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Air pollution tolerance index of *Persea bombycina*: Primary food plant of endemic muga silkworm (*Antheraea assamensis*)

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ABSTRACT

Air pollution poses a significant threat to human health, ecosystems, and the livelihood of tribal communities. This study focuses on understanding the impact of air pollution on the primary food plant som (*Persea bombycina* Kost.) of the endemic Muga silkworm (*Antheraea assamensis*) and its implications for muga silk production. The study was conducted at two sites in northeastern India, one free from atmospheric pollutants (FAP) and the other affected by pollution from an oil refinery (PAS). Various atmospheric pollutants, including particulate matter, hydrocarbons, and heavy metals, were found to be higher at the PAS site. The study investigated biochemical parameters like ascorbic acid, relative water content, total chlorophyll, and extractable pH in the leaves of *P. bombycina* to determine its air pollution tolerance index (APTI). Results showed that the ascorbic acid content in the leaves increased significantly at the PAS site ($p < 0.05$), indicating the plant's adaptation to air pollution stress. Similarly, the APTI values were higher during summer compared to winter, suggesting better tolerance during the former season. Positive correlations were found between APTI and ascorbic acid content ($p < 0.05$), emphasizing the role of ascorbic acid as an antioxidant in mitigating the effects of air pollution. The study highlights the importance of understanding the tolerance levels of *P. bombycina* to develop protective measures for sustaining Muga silk production in the face of rapid industrialization and increasing pollution. This research can aid policymakers in balancing economic growth with environmental conservation and protecting traditional practices of tribal communities.

1. Introduction

Due to rapid industrialization, air pollution continues to accelerate at far more rate than the initial industrialization periods and affecting the human health, ecosystem functioning, livelihood and also the social and cultural integrity of tribal inhabitants. The important atmospheric pollutants are airborne particulate matter (PM), Aliphatic-aromatic hydrocarbons, $PM_{2.5}$ associated nitrates, sulfates, PAHs and heavy metals (Cd, Cu, Co, Cr, Ni, Pb and Zn), SO_2, CO, H_2S, and N-oxides [1–4]. The impact of this pollution may be

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irreversible in the case of endemic species. The long-term stress may wipe out the species itself or negatively affect any other important key factor for the survival of the endemic species. Air pollutants directly/indirectly affects the growth (biological as well as physiological) of plants [5,6] through their reactive nature as well as physical presence such as dust deposition inhibits the photosynthesis, stomatal exchange and protein synthesis [7,8]. There is plenty of the research available on tolerance limits of various crops (mostly annuals), modes of action, negative effects on productivity, survival and also human health. However, investigations on the socio-economically important endemic species are highly limited [3,4,9].

Muga silkworm (*Antheraea assamensis*) is endemic to northeastern states of India. Assam alone produces ≈93.04 % of the total muga silk [10]. The second and third rank contributors are Meghalaya (5.04 %) and Arunachal Pradesh (1.22 %), respectively. Muga silk production is an important practice of neolithic culture and bears geographical indicator status. Muga silkworm is a poikilothermic insect and it is highly sensitive to unfavorable environmental conditions like high temperature, low relative humidity and environmental pollutants [11–16]. Temperature and relative humidity are considered as paramount [17], which influence all life processes of very stage of life cycle of the silkworm. Devi et al. [4], suggested that the concentration of aliphatic-aromatic hydrocarbons varied from 5.44 to 96.8 mg/kg during the pre-monsoon and 10.7–125.4 mg/kg during the post-monsoon period, which is more than the uncontaminated 3.07 mg/kg site. Devi et al. [4], also observed that the concentration of aliphatic-aromatic hydrocarbons (ranging from 26.55 to 59.42 mg/kg) in soil of *Persea bombycina* plantation is higher than the uncontaminated sites. The muga silkworm is reared outdoors throughout the year to fulfil the seed requirement for commercial production, making it highly vulnerable to air pollution [14,15]. The environmental condition also affects the rearing performance and/or crop yield due to variability in pollutant dispersal and season specific disease and pest. Muga silkworm is polyphagous in nature, but mostly reared on Som (*Persea bombycine* Kost.) and Soalu (*Litsea monopetala* Roxb.). Som is dominantly used by the local inhabitants and is abundantly distributed in the upper part of Assam, mainly Dibrugarh, Golaghat, Jorhat, North Lakhimpur, Sivasagar, and Tinsukia, and at low scale in some parts of lower Assam, Manipur, Meghalaya Nagaland, and West Bengal. The qualitative and quantitative leaf production of muga host plants chiefly depends on the soil, geological, topographic, climatic, physiological and environmental characteristics [14,15,18]. It is well evident that muga silk production is stressed due to an inadequate supply of disease-free silkworm seed, deforestation, disease infestation, unfavorable

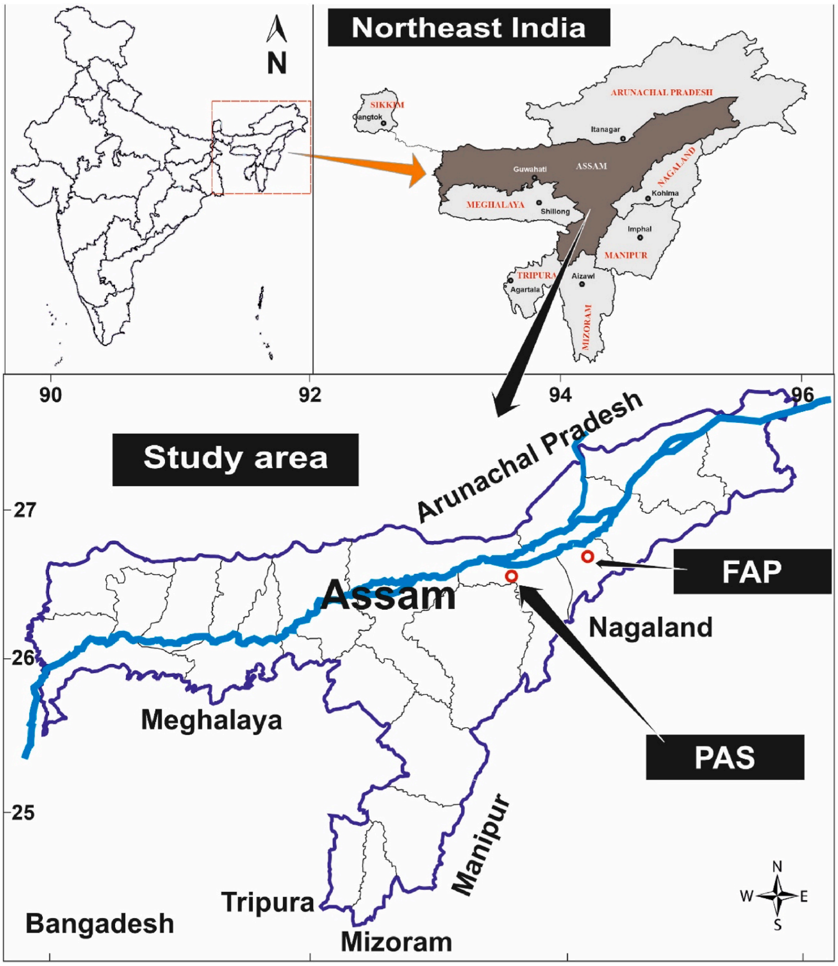

Fig. 1. Location map of study areas.

environment as well as pollutants released from tea cultivation, and environmental pollution from oil [2–4]. Oil refineries in Assam are concentrated in Upper Assam belt situated on the southeastern slope of the Brahmaputra arch and muga silkworm rearers co-exist in the close vicinity. Hence, the muga silkworm and its host plants are, exposed to different types of air pollutants mainly NO_X, SO_2, CO and aerosol for more than a decade [2–4,14,17]. The study suggested that comparatively greater number of days are required for the larval and pupal period in the oil polluted sites [12–14,17]. The crude protein content was found lower in contaminated sample than the control sample. Reduction in crude protein content of contaminated plant leaf samples might be due to the enhanced rate of protein denaturation and break down of protein to amino acid [4]. Continuous exposure of Muga Silkworm to toxic and non-toxic pollutants like hydrocarbons and heavy metals (Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb & Zn) in the atmosphere is likely to have serious effects on muga silkworm with respect to both quality and quantity [4]. Many research studies also suggested that the muga silk rearing and other lepidopteron insects is in jeopardy in petroleum contaminated areas due to release of different pollutants from extensive oil exploration and production activities of oil agencies [2,14–17,19]. Thus, ethnic and economic value of the muga silk is under stress. India is a developing country and is currently one of the fastest growing economies. To maintain the pace of the economic growth, energy is highly essential, and the petroleum industry plays a critical and vital role to fulfilling energy needs. Considering both of the limitations, it is highly required to know how much Muga sericulture can substantiate the load of atmospheric pollution. Air pollution tolerance index (APTI) is the intrinsic capacity of the plants to tolerate air pollution load. This intrinsic capacity is basically depending on morphological, physiological and biological characteristics of the plants. Based on the APTI plant species can be categorized into the sensitive to tolerant level and performance of monotonous system can also be evaluated to recommend further especially perennial species and/or formulating the protective/ameliorative measures in case of sensitive species of ecosystem. Thus, keeping this in view, the present investigation has been devised to understand the atmospheric pollution tolerance limit of the major primary host plant of muga silkworm i.e., Som and its seasonal behaviour. The present investigation will serve as a milestone for policymakers to protect the endemic muga silk production as well as its expansion in other promising areas without affecting the key industrial development project.

2. Methodology

2.1. Study area

Under the present investigation, the silkworm rearing was conducted at two sites i.e., Jorhat and Golaghat (Fig. 1). Jorhat was selected as control site (Free from atmosphere pollutants-FAP) at Central Muga Eri Research and Training Institute, Lahdoigarh rearing farms (26°47′06.04″ N; 94°20′39.0″ E). The FAP site is under full control of the activity related to atmospheric pollution. FAP is surrounded by green lush vegetation and free from industrial activity whereas, this location is more than 2.5 km away from the national highway. Other selected site was Muga Village Grazing Resource (Muga-VGR) farm at Ponka, (26°35′04.2″ N; 93°46′20.1″ E) in Golaghat district which is adjacent to Numaligarh Refinery Limited and taken as pollution affected site (PAS). Gas flaring has been continued at PAS at least for a decade. The gas flaring is a continuous and common process of oil industry at PAS which contributes

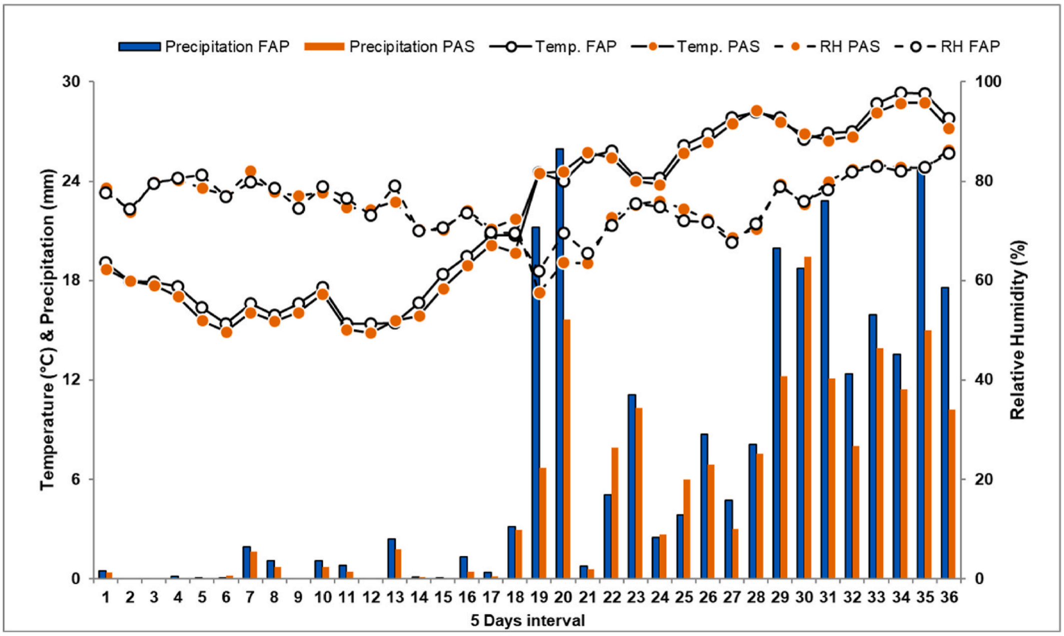

Fig. 2. Variation in temperature (°C), precipitation (mm) and relative humidity (%) during rearing seasons in winter and summer at FAP and PAS. Data is recorded at 5 days' interval. Serial no. 1 to 18 is presenting winter season and 19 to 36 is summer season.

significant amount of carbon dioxide to the atmosphere [19,20]. The gas flaring is mainly responsible for altering the morpho-physiology of plant as well as feeding performance of Lepidoptera species [14,15,19]. Sarma et al. [19], also revealed that the higher carbon dioxide (CO_2) as well as temperature was found near flaring sites.

2.2. Meteorological information

The climate of the FAP and PAS (Ponka, Golaghat) is humid subtropical. The mean air temperature was 22 °C and 21.7 °C at FAP and PAS. The mean winter (Dec to Feb) temperature recorded was 17.4 °C and 16.9 °C at FAP and PAS, respectively. The mean temperature during summer (Jun to Aug) was recorded 26.7 °C–26.5 °C at FAP and PAS, respectively. The annual relative humidity ranged from 62 to 86 % with mean of 75 % and 58–86 % with mean of 75 % at FAP and PAS, respectively (Fig. 2). It clearly shows that the annual weather constituents (Precipitation, temperature and relative humidity) are not variable at FAP and PAS sites.

2.3. Air quality at PAS site

Air quality data showed that the concentration of SO_2 ranged from 8.40 to 21.20 μg/m^3 at PAS (NRL, 2020). The range of particulate matter (10) and particulate matter (2.5) ranged from 30.0 to 51.8 μg/m^3 and 12.6–17.2 μg/m^3, respectively. The concentrations of NOx ranged from 12.1 to 20.8 μg/m^3. The concentrations of total hydrocarbons, methane hydrocarbons and non-methane hydrocarbons were 195–300 μg/m^3, 99–164 μg/m^3 and 70–137 μg/m^3, respectively. These values fall below the National Ambient Air Quality Standard's (2009) acceptable limits, however the presence of harmful compounds in the soil and air hinders the growth and development of silkworms and their host plants in the immediate vicinity and increases the mortality rate of both.

2.4. Plant sampling

The PAS site was divided into 16 equal, small sections, leading to the collection of 16 mature leaf samples from the PAS site during each of the seasons of summer (June–August) and winter (December–February), as well as the same number of leaf samples from the FAP site. Major food plant for Muga silkworm i.e., Som (*Persea bombycina*) was selected on the basis of same morphological aspects, direction of air flow, number of abundance plant at PAS. Collected leaf samples were packed in airtight polythene bags and immediately transported to the laboratory in ice box for biochemical analysis i.e., ascorbic acid, pH of leaf extract, relative water content and total chlorophyll. Total chlorophyll was estimated using equation (1) [21], ascorbic acid content was estimated as per the method described by Keller and Schwager [22], relative water content was estimated following Liu and Ding [23], using equation (2) and Leaf extracted pH was estimated following the methods used by Mina et al., [24].

$$\text{Total Chlorophyll}\left(\frac{\text{mg}}{\text{g}}\text{FW}\right) = \left[\frac{\{20.2(A645) + 8.02(A663)\}}{(1000 * W)}\right] * V \tag{1}$$

where: V is extracted volume (ml)
W is sample weight (gm)
A_{663}, A_{665} = Absorption at these wavelengths

$$RWC = \frac{FW - DW}{TW - DW}100 \tag{2}$$

where: FW is the fresh weight of the turgid leaf.
DW is the dry weight of turgid leaves after oven-drying at 115 °C for 2 h.
TW is the turgid weight of overnight immersed leaf in water.

Air Pollution tolerance index (APTI) is a very useful method to understand the plant tolerance/resistance capacity against air pollution. APTI investigations are highly useful for biomonitoring purpose [25]. Based on the APTI score, plants can be categorized into three different categories i.e., sensitive (1-11), intermediate (12–16) and tolerant (≥17) [26]. In the present study, APTI score was determined by using equation (3):

$$APTI = \frac{[A(T + P) + R]}{10} \tag{3}$$

where: A = Ascorbic Acid (mg/g); T = Total Chlorophyll (mg/g-fw)
P = pH of the leaf extract; R = Relative water content of leaf (%)

2.5. Statistical analysis

Statistical analysis was done in OPSTATE [27]. The statistical test was conducted to ascertain the significant differences among the APTI, leaf extract pH, Total Chl. and RWC using pooled analysis of variance (Pooled ANOVA). Under condition of homogenous variance and significant ANOVA, it was tested for significance of season, treatment and its interaction.

3. Results & discussion

3.1. Ascorbic acid

The mean ascorbic acid in leaves of *P. bombycina* was recorded as 13.00 and 22.64 mg/g in summer at FAP and PAS, respectively. In winter, ascorbic acid concentration reduced to 5.97 and 10.80 mg/g in leaf of *P. bombycina* at FAP and PAS, respectively (Table 1). A *t*-test was conducted, and the p-value obtained was very low, indicating a statistically significant difference between the two sites in terms of ascorbic acid content during the summer [$p < 0.05$(value 0.00001)] and winter [$p < 0.05$ (value 0.00079)] seasons. The difference in ascorbic acid content between the summer and winter seasons at both FAP and PAS sites was statistically significant [$p < 0.05$ (value 0.0000001 and 0.000002, respectively)]. The results revealed that the ascorbic acid content in PAS samples increased by 57.42 % during summer while increased by 9.49 mg/g to 16.72 mg/g in FAP and PAS, respectively. It may be due to increase of pollution load at PAS. As under stress condition, reactive oxygen species (ROS) production increases, eventually ascorbic acid production increases. Shrestha et al. [28], also suggests that the photo-oxidation of sulphur-di-oxide into sulphate, increases the ROS production. Shrestha et al. [28] supported that increase in ascorbic acid content is a tolerance indicator of plant against sulphur -di-oxide pollution. Ascorbic acid function as an anti-oxidant and provide homeostatic stability against ROS stress [29–31]. Gupta et al. [32], found significant ($p < 0.05$ & $p < 0.01$) increase in ascorbic acid content in *A. moniliformis* (3.88–5.42 mg/g), *L. speciose* (0.94–1.56 mg/g), *C. samia* (3.38–4.74 mg/g), *C. fistula* (1.62–2.65 mg/g), *T. grandis* (1.06–2.04 mg/g) and *S. robusta* (3.76–5.14 mg/g) in the industrial region. Gupta et al. [33], observed significant increase in ascorbic acid concentration in Arjun (1.42–0.93 mg/g), Morus (1.20–2.00 mg/g), Sheesham (0.27–0.50 mg/g) and Ashok (1.64–1.95 mg/g) in the Sahibabad industrial area of Delhi.

Roy et al. [31], investigated ascorbic acid levels in *Mangifera indica* grown in commercial and industrial sites. The increase ascorbic acid concentration was observed in post-monsoon season at commercial (28.13 mg/g), industrial (26.93 mg/g) than control (18.75 mg/g) site. Roy et al. [31], also observed strong correlation between APTI and ascorbic acid concentration at industrial site ($R^2 = 0.94$), commercial site ($R^2 = 0.94$) in post-monsoon season. Bharti et al. [30], also observed increases in ascorbic acid concentration at industrial sites in *C. procera* (2.00–5.00 mg/g), *H. brasiliensis* (9.70–13.30 mg/g), *S. cumini* (13.50–18.10 mg/g), *A. indicus* (8.90–12.80 mg/g), *F. religiosa* (12.80–17.30 mg/g) and *E. globlus* (10.40–19.00 mg/g) in control to industrial site. Shrestha et al. [28], study the ascorbic acid contents in 12 species grown on the roadside and conclude that increase concentration of ascorbic acid is directly contributes in the tolerance capacity of the respective plant species such as *A. julibrissin* (15.6 mg/g), *C. camphora* (30.2 mg/g) and *N. oleander* (13.0 mg/g). Shritama et al. [34], observed significant increase in the ascorbic acid (18.18–27.27 mg/g) concentration in *Murraya paniculate* at polluted sites than the reference site in Kolkatta, India.

3.2. Relative water content (RWC)

Relative water content contributes significantly in the permeability of the cell protoplasm, water losses and physiological balance. Air quality especially the vapour pressure deficient significantly control the relative water content in leaves. The higher RWC capacity induces higher tolerance into the plants against air pollution. In the present investigation, RWC was found to be 83.29 % and 84.80 % in summer season and 84.97 % and 66.57 % in winter season at FAP and PAS site, respectively (Table 1). In summer season, relative water content in leaves was similar at both sites whereas in winter season 21.65 % lower at PAS than FAP. The *t*-test indicated that there was no significant difference in RWC between the two sites during the summer (p value 0.58) and highly significant during the winter [($p < 0.05$ (value 0.000008)]. The difference in RWC between the summer and winter seasons at FAP site was not statistically significant (p value 0.40) and statistically significant [($p < 0.05$ (value 0.00007) at PAS site.

Table 1
Analytical estimation of various biochemical constituents in Som plant leaf samples from two different sites (FAP and PAS) during both the summer and winter seasons. The values presented in the table include the minimum, maximum, and mean values for each biochemical constituent.

Season/sites	SUMMER			WINTER		
Biochemical constituents	FAP (n = 16)	PAS (n = 16)	P value (at α = 0.05)	FAP (n = 16)	PAS (n = 16)	P value (at α = 0.05)
Ascorbic Acid (mg/g)	6.69–18.21 (13.00 ± 0.80)	12.52–35.14 (22.64 ± 1.46)	0.00001	1.95–12.25 (5.97 ± 1.24)	5.88–19.02 (10.80 ± 1.28)	0.00079
pH	5.15–6.49 (5.68 ± 0.16)	5.10–6.16 (5.57 ± 0.13)	0.37	5.10–6.45 (5.68 ± 0.18)	5.24–6.19 (5.80 ± 0.11)	0.31
RWC (%)	74.28–98.33 (83.29 ± 0.66)	61.37–90.94 (84.80 ± 1.00)	0.58	73.61–93.10 (84.97 ± 0.54)	47.34–90.86 (66.57 ± 1.58)	0.000008
T Chl (mg/g-FW)	1.34–2.76 (2.27 ± 0.25)	1.02–2.72 (1.92 ± 0.35)	0.03	1.18–2.57 (2.07 ± 0.30)	0.58–2.69 (1.97 ± 0.51)	0.60
APTI	13.32–23.10 (18.69 ± 0.59)	9.69–26.40 (16.80 ± 1.18)	0.17	9.86–18.33 (13.25 ± 0.72)	11.35–24.80 (15.17 ± 1.24)	0.17

#ranges and mean value along with standard error (*t*-Test: Two-Sample independent Variances).

3.3. Chlorophyll content and extractable pH

The average concentration of total chlorophyll (mg/g FW) in leaves of *P. bombycina* was recorded 2.27 and 1.92 in summer season at FAP and PAS, respectively (Table 1). The difference in pH between the summer and winter seasons at FAP site was not statistically significant. However, the difference in pH between the summer and winter seasons at PAS site was statistically significant ($p < 0.05$). The *t*-test showed that the difference in pH between the two sites during the summer and winter seasons were not statistically significant.

In winter season, total chlorophyll (mg/g FW) concentration was 2.07 and 1.97 in *P. bombycina* at FAP and PAS site, respectively. The difference in total chlorophyll content between the summer and winter seasons at both FAP and PAS sites was not statistically significant. The *t*-test showed a statistically significant difference in T Chl content between the both sites during the summer ($p < 0.05$). However, during the winter, the difference in T Chl between the both sites was not significant. Reduction in chlorophyll content reduces the photosynthetic activity, growth and biomass productivity and hence resistance capacity of the plants against air pollution. Chlorophyll degradation is widely considered as an indication of air pollution stress [35,36]. Air pollutants i.e., nitrogen dioxide, sulphur dioxide, and suspended particulate matter (PM) may enter through stomata can degrade chloroplast partially [37]. Gupta et al. [32], reported the significant ($p < 0.05$) reduction in chlorophyll content in *L. speciose* (7.84–5.26 mg/g), *C. fistula* (4.08–2.16 mg/g), *D. sisoo* (6.24–2.16 mg/g), *T. grandis* (9.66–8.47 mg/g) and *A. moniliformis* (4.56–3.36 mg/g) in the industrial region. Gupta et al. [33], also observed that chlorophyll content was significantly reduced in Arjun (2.33–1.76 mg/g fw), Morus (3.02–2.15 mg/g fw), Sheesham (2.23–1.76 mg/g fw), and Ashok foliar (3.62–3.05). Gupta et al. [33], envisaged that increase in dust fall flux may decreases the total chlorophyll content. Bharti et al. [30], have found higher photosynthetic rate in *A. nilotica* at reference site (1.49 mg/g) that the industrial site (1.39 mg/g). Woo and Je [35], suggested that lower chlorophyll content contributes to the increased sensitivity in *Thuja* sp. *(*0.192 mg/g*), N. oleander (*0.106 mg/g*), Ficus* sp. (0.14 mg/g) and *F. benjamina* (0.199 mg/g). Thus, the plant species that are able to maintain their chlorophyll levels in polluted conditions to combat air pollution are known as tolerant species [36].

Extractable pH of Som plant leaves was acidic in nature and ranged from 5.15 to 6.49 and 5.10 to 6.16 during summer season at FAP and PAS, respectively. The leaf extract pH of the Som plant ranged from 5.10 to 6.45 in FAP and 5.24 to 6.19 in PAS samples during winter season (Table 1). In the present study, all the Som plants had pH values below 7. Leaf extract pH is found more acidic in Som leaves of PAS which may be impact of environmental stress. Karmakar et al. [38], suggested that air pollution and lower extractable pH of leaf are significantly correlated. The under-leaf environment the gaseous air pollutant i.e., SO_2, NO_X forms acid radicles reacting with cellular water and induce stress to chlorophyll content [39] (Turk and Wirth, 1975). Larcher [40] also suggested that air pollutant i.e., SO_2, NO_X may lower the leaf pH and induce early stomatal closure. Pratibha et al. [41], envisaged that enhanced pH of leaf extract may be helpful to enhance the tolerance limits of plants species against air pollutants.

3.4. Air pollution tolerance index

The air pollution tolerance index (APTI) of the current study showed that there was seasonal variation in both the locations. APTI was calculated for *P. bombycina* plant species cultivated in FAP and PAS sites during summer and winter seasons and the data is presented in Table 1. APTI of *P. bombycina* ranged from 13.32 to 23.10 and 9.69 to 26.40 at FAP and PAS, respectively during summer season (Table 1). The *t*-test showed no significant difference at $p < 0.05$ in APTI between the two sites during the summer and winter seasons. The difference in APTI between the summer and winter seasons at both FAP and PAS sites was statistically significant ($p < 0.05$). During the summer season, five samples of control exhibited sensitivity towards air pollution and eleven samples are found in

Table 2
Inter-elemental matrix of biochemical constituents of FAP and PAS samples (n = 16).

Pearson Correlation Matrix of FAP					
	Ascorbic acid	pH	RWC	Total Chl	APTI
Ascorbic acid	1				
pH	0.35[a]	1			
RWC	−0.23	−0.30	1		
Total Chl	0.28	0.07	0.14	1	
APTI	0.98[b]	0.40[a]	−0.07	0.38[a]	1
Pearson Correlation Matrix of PAS					
	Ascorbic acid	pH	RWC	Total Chl	APTI
Ascorbic acid	1				
pH	−0.39[a]	1			
RWC	0.74[b]	−0.47[b]	1		
Total Chl	−0.01	−0.04	0.07	1	
APTI	0.76[b]	−0.11	0.58[b]	0.24	1
CD for Seasons		1.44			
CD for Treatments		1.93			
CD for Season x Treatments		2.72			

[a] Correlation is significant at 0.05 level.
[b] Correlation is significant at 0.01 level.

intermediate range. One sample is sensitive and eleven are found intermediate whereas four are found tolerance at PAS site during summer season. The indication of the tolerance index shows that the air stress is higher at PAS due to the release or emission of flare from the oil industry [3]. In winter season, APTI varied from 9.86 to 18.33 and 11.35 to 24.80 at FAP and PAS, respectively. It was recorded that APTI is lower in the winter season compared with the summer. The higher APTI index during summer than winter season may be due to enhanced adaptation against air born stresses. The enhancement of ascorbic acid concentration may also support the defense mechanism during summer season as also observed in other plant species. The results generated under the present study are in agreement with Rai and Panda [42] who revealed that the APTI range from 8.11 to 19.48 in plant species at control site. Achakzai et al. [43] also suggested that the positive correlation between APTI and ascorbic acid is $r2 = 0.776$, 0.801, and 0.879 at sites 1, 2, and 3, respectively. Molnár et al. [44] advocated that in future, APTI can provide an efficient tool in the pollution monitoring of air quality assessments mainly in evaluating emission levels in urban areas.

3.5. Correlation analysis of biochemical constituents of FAP and PAS samples of som plant

Statistical correlation matrix of various biochemical constituents of FAP and PAS leaf samples of som plants are depicted in Table 2. The correlation analysis indicates that there are some interesting associations between the biochemical constituents of *P. bombycina* plant leaf samples at the FAP and PAS sites. At the FAP site, there is a strong positive correlation ($p < 0.01$) between Ascorbic Acid and APTI. Additionally, at the PAS site, there are significant correlations between Ascorbic Acid and pH, as well as RWC and APTI, indicating potential relationships between these parameters in response to environmental conditions at that site, suggesting a potential role of Ascorbic Acid in air pollution tolerance.

Figs. 3 and 4 present linear regression analyses depicting the relationships between the Air Pollution Tolerance Index (APTI) and four biochemical constituents (Ascorbic Acid, pH, Relative Water Content (RWC), and Total Chlorophyll) in Som plant leaf samples collected from two different sites, FAP and PAS. Each figure contains separate linear regression graphs for the summer and winter data at the respective sites. This regression can help to identify any potential patterns, trends, or correlations between these variables, contributing to a better understanding of how the biochemical composition of the plants relates to their tolerance to air pollution at the two sites and across different seasons. A high positive correlation is found between APTI and ascorbic acid at FAP ($R^2 = 0.96$) and at PAS ($R^2 = 0.61$) during both the seasons (Figs. 3a and 4a). Whereas non-significant low correlation exists between APTI and T. chlorophyll ($R^2 = 0.15$), pH of leaf extract ($R^2 = 0.16$) and RWC ($R^2 = 0.01$) at FAP site (Fig. 3b, c, d). The non-significant low correlations were found between APTI and T. chlorophyll ($R^2 = 0.06$, pH of leaf extract ($R^2 = 0.01$) and RWC ($R^2 = 0.33$) at PAS site (Fig. 4b, c, d). The aggravated result indicates that, air pollution induced increase in ascorbic acid content also enhance the capacity of plant to tolerate air pollution.

Pai and Panda [42], observed strong positive correlation of APTI with chlorophyll ($R^2 = 0.86$) and ascorbic acid ($R^2 = 0.92$), where insignificant correlation between APTI with pH. Gupta et al. [32] found that the most of the plants near factories showed a significant

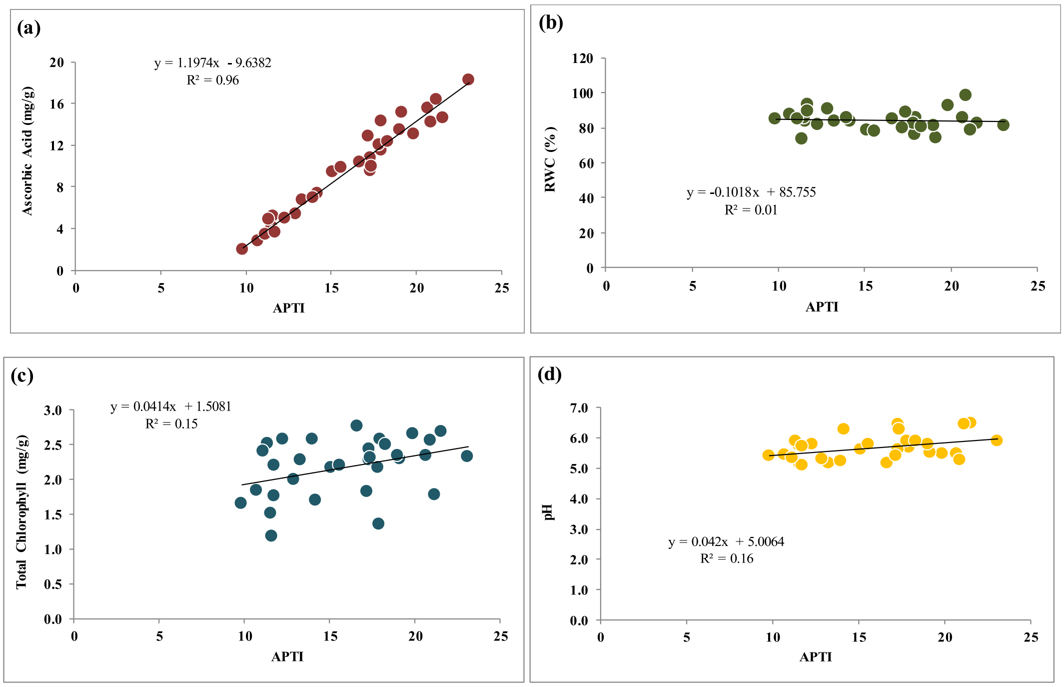

Fig. 3. Linear regression between the ATPI and four biochemical constituents in Som plant at FAP site. The linear regression graphs are prepared with summer and winter data.

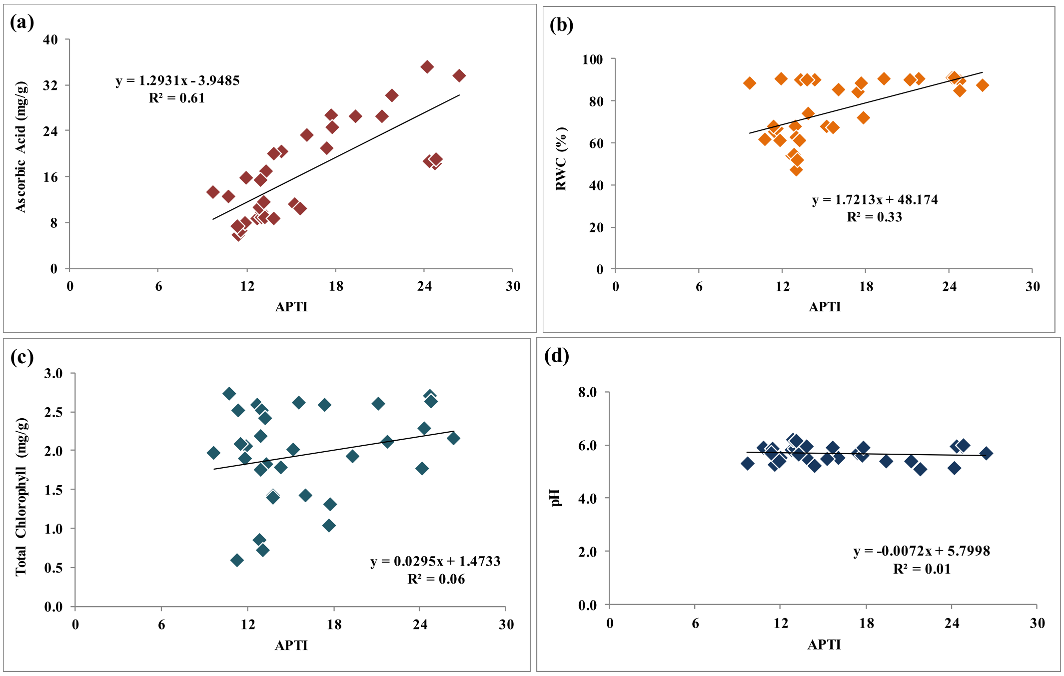

Fig. 4. The linear regression between the ATPI and four biochemical constituents in Som plant at PAS site. The linear regression graphs are prepared with summer and winter data.

increase ($p < 0.01$, <0.05) in ascorbic acid content and suggests that higher accumulation of PM associated heavy metals induces the stress and in response to tolerate the stress the ascorbic acid production increased by the plants. Bharti et al. [30], observed high positive correlation between APTI and ascorbic acid ($R^2 = 0.897$). The presence of more ascorbic acid at a polluted environment indicates that the plants there are tolerant of the pollutants. Shritama et al. [34], study directly indicated that higher APTI is dependent on the higher leaf tissue pH ($R^2 = 0.99$), ascorbic acid content ($R^2 = 0.98$), RWC ($R^2 = 0.85$) and total chlorophyll content ($R^2 = 0.78$).

Several research investigations have also testified the similar finding of correlation between four biochemical constituents and APTI [30,31,45].

4. Conclusion

The present study aimed to assess the Air Pollution Tolerance Index (APTI) of *Persea bombycina*, the primary food plant of the endemic Muga silkworm (*Antheraea assamensis*) at two locations, a control site (FAP) and a pollution-affected site (PAS) near an oil refinery. The investigation focused on various biochemical constituents such as ascorbic acid, leaf extract pH, relative water content (RWC), and total chlorophyll in the leaves of *P. bombycina* during summer and winter seasons. The results demonstrated that *P. bombycina* exhibits a certain degree of tolerance to air pollution, as indicated by its APTI scores. Notably, the higher APTI scores during summer were associated with increased ascorbic acid content, suggesting that the plant's ability to synthesize this antioxidant plays a crucial role in its adaptation to air pollution stress. The study also revealed that air pollution significantly affected various biochemical constituents in *P. bombycina* leaves. Higher levels of air pollutants, such as sulphur dioxide and nitrogen oxides near the PAS site, were associated with increased ascorbic acid content and lower total chlorophyll levels in the leaves. However, the extractable pH and relative water content showed insignificant variations between the two sites and seasons. The correlation analysis further supported the positive relationship between APTI and ascorbic acid content, signifying its importance in determining the plant's tolerance to air pollution. The results underscore the importance of monitoring and understanding the biochemical responses of plant species in polluted environments, especially those with ecological and economic significance.

In conclusion, *Persea bombycina*, the primary food plant of the Muga silkworm, exhibits a certain level of tolerance to air pollution, and its APTI scores can be influenced by seasonal variations and environmental stressors. Understanding the plant's tolerance mechanisms can aid in formulating effective protective and ameliorative measures for sustaining the Muga silk industry and conserving the ecological integrity of the region. Additionally, APTI can serve as a valuable tool in biomonitoring and assessing the impact of air pollution on various plant species, aiding in the formulation of effective protective and ameliorative measures to combat pollution-induced stress on plant life. By preserving and safeguarding key endemic species like Muga silkworm (*Antheraea assamensis*), we can strike a balance between industrial development and ecological conservation, ensuring the long-term sustainability of both.

Data availability statement

Data included in article.

CRediT authorship contribution statement

Dharmendra Kumar Jigyasu: Conceptualization, Formal analysis, Funding acquisition, Project administration, Writing – original draft. **Amit Kumar:** Conceptualization, Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing. **Aftab A. Shabnam:** Formal analysis, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Raisa Begum:** Data curation, Formal analysis. **Subadas Singh:** Funding acquisition, Resources. **Sandeep Kumar Malyan:** Software, Writing – original draft, Writing – review & editing. **Kartik Neog:** Funding acquisition, Supervision, Writing – review & editing. **K.M. Vijayakumari:** Project administration, Supervision, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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