



Assessing the Effects of Gas Flaring on the Growth Physiology of Tea Crop

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Assam is known worldwide for its production of tea; the state contributes around 52 percent to the India's tea production. Similar to tea, crude oil operations date back to the 19th century and accounts for about 15 percent of India's crude output, thus making these sectors important economically. However apart from the positive effects, crude oil operations result in gas flaring, which is already recognized as an environmental problem. Taking into context the harmful effects of gas flaring the present investigation aims to study the impact of gas flaring on physiological parameters of tea plant with respect to growth adjacent to oil field in Merbil Majuli OCS 6(WEST) in Dibrugarh district of Assam during 2019-2020. The study was designed with Randomized Complete Block Design (RCBD) accommodating five levels under distance and two levels under seasons. Plant samples were collected at random intervals and estimated for plant physiological parameters such as water saturation deficit(WSD), relative turgidity, stomatal count, polyphenol content, specific leaf weight, caffeine content, chlorophyll content, plucking point density. The present study found that gas flaring had a significant effect on the physiology of the tea crop in terms of growth. The study found a gradual decrease in relative turgidity, specific leaf weight, plucking point density, stomatal count, leaf area measurement, polyphenol content, chlorophyll content, caffeine content, but an increase in water saturation deficit of green tea leaves on plants growing closer to the flare pit.

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1. INTRODUCTION

Assam is a major contributor of tea (*Camellia sinensis* (L.) and crude oil, it contributes significantly to Indian total production. Tea has been cultivated in Assam commercially since a very long time, it also contributes significantly to the total production of tea in the country. More than half of the country's tea area is under Assam, it also contributes around 52 percent to the tea production of the country. Assam has a production of 379790 thousand kg during January to August, 2021 [1]. The tea industry of the state provides about seven lakh daily employment which is more than 60 percent of the total daily employment generated by this sector in the country [1]. Crude oil is another such sector that contributes significantly to Indian economy. Crude oil operations date back to the 19th century and accounts for about 15 percent of India's crude output, contributing around 3922000 tonnes (2019-20) [1]. The significant contributions of the sectors make them important economically. The industries though bring about a significant positive impact to the state, however, the different operations for example crude oil extraction led to production of unwanted gas, generally known as gas flaring. Gas flaring does not only destruct vegetation, wild life and ecological change in the ecosystem, but it also significantly affects the microclimate and physico-chemical properties of soils near the flare sites, which may hamper the growth of plants resulting in decrease in yield of crop [2]. The gas flaring consists of a mixture of different gases. The composition of which depends on the source of the gas that is going to the flare system. During oil-gas production, associated gases that are released mainly contain natural gas comprising of more than 90% methane (CH₄) with ethane, a small amount of other hydrocarbons and inert gases such as Nitrogen (N₂) and Carbon dioxide (CO₂). Gas flaring from refineries and other process operations commonly contain a mixture of hydrocarbons and in some cases hydrogen (H₂). Carbon dioxide (CO₂) and methane (CH₄) are the Green House Gases which increase the temperature of the atmosphere when directly released into the air [3]. The harmful effect of flaring is

widespread, particularly its effects in the ecosystem. Associated gas flaring and crude oil are the major reasons for the occurrence of acid rain [4] and the acid rain causes loss of vegetation [5]. The impact of acid rain on vegetation is more severe near the gas flare stack [6]. When leaves are washed with acid precipitation; their photosynthesis may be reduced by wearing away the protective waxy coating. Oil deposited on leaves of plants penetrates into the leaves that reduce transpiration and photosynthesis and as a result leaves become yellow [7]. Gas flaring affects on the growth of tea plants and its resistance to biotic and abiotic stresses, making them more vulnerable to pathogens. Many crude oil drilling sites around tea plantation area of Upper Assam is very common in Assam. Oil drilling operations that are done in nearby areas of tea gardens result in the contamination of crude oil, heavy metals and gas. Considering the above effects on gas flaring, an attempt has been made to study the impact of gas flaring released nearby oil fields with probable effect on growth of the tea crop.

2. MATERIALS AND METHODS

The study was carried out during the rainy and autumn season of 2019 and 2020 following the Randomized Complete Block Design (RCBD). The different parameters calculated have been mentioned below:

2.1 Collection of Plant Samples

Tea Leaf samples were collected from four different plants for each parameter separately at each replication and for both the seasons at an interval of 20 m distances at 35 -55 m, 55-75 m, 75-95 m and 95-115 m starting from the gas flaring point. Other samples were collected from four different plants from adjacent to unpolluted control plot at distance 130-150 m. For one season, a total of 60 samples were collected. All total of 120 samples were collected in polythene bags.

2.2 Experimental Details

List 1. Experimental details

Period of the experiment	September 2019-March 2020
Location	Merbil Majuli OCS 6 (WEST), Dibrugarh, Assam
Experimental Design	Randomized Complete Block Design (RCBD)
Number of factors	i. Distance (D) ii. Season (S)
Number of levels under factor D	5
Number of levels under factor S	2
Number of replication (r)	3
Total number of plots (DxSxr)	5 X2X3=30
Crop	Tea
Planting material	TV 22
Spacing of Tea	105 cm x 60 cm

2.3 Plant Physiological Parameters

2.3.1 Stomatal count

Stomatal count was estimated using Leaf impression method given by Beakbane and Mazumder [8]. The impression was taken from lower surface of fully expanded maintenance foliage using quick fix. The number of stomata per unit area of leaf was counted under high power microscope.

2.3.2 Leaf area

Measurement of leaf area was done on a metric unit graph paper. In this method, a leaf was taken and traced over graph paper, and the grids covered by the leaf are counted to give the area. It is expressed in cm² [9].

2.3.3 Specific leaf weight

Specific leaf weight was calculated by dividing the dry weight of leaves by their surface area. It is expressed in mg/cm² [10].

$$i. SLW(g / cm^2) = \frac{\text{Dry weight of leaf (mg)}}{\text{Total area of leaf (cm}^2\text{)}}$$

2.3.4 Relative turgidity

Relative turgidity was estimated using Relative water content technique given by Weatherly, 1950 [10]. From the recorded values the relative turgidity was calculated using the following formula.

$$ii. \text{Relative Turgidity (RT)\%} = \frac{\text{Fresh Weight} - \text{Dry Weight}}{\text{Saturated Fresh Weight} - \text{Dry Weight}} \times 100$$

2.3.5 Water saturation deficit

Water saturation deficit was estimated using Relative water content technique given by Weatherly, 1950 [11]. From the recorded values the relative turgidity was calculated using the following formula.

$$iii. \text{Water saturation deficit (WSD)\%} = \frac{\text{Saturated Fresh Weight} - \text{Fresh Weight}}{\text{Saturated Fresh Weight} - \text{Dry Weight}} \times 100$$

2.3.6 Chlorophyll content

The extraction was done with methanol following the method of Taylor [12] to analyze chlorophyll pigments. The spectrophotometric observations of diluted solutions were recorded at wavelength 653 nm for chlorophyll-a and 666 nm for chlorophyll-b. The spectrophotometric values were converted into the actual quantities of chl-a and chl-b using the following formulas.

$$iv. \text{Chl-a} = 15.65A_{666} - 7.34A_{653}$$

$$\text{Chl-b} = 27.05A_{653} - 11.21A_{666}$$

2.3.7 Caffeine content

The caffeine estimation and purification was done using chloroform following the method of Rapić [13] from freshly collected sample of two leaves and a bud. The amount of caffeine was calculated by taking weight of fresh tea leaves and weight of crude caffeine in gram, and then the total caffeine in tea leaves was expressed in percentage.

2.3.8 Total polyphenol content

Estimation of polyphenol was done using Folin-Ciocalteu reagent based on the reaction between phenols and an oxidizing agent

phosphomolybdate which results in formation of blue complex [14]. The value of polyphenol was expressed in percentage.

2.3.9 Plucking point density

The method used for counting plucking points was similar to the method described by Barua and Dutta [15]. A square grid of 50 x 50 cm² area was placed at the top of a bush selected at random within the experimental plot. The number of plucking points were counted and expressed in number per 50 x 50 cm² area.

3. RESULTS AND DISCUSSION

3.1 Relative Turgidity

Relative turgidity of tea leaves were significantly affected by gas flaring at distances and seasons. Relative turgidity for D₁ was calculated to be 83.40 percent in rainy season and 81.03 percent in autumn season, whereas for D_c it was calculated to be 85.77 percent and 84.82 percent for rainy season and autumn season respectively. Thus, in both rainy season and autumn season the relative turgidity of leaves decreased near to the flare site at D₁ whereas increased away from the flare site near to the control plot at D_c (Table 1). While considering seasons, it was observed that the effect was more prominent during autumn season. Increase in soil temperature and decrease in soil moisture might have adversely affected the absorption of water from the soil by the tea plants. This study corroborates the findings of Kaur et al. [16] who reported that the relative turgidity of wheat crop significantly decreased with an increase in heat stress.

3.2 Water Saturation Deficit

Water saturation deficit of tea leaves varied significantly with both distances and seasons from the gas flaring site. From the table (Table 1) it is evident that in both seasons water saturation deficit of tea leaves was seen to be decreasing as they moved away from the flare sites, for instance WSD at D₁ for rainy season was found to be 16.59 percent and at D₂ it reduced to 14.23 percent. While considering seasons, it was observed that the effect was more prominent during autumn season. Decrease in the relative turgidity might have increased the evaporation in tea leaves due to which the water saturation deficit has been affected. Kaur et al. [16] reported that water saturation deficit of wheat crop significantly increased with increase in levels of heat stress and increase in rainfall

reduced water saturation deficit as well as evaporation to some extent.

3.3 Stomatal Count

Stomatal count of tea leaves varied significantly with both distances and seasons from the gas flaring site. The stomatal count of leaves decreased near to the flare site at D₁ whereas increased away from the flare site near to the control plot at D_c (Table 1). During rainy season the stomatal count went from 16.67 stomata mm⁻² to 20.66 stomata mm⁻² in autumn season. While considering seasons it was observed that the effect was more prominent during autumn season as warm temperature evaporated the water more readily from the tea leaves, due to which the plants closed their stomata to prevent excessive water loss. In a similar finding of Shen et al. [17] reported that heat stress had a significant effect which reduced the size and percentage of stomata on Rhododendron plant.

3.4 Leaf Area

Leaf area of tea plants showed marginal variations with respect to its distance from the flare site, however the variations among the seasons was very low (Table 2). Though the leaf area was found to be lowest for D₁ and highest for D_c, however the increase in area was not proportional to the increase in distance from the gas flaring site. For instance, leaf area for autumn was 31.15 cm⁻² for D₁ and increased to 32.05 cm⁻² for D₂ but further decreased to 31.67 cm⁻² for D₃. The results obtained would be due to decreased stomatal conductance and reduced nitrogen content in plant growing under enriched air pollutants level for which the photosynthetic activities might be suppressed that led to reduction in leaf area of tea. The findings of Sarma et al. [18] also reported reduction in leaf area of *Cassia tora* near the gas flaring site. However, tea leaf area had shown no significant differences in seasons.

3.5 Specific Leaf Weight

The mean specific leaf weight was significantly ($P=0.05$) different amongst different seasons and distances from the flare pit. A notable reduction in the specific leaf weight was observed in distances closer to the flare, from 0.007 g cm⁻² in D₁ to 0.010 g cm⁻² in D_c for rainy season. Dry weight of leaf and leaf area significantly decreased under drought and combined stress as compared to control (Table 1). Decrease in dry weight and leaf area leads to subsequent

reduction in specific leaf weight. Similar observation was recorded by Misganaw et al. [19] where it was reported that heat and water stress affected the biomass of all alfalfa cultivars compared with the control and significantly reduced the shoot fresh weight and thus specific leaf weight.

3.6 Chlorophyll Content

It was found that mean chlorophyll content (mg/g) was significantly varied along distances and seasons (Table 2). Mean Chlorophyll content was recorded low in the vicinity of gas flaring site at D₁ (0.55 mg g⁻¹) and highest at D_C (0.99 mg g⁻¹). High temperature might have damaged the chloroplast structure due to which the chlorophyll content of leaves decreased. This study corroborates the findings of Naz et al. [20] reported that chlorophyll damage was directly linked with the sensitivity of potato plants to a high temperature stress with respect to their chlorophyll contents.

3.7 Total Polyphenol

Significant variation was seen in total polyphenol percentage in tea leaves with both distances and seasons from the gas flaring site. The total polyphenol content of tea leaves decreased near to the flare site at D₁ (22.24 percent) whereas increased away from the flare site near to the control plot at D_C (23.51 percent) (Table 2). While considering seasons it was observed that the

effect was more in autumn season. It might be due to the high temperature near to the flare site the polyphenol genes in the leaves got inhibited and they could not work properly. The findings of Rivero et al. [21] reported that heat stress had a significant effect on phenolic activity.

3.8 Total Caffeine

Total caffeine content of tea leaves varied significantly with distances from the gas flaring site. Total caffeine content of tea leaves decreased near to the flare site at D₁ (Table 2). The genes responsible for caffeine production in the leaf might have got inhibited that affected the caffeine content of the leaves. This study corroborates the findings of Song et al. [22] reported that the total caffeine content of green leaves of tea crop decreased with an increase in temperature.

3.9 Plucking Point Density

The plucking point density was significantly different among the different distances studied and also significantly different between the seasons. Plucking point density of tea leaves decreased near to the flare site (Table 2). Air pollutants was higher in the surrounding environment of gas flare which might reduce chlorophyll content of tea leaves that affected growth of tea bush reducing its plucking point density. This study finds similarity with the findings of Sarma et al. [18].

Table 1. Effect of gas flaring on plant physiological parameters of tea (Merbil Majuli OCS-6, west)

Distances	Relative turgidity(%)		Water saturation deficit(%)		Stomatal count(stomata mm ⁻²)		Specific leaf weight(g cm ⁻²)	
	S1	S2	S1	S2	S1	S2	S1	S2
D ₁ (35-55 m)	83.40	81.03	16.59	18.97	18.00	16.67	0.007	0.007
D ₂ (55-75 m)	84.35	81.39	15.65	18.61	18.67	17.67	0.007	0.007
D ₃ (75-95 m)	84.46	82.27	15.54	17.72	19.67	18.0	0.010	0.010
D ₄ (95-115 m)	85.67	83.74	14.33	16.26	21.00	19.33	0.010	0.010
D _C (130-150 m)	85.77	84.82	14.23	15.48	21.66	20.66	0.010	0.010
CD (factor D)	1.157		1.445		0.753		0.002	
CD (factor S)	0.818		1.022		0.533		0.001	

Table 2. Effect of gas flaring on plant physiological parameters of tea (Merbil Majuli OCS-6, west) cont

Distances	Leaf area(cm ⁻²)		Chlorophyll content (mg g ⁻¹)		Caffeine content (%)		Total polyphenol content (%)		Plucking point density	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
D ₁ (35-55 m)	31.62	31.15	0.55	0.48	1.47	1.34	22.24	21.07	21.67	21.33
D ₂ (55-75 m)	32.16	32.05	0.62	0.51	2.68	2.01	22.51	21.05	22.33	22.0

Distances	Leaf area(cm ²)		Chlorophyll content (mg g ⁻¹)		Caffeine content (%)		Total polyphenol content (%)		Plucking point density	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
D ₃ (75-95 m)	32.59	31.67	0.76	0.68	2.67	2.24	23.24	22.34	23.0	22.33
D ₄ (95-115 m)	32.83	32.52	0.99	0.77	2.66	2.31	23.35	22.79	23.67	22.67
D _C (130-150 m)	33.21	32.68	0.99	0.78	3.13	2.64	23.51	23.21	24.33	23.33
CD (factor D)	0.812		0.145		0.682		0.935		0.647	
CD (factor S)	N/A		0.102		N/A		0.661		0.458	

*Significant at 5% probability level

4. CONCLUSION

The present investigation revealed that the gas flaring had significant effect on physiology of tea crop with respect to growth. Parameters like relative turgidity, stomatal count, specific leaf weight, leaf area measurement, chlorophyll content, caffeine content, total polyphenol content, plucking point density gradually decline near flare site but an increase in water saturation deficit in the tea bushes growing in the area.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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