

Original Research Article

ASSESSING THE IMPACT OF GAS FLARING ON TEA PLANTS IN THE SOUTH OF KOTHALONI OCS IN THE DISTRICT OF DIBRUGARH, ASSAM, INDIA

ABSTRACT

Effect of gas flaring on physiology and growth of tea plants was done by using Randomized Complete Block Design (RCBD) and analysis was done accommodating two seasons and five levels of distances during September 2019-March 2020. Plant samples were collected from (40-50) metres, (50-60) metres, (60-70) metres, (70-80) metres and control site (150-160) metres away from the tea garden situated south of kothaloni OCS in rainy and autumn seasons. The experimental plot was laid out at a distance of 40 metres away from the flare pit. Plant parameters namely relative turgidity, water saturation deficit, tea leaf area, specific leaf weight, stomatal count, caffeine content, chlorophyll content, polyphenol content and plucking point density were studied.

From the study, plant parameters viz. relative turgidity, stomatal count, specific leaf weight, tea leaf area, chlorophyll-a content and chlorophyll-b content, caffeine content, polyphenol content, plucking point density were found to be decreased at distances nearer to the gas flaring site which significantly increased with distances away from the gas flaring site. This sentence is long and not comprehensible.

Water saturation deficit of tea leaves recorded highest at distances closest to the flaring site and

Keywords: Gas flaring, seasons, distance, OCS Oil Collecting Station

1. INTRODUCTION

Tea (*Camellia sinensis* (L.) O. Kuntze) is one of the ancient and most popular beverages in the world. India is one of the leading countries in the world with respect to global tea consumption, production and exports. The state of Assam in India lying on either side of the Brahmaputra river is one of the most prolific tea producing regions because of its long generous rainfall and growing season.

Assam is enriched with natural resources like natural gas, oil and coal. The petroleum industry of Assam plays an important role in the process of industrialization in the state and the crude oil is one of the valuable natural resources of Assam.

~~The excess or unwanted gases which are produced during different operations such as crude oil extraction get burnt through a vertical device and the process of burning of the unwanted gases through the vertical device can be called as gas flaring.~~

Many oil drilling sites of upper Assam are located near the tea estates.

The excess or unwanted gases which are produced during different operations such as crude oil extraction get burnt through a vertical device. This process can be called as "gas flaring", and the resulting gas is called "gas flare" (references). Gas flare consists of a mixture of different gases and its composition greatly depends on the gas source that is entering to the system of flare. During oil-gas production, associated gases that are released mainly contain natural gas and natural gas is consisted of more than 90% methane (CH₄) with ethane, a small amount of other hydrocarbons and inert gases such as N₂ and CO₂ [1]. Gas flare from refineries and other different operations in general contain a mixture of hydrocarbons, carbon dioxide (CO₂) and sometimes hydrogen (references).

Comment [U1]: Put some of these sentences of the end of this section and other in study area characteristic

Comment [U2]: Do the same

Being among the Green House Gases, CO₂ and methane (CH₄) contribute to the increase of the temperature of the atmosphere when directly released into the air (references). So, gas flare significantly impacts the microclimate in vicinity of the flare sites [2]. This fact has many consequences on biota near the flare site (references). Especially for vegetation, gas flare reduce transpiration and photosynthesis when oil penetrates into leaves in one hand, and leaves turn yellow when light is the pollution (references), in other. This gas also contribute to the acid rain, with its effects on vegetation are more severe near the flare site (references). The acid rain causes vegetation loss too. When leaves get washed with acid precipitation, the protective waxy coating of leaves get away which affects their photosynthetic activity (references). So, gas flare may reduce the growth of plants and it's resistance to abiotic and biotic stresses, making them more susceptible to pathogens (references).

On the whole, the crude oil is one of the valuable natural resources Dibrugarh district in the state of Assam (India), where the petroleum industry plays an important role in the process of its industrialization. This district is also the place of intense practice of the tea culture. This is also true for Kothaloni in Dibrugarh district , particularly South of Kothaloni Oil Collection Station (OCS). However, there is any information concerning to the effects of gas flaring on tea plants in this area. Knowing the great socio-economic role of tea in this area, it is important to carry out investigations on this fact. It is the purpose of this study.

~~So an assessment was taken to study its effect on growth of tea plants adjacent to Kothaloni OCS South in the district of Dibrugarh of Assam. Keeping in view all the above points. The present investigation has the following objective: To study the effect of gas flaring on physiology and growth of tea plants.~~

2.MATERIAL AND METHODS

Follow this plan

2.1. Characteristics of the stuy area

Mentioned here the charcateristic of the study area (geagraphic localization, area, major anthropogenic activities, vegetation, population, area used for the tea culture (tea garden), the volume of oil crude treated, the number of raffinerie, etc).

ALso, Mention on a map the laocalization of the state of Assam (give its geolocalization) and then that of Dibrugarh district, follow by that of Kothaloni

2.2. Experimental techniques

2.2.1. Sampling sites

In this section mention in the map (figure) the sampling sites 4 and (D1; D2; D3; D4 and DC) and explain the reason of their choice. Explain again the choice of the seasons using., etc

2.2.2. Measurment of physiological parameters

2.2.2.1. Relative turgidity (RT) and Water saturation Deficit (WSD)

A tea garden south of Kothaloni OCS, Dibrugarh district was selected for this study. The study was done during September 2019-March 2020. Experimental Design selected was Randomized Complete Block Design accomodating five distances namely (40-50) metres, (50-60) metres, (60-70) metres, (70-80) metres and control site (150-160) metres away from the gas flaring site and two seasons namely rainy and autumn seasons.

A pond was there in between the flare site and the selected tea garden and so the experimental plot was laid out at 40 metres away from the flare pit. The flaring site was surrounded by a concrete wall. Plots were selected at 10 metres interval within the experimental design. DC denotes control plot.

2.1. Relative Turgidity (RT) and Water Saturation Deficit (WSD)

RT was obtained according Weatherley method [5]. Its expression is given by:

$$RT(\%) = \frac{\text{Average fresh weight (mg)} - \text{Average dry weight (mg)}}{\text{Average saturation weight (mg)} - \text{Average dry weight (mg)}} \times 100 \quad (1)$$

As concerned to WSD, it was obtained following standard or (the method) (references) and its expression is:

$$WSD(\%) = \frac{\text{Average saturation weight (mg)} - \text{Average fresh weight (mg)}}{\text{Average saturation weight (mg)} - \text{Average dry weight (mg)}} \times 100 \quad (1)$$

Relative turgidity (RT) was expressed in percentage (%) and water saturation deficit (WSD) was also expressed in percentage (%) and determined using the following formula:

$$RT(\%) = \frac{\text{Average fresh weight (mg)} - \text{Average dry weight (mg)}}{\text{Average saturation weight (mg)} - \text{Average dry weight (mg)}} \times 100$$

$$WSD(\%) = \frac{\text{Average saturation weight (mg)} - \text{Average fresh weight (mg)}}{\text{Average saturation weight (mg)} - \text{Average dry weight (mg)}} \times 100$$

Do the same for all the sections below

2.2. Specific leaf weight

Fifty leaves without the petiole were selected from each planting material and their individual leaf area was measured. Specific leaf weight of tea leaves can be calculated by using the following formula: (standard or method?)

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

2.3. Stomatal count

The estimation of the stomatal count was determined by the method given by Beakbane and Mazumdar [6]. (See 2.1)

2.4. Leaf area of tea leaves (cm²)

Individual leaf was taken and tracing was done over graph paper and the covered grids by the leaf are counted to give the area. It is expressed in cm². : (standard or method?)

2.5. Chlorophyll content

The chlorophyll extraction was done using methanol given by Taylor [7]. Buds and two leaves weighing 0.5g were taken and chopped into pieces. A liquid mixture was made by adding 25 ml of methanol slowly to the chopped pieces placed in mortar and pestle. In 25 ml conical flask, 2ml of supernatant was transferred and conical flask was covered with carbon paper which was kept separately in a dark place for about 30 minutes. The mixture was then centrifuged at 5000 rpm for 5 minutes. In 2 ml of the mixture, 8 ml methanol was added after centrifuge to make a volume of 10 ml. Then spectrophotometric observation of diluted solutions was recorded in different wavelengths namely 653 nm for chlorophyll-a and 666 nm for chlorophyll-b. It was expressed as mg/g.

The sample content in chlorophyll a was obtained following this expression:

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

That in chlorophyll b was by

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

A_{666} , A_{653} the absorbance of the solution obtained after treatment at 653 and 666 nm respectively.

2.6. Caffeine content

(standard or method?)

Caffeine estimation was done by boiling 200 ml of water containing 20 g of two leaves and a bud for 30 minutes in a 400 ml beaker and should be filtered while hot. The filtrate was collected in another clean beaker and of Lead acetate of 10% aqueous solution was added in the filtrate that was taken in a clean beaker followed by centrifuged at 5000 rpm for 5 minutes. The volume of the supernatant was reduced to 25 ml by boiling and cooled it at room temperature. The mixture was extracted with 25 ml chloroform in a separating funnel after cooling the reaction. The chloroform was distilled off from the chloroform extract on a water bath using a water condenser. By using a spatula, the dried residue was scrapped from the flask and weighed was taken. Weight of fresh tea leaves and weight of crude caffeine in gram gives the amount of caffeine. The total caffeine in tea leaves was expressed in percentage (%) (what formulae have you used?).

2.7. Total polyphenol content

(standard or method?)

Polyphenol content was estimated with Folin-Ciocalteu reagent, based on the reaction between phenols and phosphomolybdate that results in the blue complex formation [8]. Give more details and the expression of formulae used

2.8. Plucking point density

The method used for counting plucking points was same as the method to the method described by Barua and Dutta [9]. A light bamboo grid of 10 cm square sides was placed on the top of the bush and in the randomly selected plant, the number of plucking points at the the end of the season was collected.

3. RESULTS AND DISCUSSIONS

Don't repeat both result in table and in the text

3.1. RT

Relative turgidity of tea leaves had shown significant differences among distances and seasons. Relative turgidity was found to be lower near the gas flaring site which increased with distances away from the gas flare. Among the distances, D2, D3, D4 and DC were significant to D1. A lowest mean value was recorded at D1 (79.68%) and the highest mean value was seen at DC (84.51%) as mentioned in Table 1. This might be due to high temperature stress that decreased fresh and dry weight of leaves of tea plant which reduced its relative turgidity. This was agreed with the findings of Naz [10], who reported that relative turgidity of potato plants were successively reduced by increased heat stress. In this study, relative turgidity of tea leaves had shown decreased mean value in autumn

season as compared to rainy season. This might be due to increased water stress in the season of autumn which led to reduction in relative turgidity of tea leaves. These results corroborates the findings of Waheed [11] who reported that relative turgidity of tea leaves were successively decreased during drought stress.

3.2. Water Saturation Deficit

Water saturation deficit was significantly varied among distances. Among the distances, D2, D3, D4 and DC were significant to D1. There was no significant variations observed in rest of the distances. Highest mean value was found in D1 (20.51%) and the lowest mean value was recorded in DC (16.01%) as mentioned in Table 2. Higher water saturation deficit near the gas flaring site as compared to far away distances might be due to high temperature stress that reduced tea plants' relative water content and it successively increased water saturation deficit of tea. The relative humidity around the gas flare was relatively reduced and might desiccate leaves of plants that are vulnerable to desiccation [12]. In this study, water saturation deficit of tea leaves was increased in autumn season than rainy season. This might be due to water stress intensified by dry season that led to increase in water saturation deficit of tea leaves.

3.3. Number of stomata

Number of stomata of tea leaves varied significantly among distances. Among the distances, variations were observed between all the distances except D3 and D4. The lowest mean stomata was recorded at D1 (19.66 stomata/mm²) which gradually increased with increase in distance from the flare site and the highest value was found in control site at 150 m-160 m (22.83 stomata/mm²) as shown in Table 3. Significant variation was observed among seasons and autumn season recorded the lowest mean value (20.93 stomata/mm²) This might be due to presence of pollutants in the surrounding environment of gas flare that changed biochemical composition along with morphology of plants leading to the nitrogen limitation for the plant. In a similar findings of Sarma [13], who reported that stomata of *Cassia tora* in Sivasagar district of Assam was reduced due to CO₂ stressed environment.

3.4. Leaf area of tea leaves

It was observed that tea leaf area varied significantly among distances. Among the distances, D4, DC are significant to D1. Between rest of the distances no significant variations were observed. D1 recorded the lowest mean value of leaf area (36.37 cm²) and the highest mean value was observed in DC (49.76 cm²) as mentioned in Table 4. This might 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 [13] 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 weight of tea leaves

Specific leaf weight varied significantly among distances. Within the distances, significant variations were found between D1 and D3, D1 and D4, D1 and DC, D2 and D3, D2 and D4, D2 and DC. The D1 and D2 recorded the lowest mean specific leaf weight (0.007 g/cm²) whereas, D4 and DC

recorded the highest mean specific leaf weight (0.011 g/cm^2) as mentioned in Table 5. This might be due to the reason that plants growing under enriched CO_2 stress might reduced leaf area of tea plants resulted in lowering its specific weight near gas flaring site. The findings of Sarma [13] also reported reduction in leaf area of *Cassia tora* near the gas flaring site.

Seasons had shown significant variation where autumn season recorded the lowest mean value of specific weight of tea leaves (0.009 g/cm^2). This might be due to the seasonal variation of air pollutants and decreased photosynthetic activities during post monsoon period [13].

3.6. Chlorophyll content

It was found that chlorophyll a content (mg/g) and chlorophyll b content (mg/g) were significantly varied along distances.. D1 recorded the lowest mean chlorophyll a content (0.96 mg/g) and highest value was found in DC (2.31mg/g) and mentioned in Table 6. Lowest mean chlorophyll b content was observed in D1 (0.42 mg/g) and highest mean value was found in DC (0.85 mg/g). Combination of both drought and heat stress might be the reason of low chlorophyll content near the gas flaring site. Chlorophyll content might be reduced due to heat alone[14] and shortage of water might disrupt chloroplast structure which might led to destruction of Chlorophyll. This is in confirmation with the findings of Isichei and Sanford [12] who reported Chlorophyll content of *Eupatorium odoratum* was decreased in the distances nearer to gas flaring site.

Chlorophyll a and chlorophyll b contents had shown significant variation along seasons. Mean chlorophyll a and mean chlorophyll b contents were recorded highest in rainy season having 1.66 mg/g and 0.67 mg/g respectively.

3.7. Caffeine content

Caffeine content in tea leaves had shown significant variations with distances. Distances nearer to gas flaring site had shown lower value as compared to distances away from the gas flaring site. Lowest mean caffeine content was observed in D1 (2.35%) and the highest mean value was found in DC (3.48%) as shown in Table 7. This might be due to drought stress that inhibited the expression of genes related to caffeine biosynthesis and thereby reducing the accumulation of Caffeine in *Camellia sinensis*. This corroborates the findings of Song [15], Lee [16] and Zhang [17] who reported caffeine levels decreased with an increase in light.

3.8. Polyphenol content

Significant variation was seen in polyphenol content (%) of tea leaves along distances and the significant variations were observed between all the distances except D2 and D3. Lowest mean value for total polyphenol content was recorded in D1 (21.37 %) and the highest mean value was observed in DC (23.27 %) as recorded in Table 8. It was also observed that mean total polyphenol content was varied significantly in seasons and was higher in rainy season (22.67 %). Polyphenol content was observed to be lowest in distances closest to the gas flaring site and also observed lowest in autumn season as compared to rainy season. This might be due to the alteration of biochemical constituents necessary for tea quality by drought stress. Erturk [18] also reported significant decrease in total polyphenol content that reduced leaf quality of *Camellia sinensis* as affected by drought stress.

3.9. Plucking point density of tea bush

It had shown significant differences with respect to distance. The highest mean value was observed in DC (41.50 no./2500 cm²) and the lowest mean plucking point density was observed in D1 (31.33 no./2500 cm²) as mentioned in Table 9. Plucking point density had also shown significant variation in seasons and was higher in rainy season (36.99 no./2500 cm²).

Plucking point density of tea bush was lowest in distances nearer to the gas flaring site. Air pollutants was higher in the surrounding environment of gas flare [19] 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 [13].

Table 1. Relative turgidity of tea leaves as affected by gas flaring

Seasons Distances (m)	Relative turgidity (%)		Mean	
	Rainy season (S1)	Autumn season (S2)		
D1(40-50)	86.45	72.90	79.68	
D2(50-60)	90.32	76.04	83.18	
D3(60-70)	88.34	78.68	83.51	
D4(70-80)	89.36	78.09	83.72	
DC(150-160)	89.86	79.16	84.51	
Mean	88.86	76.97		
Factors	C.D.	SE(d)	SE(m)	Significance
Distance(D)	2.48	1.17	0.83	S
Season(S)	1.57	0.74	0.52	S
Distance X Season(DXS)	N/A	1.659	1.17	NS

*S= Significant at 5% probability level; NS = Non Significant; N/A= Not Applicable

Table 2. Water saturation deficit of tea leaves as affected by gas flaring

Seasons Distances (m)	Water saturation deficit (%)		Mean
	Rainy season (S1)	Autumn season (S2)	
D1(40-50)	13.58	27.45	20.51
D2(50-60)	9.73	23.86	16.79
D3(60-70)	11.30	21.54	16.42
D4(70-80)	10.39	21.67	16.03
DC(150-160)	11.00	21.01	16.01

Mean	11.20	23.10		
Factors	C.D.	SE(d)	SE(m)	Significance
Distance(D)	2.46	1.16	0.82	S
Season(S)	1.56	0.77	0.52	S
Distance X Season(DXS)	N/A	1.65	1.16	NS

*S= Significant at 5% probability level; NS = Non Significant; N/A= Not Applicable

Table 3. Stomatal count of tea leaves as affected by gas flaring

Seasons Distances (m)	Stomatal count (number/mm ²)		Mean	
	Rainy season(S1)	Autumn season(S2)		
D1(40-50)	20.00	19.33	19.66	
D2(50-60)	21.00	20.33	20.66	
D3(60-70)	21.66	21.33	21.50	
D4(70-80)	22.00	21.00	21.50	
DC(150-160)	23.00	22.66	22.83	
Mean	21.53	20.93		
Factors	C.D.	SE(d)	SE(m)	Significance
Distance(D)	0.49	0.23	0.16	S
Season(S)	0.31	0.15	0.10	S
Distance X Season (DXS)	N/A	0.37	0.23	NS

*S= Significant at 5% probability level; NS = Non Significant; N/A= Not Applicable

Table 4. Leaf area of tea as affected by gas flaring

Seasons Distances(m)	Leaf area (cm²)		Mean
	Rainy season (S₁)	Autumn season (S₂)	
D ₁ (40-50)	38.66	34.08	36.37
D ₂ (50-60)	45.89	36.75	41.32
D ₃ (60-70)	44.49	43.76	44.12
D ₄ (70-80)	49.76	49.18	49.47
D _C (150-160)	50.15	49.37	49.76

Mean	45.79	42.62		
Factors	C.D.	SE(d)	SE(m)	Significance
Distance(D)	8.78	4.15	2.93	S
Season(S)	N/A	2.62	1.85	NS
Distance X Season (DXS)	N/A	5.87	4.15	NS

*S= Significant at 5% probability level; NS = Non Significant ; N/A= Not Applicable

Table 5. Specific leaf weight of tea leaves as affected by gas flaring

Distances (m)	Seasons	Specific leaf weight (g/cm²)		Mean
		Rainy season (S₁)	Autumn season (S₂)	
D ₁ (40-50)		0.008	0.007	0.007
D ₂ (50-60)		0.008	0.007	0.007
D ₃ (60-70)		0.011	0.010	0.010
D ₄ (70-80)		0.012	0.010	0.011
D _C (150-160)		0.012	0.010	0.011
Mean		0.010	0.009	
Factors	C.D.	SE(d)	SE(m)	Significance
Distance(D)	0.002	0.001	0.001	S
Season(S)	0.001	0.001	0.000	S
Distance X Season (DXS)	N/A	0.001	0.001	NS

*S=Significant at 5% probability level; NS=Non Significant; N/A=Not Applicable

Table 6. Chlorophyll content of tea as affected by gas flaring

Seasons

*S= Significant at 5% probability level; NS = Non Significant ; N/A= Not Applicable

Table 7. Caffeine content of tea as affected by gas flaring

Seasons Distances (m)	Caffeine content (%)		Mean
	Rainy season (S ₁)	Autumn season (S ₂)	
D ₁ (40-50)	2.38	2.33	2.35
D ₂ (50-60)	2.46	2.44	2.45
D ₃ (60-70)	3.01	2.71	2.86
D ₄ (70-80)	3.29	3.19	3.24

D _C (150-160)	3.54	3.43	3.48	
Mean	2.93	2.82		
Factors	C.D.	SE(d)	SE(m)	Significance
Distance(D)	0.10	0.05	0.03	S
Season(S)	0.06	0.03	0.02	S
Distance X Season (DXS)	N/A	0.07	0.05	NS

*S= Significant at 5% probability level; NS = Non Significant ; N/A= Not Applicable

Table 8. Total polyphenol content of tea leaves as affected by gas flaring

Distances (m)	Seasons	Total polyphenol content (%)		Mean
		Rainy season (S ₁)	Autumn season (S ₂)	
D ₁ (40-50)		21.72	21.03	21.37
D ₂ (50-60)		22.24	21.78	22.01
D ₃ (60-70)		22.79	22.06	22.42
D ₄ (70-80)		23.13	22.80	22.96
D _C (150-160)		23.48	23.06	23.27
Mean		22.67	22.14	
Factors	C.D.	SE(d)	SE(m)	Significance
Distance(D)	0.49	0.23	0.16	S
Season(S)	0.31	0.15	0.10	S
Distance X Season (DXS)	N/A	0.33	0.23	NS

*S= Significant at 5% probability level; NS = Non Significant; N/A= Not Applicable

Table 9. Plucking point density of tea as affected by gas flaring along

Seasons Distances (m)	Plucking point density (No./2500 cm ²)		Mean	
	Rainy season (S ₁)	Autumn season (S ₂)		
D ₁ (40-50)	32.00	30.66	31.33	
D ₂ (50-60)	33.66	32.66	33.16	
D ₃ (60-70)	37.00	36.33	36.66	
D ₄ (70-80)	40.33	38.66	39.49	
D _C (150-160)	42.00	41.00	41.50	
Mean	36.99	35.86		
Factors	C.D.	SE(d)	SE(m)	Significance
Distance(D)	1.55	0.73	0.52	S
Season(S)	0.98	0.46	0.33	S
Distance X Season (DXS)	N/A	1.04	0.73	NS

*S= Significant at 5% probability level; NS = Non Significant; N/A= Not Applicable

With all results obtained give more as partial conclusion more details of the effects of gas flaring on the physiology of these tea specie.

4. CONCLUSION AND PERSPECTIVES

Gas flaring has effects in physiology of tea plants with respect to growth in the area under study where the studied plant parameters had shown an increasing trend with distances away from the gas flaring site except water saturation deficit of tea leaves which increased with the distances nearer to the gas flaring site. **Perspectives?**

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