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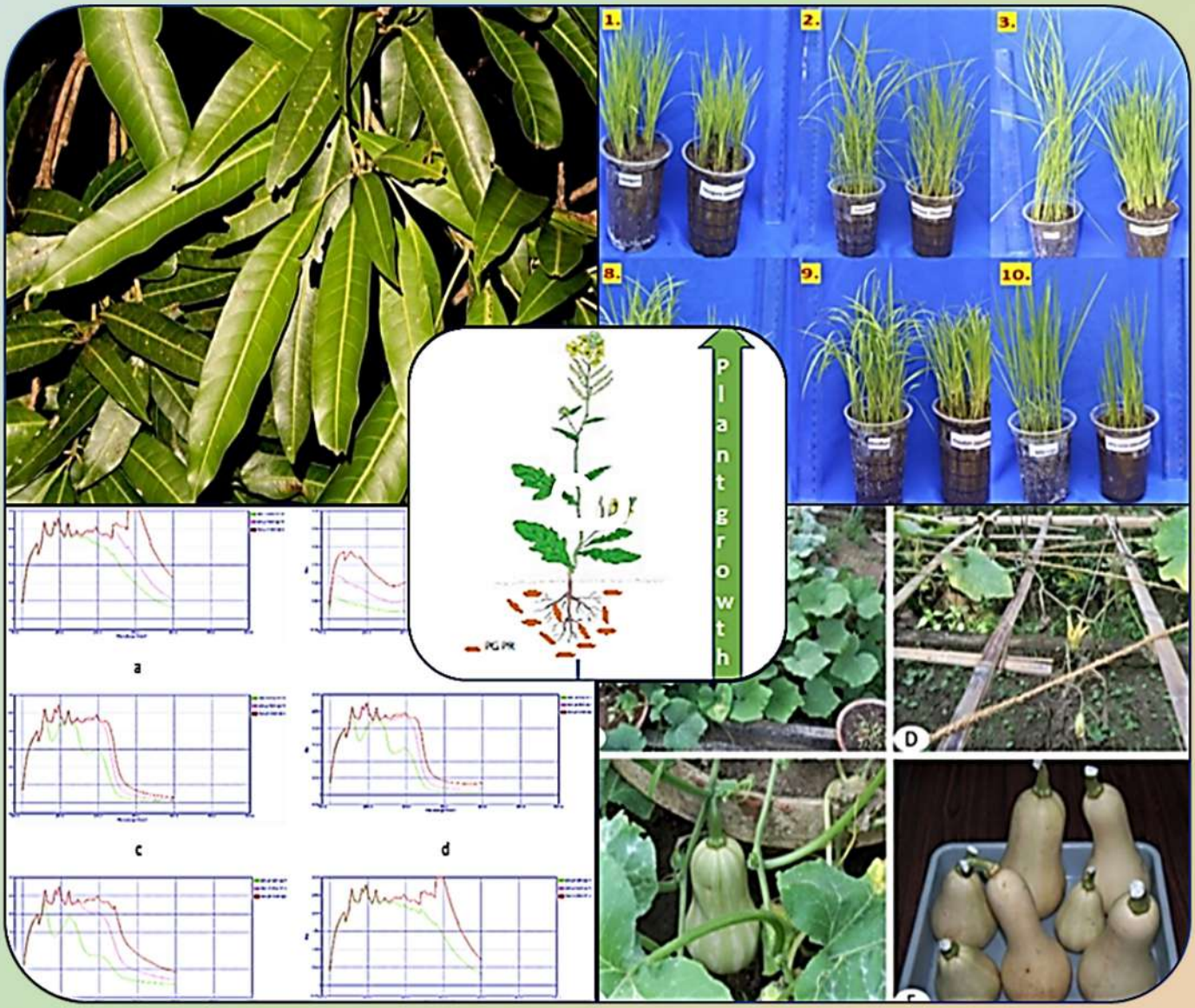

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Research Article

Effects of Automobile Emission on Morphology and Anatomy of the *Mangifera indica* L. Fruit Tree at District Samastipur, Bihar, India

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Abstract

Automobile transportation has been strongly associated with air pollution throughout various stages, like driving, fueling, production, and disposal. The emissions released from vehicle exhaust contain substantial quantities of detrimental gases, including carbon compounds, hydrocarbons, nitrogen oxides (NOx), volatile organic compounds (VOCs), and particulate matter. The reduced fertility of plants is a consequence of intense air pollution. The toxic gases emitted from vehicles endanger plants and cause changes in the physical and structural development of the *Mangifera indica* L. tree. This study aimed to explore the harmful effects of vehicle emissions on the physical structure and anatomy of Mango trees in the Samastipur district of Bihar, India. The experiment was based on the comparative study of under one year examination (2021-2022). Polluted and non-polluted sites were chosen and study was done. The result indicated that the automobile air pollution was hindering the healthy growth of *Mangifera indica* L.

Article info

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Introduction

Plants have ubiquitous and paramount significance in life of human since emergence of life on planet. Plants have been used as source of food, fodder, medicines, shelter, and aesthetics (Ishtiaq et al. 2017). Pollination is a distinctive feature of flowering plants. It operates by transferring pollen grains from one flower to the female stigma. The ultimate goal of all living organisms, including plants, is to generate offspring to ensure the continuity of the species. Angiosperms accomplish this by producing seeds. Seeds possess the genetic material required for the development of a new plant. The formation of seeds occurs through the transfer of pollen among the flowers belonging to the identical species. Pollen grains, characterized by their dusty and powdery texture, typically have a spherical shape and a diameter ranging from 25 to 50 micrometers. Pollen grains are responsible for fertilization and the fertility of plants. The amount of emissions in the air has risen because of the exponential growth in industrialization and urbanization (Shakeel et al. 2022). Although rapid industrialization and urbanization has resulted in a boom of the economy of our country, it has also contributed significantly in enhancing the problems of plant health. Vehicular exhaust adds up huge amounts of soot particles, smoke, poisonous gases like SO_2, NO_2, CO_2, and VOCs etc., heavy metals and organic molecules on the roads all over the world. All these air pollutants are known to produce adverse effects on the health of plants, animals and humans (Kaur and Nagpal 2017). Plants acts as a natural filters of air pollution either by stomatal uptake or the deposition on the surfaces of leaves. Air pollutant absorbed through stomata undergoes various interactions and enhances the tolerance capacity of the plant to fight against the stress. All these interactions lead to different biochemical, physiological and anatomical responses in plants (Tak and Kakde 2020). Air pollution due to vehicle exhaust causes irregularity in anthers, decreases the number and masculine infertility. The chemical pollutants produced by industries and traffic

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emissions not only put damaging effects on morphological and physiological parameters of plants but also comprise alterations and chromosomal impairment. These irregularities are contingent on ecological and hereditary issues and may eventually go to alteration in propagative capability of plant species. The air pollution adversely impacts the photosynthetic pigment and consequently reduces productivity. Leaf chlorophyll, carotene, proline, and proteins are found to be influenced adversely by air pollutants (Singh et al 2020). The air pollution influences stomatal functioning and leaf thickness of urban plantation. Ascorbic acid is one of the strong indicators of stress and is found to be related to air pollution levels Several investigations have indicated vehicular emission effects on plant leaves stomatal apertures, viability of pollen and development of plants (Leghari et al 2018). Many studies have shown effects of automobile emissions on leaves, stomatal apertures, and pollen viability and growth patterns of plants (Kaur and Nagpal 2017). Chlorophyll measurement is an important tool to evaluate the effects of air pollutants on plants as it plays an important role in plant metabolism and any reduction in chlorophyll content corresponds directly to plant growth (Wagh et al. 2006). Leaf chlorophyll content and carotenoids thus can provide valuable information about physiological status of plants (Joshi and Swamy2009). When flower buds release pollen grains into the air that is contaminated with pollutants, the grains absorb both moisture and specific pollutants. Pollen grains' viability and plant reproduction are adversely impacted by the existence of contaminants such as heavy metals, fluorides, and pesticides on their surfaces. Palynoindication, as one of the promising and new methods of the assessment of quality of the environment, is based on determination of the proportions of normal and abnormal pollen, when the quality of environment assessed by the share of normal pollen in the samples (Vasilevskaya 2022). The main aim and objective of this study was to explore the impact of automobile emissions on the physical characteristics and internal structure of *Mangifera Indica L*. fruit trees in the Samastipur district, Bihar. This work aimed to demonstrate the extent of damage caused by vehicle exhaust pollution on the fruit-bearing plants *of M. indica L*. by examining various aspects of plant organs.

Materials and Methods

Study Area

Urban areas of Samastipur district are extremely very much polluted due to vehicular exhaust pollution (Table 1). Trees act as an important and cost-effective solution to combat air pollution. However, different trees have varying levels of combating capacity distinguished with their adaptation and mitigation potential. Urban green belts or urban roadside plantation act as a sink for particulate and gaseous emissions in a city and are often termed as “the lungs of the City”. Trees act as a sink for CO_2 by fixing carbon during photosynthesis and storing carbon as biomass. Thus, the roadside plantations are expected to combat air pollution (Singh et al. 2020). The city area of Samastipur town and the rural village of the Satmalpur was selected to calculate the effect of automobile emissions on pollen grains of *M. indica* L (Fig. 1). The distance between Samastipur towns to the Satmalpur village is approximately 7 km. Mango, Lychee, Banana and Guava trees and some flowering plants like hibiscus, sunflower and rose, orchid was grown.

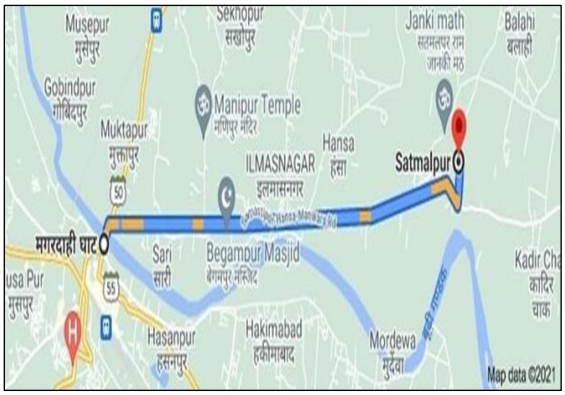

Figure 1. Google Map of Study Area

Table 1. The Air Quality Index of Samastipur

Level of Air pollution	Air quality index	Main pollutant
Very unhealthy	140* US AQI	PM2.5

The climate of the Study Area

Indo-Gangetic Plains (IGP) witnessed the green revolution and is amongst the world’s most fertile alluvium **(**Mishra et al. 2022). The area of samastipur district is 2,904 square kilometers. The samastipur is situated in the Bihar state of India. It is surrounded by different geographical features. It is bordered by the Bagmati River in the north, which separates it from Darbhanga district. To the west, it shares boundaries with Vaishali and a portion of the Muzaffarpur district. The river Ganges marks the southern border, while the eastern boundary is formed by Begusarai and a portion of the Khagaria

district. Main administrative center of the district is situated in the town of Samastipur. The area is crisscrossed by multiple rivers, namely Budhi Gandak, Baya, Kosi, Kamla, Kareh, Jhamwari, and Balan, all of which flow into the Burhi Gandak River. Furthermore, the Ganges runs alongside the district's southern border. Samastipur is positioned at 25°55'N latitude and 85°50'E longitude. Agriculture serves as the predominant economic activity in the district, supporting around 83% of the working population. Floriculture is also a significant source of income. Samastipur is situated in the North-West Alluvial plains, which is designated as agro-ecological zone-I in the state. The region is renowned for its fertile alluvial soil and the cultivation of Rabi crops. Additionally, Samastipur has historically been a prominent hub for the indigo industry. This region also cultivates wheat, pulses, and edible oil seeds. The soil's texture is sandy loamy and has a sufficient quantity of organic material, which makes it appropriate for cultivating vegetables and spices. The Samastipur district is renowned for its spice production, particularly Turmeric and Garlic. The Turmeric cultivated in this region has the capacity to become a well-known brand worldwide because of its abundant curcumin levels.

Plant Material Collected

The mango (*Mangifera indica L.*) is native to South and Southeast Asia, from where it has been distributed worldwide to become one of the most cultivated fruits in the tropics. It is the national fruit of India. In India, harvest and sale of mangoes take place during March-May and the fruits have high economic value in India **(**Saran et al 2015). Flower sample of *M indica* L. were gathered from both the contaminated and uncontaminated regions within the Samastipur district (Fig. 2). For the polluted area, the sample was taken from the Samastipur Magardahi Ghat road which is a heavily polluted area of the district, for non-polluted areas, Satmalpur village was selected for sample collection which is 7 km away from the Ghat road (Table 2).

Table 2. Name and description of site areas

Site Areas	Distances
Samastipur Magardaahi ghat (polluted area)	15 Km away from city center
Satmalpure Village area (non-polluted area)	7 Km away from Magardaahi ghat

Soil characteristics were similar of both sites. Anthers were collected from plants in a random manner. Statistical analysis was done. The soil samples were taken simultaneously from both the polluted site and the control site. In Table. 3, it is indicated that the soil pH at the polluted site was recorded as 7.2, whereas at the control site, it was slightly lower at 7.1. Additionally, the specific conductivity of the polluted site soil was measured to be 5.30 × 10-4, while at the control site, it was slightly lower at 5.20 × 10-4. The organic matter content in the polluted site soil was found to be 0.3, whereas at the control site, it was slightly higher at 0.4. Both the polluted and control sites exhibited alluvial soil texture. Table 1 shows the unhealthy level of air pollution for plants including all living beings." with a recorded Air Quality Index of 140*. The PM 2.5 was main pollutant. It is a fine particulate matter with a diameter of 2.5 micrometers or less than 2.5 micrometers.

Air Sampling and Analysis

Air quality index (AQI) is used worldwide to inform the public about levels of air pollution (degradation or improvement) and associated to different biological effects. Different types of anthropogenic activity mainly transportation have an enormous impact on the ambient air quality in several ways. The higher the AQI value, the greater the level of air pollution and greater the health concern (Shweta and Jain 2018). Air sampling and analysis were conducted at selected sites. Duration of sampling and analysis was done for one year viz. December 2021 to November 2022. The air quality was analyzed considering parameter like SO_2, NO_2, RSPM and SPM. The State Pollution Control Board in Patna, Bihar monitored air pollutants by collecting

Table 3. Soil samples of the chosen site areas

Experimental Site	Soil Parameter			
	pH level of Soil	Specified conductivity	Organic matters	Texture of the soil
Polluted Site	7.2	5.30×10^{-4}	0.3	*Alluvial Soil*
Control Site	7.1	5.20×10^{-4}	0.4	*Alluvial Soil*

Figure 2. Study Area (The Polluted site and the non-polluted Sites)

data from the SPCB. Air pollutants were quantified at the control site utilizing the RDS APM 460 apparatus. This involved drawing air into a suitable reagent for a period of 24 hours, repeated every 30 days. The APM 460 Respirable Dust Sampler was employed to examine the amount of Suspended Particulate Matter and Respirable Suspended Particulate Matter. It typically functioned with a flow rate that varied between 1.0 and 1.5 cubic meters per minute. Standard methods were followed using preweighed glass fiber filters (GF/A) from the brand Whatman. For the collection of SO_2 (Sulfur Dioxide) and NO_2 (Nitrogen Dioxide), the sample was fizzed through a specialized absorbing solution containing sodium tetrachloromercuate for SO_2 and sodium hydroxide for NOx, at an average flow rate of 0.2-0.5 min^{-1}. Samples collected in the infringe area were promptly placed in the cold closet and transferred to a refrigerator until they were ready to be analyzed.

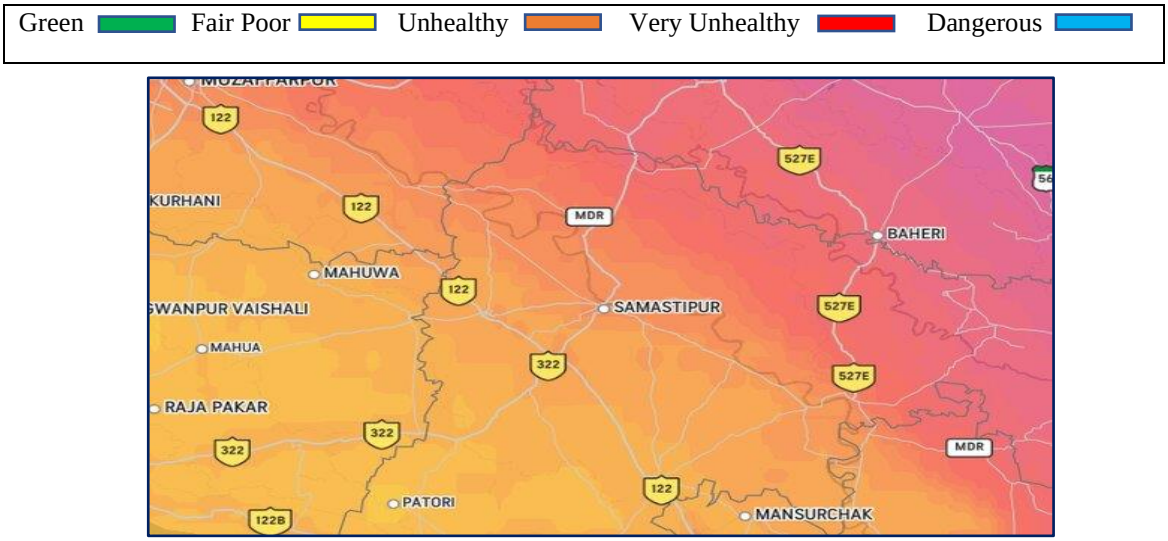

Figure 3. Present air quality of Samastipur

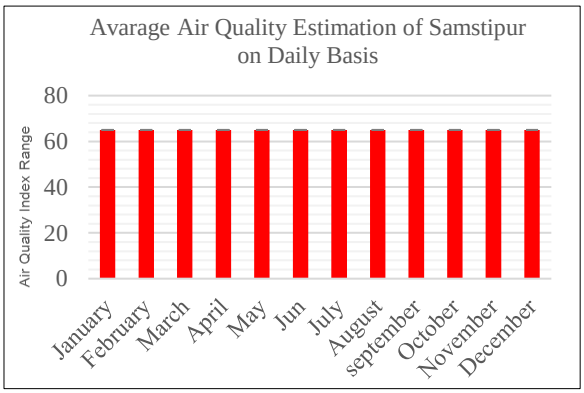

Figure 4. Chart shows the Average Air Quality Index of the City (Daily Basis)

They were then brought to the laboratory on the same day and analyzed immediately to determine their concentration levels. The concentration of NO_x was measured using the Modified Jacobs-Hochheiser method (1958), while SO_2 was measured using the Modified west and Geake method (1956). According to the graph, the air quality in Samastipur is heavily polluted and poses a health risk to sensitive groups, including plants. The measurements of Suspended Particulate Matter and Respirable Suspended Particulate Matter were obtained using filter paper methods. The sampling apparatus was positioned 2 meters above the ground surface. On the basis of collected data on pollutants, the air quality index was calculated.

Sampling and Analysis of plants

Plant sample collection was done to evaluate the consequences of automobile emissions on the morphology and anatomy of the *Mangifera indica* L. fruit tree at district samastipur. Matured leaves or leaflets of the same age and size were collected from the chosen tree plants at the morning time (5am-9 am) and having the same ecological conditions of

soil, water and light with extreme caution. Selected plant samples collected from the respective sites were used and wrapped in polythene bags (to minimize evaporation losses). It was brought to the laboratory for further examination. The fresh leaf sample was analyzed for various morphological and anatomical parameters. Air quality parameters were also analyzed during the study period. Fresh leaf samples were obtained from both polluted and control sites, collected from mature and well-aged trees. The samples were gathered from trees, specifically at an elevation ranging from 5 to 8 feet above the earth's surface. The standardized procedure established by Maclachlan and Zalik in 1963 was used to evaluate the amount of chlorophyll-a, chlorophyllv-b, and total chlorophyll. Carotenoid levels were determined using the method described by Duxbury and Yentsch in 1956. Ascorbic acid level was measured utilizing the technique outlined by Sadashivam and Manikam in 1991. For each date of sampling, ten samples were analyzed from every Mango tree.

Morphological changes in plant leaves due to pollution

The leaves of *M. indica* plants of the polluted area were found to be discolored, dusty, and wrinkled when compared to non-polluted area.

Leaf color **-:** The color of leaves was visually observed on randomly collected samples from both reference sites and polluted sites to record their leaf color.

The length and the breadth of the leaf **-:** The ruler was used to measure the size of the leaves selected from both the reference and polluted sites.

Leaf area **-:** The leaf area was determined by measuring the length and width of the leaves and subsequently multiplying those measurements by a fixed factor. Constant was obtained by comparing the leaf area calculated through the paper graph method, which involves measuring the leaf area using a graph paper, with the measured length and width of the leaf.

$$L \times B \times C = \text{Leaf area (A) or } C = A \div L \times B$$

Where, L-length of leaf, B-breadth of leaf, C-constant of the area of leaf, A-the leaf area

The length of the Petiole **-:** The length of the petiole was measured with the help of a ruler.

Micro-Morphological Parameters Examination

Microscopic investigations were performed by preparing a temporary slide of the peeled epidermis from a fully developed leaf. The procedure involved the utilization of a thin needle and a set of forceps to delicately extract the outermost layer of skin, known as the epidermis. The epidermis was then placed in water on a glass slide. Slide was then examined under a light microscope at a magnification of 400X. To determine the stomatal density and epidermal cells the following formula was used.

Number of stomata/mm^2 = the average number of stomata per microscopic field × the total number of microscopic field/mm^2

Number of epidermal cells/mm^2 = the Average number of epidermal cells per microscopic field × the total number of microscopic field/mm^2

Stomatal index (S.I.)

To determine the stomatal indices of specific plant species by measuring the density of stomata per given area and the density of epidermal cells per the same area (Salisbury's formula from1927) was employed. This formula allowed for the quantification of stomatal indices, which provide valuable insights into the plant's adaptation to various environmental conditions**.**

$$\textbf{S. I.} = S \div (S + E) \times 100$$

Where, S.I. -: stomatal index, S- number of stomata per unit area, E- number of epidermal cells per unit area, 100- percentage proportion

Stomatal and epidermal cells size **-:** The ocular micrometer was utilized to conduct the measurements, and its calibration was achieved by comparing it to a stage micrometer under a magnification of 400X.

Anatomical Parameters

Anatomical investigations were carried out by obtaining slender vertical slices from chosen leaves, taken from both uncontaminated and contaminated locations. These slices were placed in water on a glass slide and examined under a magnification of 100X. The objective was to measure the thickness in a specific area using a standardized ocular micrometer. Twenty observations were made from each of the selected plants to represent the results.

1. Midrib region
2. Midrib adjoining region
3. The Palisade tissues
4. Spongy tissue
5. Upper epidermis
6. Lower epidermis
7. Vascular bundles

Data analysis: SAS (version 9.4) software was used for arithmetical analysis of the data.

Results

The evolution of plants biomarkers uses as a tool to monitor and evaluate the environmental state is closely linked to progress in our knowledge of molecular toxicity mechanisms of pollutants in different plant species in the ecosystem (Azzazy 2016). Air pollution control and prevention are critical for human survival, but they are also an essential first step in the development of the economy (Sneha and Sunil 2023). The air quality at the chosen sites were observed to record the impact of air pollutants on plants (Fig. 3, 4, 5). The physical characteristics, microscopic features, and internal structures of the plant samples were examined and analyzed. The findings of the air quality assessment were shown in Table 4 and the discussion was based on the analysis of different morphological, micro-morphological, and anatomical characteristics of chosen plant species from both the reference and polluted sites.

Table 4. Seasonal variations of SO2, NO2, RSPM, SPM, TSPM at study sites in Samastipur for the study period (2021-2022)

Sites	Seasons	Parameters	Minimum (µg/m3)	Maximum (µg/m3)	Average
Magardaahi ghat road in samastipure (Polluted Area)	Summer	SO_2	26.40	35.50	32.40±6.45
		NO_2	33.35	65.03	48.35±15.91
		RSPM	75.90	92.40	86.50±6.92
		SPM	103.65	123.74	130.92±11.02
		TSPM	240.15	295.75	271.12±24.69
	Monsoon	SO_2	21.90	37.27	30.09±7.16
		NO_2	25.84	40.85	34.46±7.75
		RSPM	57.78	75.99	68.62±8.65
		TSPM	135.65	175.56	150.15±25.35
		SPM	280.06	407.20	355.70±65.25
	Winter	SO_2	35.75	45.94	41.99±4.11
		NO_2	20.15	36.02	25.21±6.93
		RSPM	284.50	340.01	316.17±28.30
		SPM	142.65	175.29	115.75±25.48
		TSPM	135.65	175.58	150.11±21.35
Satmalpure Village (Non-polluted Area)	Summer	SO_2	7.89	10.99	9.29±1.57
		NO_2	10	22.51	15.56±6.37
		RSPM	79.91	91.97	87.01±6.31
		TSPM	184.55	214.90	199.98±15.18
		SPM	140.67	176.28	153.37±19.88
	Monsoon	SO_2	5.79	8.26	6.86±1.26
		NO_2	5.42	9.17	7.08±1.91
		RSPM	113.30	183.33	143.67±35.93
		TSPM	260.18	336.23	307.43±41.25
		SPM	58.78	75.96	66.68±8.65
	Winter	SO_2	11.63	15	12.73±1.2
		NO_2	19.17	35.01	27.23±7.92
		RSPM	113.30	183.33	143.67±35.93
		TSPM	152.38	197.37	174.42±22.51
		SPM	152.38	197.37	174.42±22.51

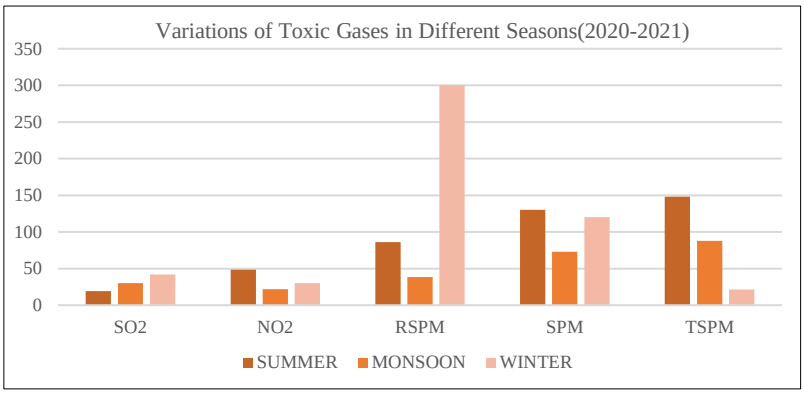

Figure 5. Graph represents variation of toxic gases in different seasons in polluted area of Magadaahi ghat road of Samastipur

The effect of pollutants on plants

Morphological Parameters

*Leaf colour***:** The Table 5 & 6 provides information about the growth of *Mangifera indica* L. (Mango tree) across different seasons and its appearance in both polluted and non-polluted sites (Fig. 6-10). In summer and monsoon seasons, the plant appears as "Dark Green" in both polluted and non-polluted sites. However, during the winter season, it exhibits different characteristics in polluted sites, it appears as "Light Green." In non-polluted sites, it appears as "Light Green with Brown Spots."

Table 5. Leaf colour of *M. indica L.* plant species during different seasons from reference and polluted site

Plant Species	Season	Polluted site	Non- Polluted Sites
Mangifera indica L.	Summer	Dark Green	Dark green
	Monsoon	Dark green	Dark green
	Winter	Light green	Light green with Brown spots

Figure 6. Dark green leaves color of *M. indica* in Control and polluted sites in summer and Monsoon season

Figure 7. Light green leaves colour of *M. indica* in polluted sites in winter season

Figure 8. Brown spotted leaves of *Mangifera indica* L (non-polluted site)

Table 6. Morphological parameter of *M. indica* L. plant species during different seasons from reference and polluted site (Mean ± S.D.)

Morphological parameter	Season	Polluted site	Non-polluted site	Decrease (%)
Leaf Length (cm)	Summer	37.133±0.23	38.433±0.45	3.38
	Monsoon	37.6 ±0.26	36.633± 0.5	2.57
	Winter	16.533±0.611	16.233 ±0.49	1.81
Leaf Breadth(cm)	Summer	21.5 ±0.56	20.767±0.40	3.41
	Monsoon	21.5 ±0.56	20.767±0.40	3.41
	Winter	20.633±0.90	19.933±0.60	3.39
Leaf Area	Summer	95.667 ±3.05	100.67±6.03	4.97
	Monsoon	100.33 ±4.04	108.33±8.02	7.38
	Winter	93.67 ±2.52	98.667±2.52	5.07
Petiole Length	Summer	12.467 ± 0.15^{Ay}	12.967 ± 0.15^{Ax}	3.86
	Monsoon	13.333 ± 0.40^{B}	13.867 ± 0.15^{B}	3.85
	Winter	11.267 ± 0.25^{Cy}	12.033 ± 0.35^{Cx}	6.37
Micro-Morphological Parameters				
Stomatal Width	Summer	13.9 ± 2.40^{y}	16.68 ± 0.00^{x}	16.67
	Monsoon	16.68±0.00	18.07 ±2.41	7.69
	Winter	11.12 ± 2.41^{y}	12.51 ± 0.00^{x}	11.11
Stomatal size	Summer	231.85 ± 0.00^{Ay}	324.59 ± 0.00^{Ax}	28.57
	Monsoon	276.29 ± 5.79^{Ax}	121.72 ± 0.00^{Ay}	55.94
	Winter	328.46 ± 5.80^{Bx}	200.94 ± 5.80^{By}	38.82
Stomatal number (per mm^2)	Summer	33 ± 3^{Ay}	39 ± 3^{ABx}	15.38
	Monsoon	38 ± 1.73^{B}	40 ± 1.73^{B}	5.00
	Winter	48 ± 6^{Bx}	31 ± 4.58^{Ay}	35.42
Epidermal cell length	Summer	18.21 ± 0.24^{Ay}	19.04 ± 0.24^{Ax}	4.38
	Monsoon	19.32 ± 0.24^{A}	18.90 ± 0.24^{B}	2.16
	Winter	26.41 ± 0.87^{ABy}	29.05 ± 0.64^{x}	9.09
Epidermal cell width	Summer	12.51 ± 0.00^{y}	16.68 ± 0.00^{x}	25.00
	Monsoon	15.29 ±2.41	18.07 ±2.41	15.38
	Winter	12.51 ± 0.00^{y}	18.07 ± 2.41^{x}	30.77
Epidermal cell size	Summer	16.68 ± 0.00^{Ay}	19.46 ± 2.41^{x}	14.29
	Monsoon	227.79 ± 0.00^{Ay}	317.64 ± 0.00^{ABx}	28.28
	Winter	224.32 ± 0.00^{A}	258.90±1.53	13.36
Epidermal cell number (per mm2)	Summer	79 ± 4.58^{Ay}	115 ± 21.07^{Ax}	31.30
	Monsoon	35 ± 2.65^{B}	104 ± 10.54^{A}	10.58
	Winter	56 ± 7.55^{Ay}	$72\pm 6B^{x}$	22.22
Stomatal index value	Summer	29.48 ± 2.79^{A}	25.58 ± 2.84	-15.27*
	Monsoon	22.82 ± 2.31^{AB}	22.30 ±1.26	-2.30*
	Winter	26.59 ± 2.79^{B}	25.36±0.63	-4.83*

***Values bearing different superscripts in small alphabets across the columns (x, y, z) differ significantly ($p\leq0.05$) and depict variations between reference and polluted site.

Values bearing different superscript in capital alphabets (A, B, C) down the row differ significantly ($p\leq0.05$) and depict variations among summer, monsoon and winter seasons.

Decrease (%) was calculated as: {(Reference site value- Polluted site value)/ Reference site value} ×100

Values bearing different superscripts in small alphabets across the columns (x, y, and z) differ significantly ($p\leq0.05$) and depict variations between reference and polluted site.

Value in asterisk indicate increase in the value of stomatal index in (%)

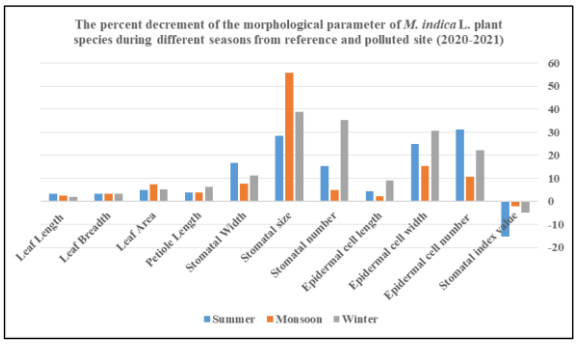

Figure 9. Graph shows the percent decrement of all morphological parameter of *M. indica* L. tree from all seasons of the year (2021-2022)

Figure 10. Shows the length of leaves taken from the Magardaahi ghat road (polluted Area)

Anatomical parameters

Table 7. Anatomical features of *M. indica L*. during different seasons from reference and polluted site (Mean ± S.D.)

Anatomical parameter	Season	Polluted site	Non- polluted site	Decrease (%)
Midrib region	Summer	1344 ± 0.00^{Ay}	1354.67 ± 9.23^{Ax}	0.79
	Monsoon	1370.67 ± 9.24^{Ax}	1354.67 ± 9.23^{Ay}	1.17
	Winter	1317.33 ± 9.23^{By}	1338.67 ± 9.23^{Bx}	1.59
Midrib adjoining region	Summer	352 ± 0.00^{Ay}	362.67 ± 9.23^{Ax}	2.94
	Monsoon	1338.67 ± 9.23^{Bx}	1317.33 ± 9.23^{By}	1.59
	Winter	325.33 ± 9.24^{Cy}	346.67 ± 9.24^{Cx}	6.15
The Palisade tissue thickness	Summer	117.33 ± 9.23^{Ay}	133.33 ± 9.23^{Ax}	12.00
	Monsoon	133.33 ± 9.24^{By}	144 ± 0.00^{Ax}	7.41
	Winter	106.67 ± 9.23^{Ay}	128 ± 0.00^{Bx}	16.67
Spongy tissue	Summer	197.33 ± 9.2^{Ay}	218.67 ± 9.23^{Ax}	9.76
	Monsoon	234.67 ± 9.24^{By}	250.67 ± 9.24^{Bx}	6.38
	Winter	170.67 ± 9.23^{Cy}	181.33 ± 9.2^{Cx}	5.88
Upper epidermis	Summer	197.33 ± 93.23^{Ay}	208 ± 0.00^{Ax}	5.13
	Monsoon	170.67 ± 9.23^{Cy}	192 ± 0.00^{Cx}	11.11
	Winter	117.33 ± 9.23^{B}	138.67 ± 9.24^{B}	15.38
Lower epidermis	Summer	245.33 ± 9.24^{Ay}	256 ± 0.00^{Ax}	4.17
	Monsoon	250.67 ± 9.23^{Ay}	266.67 ± 9.23^{Bx}	6.00
	Winter	202.67 ± 18.47^{By}	234.67 ± 9.24^{Cx}	13.64
Vascular bundles	Summer	522.67 ± 9.23^{Ay}	549.33 ± 9.23^{Ax}	4.85
	Monsoon	565.33 ± 9.2^{By}	586.67 ± 9.2^{Bx}	3.64
	Winter	490.67 ± 9.23^{Cy}	538.67 ± 9.24^{Ax}	8.91

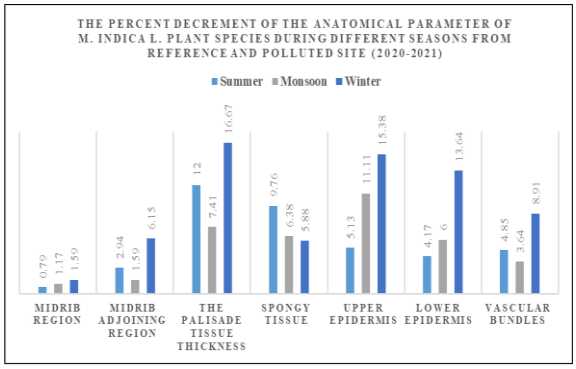

Figure 11. Graph shows the percent decrement of all anatomical parameter of *M. indica* L. tree from all seasons of the year (2021-2022)

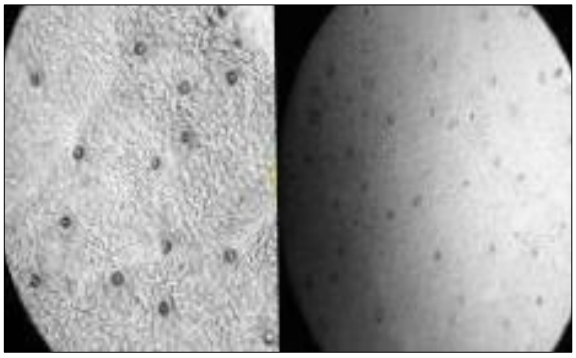

Figure 12. The leaf structure of Mangifera indica from the polluted region, under Light Microscope exhibits abnormal stomatal shapes or irregular stomatal structures. In contrast, the leaves from the unpolluted area display normal stomatal features.

Discussion

The Table 4 provides air quality data for two different locations, Magardaahi Ghat Road in Samastipur (a polluted area) and Satmalpure Village (a non-polluted area), for different seasons (summer, Monsoon, and winter) and various air quality parameters (SO_2, NO_2, RSPM, SPM, and TSPM). The parameters are measured in micrograms per cubic meter, and the table presents the minimum, maximum, and average values for each parameter in each season for both locations. At Polluted Area (Magardaahi Ghat Road) in summer SO_2 levels range from a minimum of 26.40 µg/m³ to a maximum of 35.50 µg/m³, with an average of 32.40 µg/m³. NO_2 levels range from a minimum of 33.35 µg/m³ to a maximum of 65.03 µg/m³, with an average of 48.35 µg/m³. RSPM levels range from a minimum of 75.90 µg/m³ to a maximum of 92.40 µg/m³, with an average of 86.50 µg/m³. SPM levels range from a minimum of 103.65 µg/m³ to a maximum of 123.74 µg/m³, with an average of 130.92 µg/m³.

TSPM levels range from a minimum of 240.15 µg/m³ to a maximum of 295.75 µg/m³, with an average of 271.12 µg/m³. In Monsoon, SO_2 levels range from a minimum of 21.90 µg/m³ to a maximum of 37.27 µg/m³, with an average of 30.09 µg/m³. NO_2 levels range from a minimum of 25.84 µg/m³ to a maximum of 40.85 µg/m³, with an average of 34.46 µg/m³. RSPM levels range from a minimum of 57.78 µg/m³ to a maximum of 75.99 µg/m³, with an average of 68.62 µg/m³. SPM levels range from a minimum of 280.06 µg/m³ to a maximum of 407.20 µg/m³, with an average of 355.70 µg/m³. TSPM levels range from a minimum of 135.65 µg/m³ to a maximum of 175.56 µg/m³, with an average of 150.15 µg/m³. In winter, SO_2 levels range from a minimum of 35.75 µg/m³ to a maximum of 45.94 µg/m³, with an average of 41.99 µg/m³. NO_2 levels range from a minimum of 20.15 µg/m³ to a maximum of 36.02 µg/m³, with an average of 25.21 µg/m³. RSPM levels range from a minimum of 284.50 µg/m³ to a maximum of 340.01 µg/m³, with an average of 316.17 µg/m³. SPM levels range from a minimum of 142.65 µg/m³ to a maximum of 175.29 µg/m³, with an average of 115.75 µg/m³. TSPM levels range from a minimum of 135.65 µg/m³ to a maximum of 175.58 µg/m³, with an average of 150.11 µg/m³. At Non-polluted Area of Satmalpure Village, In Summer, SO_2 levels range from a minimum of 7.89 µg/m³ to a maximum of 10.99 µg/m³, with an average of 9.29 µg/m³, NO_2 levels range from a minimum of 10 µg/m³ to a maximum of 22.51 µg/m³, with an average of 15.56 µg/m³. RSPM levels range from a minimum of 79.91 µg/m³ to a maximum of 91.97 µg/m³, with an average of 87.01 µg/m³. TSPM levels range from a minimum of 184.55 µg/m³ to a maximum of 214.90 µg/m³, with an average of 199.98 µg/m³. SPM levels range from a minimum of 140.67 µg/m³ to a maximum of 176.28 µg/m³, with an average of 153.37 µg/m³. In Monsoon, SO_2 levels range from a minimum of 5.79 µg/m³ to a maximum of 8.26 µg/m³, with an average of 6.86 µg/m³. NO_2 levels range from a minimum of 5.42 µg/m³ to a maximum of 9.17 µg/m³, with an average of 7.08 µg/m³. RSPM levels range from a minimum of 113.30 µg/m³ to a maximum of 183.33 µg/m³, with an average of 143.67 µg/m³. TSPM levels range from a minimum of 260.18 µg/m³ to a maximum of 336.23 µg/m³, with an average of 307.43 µg/m³. SPM levels range from a minimum of 58.78 µg/m³ to a maximum of 75.96 µg/m³, with an average of 66.68 µg/m³. In winter, SO_2 levels range from a minimum of 11.63 µg/m³ to a maximum of 15.00 µg/m³, with an

average of 12.73 µg/m³, In NO_2 levels range from a minimum of 19.17 µg/m³ to a maximum of 35.01 µg/m³, with an average of 27.23 µg/m³. RSPM levels range from a minimum of 113.30 µg/m³ to a maximum of 183.33 µg/m³, with an average of 143.67 µg/m³.

TSPM levels range from a minimum of 152.38 µg/m³ to a maximum of 197.37 µg/m³, with an average of 174.42 µg/m³. SPM levels range from a minimum of 152.38 µg/m³ to a maximum of 197.37 µg/m³, with an average of 174.42 µg/m³. These values represent the air quality in the specified areas and seasons, with higher values indicating more pollution in the air. Air pollution has increased tremendously that is affecting the proper growth of plants in its vicinity. The rapid addition of toxic substances to environment is responsible for altering the ecosystem. Plants growing in heavy trafficular area are thus exposed to variety of pollutants such as SMP, RSMP, NOx, & SO_2 etc. (Giri et al 2013). Leaf is the most sensitive part to be affected by air pollutants instead of all other plant parts such as stem and roots. The sensitivity rests on the fact that the major portions of the important physiological processes are concerned with leaf. Therefore, the leaf at its various stages of development, serves as a good indicator to air pollutants. Pollutants came from the auto emission can directly affect the plant by entering in to the leaf, destroying individual cells, and reducing the plant ability to produce food (Lighari and Zaidi 2013). The Table 6, presents morphological and micro-morphological parameters of a plant, measured during different seasons in both polluted and non-polluted sites. In morphological Parameters the length of leaves was measured in centimeters during summer, monsoon, and winter. In the polluted site, leaf length decreased by 3.38% in summer, 2.57% in monsoon, and 1.81% in winter compared to the non-polluted site. Leaf breadth was measured in centimeters during the same seasons. It showed a decrease of 3.41% in both summer and monsoon, and 3.39% in winter in the polluted site compared to the non-polluted site. Leaf area was measured in square centimeters during the seasons. In the polluted site, leaf area decreased by 4.97% in summer, increased by 7.38% in monsoon, and decreased by 5.07% in winter compared to the non-polluted site. Petiole length was measured in centimeters during the seasons. In the polluted site, it decreased by 3.86% in summer, 3.85% in monsoon, and 6.37% in winter compared to the non-polluted site. In Micro-Morphological Parameters stomatal width was measured in micrometers. In the polluted site, it decreased by 16.67% in summer, increased by 7.69% in monsoon, and decreased by 11.11% in winter compared to the non-polluted site. Stomatal size was measured in square micrometers. In the polluted site, it increased by 28.57% in summer, decreased by 55.94% in monsoon, and increased by 38.82% in winter compared to the non-polluted site. Stomatal density was measured per square millimeter. In the polluted site, it increased by 15.38% in summer, 5.00% in monsoon, and decreased by 35.42% in winter compared to the non-polluted site. Epidermal cell length was measured in micrometers. In the polluted site, it increased by 4.38% in summer, decreased by 2.16% in monsoon, and increased by 9.09% in winter compared to the non-polluted site. Epidermal cell width was measured in micrometers. In the polluted site, it increased by 25.00% in summer, 15.38% in monsoon, and 30.77% in winter compared to the non-polluted site. Epidermal cell size was measured in square micrometers. In the polluted site, it increased by 14.29% in summer, 28.28% in monsoon, and 13.36% in winter compared to the non-polluted site. Epidermal cell density was measured per square millimeter. In the polluted site, it increased by 31.30% in summer, decreased by 10.58% in monsoon, and increased by 22.22% in winter compared to the non-polluted site. Stomatal index value represents the ratio of stomata to epidermal cells. It decreased by 15.27% in summer, 2.30% in monsoon, and 4.83% in winter in the polluted site compared to the non-polluted site. In summary, these parameters provide detailed insights into how the plant's morphology and micro-morphology vary in different seasons and between polluted and non-polluted environments. The growth of plant species collected from uncontaminated sites is superior in terms of their morphological parameters compared to those collected from contaminated sites. Plants can hinder their growth due to their capacity to absorb, retain, and incorporate pollutants on their leaves. The toxic gases emitted by vehicles can combine with pollen grains from different plant species. These interactions conduct changes in the ontogeny, physiology and morphology in pollen grains of a plant. Alterations in chemical compounds of protein bring changes in the nature of biochemical and morphological structure of pollen grains and effect badly in its amino acids and flavonoids (Naira and Anzer 2022). Table 7 represent the changes in various anatomical parameters of plant leaves in different seasons and between polluted and non-polluted sites, along with the percentage decrease in each case. The midrib region thickness varies seasonally, with a slight decrease in summer (0.79%), an increase in Monsoon (1.17%), and a

larger increase in winter (1.59%). This region exhibits a thickness increase in summer (2.94%), a slight increase in Monsoon (1.59%), and a significant increase in winter (6.15%). Palisade tissue thickness increases in summer (12.00%), decreases in Monsoon (7.41%), and decreases further in winter (16.67%). The thickness of spongy tissue increases in summer (9.76%), Monsoon (6.38%), and winter (5.88%). The upper epidermis thickness increases in summer (5.13%), significantly increases in Monsoon (11.11%), and even more in winter (15.38%). The lower epidermis thickness increases in summer (4.17%), Monsoon (6.00%), and winter (13.64%). Vascular bundles increase in summer (4.85%), Monsoon (3.64%), and winter (8.91%). Overall, these findings suggest that seasonal variations and pollution levels impact the anatomical parameters of the plant leaves, with some parameters showing increases while others decrease under different conditions. Study highlights the detrimental impact of automobile emissions on the physical characteristics and structure of *M. indica* trees obtained from both polluted and unpolluted regions within the Samastipur district. So, it is suggested that to promote afforestation and reforestation initiatives within the city and its surroundings. There should be a heightened focus on developing green belts along highways and roads. It's crucial to closely monitor the condition of vehicles on the road, especially older and poorly maintained ones, as they contribute significantly to pollution in the surrounding environment. Increasing public awareness about these eco-friendly practices is essential.

Conclusion

Present study revealed the concentration of all the air quality parameters viz. SO2, NO2, RSPM and SPM to be higher at the polluted site of magardaahi ghat than the reference site of the satamalpure village area in all the seasons for the one years of the study period. The presence of air pollution negatively impacted all the parameters studied in plants at the polluted site, leading to a decrease in values for both morphological and anatomical characteristics compared to those at the non-polluted site. Based on the findings, it is clear that the roads in Samastipure district require regular maintenance. Issues like encroachments, potholes, and narrow roads contribute to increased fuel consumption and, subsequently, higher pollution levels. Rapid deterioration of ambient air quality has been witnessed over the years due to economic growth, higher industrialization and consistent population rise. Automobile emissions contribute about 57-75% of total emissions in urban areas **(**Pourkhabbaz et al. 2010). Constant awareness and enforcement drives should be conducted for the people to follow the traffic rules. Encouraging afforestation and reforestation initiatives within and around the city is recommended. It's essential to prioritize the development of green belts along highways and roads. Monitoring the condition of vehicles on the road is crucial because old and poorly maintained vehicles contribute to increased pollution in the surrounding environment. Promoting awareness about these environmentally friendly practices among the general public is also essential.

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Research Article

Physiological Adaptive Capabilities of Fifteen Different Local Rice Cultivars Under Salinity Condition

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Abstract

Rice is a major cereal contributing to the world's calories consumption and staple food crop over for one-third of the world's population. At present salinity is the second most widespread soil problem after drought and is considered as a serious constraint to increase rice production. Soil salinity affects plants through osmotic effects, ion-specific effects and oxidative stress. The effect of salinity stress in plants is mediated at least in part by an enhanced generation of active oxygen species, especially in chloroplast and in mitochondria which cause lipid peroxidation and membrane injury, protein degradation and enzyme inactivation. Plants have developed a complex anti-oxidant complex which mitigates and repairs the damage initiated by reactive oxygen species, toward enzyme synthesis to protect the cellular and subcellular system degradation. The seedling stage is one of the most sensitive stages to salt stress in rice and studies on salt tolerance during this stage could probably provide insights for enhancing tolerance throughout the plant life cycle. The present investigation was undertaken to examine the influence of NaCl on metabolic status of chlorophyll, protein, starch, soluble sugar and salt-tolerant capabilities among different rice cultivars.

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Introduction

Salinity causes reduction in percentage of germination, seedling vigor and other growth attributes (Farsaraei et. al. 2021). The main problem exerted by the salinity stress in rice plant is the accumulation of sodium and chloride ions at toxic level which in turn produces reactive oxygen species (ROS), free radicals and subsequently damages various photosynthetic pigments viz. chlorophyll, carotenoid, xanthophyll. Saline soil also restricts the absorption of potassium ions and thereby imbalances the normal Na^+/K^+ ratio within the plant body (Khare et. al. 2015). There are 3 basic stages of seed germination; water imbibition, activation of metabolic process and final germination phase. It has been reported that salinity adversely affected the first stage of germination i.e. water absorption phase by establishing osmotic pressure and contriving the symptoms related to drought like insufficient water availability, wilting and reduced growth (Ucarli et. a. 2020). Disruption of ionic balance as a result of salinity also negatively influences the production of vital hormones and enzymes involved during seedling establishment (Kumar et. al. 2021). One of these enzymes is α-amylase, functions in the hydrolysis of stored form of carbohydrate i.e., starch into the less complex utilizable form (e.g., glucose, maltose etc.) during germination (Apar et. al. 2004). Salinity stress strongly affected the activity of hydrolytic enzymes (Liu et. al 2018). It has been observed that the immediate response of plant against high salt concentration is to adjust osmotic pressure by reducing the expansion and division of cell. The reduction in turgor pressure is also mediated by closing of stomata and minimization of leaf surface area to prevent transpiration. This results in declined photosynthesis and stunted plant growth (Sarker et. al. 2020). As glycophytic plant, rice is

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much more sensitive to saline soil as compared to other cereal crops. Several reports have been made that rice have developed a number of salinity-tolerant mechanisms up to a certain degree (Horie et. al. 2012). One of the physiological indicators to this salinity-adaptability is the accumulation of compatible osmolyte or solutes. The compatible osmolytes or solutes are a diverse group of chemical compounds which are polar, uncharged and soluble in nature. The main function of accumulated osmolyte is to maintain osmotic balance through continuous water inflow and thereby protecting the cell's structural integrity (Chen et. al. 2021). The major advantage of osmolyte accumulation is that, these compounds are unreactive to any cellular metabolic process even if present at higher concentration. Proline is such an amino acid group of organic osmolyte present in diverse taxonomic class including rice. There are two possible ways of maintaining the concentration of compatible osmolytes within the plant body either by continual synthesis of compounds or by combined synthesis with degradation (Gupta et. al. 2014). The accumulated intracellular proline provides tolerance-capability under salinity by supplying organic nitrogen reserve for stress recovery (Kaur et. al. 2015). Endosperm utilization during early seedling growth is greatly hampered under salinity stress (Blum et. al. 1994). It is largely depended on the capabilities of specific seed hydrolytic enzymes to produce soluble sugar and support seedling growth. Relative water content (RWC) is another important physiological parameter related to salt tolerance. Relative water content is the measurement of maximum amount of water that plants leaf can take up under full turgid condition and hence it is considered as an appropriate determinant of plant water status under stress. Relative water content is efficient over leaf water potential which estimates the plant water status only during conduction between soil-water-atmosphere interface, but it does not take osmotic adjustment (OA) under consideration. OA is an influential mechanism for conservation of cellular hydration status under water scarcity. Hence RWC is most crucial in estimating water status as it accounts both OA and leaf water potential experienced by plants at early seedling stage (Suriya-arunroj et. al. 2004). So, the salt-tolerant cultivars could be selected on the basis of their ability to maintain the higher RWC during initial stages of salt stress.

Materials and methods

Seed sample collection

Matured seeds (caryopses) of fifteen local rice cultivars viz., Amalmona (AMA), Chinigura (CHI), Dudheshwar (DUD), Gitanjali (GIT), Gobindobhog (GOB), Harinakhuri (HAR), Kalojira (KAL), Kanakchur (KAN), Kartick khas (KAR), Khas dhan (KHA), MTU-1153 (MTU), Radhunipagal (RAD), Sabita (SAB), Satabdi IET-4786 (SAT), Sukumar C (SUK) were collected from two research stations i.e., Bidhan Chandra Krishi Vishwavidyalaya, Mohanpur, Nadia, West Bengal, and Indian Council of Agricultural Research (ICAR), Dighirpar, Canning, West Bengal. The collected mature seeds were categorized into two subtypes, viz., aromatic and high yielding cultivars.

Growth condition for seedling

For biochemical assessment at seedling stage, seeds were surface sterilized with 1% sodium hypochlorite solution and imbibed in distilled water for 24 h. Then germinated seedlings were raised in soil up to 14 days. Seedlings were maintained in natural conditions (Rice experimental field, University of North Bengal, attitude 26˚84' North, longitude 88˚44' East) at 28 ± 1˚C, 70-80% relative humidity and 12h day/night photoperiod in the month of July 2022. For salinity treatment, aqueous solution (200 mM) of NaCl was prepared. Soil was saturated with NaCl solution at 7 days' stage. Control sets were treated with distilled water.

Salinity stress indices

Shoot and root phyto-toxicity was calculated according to the formula described by Asmare 2013. Relative water content was measured using procedure described by Suriya-arunroj et. al. 2004. Endosperm utilization was calculated by method used by Blum et. al. 1994.

Biochemical procedures

For assessment of chlorophyll content, the method described by Arnon was followed. Protein content was estimated as described by Lowry et. al. For starch and total soluble sugar, the method by Hedge et. al. (1962) was followed. Proline content was estimated as described by Bates et. al. (1973). Endosperm utilization efficiency (EUE) was calculated according to Blum et. al. (1994). Shoot and root Phytotoxicity was calculated as described by Asmare et. al. (2013).

Results and Discussion

Table 1. Morphological indices of different cultivars under salinity stress

Cultivar	Treatment	Germination (%)	Shoot length (cm)	Root length (cm)
Kalojira	Control	86.67^{f} ± 3.53	8.08 ^{kn} ± 0.12	8.9 ^{eg} ± 0.03
	NaCl	61.33^{n} ±1.33	4.58 ^{P} ± 0.18	3.31^{mn}± 0.48
Kanakchur	Control	93.33 ^{e}± 1.33	12.26^{eg} ± 0.12	12.58^{b} ± 0.15
	NaCl	50.67^{P} ± 1.33	6.18^{mP} ± 0.30	3.5^{mn} ± 0.18
Harinakhuri	Control	88.00^{ef} ± 2.31	12.28^{eg} ± 0.82	7.74^{fj} ± 0.12
	NaCl	54.67^{O} ± 1.33	7.16 ^{O} ± 0.17	1.94^{n} ± 0.20
Gobindobhog	Control	81.33^{gh}± 3.53	10.26 ^{hi}± 0.63	9.56^{df} ± 0.44
	NaCl	40.00^{n} ± 2.31	4.38^{P} ± 0.51	6.26 ^{l}± 0.33
Kartick khas	Control	82.67^{g} ± 3.53	9.24 ^{k} ± 0.64	9.4^{ef} ± 0.20
	NaCl	76.00^{j} ± 2.31	6.82^{lO} ± 0.60	9.02^{eg} ± 0.86
Khas dhan	Control	90.67 ^{d}±3.53	9.6 ^{k} ± 0.31	9.88^{de} ± 0.25
	NaCl	76.00^{j} ± 2.31	5.6^{OP} ± 0.20	4.72^{lm} ± 0.09
Sabita	Control	96.00^{b} ± 4.00	21.64^{a} ±0.73	12.98^{b} ± 0.25
	NaCl	65.33^{m} ±1.33	13.22^{df} ±0.49	10.38 ^{ce}±0.37
Gitanjali	Control	94.67^{bc} ±1.33	13.52^{df} ±0.63	11.96^{a} ± 0.19
	NaCl	64.00^{m} ±2.31	6.62^{mO} ± 0.36	10.16^{bd} ±0.28
Amalmona	Control	89.33^{de} ± 1.33	19.44^{b} ± 0.54	18.84^{b} ± 0.61
	NaCl	76.00^{j} ± 2.31	13.96^{de} ± 0.50	11.32^{f} ±1.47
Dudheshwar	Control	98.67^{a} ± 1.33	16.94^{c} ± 0.54	12.76^{b} ± 0.61
	NaCl	80.00 ^{h}± 2.31	14.5 ^{d} ± 0.21	7.8 ^{jl} ± 0.15
Chinigura	Control	86.67^{f} ± 3.53	11.72^{fh} ±0.24	12.48^{ef} ±0.33
	NaCl	69.33^{l} ± 1.33	3.42^{P} ± 0.48	5.82^{gk} ± 0.11
Radhunipagal	Control	82.67^{g} ± 1.33	11.08^{g} ±0.56	9.3 ^{l} ± 0.53
	NaCl	72.00 ^{k} ± 2.31	5.38^{OP} ± 0.38	7.32^{ef} ± 0.23
MTU-1153	Control	78.67 ^{h}± 3.53	13.9 ^{de} ± 0.42	5.88^{gk} ± 0.19
	NaCl	49.33^{P} ± 3.53	9.56 ^{k} ± 0.58	6.9 ^{l} ± 0.79
Satabdi IET-4786	Control	86.67^{f} ± 5.33	7.7^{kn} ± 0.23	7.7^{hk} ± 0.15
	NaCl	54.67^{O} ± 5.81	5.4^{OP} ± 0.17	5.4^{fj} ± 0.21
Sukumar C	Control	76.00^{j} ± 2.31	8.68 ^{jl} ± 0.73	8.68 ^{kl} ± 0.52
	NaCl	64.00^{m} ± 2.31	5.82^{OP} ± 0.59	5.82^{jl} ± 0.24

Note: Values are mean ± SE of three independent determinant

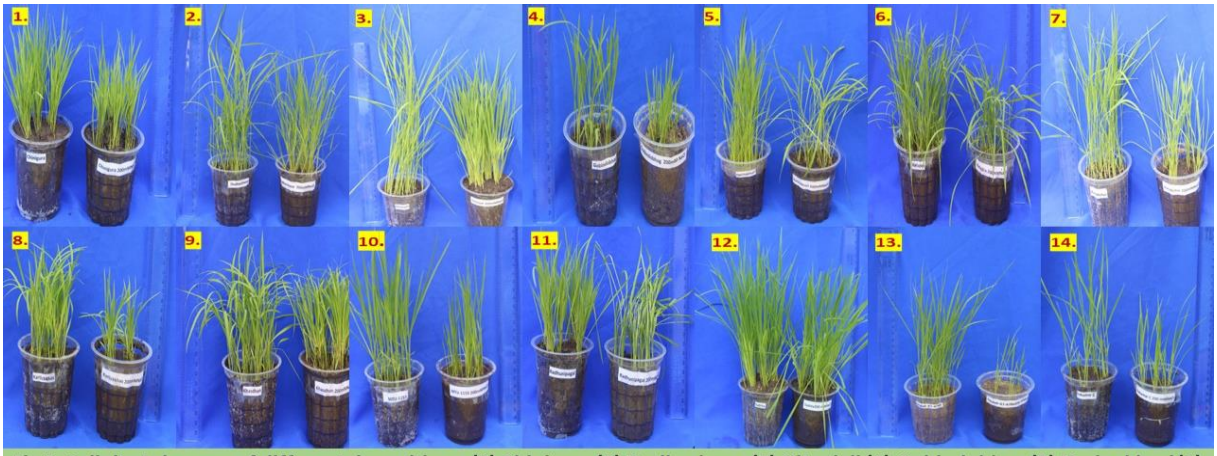

Fig 1: Salinity tolerance of different rice cultivars (1) Chinigura (2) Dudheshwar (3) Gitanjali (4) Gobindobhog (5) Harinakhuri (6) Kalojira (7) Kanakchur (8) Kartickkhas (9) Khasdhan (10) MTU-1153 (11) Radhunipagal (12) Sabita (13) Satabdi (14) Sukumar-C

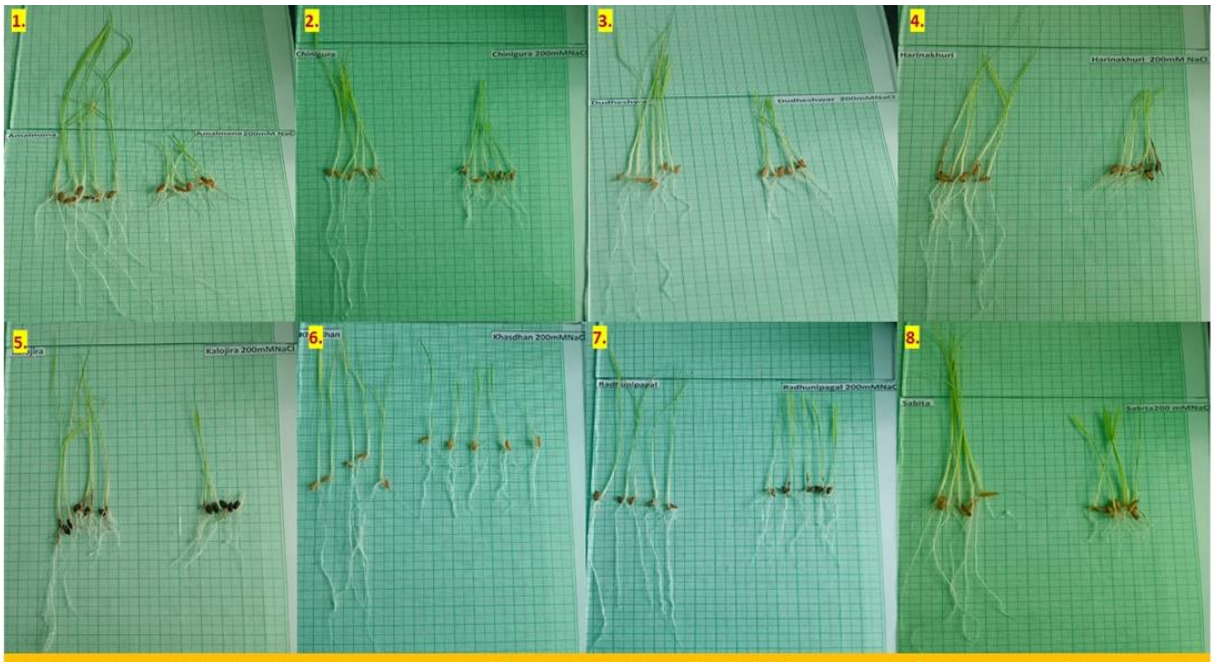

Fig 2 : Salinity treatment in 14 days seedling stage-(1) Amalmona (2) Chinigura (3) Dudheshwar (4) Harinakhuri (5) Kalojira (6) Khasdhan (7) Radhunipagal (8) Sabita

The salinity-induced decline in growth may be due to the creation of osmotic stress that inhibits transport and absorption of water as shown by salt-sensitive cultivar chinigura (CHI), kanakchur (KAN), harinakhuri (HAR) (Table 1 & Fig. 1). Considering the proline content, it is shown to be highest in kanakchur (KAN). As proline is a stress marker, most of the energy get utilized in survival mechanism and less energy is allocated for growth purpose. As a result of which reduced root and shoot length can be seen as represented in Fig. 2. It has been reported that proline not always make reduction in growth but also provides nitrogen source to overcome the saline toxicity and support growth as shown by cultivars amalmona (AMA), sabita (SAB) and kalojira (KAL) supported by result of Cha-Um (2009). The remaining cultivars do not show any significant changes in proline content. If we consider the chlorophyll content, it is severely affected during salinity imposition. Salinity stress generates reaction species that cause oxidative damage to chlorophyll pigment, thereby reduce the photosynthetic potentiality according to the report of Gharsallah (2016). To cope up with this deadly photo-oxidative damage, plants possess a diverse phenolic group of non-enzymatic anti-oxidant molecules, that function in neutralizing free radicals and detoxifying its effect up to a certain level supported by results of Yan (2022). Cultivars MTU-1153, satabdi (SAT), amalmona (AMA) were found to have quite higher phenol content, could scavenge the reactive oxygen species (ROS) molecules and maintain the pigment component to a static level. As we know that starch, the ultimate storage product of vital metabolic

Table 2. Stress tolerance indices of different rice cultivars

Cultivar	Treatment	Relative water content (%)	Endosperm utilization efficiency (EUE)	Shoot-phytotoxiciity	Root-phytotoxicity
Kalojira	Control	69.7	73.2	43.3	62.8
	NaCl	62.5	56.1		
Kanakchur	Control	84.4	88.9	49.6	72.2
	NaCl	80.9	60.5		
Harinakhuri	Control	80.9	45.7	41.7	74.9
	NaCl	71.4	60.9		
Gobindobhog	Control	73.9	79.2	57.3	34.5
	NaCl	43.8	58.9		
Kartickkhas	Control	83.9	85.7	26.2	4
	NaCl	63.6	56.3		
Khasdhan	Control	80.6	87.9	41.7	52.2
	NaCl	90.9	58.2		
Sabita	Control	81.8	88.0	38.9	20
	NaCl	78.9	69.6		
Gitanjali	Control	87.3	93.2	51	15.1
	NaCl	66.3	81.2		
Amalmona	Control	88.7	86.3	28.2	39.9
	NaCl	79.2	76.1		
Dudheshwar	Control	73.7	81.7	14.4	38.9
	NaCl	70.0	70.9		
Chinigura	Control	83.8	65.7	70.8	53.4
	NaCl	48.6	43.8		
Radhunipagal	Control	71.4	77.8	51.4	21.3
	NaCl	50.0	54.2		
MTU-1153	Control	64.5	84.2	31.2	17.3
	NaCl	59.3	69.3		
Satabdi-IET 4786	Control	78.2	71.5	29.9	6.8
	NaCl	74.6	60.2		
Sukumar C	Control	67.5	70.5	32.9	2.7
	NaCl	63.5	59.6		

Fig 3: 14 days old seedling under salinity (A) Chlorophyll content (B) Starch content

Fig 4: Seedling content under salinity stress- (A) Total phenol content (B) Soluble sugar content

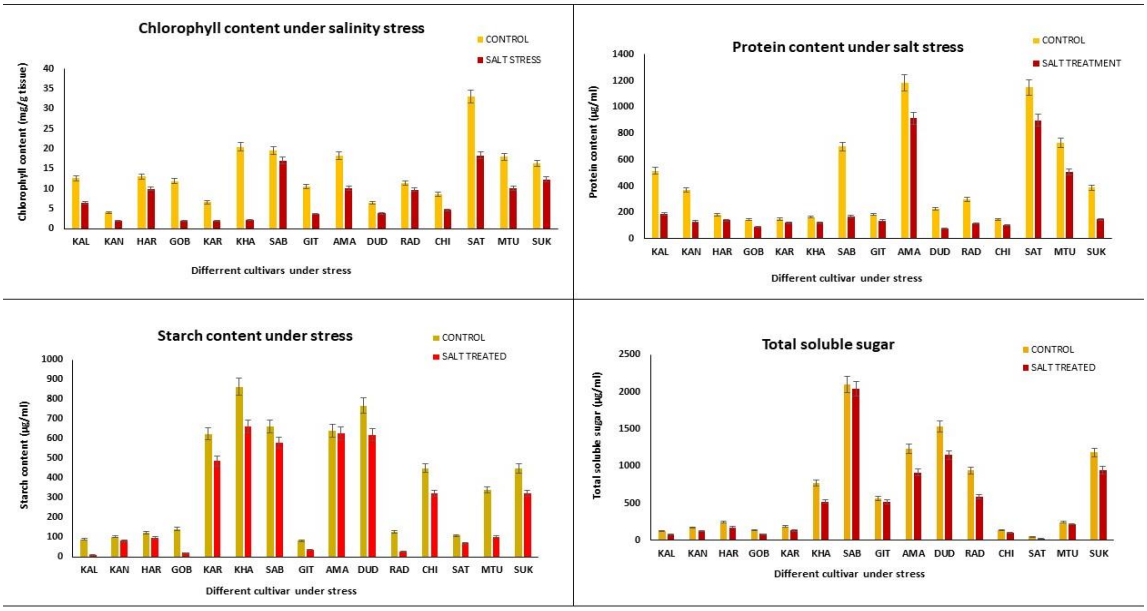

Fig 5: Biochemical attributes of seedling of different cultivars under salinity stress

process photosynthesis, the accumulation of starch is directly related to the principal pigment chlorophylls (chl-a and chl-b) as represented in Fig. 3 & 5. Direct proportionality between chlorophyll pigment and starch product can been seen in cultivars khasdhan (KHA), amalmona (AMA), MTU-1153, satabdi (SAT); The remaining cultivars represent moderate changes under saline condition. Upon endo-hydrolytic enzyme activity, stored starch gets hydrolyzed into soluble form and mobilized into the germinating seeds to support its seedling growth.

Such higher level of soluble sugar can be observed in case of cultivars sabita (SAB), dudheshwar (DUD), amalmona (AMA) and khasdhan (KHA) (Fig. 4 & 5). But this carbohydrate mobilization can be severely affected by salinity stress and negatively influence the seedling growth as shown by cultivars kalojira (KAL), gobindobhog (GOB), chinigura (CHI).

Endosperm content utilization efficiency was shown to be reduced in salt sensitive cultivars as documented in Table 2.

Conclusion

In the present study, salt treatment resulted in the reduced level of chlorophyll, protein and soluble sugar content. This happens perhaps due to the generation of excess ROS, which may lead to the oxidative degradation of chlorophyll pigment and ultimate accumulation of less photo-assimilates during photosynthesis. In our study proline content is significantly increased under salt-treatment, a key indicator representing salt-tolerance capabilities among different rice cultivars. Furthermore, our finding demonstrated a positive interdependent correlation between chlorophyll content and dry matter accumulation.

Conflicts of interest

There are no actual or potential conflicts of interest to declare.

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Review Article

Role of Polyamines in the Physiological Responses of Plants

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Abstract

Polyamines (PAs) are aliphatic nitrogenous bases containing two or more amino groups. These organic compounds have a low molecular weight and play essential role in the growth and development of plants. They contribute to the tolerance of plants against all abiotic and biotic stresses. They occur in the free form as cations, but are often found in the conjugated form to different macromolecules such as proteins and nucleic acids and to small molecules like phenolic acids. They are produced by plants during metabolism and are ubiquitous in plant cells. They are considered to be a new kind of plant biostimulant because they are intimately associated with a wide range of metabolic process in plants, ranging from cell division and organogenesis to protection against abiotic and biotic stress. Their chemistry, biosynthetic pathway and metabolism are now well characterized. Their titer varies and depends on the environmental conditions, especially stress. With the development of molecular biotechnology, genes for several key biosynthetic enzymes of the PA pathway have been cloned from different plants species, and antibodies to some of the genes are now available. The antisense transgenic approaches and over-expressed PA biosynthetic genes have given further evidence that PAs are required for plant growth, productivity and development of stress tolerance. This paper aims to review the various physiological responses of plants to PA with special emphasis to abiotic stress response and to provide a basis for future research on the role of polyamines in plant physiology.

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Introduction

Polyamines are widely distributed in eukaryotic and prokaryotic cells (Liu et al., 2017; Mustafavi et al., 2018). PAs have low molecular weight and are aliphatic nitrogenous bases containing two or more amino groups which have potent biological activity (Xu et al., 2009; Vuoksu et al., 2018). They may exist freely in living organisms (F-PAs) or in covalently conjugated form (CC-PAs) or non-covalently conjugated (NCC-PAs) forms (Gholami et al., 2013). The history of PA biochemistry goes back to more than 300 years. It was Antoni van Leeuwenhoek who first observed depositions of star shaped crystals in aging sperms when he was observing human semen through his primitive microscopic lenses in 1678. Almost more than 200 years later, the basic component of these star shaped phosphate crystals was named Spermine. By mid 1920s the correct chemical composition and structure was determined. Spermidine was also discovered around the same time. Since then, PAs remained interesting mainly to chemists for about next half a century. Later Cohen's book directed the attention to possible biological importance of these compounds and initiated research in many areas, including plant physiology. A definitive work on plant polyamine biochemistry came from Terence A Smith at Long Ashton Research Station of the University of Bristol. Later in 1973, a paper delivered at plant growth hormone symposium in Tokyo suggested that polyamines have a regulatory action in plants. Since then, research on polyamines has spread to many countries of the world. Polyamines are essential for the growth and development in prokaryotes and eukaryotes (Tabor and Tabor, 1984; Tiburcio et al., 1990). In plant

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cells, the diamine putrescine (Put), triamine spermidine (Spd) and tetramine spermine (Spm) constitute the major PAs. They remain associated with macromolecules such as proteins and nucleic acids and stimulate DNA replication and protein synthesis. They participate in wide range of biological processes related to growth and development of plants such as senescence, embryo development, environmental stress and also biotic stress such as infection by fungi and viruses. Their biological activity is mainly attributed to their cationic nature. Recently use of PA biosynthetic inhibitors has shown a causal relationship between changes in the endogenous levels of PA and growth responses in pants. These observations have enhanced further studies in understanding the mode of PA action. Since PAs are involved in numerous biological interactions in plant systems like stabilizing membranes, scavenging free radicals, affecting nucleic acid and protein synthetics, enzyme activities, hormone interactions etc, it has been difficult to determine their precise role in plant growth and development. However recent investigations into the molecular genetics of plant PAs helped to isolate number of genes encoding enzymes of PA biosynthetic pathway. Antibodies of some of the genes have also been developed. Genomic and proteomic approaches are being used to find out the role of PAs in plant developmental processes. This review article highlights the role of PAs in plants with particular emphasis on its role in abiotic stress responses.

Distribution of Polyamines

Polyamines are ubiquitous in eukaryotic and prokaryotic cells (Liu et al., 2016, 2017). They are also found in plant tumors and in plant RNA viruses. PAs exist in variety of forms with potent biological activities. In higher plants, PAs are present in their free form. The most common types of PAs found in higher plants include Put, Spd, Spm, Tspm (thermospermin) etc. (Kim et al., 2014; Takahashi et al., 2017) and also Cadaverine (cad) (Regla – Marquez et al., 2015; Nahar et al., 2016). Other types of PAs are found only in certain plants or may be under special conditions only. The polyamines are organ specific and tissue specific in distribution. It was found that the most abundant PA in leaves was Put whereas Spd was found at elevated levels in other organs (Takahashi et al., 2017). Different type of PAs shows different localization pattern within cells. In the cells of carrot, Put was found to accumulate in the cytoplasm and Spm in the cell wall (Cai et al, 2006). It is concluded that the distribution pattern of PAs is very much related to its unique functions. The more vigorous plant growth and metabolism is directly proportional to greater PA biosynthesis (Zhao et al., 2004; Cai et al., 2006).

Metabolism of polyamines

The central product of the common PA biosynthetic pathway is Putrescine which contains two amino groups and is a synthetic precursor of Spd and Spm (Xu et al., 2009). Basically there are three different routes of Put biosynthesis in plants. In the first route, Arginine (Arg) loses its No. 8 carbon atom by arginine decarboxylase to from agamatine (Agm) and CO_2. Agmatine next loses Nitrogen at No.2 position to from N- carbamoyl Put (NCPA) and ammonia. NCPA is further hydrolyzed by N-carbamoyl putreseine amidohydrolase (NCPAH) and its carbamoyl is removed to form Put, CO_2 and NH_3. This is the main Put synthesis pathway in plants (Docimo et al., 2012; Pegg, 2016). In the second route, Arginine is converted to Ornithine (Orn) by Arginase and then ornithine decarboxylase removes the carboxyl group of No.1 carbon atom of ornithine to produce Put and CO_2 (Hanfrey et al., 2010). In the third route Arginine is first converted to Citrulline (Cit) which is further decarboxylated by citrulline decarboxylase to form Put (Han, 2016). The first two pathways are more common in plants. The third Citrulline pathway has been found only in sesame, till date. Spd and Spm are produced from Put and Amino propyl residues, which are gradually provided by methionine (Vuosku et al., 2018). The PAs are broken down in plants by the action of amine oxidases (Agudelo-Romero et al., 2013). The diamine oxidase (DAO) and PA oxidase (PAO) are the key players.DAO catalyses the formation of H_2O_2, ammonia and 4-aminobutanal from Put. The 4-aminobutanal undergoes cyclization to form pyrroline (PYRR), which is next converted to ɣ- amino butyric acid (GABA) by the action of pyrroline dehydrogenase (Hu et al., 2015). Finally GABA is converted into Succinate which enters into Krebs cycle in mitochondria. Dicots contain high levels of DAO (Cona et al., 2006) and PAO is found at elevated levels in Monocots (Tian, 2012; Takahashi et al., 2017). Its substrates are Spd, Spm and Tspm. There are multiple PAO families in plants (Tian, 2012; Liu et al., 2014, Takahashi et al., 2017). The metabolism of PAs in plants has quite significance. The H_2O_2 produced by oxidation of PA functions in the signal

transduction process in plants during biotic and abiotic stress responses (Freitas et al., 2017; Mellidou et al., 2017). It also affects stomatal closure induced by Abscisic acid. (Cona et al., 2006; Tim et al., 2006; An et al., 2008). The S-adenosyl methionine produced in the PA biosynthesis route is also a precursor for ethylene synthesis (Chen et al., 2014). In addition, PA metabolism is related to NO production (Pal et al., 2015). NO is an essential signaling component for plant growth (Krasuska et al., 2013; Agurla et al., 2017). So the roles of PAs in plant growth and development and the mechanism of how they function can be discovered by studying the relationship of plant hormones and PA metabolism and also the effect of the later on plant signaling substances.

Polyamines in plant growth and development

PAs are involved in many plant developmental processes. With the availability of specific inhibitors of PA biosynthesis, it became easy to investigate the mechanisms involved in PA interactions to some extent. Clearly, PAs are involved in developmental processes like cell division, embryo development, reproductive organ development, growth of root, floral initiation and development, fruit development and ripening, leaf senescence and abiotic stresses (Sawhney et al., 2003). It has been found that changes in free and conjugated PAs and their biosynthetic enzymes like ADC, ODC and SAMDC have been found to occur during these developmental processes. In general, cells undergoing division have high levels of free PAs synthesized via ODC, and cells undergoing expansion and elongation contain low level of free PAs synthesized via ADC. High levels of endogenous PAs and their conjugates have also been found in apical shoots and meristems before flowering (Cabbane et al., 1981) and flower parts of many plants (Martin-Tanguy, 1985; Ahmed et al., 2017). Callus cultures derived from explants of tobacco inflorescence show that endogenous Spd increases more rapidly than other PAs in floral buds than in vegetative buds. Addition of Cyclohexylamine (CHA), an inhibitor of Spd synthesis, switches vegetative bud development instead of floral bud (Sawhney et al., 2003). This inhibition could be reversed by addition of exogenous Spd (Kaur-Sawhney et al., 1988). Flower bud differentiation is a complex process of morphogenesis. It is triggered by various factors such as photoperiod, nutrition, vernalization and water status, and is accomplished by the interaction and coordination of hormones and PAs (Xu, 2015). Exogenous PAs accelerate the process of flower bud differentiation. In *Arabidopsis*, PAs were found to be more abundant in flowers than in other organs and addition of exogenous PAs stimulated flowering response (Applewhite et al., 2010). Lower content of PAs, mainly Put & Spd, were found to lower floral bud initiation in rapeseed while increased PA content promoted differentiation of floral bud. Many growth and developmental processes of plants regulated by phyto hormones such as Auxins, 2, 4-D, GA and ethylene have also been correlated with PA metabolism (Sawhney et al., 2003). These changes occur both on the endogenous levels of PAs and also in the level of their biosynthetic enzymes and appears to be tissue specific. Thus PAs which may or may not migrate can serve as intracellular mediators of hormone actions (Galston and Kaur-Sawhney, 1995). Amongst these, ethylene has been most extensively studied with respect to PA metabolism. PAs and ethylene play antagonistic roles in plant processes. While PAs inhibit senescence in leaves and fruit ripening, ethylene promotes these processes. PAs and ethylene regulates each other's synthesis, either directly or through metabolic competition for SAM, a common precursor for their biosynthesis. PAs inhibit ethylene biosynthesis perhaps by blocking the conversion of SAM to ACC and of ACC to ethylene (Apelbaum et al., 1981; Suttle 1981). During senescence, chlorophyll content decreases, activities of ADC and ODC decrease, while activities of PAO and hydrolases like Proteases and Ribonuclease increases rapidly(Bagni and Tassoni, 2006; Chen et al, 2019). All these changes can be inhibited by application of exogenous PAs (Duan, 2000, Cai, 2009). So polyamines delay senescence by inhibiting ethylene biosynthesis (Woo et al., 2013, Anwar et al., 2015).

Polyamines bind to negatively charged nucleic acids, proteins and phospholipids by ionic and hydrogen bonds through their amino and imino groups and help in establishing the zygote polarity during embryo development and also promotes cell layer differentiation and establishment of the meristem (Chen et al, 2019; Chen and Lv, 2000). Polyamines are considered to regulate the embryogenesis in both gymnosperms and angiosperms (de Oliveira et al., 2016; Kevers et al., 2000) and an increase in PA content is required for the process. However, the types and abundance of PAs vary in different stages of embryonic

development. PAs more abundant in embryogenic callus and somatic and zygotic immature embryos than in mature and germinating embryos (Cao, 2010). Putrescine stimulates somatic embryogenesis and reduced level of Put and Spd result in fewer somatic embryos (Chen et al, 2019). Polyamines and abiotic stress responses: Polyamines play a crucial role in the physiological responses of plant against stress. Stress may be either biotic or abiotic in nature. In fact there are several factors that causes abiotic stress in plants-

i) Polyamines and Temperature Stress

Temperature stress is generally of two categories- low and high temperature stress. Low temperature stress is again of two types- cold stress and freezing stress. Few studies have been conducted till date to focus on the physiological functions of PAs in plants under high temperature stress (Chen et al, 2019). High temperature stress affects PA synthesis in the leaves by increasing the Put content but the increase is not sustained for a longer period of time (Yang and Yang, 2002). PAs promote photosynthesis and increases antioxidant capacity and osmotic adjustment capabilities of plants under high temperature stress (Tian, 2012; Guo et al., 2015). The antioxidant enzymes scavenge ROS to prevent membrane lipid per oxidation and stabilize membrane structure (Zhuo et al., 2018). Shao et al. (2015) reported that heat tolerance of alfalfa was because of higher Spd content and lower Put and Spm content (Shao et al., 2015). However, the main physiological mechanism of high temperature tolerance differs among plant species. PAs can bind to the phospholipid site of the cell membrane to prevent cyclosis and improve cold resistance (Li and He, 2012). However, the relationship between Put and plant chilling stress is debatable. Sweet pepper and Zucchini fruits, when stored in chilling temperature, shown an exponential increase in Put content accompanying by chilling damage. Again increased Spm level may be a defense response to cold damage by lowering Put accumulation and thereby reducing chilling damage (Zhan et al., 2000; Roy and Wu, 2001). Sun et al. studied the effect of Put and D-Arg on the physiological and biochemical indexes of *Anthurium andraeanum* under chilling stress at 6° C in winter. They found that Put application resulted in increased antioxidant enzyme activities, nitrogen metabolism, chlorophyll and proline content. Similar results are found in *Stevia* plants where PA supplementation increases tolerance to cold conditions (Peynevandi et al., 2018). Recent studies suggest that abiotic stress tolerance is mainly affected by role of PAs in signal transduction rather than their accumulation. (Pal et. al., 2015).

ii) Polyamines and Water Stress

Majority of the work on the relationship between PAs and water stress has focused on drought resistance (Ebeed et al., 2017) and little attention has been given on water logging resistance. Polyamines have been found to regulate the size of K^+ channel and pore size in the plasma membrane of the guard cells, thereby regulating the opening and closing of the pore. This is how PAs can control water loss in plants (Liu et al., 2000). Several other studies have shown that application of Put, at an appropriate level can affect the biosynthesis of osmotic adjustment substances like soluble sugars, amino acids and proline. This may compensate for the negative impacts of drought stress on plant biomass. In alfalfa, treatment with Put have shown to improve seed germination, growth of hypocotyl length etc. under drought stress caused by various concentrations of polyethylene glycol (PEG), both invitro and in a pot experiment (Zeid and Shedeed, 2006). A mutant of *Arabidopsis* acl5/spms was cured which is hypersensitive to drought due to Spm deficiency. (Yamaguchi et al., 2007). These results indicate that function of PAs differs amongst different plants and even in different parts of the same plant, whether under osmotic stress of water stress (Sun et al., 2018). It can be concluded that response of plants to exogenous PAs under water stress and osmotic stress is species specific.

iii) Polyamines and Salt Stress

Like drought stress, salt stress also lead to reduced water potential in plants. Salinity is a complex environmental constraint on plants. A higher concentration of salt reduces membrane integrity, decreases the activity of various enzymes and also harms the function of photosynthesis apparatus. So plants adjust to such extreme environmental conditions by accumulating osmolytes of low molecular weight like PAs and proline. The application of different types of exogenous PAs, at different concentrations, have shown to reverse the effects of NaCl stress and reduce damage in various plants (Verma and Mishra, 2005, Li et al., 2008). Plants rich in PAs have strong salt tolerance. Li and He (2012) suggested that Spm level in plants is

an important indicator of salt tolerance. Exogenous PAs especially Spm and Spd increases the metabolism of reactive oxygen and photosynthesis, thereby improving plant growth and reduces the inhibitory effects of salt stress (Meng et al., 2015; Baniasadi et al., 2018). Li et al. produced a cucumber line with greater SAMDC expression and lower ADC and ODC expression, resulting in greater Put accumulation during salt stress. As a result, inhibition of plant growth under salt stress was reversed in transgenic seedlings (Li et al., 2011; Takahashi et al., 2017; Takahashi et al., 2017b). Sun et al. showed that PAs and ABA together alleviated salt stress in grape seedlings (Sun et al., 2018). Recent studies have discovered the relationship between PAs and salt stress resistance by using genetic engineering techniques. Malabika et al. transformed the ADC gene of oat into rice and found that ADC activity, biological yield and Put contents were higher in transgenic rice and its progeny under NaCl stress (Roy and Wu, 2001).

iv) Polyamines and Oxidative Stress

Polyamines play a very complicated role in plant oxidative stress. (Minocha et al., 2014). Polyamines increase the activity of various antioxidant enzymes in plants which can effectively regulate oxidative stress caused by several environmental factors. Increased tolerance to oxidative stress induced by paraquat was overcome by pretreatment of leaves with Spm and Put in Maize (Durmu and Kadioglu, 2005). Application of Spd significantly increase Spd and Spm levels and reduce Put level in roots of cucumber seedling under hypoxia stress. An increased antioxidant enzyme activity, enhanced ROS scavenging ability and less membrane lipid per oxidation were some of the changes which ultimately led to increased hypoxia stress tolerance (Jia et al., 2008; Wu et al., 2018). It was reported that during cadmium and copper induced oxidative stress, lipid per oxidation increases in sunflower leaf and activities of glutathione reductase and superoxide dismutase decreases (Groppa et al., 2001; Gholami et al., 2013). On the other hand, PAs are also source of ROS because their catabolism produces strong oxidizers H_2O_2 and acrolein. So PAs can cause cellular harm under stress condition (Minocha et al., 2014. However H_2O_2 being a signal molecule enter stress signal transduction chain and activate antioxidant defense response (Groppa and Benavides, 2008). Therefore we can say that PAs are regulators of the redox homeostasis that play a dual role in plant oxidative stress (Saha et al. 2015). Besides the above mentioned abiotic stresses, plants can be affected by acid, radiation, wound and heavy metal stress. Few studies have been conducted on these topics, but the current idea is that PAs are important in the response to these stresses. Applications of exogenous Put regulates the balance of active oxygen metabolism under acid stress and stabilizes membrane system structure and hence protect plant from acid stress and improve acid resistance (Li et al., 1995). Mechanical injury and wounding of the leaves have shown to increase expression of ADC2 (Perezamador et al., 2002) and increase in free Put content (Cowley and Walters, 2010). Treatment with heavy metals Hg^{2+} and Cr^{6+} led to reduction in Spm and Spd content with decreased activities of SOD, catalase and peroxidase leading to excessive accumulation of membrane lipid peroxides and sharp decrease in chlorophyll and soluble protein contents. Application of exogenous Spd helped to overcome these negative effects of Hg^{2+} and Cr^{6+} (Wang et al., 2003; Wang and Shi, 2004).

Conclusion

This article represents a detailed and comprehensive review of the published literature dealing with the relationship between PAs and plant growth, development and abiotic stress tolerance. The role of polyamines in plant developmental processes ranging from flowering to senescence, embryo development has been discussed. These informations will surely provide a reference for future research work on the regulatory mechanism of PAs and on the significance of the use of exogenous PAs to regulate plant growth and production. Application of the technique of endogenous PA production is becoming increasingly popular via genetic manipulation to regulate plant growth. Still many questions are left to be answered regarding the roles of PAs in regulating plant growth and development. Knowledge about the different biosynthetic and catabolic pathways and their regulation at differ levels are yet to be deciphered. Further research to uncover the exact mechanism of PA accumulation, in order to improve plant stress resistance needs to be done. Moreover, there is still much left to be discovered about the metabolic relationship between PAs and other phytohormones during growth and development of higher plants, more specifically the relationship between PAs and ethylene. With the advancement of molecular

biology techniques and transgenic methods, PA metabolism can now be manipulated and has become a good tool to study the physiological responses of PAs in higher plants. Besides the known PAs, many unusual PAs are found in nature too like the Tspm from bacteria residing in hot springs having enzymes resistant to heat denaturation. Future research on this aspect could also be eye opener.

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Review Article

Underutilized Fruits of Northeast India and its Potential Benefits on Human Health - Review

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Abstract

The Indian Himalayas, a global biodiversity hotspot, is home to 2532 species from temperate regions of Europe, China, Burma, the Sahara, and Africa. India's plant biodiversity includes 21 agroecosystems, including farmed fruit and wild, under-utilized fruit crops. These underutilized fruit crops have potential but are rarely planted, infrequently available on the market, or not farmed commercially. They are disease-resistant and adapted to heat and cold extremes, blessing tropical nations like India. Underutilized fruit crops have medicinal properties and are often used by Native Americans to heal ailments and for the financial well-being of tribal people in rural regions. The use of wild fruits as nutritional supplements or less expensive alternatives to commercial fruits is growing worldwide. Identifying and utilizing underutilized species is crucial for a diverse and nutritious diet, especially for rural poor and socially vulnerable populations in emerging countries. India's North-Eastern Hill region is an agrobiodiversity hub characterized by diverse ethnic and cultural backgrounds. It is rich in wild agricultural plant relatives, particularly underutilized fruit crops, and mixed temperate, tropical, and subtropical fruits from various genera. Fruits, vegetables, and other plants naturally produce important polyphenol metabolites that influence their sensory and nutritive qualities, potentially curing various conditions.

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Introduction

The Indian Himalayas, a global biodiversity hotspot, is divided into four main areas: the eastern and north-eastern Himalayas, the eastern flank, and the northwest Himalayas. The Himalayas have the rarest crop biodiversity and the highest agroecosystem on Earth. India's plant biodiversity includes 2532 species from temperate regions of Europe, China, Burma, the Sahara, and Africa. 6.5% of the 2252 genera found in India are indigenous. The Himalayas are home to five identified 21 agroecosystems, including farmed fruit and wild, under-utilized fruit crops. (Lata et al., 2023). Underutilized fruit crops have potential but are rarely planted, are infrequently available on the market, or are not farmed for commercial purposes (Agent, 1994). Scarcer, less often consumed, or region-specific fruits can also be underutilized (William and Haq, 2002). The popularity of these fruit crops varies from crop to crop and region to region; publicity can, however, boost them more. Native Americans generally use underutilized fruits to heal a range of ailments. Still, they are also essential to the financial well-being of tribal people in rural regions since they may be used to manufacture furniture, firewood, fodder, dyes, oils, and other high-value commodities. The underutilized fruits are naturally disease-resistant and adapted to heat and cold extremes, barely equatorial temperature ranges. A range of underutilized fruits that are naturally cultivated is a blessing for tropical nations like India (Dutta et al., 2018).

It is common knowledge that fruits from tropical and subtropical climates have medicinal properties. In addition to the more popular fruits, other less popular fruits are included in traditional meals, especially in rural regions. As sources of antioxidants, they have yet to receive as much attention as commercial fruits since they are less

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famous, untested, and unknown (Loganayaki & Manian, 2010). Worldwide, the use of wild fruits as possible nutritional supplements or less expensive alternatives to commercial fruits is growing in popularity (Rawat et al., 2011; Zhang et al., 2017).

Identifying and utilizing underutilized species is crucial for a diverse and nutritious diet, especially for rural poor and socially vulnerable populations in emerging countries. Reclaiming these species' potential can significantly enhance nutrient intake as local knowledge diminishes, especially for rural poor and socially vulnerable populations. (Kour et al., 2018).

India's North-Eastern Hill (NEH) region is an agrobiodiversity hub characterized by diverse ethnic and cultural backgrounds. It is rich in wild agricultural plant relatives, particularly underutilized fruit crops, and mixed temperate, tropical, and subtropical fruits from various genera. The region's biological richness is significant. Various genera are available in the northeastern region such as *Pyrus, Rubus, Prunus, Garcinia, Phyllanthus, Averrhoa, Persia, Elaeagnus Myrica Passiflora Calamus, Dimocarpus, Annona, Rubus Dillenia, Baccaurea and others* (Deka et al. 2012, 2014; Singh et al. 2014 Bachheti et al. 2023 Hazarika et al. 2015).

Most studies have discussed several indigenous fruits' geographical distribution and ethnomedical uses. These fruits have several bioactive compounds, although few have been identified. Fruits are rich in polyphenols, which have antioxidant and redox properties. These chemicals help combat reactive oxygen and nitrogen species (ROS and RNS), essential for physiological functions. However, excessive ROS can lead to oxidative stress, increasing the risk of diseases like diabetes, cancer, obesity, and cognitive issues. In hypoxic conditions, nitric oxide can produce RNS, leading to lipid peroxidation and reactive aldehydes. Oxidative excess can cause cancer and inflammation, resulting from altered transcriptional parameters, protein modification, and DNA damage. (Dutta et al. 2018). Fruits, vegetables, and other plants naturally produce important polyphenol metabolites that influence their sensory and nutritive qualities. They are primarily responsible for the antioxidant activity in many fruits (Li et al. 2012). The prevention of gastrointestinal illnesses, colon cancer, obesity, and heart disease has been related to polyphenols. They also fight against oxidizing substances and free radicals, which preserve fatty acids from oxidative degradation (Ignat et al. 2011). It is firmly considered that consistent consumption of phytochemicals produced from plants may tip the scales in favor of the body's proper antioxidant state [Mahomoodally et al. 2012].

Polyphenols, including flavonoids, phenolic acids, and tannins, are essential antioxidants in citrus fruits, tomatoes, and aromatic plants. Flavonoids protect plants from harmful UV rays, fungi, and oxidative cell damage. Anthocyanins, flavones, isoflavones, flavanones, and flavonols are different types of flavonoids. Anthocyanins are water-soluble pigments found in plant tissues that act as antioxidants by halting the growth of new radicals. Phenolic acids, including hydroxycinnamic and oxybenzoic acids, impact biological systems and prevent degenerative illnesses. Fruits with higher phenolic content have more potent antioxidant qualities. These phytochemicals have been investigated for their potential to cure various conditions, including cancer, chest pains, epilepsy, leucorrhea, hemoptysis, hepatic disorders, skin disorders, infammation, leucorrhoea, joint pains, and dysentery. (Ignat et al. 2011; Bachheti et al. 2023).

Interest in natural antioxidants, particularly plant-derived ones, has increased recently, leading to their inclusion in preservation technology and modern healthcare. This review article aims to gather information on the therapeutic, phytochemical, and nutritional value of underutilized plant fruits, presenting relevant research findings and potential commercial applications.

Morphology and general distribution of some underutilized fruits

Baccaurea ramiflora Lour. (Latkan)

The plant is native to Southeast Asia, namely the sub-Himalayan region that stretches from Nepal through Sikkim, Darjeeling Hills, Arunachal Pradesh, Tripura, Assam, Bhutan, Burma, Peninsular Malaysia, Tibet, and the Andaman Islands. *Baccaurea ramifora* Lour., syn. Baccaurea sapida (Roxb.) Muell. Arg. is under the family Phyllanthaceae. The name of the genus is derived from the Latin word "*Baccaurea*" and relates to the golden-yellow color of the fruits (Goyel et al. 2022). It is a small to medium-sized, ten m-tall, semi-deciduous tree. Ripe fruits are first yellow and edible before changing to ivory, yellowish, pinkish-buff, or even brilliant red. Near the seeds, the pulp is pale and occasionally deep pink; its flavor can be either acidic or sweet. (De et al. 2017)

***Passiflora edulis* Sims. (Passion fruit)**

Passion fruit is the most prominent genus in the Passifloraceae family, with roughly 500 species. The *Passiflora edulis* stands out among them due to its commercial and therapeutic significance., It is grown in some areas of the Northeastern region, like Mizoram, Manipur, Nagaland, and Sikkim. The diameter of the golden passion fruit is 4–7 cm, and its length is 6–12 cm. The peel is thick, thick, and a brilliant yellow color. Brown marks may be seen on the seeds. The pulp has a robust, fragrant flavor and is acidic. The purple passion fruit is tiny (4–9 cm long and 3–7 cm in diameter). The seed is black, while the peel is purple (He et al. 2020).

***Phyllanthus acidus* (L.) Skeels (Star aonla)**

Phyllanthus is one of the largest genera of the Phyllanthaceae family, represented worldwide by some 700 well-known species, mainly distributed in the tropics and subtropics **(**Banerjee et al. 2022). It is present in the southern and northeastern regions, especially in Mizoram. It is a 2–9 m tall shrub or tree with a spreading, bushy crown and rough main branches that are appealing and striking. Dioecious or monoecious flowers with many bracts are borne alone or in pairs in axillary fascicles (De et al. 2017).

***Elaeagnus pyriformis* Hook.f. (silverberry)**

E. pyriformis, often known as Silverberry or Oleaster, is a member of the family Elaeagnaceae. The only Elaeagnus species identified in India are E. pyriformis, E. angustifolia, E. latifolia, and E. umbellata, according to Sharma and Kumar (2006). A deciduous shrub, *E. pyriformis*, is mainly found in northeastern India. In the Himalayan area, these species are found at heights of 1500 meters. It is common in Sibsagar (Dikho valley of Assam), Naga Hills (Nagaland), Khasi, Jaintia Hills of Meghalaya, and Sikkim, all Northeastern states. It is a big, woody, evergreen shrub with rusty-shiny thorns. Bees pollinate the hermaphrodite blooms to produce them. When fully ripe, the fruits of *E. latifolia* are rectangular and dark pink, while *E. pyriformis* is pyriform and have minor points on both ends. (De et al. 2017).

***Prunus bracteopadus* Koehne *(Prunus nepalensis* Hook.f.) (Khasi cherry)**

The Rosaceae family includes the underutilized wild fruit *Prunus nepalensis*, abundantly grown in the Indian state of Meghalaya's Khasi and Jaintia Hills. It is a dark purple fruit resembling a cherry with distinct organoleptic qualities (color, aroma, and flavor). It is also known as the Khasi cherry and may be processed or eaten raw. Even though it is very popular as a nutritious fruit among the local tribes, there is no scientific information available on the physicochemical characterization and value-addition of the fruit. It is naturally distributed in East Khasi Hills, West Khasi Hill, and Jaintia Hills district of Meghalaya between 1500 and 2000 m altitude. The highest diversity of Sohiong trees is observed in the East Khasi Hills District. It is distributed in the Khadar shnong area comprising villages like Dewlieh, Nongstraw, Wah Sohra, Diengsong, Tyngiar, Mawtuli, Kshaid, Phong Shnongpdei, Kharang, Krohiawhiar, Puhbsein and Nohshut. It is also observed in Mawsynram, Mawkynrew, Mylliem, Mawphlang, Mawklot, Pynursla, Pongkung, and Mawryngkneng. Some trees are also found in adjoining areas of Shillong in an isolated manner. It is an essential indigenous, nutritionally rich, underutilized fruit of temperate areas. The fruit is locally called Sohiong in Khasi (Meghalaya). The tree is medium to tall and evergreen, grown to 15-20 m. It starts bearing fruits after seven years of planting. Flowers are white, borne in terminal racemes or auxiliary. Fruits are drupe, fleshy, dark purple at full ripe, and green to pinkish in the immature stage. The fruit surface is smooth and round. Usually, the fruit shape resembles black grapes. Stone is hard and round with a smooth surface, but some other genotypes grown in mid-hills have rough stone surfaces, and the seed looks just like a peach. The stone size varies with genotype. (De et al. 2017).

Table 1. General information of some underutilized fruits

FRUIT NAME	LOCAL NAME	FLOWERING SEASON	FRUITING TIME
Baccaurea ramiflora Lour.	Latkan	during the summer months.	rainy season, i.e., July to August month
Passiflora edulis Sims.	Soh-brab	April - June	June - September
Phyllanthus acidus (L.) Skeels	Arbari	March-April	December -January
Elaeagnus pyriformis Hook.f.	Soshang	September-December	March-April
Prunus bracteopadus Koehne (= Prunus nepalensis Hook.f.)	Sohiong	November-March	July-October

Phytochemical constituents and nutritional status of underutilized fruits

Fresh *Baccaurea ramiflora* fruit and peel were described as fresh, juicy, tropical, grassy, fruity, and green and were noted as being predominantly sensory. It also had a smell that was evocative of green coconut, melon, cherry, and berries. It was discovered that lauryl alcohol significantly contributes to the fruit's fragrance characteristic and gives it a flowery scent after dilution. It has been utilized as a food ingredient that enhances the flavor and produces medicinal surfactants and monolithic polymers. The fragrance, flavor, and cosmetic sectors can all benefit from using more chemical constituents (Mann et al. 2016). The fruits were discovered to be a possible source of sapidolide A, picrotoximaesin, and ramifoside. These fruits are rich in nutritious components and famous for their sweet-sour flavor. Fruits of *B. sapida* have been shown to contain oleic and palmitic acids (Goyel et al. 2022). From the MeOH extract of fresh fruit pulp and peel, several other phytoconstituents, including nonanoic acid, octadecanoic acid, lauric acid, isovaleric acid, D-allose, and D-galactose, were discovered. People are increasingly turning to plant-based foods to fulfill their daily needs, and *B. ramifora* is among them. Over time, people have been interested in the fruit of *B. ramifora* because it is sweet-sour. The fruit is a good source of vitamin C and nutritional fiber. The fruits have low levels of fat, ash, and protein. However, they are rich in a variety of minerals, including calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na), iron (Fe), molybdenum (Mo), zinc (Zn), copper (Cu), and manganese (Mn). If included in the diet, *B. ramifora fruit*, a good provider of nutritious components, can assist in reducing the adverse effects of malnutrition. (Goyel et al. 2022).

Passion fruit commonly called "the king of fruits," is consumed raw or juiced. High quantities of polyphenols, fiber, and trace elements in the peels make them ideal for processing feed and wine or tea, cooking, and extracting pectin and other therapeutic compounds. The edible seeds are rich in protein and oil mainly composed of linoleic, oleic, and palmitic acids. Several pharmaceutical preparations based on components have been produced and utilized in folk medicine in addition to being a culinary item. Polyphenols, triterpenes and their glycosides, carotenoids, cyanogenic glycosides, polysaccharides, amino acids, essential oils, microelements, and squalene are the main components of *P. edulis*. Among these compounds, luteolin, apigenin, and quercetin derivatives are the most reported. Most importantly, passion fruit contains nutritionally valuable compounds like vitamin C, dietary fiber, B vitamins, niacin, iron, phosphorus, etc. (He et al. 2020)

Due to their high acid content, mature fruits are often acidic and sour. Fruits are a source of several nutrients and have a high moisture content. The fruit contains acids, sugars, and phenolics. The fruit also contains traces of vitamin B (thiamine, 0.01 mg/100 g, riboflavin, 0.05 mg/100 g), ascorbic acid (36.7 mg/100 g), and other nutrients (Brooks et al. 2020). Minerals can be found in abundance in *P. acidus* fruit. Numerous study teams revealed the existence of microelements, including calcium, magnesium, potassium, and phosphorus, as well as microelements like iron, copper, zinc, and manganese. There are reports of significant phytochemicals such as beta-sitosterol and D-glycoside. (Tan et al. 2020)

In terms of nutrition, *E. pyriformis* contains a sizable amount of macro elements like nitrogen, phosphorus, potassium, calcium, magnesium, and sodium, as well as microelements like iron, zinc, copper, and manganese that have the potential to have enormous therapeutic significance (Valvi & Rathod, 2011; Uprety et al. 2016). Numerous phyto-compounds, including the sugar (D-allose), ketone (Furylhydroxymethyl ketone), aldehydes (4-Methoxymethoxy-4-methyl-hex-2-ynal), fatty acids (3-Hydroxydecanoic acid), phenolics (4-Mercaptophenol), and flavonoids (4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl) were reported. Kar et al. 2016.

It is said to be an excellent source of dietary bioactive substances. Total polyphenol concentration (1131.30, mg/100 g GAE), anthocyanin content (293.33mg/100gm), beta-carotene, and antioxidant capacity (IC50 0.612 mg/mL) are high in sohiong. Fruits like sohiong are quite acidic; their pH ranges from 3.50 to 3.60. It was discovered that Sohiong has much glucose (1.52g/100gm) and fructose (1.64g/100gm). Sohiong was discovered to have high ascorbic acid (46 mg/100 g) and beta-carotene (0.215 mg/100 g) levels compared to all other peach and plum types. Sohiong was discovered to have unusually high concentrations of minerals, including calcium, magnesium, zinc, and iron. Numerous minerals, such as calcium, are essential for the body's regular operation, which is crucial for strong bones and teeth, blood pressure control, blood clotting, and good neuron function. Magnesium is necessary for protein production, nerve conduction, and muscle

contraction. Many bodily cellular processes, including sperm formation and sexual development, depend on zinc. Iron is a component of the hemoglobin in red blood cells, which transports oxygen throughout the body. Because sulfur is present in protein molecules, the high sulfur level of Sohiong may be the source of its high protein concentration. The fruit's phenolic components, which include flavonoids, other phenolic acids, and anthocyanidins, may have a higher antioxidant activity (Vivek et al. 2018).

Medicinal and health-beneficial role of some underutilized fruits

Fruits are the most prevalent sources of vital micronutrients, including vitamin C, tocopherol, carotenoids, polyphenolics, flavonoids, etc., which have nutritional value, protect the body from oxidative damage, and give health benefits. Fruit polyphenols have a variety of roles due to their redox characteristics, including hydrogen donation, singlet oxygen quenching, metal chelation, and reducing agents. Residents of the Morang District of Assam, India, consume the fruit juice of *B. ramifora* orally as an antidote for snake venom.

B. ramiflora fruit juice is a pleasant beverage with excellent health advantages. Its antioxidative capabilities showed its promise as a reasonably priced health beverage in various settings. Due to its limited cytotoxic and hemolytic impact, BRJ has few side effects. (Saha et al. 2016). Therefore, the current study demonstrates that Latkan fruit, like other commonly used berries, is suited for creating decent wine. Additionally, since fruit has a relatively limited shelf life, creating wine is an excellent solution to avoid fruit waste. The current study also clarifies that wine is a good source of natural antioxidants such as phenols, flavonoids, and proanthocyanidins, which can benefit health if eaten in moderation. (Goyel et al. 2013)

Passion fruit is especially well-liked due to its alluring nutritional and sensory features for consumer health and well-being worldwide. Due to its multiple health advantages, high commercial worth, and application in food, cosmetics, and medicine, secondary metabolites in passion fruit have drawn much interest. Due to their abundance in biologically active chemicals, passion fruit peels, which make up about 50% of the entire fruit, have a significant potential for usage as functional additives. Numerous nutritional and medicinal advantages of passion fruit have been seen and documented due to its distinctive bioactive components.

The different extracts from the various parts of *P. edulis* showed a wide range of pharmacological activities, including antioxidant, analgesic and anti-inflammatory, antimicrobial, anti-hypertensive, hepatoprotective and lung-protective, anti-tumor, antidiabetic, hypolipidemic, antidepressant and anxiolytic-like capacities, and are therefore used in phytotherapeutic remedies. According to research on acute and subacute toxicity, a rationalized daily amount of passion fruit is most likely safe to eat. These remarkable findings imply that passion fruit may provide a variety of health advantages, including managing neurological and inflammatory conditions and preventing some chronic illnesses, including hypertension and hyperlipidemia (He et al. 2020).

The herb has long been utilized in several folk remedies to cure various human illnesses. Pharmaceutical Applications Traditional medicine uses *P. acidus* to treat various illnesses. These include rheumatism, diabetes, hypertension, hepatic illness, bronchitis, asthma, and respiratory disorders. Fruit extracts have qualities that are hypoglycemic, anti-diarrheal, analgesic, antibacterial, and anesthetic. The fruit is consumed as a liver tonic in India, while it is used as a laxative in Myanmar. According to (Banerjee et al. 2022), fruit juice can potentially alleviate gentamicin-induced renal dysfunction and kidney disorders.

Native inhabitants of this area (from Assam, Meghalaya, Arunachal Pradesh, Nagaland, etc.) take these fruits to improve their diets and fend off numerous illnesses. Elaeagnus is highly well-liked by indigenous people. E. pyriformis, often known as Oleaster or Silverberry, is a member of the Elaeagnaceae family (Banerjee et al. 2022). These plants are crucial for medicine and commerce in addition to being actinorhizal. Kar et al. 2016 reported that *Elaeagnus pyriformis* has several bioactive compounds (4H-Pyran-4-one, 2,3-dihydro-3,5- dihydroxy-6-methyl, D-Allose, 5-Hydroxymethylfurfural, n-Hexadecanoic acid, Fumaric acid) which sows anti-microbial, anti-inflammatory, anti-cancer, antioxidant, Hypocholesterolemia, anti-fungal activities. Elaeagnus pyriformis also has neuroprotective activity against renal injury (Kar et al. 2019).

Important bioactive substances found in *Prunus nepalensis* include Quercetin, quinic acid, rutin, scopoletin, naringenin, and palmitoleic acid. With its distinctive taste, flavor, and color, the Sohiong fruit is popular among locals. It also makes squash, ready-to-serve drinks, jams, preserves, and wine.

The fruits are also abundant in phytochemicals such as rutin, purpurin, tannic acid, methyl gallate, reserpine, gallic acid, ascorbic acid, and catechin that can chelate iron and have the exceptional ability to scavenge free radicals, delaying the start and progression of degenerative illness. (Lata et al. 2023).

Due to the existence of pigments and other bioactive substances, the food, pharmaceutical, and textile sectors may benefit. Numerous degenerative disorders, including edema and diuresis, are routinely treated with these fruits (Vivek et al. 2017).

Conservation strategies of underutilized fruit of Northeast India

Indo-Burma Region in Northeast India is a biodiversity hotspot, with underutilized and unexploited fruits being beneficial for health benefits, revenue-generating, and eradicating poverty. Climate change, deforestation, changing farming, urbanization, and construction projects, however, are endangering these resources. The scientific community must research all plant resources and create techniques for culture, multiplication, regeneration, and propagation to preserve these genetic riches.*Ex situ* and *in situ* conservation techniques should be used to safeguard vulnerable species and help them recover. Cryopreservation, *in vitro* seed storage, and field gene banks are examples of *ex-situ* conservation techniques. On-farm conservation, natural reserves, gene sanctuaries, and the integration of underutilized and unexploited fruit species in the social forestry system are examples of *in situ* conservation initiatives. The protection and sustainable use of these priceless plants require implementing a national strategy, action plan, and program. A comprehensive inventory and documentation of the species that are currently accessible, their chemical components, antioxidants, habitats, and potential use as raw materials should be prioritized.

Conclusion

These fruits, not used as much yet, have excellent nutritional and therapeutic significance. However, despite the abundance of germplasm in India, the creation of standard cultivars remained constrained. They can survive in challenging climatic and edaphic circumstances thanks to their flexibility and tolerance. These fruits have the potential to aid in sustainable farming. Large populations of individuals in poor nations have long used herbal medications as an essential component of their healthcare systems. However, in recent years, affluent nations have also begun to favor herbal treatments due to a perception that they are secure. The market is currently flooded with several medications made from plants. These underutilized fruits do have a wide range of pharmacological effects. The information now available on this medicinal plant may be the foundation for further research into its mechanism of action, safety effectiveness, toxicity, therapeutic relevance, and potential for developing innovative pharmaceuticals. Research and development efforts, farmer knowledge, and the viability of cultivating these lesser-known fruits should be considered.

Author contributions

Swarnendra Banerjee: Investigation, Writing – original draft. **Arnab Sen:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

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Review Article

Unravelling the Roles of Plant Growth Promoting Rhizobacteria (PGPR) in Growth Promotion, Phytoremediation and as Biocontrol Agents to Suppress Plant Diseases

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Abstract

Agriculture in the twenty-first century has several issues, including soil fertility, climate changes, environmental degradation, urbanisation & rising food consumption to feed the world's growing population. Meanwhile, scientists are grappling with major obstacles in expanding food yield from the present land base. Traditional farming has seen increased per-acre crop yields due to the haphazard and injudicious use of agrochemicals, such as pesticides and synthetic fertilisers, but at a significant environmental cost. Crop pests developing pesticide resistance is another big worry in modern agriculture. Therefore, alternative ecologically friendly crop yield-increasing techniques are necessary for the future of sustainable crop production. Scientists are very interested in utility of rhizobacteria, particularly PGPR, as an alternative to pesticides. These rhizobacteria employ a range of tactics to encourage plant growth, thwart plant pests, and foster resilience to abiotic stresses. The mechanisms of rhizobacteria involved in soil bioremediation, pest biocontrol, and plant growth promotion are reviewed in this article. It also looks at how PGPR vaccination affects plant growth and survival in challenging conditions. An in-depth examination is also given of the benefits and drawbacks of rhizobacterial application as well as potential solutions for rhizobacteria's long-term use in agriculture.

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Introduction

The persistent use of chemical fertilisers to boost fertility and feed the world's rising population growth has led to a slew of environmental hazards. To feed the world's rising population, the total fertiliser nutrient demand (N, P, and K) is expected getting there 200 million tonnes by 2020 (www.fao.org). Traditional nutrient management, on the other hand, depends on exogenous chemical input, which results in lower nutrient utilisation efficiency and increased environmental risks. There is a push to develop an alternative to make better use of land, use less fertiliser, maintain soil health, and maintain ecological balance (Hasler et al., 2017). The goal is to maximise the capacity of soil and plant systems for biological activity to achieve the vision of a healthy environment. The decreased carbon components that makeup soil are a source of a variety of microbial communities in general (Backer et al., 2018). Plants with root-knot nematodes show symptoms above and below ground. The aboveground indicators are needful development and smaller, pale green leaves that fade in hot weather. (Elnahal et al., 2022). A sophisticated and organised microbial community is associated with a plant growing in the soil. However, due to its numerous beneficial benefits, Agriculture benefits more from the microbiota linked to roots. The close relationship between plant roots and bacteria has a wide range of effects, including improved plant physiology, signal exchange, resistance, and so on. PGPR are an essential tool for protecting the plant's health through a variety of mechanisms. They consume the root's nutrient-rich exudates and repay the

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favour with biofertilizers and biocontrolling. In order to shift to environmentally friendly agriculture, a complete understanding of these interactions is crucial for improving soil fertility as well as plant health.

Roles of PGPR in various aspects

According to Bhattacharyya et al. (2012), PGPR was first utilised in order to boost yields and protect plants from environmental challenges including floods, droughts, high salt, phytopathogens and other environmental variables. PGPR will be enjoyed by heavy metal lovers. High levels of heavy metal content in the soil must be tolerated by phytoextraction. To develop this tolerance, mechanisms that reduce metal ion toxicity must be put in place. The transformation of metal ions into less harmful forms or their encapsulation in extracellular or intracellular polymers are examples of these processes (Rajkumar et al., 2013). Metal-resistant Increased plant tolerance to high levels of heavy metals in soil is the main result of PGPR, which controls ethylene concentrations by producing ACC deaminase (Hrynkiewicz et al., 2011). Gibberellins, cytokinins, and auxins, as well as siderophores that change nutrient and metal bioavailability, are produced by plants as a result of PGPR. Plant growth-promoting endophytes (PGPE) are microbes that inhabit plants and provide the nutrients they need to develop and survive (Lodewyckx et al. 2002). Most of the time, PGPR employed in phytoextraction investigations is isolated from rhizosphere of plants growing in contaminated soils (Mendoza-Hernández et al., 2019, Jinal et al,. 2019)andis therefore adapted to high metal concentrations, while PGPE utilised to improve phytoextraction is often isolated from hyperaccumulator plants or other polluted soil-growing plants (Tang et al. 2020). Examples of recent PGPR/PGPE utilisation in phytoremediation employing Brassica species are shown in Table 1.

Mechanisms exhibited by PGPR

PGPR can boost various ways, either directly or indirectly, to evolution of plants (Figure 1 & Figure 2) (Ahmed et al., 2017). Vitamins, phytohormones, HCN, ammonia, siderophore synthesis, phosphorus solubilization and nitrogen-fixing (such as auxin, cytokinin, and gibberellins) are examples of direct mechanisms, whereas indirect mechanisms are those that are not directly involved in growth promotion but play a role in the synthesis path. ACC deaminase activity, antibiotic synthesis, hydrolytic enzymes, and phytopathogen ISR are examples of indirect processes (Aloo et al. 2019).

Direct Mechanisms of action

Biological Nitrogen Fixation

The growth of plants depends on the presence of nitrogen. Among other things, it can be found in proteins, enzymes, and nucleic acids. Unfortunately, plants and animals cannot access nitrogen, a gas that predominates in the atmosphere. The conversion of atmospheric nitrogen to ammonia is required for plant nitrogen absorption. Nitrogen-fixing bacteria that include an enzyme complex termed nitrogenise facilitate the process, which is known as biological nitrogen fixation (Smith et al., 2013). A symbiotic relationship is a mutualistic relationship in which both microbes and plants benefit (Ahemad et al., 2012). *Rhizobium* and *Mesorhizobium* create symbiotic relationships with *leguminous* plants, but *Frankia* forms symbiotic relationships with non-leguminous trees & shrubs (Zahran et al., 2001). *Cyanobacteria* (*Nostoc*, *Anabaena*), *Azotobacter*, *Gluconacetobacter* & *Pseudomonas* form a non-symbiotic relationship that can be both free-living and endophytic (Meena et al., 2021). Thus, inoculating seeds, seedlings, or soil with nitrogen-fixing microorganisms stimulates plant growth, improves soil quality, and maintains nitrogen levels in the soil (Damam et al. 2016). Encouragement of plant growth is done by PGPR in a number of direct and indirect methods. The most advantageous growth strategy for PGPR is biological nitrogen fixation, and molecular analysis of isolates of PGPR that fix nitrogen has revealed the presence of several *nif* genes, which encode the nitrogenase enzyme. A membrane complex that aided in electron transfer to the nitrogenase enzyme was made by the fixABCX gene, which was discovered in nitrogen-fixing Rhizobium species & other diazotrophs, in addition to *nif* genes (Mahmud et al., 2020).

Solubilisation of phosphate

A crucial component for the growth and development of plants is phosphorus. It participates in almost all of a plant's metabolic activities, including photosynthesis; respiration, signal transduction and energy transfer (Ahmed et al., 2017). The majority of phosphorus is contained in the soil as insoluble organic and inorganic phosphate. Phosphate solubilizing bacteria (PSB) is significant in this area since they can release phosphates from organic molecules and solubilize insoluble inorganic phosphate. Plants can only absorb monobasic and dibasic phosphate ions (HPO4−and H2PO42−, respectively) (Gouda et al.,

2018). Phosphorus is extracted from organic molecules using several methods. Phosphatases break down phospho-ester linkages; phytases liberate phytic acid; phosphonatases employ magnesium (II) as a cofactor to catalysethe hydrolysis of phos-phonoacetaldehyde to produce acetaldehyde and phosphate and C-P lyases catalyse the C-P cleavage of phosphonates. (Morais et al., 2000).

Table 1. Represents the uses of PGPR in phytoremediation with *Brassica* species

Host	Bacteria	PGPR Effect	Reference
Brassica juncea	*Bacillus* sp. PZ-1	Increased biomass (up to 35%) Pb absorption by roots (28.3-83.6%) and shoots (52-106%) increased. A higher TFroot-shoot (12–55%)	Yu et al. (2017)
Brassica juncea	*Bacillus toyonensis* (MG430287) *Rhodococcus hoagii* (MG432495) *Lysinibacillus mangiferihumi* (MG432492) *Lysinibacillus fusiformis* (MG430290)	Increased rot length (47–106%) lengthened shoots (by 49–71%) Enhanced absorption of Fe (57.91-128%) production of antioxidant compounds has increased.	Jinal et al. (2019)
Brassica napus	*Bacteroidetes bacterium, Pseudomonas fluorescens Variovorax* sp.	Biomass has not increased. Increased roots and shoots Cd uptake (up to 12% and 10%, respectively) greater uptake of Zn (18% in shoots and 8% in roots)	Tang et al. (2020)
Brassica juncea	Isolates SMV242, SMV244, SMV248, SMV250, and Actinobacteria, Proteobacteria, and Firmicutes are the three phyla that SMV251 belongs to.	Biomass has not increased. As uptake increased only in roots (55%) Only when the mobilising chemical K2HPO4 (150%) is present do shoots exhibit increased As absorption.	Franchi et al. (2018)
Brassica juncea	*Burkholderiaphytofirmans*PsJNT	No increase in biomass Increased shoot uptake of Cd (22%) and Zn (38%)	Konkolewska et al. (2020)
Brassica juncea	*Rhizobium leguminosarumbv. I strain RCAM1066, Variovorax. paradoxus strain 5C-2, and Glomus sp. strain 1Fo of the AMF are three examples.*	Biomass has not increased. Uptake of Cd increases (by up to 10%)	Belimov et al. (2020)

Production of siderophores

Surface iron in aerobic conditions is converted into an insoluble form like oxyhydroxide, which leads to the production of ferric oxide, microbes have trouble getting enough iron to maintain their growth in the rhizosphere. Iron is needed for enzyme cofactors, oxygen metabolism, electron transport, DNA and RNA synthesis, as well as biofilm formation (Patel et al., 2018). PGPR-produced siderophores help plants get the iron they need by making it soluble and chelating it from accessible complex organic and inorganic iron (Singh et al. 2017). Some microbes create a siderophore that chelates available iron and competes with phytopathogens for iron feeding (Shaikh et al., 2016). *Alcaligenes*, *Pseudomonas*, *Bacillus* and *Rhizobium* all produce siderophore (Shaikh et al., 2015). The ability to colonise roots and exclude other bacteria due to siderophore synthesis gives PGPR a competitive advantage. The ability to obtain iron via siderophores may decide the outcome of competition for diverse carbon sources accessible as a result of root exudation andrhizodeposition under highly competitive situations (Tsegaye et al., 2017).

Production of IAA

80 % of PGPR produces IAA, an auxin that is physiologically active and encourages several growths including cell division, elongation, and differentiation (Ahmed et al. 2017). The most prevalent genera of bacteria involved in the production of IAA rhizosphere of different crops are *Acinetobacter*, *Rhizobium*, *Bacillus* and *Klebsiella* (Choudhary et al. 2018). *Pseudomonas spp*. is the most powerful producer of IAA among these bacterial genera, with *Pseudomonas putida*

producing more IAA than *Pseudomonas fluorescens* (Singh et al., 2019).

Indirect Mechanisms of Action

Production of hydrogen cyanide

HCN synthesis is required for strains that encourage plant growth to function. Due to its great toxicity against plant diseases, ability to chelate metal ions, and indirect ability to increase the availability of phosphate, In the agricultural production system, hydrogen cyanide is commonly used as a biocontrol agent (Rijavec et al., 2016). HCN-producing PGPR and their usage as a biofertilizer for promoting growth, increasing yields, and preventing disease have been described by a number of studies (Ahmed et al., 2017). Numerous bacterial taxa, such as *Aeromonas*, *Pseudomonas*, *Bacillus*, and *Enterobacter*, have been found to emit HCN in the rhizosphere (Vaikuntapu et al,.2014).

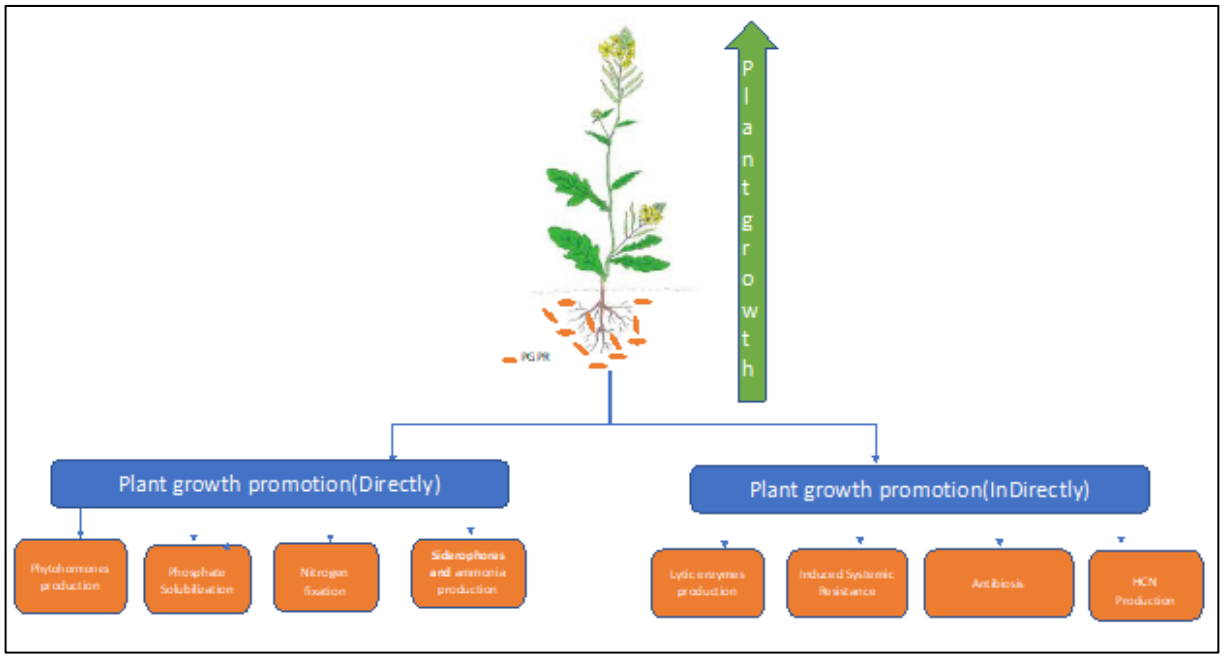

Figure 1. Direct and indirect plant growth promotion in a diagram (Mhatre *et al.* 2019)

Induced systemic resistance (ISR) and antibiotic production

PGPR strains produce antibiotics like phenazines, pyrrole-type compounds, butyrolactones, 2,4-diacetyl phloroglucinol, pyrrolnitrin, polyketides, and peptides that strengthen plant defence systems against infections (Govind et al., 2015). Numerous plant diseases have been discovered to be suppressed by PGPR strains that produce antibiotics, including *P. fluorescens* MKB 100, B. subtilis BMB 26, and *P. fluorescens* BL915 (Khan et al., 2005). Induced systemic resistance, also known as rhizobacterial strains, has been proven to give plants protection against pathogenic fungus, bacteria, viruses, nematodes, and pests (ISR) (Lugtenberg and Kamilova, 2009). The host plant's defence response is triggered when it is subjected to a high level of pathogenic infections by the PGPR increasing jasmonate/ethylene reliant on ISR-specific signals, which either induces specific jasmonic acid sensitive genes or upregulates ISR-associated genes (Glick, 2012).

PGPR is employed as a biocontrol agent

Several PGPR strains are utilised to manage a wide range of plant diseases by secreting a number of chemicals, including phenazine, DAPG, viscosinamide and tensin, which are frequently found to be disease suppressors. *Pseudomonas*, *Azotobacter*, *Bacillus* and *Streptomyces* are among the bacteria (Bharti et al., 2016). *Rhizobacteria* can inhibit the growth of several phytopathogens by competing for nutrients and space, creating lytic enzymes, bacteriocins, antibiotics, and siderophores, among other things (Tariq et al., 2017).

Biofertilizers

The words "biofertilizer" and "bioinoculant" have been derived in a variety of ways as a result of the remarkable advancements made in the study of the interaction between microorganisms and plants during the past 20 years. According to Vessey et al. (2003), "a material containing living

microorganisms that, when applied to seed, plant surfaces, or soil, colonises the rhizosphere or the inside of the plant and encourages growth."A revised definition of biofertilizers was later proposed by Dineshkumar et al. (2018) as "products (carrier or liquid based) containing living or dormant microbes (bacteria, actinomycetes, fungi, algae) only or in combination, which helps in fixing

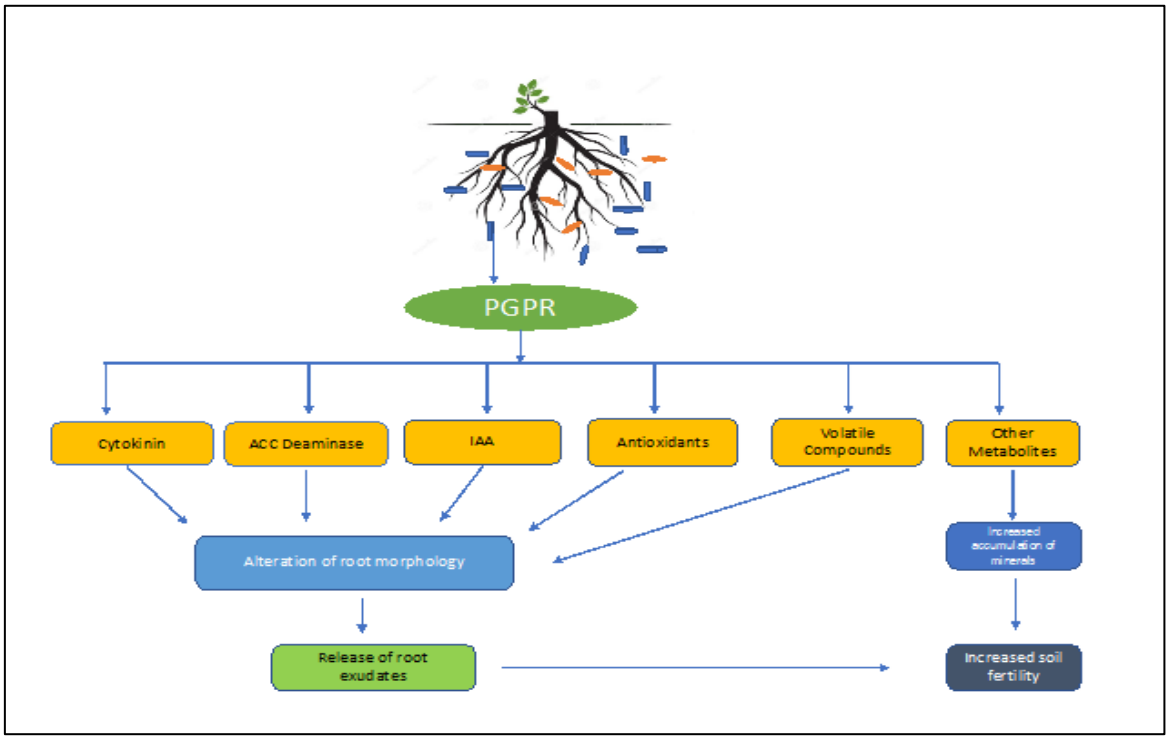

Figure 2. PGPR plays in creating sustainable crop production systems (Sharma et al. 2017)

atmospheric nitrogen or solubilizing soil nutrients further to secreting growth promoting substances for improving crop growth and yield." The microorganisms in biofertilizers use a number of techniques to assist agricultural plants. They may be adept in promoting plant growth, phosphate solubilization, and nitrogen fixation, or they may combine these abilities. (Mahanty et al., 2017). Compared to chemical inoculants, microbial inoculants provide a variety of benefits. (Meena et al., 2020). They are trustworthy sources of renewable nutrients needed for soil biology and wellness that are also environmentally benign (Sun et al., 2020). Additionally, they defend against different crop diseases and combat abiotic stresses (Ilangumaran et al., 2017). A number of microbial taxa have been employed commercially as efficient biofertilizers because of their ability to draw nutrients from the soil, fix atmospheric N2, enhance nutrient solubilization, and act as biocontrol agents (Schütz et al., 2018).

Ideal PGPR Characteristics

(1) It ought to be rhizosphere-capable and environmentally friendly.
(2) After inoculation, it should colonise the plant roots in substantial quantities.
(3) It should be capable of encouraging plant development.
(4) It should have a wide range of actions.
(5) The bacteria in the rhizosphere must get along with one another.
(6) Physical and chemical factors including heat, humidity, radiation, and oxidants must not harm it.
(7) It should outperform existing rhizobacterial communities in terms of competitive abilities.

PGPR's Mechanisms

Being the dominant microbial population in the rhizosphere, PGPR either actively or passively contributes to the support of plant growth. By enhancing biotic and abiotic stress tolerance and supplying nutrients to host plants, they can act as biofertilizers, boosting plant growth and development (Sagar et al. 2020, Mahdi et al. 2020). These helpful bacteria defend plants and aid in their growth through a number of processes, including root colonisation, favourable effects on plant physiology and growth, biofertilization, inducing systemic resistance, and biocontrol of

phytopathogens, among others. The precise mechanisms of PGPR action and their distinctive role in promoting plant growth have been widely investigated (Swarnalakshmi et al. 2020). According to general definitions, the direct and indirect ways that PGPR promotes plant growth take place inside and outside of the plant, respectively (Goswami et al. 2016).

Commercialization of PGPR

In addition to promoting plant development, improving soil fertility, and controlling phytopathogens, PGPR is used as a nematode biocontrol agent and as an ecologically friendly substitute for synthetic agrochemicals like chemical fertilisers and pesticides promoting sustainable agriculture. PGPR-based biocontrol agent development and commercialization guidelines. Although different strains of PGPR are already offered as biological nematicides on the market, a straightforward query (i.e., repeatability) needs to be resolved before PGPR may be commercialised. However, more research on these products' efficacy is required. To be profitable, PGPR products need to have a variety of uses, a long shelf life, safety during use, a viable market, accessibility, consistency in terms of efficacy, and a cheap investment cost.

Plant Gene Expression and the PGPR

In addition to nitrogen fixation, phosphate solubilization is a well-known property of PGPR isolates. The six core genes of the PQQ operon, which codes for the membrane-bound enzyme glucose dehydrogenase and its enzymatic cofactor pyrroloquinoline quinine (PQQ), namely *pqqA*, *paqB*, *pqqC*, *pqqD*, *pqqE*, and *pqqF*, solubilize mineral phosphates (Matsushita et al., 1982). Another key aspect of PGPR is the creation of a siderophore, which aids plant growth by solubilizing and transferring iron through the generation of soluble Fe3+.The up-regulation of the sid gene by PGPR is said to be responsible for siderophores synthesis (Ovaa et al., 1995). Plant gene expression is altered by PGPR, which controls genes involved in metabolism, stress response, defence, and phytohormones. Plant exudates operate as signalling chemicals, influencing microbiont gene expression (Sharma et al., 2019), ISR and hormonal homeostasis were associated with the majority of differentially regulated activated genes. The gene expression of the nitrate and ammonium absorption genes in Arabidopsis thaliana was altered by PGPR, as demonstrated by Calvo et al. (2019). Reduced expression of the cell wall and root defence mechanism genes was observed after B. subtilis colonisation of A. thaliana plants (Blake et al., 2021). During the colonisation of rice plantlets' roots, B. subtilis RR4 has been shown to repress several defense-related genes in order to enhance plant immunity (Rekha et al., 2018). The molecular processes by which PGPR isolates stimulate plant growth are still being researched, and further research is needed to confirm how PGPR regulate phytobeneficial features during plant colonisation, there is gene regulation between bacteria and plants.

Conclusions and Implications for the Future

Among the several sectors of the economy in a nation, the agriculture sector not only ensures its citizens' subsistence moreover helps the nation satisfy its export and population growth demands. Following the Green Revolution, the agroindustry has experienced a number of technological advances that have enhanced agricultural productivity but come with negative environmental effects. While biofertilizers are natural products with no environmental danger, chemical fertilisers are bad for the health of the soil and the environment. Therefore, in terms of maintaining long-term soil fertility and crop yield, fertilisers made from natural products demonstrate that they are a crucial and integrated part of sustainable agriculture. A revolution has unavoidably occurred in the preceding ten years due to the growing usage of biological inoculants in place of agrochemicals for sustainable agriculture. In addition to being essential for overall crop plant development and production. Our planet's health and appropriate biogeochemical cycling depend on the interactions between the bioinoculant microorganism(s), local soil microbiota, and host plant(s).

Farmers' emphasis is predicted to shift toward organic farming and adoption of sustainable agricultural practises as concerns about food safety and the need to control food production quality to meet shifting customer demand develop. As a result, when looking for environmentally acceptable alternatives to harmful chemicals, it's important to keep the three "Ps" in mind: people, prosperity, and the environment. Biofertilizers should be promoted by governments and federal agencies as environmentally acceptable crop development choices. Entrepreneurs ought to invest more in the biofertilizer sector and support startups financially. To secure a greener future, it is additionally necessary to raise general public awareness in order to inform farmers and

consumers about the advantages of using microbe-based biofertilizers.

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Research Article

Acaricidal and Ovicidal Effects of *Vitex negundo*, Against *Oligonychus coffeae*, A Common Pest Found in Tea Gardens of North Bengal, India

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Abstract

Tea, a widely consumed and economical beverage across 65 nations, confronts substantial challenges from pests, notably the destructive Red spider mites, which pose significant hurdles for the industry due to their resilient nature and severe impact. Chemical pesticides, while effective against pests in tea production, degrade tea quality, prompting exploration into natural alternatives like floral diversity for pest control. A study in North Bengal, India, aims to the pest-controlling abilities of common weed found in tea gardens in the Terai and Dooars regions.The aqueous extracts of Vitex negundo L. as biocide employed in this study demonstrated substantial acaricidal and oviposition deterrent action against the tea Red Spider Mite Oligonychus coffeae after 48 hours of application. In the future, this aqueous extract could prove to be a fairly priced and efficient acaricide.

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Introduction

The majority of people in the world nearly two-thirds consume tea, which is their second favourite beverage after water. India produces a significant amount of tea, with North East India producing over 75% of all the country's tea (Roy et al.2020). India is also the world's greatest consumer of tea, with 90% of Indian families reporting regular tea consumption (Jain, 2012). Tea plants are subjected to many pest attacks though only around 380 species of phytophagous insects and mites pests are documented from India, it is predicted that more than 1000 species of arthropods infest tea throughout the world as pests, incidental visitors, as well as predators and parasitoids of pests (Hazarika et al. 2009a) (Roy et al. 2014). Pest-related crop loss is from 15% to 20%. (Muraleedharan and Selvasundaram 2002). Given the increased productivity and output, the magnitudes of losses are certain to be bigger today. Due to the frequent and uncontrollable reappearance of the pest and the prolonged usage of conventional pesticides, the mites developed high chemical resistance (Roy et al. 2019), resulting in crop loss and a consequent economic blow to India's tea trade. In order to combat significant pests of tea in Assam, Darjeeling, and West Bengal, (Gurusubramanian et al. 2008) utilized the ovicidal, antifeedant, and insecticidal or acaricidal properties of solvent extracts from a variety of plants, including *Heliotropium indicum* L., *Spilanthes calva* DC., *Polygonum* h*ydropiper* L., *Pogostemon parviflorus* Benth., *Polygonum glabra* (Willdenow) M. Gómez, *Azadirachta. Indica* A.Juss. etc against *Adalia bipunctata*. Similar *to P. hydropiper*, *Annona squamosa*, *Clerodendrum viscosum*, *Argyreia speciosa*, and *Leucas aspera*, aqueous extracts of these plants have demonstrated varying degrees of control over the black-inch looper *Hyposidra talaca* (Roy et al. 2015).

In *Vitex negundo* L. there are several secondary phytochemical metabolites present in every part of the plant (Vishwanathan and Basavaraju, 2010), from the root to the fruit, giving it an unheard-of range of medical benefits. Fresh leaves of *V. negundo* L. have anti-inflammatory, analgesic, and antihistamine properties (Dharmasiri et al. 2003). There is not much work regarding the efficacy of Vitex negundo against *Oligonychus coffeae* Neitner

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done in thease North Bengal region except a few (Deka et al.2017). Therefore, the present work has been designed to primarily evaluate the efficacy of aqueous extracts of *V. negundo* L. to control the tea red spider mite (*O.coffeae*) as a part of integrated pest management with inquisitive emphasis on adulticidal and ovicidal effects.

Materials and methods

Red Spider Mite (RSM) rearing

The detached leaf culture method of Roy et al. 2010 was used to maintain a red spider mite culture on a sensitive tea clone, like TV 25, and TV 26 in the lab at 25C and 70-80% relative humidity.

Preparation of botanicals

Locally available leaves and succulent stems of *Vitex negundo* L. (Lamiaceae) were collected in North Bengal, India. After drying in the shade, the plant material was pulverized with an electric grinder. Using soap nut powder as a surfactant, aqueous plant extracts from powder samples were created using the cold percolation technique. Using Whatman (no. 1) filter paper, the resulting extract was collected, filtered, and the volume was adjusted to produce the desired concentrations.

Bioassay studies of botanical aqueous extracts on Oligonychus coffeae adults

Twenty red spider mites, healthy gravid females, were introduced on Tea leaf discs with a 2 cm diameter, dipped in various extract strengths, and dried. Then, filter paper and a damp cotton bed are laid on top of the leaf discs. Leaf discs that had been wetted were utilized as a control. Each concentration of the investigated plants was subjected to three separate iterations of the experiment. We studied the leaf discs after 24 and 48 hours (Fig.1).

Statistical analysis

Probit analysis and IBM SPSS version 21 were used to find the LC50 values or lethal doses that caused 50% of the larvae to die. Results were considered statistically significant if they met the threshold of 0.05.

Ovipositional deterrence

By using the technique developed by Roy et al. 2011, the ovipositional deterrent generated by aqueous extract after 48 hours was investigated.MS Excel was used to determine the discrimination quotient using the formula provided by Roobakkumar et al. 2010. On each of the variably treated leaf discs, five gravid females were transplanted and permitted to oviposit. After transferring all the mites, the number of eggs laid was counted. As a baseline for all tests, water-treated leaf discs were used.

Results and Discussion

The mite populations in the Terai and Dooars showed high resistance to widely used acaricides like Fenazaquin 10 EC and Propargite 57 EC because they were used for a long period. The present bioassay and ovipositional deterrence studies were done by using pieces of tea leaves dipped in different concentrations of aqueous extracts of *Vitex negundo* against *O. coffeae* and LC50 and LC90 values were determined which shows the lethal concentration of mite mortality. By using probit analysis, it was discovered that adult O. coffeae mortality was linearly proportional to the length of time following treatment for each increasing concentration. the LC50 and LC90 values after 48 hours for *Vitex negundo* aqueous extract is 19.551 mg/ml and 93.437 mg/ml. In control the mortality was null (Fig 1 and 2).

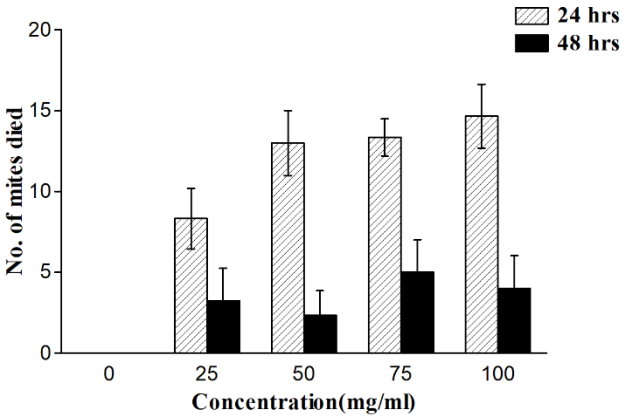

Figure 1. Mortality rate of *O. coffeae* with the treatment of botanicals

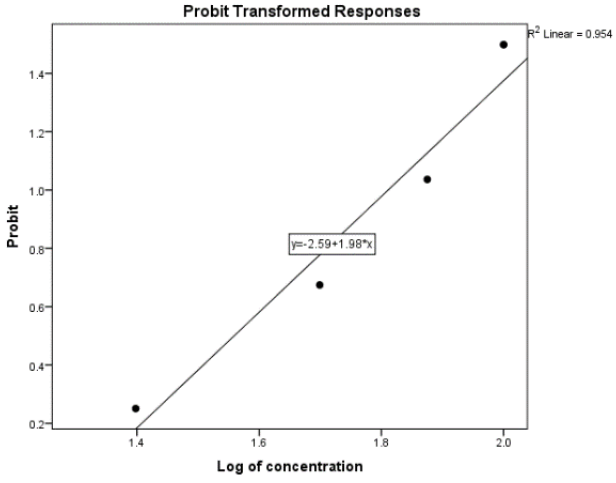

Figure 2. Probit analysis of *Vitex negundo* plant extract

The botanicals demonstrated ovipositional deterrents, and their discrimination quotient was assessed (DQ). The discriminating quotient, which runs from 0 to 1.0, is a technique for assessing how drugs affect insects' egg-laying behavior. The mites made a distinction between the treated leaves based on the number of eggs deposited when the leaves were treated with botanicals. On leaf discs treated with various doses of the botanicals' aqueous extract, red spider mites did not produce eggs. The value of the Ovipositional Discrimination and Deterrence Quotient is stated in Table 1.

Table 1. Ovipositional deterrence and discrimination quotient (DQ) value of *Vitex negundo* extracts on *Oligonychus coffeae*

Treatment	Dosages mg/ml	No. of egg laid	DQ value
Vitex negundo	25	8.3	0.566
	50	5.1	0.709
	75	4	0.764
	100	2	0.875
Control	0	30	

The number of mite eggs placed on leaf discs treated with the strongest aqueous extract of *Vitex negundo* during the investigation of ovipositional deterrence was zero, and the DQ value it provided was 1.0, which is consistent with the findings of (Thanigaivel et al. 2017). The cost of making botanicals from weeds that occur often in tea plantations is virtually nothing, therefore, economically; botanicals can also reduce the cost load of controlling red spider mites at least impeding them from reaching the economic threshold level.

Conclusion

The importance of biopesticides has increased due to the risk or adverse effects of chemical pesticides and their growing resistance to pests. Thus, the use of traditional chemical pesticides is declining daily as a result of increased awareness of the deleterious effects of chemicals. Significant acaricidal and ovipositional deterrent effects were found in the aqueous extracts of *V. negundo* L which was used for this study. However, since the experiments were conducted in a lab setting, its effectiveness in the plantations of the North Bengal region is still unknown. Further studies are required to validate the findings in field conditions, with respect to the present climate changes.

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Conflicts of interest

There are no actual or potential conflicts of interest to declare.

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Research Article

A Pilot Study of Sun Protective Factor of Selected Lichens from Himalayan Region

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Abstract

The Sun's ultraviolet light can causes early aging of the skin, leathery skin, Wrinkles, actinic keratosis and liver spots on our skin. Every plants contain many active constituents that can protect our skin form sun burn. There are many synthetic sunscreen are offered in market, but formulation of natural sunscreen is an important aspect in cosmetic industry. Thus the aim of the present study is to inspect the presence of UV light absorption ability of the selected lichens.

Keywords: Lichens, Sun Protection Factor, Sunscreen, UV light

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Introduction

Exposure to Sun rays may lead to skin damage and skin cancer also. Because skin is susceptible to the photo damage due to direct exposure to the solar radiation. However, UV also welfares human health by facilitating natural production of vitamin-D and endorphins in our skin, hence UV has complex and diverse effects on our health. UV radiation is divided into three regions, UV-A (400-320nm), UV-B (320-290nm), UV-C (290-200nm). UV-A can causes indirect damage to the microbe's DNA and causes darkening and tanning effect on human beings. UV-B mostly absorbed by the ozone layer and only a small amount reaches to the earth. But it is one thousand times more responsible to cause skin cancer and highly dangerous for human skin. UV-C rays are completely absorbed by ozone layer and not causing any more harmful effect on the skin (Nohynek et al. 2010). So, the protection from UV-A & UV-B is most important. Though there are many chemical products are available in the market but they pose many adverse effects. Therefore, evaluation of plant-based sunscreen is important. Lichen communities are familiar for their great diversity in high-altitude terrestrial ecosystems and they face the threats of UV radiation rising from the polar O_3 depletion (Marie et al. 2015). In presence of polar UV radiation, they increase the production and accumulation of different secondary biochemical in their body to decrease UV penetration (Bjorn 2006). In lichen UV protectant phytochemicals are produced by both the photosynthetic and fungal partner (Nguyen et al. 2013). To keep this view in mind, the goal of the present work is to find out the potency of seven selected lichen species as a skin protective agents and comparative SPF (Sun Protective Factor) values of them are also evaluated using Mansur equation.

Materials and Methods

Chemicals and Reagents

Methanol of analytical grades and procured from Merck, India Ltd.

Collection and Authentication of Plants

The Lichens were collected from different places of India. After the collection the specimens were dried properly and then studied morphologically (i.e. forms, size, structure etc.), anatomically (i.e. cellular structutre) and chemically (by color spot tests) to identify. Finally, we select seven lichen specimens to study (Table 1).

Extraction of Plant Material

After sample collection, the materials were washed properly and then shade dried. Then the dried lichens were grounded into powder. Then the dried lichens were grounded into powder. Then 10g powdered lichen added to 100ml methanol and kept on a rotary shaker for 24h. at 150 rpm at 25 ºC and then filtered through whatman No.1 filter paper. Next day extracts were concentrated under reduced pressure on a rotary evaporator at 40°C. Then the concentrated were kept in a desiccator until the traces of solvents was evaporated completely.

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Table 1. Details on lichens collection

SL No.	Lichen Name	Family	Habit (Thallus type)	Habitat (Based on substrate)	Place of collection	Date of collection
LS1	*Parmotrema austrosinense* (Zahlbr.) Hale	Parmeliaceae	Foliose	corticolous	Kalimpong Hills	March,2019
LS2	*Parmotrema sancti-angeli* (Lynge) Hale	,,	,,	,,	Nainital	Oct,2019
LS3	*P. tinctorum* (Nyl.) Hale	,,	,,	,,	Sikkim	Jun,2020
LS4	*Flavoparmelia caperata* (L.) Hale	,,	,,	,,	Nadia	July,2020
LS5	*Parmelia sulcata* Taylor	,,	,,	,,	Burdwan	,,
LS6	*Evernia prunastri* (L.) Ach.	,,	Fruticose	,,	Kalimpong Hills	Sept,2020
LS7	*Usnea florida* (L.) F. H. Wigg.	,,	,,	,,	,,	,,

Finally, the dried crude samples were kept in air tight vial and stored at 4 ºC for further studies.

Absorbance Capacity of Extracts in UV Region

The antisolar activity was executed by UV-visible spectrophotometry. Photoprotective elements that have specific absorbance at specific UV spectrum. To observe the UV absorbing property of the lichen extracts spectrophotometric readings were taken from 200 to 400 nm with 50 nm variation.

Sample preparation

1 mg of each extract dissolved in 1 ml of methanol dissolved in methanol to prepare stock solution (1mg/ml). Then made different concentrations such as 0.5mg/ml & 0.25mg/ml by serial dilutions.

Determination of SPF (Sun Protection Factor) of Lichen extract

The efficacy of sunscreen agent is usually expressed by the SPF, which is defined as the UV energy required to yield a MED (minimal erythema dose) on protected skin, divided by the amount of UV energy necessary to produce a MED on the undefended skin.

SPF = Minimal Erythemal Dose of Protected Skin / Minimal Erythemal Dose of Unprotected Skin.

To determine the SPF value of different concentration at 290-320 nm at 5 nm interval. The measurements were performed in triplicate for each concentration using 1 cm quartz cell and methanol was used as blank. Mansur equation (Kaur and Saraf 2010) was used to determine the SPF values of the formulations by using a UV spectrophotometer. The Equation is,

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where, CF = Correction Factor (10)
EE (λ) = Erythrogenic Effect of radiation
I (λ) = Solar Intensity spectrum
Abs (λ) = Spectrophotometric absorbance value.
The standards of EE x I are shown in Table 2.

Table 2. Normalized product function used in calculation of SPF

No	Wavelength (λ)	EE X I (normalized)
1	**290**	**0.0150**
2	**295**	**0.0817**
3	**300**	**0.2874**
4	**305**	**0.3278**
5	**310**	**0.1864**
6	**315**	**0.0839**
7	**320**	**0.0180**
Total		**1**

Results and Discussion

SPF value has become a global quantitative measurement of for determining the efficiency of sunscreen formulation. It provides a knowledge about how lengthy we can stay in the sunlight starved of getting hurt by the sun. The SPF number of methanol extracts of the lichen specimens was deliberate by applying mathematical equation. The absorbance (between 290-320 nm) nm and SPF values of the samples determined through the UV-Spectrophotometric method are revealed in Table 3-5. Table 3 represents the SPF Values of 1000µg/ml concentration of different lichen extracts. Table 4 represents the SPF Values of 500µg/ml concentration and Table 5 represents the SPF Values of 250µg/ml concentration of the lichen extracts. SPF value was calculated by absorption spectroscopy using the Mansur equation.

Table 3. Determination of *in vitro* SPF at 1000 µg/ml concentration of lichen extract

No	Wave length (λ)	EExI	L1		L2		L3		L4		L5		L6		L7	
			Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs
1	290	0.0150	2.438	0.0372	0.917	0.0138	2.341	0.0351	2.383	0.0357	2.394	0.0359	2.482	0.0372	2.429	0.0364
2	295	0.0817	2.468	0.2016	0.953	0.0779	2.372	0.1938	2.414	0.1972	2.423	0.1980	2.516	0.2056	2.461	0.2011
3	300	0.2874	2.455	0.7056	1.003	0.2883	2.358	0.6777	2.395	0.6883	2.409	0.6923	2.502	0.7191	2.442	0.7018
4	305	0.3278	2.357	0.7726	1.022	0.3350	2.257	0.7398	2.301	0.7543	2.318	0.7598	2.410	0.7899	2.351	0.7707
5	310	0.1864	2.377	0.4431	0.990	0.1845	2.268	0.4228	2.313	0.4311	2.329	0.4341	2.435	0.4539	2.373	0.4423
6	315	0.0839	2.314	0.1941	0.902	0.0757	2.118	0.1777	2.176	0.1826	2.228	0.1869	2.370	0.1988	2.308	0.1936
7	320	0.0180	2.475	0.0446	0.809	0.0146	1.654	0.0298	1.701	0.0306	2.154	0.0388	2.526	0.0455	2.456	0.0442
SPF				2.3988		0.9898		2.2767		2.3198		2.3458		2.4500		2.3901

Table 4. Determination of *in vitro* SPF at 500 µg/ml concentration of lichen extract

No	Wave length (λ)	EExI	L1		L2		L3		L4		L5		L6		L7	
			Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs
1	290	0.0150	2.396	0.0359	0.460	0.0069	2.292	0.0439	2.293	0.0344	2.034	0.0305	2.426	0.0364	2.212	0.0332
2	295	0.0817	2.422	0.1979	0.478	0.0391	2.329	0.1903	2.339	0.1911	2.038	0.1665	2.454	0.2005	2.127	0.1738
3	300	0.2874	2.404	0.6909	0.504	0.1448	2.310	0.6639	2.318	0.6662	2.026	0.5823	2.441	0.7015	2.055	0.5906
4	305	0.3278	2.307	0.7562	0.516	0.1691	2.188	0.7172	2.190	0.7179	1.932	0.6333	2.348	0.7697	1.989	0.6520
5	310	0.1864	2.322	0.4328	0.498	0.0928	2.036	0.3795	1.993	0.3715	1.788	0.3333	2.370	0.4418	1.977	0.3685
6	315	0.0839	2.251	0.1889	0.453	0.0380	1.518	0.1274	1.445	0.1212	1.506	0.1264	2.308	0.1936	1.895	0.1590
7	320	0.0180	2.365	0.0426	0.405	0.0073	0.954	0.0172	0.893	0.0161	1.188	0.0214	2.453	0.0442	1.819	0.0327
SPF				2.3452		0.4980		2.1394		2.1264		1.8937		2.3877		2.0098

Table 5. Determination of *in vitro* SPF at 250 µg/ml concentration of lichen extract

No	Wave length (λ)	EExI	L1		L2		L3		L4		L5		L6		L7	
			Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs
1	290	0.0150	2.187	0.0328	0.200	0.0030	1.398	0.0210	1.304	0.0196	0.876	0.0131	2.249	0.0337	0.979	0.0147
2	295	0.0817	2.116	0.1729	0.208	0.0170	1.468	0.1199	1.382	0.1129	0.869	0.0710	2.211	0.1806	0.885	0.0723
3	300	0.2874	2.064	0.5932	0.219	0.0629	1.495	0.4297	1.377	0.3957	0.863	0.2480	2.178	0.6259	0.838	0.2408
4	305	0.3278	1.996	0.6543	0.224	0.0734	1.310	0.4294	1.234	0.4045	0.821	0.2691	2.121	0.6953	0.818	0.2681
5	310	0.1864	1.972	0.3676	0.216	0.0403	1.035	0.1929	0.968	0.1804	0.733	0.1366	2.130	0.3970	0.803	0.1497
6	315	0.0839	1.870	0.1569	0.197	0.0165	0.691	0.0580	0.642	0.0539	0.598	0.0502	2.061	0.1729	0.770	0.0646
7	320	0.0180	1.792	0.0323	0.175	0.0032	0.414	0.0075	0.382	0.0069	0.458	0.0082	2.070	0.0373	0.711	0.0128
SPF				2.0100		0.2163		1.2584		1.1739		0.7962		2.1427		0.8230

SPF of the lichen extracts was determined by taking diverse concentrations of the methanolic extracts at 290–320 at 5 nm interval. It was detected that a rise in absorption is concentration reliant. The calculated SPF values were ranges between 0.2 to 2.4 (Fig 1). Among the seven lichens used in this study, the 1000µg/ml concentration of lichen extract of L6 offered highest SPF activity, i.e.,

2.4500 and 250µg/ml concentration of L2 lichen showed the lowest SPF activity when compared to the other remaining lichen extracts. The SPF activity of the different concentration of selected lichens with their graphical representation is displayed in Fig 2.

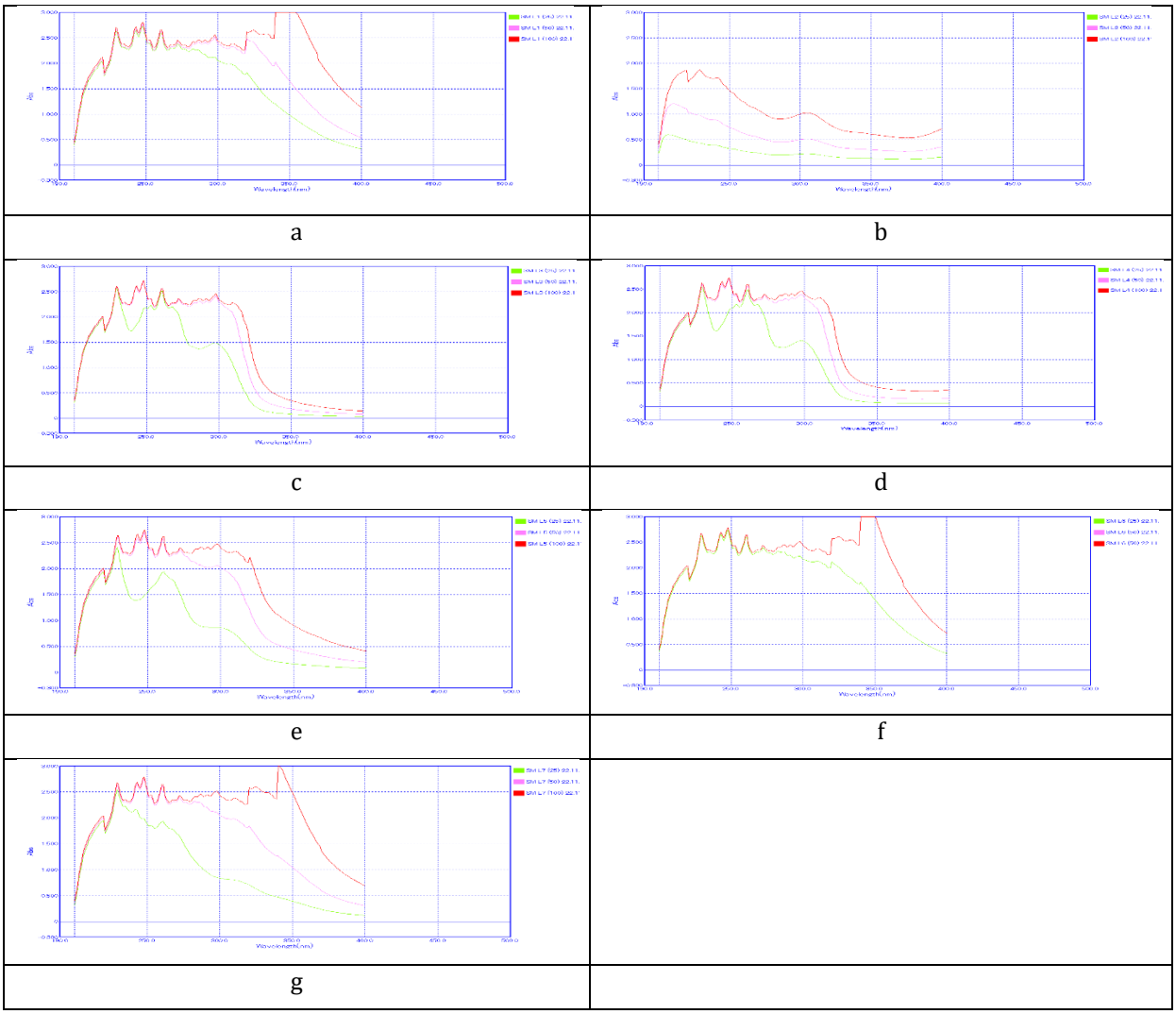

Figure 1. UV-VIS spectra of different Lichen extracts at various concentrations. a.UV-VIS spectra of L1, b. UV-VIS spectra of L2, c. UV-VIS spectra of L3, d. UV-VIS spectra of L4, e. UV-VIS spectra of L5, f. UV-VIS spectra of L6, g. UV-VIS spectra of L7.

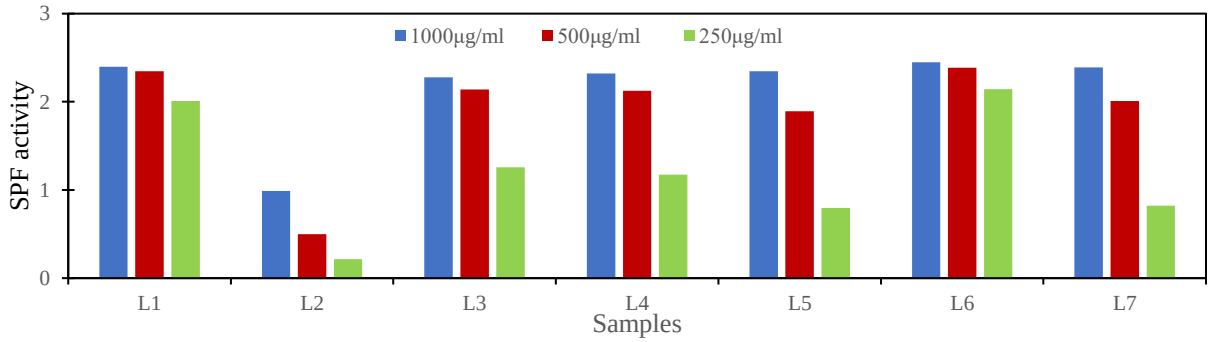

Figure 2. Graphical representation of SPF activity of different lichens at different concentrations

Although there is various synthetic sunscreen in the market, their application is inadequate because of their destructive effects on human skin (Mbanga et al. 2015). A sunscreen must have enough amounts of photoprotective agents to offer a high-level shield. Herbal products are known to be safe and have been universally accepted by clients. They can stimulate the immune response, detoxify carcinogens and block oxidative damage of DNA (Guyer et al. 2003). Thus, these natural products perform several roles in amending the route of carcinogenesis. Therefore, these natural or harbal products preparations at ideal concentrations could yield numerous valuable effects to our skin. The available literature explains that there is a positive correlation among the SPF and phenolic phytochemicals (Yasmeen and Gupta 2016) UV ray is extremely genotoxic and this causes the earliest phase of skin cancer. Sunscreen gives the protections to sunburn and numerous skin damage (Svobodova et al. 2003). Another study conducted by Mishra et al., has exhibited that flavonoid and phenolic compounds have excellent photoprotective properties (Mishra et al. 2012). T. Nguyen and his coworkers showed the SPF activity of extract of ***Parmotrema sancti-angeli*** and ***P. tinctorum*** and said that the SPF value to be 4.1 and 2.1 which is more or less similar to our result obtained from the present work (Nguyen et al. 2010). There are already many products processed from ***Evernia prunastri*** are available in market. Therefore, the present study displays that these lichen formulations at ideal concentrations could yield several valuable effects to our skin as an UV filter. In future laboratory studies could evaluate different quenching process in different lichens to estimate the degree of protection during threat by UV radiation, as has been done by Veerman et al. 2007 for *P. sulcate*.

Conclusion

The pilot findings of the present study reveal that the SPF values of the methanolic extracts of some lichen sources were calculated. It was established that all of the studied lichen has the UV protection abilities and can be used in sunscreen formulations. These lichens could become a decent, inexpensive and easily available components used in sunscreen products.

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Research Article

Experimental Farming of *Cucurbita moschata* Duchesne – An Exotic pumpkin at NBU Medicinal Plant Garden

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Abstract

Cucurbita moschata Duchesne (Butter Squash) is a high food value exotic vegetable of Cucurbitaceae with its origin in Columbia, South America. It is widely cultivated in South America, Central America and some of the Asian countries and Australia, and consumed as cooked or raw vegetable. This crop is notably promising with high yield and low cost of production. It is close to Pumpkin (*Cucurbita maxima* Duchesne) which is largely grown in India, particularly in Bengal as an important vegetable for preparing curries. There is no report of introduction and cultivation of Butter Squash in Bengal. An experimental cultivation plot was set up in the nursery of NBU medicinal plant garden when some seeds were obtained from England for experimental cultivation.

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Introduction

Butter Squash (*Cucurbita moschata* Duchesne) from the family Cucurbitaceae is a high value exotic vegetable and is native to Columbia, South America. It is widely cultivated in Mexico, Central America, South America, some of the Asian countries and Australia (Hui, Yiu H. 2006; Mondal & Basu 2012). It is close to Pumpkin (*Cucurbita maxima*) which is largely grown in India, and similar to a number of major and minor cucurbits which are cultivated in several commercial cropping systems as popular kitchen garden crops (Setiawan et. al 2001). Cucurbits share about 5.6% of the total vegetable production of India as estimated by FAO. Apart from developing the cultivation technique of this newly introduced crop, detailed taxonomical, anatomical and phenological studies were carried out in our laboratory and experimental plot. The nutritional value per 100 gm edible portion of Butter Squash (*Cucurbita moschata*) contains carbohydrate 11.69 gm, fat 0.10 gm, protein 1.0 gm, Vitamin 'C' 21.0 mg, and Vitamin 'B' 60 mg (Uchanski & Mason 2018; Thomas & Bemis 1975). There is no report of introduction and cultivation of Butter Squash in Bengal, thus a trial plot was set up in the nursery at NBU medicinal plant garden when some seeds were obtained from England for experimental cultivation.

Materials and Methods

Semidried seeds were sown in mist chamber under different seed pans each 40 cm in diameter, and 10 cm deep containing equal proportion of germinating medium such as pulverized garden loam and leaf mould dust with small quantity of cow dung manure (1:1:1). The soil mixture was sterilized by sunlight. Seed pans were placed in the mist chamber of the NBU medicinal plant laboratory under diffused sunlight. Upper surface of the medium was soaked with water. Seedlings were transplanted at the four leaved stages in to growing medium of soil, leaf mould and cow manure. 5 numbers of trial plots $3^{ft} \times 3^{ft}$ (A^1, A^2, A^3, A^4, A^5 are prepared with) make shift small trellis of 2 sapling each plot and 5 numbers 5 trial plots of $3^{ft} \times 3^{ft}$ (B^1, B^2, B^3, B^4, B^5) prepared of 2 sapling each plot. The plants with the help of tendrils got the support of the trellis. Harvestable fruits were obtained after about 3 months from transplantation. Apart from developing the cultivation technique of

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this newly introduced crop, detailed taxonomical, anatomical and phenological studies have been done in our laboratory and experimental plot.

Taxonomic description

Habit prostrate herb, habitat- terrestrial, leaf- Simple, alternate, extipulate, green in colour, usually palmately 5 lobed, hair present on both surface, glabrous, margin serrated, reticulate multicostate divergent venation, apex acute, petiole long 4.2–5.5 cm hollow (Fig. 1). Mature leaf 6.5–7 cm length and 9–9.5 cm breath. Length of the first cotyledonary leaf 3.8 cm and breath 2.2 cm. Stem round, green, hollow, branched, to 3.142 cm in diameter. Inflorescence racemose; male flowers on pedunculate raceme with 10–15 flower heads or sometimes solitary with very long peduncule, female flower solitary, unisexual, actinomorphic, epigynous, yellow coloured. Calyx; Sepal 5, poly-sepalous**,** imbricate, densely hairy, pedicellate, pedicel long 4.3–6.3 cm. Corolla; Petal5, campanulate, petals united at the base, aestivation valvate, 3- ribbed, hair present on both surface, 68.5 cm long. Stamens 3, anther lobed, anther lobes variously curved, filament short, basifixed. Gynoecium carpel usually 3, connate, ovary inferior, 3 chambered, many ovule in a chambered, ovule anatropous, style short, stigma 3. Fruit- whitish cream in colour, 5 angled stalk, enlarged at fruit attachment and sunken. Weight; 1–1.5 kg, epidermis 0.3 cm thick, upper portion of the fruit 3.7 cm wide and lower portion of the fruit 7.8 cm wide, fruit avg. 12.5 cm long, ovary 2 chambered. Seed white, 1.9 cm long and 0.8 cm wide.

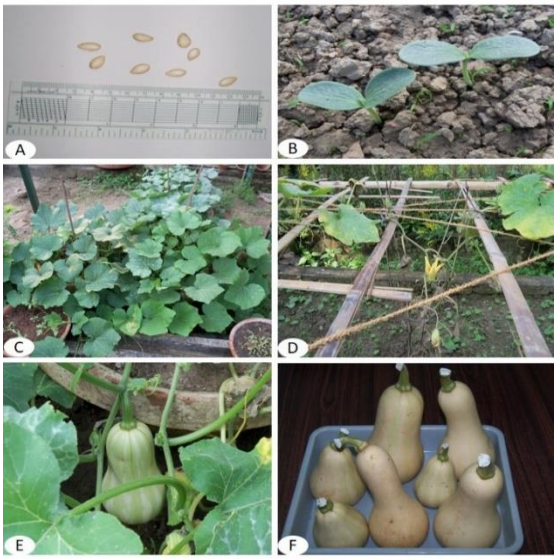

Figure 1. *Cucurbita moschata* Duchesne **A.** Seeds, **B**. Cotyledonary leaf, **C.** Prostrate view, **D.** Male Flowers, **E.** Raw fruit, **F.** Harvesting fruit

Uses

Butternut squash is a fruit that can be baked and toasted and also be squashed or mashed into soups, breads, casseroles, and muffins. It is a good source of fibre, vitamin C, magnesium, manganese, and potassium. It is also an excellent source of vitamin 'A' and 'E'. *Cucurbita moschata* has several medicinal applications in China and Thailand. Crushed fresh seeds are used as an anthelmintic, and are also applied to skin infections and inflammations (Castetter 1930).

Observation

First visible germination was observed after five days of sowing. About 80% seeds germinated after 10 days. After 3 weeks it was found that the plants that were allowed to grow in the plots B^1, B^2, B^3, B^4, B^5, were much vigorous in their growth and A^1, A^2, A^3, A^4, A^5 dried. First flowering was noted 60 days of transplantation but the initial flowers were male followed by female flowers. Female flowers started developing after anthesis. The ratio of male to female flowers is around 20:1. The ratio is influenced by the growing conditions. Movements of insects, small beetles were noted at the period of anthesis. About 4–5 fruits weiging 1–1.5 kg was obtained from each plant.

Conclusion

There is no report of butter squash cultivation in West Bengal, from the present field study it can be suggest that this vegetable crop can easily be cultivated in soils of lower Bengal just like Pumpkin. This crop according to our observation can be cultivated economically by our farmers. As duration from transplantation of seedling to production of fruit each about 3 month. As it is a trailing plant these can also be cultivated as an inter crop with other long duration vegetable crops. Thereby reduce overall land requirements. As a baseline crop development of *Cucurbita moschata* in West Bengal, further agronomic information is essential including studies on variability, propagation, land preparation, weeding pruning and harvesting intervals. The on farm indicates that this is a feasible activity with the potential to improve both income and nutrition level of farmers in Bengal.

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