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Response of Gas Exchange Activity and Relative Water Content of Neem in Relation to Weather

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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

Neem is known for its medicinal values and is a hardy plant that can grow in a wide variety of environmental conditions. However, productivity is impacted by unfavourable weather conditions due to different geographical locations. The present study was conducted to study the influence of prevailing weather conditions on certain physiological parameters deciding the reproductive ability of neem trees. The study was conducted in two different locations at different agro-climatic regions of Tamil Nadu, India. Gas exchange parameters like photosynthetic rate, stomatal conductance, transpiration rate and leaf temperature and relative water content during pre-flowering, flowering and post-flowering stages were measured and correlated with temperature, relative humidity, precipitation and wind speed observed from the corresponding location. In both locations, the neem trees recorded significantly higher values for these physiological parameters during the pre-flowering and fruiting stages compared to the flowering stage. The trees with higher Diameter and

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breast height (DBH) showed higher values for gas exchange parameters compared to the trees with lower DBH.

Keywords: Neem; Photosynthetic rate; Transpiration rate; Stomatal conductivity; Weather.

1. INTRODUCTION

Neem (*Azadirachta indica* A. Juss.) is an evergreen, tropical forest tree that belongs to the *Meliaceae* family and is native to the Indian subcontinent and southeast Asia. Neem is known for its antifungal, antiseptic, antiviral, antipyretic and anti-inflammatory properties present in all parts of the plant such as bark, leaves, flowers, fruits, seeds and roots. Among these, the most significant is the eco-friendly pest management and medicinal properties that make neem unique [1]. The habitat of neem extends to about 72 countries of various continents. There are an estimated 25 million trees in India with Uttar Pradesh (55.7%) and Tamil Nadu (17.8%) ranking top and second, respectively and Karnataka (5.5%) ranking third [2].

Neem is usually propagated through seed and it reaches the reproductive stage after five years from planting. The flowering usually occurs from January to April and attains maturity between May and August. Fruits are about 2cm in length occurring in drooping panicles. Browny oil kernel is surrounded by yellow and fleshy pericarp with a hard white shell [3].

Neem being a hardy tree species grows in varied soil and climatic conditions. The ideal temperature range for a neem tree is around 33^{o}C. It can grow in temperatures as low as 10-14^{o}C and a maximum of 53^{o}C [4-7]. In India and specifically in Tamil Nadu, neem is widely grown in rainfed semi-arid regions and other areas under non-irrigated conditions. The rainfed semi-arid areas are known for weather vagaries and the pattern of rainfall, temperature, humidity and wind speed are ever-changing and often not favourable for the growth and development of plants. Since neem is adapted to such conditions, it can thrive well under such unfavourable conditions but it is observed that the growth and reproductive capacity of neem is affected significantly due to the variation or deviation in the prevailing weather parameters such as temperature, relative humidity, precipitation and wind speed. Though such variation in growth and yield due to weather vagaries was observed over a period of time by the farmers and scientists, there was no scientific study to explain and confirm as for as neem is concerned. Hence, this study was proposed to extensively observe the changes in physiology and yielding capacity of neem in different agro-ecological zones of Tamil Nadu in relation to the prevailing weather conditions in such zones. This article correlates how the changing weather conditions affect the leaf temperature, gas exchange parameters namely photosynthetic rate, stomatal conductance and transpiration rate of neem trees growing in different agro-climatic conditions.

Weather parameters play a significant role in the growth and productivity of the plants. Gas exchange parameters in neem were showing decreased trend under water-stressed conditions of various provenances in China [8]. Next to water status, temperature a predominant environmental factor plays a significant role in the activity of enzymes which is the major determining factor for all biological reactions, cellular respiration and stomatal conductivity in plants [9]. Atmospheric moisture also influences transpiration rates from leaf stomata there by affecting soil water storage and hence, the water status of trees [10].

It is imperative that understanding the influence of variations in weather parameters on certain physiological characters especially gas exchange parameters on different phenological stages of neem, may enable us to make decisions to improve its productivity. Hence, this study was planned and undertaken.

2. MATERIALS AND METHODS

2.1 Location and Selection of Trees

This study was carried out in two agro-climatic regions –Tamil Nadu Agriculture University (TNAU) Coimbatore and Regional Research Station (RRS), Paiyur. Coimbatore (11.02^{0}N, 76.92^{0}E) is characterised with clay loamy soil having low nitrogen and medium phosphorous and potassium with an average rainfall of 643mm and mean maximum and minimum temperature is 32^{0}C and 20.1^{0}C. Regarding Paiyur (12.38^{0}N, 78.22^{0}E), the Krishnagiri district is characterised by non-calcareous red loamy sand to sandy loam soil type and with fertility status of low N, medium

P&K. Paiyur recorded an average rainfall of 917 mm and the mean maximum and minimum temperature is 33.9^{0}C and 21.9^{0}C.

2.2 Description of Trees

In each location, sixteen trees were selected based on the performance in previous years and growing individually in open places and were grouped into four with four trees in each group based on their girth size (DBH-diameter at breast height) as 45-60 cm (T1), 60-75 cm (T2), 75-90 cm (T3) and 90 cm & above (T4)which was considered as treatment and three types of crown shapes as Cylindrical, Oblong and Irregular were selected and studied during 2020-21 (Table 1). General biometric observations such as tree height, crown width, DBH were measured using Blume-Leiss dendrometer and standard measuring tapes respectively.

2.3 Physiological Parameters

2.3.1 Measurement of relative water content

Relative water content (RWC) was evaluated by analyzing twenty leaf disks of 1.5 cm^2 and weighed immediately to get fresh weight (W), and then make the leaf disks fully turgid by dipping them in deionized water at normal room temperature for about 4 Hrs. Subsequently, they were taken out of the water and dried gently to remove the surface moisture using filter paper. The weight of each sample was measured to obtain a fully turgid weight (TW) [11]. Samples were placed in a pre-heated oven at 80°C for 48 hours distinctly and weighed to obtain dry weight (DW). Then, relative water content was measured in percentage by using this formula,

$$RWC\ (\%) = [(W-DW)/ (TW-DW)] \times 100$$

W = Sample fresh weight (g)
TW = Sample turgid weight (g)
DW = Sample dry weight (g)

2.3.2 Measurement of gas exchange parameters

Gas exchange parameter includes photosynthetic rate (µmol CO_2 m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}), stomatal conductance (mol H_2O m^{-2} s^{-1}) and leaf temperature (^{0}C) were measured at three stages *viz*. Before flowering (P1) - first week of February (5th standard meteorological week); During flowering (P2) - second week of April (15th standard meteorological week); During fruiting stage (P3) - third week of June (25th standard meteorological week) [12]. Gas exchange parameters were assessed using an infrared gas analyzer [Portable Photosynthetic System (PPS) - Model LCi-SD of ADC Bio-scientific Ltd., Great Am-well, Hertfordshire, UK.] between 9 and 11 AM. For photosynthetic gas exchange measurements, four fully grown and exposed leaves in the middle of the canopy from all the sides of the trees were selected, measured and averaged.

2.3.3 Weather parameters

Weather parameters *viz*., maximum and minimum temperature, rainfall and relative humidity were measured using a maximum and minimum thermometer, ordinary raingauge, dry and wet bulb thermometers, respectively and collected from the observatories located in Coimbatore and Paiyur throughout the study period (December 2020-July 2021) (Fig 1).

Table 1. Biometric observations of selected trees

	DBH (cm)	Crown width (m) Average of N-S,E-W	Trunk Height (m)	Crown Height (m)	Tree Height (m)
L_1T_1	50.7 ± 2.6	4.9 ± 0.2	3 ± 0.2	5 ± 0.4	8 ± 0.4
L_1T_2	67.7 ± 1.6	5.9 ± 0.2	3.7 ± 0.5	4.5 ± 0.4	8.2 ± 0.2
L_1T_3	78 ± 0.8	8.5 ± 0.4	4 ± 0.2	6.5 ± 0.4	10.5 ± 0.4
L_1T_4	102.7 ± 6.6	9.8 ± 0.4	4 ± 0.2	7.7 ± 0.1	11.7 ± 0.3
L_2T_1	50.7 ± 2.6	4.9 ± 0.2	3 ± 0.2	5 ± 0.4	8 ± 0.4
L_2T_2	67.7 ± 1.6	5.9 ± 0.2	3.7 ± 0.5	4.5 ± 0.4	8.2 ± 0.2
L_2T_3	78 ± 0.8	8.5 ± 0.4	4 ± 0.2	6.5 ± 0.4	10.5 ± 0.4
L_2T_4	102.7 ± 6.6	9.8 ± 0.4	4 ± 0.2	7.7 ± 0.1	11.7 ± 0.3

L_1-Paiyur, L_2-Coimbatore, DBH- Diameter at Breast Height, an average of each class trees

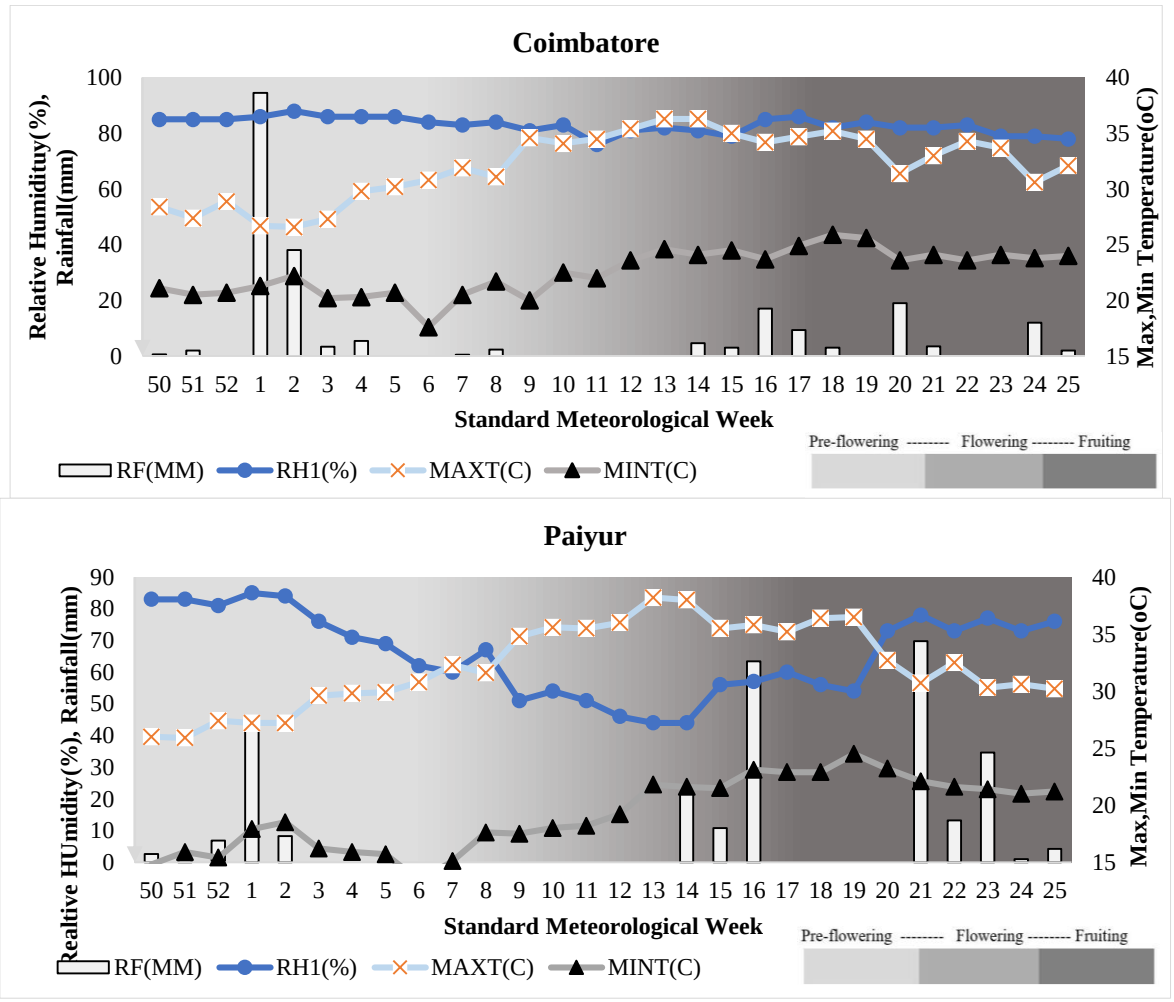

Fig. 1. Weather condition prevailed during study period in Coimbatore and Paiyur

2.4 Statistical Analysis

The experimental results were analyzed in STAR [Statistical Tool for Agricultural Research] with four replications and the age category of trees was considered as treatments. Means were compared using the least significant difference (LSD) at a 5% level of confidence using STAR Tool [13].

3. RESULTS and DISCUSSION

3.1 Physiological Parameters

3.1.1 Relative water content (RWC)

The water status of the plants is determined by measuring RWC and it also indicates the balance between leaf water supply and transpiration rate [14]. From Fig 2 the RWC was the highest in T_1P_2 (91.4%) and the lowest was recorded with T_3P_1 (80.6). The results revealed that RWC was higher during the flowering stage than the pre-flowering stage. Also, higher RWC coincided with the lower transpiration rate at the flowering stage. During the pre-flowering stage, the temperature and solar radiation were relatively lesser compared to the flowering stage which would have an impact on the RWC observed in this study. Rodríguez *et al.*,(2001) [15] also reported the same results as increased relative water content with declined transpiration rate.

3.1.2 Photosynthetic rate

Photosynthesis is the physicochemical process by which plants use light energy to drive the synthesis of organic compounds and it is the basis of plant production which is measured in terms of photosynthetic rate [16]. The photosynthetic capacity of the plant at different phenological stages is one of the determining traits of normal growth and development [5].

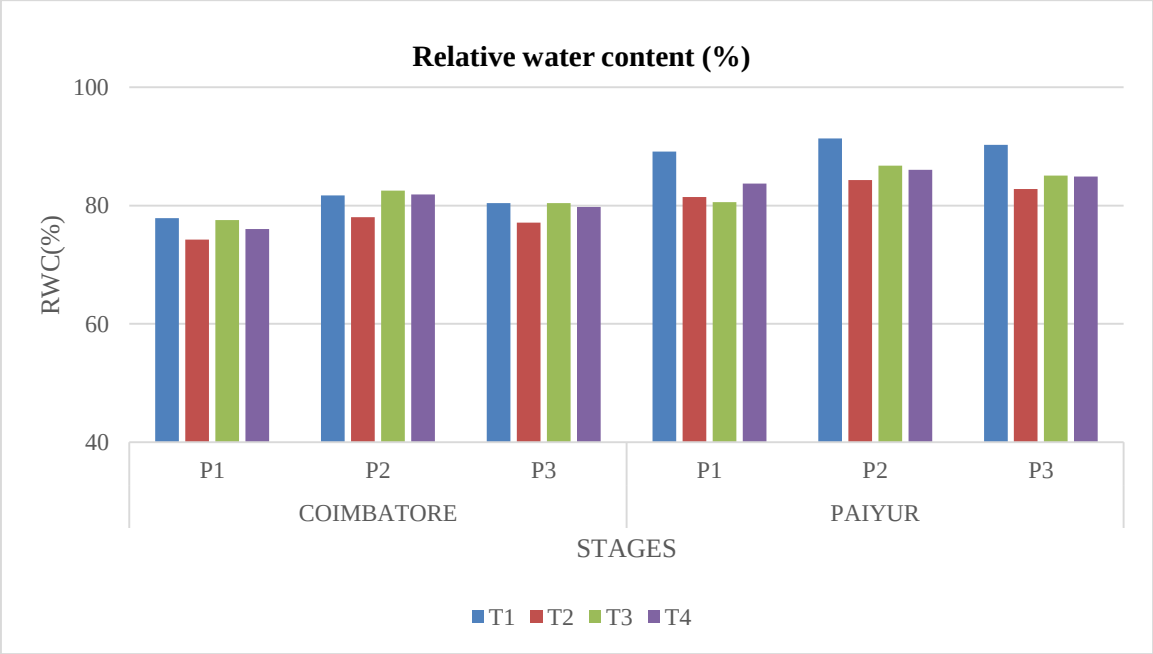

Fig. 2. Relative water content status at different stages

Photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) of neem trees measured at pre-flowering, flowering and fruiting stages at Coimbatore and Paiyur locations (Table 2).

Among different ages of trees based on DPH, the photosynthetic rate ranged between 3.01 (T_1P_2) to 12.22 (T_4S_1) in Paiyur and 2.58 (T_1P_3) to 10.68 (T_4S_1) in Coimbatore. The rate of photosynthesis was found to be higher in T_4 category compared to other age groups in all the stages of observation (L_1P_1-12.22, L_1P_2-5.84, L_1P_3-7.87 and L_2P_1-10.68, L_2P_2-3.06, L_2P_3-5.03) and the lowest photosynthetic rate was measured in T_1 (L_1T_1 - 4.70 and L_2T_2 - 1.86) followed by T_2 (L_1T_2 - 5.47 and L_2T_2 - 2.87) among different age groups categorised based on their DPH. Results showed that photosynthetic rate was higher during the pre-flowering stage and gradually decreased during flowering but there was a significant increase in photosynthetic rate after flowering *i.e.,* during the fruiting period but the rate of increase was not as much as that of the pre-flowering stage. Also, it was observed that between the two locations the neem trees at Paiyur recorded significantly higher values for photosynthesis in all the age groups compared to the trees selected at Coimbatore.

Generally, the demand for the sink increases during the flowering and fruiting stages of crops than that required for the normal functioning of the crops [17]. However, our result showed a decrease in the photosynthetic rate of the crops during the flowering stage and increases during the fruiting stage of the crop which was in accordance with the results of [18]and [19] in mango trees and [20] in apple trees.

A decrease in photosynthetic rate during the flowering stage of the trees in this study might be due to the coincidence of flowering with summer months (March and April) which usually registered higher temperatures and solar radiation which induces higher photorespiration and thus reduced photosynthetic rate. The weather parameters observed during this period in both locations supported this observation in this study. Similarly, [21] Urban *et al.*, (2008) observed the decline in photosynthetic rate with a simultaneous increase in the photorespiration in mango trees. Also, the RWC of leaves plays a major role in metabolic activities such as respiration and photosynthesis. In this study, the fluctuations in RWS during the periods of observation might also be contributed to the variation in photosynthesis and photorespiration.

3.1.3 Transpiration rate

The transpiration rate of the neem trees observed during different stages followed the same trend as that of photosynthetic rate (Table 3) in both locations. Transpiration rate among the neem trees of different age groups ranged between 1.1 (T_1P_3), 0.16 (T_1P_2) to 2.73 (T_4P_1),

2.1 (T_4P_1) at Paiyur and Coimbatore, respectively. Among the four categories of neem trees, the transpiration rate was the highest in T_4 and the lowest was observed in T_1. Results showed that the transpiration rate was higher during the pre-flowering stage and subsequently decreased. The transpiration rate during the flowering stage was found lower than pre-flowering and fruiting stages which impacted the photosynthetic activity of trees in the respective stages as was observed in this study. The data revealed that among the two locations, the trees at Paiyur recorded a higher transpiration rate with a mean of 1.66 compare to the trees at Coimbatore in which the average was 1.33.

In general, transpiration rate is an indirect indicator of the rate of photosynthesis in leaves and has a positive correlation with photosynthesis [22]. In this study also the data on transpiration and photosynthesis followed the same trend in all the stages of observation.

3.1.4 Stomatal conductivity

The mean of the neem trees observed at Paiyur and Coimbatore were ranged between 0.153 (T_4P_1), 0.257 (T_3P_1) to 0.057 (T_1P_2), 0.03 (T_2, T_3P_2), respectively and there was a significant difference among the locations and stages of observation (Table 4). However, the stomatal conductivity observed during the pre-flowering stage did not show significant variation among the different DBH categories of neem trees at Paiyur and in Coimbatore, during the flowering stage, there were no significant differences among the categories for stomatal conductivity. From the data, it was observed that among the three stages pre-flowering stage recorded the highest values and decreased in succeeding stages. Among the two locations, the trees at Paiyur registered higher mean stomatal activity (0.117) than in Coimbatore (0.115).

Table 2. Photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) of neem at three stages in two locations

Stages vs Age	PAIYUR				COIMBATORE			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
T_1	7.74 ^{d}	3.01 ^{c}	3.35 ^{d}	4.70	1.67 ^{d}	1.32 ^{d}	2.58 ^{c}	1.86
T_2	8.46 ^{c}	3.17 ^{c}	4.79 ^{c}	5.47	3.42 ^{c}	1.64 ^{c}	3.54 ^{b}	2.87
T_3	11.29 ^{b}	4.44 ^{b}	6.52 ^{b}	7.41	5.60 ^{b}	2.26 ^{b}	4.90 ^{a}	4.25
T_4	12.22 ^{a}	5.84 ^{a}	7.87 ^{a}	8.64	10.68 ^{a}	3.06 ^{a}	5.03 ^{a}	6.26
Mean	9.93	4.11	5.63	6.56	5.34	2.07	4.01	3.81

Among rows means with the same letters indicates no significant difference between treatments at 5% levels of LSD

Table 3. Transpiration rate (mmol H_2O m^{-2} s^{-1}) of neem at three stages in two locations

Stages vs Age	PAIYUR				COIMBATORE			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
T_1	2.14 ^{c}	1.26 ^{c}	1.10 ^{c}	1.50	0.97 ^{c}	0.16 ^{c}	1.60 ^{b}	0.91
T_2	2.45 ^{b}	1.45 ^{bc}	1.22 ^{c}	1.71	2.10 ^{a}	0.95 ^{b}	1.20 ^{c}	1.42
T_3	2.58 ^{ab}	1.49 ^{ab}	1.46 ^{b}	1.84	1.47 ^{b}	1.22 ^{a}	1.44 ^{b}	1.38
T_4	2.73 ^{a}	1.69 ^{a}	1.69 ^{a}	2.04	2.11 ^{a}	1.13 ^{ab}	1.82 ^{a}	1.69
Mean	2.47	1.47	1.37	1.77	1.66	0.87	1.52	1.35

Among rows means with the same letters indicates no significant difference between treatments at 5% levels of LSD

Table 4. Stomatal conductance (mol H_2O m^{-2} s^{-1}) of neem at three stages in two locations

Stages vs Age	PAIYUR				COIMBATORE			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
T_1	0.12 ^{b}	0.057 ^{c}	0.16 ^{b}	0.112	0.157 ^{c}	0.04 ^{a}	0.087 ^{c}	0.094
T_2	0.143 ^{a}	0.073 ^{ab}	0.18 ^{a}	0.132	0.19 ^{b}	0.033 ^{a}	0.117 ^{b}	0.113
T_3	0.147 ^{a}	0.067 ^{bc}	0.107 ^{c}	0.107	0.257 ^{a}	0.033 ^{a}	0.13 ^{b}	0.140
T_4	0.153 ^{a}	0.083 ^{a}	0.11 ^{c}	0.116	0.123 ^{d}	0.043 ^{a}	0.17 ^{a}	0.112
Mean	0.141	0.070	0.139	0.117	0.182	0.037	0.126	0.115

Among rows means with the same letters indicates no significant difference between treatments at 5% levels of LSD

Net photosynthesis and stomatal conductance are often related to each other [9]. The observed decrease in the photosynthetic rate during the flowering stage in this study might be due to a reduction in stomatal conductivity and was also explained by [23] in olive trees. Photosynthetic rate and stomatal conductivity were found to decrease due to advancement in phenological stages were reported by [24], But in this study, these parameters showed an increasing trend with respect to different phenological stages of the trees.

3.2.5 Leaf temperature

Temperature is one of the main environmental factors that affect plant metabolism [25]. Variation in leaf temperature of different categories of neem trees observed at various stages in Paiyur and Coimbatore was given in Table 5. The data on leaf temperature showed that there was no significant variation among the different categories of neem trees selected for the observation and also between the locations but some trees showed lesser leaf temperatures which might be due to the higher water status of plants which was noticed from the RWC on the trees observed in this study.

In general, there was a positive and exponential relationship reported among photosynthetic rate, transpiration rate and leaf temperature [26], however, in this study, no such relationship was observed with the leaf temperature. [27] Gates (1968) also reported that there was no significant difference among air temperature, leaf temperature and metabolic activities but it did not occur on all the conditions as it depends on radiation, water status and other factors. However, this study noted that the air temperature of the study area and leaf temperature showed significantly higher variation between them. But it is interesting to note that [28] Miller and Saunders (1923) stated marked disagreement with the work of others reporting large differences between air and leaf temperatures.

3.2.6 Correlation between weather parameters and gas exchange parameters

Correlation analysis between weather parameters during the study period and the gas exchange parameters was done and the results were depicted in Table 6. In this study, the correlation matrix revealed that maximum and minimum temperatures negatively correlated with all the gas exchange parameters observed, however, there was no significant correlation for any of the observed parameters at a 5% confidence interval. Rainfall and relative humidity registered a positive correlation with the gas exchange parameters and leaf temperature. Stomatal conductivity showed a higher positive correlation with Karl Pearson correlation coefficient of 0.0862 and was found significant at a 5% confidence interval.

The gas exchange parameters showed a positive correlation among themselves and them, photosynthetic rate found to be highly correlated with transpiration rate with a correlation coefficient of 0.948 and found significant at 1% confidence interval. Leaf temperature recorded a positive correlation with photosynthetic rate and stomatal conductivity with a correlation coefficient of 0.248 and 0.381, respectively. As net photosynthesis and stomatal conductance are positively correlated with each other, the stomatal regulation influences the photosynthetic performance of the plants. Typically, net photosynthetic rates increase when stomata open and decrease when they close [29] and [30] as was observed in this study. Changes in stomatal conductance may cause changes in photosynthetic rates [31]. The significance of stomatal conductance and its positive influence on net photosynthesis were also reported in fig and peach trees [32] and in neem [33]. In this

Table 5. Leaf temperature (^{0}C) of neem at three stages in two locations

Stages vs Age	PAIYUR				COIMBATORE			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
T_1	35.27 ^{c}	35.33 ^{d}	32.57 ^{d}	34.39	38.87 ^{a}	33.56 ^{b}	34.30 ^{b}	35.58
T_2	35.47 ^{b}	36.13 ^{b}	33.17 ^{c}	34.92	38.73 ^{ab}	33.63 ^{b}	34.63 ^{a}	35.67
T_3	35.70 ^{a}	36.53 ^{a}	34.23 ^{a}	35.49	38.77 ^{ab}	33.27 ^{c}	34.43 ^{b}	35.49
T_4	35.80 ^{a}	35.73 ^{c}	33.77 ^{b}	35.10	38.63 ^{b}	34.67 ^{a}	32.63 ^{c}	35.31
Mean	35.56	35.93	33.43	34.98	38.75	33.78	34.00	35.51

Among rows means with the same letters indicates no significant difference between treatments at 5% levels of LSD

Table 6. Correlation matrix among the Gas Exchange Parameters and with the weather parameters

	MAXT(^{0}C)	MINT(^{0}C)	RF(mm)	RH(%)	PS	TR	Stomatal	LEAF TEMP	RWC
MAXT(^{0}C)	1								
MINT(^{0}C)	0.438	1							
RF(MM)	-0.5548	0.1176	1						
RH1(%)	-0.5618	0.3389	0.2285	1					
PS	-0.7396	-0.6689	0.3478	0.0602	1				
TR	-0.7416	-0.6749	0.2323	0.0899	0.9485**	1			
SC	-0.7593	-0.0339	0.8626*	0.3728	0.605	0.6039	1		
LEAF TEMP	-0.6517	-0.423	0.4155	0.1658	0.2475	0.3813	0.5114	1	
RWC	-0.6057	-0.3282	-0.0974	-0.8832*	0.1778	0.0564	-0.3965	-0.441	1

significant corelation at 5%* * significant corelation at 1%*

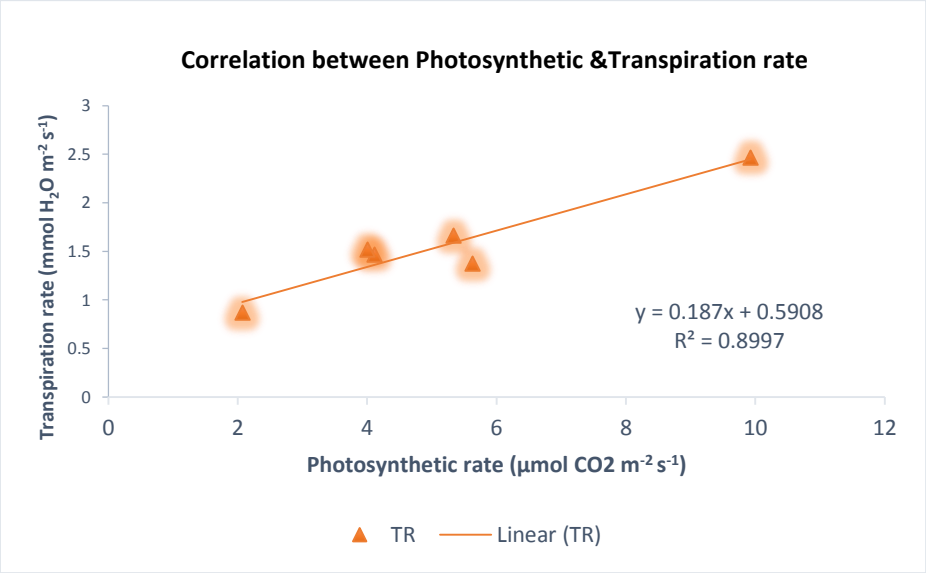

Fig. 3. Correlation between photosynthetic &transpiration rate

study also a positive correlation between photosynthetic rate and stomatal conductance was observed with a correlation coefficient of 0.605 but there was no significance observed at a 5% confidence level. Response of plants to environmental conditions can easily be characterized by the leaf photosynthetic and gas exchange parameters. Photosynthesis depends on the water status of the plants, which affects the metabolic and photochemical reactions of the leaves and indirectly affects the stomatal opening function and both of these characteristics alter the leaf growth and leaf surface area [6].

The results of this study had a positive correlation of 0.89 as the coefficient of determination in linear regression for photosynthetic rate and transpiration rate (Fig 3). The photosynthetic rate was found highly correlated with transpiration rate. When the transpiration rate increases, the gas exchange activities tend to increase but based on the other environmental conditions like water status, temperature, relative humidity and wind speed, the photosynthetic rate may increase or decrease. The same observation was recorded in the present study which was earlier reported by [34].

Stomatal conductance is based on the degree of stomatal opening and closing which can be used as an indicator of plant water status. Reduction in stomatal conductance is the consequence of the decrease in water potential and transpiration. The reduction in water potential induces stomatal closure which leads to a decrease in stomatal conductance [35]. Water stress determines the extent of growth, development and production of the neem trees [7]. Also, a positive correlation between rainfall and stomatal conductance with 0.744 as the coefficient of determination was observed in this study.

4. CONCLUSION

Neem trees growing in two different agro-ecological zones of Tamil Nadu showed significant variation for physiological parameters such as photosynthetic rate, stomatal conductivity and transpiration rate due to the variability observed in prevailing weather parameters. It was observed that the neem trees recorded significantly higher values during these physiological parameters pre-flowering and fruiting stages compared to the flowering stage in both locations. Among the different categories based on DBH, the trees with higher DBH were found to have a higher photosynthetic rate, stomatal conductivity and transpiration rate when compared to the trees with lesser DBH. Also, in this study, a positive relationship was observed among photosynthetic rate, transpiration rate, plant water status and weather parameters. Among weather parameters relative humidity and rainfall influences relative water content and temperature influences the gas exchange activities which favourably influenced the flowering, fruit formation and yield of neem trees in both locations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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