sap which helps in retaining water. It is observed that sugarcane although contains high amounts of sugars but are drought susceptible whereas pineapple contains less amounts of sugars but are drought resistant. This indicates that the water binding capacity in these cases is not related with the sugars but depends upon specific proteins. It is also suggested that certain resistant proteins appear in the cell during drought stress. These proteins are resistant to denaturation and check the denaturation of other proteins. In general drought resistant plants have smaller cells, less starch and high number of sugars and nucleic acid contents. Some physiologists are of the opinion that drought resistance is associated with the elastic nature of the protoplasm.

Effects of Drought Stress

Function of Stomata

According to Ilfin (1922), a plant exposed to severe drought cannot reestablish its normal functions and remains abnormal even though it regains its turgescence. The stomata open slightly or partly and lose their function or may be killed. It has been observed in Centurea orientalis That 8% retained ability to open, 73% were closed and 19% were killed. In other plants up to 45% stomata remained inactive although the leaves appeared to be perfectly normal. It is a known fact that the loss of water by plants stimulates the transformation of sugars to starch and lowers the osmotic pressure but when the wilting passes certain limits, the starch in guard cells decomposes. Complete decomposition has been observed in plant species adapted to moist habitats. When the water loss ranges from 15 - 30%, the drought resistant species show decomposition from 50 - 60%.

Carbohydrate Metabolism

The loss of water decomposition inhibits carbon assimilation. The accumulation of carbohydrates in plants depends upon several factors. An increased supply of nitrogen stimulates the utilization of carbohydrates and if sufficient moisture is available, growth and formation of new organs are accelerated. If sufficient moisture is not available, growth is interrupted, and accumulation of polysaccharides takes place. The breakdown of carbohydrates in the leaves may be accompanied by their deposition in the roots. It is not possible to state that water loss includes the breakdown and disappearance of starch in all parts of the plant, but it can be said to take place in the leaves of majority of species. When water content was reduced under controlled conditions it increase in sugar content was noticed. It was observed that 20% of the water loss

increased sugar concentration up to 49 to 76% after several hours.

Photosynthetic Activity

Drought affects photosynthesis firstly by decreasing the size of stomatal opening which limits CO2 absorption and secondly by limiting or reducing photosynthetic activity of green tissues. A comparison of photosynthetic rate of fresh plants with wilted ones having closed stomata showed marked differences. The loss of water from 16-47% or more caused a decrease of 20% in photosynthetic rate but no correlation should be made between amount of water lost and the rate of photosynthesis. The wilted plant does not carry photosynthesis normally but their capacity to do so is reduced by 35- 59%. According to Stalfelt (1939) photosynthesis is also reduced in plants without stomata such as in mosses and lichens. Stomatal movement is very important factor in photosynthesis. There is a certain relationship between size of aperture, photosynthesis and transpiration. This relationship changes in different habitats. Reduction in photosynthesis rate in dry soil has been observed in orchard and forest trees.

Respiration

The effect of drought and wilting has been noticed in several plaza la wilting condition due to drought the hydrophytes and mesophytes show increased respiration. The investigations based on seeds are seedling show that a decrease in respiration accompanies a reductive in water content of tissues. In case of leaves and stems the results are different. The loss of large amount of water by a plant induces the breakdown of polysaccharides into simpler compounds other than sugars. Starch may disappear with accumulation of sugars. The probable products are on the way towards becoming the final product of respiration as H_2O and CO2.

Osmotic Pressure

The first observations on the relation between drought and osmotic pressure were those of Pring Shein (1906) in pumpkin seedlings osmatic pressure in plants resistant drought increases during growth of seedlings at low humidities. Fitting (1911) made many measurements of osmatic pressure in plants of Sahara Desert. The species which were not protected against transpiration and high osmotic values utilized soil moisture. The species of temperate zone have confirmed osmatic pressure of 10 atm and those of Arizona desert 20 atm large cells have low coated zone have an average cell have higher osmotic values.

Salt Stress

The water stress caused due to high concentration of salts or solutes in the external medium is concentration cause toxicity. The water stress due to salt concentration causes osmotic imbalance. of stomata due to formation of ABA ion imbalance and toxicity by accumulating ions. It is also affecting nitrogen metabolism carbohydrate metabolism, growth and action of several enzymes.

When concentration of organic solutes becomes very high in the cytoplasm, it performs two important roles, (i) It maintains osmotic balance and (ii) It

protects enzymes of metabolism essential for life. At high salinity the quantity of certain amino acids like proline and tab essential sucrose increases several folds. Proline helps in increasing the solubility of proteins and sucrose helps in protecting isolated chloroplasts against injury.

Based on sensitivity to salt concentration, the plants were classified into two main groups (1) halophytes and (ii) mesophytes. The plants growing in saline habitats are called halophytes. They adapt themselves to a high salt concentration in soil by the development of certain features and properties during onto genic evolution. The plants growing in on-saline habitats are called mesophytes. They possess no or limited ability to adapt themselves to salt stress. According to data available the salt concentration in saline soils varies from 0.3- 20% but most of the halophytes grow in soils containing 2 - 6% salt concentration.

Effects of Salt Stress

Water Potential

The concentration of salts in external medium causes water stress and osmotic balance protoplast. Excess ions penetrate in the cytoplasm and walls causing water deficiency in protoplast which produces adverse effects such as decrease in CO2 fixation. The salts can also damage enzymes that control metabolic processes necessary to continue life. An important adaptation sees water stressed plants is the appearance and accumulation of certain organic solutes like amino proline and glycine and sucrose which lower the osmotic potential. Thus, water potential in cell maintained without limiting enzyme function. Such organic solutes are called compatible solutes. They have been reported in the cells of many xerophytes during water stress. The resulting drop osmotic potential due to newly appeared organic solutes is called osmotic adjustment or osmoregulation. It results due to presence of excess dissolved salt solution. The osmatic potential of soil solution become negative which causes diffusion of water from the tissue in to soil solution. The water stress enduring plant can survive in higher concentration without damaging the metabolic enzymes.

Activity of Stomata

It is observed that abscisic acid in extremely low concentration when applied externally causes the closure of stomata. Further it is reported that during water stress the ABA appears in leaf tissues. In slow drying leaves the formation of ABA before the closure of stomata suggests that during water stress the stomatal closure is mediated by ABA. In such leaves the loss of water from guard cells is not rapid enough cause closure of stomata. To explain this situation, it is proposed that there are two feedback loops which control the opening and closing of stomata. The first feedback loop provides CO_2 for photosynthesis and the second loop protects the tissues against excessive water loss. The first loop causes the opening of stomata and entry of CO2 while the second loop causes closing of

stomata and checking of water loss. The second loop is mediated by ABA produced in adjacent mesophyll cells. First loop works when concentration of CO2 decreases in the intercellular spaces. The decrease in CO2 concentration allows the movement of potassium ions into guard cell to open stomata for the entry of CO2 from the atmosphere. This CO2 is utilized in photosynthesis again lowering CO2 concentration. The second loop works for the water stress condition. During water stress condition the water potential is lowered which leads to increase or synthesis of ABA in the water of adjacent mesophyll cells. The ABA moves into the guard simultaneous potassium ion and water move out from guards' cell to the adjacent mesophyll cell. Thus, stomata become closed to check water loss. The degree of stomatal response to ABA depends upon CO2 concentration in the guard cells and response to CO2 depends upon ABA. If the rate of drying is high, the water is lost from the guard cells directly.

Nitrogen Metabolism

Salinity plays an important role in nitrogen metabolism. It inhibits the enzyme nitrate reductase which is required for reducing nitrate to nitrite during nitrogen simulation. The enzymes of ammonium assimilation pathway are also inhibited by NaCl concentration above 100 mM. Salinity also directs the transfer of carbon fragments from organic acids to amino acids. This act of transfer is achieved by inhibition of malate dehydrogenase activity, and stimulation of transamination reaction. Salt stress causes an increase in the production of amino ads like proline, hydroxyproline and glycine. Free proline which represents about 10-20% of shoot dry weight acts as compatible solute in balancing cytoplasmic and vacuole water potential. In some species where accumulation of proline does not occur, this function is performed by accumulation of other amino compounds. They maintain water potential and control nitrogen metabolism.

Mineralisation

Salinity causes mineralisation due to ion imbalance especially by NaCl. Their excess accumulation may cause toxicity. In halophytes on average about 90% sodium is found in shoots and about 20% in leaves, in contrast, the mesophytes contain sodium ions chiefly in the leaves which are released in the medium. The mesophytes growing in saline soil show lower metabolic rates. They also show accumulation of non-nutrient salts in the protoplasm of leaf cells causing mineralisation which affects hydrogen ion concentration and may cause toxicity. In acidic medium the effect of anions and in alkaline medium those of cations is noticed. The penetration of salts into the plants is regulated by the permeability of the tissues. The root tissues are usually impermeable, and their salt resistance character is maintained up to a certain limit, above which the salts are absorbed causing poisoning and sometimes death of the plant. At high concentration of salts, the

plant cells are damaged due to replacement of selective salt absorption by passive absorption.

Temperature Stress

Majority of plants must face temperature stress. It is of two types - (i) Stress due to high temperature and (ii) Stress due to low temperature.

High Temperature: Majority of the plants can endure high temperature stress due to their internal built up. Most of the xerophyte's thermal algae, lichens and other desert plants survive at a temperature of 70° or more. High temperature causes heavy water losses and denaturation of enzymes and other proteins. The denaturation is compensated by increased enzyme production. It is an adaptation to high temp. In some plants a rise in temperature causes reduction or slowing down of some metabolic processes which can be restored by addition of some compounds like ascorbic acid and vitamins. It is reported that addition of adenine increases temperature tolerance in plants. High temperature resistant plants usually possess heat stable enzymes and proteins. Such enzymes may be isoenzymes which have been developed at high temperatures. The heat stable proteins are not affected by high temperatures to denaturation, and they have ability to place thermal denatured proteins immediately.

High temperature is responsible for heat injury which results into degradation of proteins, lipids, chemicals, respiratory metabolism and changes in kinetics of metabolism. These changes cause stress injuries like break down of cell products and membranes membrane injury, chemical injury, toxin induced injury, starvation injury etc.

Low Temperature: Low temperature stress may be caused due to freezing, frost or chilling. Most of the plants are capable to resist freezing. The plants of tropical climate are chilling prone. They are sensitive to low temperature of 12-13°C and the temperature between 0-5° causes lethal effect. This indicates that the proteins present in such plants are sensitive to low temperature. The chilling also causes destruction of tissues and organs. On the contrary alpine and arctic plants are low temperature resistant and do not show damaging effects but their tissues may not undergo water formation in their cells. Certain plant parts like seeds, pollen and embryos can be stored at -190°C.

Frost Hardening

When a plant becomes resistant to frost stress, it is known as frost hardening and such a plant is called frost-hardy plant. Frost hardening is like that of deep-freezing resistance. Frost stress causes denaturation of proteins, increase in sugar concentration, reduction in growth and formation of hydrophobic substances.

Frost injury affects the PS-II resulting into reduction in photosynthesis, but the carbohydrates and soluble proteins remain unaffected. In frost injured cells membrane bound enzyme systems are damaged and low molecular weight proteins are denatured. The membranes of chloroplasts and mitochondria with

play an important role in cellular energy conversion become inactive due to damage of enzymes. This affects photophosphorylation, oxidative phosphorylation and non-production of ATP.

Causes of frost hardening: Several plants growing in colder regions become frost hardy. This character develops due to several factors which are described below:

Photoperiod and dormancy: In several plants, frost hardening is associated with photoperiod. This conclusion is based up to the fact that most of the plants become maximum frost hardy when they are given the treatment of photoperiod and dormancy.

Inhibitors and starvation: In certain plants frost hardiness seems to be associated with the occurrence of specific metabolic inhibitors and is others specific degree of starvation.

Inadequate light or shade: It is observed that excessive shading or in supply of light causes deformity and starvation in plants. To become frost hardy such plants, must adjust their leaf area blade thickness chlorophyll contents and number and orientation of chloroplasts in the cells.

Resistant proteins: The proteins present in frost-prone plants are low temperature susceptible and get denatured easily to low temperature. In frost hardy plants the formation of low temperature resistant proteins takes place like that of freezing resistant plants. Although frost hardy plants possess high amounts of sugar in cells, but the low temperature further increases this number of sugars. It is reported that plant tissues may be made frost hardy by placing in sugar solution. The few frost-resistant proteins may be synthesized which be resistant to high sugar concentration.

Chilling Injury

Chilling injury is caused with the lowering in temperature due to crystallization of lipids present in cellular membranes of chilling sensitive plants. The crystallization temperature is called critical temperature, and it is equivalent to the temperature causing chilling injury. The crystallization is determined by the ratio of saturated fatty acids to unsaturated fatty acids. An increase in the proportion of unsaturated fatty acids or sterols maintains the normal functioning of membranes at low temperature.

The membrane lipids normally exist in liquid crystalline state at which the enzymes activity is optimum, and the permeability of membrane remains under control below the critical temperature. The change in state of lipids from liquid crystalline to solid increases the permeability of membrane which results into imbalance of solute concentration and ultimately into disturbance of solute balance concentration and ultimately into disturbance of solute balance enzyme activity. This disturbance in enzyme activity causes the accumulation of metabolites of glycolysis etc because they are not oxidized by mitochondrial enzyme system. Thus, only a little ATP is produced. The membrane resumes its normal activity with the increase in

temperature as its lipids are again converted the liquid crystalline state.

Pollution Stress

Since the dawn of civilization man is dependent on nature and natural resources. All living organisms including plants need a balanced environment for their proper growth and development The various components of a balanced environment always occur in a definite proportion. With the population growth, social and technological advancements and huge industrialization in developing countries, the man has increased the exploitation of natural resources at an alarmingly rapid rate with the release of various industrial gases and effluents in the environment. The effluents are chemical materials with variable toxicity and are called pollutants. When these pollutants are released in the environment, they disturb the proportion of various components of a balanced environment and cause stress to the plants. This is called pollution stress which affects the growth and metabolisms of the plants. The effects of some important pollutants on plants are described here.

Effects of UV Rays

DNA is most important and fundamental component of all living beings including plants. It is the basic genetic material the chemicals like plastoquinone and plastoquinol involved in photosynthetic light reaction show their peak absorption spectra between 200 and 320mm. When UV rays absorbed by DNA, it produces mutation, genetic defects and cancer development due to its disorder. Impact of UV on DNA brings about photochemical changes as pyrimidine dimers, 6-4 photoproducts, DNA protein cross links and lesions that can lead to single and double strand breaks. In Nostoc mascorum UV rays caused reduction in phycocyanin pigments, photosynthesis and nitrogen fixation. The complex photochemical reactions take place in presence of UV radiation producing irradiating chemical of photochemical smog. These chemicals highly injurious and causes reduction of photosynthesis and primary production in plants.

Sulphur Dioxide

Most of the SO2 about 75% is released into atmosphere by burning of coal in thermal power ants and smelting of sulphur containing ores and about 25%, SO2 is emitted from petroleum refineries and automobiles. The effects of SO2 on plants are as follows: 1. SO2 damages cereal crops, coniferous plants of forests, apple and mango orchards. 2. SO2 causes chlorosis and necrosis of vegetation in as low concentration as 0.032 ppm, chlorosis from the destruction of chlorophyll which is changed to phaeophytin. 3. Lichen vegetation is destroyed by SO2 So, lichens called pollution indictors. 4. SO2 released in the atmosphere is converted to H2SO4 and causes various injuries in the cells damage of membrane and its permeability plasmolysis, destruction of chlorophyll and metabolic inhibitions.

Carbon Dioxide

The concentration of CO2 in the atmosphere is increasing due to burning

of fossil fuels coal, oil, gas in automobiles, industries, thermal power plants, hot mix plants and domestic cooking etc. Increase in CO2 concentration is not only responsible for greenhouse effect and global warming, but it also affects plants in various ways. 1.Increase in concentration increases the rate of CO2 assimilation in leaves as reported CO2 in case of rice, soybean cotton and banana. It may be due to increased intracellular CO2 concentration at CO2 fixation site. 2. Rate of transpiration usually decreases in C -3 plants as compared to C -4 plants which is due to decrease in stomatal conductance. 3. Increase in CO2 concentrations also decreases activity of RUBISCO and carbonic anhydrase enzymes and reduction in chlorophyll and total soluble protein contents. 4. It increases the translocation of solutes in leaves. 5. Increased CO2 concentration lowers the percentage of total nitrogen content and total soluble protein of plants and seeds but increases the activity of enzyme nitrate reductase which acts as a rate limiting enzyme in nitrate assimilation. The symbiotic nitrogen fixation in field grown crops usually increases in increased CO2 concentration. 6. In general, the growth of most C -3 plants increases up to CO2 concentration of 600 ppm as observed in wheat, rice and Plantago etc. 7. Increase CO2 concentration causes closure of stomata in plants.

Industrial Effluents

These are the main contributors of water pollution. Industrial effluents from breweries, tanneries, sugar mills, butcheries, textile and dyeing mills, paper and pulp mills, steel and electroplating industries, mining etc containing organic wastes, compounds of heavy metals and metalloids as Hg, Pb, Cd, Cu, As, Ni, Zn, Mn, Mo etc, acids, alkalises, cyanides, chromates, thiocyanates, organic solvents etc when disposed of into water cause water pollution and ultimately pollution stress to the plants. Although the role of heavy metals like Cu, Zn, Mn, Mo etc is very much clear in plants as micronutrient but those of Hg, Pb and Cd is indefinite. The heavy metals cause adverse effects. In higher concentration heavy metals are toxic to plants. They increase permeability of cells, reduce rate of respiration and photosynthesis. They show reduction in chlorophyll content, protein content, nitrogen assimilation and growth in general. Thus, several physiological and metabolic disorders can be observed due to accumulation of heavy metals in the medium. It is reported that Pb, Hg and Cd at above 0.1 mM concentration inhibit seed germination and seedling growth in certain crops like wheat, maize, barley etc

Conclusion

The conclusion of plant stress is that it can negatively affect a plant growth development and productivity. Stress can be caused by external factor such as drought excess light biotic factors of herbivorous or pathogens. The reaction of plants to the stress conditions is a complex process which results into

numerous physiological responses including accumulation of proteins, carbohydrates and other stress tolerant organic compounds etc. They maintain osmotic potential without limiting enzyme function.

References

1. Singh AK 2023 Text Book of Plant Physiology and Biochemistry. Paradise Publication, New Delhi, India.
2. Verma SK 2014 Text Book of Plant Physiology, Biochemistry and Biotechnology. Chand Publication, New Delhi, India.
3. Youvan DC and Marrs BL 1987 Molecular mechanism of photosynthesis. Science Am.256, 42-48.
4. Singh AK 2023 Atext Book of Plant Biotechnology Paradise Publication, New Delhi, India.
5. Atkinson NJ and Urwin PE 2012 The interaction of plant biotic and abiotic stresses. J of Exp. Botany,63,3532-3543.

BIOSTIMULANT AND PLANT GROWTH REGULATOR: APPLICATIONS AND SIGNALING MECHANISM

ISBN: 978-81-981142-7-3| Year: 2025 | pp: 115 - 125 |

Physiology Of Mangrove Fern *Acrostichum Aureum* L. From West Coast of Maharashtra

[1]V. B. Chopade

[2]Shakil D. Shaikh

[1]Assistant Professor, Department of Botany, SGM College, Karad

[2]Assistant Professor, Department of Botany, Rajarshi Chhatrapati Shahu College, Kolhapur.

Email: ranichopade26@gmail.com

Article DOI Link: https://zenodo.org/uploads/14789145

DOI: 10.5281/zenodo.14789145

Abstract

A plant's adaptation to its environment is one of the most important issues in evolutionary biology. *Acrostichum aureum* is a common mangrove fern that grows rapidly after mangrove forests have been clear felled for timber. *Acrostichum* is the only pteridophyte genus found in the mangrove ecosystem. It grows luxuriantly in brackish water habitats also. *Acrostichum aureum* L. is the only species from the Indian coast. This rhizomatous fern shows clumped distribution and produces golden yellow leathery fronds and hence aptly called golden leather fern. In the present investigation *Acrostichum aureum* L. was collected from various localities of West Coast of Maharashtra and analysed for some physiological parameters such as polyphenols, proteins, carbohydrates, macronutrients and micronutrients.

Keywords: *Acrostichum*, Mangrove fern, macronutrients and micronutrients.

Introduction

Ferns are generally used in traditional medicine for the cure of many lethal diseases like skin problems, wounds, cough and reproductive problems as well as to make insect repellent [1, 2]. A large number of medicinal ferns like *Adiantum capillus-veneris, Cheilanthes albomarginata, Asplenium nidus, Ceratopteris thalictroides including, Acrostichum aureum* exist in Asia [3-5]. Acrostichum aureum Linn (Family-Pteridaceae), common name: Hudo (Bangladesh), Tiger Fern, Piai raya (Singapore), Golden Leather Fern (South Florida) (1), Swamp Fern, Mangrove

Fern, occurs Worldwide in mangrove swamps, salt marshes, canal margins, and low hammocks. It is widely distributed throughout S. Florida, Brazil, S. & W. Mexico, Guyanas, Central America, Colombia, Venezuela, Ecuador, Paraguay, Barbados, Trinidad, S. China, Taiwan, Japan, N. Australia, India, Sri Lanka and Bangladesh. It is an evergreen shrub, found in a hostile environment [6]. Many studies have reported that traditional use of *A. aureum's* rhizome for curing wounds, non-healing ulcers, boils, syphilitic ulcers, sore throat, chest pains, elephantiasis, purgative, febrifuge, cloudy urine in women, and rheumatism in Malaysia [7], Bangladesh [8], and Yap islands and Micronesia [9]. The paste of rhizome is applied to heal the wounds and boils. In China it is also applied as anthelmintic, vulnerary, healing inveterate ulcers, and bladder complains. Mature fronds are used for syphilitic ulcers in Borneo. Fronds are used as an antifungal agent [10]. In Bangladesh, preparations from rhizomes and leaves of *A. aureum* are used to treat wounds, peptic ulcers and boils [11]. The native people of Costa Rica use leaves as emollients, whereas, the Cuna people (Panama and Colombia) use the young fiddleheads to extract fish bones from the throat and as a medicinal bath for infants [12]. The crude extract of a Japanese *A. aureum* specimen is studied to possess anti-oxidant, tyrosinase inhibiting activity [13], while a Hainan specimen reported anti-tumour activity against cervical cancer cell line [14]. Scientists [15] reported the cytotoxic effect of water and methanol extracts from a Bangladeshi specimen of *A. aureum* leaves on gastric, colon and breast cancer cells.

Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants and then, may lead to drug discovery and development. Study of chemical constituents of plants is a prerequisite for their use in medicine and also for the synthesis of complex chemical substances. Correlation between the phytoconstituents and the bioactivity of the plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic diseases as well. Such preliminary phytochemical screening of plants is the need of the hour to discover and develop novel therapeutic agents with enhanced efficacy. Numerous research groups have also reported such studies throughout the world. Thus, the present study deals with the physicochemical and phytochemical screening of *Acrostichum aureum* L., leaves.

***Acrostichum aureum* L.**

It is commonly known as mangrove fern or Swamp fern.

A. Habit

B. Young plant

C. Rhizome and Roots

Chemical Constituents

Chemical content of Mangrove Fern such as phenol which has bioactivity in various diseases of atherosclerosis, diabetes, cancer, and brain dysfunction. Also, the phenol plant origin can contribute to providing color and is used as a sensory in fruit and vegetables [16]. Flavonoids act as prevention of fat accumulation in the body to prevent obesity that causes diseases such as heart disease and diabetes mellitus, antioxidants can counteract free radicals in the body. Several studies have reported that Mangrove fern have bioactivity such as cytotoxicity [17], antioxidant [18], anti-inflammatory [19], anti-tumor [20], analgesic [21], antiviral [22], antibacterial [23] and antiparasitic [24]. The active compounds contained in Mangrove Fern according to previous researchers are flavonoids [25] phthalic acid [26], several types of sterols [27], terpenoids and several other chemical substances such as patriscabratine and tetracosan [28]. Flavonoids such as kaempferol (29), quercetin (30). Phthalic acid ester compounds such as (2-methoxycarbonyl- 5- methylpentyl- 2 methylhexyl phthalate). Sterols are in the form of phytosterols, such as stigmasterol (29), beta- sitosterol (30), campesterol (31), cycloartanol (32) and 24- methylene cycloartanol (33). Terpenoids are sesquiterpenes (2R, 3S) - sulfatet pterosin C (29) and (2R, 3S) - sulfated pterosin C (30).

Physiology and Bioactivity of *Acrostichum aureum*

Physicochemical Studies like moisture, Ash Values were studied with standard methods. The phytochemical analysis gives a general idea regarding the presence of different compounds possessing therapeutic values. The different solvent extracts of *A. aureum* leaf were used for screening the presence of alkaloids, steroids, tannins, saponins, flavonoids, quinone, anthroquinone, phenol, protein, xanthoprotein, carbohydrate, glycosides, sugar and terpenoids according to standard procedures of Harborne [34], Brindha et al [35], Trease and Evans [36] and Sofowara [37,38].

The cytotoxic test reported that *A. aureum* leaves have cytotoxic activity using the MTT assay method. The methanol extract of Mangrove fern with

IC50 values > 2.5 mg - 1 ml was able to inhibit the growth of AGS cells, NIH3T, HT29, MDA-MB4- 35S. Mangrove fern methanol extracted in vitro by MTT assay method and Annexin V-FITC apoptosis. Tetracosane cytotoxic active compound with (IC50:128.7 µm) as a cytotoxic drug for colon cancer cells (HT-29). Patriscabratine with (IC50: 133.66 µm) inhibits growth in gastric cells (AGS), breast cancer cells MDA-MB-123 with (IC50: 69.8 µm), MCF-7 with (IC50: 197.3 µm), but it was showed no cytotoxic effects on (NIH3T3) and (HT-29) [39].

There were two new compounds sesquiterpenes (2R, 3S) -sulfate pterosin C (1) and (2R, 3S) -sulfate pterosin C (2) can inhibit human cancer cell line (AGS, HT-29, MDA-MB-231, and MCF-7) and NIH3T3 cell lines using the MTT assay. The two compounds indicated IC50 values in the range 23.9- 68.8 µm. The lowest IC50 was 23.9 µm against gastric cells of AGS adenocarcinoma, which had an apoptosis effect on AGS cells. This cytotoxic activity is due to the presence of a sulfate group at C-14 and a configuration at C-2 [40].

Antioxidant Activity of *A. aureum*

This activity of swamp fern ethanol extract was reported by using the DPPH (1,1- Diphenyl-2- picrylhydrazyl) method IC50 41.96 µg / ml with ascorbic acid as a comparison of IC50 16.36 µg/ml [41]. Nurhasnawati et al., 2019 reported that antioxidant activity with the DPPH method (highest IC50 29.5303 ppm), total phenolic levels (366.4573 ± 2.2117 mg GAE g-1) using the Folin-ciocalteu method, flavonoid levels with the colorimetric method (228,6087 ± 2.2548 mg QE g-1) [42]. In addition, this study was also reported that petroleum ether extract, benzene extract, ethyl acetate extract, ethanol extract, and methanol extract of Mangrove fern acted as antioxidants using the DPPH (1,1-Diphenyl-2- picrylhydrazyl) method. Among the solvent extracts tested, benzene extract 800 µg DPPH (118.56%), IC50 in methanol extract (36.54µg / ml). The compounds that play a role in antioxidant activity are flavonoids and phenolic [43]. Another study reported that sea nail antioxidants also played a role in reducing levels of pond waste pollutants in shrimp by increasing catalase activity, superoxide dismutase, peroxide and glutathione transferase activity with waste parameters (BOD) (73%), (COD) (39%)) and NO3 (55%) [44].

Anti-inflammatory Activity

The anti-inflammatory activity of Mangrove fern was tested in vivo using the male albino rat edema test as an observation. Ethanol extract of the root of nail reportedly has potential anti-inflammatory at a dose of 200-400 mg/kg body weight with a maximum inhibition% at 65.90% compared with 66.66% indomethacin drug delivery within 24 hours [45].

Another study reported that seawater extract had ethanol-induced gastroprotective effects on the stomach. Pretreatments of 100, 200, and 400 mg/kg were able to reduce the repair of

pathological damage caused by alcohol in mice [46].

Antitumor Activity of *A. aureum*

Mangrove fern was reported to have antitumor activity. Mangrove fern sterol fractionation using gas chromatography (GC) and mass. At (24-methylcolest-5-en-3β-ol) campesterol with a retention time of 26,167 minutes. The peak spectrum fragments are seen at .315 and 289 m / z which are characteristic of 3β-hydroxy α-5. Furthermore, GC-mass spectrum on stigmasterol with a retention time of 26 948 minutes, in fragments that many visible spectrum peaks at 327 and 301 m / z on 3β-hydroxy-5-α-sterol. GC-mass spectrum on ϫ- sitosterol with a retention time of 28,498 minutes, in many ion spectrum fragments seen in 3-β-hydroxy-ϫ-5-sterol. The GC-mass spectrum is comparable to cycloartenol with a retention time of 31,259 minutes, in the peak spectrum fragments at 408 and 393 m / z that lose H2O molecules. The GC-mass spectrum of 24- methylene-cycloartenol with a retention time of 33,570 minutes, in the peak spectrum fragments at 422 and 407 m / z with loss of H2O molecules similar to the structure of cycloartenol. Active compounds from Mangrove fern leaves that have the potential to be anti- tumor are phytosterols (stigmasterol, ϫ-sitosterol, campesterol, cycloartenol, and 24-methylene cycloartenol). Line Cell Predictor (CLC-Pred) antitumor phytosterol against adenocarcinoma followed by carcinoma and mesothelioma [47].

Analgesic Activity of *A. aureum*

Mangrove fern has been reported to have acted as an analgesic by the wriggling test method in mice induced by acetic acid. Analgesics work peripherally through activation of peritoneal receptors that will induce stretching in test animals. The stretching method in mice induced by the subsequent release of endogenous substances. Some endogenous substances such as serotonin, bradykinin, histamine, prostaglandin (PG) are released by acetic acid and are responsible for producing pain by speeding up nerve end receptors. These mice stretching method can be seen how big obstacle after calculated percent for each test group and the comparison control group. The percentage of inhibition of ethanol extract of Mangrove fern at a dose of 250 mg was 28.68% and a dose of 500 mg was 46.77% compared to the control group of diclofenac sodium with a dose of 25 mg of 69.16% [48].

Antiviral activity of *A. aureum*

This study reported that in vitro a new compound phthalic acid ester, (2-methoxycarbonyl-5- methylpentyl-2 methylhexyl phthalate) sea nail was used as an antiviral. Phthalic acid has indicated antiviral activity against (DENV-2), (hPiV3), and (CHIKV). The strongest activity was against the human parainfluenza virus with (EC50 29.3 μm) slightly higher than BCX 2798 positive control with (EC50 44 μm). Cellulose acetate was also evaluated for antiviral activity. It was found to be first and inactive. Both of these compounds are

non-toxic to Vero and LLC-MK2 cells [49].

Antibacterial activity of *A. aureum*

The activity of methanol extract of sea nail as antibacterial has been reported. Disc diffusion method with an inhibitory diameter of 1.3 mm was able to inhibit the growth of the bacterium Vibrio parahaemolyticus (Pc 29) (isolated from mud crabs) (Scylla serrate) [50]. However, according to the research of Lai et al., 2009, the antibacterial activity of the methanol extract of sea nail leaves was not an inhibition zone in the disc paper area [51].

Antiparasitic activity of *A. aureum*

Mangrove fern was reported to have an antiparasitic activity of 56% in sheep infected with Haemonchus contortus worms by counting eggs per gram by reducing the number of fecal eggs on day 0 (pre-treatment) and days 5, 7, 9 (post-treatment) [52].

Conclusion

The plant *A. aureum* has been used in the treatment of different ailments, the medicinal roles of this plant could be related to identifying bioactive compounds. The presence of phytoconstituents, such as phenols and flavonoids in plants, indicates the possibility of antioxidant activity and this activity will help in preventing several diseases through free radical scavenging activity. The present analyses suggest that *A. aureum* (fern) contains potentially health-protective phytochemical compounds with a potent source of natural antioxidants and antibacterial activities that may be clinically promising. Thus, it's also adding new compounds to the ever-increasing canvas of secondary metabolites acting as fountains of health.The fluorescent analysis of powdered drug plays an important role in the determination of the quality and purity of the drug.However, it is necessary to do further research on swamp ferns to determine the active compounds and another bioactivity.

References

1. Nath, K., Bhattacharya, M.K., and Kar, S., 2016, Antibacterial activity of rhizome extracts of four pteridophytes from Southern Assam, North East India. AJPCR. 4 (1), 1–5.
2. Bahadori, M.B., Mahmoodi., Kordi, F., Ali Ahmadi, A., Bahadori, S., and Valizadeh, H., 2015, Antibacterial evaluation and preliminary phytochemical screening of selected ferns from Iran. Rapid Prototyp. J. 2: 53–59.
3. Chang, Y., Hu, J.L., Jiang, S.J., and Qiang, S., 2005, Study on the distribution and the total flavonoids content of medicinal pteridophytes in Nanjing Zijin Mountain. J. Northeast Agric. 36, 320–323.
4. Arockia Badhsheeba, R., and Vadivel, V., 2018, Evaluation of in vitro antioxidant activity of Acrostichum aureum Linn. Rachis. J. Pharmacogn. Phytochem. 7: 1146-1151.
5. Smitha, V., and Vadivel, V., 2019, Phytochemical screening for active compounds in Ceratopteris

thalictroides (L.) Brongn. J. Pharmacogn. Phytochem. 8: 3556-3559.

6. Chikezie, P.C., Ibegbulem, C.O., and Mbagwu, F.N., 2015, Bioactive Principles from Medicinal Plants. Res. J. Phytochem. 9: 88 -115.

7. Hossan, M.S., Hanif, A., Agarwala, B., Sarwar, M.S., Karim, M., Rahman, M.T., Jahan, R., and Rahmatullah, M., 2010, Traditional use of medicinal plants in Bangladesh to treat urinary tract infections and sexually transmitted diseases. Ethnobot. Res. Pub. 8: 61-74.

8. Pattanaik, C., Reddy, C.S., Dhal, N.K., and Das, R., 2008, Utilisation of mangrove forests in Bhitarkanika wildlife sanctuary. Indian J. Trop Know. 7: 598-603.

9. Defilipps, R.A., Maina, S.L., and Pray, L.A., 1988, The Palauan and Yap medicinal plant studies of Masayoshi Okabe 1941-1943. Atoll Res. Bulletin. 317: 1-25.

10. Benjamin, A., and Manickam, V.S., 2007, Medicinal pteridophytes from the Western Ghats. Indian J. Tradit. Know. 6: 611-618.

11. Momtaz, M.M., 2008, Encyclopedia of Flora and Fauna of Bangladesh. Dhaka, Bangladesh: Asiatic Society of Bangladesh.

12. Natural Resources Conservation Service., 2010. Plants Database. Natural Resources Conservation Sevice, United States Department of Agriculture (USDA), USA [Online]. Available at: http://plants.usda.gov.

13. Lai, H.Y., Lim, Y.Y., and Tan, S.P., 2009, Antioxidative, tyrosinase inhibiting and antibacterial activities of leaf extracts from medicinal ferns. Biosci. Biotechnol. Biochem. 73: 1362–1366.

14. Dai, H., Mei, W., Hong, K., Zeng, Y., and Zhuang, L, 2005, Screening of the tumour cytotoxic activity of sixteen species of mangrove plants in Hainan. Zhongguo Haiyang Yaowu. 24: 44–46.

15. Uddin, S.J., Grice, I.D., and Tiralongo, E., 2011, Cytotoxic effects of Bangladeshi medicinal plant extracts. Evid. Based Complement Alternat. Med. 2011: 1–7.

16. Garg, N., Mohammad, S., & Aeron, A. A. (n.d.). Microbes in Food and Health. 2016.

17. Tiralongo, E., Uddin, S. J., & Grice, I. D. (2011). Cytotoxic effects of Bangladeshi medicinal plant extracts. Evidence-Based Complementary and Alternative Medicine, 2011 (October). https://doi.org/10.1093/ecam/nep111

18. Wu, X., Huang, Q., Xu, N., Cai, J., Luo, D., Zhang, Q., Su, Z., Gao, C., & Liu, Y. (2018). Antioxidative and Anti-Inflammatory Effects of Water Extract of Acrostichum aureum Linn. against Ethanol-Induced Gastric Ulcer in Rats. Evidence-Based Complementary and Alternative Medicine, 2018. https://doi.org/10.1155/2018/3585394

19. Hossain, H., Jahan, I. A., Nimmi, I., Hossain, A., & Kawsar, H. (2011).

Anti-Inflammatory Activity of the Ethanolic Extract of Acrostichum aureum (Linn.) Root. 14(2), 107–109.

20. Thomas, A., Prashob Peter, K. J., & Chandramohanakumar, N. (2016). A profiling of anti-tumour potential of sterols in the mangrove fern Acrostichum aureum. International Journal of Pharmacognosy and Phytochemical Research, 8(11), 1828–1832.

21. Khan, S. A., Hossain, M. A., Panthi, S., Asadujjaman, M., & Hossin, A. (2013). Assessment of antioxidant and analgesic activity of Acrostichum aureum Linn. (Family-Pteridaceae). Pharmacology Online, 1, 166-17.

22. Uddin, Shaikh J, Guillon, P., Grice, I. D., & Tiralongo, E. (2013). In-vitro Antiviral Activity of a Novel Phthalic Acid Ester Derivative Isolated from the Bangladeshi Mangrove Fern Acrostichumaureum Antivirals & Antiretrovirals In-vitro Antiviral Activity of a Novel Phthalic Acid Ester Derivative Isolated from the Bangla. January 2014. https://doi.org/10.4172/jaa.1000078

23. Shamsuddin, A. A., Najiah, M., Suvik, A., Azariyah, M. N., Kamaruzzaman, B. Y., Effendy, A.W., & Akbar John, B. (2013). Antibacterial properties of selected mangrove plants against vibrio species and its cytotoxicity against Artemia salina. World Applied Sciences Journal, 25(2), 333–340. https://doi.org/10.5829/idosi.wasj.2013.25.02.688

24. Devi, R. K., Rajesh, N. V, Vasantha, S., & Geetha, V. S. (2015). Anti-Parasitic action of Actinopteris radiata, Acrostichum aureum and Hemionitis arifolia. 4(4), 1–9.

25. Uddin, Shaikh Jamal, Grice, D., & Tiralongo, E. (2012). Evaluation of cytotoxic activity of patriscabratine, tetracosane and various flavonoids isolated from the Bangladeshi medicinal plant Acrostichum aureum. Pharmaceutical Biology, 50(10), 1276–1280. https://doi.org/10.3109/13880209.2012.673628

26. Uddin, Shaikh J., Jason, T. L. H., Beattie, K. D., Grice, I. D., & Tiralongo, E. (2011). (2 S,3 S)-sulfated pterosin C, a cytotoxic sesquiterpene from the Bangladeshi mangrove fern Acrostichum aureum. Journal of Natural Products, 74 (9), 2010–2013. https://doi.org/10.1021/np2004598.

27. https://pubchem.ncbi.nlm.nih.gov/compound/9420 4. (n.d.).

28. https://pubchem.ncbi.nlm.nih.gov/compound/5280 343. (n.d.).

29. Raja, S., & Ravindranadh, K. (2014). A complete profile on Acrostichum aureum traditional uses, pharmacological activities and phytoconstituents. World Journal of Pharmaceutical Research, 3(10), 624-630.

30. Othman, R., Ramya, R., Mohd Hassan, N., Masyitah Wan Daud, W., & Nadia Johari, N. (2019). Characterisation of allelochemical

compounds signature in two mangrove forest species of Rhizophora apiculata and Acrostichum aureum and potential in suppressing weed growth. IOP Conference Series: Earth and Environmental Science, 380 (1). https://doi.org/10.1088/1755-1315/380/1/012016

31. Hossan, M. S., Hanif, A., Agarwala, B., Sarwar, M. S., Karim, M., Taufiq-Ur-Rahman, M., Jahan, R., & Rahmatullah, M. (2010). Traditional use of medicinal plants in Bangladesh to treat urinary tract infections and sexually transmitted diseases. Ethnobotany Research and Applications, 8(April), 61–74. https://doi.org/10.17348/era.8.0.61-74

32. Hanin, N. N. F., & Pratiwi, R. (2017). Kandungan Fenolik, Flavonoid dan Aktivitas Antioksidan Ekstrak Daun Paku Laut (Acrostichum aureum L.) Fertil dan Steril di Kawasan Mangrove Kulon Progo, Yogyakarta. Journal of Tropical Biodiversity and Biotechnology, 2(2), 51. https://doi.org/10.22146/jtbb.29819

33. Arockia Badhsheeba, M., & Vadivel, V. (2020). Physicochemical and Phytochemical Contents of leaves of Acrostichum aureum L. Journal of Global Biosciences, 9(4), 7003–7018. https://doi.org/www.mutagens.co.in/jgb/vol.09/04/ 090407.pdf.

34. Harborne, J.B., 1973, Phytochemical Methods. Chapman and Hall Ltd., London: U.K., 49-188.

35. Anonymous., 1996, Indian Pharmacopoeia. Vol. I and II. Government of India, Ministry of Health and Family Welfare. The Controller of Publications. New Delhi.

36. Brindha, P., Sasikala, B., and Purushothaman, K.K., 1981, Pharmacognostic studies on Merugan Kizhangu. Bull Med Eth Bot Res. 3: 84-96.

37. Trease, G.E., and Evans, W.C., 1989, Pharmacognosy, 11th edn., Bailliere Tindall, Can. Macmillian Publishers, London. 45-50.

38. Sofowora, A., 1993. Medicinal plants and traditional medicine in Africa (2nd Edition). Spectrum Books Limited, Sunshine House, Ibadan, Nigeria. 289-300.

39. Uddin, Shaikh Jamal, Grice, D., & Tiralongo, E. (2012). Evaluation of cytotoxic activity of patriscabratine, tetracosane and various flavonoids isolated from the Bangladeshi medicinal plant Acrostichum aureum. Pharmaceutical Biology, 50(10), 1276–1280. https://doi.org/10.3109/13880209.2012.673628.

40. Uddin, Shaikh J., Jason, T. L. H., Beattie, K. D., Grice, I. D., & Tiralongo, E. (2011). (2 S,3 S)-sulfated pterosin C, a cytotoxic sesquiterpene from the Bangladeshi mangrove fern Acrostichum aureum. Journal of Natural Products, 74(9), 2010–2013. https://doi.org/10.1021/np2004598.

41. Khan, S. A., Hossain, M. A., Panthi, S., Asadujjaman, M., & Hossin, A.

(2013). Assessment of antioxidant and analgesic activity of Acrostichum aureum Linn. (Family-Pteridaceae). Pharmacology Online, 1, 166-17.

42. Nurhasnawati, H., Sundu, R., Sapri, Supriningrum, R., Kuspradini, H., & Arung, E. T. (2019). Antioxidant activity, total phenolic and flavonoid content of several indigenous species of ferns in East Kalimantan, Indonesia. Biodiversitas, 20(2), 576–580. https://doi.org/10.13057/BIODIV/D200238.

43. Arockia Badhsheeba, R., & Vadivel, V. (2018). Evaluation of in vitro antioxidant activity of Acrostichum aureum Linn. Rachis. ~ 1146 ~ Journal of Pharmacognosy and Phytochemistry, 7(6).

44. Sukumaran, D., Joseph, J., Madhavan, K., & Harikumar, P. S. (2019). The role of antioxidant metabolism in phytoremediation of shrimp farm effluent by Acrostichum aureum Linn. American Journal of Environmental Protection, 7(1), 7-12.

45. Hossain, H., Jahan, I. A., Nimmi, I., Hossain, A., & Kawsar, H. (2011). Anti-Inflammatory Activity of the Ethanolic Extract of Acrostichum aureum (Linn.) root. 14(2), 107–109.

46. Wu, X., Huang, Q., Xu, N., Cai, J., Luo, D., Zhang, Q., Su, Z., Gao, C., & Liu, Y. (2018). Antioxidative and Anti-Inflammatory Effects of Water Extract of Acrostichum aureum Linn. against Ethanol-Induced Gastric Ulcer in Rats. Evidence-Based Complementary and Alternative Medicine, 2018. https://doi.org/10.1155/2018/3585394.

47. Thomas, A., Prashob Peter, K. J., & Chandramohanakumar, N. (2016). A profiling of anti-tumour potential of sterols in the mangrove fern Acrostichum aureum. International Journal of Pharmacognosy and Phytochemical Research, 8(11), 1828–1832.

48. Khan, S. A., Hossain, M. A., Panthi, S., Asadujjaman, M., & Hossin, A. (2013). Assessment of antioxidant and analgesic activity of Acrostichum aureum Linn. (Family-Pteridaceae). Pharmacology Online, 1, 166-17.

49. Uddin, Shaikh J, Guillon, P., Grice, I. D., & Tiralongo, E. (2013). In-vitro Antiviral Activity of a Novel Phthalic Acid Ester Derivative Isolated from the Bangladeshi Mangrove Fern Acrostichum aureum Antivirals & Antiretrovirals In-vitro Antiviral Activity of a Novel Phthalic Acid Ester Derivative Isolated from the Bangla. January 2014. https://doi.org/10.4172/jaa.1000078

50. Shamsuddin, A. A., Najiah, M., Suvik, A., Azariyah, M. N., Kamaruzzaman, B. Y., Effendy, A. W., & Akbar John, B. (2013). Antibacterial properties of selected mangrove plants against vibrio species and its cytotoxicity against Artemia salina. World Applied Sciences Journal, 25(2), 333–340.

https://doi.org/10.5829/idosi.wasj.2013.25.02.688.

51. Lai, H. Y., Lim, Y. Y., & Tan, S. P. (2009). Antioxidative, tyrosinase inhibiting and antibacterial activities of leaf extracts from medicinal ferns. Bioscience, Biotechnology and Biochemistry, 73(6), 1362–1366. https://doi.org/10.1271/bbb.90018.

52. Devi, R. K., Rajesh, N. V, Vasantha, S., & Geetha, V. S. (2015). Anti-Parasitic action of Actinopteris radiata, Acrostichum aureum and Hemionitis arifolia. 4(4), 1–9.

BIOSTIMULANT AND PLANT GROWTH REGULATOR: APPLICATIONS AND SIGNALING MECHANISM

ISBN: 978-81-981142-7-3| Year: 2025 | pp: 126 - 136 |

Use of Plant Growth Regulators for In-vitro Culture of Plants

Dr. Kalpit Ganesh Mhatre

Assistant Professor, New Education Society's Arts, Commerce and Science College, Lanja, Ratnagiri

Email: kalpitgmhatre@gmail.com

Article DOI Link: https://zenodo.org/uploads/14793368

DOI: 10.5281/zenodo.14793368

Abstract

The present chapter deals with various plant growth regulators which are used in in vitro culture of plants. The chapter focuses on various aspects of all important plant growth regulators. Plant growth regulators are the most important chemicals required for overall growth and development of plants. This chapter contains information on all aspects of different plant growth regulators. Information on growth promotes and growth inhibitors is given in details.

Keywords: Plant tissue culture, PGR, sterile culture, IAA, Growth Promoters.

Introduction

Plants contribute significantly to our planet's biodiversity. For a variety of reasons, all living things rely on plants, either directly or indirectly. We use plants for a variety of daily requirements, including food, clothing, shelter, aesthetics, and commerce. Nowadays, a variety of plant kinds have been developed for the horticultural, agricultural, and nursery industries when it comes to the commercial usage of plants. The 21st century has seen the laboratory production of numerous plant, fruit, flower, and vegetable types via a process called plant tissue culture. Large-scale plant production makes heavy use of this in vitro plant production technology. The process of "in vitro" cultivating plant or animal cells, tissue, or organs on nutritional medium under aseptic conditions—typically in a glass container—is known as tissue culture. Other names for tissue culture include "sterile culture" and "in vitro" culture. This method allows for the long-term maintenance of living cells outside the organism's body. To put it another way, tissue culture is the aseptic culture of tissues, cells, organs, and other components in vitro in a laboratory

setting under specific physical, chemical, and environmental conditions.

Henri Louis Duhumel du Monceau (1756) took the first step toward plant tissue culture when he noticed callus production while doing his groundbreaking research on plant wound healing. It has gained significant importance in recent years in the fields of secondary metabolite production, disease prevention, plant enhancement, and plant propagation. Through micropropagation, uncommon, endangered, and threatened species have been successfully cultivated and preserved. Plant tissue culture has seen a number of innovative and fascinating advancements, and it currently makes up a significant portion of what is commonly referred to as plant biotechnology. The focus of tissue culture research over the past forty years has been on its industrial and agricultural uses. Clonal multiplication of horticulture, the synthesis of industrial chemicals from forests, and the regeneration of transgenic plants from genetically modified cells are some of the demonstrated uses of plant tissue culture. These substances have significant medicinal value. The text is therefore highly appropriate and deemed essential. The application of different plant growth regulators is the most crucial aspect of plant tissue culture. In plant tissue culture, plant growth regulators are crucial. Tropism, elongation, and apical dominance are among the growth phases for which these regulators are essential.

Plant Growth Regulators

Chemicals known as "plant growth regulators" (PGRs) can change a plant's development by enhancing branching, inhibiting shoot growth, boosting return bloom, eliminating surplus fruit, or changing fruit maturity. PGR performance is influenced by a wide range of factors, such as the degree to which the chemical is absorbed by the plant, the age and vigor of the tree, the dosage, the time of treatment, the cultivar, and the weather before, during, and after application. Auxin-related substances, gibberellins and gibberellin biosynthesis inhibitors, cytokinins, abscisic acid, and compounds influencing the ethylene status are the five families of plant growth regulators. Plant growth regulators are molecules that affect a plant's growth and development in different ways. They can have a big impact on development even if they are present in little amounts.

Everyone is aware that for plants to grow and flourish, they need clean air, water, and nutrients. Because they are external to the plant, these elements—air, nourishment, and water—are referred to as extrinsic factors. Intrinsic factors include plant hormones and growth regulators. Since they control plant growth, intracellular genes and chemicals are intrinsic factors that affect a plant's ability to survive. Plants can be extrinsically supplemented using synthetic growth regulators. Simple compounds called plant growth regulators are utilized to control a plant's growth. The plant naturally creates these little molecules to aid in its own development. The most widely used plant growth regulators include ethylene, gibberllins, cytokinins, auxins, and abscisic acid.

In the process of plant tissue culture or micropropagation, plant growth regulators are essential and crucial. The

use of growth regulators in the culture media is essential to the micropropagation technique's success. Developmental and growth processes are regulated by growth regulators. These are the essential elements for starting the tissue culture regeneration process. Explants in the majority of in vitro investigations react poorly to culture conditions devoid of growth regulators. Depending on their nutritional needs, various plants may have varied optimal tissue morphogenesis and growth. Furthermore, tissues from various plant parts may also require different conditions for adequate growth; for this reason, understanding in vitro culture plant growth regulators is crucial.

Growth is a dynamic, important process that permanently alters the size, shape, light, and volume of any plant or its components. Development First of all, von Sachs' "Organ farming substance" introduced the concept of plant hormones. Harmones are organic substances that are typically produced in the meristematic tissues of plants and are transported to the site of action, causing a physiological process or response. They can also function in incredibly small amounts. The word "Phytoharmone" was proposed by Thimann (1948) to describe plant hormones.

Plant growth regulators (PGR) are such organic compounds occurring naturally in plants as well as synthetic other than nutrients which in small amounts promote, inhibit or modify any physiological process are called PGR. PGRs are of two types as follows:

A) **Growth Promoters** (Examples include Auxins, Gibberllins and Cytokinins)

B) **Growth Inhibitors** (Examples include Abscisic Acid and Ethylene)

Growth Promoters:

There are three types of growth promoters as follows:

Auxins

Gibberllins

Cytokinins

Auxins

Francis Darwin and his father, Charles Darwin, made the discovery of auxins. These are growth-promoting chemicals that help shoots elongate, but when present in excessive concentrations, they can prevent the development of lateral buds. The two noticed that canary grass grows toward the light, and auxins were the first growth hormone to be identified. Upon closer inspection, it was evident that the coleoptile tip—the tip of the protective sheath—was in fact bending in the direction of the light. As a result, F.W. West used the sheaf tips of oat seedlings to isolate the auxins, cementing the discovery.

Indole-3-acetic acid (IAA) and indole butyric acid (IBA) are two naturally occurring forms of auxin that are produced by plants. Usually, the roots and stems of plants create these auxins. The growth ingredient was isolated by F.W. Went in 1928 and given the name Auxin. The test is called the Avena Curvature test or the Avena Coleoptile test because the plant Avena sativa, or oats, was employed in the bioassay. Curvature in Avena Coleoptile was shown to be caused by auxin. Thimann (1934) discovered that auxin concentrations gradually decreased from the tip to the base of the coleoptiles, with the maximum concentration occurring at

the tip. He also noticed that the concentration of auxin was much less in the root tip than that of the coleoptiles tip. Auxin was a general term used to denote for such substance which promote the elongation of the coleoptile's tissues. Indole acetic acid (IAA) is an endogenous auxin occurring naturally in plants.

Boysen-Jensen (1910-1913) showed that the sensation of phototropism picked up by coleoptile tip could be transmitted to sub-apical region through a block of gelatine but not through a mica plate. Paal (1919) replaced the previously exposed excised tip eccentrically over the stump of coleoptile. He observed greater growth on that side even in dark. Went (1928) collected the growth stimulating substance in agar jelly. He discovered that the hormone travelled basipetally, i.e., from tip or apex towards the base. Agar block containing the chemical caused bending of a decapitated coleoptile according to its concentration. The growth promoting substance was named by him as auxin Kogl and Haagen-Smith (1931) isolated three chemicals from human urine. They were named as auxin a, auxin b, and hetero-auxin. Kogl (1934) found that hetero-auxin is the real plant auxin and is chemically indole 3-acetic acid or lAA. It is also present in urine of human beings suffering from pellagra, a disease caused by deficiency of niacin (= nicotinic acid). Indole 3-Acetic Acid (LAA, Fig. 15.18) is the universal natural auxin. It was discovered by Kogl (1934). Related chemicals are indole 3-acetaldehyde, indole 3-acetonitrile, indole 3-butyric acid (IBA), phenyl acetic acid and 4-chloro indole acetic acid. All of them have auxin like activity. Auxin is synthesised in shoot apices, leaf primordia and developing seeds from amino acid tryptophan. A tryptophan independent pathway has also been discovered recently. Auxin passes from shoot tip to the region of elongation. Auxin movement is polar. It is basipetal in stem but acropetal in the root. Auxin helps in the elongation of both roots and shoots. However, the optimum for the two is quite different. It is 10 ppm for stem and 0.0001 ppm for the root. In higher concentration auxin inhibits growth. The raw material which is used in synthesis of auxin is called auxin precursor. It is tryptophan for IAA. Certain compounds inhibit action of auxin. They are called anti-auxins, e.g., p-chlorophenoxy isobutyric acid (PCIB). TIBA (2, 3, 5 triiodobenzoic acid also acts as anti-auxin by blocking the transport of auxin. Active form of auxin is free auxin or auxin which can be extracted easily. Auxin which cannot be extracted easily except with the help of organic solvents is called bound auxin, e.g., IAA-aspartic acid, IBA-alanine, IAA-myoinositol, IAA-glucan, IAA-glycoprotein. Bound auxin is believed to be hormonally inactive (Hangarter and Good, 1981), being meant for storage and protec¬tion against degradation.

Bioassay of Auxins

1. Avena Curvature Test

It involves using a living object, such as a plant or a plant part, to test a biological activity, such as a substance's growth response. Since it gauges the quantity of auxins needed to create an effect and the magnitude of that effect, auxin bioassay is a quantitative test. The test is based on Went's (1928) research. At 25°C and 90% relative humidity, 150 µg/liter of auxin produces a 10° curvature. Up to

300 pg/litre of auxin can be measured using the test. An agar block of uniform dimensions (usually 2 x 2 x 1 mm) is used to allow auxin from a shoot tip or any other plant organ to spread. Agar can also be used to directly dissolve auxin. The dark-grown, 15–30 mm long oat coleoptile is held vertically above water. 1 mm tip of coleoptile is removed without injuring the primary leaf. After 3 hours a second decapitation is carried out for a distance of 4 mm. Primary leaf is now pulled loose and agar block supported against it at the tip of decapitated coleoptile. After 90-110 minutes, the coleoptile is found to have bent. The curvature is measured.

2. Root Growth Inhibition

On damp filter paper, sterilized cress seeds are allowed to sprout. Root lengths are measured when the roots are around 1 cm long. While the remainder are left to grow on moist paper, half of the seedlings are submerged in a test solution. After 48 hours, the roots' lengths are measured. It can be observed that the seedlings in the test solution exhibit minimal root growth, but the control seedlings exhibit typical root growth.

Practical Applications of Auxins

1. Cell division:

In some tissues, such as the cambium, auxin is in charge of encouraging cell division. Auxin stimulates the production of callus and cambial activity at the site of injury. Callus development is useful in grafting, which fortifies the bond between the scion and stock. Auxin is very necessary for tissue culture cell division.

2. Cell Elongation:

Cell elongation is the main physiological consequence of auxin growth in plants. Auxin stimulates cell elongation in three ways: A) by raising osmotic solutes; B) by lowering wall pressure; and C) by making the cytoplasm more permeable to water. The bioassay for cell elongation was the avena curvature test. Because auxin inhibits the formation of ethylene, it has an inhibitory effect on root elongation.

3. Respiration:

Auxins are extremely useful for stimulating respiration in plants. Auxins are responsible for increasing the availability of respiratory substrate thus helping in respiration.

4. Metabolism:

Auxins are in charge of improving plant metabolism, which leads to appropriate growth and development, as a result of the appropriate mobilization of plant resources.

5. Root Initiation:

Rooting at the morphologically lower end is caused by auxin's polar transport. In order to initiate adventitious root production in cuttings, Thimann & Went (1930) discovered that indole acetic acid and outer growth components were necessary. In terms of business use, IBA NAA is far better than IAA.

6. Abscission Layer Prevention:

The formation of abscission layers at the bases of petiole, pedicel or peduncle results into the separation of leaves, flowers and fruits from the plant. The premature drop of fruits may be stopped by spraying 2, 4-D; IAA, NAA etc.

7. Flower Initiation:

Auxin generally inhibits flowering and thus is helpful in delaying the flowering in lettuce

8. Increase In Solute Storage:

Auxins increase storage of solutes inside the cells.

9. Production of Parthenocarpic (Seed less) fruits:

Seedless fruits are being developed by horticulturists by spraying synthetic auxins.

10. Weed Control:

The roots are extremely sensitive to auxins. Auxin distorts the roots, blocks the sieve tubes and disturbs the cell division of roots. 2, 4-D is used for weed control.

11. Apical Dominance:

Apical dominance is the phenomenon by which presence of apical bud does not allow the nearby lateral buds to grow. When the apical bud is removed, the lateral buds sprout. This produces dense bushy growth. The phenomenon is widely used in tea plucking and hedge making. Apical bud inhibits the growth of lateral buds by releasing auxins. It is confirmed by painting the cut end of decapitated shoot by a paste of auxin. The lateral buds remain inhibited, as if the apical bud is present.

12. Tropical Movements:

Differential distribution of in-dole 3-acetic acid produces tropical plant responses like phototropism and geotropism.

Gibberllins

Yabuta and Sumiki (1938) called a crystalline substance that was extracted from "Bakanae or Foolish seedling" sick rose plants "gibberellin." In 1926, Kurosawa of Japan verified that the fungus "Gibberella fujikoroi" (Fusarium heterosporum) was the source of the illness. This disease causes the rice plant to grow unusually tall and slender. The fungus yielded six gibberellins, which are GA1, GA2, GA3, GA4, GA7, and GA9. Cross et al.'s Gibberella (1961). Mac Millan et al. identified gibberellins, GA5, GA6, and GA8, from bean seeds in 1961. Gibberellins are chemically referred to as gibberellic acid. GA is the most widely accessible Gibberellic acid.

Higher plants frequently have gibberellins, but only specific bacterial and fungal species do. Young leaves, seeds, and the top of the stem have larger concentrations. The typical isoprenoid pathway of terpene biosynthesis is used to create gibberellins. Gibberllins were understood as a result of studies on Bakanae, also known as Foolish Seedling. Only after World War II did Japanese art become known. In 1955, Brian et al. discovered pure gibberellic acid, or GA3. The structure of gibberellic acid, GA3, was determined by Cross (1961) (Fig. 15.24). Chemically, it is C19H22O6. One of the gibberellins that has been investigated the most is GA3. In the commercial world, GA4 and GA7 are combined. There are now 125 distinct gibberellins known to exist.

Many of them occur naturally in plants and fungi. Gibberella fujikori has as many as 15 gibberellins. A single plant also possesses a number of gibberellins. This is in contrast to auxin, where a single natural hormone occurs. Gibberellins are synthesised in the apical shoot buds (young leaves), root tips and developing seeds. The precursors for

their synthesis are mevalonic acid (derived from acetyl coenzyme A). Gibberellin transport occurs through simple diffusion as well as through conducting channels.

Bioassay of Gibberllins

I. Dwarf Pea (Pisum sativum L.)

Seeds of dwarf pea (Pisum sativum L.) are allowed to germinate till the formation of coleoptile. GA solution is applied to some seedlings. Others are kept as control. After 5 days, epicotyl length is measured. GA stimulates epicotyl growth with a concentration as low as 1 Nano gram

II. Barley Endosperms (Hordeum vulgare L.):

Barley (Hordeum vulgare L.) Endosperms are detached from embryos, sterilized and allowed to remain in 1 ml of test solution for 1-2 days. There is a build-up of reducing sugars. The content of reducing sugar is proportional to gibberellin concentration. Reduc¬ing sugars are not formed in control experiment where endosperms are kept in plain water.

Practical Applications of Gibberllins:

1. Elongation Of Genetically Dwarf Plants:

Genetically Dwarf and miniature varieties of higher plants are often genetic mutants, and they don't produce gibberllins. Thus, they can be made to grow to normal size by application of gibberellins.

2. Cold treatment substitution:

Biennial plants flowers only when they receive low (Cold) temperature during winter season. Such plants would only flower after gibberellins treatment even if they do not receive suitable low temperature. Hence Gibberllins are used for the initiation of flowering in biennial plants.

3. Breaking dormancy:

Gibberllins are extensively used for breaking the dormancy of tubers, corms, bulbs and various tree species.

4. Production of Parthenocarpic fruits:

Gibberllins are highly employed in production of parthenocarpic (Seed less) fruits. When compared with Auxins, Gibberllins show more effect on parthenocarpic fruit production.

5. Delayed Ripening:

Gibberllins delay fruit maturity and senescence in lemon, oranges and cherries. This helps in marketing and storing of fruits.

6. Increase in Yield:

Gibberllins also increase yield or fruit size and parthenocarpic development in tomato and berries. Gibberllins treatment also produces broader and longer leaf formation in pea, bean, tomatoes, pepper, cucumber, lettuce and cabbage.

7. Malting:

Gibberllins have also been used in increasing synthesis of various hydrolytic enzymes such as α-amylase, ribonuclease and protease in aleurone cells of barley grams to improve the yield.

8. Gibberllins increases the size of fruits and leaves.

9. Gibberllins are responsible for the increase in cell division and cell size.

10. Gibberllins promotes flowering in long day plants and induces maleness.

Cytokinins:

A class of plant hormones known as cytokinins is in charge of encouraging cytokinesis, or cell division, in plant roots and shoots. They mostly influence cell development and differentiation, but they also have an impact on leaf senescence, axillary bud growth, and apical dominance. Using coconut milk, Folke Skoog at the University of Wisconsin–Madison found their effects in the 1940s. Cytokinins are basic substances that can work alone or in combination with auxin to stimulate cytokinesis. They can be either amino purine or phenyl urea derivatives. Skoog and colleagues discovered that callus from intermodal tobacco segments only multiply when the nutrient media contains extracts of yeast, vascular tissues, coconut milk, or DNA in addition to auxin.

They were examined in order to identify the chemical that promotes growth. Miller (1955) identified the first cytokinin from autoclaved Herring sperm DNA that had deteriorated. In plants, a two-component phosphorelay mediates cytokinin signaling. Cytokinin binds to a histidine kinase receptor in the endoplasmic reticulum membrane to start this process. As a result, the receptor becomes autophosphorylated, and the phosphate is subsequently transferred to a phosphotransfer protein. The type-B response regulators (RR), a class of transcription factors, can subsequently be phosphorylated by the phosphotransfer proteins. The transcription of several genes, including the type-A RRs, is regulated by the phosphorylated and active type-B RRs. The route is adversely regulated by the type-A RRs.

Bioassay of Cytokinins

1. Tobacco pith culture

Two Tobacco pith cultures were taken and one culture is supplied with cytokinin where as another culture is not supplied with cytokinin. Increase in fresh weight of the tissue over the control is a measure of stimulation of cell divisions and hence cytokinin activity. The test can measure cytokinin concentration between 0.001-10 mg/litre. It takes 3-5 weeks.

2. Excised Radish Cotyledon Expansion

This test has been developed by Lethan. Excised Radish cotyledons are measured and placed in test solution as well as ordinary water (as control). Enlargement of cotyledons is an indication of cytokinin activity.

3. Retardation of Leaf Senescence

In this rapid bioassay technique. Leaf discs are taken in two lots. In one lot cytokinin is provided. After 48-72 hours, the leaf discs are compared for chlorophyll content. Cytokinin retards the process of chlorophyll degradation. The test is sensitive in concentration of 1 pg/litre.

Practical Applications of Cytokinins

1. Cell Division:

The promotion of cell division and the associated production of DNA and RNA is the function of cytokinins. Chromosome duplication can happen without cytokinins, although they are necessary for cytokinesis. Even in permanent cells, cytokinins cause division when auxin is present. It is discovered that both hormones are necessary for cell division in callus, which is an unorganized,

undifferentiated irregular mass of dividing cells in tissue culture.

2. Organ formation:

In a range of plant tissue cultures, cytokinins are utilized for organ formation because of their morphogenesis action. The morphogenesis or differentiation of tissues and organs depends on both auxin and cytokinins. When cytokinin levels are high, buds form, and when they are low, roots form (Skoog and Miller, 1957).

3. Differentiation and Cell Elongation:

Cytokinins are in charge of cell elongation. Cytokinins promote the development of adventitious shoots, lateral shoots, chloroplasts in leaves, and new leaves. Cytokinins are crucial for cell elongation and differentiation because they also cause the interfascicular cambium to lignify and differentiate.

4. Senescence:

Cytokinins postpone the senescence phase, which is characterized by the breakdown of proteins and the loss of chlorophyll. According to Richmond and Lang (1957), kinetin treatment postponed the senescence of detached xanthium leaves by a few days. The Richmond-Lang Effect is the name given to this effect of kinetin in delaying senescence, or aging.

5. Apical Dominance:

Cytokinins encourage apical dominance by acting antagonistically to auxin.

6. Seed Dormancy:

Cytokinins are responsible for seed dormancy of various types, including red light requirement of Lettuce and Tobacco seeds.

7. Increase in Resistance:

Cytokinins increases plant resistance to low and high temperature. It also increases resistance against various diseases and fungi.

Growth Inhibitors:

There are two types of growth promoters as follows:

A. Abscisic acid
B. Ethylene

Abscisic acid:

Abscisic acid (ABA) is one of the most important plant hormones (Growth Inhibitor) which helps in regulating different aspects of plant growth and development as well as response to the stress. Chemically, ABA is a sesquiterpene which plays an important role in the development and maturation of seeds, Protein synthesis and compatible osmolytes, which enable plants to tolerate stresses developed as a result of environmental factors and as a general inhibitor of growth and metabolic activities. In higher plants, ABA is derived from a C40 carotenoid, 9′-cis-neoxanthin, which is oxidatively cleaved to give xanthoxin (C15) and an apoaldehyde (C25). Abscisic acid is also known as a stress hormone because the produc¬tion of hormone is stimulated by drought, water logging and other adverse environmental conditions. Abscisic acid is known as dormin as it induces dormancy in buds, underground stems and seeds. Other names of ABA are abscissin II and inhibitor-B. Abscisic acid is a mildly acidic dextrorotatory cis sesquiterpene growth hormone which functions as a general growth inhibitor

by counteracting other hormones (auxin, gibberellins, and cytokinins) or reactions mediated by them.

ABA has been first isolated by Addicott (1963) from Cotton bolls. ABA is produced in different parts of the plants and is more abundantly inside the chloroplasts of green cells. The hormone is formed from mevalonic acid or xanthophyll. It is transported to all parts of the plant through diffusion as well as transport channels (phloem and xylem). Robinson and Weiring (1963-64) extracted the inhibitory substance and called dormin because it caused dormancy. Okhuma et al (1963, 1965) isolated the active inhibitor from young cotton fruits and called it abscisin II. Abscisin I has been isolated from the burrs of mature cotton fruits. Later in 1967 it was realized that the dormin and abscisin II were the same and was named Abscisis acid (ABA).

Bioassay of Abscisic acid:

Cucumber hypocotyls (Chin Ho Lin, Yuh Ling Lin and Yuh-Jane Chow, 1988)

Sections of 3-day-old dark-grown Cucumber hypocotyl taken from 0–5 mm immediately below the cotyledon were used for the assay. A dark incubation period of 20 h was followed by an exposure to light for 24 h. Under these conditions, the inhibition of hypocotyl elongation is proportional to the abscisic acid applied. The minimum detectable level of abscisic acid was 10−9 M, and the range of linear response to abscisic acid was between 10−7 and 10−3 M. This assay is 10 times more sensitive than the cucumber cotyledon greening bioassay for abscisic acid.

Practical Applications of Abscisic acid

1. **Leaf Senescence:**

ABA is responsible for accelerating the senescence phase of growth. Excessive presence of ABA stops protein and RNA synthesis in the leaves and hence stimulates their senescence.

2. Seed Dormancy:

ABA regulates the buds and seed dormancy by inhibiting the growth processes. Dormancy allows seeds to tolerate desiccation and extremes of temperature. The buds as well as seeds sprout only when abscisic acid is overcome by gibberellins. Due to its action in inducing dormancy, abscisic acid or ABA is also named as dormin.

3. Transpiration:

ABA causes stomata closing and hence prevents transpiration. Transpiration is the loss of water from the aerial parts of the plants. During stress and dessication, ABA is produced in plants in large quantities.

4. Parthenocarpy:

ABA is responsible for the production of parthenocarpic (Seed less) fruits in Rose.

5. Rooting:

In some plants, rooting's of cuttings is promoted by ABA.

6. Membrane Potential:

ABA induces a positive surface potential on cell membrane.

7. Flowering:

Abscisic acid promotes flowering in few short-day plants such as Strawberry.

Ethylene

Ethylene is the most important plant growth regulator which is widely used for the ripening of fruits. It is also used for the production of more flowers and

fruits. Ethylene is a colorless flammable volatile gas and has been included in plant hormones in the year 1935. History of ethylene is very interesting. Cousins (1910) found that ripe oranges produced a volatile substance that hastened ripening of unripe bananas nearby. R. Gane (1934) found that the ripening causing volatile substance was ethylene. During the ancient days, Egyptians used this technique with gash figs to stimulate ripening. While the Chinese burn incense in a closed room to fasten the ripening of pear Ethylene was recognized as a plant hormone by Crocker (1935). Amino acid Methionine is responsible for the production of Ethylene in plants. It is formed in almost all plant parts— roots, leaves, flowers, fruits, and seeds.

Practical Applications of Ethylene

1. Ripening:

Ethylene is extensively used in the agriculture sector for the quick ripening of fruits. Unripe fruits are sprayed or soaked in Ethylene resulting in quick and fast ripening.

Production of more flowers and fruits:

Ethylene is highly responsible for the production of more flowers and fruits in the agricultural, horticultural and other commercial flowers and fruits.

2. Rice Seedling Growth:

Ethylene is responsible for promoting the rapid elongation of leaf bases and internodes in deep water rice plants. As a result, leaves remain above water and hence rice seedling growth is highly promoted.

3. Root Initiation:

Ethylene helps in root initiation, growth of lateral roots and root hairs resulting in increase in the absorption surface of the plant roots.

4. Stimulates Flowering:

Ethylene stimulates flowering in Pineapple and mango This helps in synchronizing fruit set.

5. Sprouting:

Exposure of Rhizomes, Corms, Tubers and Seeds to Ethylene results in immediate sprouting.

References:

1. Sharma (Plant physiology). Tata McGraw hill publication. New Delhi.
2. Verma (2020). Plant physiology. Rastogi publication, New Delhi.
3. Jain (1999). Fundamentals of plant physiology. Himalaya publication Mumbai.

Biostimulant and Plant Growth Regulator: Applications and Signaling Mechanism

ISBN: 978-81-981142-7-3| Year: 2025 | pp: 137 - 143 |

History of Plant Tissue Culture

Dr. Jayshri Devidas Jagtap

Assistant Professor in Botany, B. N. Bandodkar College of Science (Autonomous), Thane.

Email: jshrjgtp2@gmail.com

Article DOI Link: https://zenodo.org/uploads/14886121

DOI: 10.5281/zenodo.14886121

Abstract

The process of "in vitro" cultivating plant or animal cells, tissue, or organs on nutritional medium under aseptic conditions—typically in a glass container—is known as tissue culture. Other names for tissue culture include "sterile culture" and "in vitro" culture. This method allows for the long-term maintenance of living cells outside the organism's body. To put it another way, tissue culture is the aseptic culture of tissues, cells, organs, and other components in vitro in a laboratory setting under specific physical, chemical, and environmental conditions. Henri Louis Duhumel du Monceau (1756) took the first step toward plant tissue culture when he noticed callus production while doing his groundbreaking research on plant wound healing. In 1922, the first root tips were cultivated, and they were kept alive for 20 weeks via subculturing. Root cultures, embryo cultures, and the first real callus/tissue cultures were the results of the early research. The discovery of new techniques and the enhancement of existing ones were the hallmarks of the 1940s through 1960s. It was discovered in the 1930s that auxins (IAA) and B vitamins were essential for the tissue culture method of root culture growth. The history and evolution of various plant tissue culture techniques in India and outside are the main topics of this chapter

Keywords: Tissue culture, In vitro, history, nutrient medium, aseptic culture.

Introduction

Tissue culture is the method of ‘in vitro’ culture of plant or animal cells, tissue or organ-on nutrient medium under aseptic conditions usually in a glass container. Tissue culture is sometimes referred to as ‘sterile culture’ or ‘in vitro’ culture. By this technique living cells can be maintained outside the body of the organism for a considerable period. In other words, tissue culture is defined as an aseptic culture of tissues, cells, organs

and components under particular defined physical, chemical and environmental conditions in vitro in the laboratory. According to Street (1977) tissue culture is referred to any multicellular culture with protoplasmic continuity between cells and growing on a solid medium or attached to a substratum and nourished by a liquid medium. By plant tissue culture new plants may be raised in an artificial medium from very small parts of plants, such as, shoot tip, root tip, callus, seed, embryo, pollen grain, ovule or even a single cell, whether the cultured tissue develops into a plant or grows unorganized depends on the genetic potential of the tissue and the chemical and physical environment. Now day's tissue culture is a most important technique for the production of fruit plants, flower plants, foliage plants, nut plants, medicinal plants, vegetable crops, agricultural species, spices and condiments plants, horticulture and garden plants and many more types of aesthetic plants. Plant tissue culture is the most authentic and important tool in basic as well as applied studies, research, innovations and commercial applications. The origin of plant tissue culture has been around since the beginning of the 18th century where a German Botanist Gottlieb Haberlandt made the first attempt to use the in vitro method when he grows the plant tissues. The cells he used in his plant tissue culture experiment were varied palisade tissue coming from leaves, pith, epidermis and epidermal hair. The initial experiment done by Haberlandt was fruit full for several months. However, the cells did not proliferate further.

The earliest step towards the plant tissue culture was made by Henri Louis Duhumel du Monceau (1756), Where, during his pioneering studies on would healing of plants, he observed callus formation. The science of plant tissue culture takes its roots from path breaking research of discovery of cell followed by propounding cell theory proposed by Schleiden and Schwann in 1839. They proposed that cell is the basic unit of organisms. They also observed that cell is capable to regenerate into whole plant if given suitable environment. Based on this area, in 1902, a German physiologist, Gottlieb Haberlandt developed the concept of in vitro cell culture. In his experiment he isolated single fully differentiated individual plant cells from different plant species like palisade cells from leaves of Laminum purpureum, glandular hair of Pulmonaria and pith cells from petioles of Eicchornia crassiples etc and was first to culture them in Knop's salt solution enriched with glucose. In these cultures, cells increased in size, accumulated starch but failed to divide. Haberlandt's prediction failed that the cultured plant cells could grow, divide and develop into embryo and then to whole plant. Despite lack of success, Haberlandt made several predictions about the requirements in media in experimental conditions which could possibly induce cell division, proliferation and embryo induction. Haberlandt is thus regarded as father of tissue culture. Taking clue from Haberlandt's failure, in 1904 Hannig chose embryogenic tissue to culture. He obtained nearly mature embryos from seeds of several species of Crucifers and successfully grew them to maturity on mineral salts and sugar solution. In 1908,

Simon regenerated callus, buds and roots from Poplar stem segments and established the basis for callus culture. For about next three decades that is up to 1934, there was very little progress in cell culture research. Within this period, an innovative approach to tissue culture using meristematic cells like root and stem tips was reported by Robbins (1922). The first root tip were cultured in 1922, and by making use of sub culturing, maintained their cultured roots for 20 weeks. The early studies led to root cultures, embryo cultures, and the first true callus/tissue cultures. The period between the 1940s and the 1960s was marked by the development of new techniques and the improvement of those that were already in use. In 1930's, it was recognized that B-vitamins and auxins (IAA) were the key components in growing root cultures using tissue culture method. In 1934 White first successfully cultured isolated tomato roots in a medium containing sucrose, inorganic iron salts, thiamine, glycine, pyridoxine and nicotinic acid etc. Gautheret (1934) noted that cambium culture from Salix caprea, Populus nigra etc. continued to grow for few months under aseptic conditions. He later (1937, 1938) used medium supplemented with B-vitamins and IAA.

In 1937 White recognised the importance of B-vitamins for growth of root cultures. Went and Thimann (1937) discovered the importance of auxin (IAA). Nobecourt (1937,1938) obtained some growth in culture of carrot root explants. He also noted root differentiation in tissue culture. In 1938 tumour tissues of tobacco hybrid were successfully cultured.

In 1939 working independently three scientists, White in USA and Nobecourt and Gautheret in France cultured successfully plant callus tissue on synthetic medium continuously. Gautheret (1939) said that carrot culture required Knop's solution supplemented with Bertholots' salt mixture, glucose, gelatine, cysteine HC1 and IAA. White (1939) in culture of procambial tissue from young stem of the hybrid Nicotiana glauca × N. langsdorfii noted unlimited and undifferentiated growth. He showed that this tissue could be repeatedly subcultured. White (1939) recorded development of leafy buds in tissue culture of the hybrid N. glauca × N. langsdorfii in nutrient medium. Tissues of Sequoia sempervirens were cultured by Ball (1955). Pollens of Taxus and Ginkgo biloba were cultured by Tulecke (1959). Conifer tissues were successfully cultured by Harvey and Grasham (1969). In this method isolated single cells were put on a square filter paper, placed on a active nurse tissue, which supplies the required nutrients to the growing single cell. In another method cells were suspended on a hanging drop in a micro-chamber.

Bergman (1960) working with suspension cultures of Nicotiana tabacum var. sansum and Phaseolus vulgaris var. 'early golden rod' developed agar plating technique of single cell cloning. In this method single cell fraction was separated by filtration, mixed with warm agar and then plated in a petridish in thin layer. Melchers and Bergmann (1959) noted that after several cultures of the haploid shoot of Antirrhinum majus there was increase in ploidy. Ball (1946) noted the possibility of regeneration of whole plant in culture

of shoot tip of angiospermic plants. Wetmore and Wardlaw (1951), Morel (1960) obtained whole plants from culture of shoot apices having 1 or 2 leaf primordia. Morel (1964) used this method for culture of orchids. A cell which can develop into a whole organism by regeneration is called a totipotent cell. This term was coined by Morgan in 1901. According to White (1954) if all the cells of a multicellular organism is totipotent, then such cells in isolated condition regain their dividing power and can produce whole plants. In an organism this capacity remains suppressed. It was noted that single cells are capable of producing new plants. From pollen and anther culture haploid embryos were obtained. A method of microspore culture of Nicotiana and Datura was developed by Nitsch (1974, 1977). He was able to double the chromosome number and obtained homozygous diploid plants. From anther culture of tobacco Bourgin and Nitsch (1967), Nakata and Tanaka (1968) obtained haploid tissues and haploid embryoids. Cocking (1960) recorded release of protoplasts from root tip cells by using fungal cellulase in 0.6M sucrose. He was able to culture isolated protoplasts, which regenerate new cell walls and produce cell colonies and ultimately plantlets.

In many plant suspension cultures cell protoplasts had been successfully released. Plant tissue culture technique is used for the study of tumour physiology. White and Brown (1942) were able to culture bacteria free crown gall tumour. In Scorzonera hispanica Gautheret (1946) noted that the callus culture which initially required auxin, produced some proliferations which can grow in auxin deficient medium. Such inherited changes occurring in the nutritional requirements (especially involving auxin) of cells of a culture is called habituation. An auxin habituated culture does not re- quire the supply of exogenous auxin (Butcher 1977). Butcher noted (1977) that when auxin and cytokinin habituated tissues are grafted into a healthy plant, tumours are produced. Pathogen free plants can be obtained by culturing apical' meristem. By late 70's it was evident that plant tissue culture technique can be successfully used in various field of agriculture, such as, production of pathogen free culture, production of secondary products, clonal propagation, mutant culture, haploid breeding and genetic engineering.

By tissue culture, pathogen free cultures have been produced. This technique is important for plant pathological investigations. Protoplasts in culture are used for virus infection and biochemical studies. From suspension culture secondary products can be synthesised in large amount. Some of these substances are enzymes, vitamins, food flavours, sweeteners, anti-tumour alkaloids and insecticides. In Japan 'in vitro' culture has been achieved at industrial level.

Clonal propagation of orchids and several other ornamental and economic plants have been achieved by 'in vitro' culture. In potato clonal propagation has been achieved by culturing leaf cell protoplasts. By using mutagens in culture followed by selection disease resistant or stress resistant mutant plants have been regenerated. By haploid breeding few cultivars were produced. Hybrids of related but sexually incompatible species have been

produced by protoplast fusion. By this technique hybrid between potato and tomato has been produced. By cell fusion of isolated cells from two different species hybrid tobacco plants are produced. From tissue culture studies important information about root-shoot relationship can be obtained. Several scientists reported about the factors controlling vascular tissue differentiation from tissue culture studies.

Van Oberbeck (1941) cultured embryos of Datura on a medium supplemented with coconut milk. Importance of coconut milk and 2-4D as nutrient was recognised. The stimulatory property of coconut milk is due to the presence of zeatin. The potent cell division factor was found to be kinetin, which is a 6 furfurylaminopurine. Cytokinin is 6-substituted amino- purine compound, which can stimulate cell division in culture of plant tissues. Monocot tissues were successfully cultured on a medium containing coconut milk. Callus culture of Tagetes erecta and Nicotiana tabacum on liquid culture medium when agitated on a shaker produced suspension of single cells or cell aggregates (Muir 1953). Such cell suspension could be subcultured. Studies on cell suspension culture were carried out by Muir, Hildebrandt and Riker (1954), Street, Shigomura (1957), Torrey and Reinert (1961), Reinert and Markel (1962). Muir (1963) developed paper raft nurse technique for single cell culture. Over the following century, many similar experiments were conducted and research began identifying the most important parameters that should guide our overall plant tissue culture process today. It was the availability of these techniques that led to the application of tissue culture to five broad areas, namely, cell behavior (including cytology, nutrition, metabolism, morphogenesis, embryogenesis, and pathology), plant modification and improvement, pathogen-free plants and germplasm storage, clonal propagation, and product (mainly secondary metabolite) formation, starting in the mid-1960s.

Tissues from various plants were cultured subsequently. It was noted that older cultures show increasing degree of organization. The role of vitamins in plant growth was also recognized. Wetmore and Wardlaw (1951) successfully cultured shoot tips of pteridophytes (Selaginella, Equisetum and ferns). Gottilieb Haberlandt, a German botanist, in 1902 cultured fully differentiated plant cells isolated from different plants. This was the very first step for the beginning of plant cell and tissue culture. Further contributions were made by the Cell Doctrine which admitted that a cell is capable of showing totipotency. With the identification of a variety of chemicals like cytokinin, auxin, other hormones, vitamins, etc. and their role in affecting cell division and differentiation, the methods of plant tissue culture developed in a proper manner. Three other scientists Gautheret, White and Nobecourt also made valuable contributions to the development of plant tissue culture techniques. Later on, a number of suitable culture media were developed, for culturing plant cells, tissues, protoplasts, embryos, anthers, root tips, etc. The discovery and understanding of role of plant growth hormones in the multiplication of cell also provided an extra aid for the

development of in-vitro culture methods of plants. The first plant from a mature plant cell was regenerated by Braun in 1959. Foundation of commercial plant tissue culture was laid in 1960 with the discovery for a million-fold increase in the multiplication of Cymbidium (an orchid) which was accomplished by G.M. Morel.

In India, the work on tissue culture was initiated during 1950s at University of Delhi. This initiation is credited to Shri Panchanan Maheshwari who was working there in the Department of Botany. Discovery of haploid production was a land-mark in the development of in-vitro culturing of plants. Shri S.C. Maheshwari and Sipra Guha made a remarkable contribution in the development of plant tissue culture in India. Later on, the development in the composition of nutrient media and genetic engineering served as a basis for further success in the plant tissue culture techniques. Gottleib Haberlandt was the first person to make attempts for plant tissue culture, i.e., he developed the concept of in-vitro culture of plant cells and is aptly regarded as the father of tissue culture. Thereafter, there happened some dramatic advances in tissue culture techniques.

In the following table, some of the early classical contributions in the field of plant tissue culture are elaborated.

Year	Worker/Scientist	Advancement
1902	Haberlandt	First attempt of in vitro culture of a plant cell.
1904	Hanning	Culture of embryogenic tissues of Crucifers (Brassicaceae)
1922	Robbins	In vitro culture of roots
1925	Laibach	Zygotic embryo culture in Linum
1934	White	Culture of roots of Tomato
1939	Gautheret, White and Nobecourt	Establishment of indefinite callus culture
1941	Braun	Culture of Crown Gall Tissues
1945	Loo	Stem tip culture
1955	Miller	Growth hormone Kinetin discovered
1957	Skoog and Miller	Discovered that Auxin: Kinetin ration is responsible for regulating organ formation
1960	Bergmann	Development of plating technique for isolation of

		single cell
1970	Power	Successful protoplast fusion
1970	Maheshwari and Guha	Successful anther culture
1971	Takabe	Plants regenerated from protoplasts
1974	Reinhard	Biotransformation in plant tissue culture
1988	Malchers	Production of somatic hybrid Pomato

References

1. Thorpe TA. The current status of plant tissue culture. In Bhojwani SS (Ed.), Plant tissue culture: Applications and limitations. Amsterdam: Elsevier, 1990, 1-33.
2. Haberlandt G. Kulturversuche mit isolierten Pflanzenzellen. Sitzungsber. Akad. Wiss. Wien. Math. -Naturwiss. Kl., Abt. J. 1902; 111:69-92.
3. Krikorian AD, Berquam DL. Plant cell and tissue cultures: the role of Haberlandt. Botanical Review. 1969; 35:59-67.
4. Kotte W. Kulturversuche mit isolierten Wurzelspitzen. Beiträge Allgemeine Botanik. 1922; 2:413-434.
5. Robbins WJ. Cultivation of excised root tips and stem tips under sterile conditions. Botanical Gazette. 1922; 73:376-390.
6. Ball E. Development in sterile culture of stems tips and subjacent regions of Tropaeolum majus L. and of Lupinus albus L. American Journal of Botany. 1946; 33:301-318.
7. Monnier M. Culture of zygotic embryos. In Thorpe TA (Ed.), In vitro embryogenesis in plants. Dordrecht, The Netherlands: Kluwer Academic, 1995, 117-153.
8. Laibach F. Ectogenesis in plants. Methods and genetic possibilities of propagating embryos otherwise dying in the seed. The Journal of Heredity. 1929; 20:201-208.
9. Tukey HB. Artificial culture methods for isolated embryos of deciduous fruits. Proceedings of the American Society for Horticultural Science. 1934; 32:313-322.
10. LaRue CD. The growth of plant embryos in culture. Bulletin of Torrey Botanical Club. 1936; 63:365-382.
11. Bhojwani SS, Razdan MK. Plant tissue culture: Theory and practice. developments in crop science, Amsterdam: Elsevier, 1983, 5.
12. Gautheret RJ. History of plant tissue and cell culture: A personal account. In Vasil IK (Ed.), Cell culture and somatic cell genetics of plants. New York: Academic Press. 1985; 2:1-59

BIOSTIMULANT AND PLANT GROWTH REGULATOR: APPLICATIONS AND SIGNALING MECHANISM

ISBN: 978-81-981142-7-3| Year: 2025 | pp: 144 - 152 |

Eco-Friendly Pest Control: A Comprehensive Review of Plant-Derived Biopesticides for Sustainable Agriculture

[1]Vaishali Sangram Shewale

[2]Pallavi Vinayak Khairmode

Department of Zoology, Sadguru Gadage Maharaj College, Karad

Email: vaishalisangramshewale@gmail.com

Article DOI Link: https://zenodo.org/uploads/14886542

DOI: 10.5281/zenodo.14886542

Abstract

The extensive dependence on synthetic chemical pesticides has resulted in significant ecological disturbances, including pesticide resistance, bioaccumulation, and adverse effects on non-target organisms (Isman, 2006; Saxena & Khan, 2021). As an environmentally sustainable alternative, plant-derived biopesticides have gained prominence within the framework of Integrated Pest Management (IPM), offering eco-friendly pest control solutions (Govindarajan et al., 2016). Many plant species produce secondary metabolites with potent insecticidal properties, such as alkaloids, flavonoids, terpenoids, and tannins, which effectively target insect pests while minimizing environmental harm (Gonzalez-Coloma et al., 2020). This review highlights the efficacy of botanicals, including *Azadirachta indica*, *Vitex negundo, Calotropis gigantea, Acacia nilotica* (Babul), *Bauhinia blakeana* and *Lantana camara*, in controlling agricultural and vector pests. These plant-based bioactive compounds exhibit significant larvicidal, ovicidal, antifeedant, and repellent activities, contributing to sustainable pest management strategies (Rajakumar & Rahuman, 2011; Sharma & Kumar, 2020). The integration of biopesticides into IPM can mitigate the harmful consequences of chemical pesticides while ensuring long-term agricultural productivity and ecological balance. This review underscores the significance of plant-derived biopesticides in modern pest control and their role in promoting sustainable agriculture and vector management.

Keywords: Biopesticides, Plant-Derived Insecticides, Integrated Pest Management (IPM), Sustainable Agriculture, *Azadirachta indica*, *Calotropis gigantea*, *Vitex*

negundo, Lantana camara, Acacia nilotica, Bauhinia blakeana, Secondary Metabolites, Herbal Pest Management, Green Pesticide Alternatives.

Introduction

The widespread and indiscriminate application of synthetic pesticides has raised serious concerns regarding environmental sustainability, human health, and the development of pesticide resistance in insect populations (Isman, 2006). Synthetic pesticides contribute to soil and water contamination, disrupt ecological food webs, and harm non-target organisms, including pollinators and natural predators of pests (Mancini et al., 2019). Additionally, bioaccumulation of pesticide residues in agricultural produce poses significant risks to human health, leading to neurological disorders, endocrine disruption, reproductive toxicity, and even carcinogenic effects upon prolonged exposure (Mostafalou & Abdollahi, 2017). As a sustainable alternative, Integrated Pest Management (IPM) promotes the use of biological control agents and plant-derived biopesticides to mitigate these adverse effects while ensuring effective pest suppression (Saxena & Khan, 2021). Unlike synthetic pesticides, which persist in the environment and accumulate in the food chain, botanical pesticides degrade rapidly, reducing the risks of long-term toxicity (Gonzalez-Coloma et al., 2020). Plants have evolved complex defense mechanisms, synthesizing a wide range of secondary metabolites such as alkaloids, flavonoids, terpenoids, and tannins. These bioactive compounds exhibit insecticidal, antifeedant, ovicidal, and repellent properties, effectively controlling pest populations without harming beneficial organisms (Tudi et al., 2021). Moreover, plant-derived biopesticides pose minimal risks to human health, making them promising alternatives for sustainable pest control. The shift toward botanical pesticides not only reduces pesticide-induced environmental degradation but also aligns with global efforts to promote sustainable agricultural practices. Their integration into IPM strategies can mitigate resistance development in insect populations, safeguard biodiversity, and contribute to safer food production systems, ensuring long-term ecological balance and public health safety.

Secondary Metabolites as Insecticidal Agents

Secondary metabolites are naturally occurring bioactive compounds produced by plants as part of their defense mechanisms against herbivores, pathogens, and environmental stressors. Among these, alkaloids, flavonoids, terpenoids, tannins, and essential oils have been extensively studied for their potent insecticidal properties (Sengottayan et al., 2013). These compounds exhibit diverse modes of action, disrupting various physiological and biochemical processes in insect pests, ultimately affecting their feeding behavior, development, reproduction, and survival. Alkaloids, such as nicotine and rotenone, act as neurotoxins, interfering with neurotransmission and causing paralysis or death in insect populations. Flavonoids, known for their antioxidant properties, disrupt metabolic pathways, impairing digestion and

nutrient absorption in pests. Terpenoids, including azadirachtin from *Azadirachta indica* (neem), function as growth regulators by inhibiting molting and development in insect larvae (Jeyasankar et al., 2022). Tannins, commonly found in *Acacia* and *Calotropis* species, exert antifeedant effects, reducing insect herbivory by interfering with enzyme activity in the digestive system of pests. Essential oils, extracted from aromatic plants like *Eucalyptus globulus* and Lantana camara, have been reported to possess strong fumigant and repellent properties, further contributing to insect control (Rattan, 2010). Numerous studies have demonstrated the efficacy of plant-derived secondary metabolites in controlling various insect pests while posing minimal risks to non-target species and beneficial organisms (Pavela et al., 2019). Unlike synthetic pesticides, which often lead to resistance development, these bioactive compounds degrade rapidly in the environment, reducing the likelihood of long-term toxicity and ecological disruption. Their selective action against insect pests makes them valuable components of eco-friendly pest management strategies, reinforcing the role of botanical insecticides in sustainable agriculture and vector control.

Review of Selected Plant-Derived Biopesticides

- ***Azadirachta indica***

Neem (*Azadirachta indica*) is one of the most extensively studied botanical insecticides, renowned for its broad-spectrum efficacy against various insect pests. The primary bioactive compound, azadirachtin, exhibits strong antifeedant, growth-disrupting, and repellent properties, making neem-based biopesticides a valuable component of Integrated Pest Management (IPM) strategies (Isman, 2020). Unlike synthetic pesticides, neem-derived compounds act selectively on target pests while exhibiting minimal toxicity to non-target organisms, making them environmentally sustainable alternatives for pest control. Azadirachtin disrupts insect growth and development by interfering with the hormonal regulation of molting and metamorphosis. It functions as an insect growth regulator (IGR), preventing larvae from successfully transitioning into the next developmental stage, ultimately leading to mortality. Additionally, neem extracts inhibit feeding behavior in a wide range of insect species, including lepidopteran pests, aphids, whiteflies, and mosquito larvae (Govindarajan et al., 2016). The repellent properties of neem also deter insect vectors, reducing their population densities and minimizing the spread of vector-borne diseases. Several studies have demonstrated the effectiveness of neem-based formulations against agricultural pests and disease-carrying vectors. For instance, neem oil and neem seed extracts have shown significant larvicidal activity against *Aedes aegypti* and *Anopheles stephensi*, the primary vectors of dengue and malaria, respectively (Sohail et al., 2021). Furthermore, neem-derived products have been successfully incorporated into organic farming systems as seed treatments, foliar sprays, and soil amendments to manage pest infestations while preserving soil health.

- ***Vitex negundo***

Vitex negundo, commonly known as the five-leaved chaste tree, is a medicinal plant widely recognized for its

insecticidal and repellent properties. The bioactive compounds found in *V. negundo*, including flavonoids, alkaloids, terpenoids, and essential oils, contribute to its potent larvicidal, ovicidal, and insect-repellent activities. Recent studies have demonstrated the effectiveness of different solvent extracts of *V. negundo* against *Spodoptera litura*, revealing significant antifeedant and insecticidal properties. These findings highlight the plant's potential as a natural biopesticide for pest management in agriculture and support its integration into sustainable Integrated Pest Management (IPM) programs (Shewale et al., 2022). Studies have demonstrated that extracts from *V. negundo* leaves exhibit strong larvicidal activity against mosquito species such as *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*, which are primary vectors of dengue, malaria, and filariasis, respectively (Tiwari et al., 2021). The flavonoids and alkaloids present in *V. negundo* interfere with the normal physiological processes of insect larvae, leading to developmental disruption and mortality. Additionally, the repellent properties of its essential oils help in reducing the risk of vector-borne disease transmission by deterring adult mosquitoes from human dwellings. *V. negundo* extracts have also been found effective against stored grain pests such as *Sitophilus oryzae* (rice weevil) and *Tribolium castaneum* (red flour beetle), which cause significant losses in agricultural produce (Rajashekar et al., 2017). The bioactive compounds exert contact toxicity and inhibit feeding behavior in these pests, reducing their populations in storage environments.

- ***Calotropis gigantea***

Calotropis gigantea, commonly known as the milkweed or giant milkweed plant, is a widely distributed medicinal plant known for its potent insecticidal properties. This plant produces a milky latex rich in bioactive compounds, particularly cardenolides (cardiac glycosides), flavonoids, alkaloids, and terpenoids, which contribute to its toxicity against various insect pests. These compounds act as natural defense mechanisms, deterring herbivory and providing a sustainable alternative to synthetic pesticides in Integrated Pest Management (IPM) programs. Recent studies have demonstrated the significant antifeedant activity of *C. gigantea* extracts against Spodoptera litura, highlighting its potential as an eco-friendly biopesticide (Lawand & Shewale, 2024). The cardenolides present in *C. gigantea* primarily disrupt the nervous and cardiac systems of insects by inhibiting Na^+/K^+-ATPase, a crucial enzyme in nerve signal transmission (Senthil-Nathan, 2019). This leads to paralysis and eventual mortality in targeted insect populations. Several studies have demonstrated that extracts derived from different parts of *C. gigantea*, including leaves, flowers, and latex, exhibit strong insecticidal activity against mosquitoes such as *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Rajakumar & Rahuman, 2011). These mosquito species are responsible for transmitting lifethreatening diseases such as dengue, malaria, and filariasis, making C. gigantea a valuable plant for vector control strategies.

C. gigantea extracts have also shown remarkable toxicity against lepidopteran larvae, such as *Spodoptera litura* and

Helicoverpa armigera, which are major agricultural pests (Murugan et al., 2012). The latex-derived compounds interfere with larval growth and development, causing feeding inhibition, developmental deformities, and increased mortality rates. Additionally, the plant's secondary metabolites exhibit antifeedant and repellent properties, reducing crop damage by deterring insect herbivores.

- ***Acacia nilotica***

Acacia nilotica, commonly known as babul or gum arabic tree, is a leguminous plant widely recognized for its potent insecticidal, antimicrobial, and medicinal properties. The plant is particularly rich in tannins, flavonoids, alkaloids, and phenolic compounds, which contribute to its strong bioactivity against a range of insect pests. The high tannin content in A. nilotica plays a crucial role in disrupting insect digestive processes by forming complexes with proteins, thereby reducing nutrient absorption and leading to growth inhibition and mortality (Ali et al., 2018). The presence of these bioactive compounds makes *A. nilotica* a promising botanical pesticide, offering an eco-friendly alternative to conventional chemical insecticides.

Several studies have demonstrated the efficacy of *A. nilotica* extracts against key agricultural and vector pests. Its leaf and bark extracts have shown significant toxicity against cotton pests such as *Helicoverpa armigera and Spodoptera litura,* which are major threats to cotton crops (Kumar et al., 2019). Additionally, *A. nilotica* extracts exhibit strong repellent and larvicidal effects against stored grain pests like Sitophilus oryzae (rice weevil) and *Callosobruchus chinensis* (pulse beetle), reducing post-harvest losses in storage systems (Sharma & Gupta, 2021). Furthermore, aqueous and ethanol extracts of *A. nilotica* have demonstrated substantial larvicidal activity against mosquito species such as *Aedes aegypti and Culex quinquefasciatus,* which are responsible for transmitting diseases like dengue and filariasis (Ali et al., 2018). These findings highlight the potential of *A. nilotica* in sustainable pest management, supporting its integration into organic farming and vector control programs.

- ***Bauhinia blakeana***

Bauhinia blakeana, commonly known as the Hong Kong orchid tree, is a flowering plant that has gained attention for its bioactive properties, particularly in insect pest management. The floral extracts of *B. blakeana* are rich in secondary metabolites such as flavonoids, tannins, and alkaloids, which contribute to its strong insecticidal effects. Studies have demonstrated that these bioactive compounds interfere with insect growth and development, causing physiological disruptions that lead to high mortality rates in target pests (Ganesan et al., 2014). Among its notable applications, *B. blakeana* extracts have exhibited strong larvicidal activity against mosquito species, making it a promising natural alternative for controlling vector populations. Research indicates that the chloroform and acetone extract of *B. blakeana* flowers effectively target late third- and early fourth-instar mosquito larvae, causing significant mortality within a short exposure period. This suggests that the plant's bioactive compounds affect larval metabolism, ultimately leading to

death (Kumar et al., 2018). The eco-friendly nature of *B. blakeana* extracts presents an advantage over synthetic insecticides, as they pose minimal risk to non-target organisms and the environment. With further studies and formulation advancements, *B. blakeana* could be integrated into sustainable vector control programs, offering a plant-based alternative for mosquito management.

- ***Lantana camara***

Lantana camara, an invasive shrub known for its medicinal and pesticidal properties, has been extensively studied for its insecticidal potential. The plant produces a variety of bioactive compounds, including essential oils, flavonoids, alkaloids, and phenolic compounds, which contribute to its effectiveness against insect pests. Essential oils extracted from *L. camara* leaves have been shown to exhibit strong repellent and toxic effects against mosquitoes, particularly *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*—all known vectors of major diseases like dengue, malaria, and filariasis (Sharma & Kumar, 2020). Additionally, its insecticidal activity extends to agricultural pests such as aphids, whiteflies, and stored grain pests, making it a valuable natural alternative to synthetic pesticides. The mode of action of *L. camara* extracts is largely attributed to its phenolic and terpenoid compounds, which interfere with insect feeding, molting, and overall development. These compounds act as antifeedants, growth regulators, and neurotoxins, disrupting the normal physiology of target insects and ultimately causing mortality (Gupta et al., 2019). Studies have shown that the leaf extracts not only reduce pest populations but also deter future infestations by affecting insect oviposition behavior.

Efficacy of plant-derived biopesticides against insect pests

Plant-derived biopesticides have gained significant attention as effective alternatives to synthetic chemical pesticides, providing sustainable pest management solutions with minimal environmental impact. Numerous studies have demonstrated the efficacy of botanical extracts against a wide range of agricultural and public health pests, including mosquitoes, aphids, lepidopteran larvae, and stored grain pests. The bioactive compounds present in these plantbased formulations, such as alkaloids, flavonoids, terpenoids, and tannins, exhibit insecticidal, antifeedant, repellent, and ovicidal properties that disrupt the life cycle of various insect species (Rajakumar & Rahuman, 2011). The efficacy of these botanical pesticides depends on the plant species, extraction method, and target pest, with some showing remarkable pest control potential comparable to synthetic insecticides. Among the most effective plant-based biopesticides, *Azadirachta indica* (Neem) and *Calotropis gigantea* have demonstrated over 80% mortality rates against mosquito larvae, significantly reducing vector populations responsible for transmitting diseases like malaria and dengue (Rajakumar & Rahuman, 2011). Similarly, Vitex negundo and Lantana camara extracts have shown strong antifeedant and ovicidal effects against stored grain pests such as *Sitophilus oryzae* and *Callosobruchus chinensis*, effectively

preventing post-harvest losses (Tiwari et al., 2021). These plant-based biopesticides not only provide effective pest control but also reduce the risk of pesticide resistance and ecological damage associated with synthetic chemicals.

Conclusion

Plant-derived biopesticides offer an eco-friendly and sustainable alternative to synthetic pesticides, reducing environmental contamination, pesticide resistance, and health risks. The bioactive compounds in botanicals such as *Azadirachta indica, Calotropis gigantea, Vitex negundo, Lantana camara, Acacia nilotica*, and *Bauhinia blakeana* exhibit potent insecticidal, antifeedant, ovicidal, and repellent properties, making them valuable tools in Integrated Pest
Management (IPM).

Despite their proven efficacy, challenges such as formulation stability, extraction efficiency, and large-scale commercialization need to be addressed. Advancements in formulation technology and field validation will enhance their effectiveness and widespread adoption. Future research should focus on optimizing their application, exploring novel bioactive compounds, and promoting their integration into mainstream pest management. By overcoming these barriers, plant-derived biopesticides can contribute significantly to sustainable agriculture and public health pest control while preserving ecological balance.

References

1. Ali, M. Y., Rauf, A., Kaur, N., Khan, H., & Mubarak, M. S. (2018). Acacia species: A review of phytochemistry and pharmacological properties. Frontiers in Pharmacology, 9, 225. https://doi.org/10.3389/fphar.2018.00225
2. Ali, M. Y., Singh, S., & Akhtar, S. (2018). Efficacy of Acacia nilotica extract against stored grain pests. International Journal of Agricultural Sciences, 14(2), 110–118.
3. 3. Batish, D. R., Singh, H. P., Kohli, R. K., & Kaur, S. (2008). Eucalyptus essential oil as a natural pesticide. Bioresource Technology, 99(21), 8730–8734. https://doi.org/10.1016/j.biortech.2008.04.048
4. Ganesan, R., Baskar, K., & Vasantha-Srinivasan, P. (2014). Larvicidal efficacy of Bauhinia blakeana floral extracts against mosquito vectors. International Journal of Mosquito Research, 1(3), 1–5.
5. Ganesan, S., Suresh, K., & Kesavan, D. (2014). Phytochemical composition and pharmacological potential of Bauhinia species: A review. International Journal of Pharmaceutical Sciences and Research, 5(3), 829–838.
6. Gonzalez-Coloma, A., Reina, M., Díaz, C. E., Fraga, B. M., & Lopez-Rodríguez, M.(2020). Natural product-based biopesticides for insect control. Pest Management Science, 76(11), 3890–3897. https://doi.org/10.1002/ps.5921
7. Govindarajan, M., Benelli, G., & Rajeswary, M. (2016). Neem-based formulations for mosquito control. Parasitology Research, 115(12), 4415–4423.

https://doi.org/10.1007/s00436-016-5242-7

8. Gupta, R., Bajpai, P., & Sharma, A. (2019). Phytochemical composition and pesticidal potential of Lantana camara extracts. International Journal of Tropical Insect Science, 39(2), 167–175. https://doi.org/10.1017/S1742758419000246
9. Isman, M. B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture. Annual Review of Entomology, 51, 45–66. https://doi.org/10.1146/annurev.ento.51.110104.151146
10. Isman, M. B. (2020). Commercial development of plant essential oils and their constituents as active ingredients in bioinsecticides. Phytochemistry Reviews, 19(5), 235–244. https://doi.org/10.1007/s11101-019-09665-1
11. Jeyasankar, A., Jesudoss, V. A. S., & Parisad, V. C. (2022). Plant-derived secondary metabolites as eco-friendly biopesticides against insect pests: A review. Environmental Science and Pollution Research, 29(12), 17821–17845. https://doi.org/10.1007/s11356-021-17045-2
12. Kumar, P., Senthil-Nathan, S., & Prakash, S. (2018). Botanical larvicides and their impact on mosquito control: A review. Environmental Science and Pollution Research, 25(10), 9650–9661. https://doi.org/10.1007/s11356-018-1458-6
13. Mostafalou, S., & Abdollahi, M. (2017). Pesticides and human chronic diseases: Evidences, mechanisms, and perspectives. Toxicology and Applied Pharmacology, 325, 41–51. https://doi.org/10.1016/j.taap.2017.03.008
14. Murugan, K., Benelli, G., Panneerselvam, C., Subramaniam, J., & Nicoletti, M. (2012). Calotropis gigantea latex-based green synthesis of silver nanoparticles with high mosquito larvicidal potential. Parasitology Research, 111(2), 555–562. https://doi.org/10.1007/s00436-012-2870-y
15. Pavela, R., Maggi, F., Cianfaglione, K., Bruno, M., Benelli, G., & Canale, A. (2019). Efficacy of plant essential oils against insect pests and vectors. Industrial Crops and Products, 129, 446–456. https://doi.org/10.1016/j.indcrop.2018.12.073
16. Rajakumar, G., & Rahuman, A. A. (2011). Efficacy of Calotropis gigantea against mosquito vectors. Acta Tropica, 118(3), 196–203. https://doi.org/10.1016/j.actatropica.2011.03.008
17. Rajashekar, Y., Raghavendra, A., & Bakthavatsalam, N. (2017). Botanicals as grain protectants. Journal of Stored Products Research, 71, 34–40. https://doi.org/10.1016/j.jspr.2016.12.002
18. Rattan, R. S. (2010). Mechanism of action of insecticidal secondary metabolites of plant origin. Crop Protection, 29(9), 913–920.

https://doi.org/10.1016/j.cropro.2010.05.008

19. Saxena, R. C., & Khan, Z. R. (2021). Plant-derived insecticides in IPM. Journal of Pest Science, 94(3), 567–580. https://doi.org/10.1007/s10340-020-01300-6
20. Senthil-Nathan, S. (2019). A review of biopesticidal and insecticidal activity of plantderived secondary metabolites. Acta Tropica, 196, 78–89. https://doi.org/10.1016/j.actatropica.2019.05.017
21. Sengottayan, P., Kovendan, K., Murugan, K., & Vincent, S. (2013). Biopesticidal effect of plant-derived secondary metabolites against mosquito vectors. Parasitology Research, 112(4), 1331–1343. https://doi.org/10.1007/s00436-013-3279-7
22. Sharma, P., & Kumar, S. (2020). Insecticidal and repellent properties of Lantana camara against vector and agricultural pests. Journal of Pest Science, 93(4), 1125–1136. https://doi.org/10.1007/s10340-020-01243-1
23. Shewale, V. S., Khairmode, P. V., Lawand, S. T., & Bhosale, A. R. (2022). Antifeedant and insecticidal activity of different solvent extracts of Vitex negundo (L.) against cotton leafworm Spodoptera litura (Fab.). Asian Journal of Research in Zoology, 5(4), 1–8.
24. Lawand, S. T., & Shewale, V. S. (2024). Evaluation of antifeedant activity of Calotropis gigantea extracts against Spodoptera litura. Journal of Entomology and Zoology Studies, 12(3), 45–50.
25. Netam, V. S., & Shewale, V. S. (2022). Study of biology of Leucinodes orbonalis Guenee (EFSB) under laboratory condition fed on two different diets. Zeichen Journal, 8(6), 123–130.
26. Tiwari, N., Singh, P., & Gupta, S. (2021). Larvicidal and antifeedant properties of Vitex negundo against insect pests. Indian Journal of Entomology, 83(1), 45–52. https://doi.org/10.5958/0974-8172.2021.00009.3
27. Tiwari, R., Singh, S., & Mishra, K. (2021). Role of botanicals in insect pest management: A sustainable approach. Journal of Applied Entomology, 145(6), 475– 489. https://doi.org/10.1111/jen.12860

ABOUT THE EDITORS

Dr. Vijaya K. Nikam

She is currently working as Associate Professor, Department of Botany, Sadguru Gadage Maharaj College, Karad (Dist. Satara) - 415 124, Maharashtra, India. She accomplished M.Sc. (Botany with specialization in Plant Physiology) course in the Botany Department at Shivaji University, Kolhapur, in 2002. She had been awarded a Ph.D. from Shivaji University, Kolhapur, for her research work on the physiology of a medicinal plant, *Chlorophytum borivilianum*. She has been involved in teaching and research programs in senior colleges of the renowned educational institute Rayat Shikshan Sanstha (Maharashtra) for the last 14 years. She has co-authored 20 textbooks for undergraduate courses in botany. She also published a reference book on her research and a patent. She has successfully conducted two minor research projects in plant science. She has recognized research guide affiliated to Shivaji University, Kolhapur.

Dr. Swarupa V. Agnihotri

She is currently working as Assistant Professor in department of Botany, B. N. Bandodkar College of Science (Autonomous), Thane. She had done masters in Botany in specialization of Plant Physiology. Her area of research is 'Stress Physiology'. During M.phil and Ph.D. she had studied physiological effect of water logging stress on Finger millet. She has published book chapters and research papers in various national and international publications. She has more than ten years of teaching experience.

Dr. Pratishtha N. Nagane

Presently she is working as an Assistant Professor in the Department of Botany, Rayat Shikshan Sanstha's, Balwant College, Vita, Tal- Khanapur, Dist.- Sangali (MH.). She was completed her B. Sc. in Botany from The New College, Kolhapur, M.Sc. M. Phil. and Ph.D. from Department of Botany, Shivaji University Kolhapur. She has a teaching experience of 22 years at under graduation & post graduation. Dr. Nagane contributed a lot to the research field also, she published more than 15 Research Papers with impact factors, h-index 3, and more than 23 citations. Her area of research specialization is Mycology, Plant Pathology, Plant Physiology, Biotechnology, Cytology, Plant Breeding, etc. She published 9 book chapters so far and has been granted one patent by the Australian Government. She is recognized as a PG Teacher & Ph. D. guide by Savitribai Phule Pune University, Pune.

Dr. Manasi S. Patil

She is working as Assistant Professor, Department of Botany in Rayat Shikshan Sanstha's, Sadaguru Gadage Maharaj College Karad. She has received her doctoral degree from Shivaji University Kolhapur. She has more than 10 years teaching experience and research on Plant Physiology and Phytochemical study of medicinal plants. She is recognized Research Guide of Shivaji University Kolhapur for M.Phil/ Ph.D. Presently three students are pursuing for Ph. D under her guidance. She has completed One Minor Research Project sponsored by Research Initiation Scheme of Shivaji University, Kolhapur and Two research projects sponsored by SGM College under RUSA scheme. She has published Thirty eight research papers in reputed peer reviewed UGC care national and international journals. She has presented her research work in number of national and international seminars and conferences. She is a recipient of Young Plant Scientist award presented by Plantica foundation, Uttarakhand and also Best Teacher Award from Avishkar Foundation, Kolhapur. She has worked as resource person for many academic Programmes, like Avishkar, Inspire Project, Research Methodology etc.

Nature Light Publications

309 West 11, Manjari VSI Road, Manjari Bk.,
Haveli, Pune- 412 307.
Website: www.naturelightpublications.com
Email: naturelightpublications@gmail.com
Contact No: +919822489040 / 9922489040

NP
Nature Light Publications

Nature-34

ISBN: 978-81-981142-7-3

Price- 720/-