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Research Paper

Weather induced physiological responses on the flowering habits of neem trees (*Azadirachta indica*)

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ABSTRACT

Adaptability and significant economic value of the neem tree are well-known, as it can flourish in a variety of environmental conditions. While the neem seed production is highly sensitive to prevailing weather conditions during the reproductive phase and flowering behaviour of the tree. A study was conducted at Tamil Nadu Agricultural University, Coimbatore in 2023 with the primary objective of validating the weather influence on neem seed production using the logics of physiological responses, as a continuation of research conducted the previous year (2022). During the pre-flowering and flowering stages, diverse weather conditions led to notable changes in the physiological traits of neem trees, which displayed varying patterns of flowering. Trees that flowered consistently showed elevated levels of indole acetic acid (IAA) oxidase, relative water content, and nitrate reductase compared to those that lacked flowers or produced intermittently. In the flowering stage, the neem trees responded positively in terms of physiological aspects like IAA oxidase, relative water content, nitrate reductase, and exhibited lower proline levels, which can be attributed to the optimal maximum temperature, rainfall, and soil moisture. Proline levels rose during the pre-flowering stage due to soil moisture deficits but fell during the flowering stage with the onset of rain. These physiological changes, driven by climatic factors, are likely to enhance the flowering, fruiting, and overall yield of neem trees.

***Keyword**:* Weather, Physiological response, Neem, Flowering habits, Relative water content, IAA oxidase

Neem (*Azadirachta indica A. Juss*.) is an indigenous tree to the Indian subcontinent and characterised by its rapid growth, significant economic worth, and wide range of applications. More than 50% of the world's countries already have neem trees growing there, and the number of neem trees worldwide is steadily rising. Neem is a multipurpose tree, also known as "wonder tree" due to its numerous uses, which have been recognised in India since the Vedic period. Neem trees has showed potential as a natural source of insecticides, pesticides, and agrochemicals, making it valuable in a variety of industries in addition to its therapeutic effects. The neem tree, also referred to as 'Indian lilacs,' is notable for its rapid growth, reaching heights of up to 25 metres and living for more than 200 years (Ragit *et al.,* 2011).

The neem tree demonstrates its extraordinary ability to thrive in a variety of climatic, topographical, and soil conditions, enabling it to withstand extremely high and low temperatures, prolonged periods of drought, and even salinity (Brahmachari, 2004; Vishnu Shankar, 2022). Neem begins reproducing after four to five years of maturity. Typically, the neem tree blossoming cycle begins in March and ends in May, subsequently, fruits ripen from May to August. South India's neem blossoms appear 2 to 5 weeks earlier than North India's (Narong and Sobon, 2014; Prabakaran *et al.,* 2019). Reproductive phase of the neem tree is extremely susceptible to weather conditions, which are influenced by the tree's altered physiology. Relative Water Content (RWC) is an important metric for assessing the water level and overall health of trees. Drought can directly impact RWC and vital activities like photosynthesis, impacting parameters including carbon fixation, stomatal conductance, and photosynthetic pigments (Ashraf and

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Harris, 2013), as well as influencing neem tree flowering.

The plant hormone Indole Acetic Acid (IAA) regulates cell division, elongation, differentiation, root initiation, apical dominance, and responses to diverse stimuli (Gangwar *et al.,* 2011). In many instances, an increase in IAA-oxidase activity has been observed as fruit maturation progresses (Frenkel, 1972). Proline, an amino acid, exhibits a tendency to accumulate at elevated concentrations as a physiological response when plants are exposed to unfavourable conditions such as high soil salinity or limited soil moisture (Yaish, 2015). Nitrate reductase being a key enzyme in the assimilation of nitrogen, has attracted widespread attention both from the fundamental as well as its applied aspects, as applicable to plant productivity (Chacko, 1983). Temperature had a positive effect on nitrate content, yet relative humidity had a negative influence. (San Bautista *et al.,* 2020).

Although there have been anecdotal reports from farmers and academia on the impact of weather parameter on neem growth and yield, there is currently a lack of scientific studies specifically investigating this phenomenon. In this context, a study was undertaken at Tamil Nadu Agricultural University, Coimbatore by Pugazenthi *et al*., (2021). In light of this, the study objective was set to investigate the weather-induced physiological responses in three flowering habituated (flowering, dormant flowering and alternating flowering) neem trees in Tamil Nadu.

MATERIALS AND METHODS

A study was conducted at Tamil Nadu Agricultural University, Coimbatore in 2023 to further investigate the impact of weather conditions on neem seed production. The primary objective of this research was to validate the relationship between weather variability and neem seed production by analysing physiological responses. This study builds upon previous research conducted in the preceding year (2021).

Current study period and location

Study was conducted during January to August 2023 at Tamil Nadu Agricultural University (TNAU) in Coimbatore, situated in the western Agro Climatic Zone of Tamil Nadu. The sampling trees were selected from the 20 hectares around the geographical coordinates of 11.0125°N 76.9714°E to avoid variation in the environment. The reproductive phase of the neem tree is generally start by February and end by July month in the study area and observations were made accordingly. Biometric observation were made at the start of the study and physiological analysis sample collections were made thrice during the pre-flowering (5^{th}, 8^{th} and 12^{th} SMW) and flowering (15^{th}, 19^{th} and 23^{rd} SMW) phases of regular flowering neem trees and the averaged value of that three observations were presented. In dormant and alternate flowering trees, observation had been made at the same time frame as flowering trees. The soils in the area had moderately deep and sandy clay loam in texture with low N, medium P and K.

Weather parameter

Daily weather parameters such as maximum and minimum temperature (°C), rainfall (mm), wind speed (km/h), global solar radiation (cal/cm^2/day) and soil moisture (%) were collected for the study period (February 2023 - June 2023) from the TNAU's principal observatory, Coimbatore (11° N and 77° E), which is situated very nearer to the study area. The maximum and minimum thermometer, ordinary rain gauge (mm), Robinson cup anemometer and Bellani's pyranometer were used to observe the above weather parameters, respectively. The solar radiation values in cal/cm^2/day were converted to watt/m^2. Soil moisture was measured every week at two meters away from the trunk with Delta-T Theta probe with HH2 soil moisture meter. The daily data were converted to Standard Meteorological Week (SMW) and is displayed in Fig. 1.

Pre-flowering phase (5 -14 SMW)***:*** The study location received 17 mm of rainfall and had average soil moisture of 17.8 per cent during the pre-flowering stage. The maximum, minimum and average temperature of study location was 37.1, 21.2 and 30.3°C, respectively. The average solar radiation received at the study location during the pre-flowering phase was 376.6 watt/m^2.

Flowering phase (15 -24 SMW)***:*** The study location received 231 mm of rainfall and had average soil moisture of 25.5 per cent during the flowering stage. The maximum, minimum and average temperature of study location was 38.7, 24.4 and 33.5°C, respectively. The average solar radiation received at the study location during the flowering phase was 354.6 watt/m^2.

Selection of trees

The 20 hectare study area was partitioned into four separate groups: East, West, North, and South and these four groups were treated as replications. In each replication, nine neem trees that had different flowering frequency and Breast Height Diameter (DBH) were chosen as nine treatments for the investigation.

The three flowering frequency types used in this study were regular flowering trees (F1) that blooming every year, dormant flowering trees that had not yet bloomed after six years (F2), and alternate flowering trees that bloomed every other year (F3). The three DBH values considered for the study were >40 cm (D1), 30-40 cm (D2), and <30 cm (D3). In each tree, eight branches had marked as observational points, and the mean values were used to represent the treatment value. Basic growth parameters such as tree height, crown width, and DBH were measured using Blume-leiss Dendrometer which is in medium size and less weight for easy carrying as described by Cailliez and Alder, (1980) and it works on the concept of trigonometric laws to estimate height, as described by Waring and Schlesinger (1985). and standard measuring tapes (Table 1).

Statistical analysis

Experiment was conducted in Factorial Randomized Block Design (FRBD) with four replications (Four directions) and two factors viz., flowering habitat and Diameter at Breast Height (DBH). The field trial had totally nine treatment combination (3 F x 3 D). Analysis of Variance (ANOVA) and correlation between meteorological and physiological parameters were performed using "agricolae" package in "R" program and statistical software "GRAPES" tool (Gopinath *et al.,* 2021).

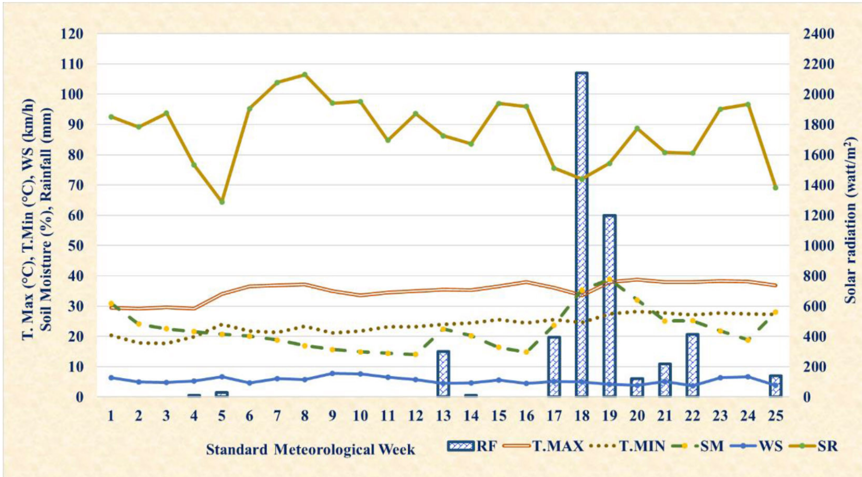

Fig. 1: Weather conditions prevailed during the study period in Coimbatore

Table 1: Biometric observations of selected trees

Factors	D1	D2	D3	Mean	D1	D2	D3	Mean
	Diameter at Breast Height (DBH, cm)				**Trunk height (m)**			
F1	41.8	34.2	26.4	34.1	3.5	3.5	2.5	3.2
F2	42.6	32.4	25.2	33.4	3.5	3.5	2.5	3.2
F3	43.1	32.4	26.1	33.9	3.5	3.5	2.5	3.2
Mean	42.5	33.0	25.9	33.8	3.5	3.5	2.5	3.2
	Crown height (m)				**Tree height (m)**			
F1	11.5	10.0	9.0	10.2	15.0	13.5	11.5	13.3
F2	11.0	9.0	8.5	9.5	`14.5	12.5	11.0	11.8
F3	12.0	10.5	9.5	10.7	15.5	14.0	12.0	13.8
Mean	11.5	9.8	9.0	10.1	15.3	13.3	11.5	13.4

F1 - Regular flowering, F2 - Dormant flowering, F3 - Alternate flowering, D1 - DBH <30 cm, D2 - DBH 30 -40 cm, D3 - DBH >40cm

Relative water content (RWC)

A total of 10 leaf discs, each measuring 1.5 cm^2 were obtained from 3rd leaf of tagged twigs of the neem tree and used to assess the relative water content (RWC). After determining the fresh weight (W), each leaf disc had been immersed separately in deionized water for 4 hours to attain full turgidity, which was maintained at normal ambient temperature and light. After removing the leaf discs from the water, the surface moisture was carefully cleansed using filter paper and each disc was weighed individually to estimate its fully turgid weight (TW). Individual leaf discs were placed in a separate container and dried in an oven at 80°C for 48 hours before being weighed to determine their dry weight (DW). The RWC was calculated and reported in percentage using the equation below (Ahmed *et al.,* 2008).

$$RWC(\%) = \frac{(W-DW)}{TW-DW} * 100$$

where, W = fresh weight (g); TW = turgid weight (g); DW = dry weight (g).

Indole acetic acid (IAA) oxidase activity

IAA oxidase activity in neem leaves was measured using the Parthasarthi *et al.,* (1970) methodology and expressed as moles of unoxidized auxin $g^{-1}h^{-1}$. Neem leaves weighed 2g was macerated in 10 ml of cold distilled water and the extract was filtered, diluted with cold water to a volume of 25 ml, and centrifuged for two minutes. Each one ml of phosphate buffer, IAA solution (100 micrograms/ml solution in 0.1 M sodium bicarbonate), and water were added to the extract, thoroughly mixed and incubated in the dark for 2 hours at 25°C. After the incubation period, the reaction was halted by placing the test tube in boiling water bath for three minutes. The concentration of IAA remaining in the reaction mixture was determined by combining 8 ml ferric chloride-sulfuric acid reagent with two ml reaction mixture and quantifying the resulting colour intensity at 540 nm. The enzyme activity was calculated as the difference between the amount of IAA utilised and that remained.

Proline content

Proline was determined following the methodology used by Bates *et al.,* (1973). About 0.5gm of plant material was homogenised in 10 ml of three per cent aqueous sulfosalicylic acid, and the resulting homogenate was then filtered through Whatman 2 filter paper. Each two ml of filtrate, acid ninhydrin, and glacial acetic acid were added to a test tube, and the reaction was allowed

Table 2: Intra-seasonal variability of weather on the relative water content, indole acetic acid activity, nitrate reductase, and proline content of the neem trees

Factors	Pre-flowering phase				Flowering phase			
	D1	D2	D3	Mean	D1	D2	D3	Mean
				Relative water content (RWC, %)				
F1	73.1	72.6	74.3	73.3	77.0	76.0	77.7	76.9
F2	73.9	67.2	65.2	68.8	76.4	74.7	70.6	73.9
F3	73.2	70.5	68.4	70.9	76.2	74.0	70.1	73.4
Mean	73.4	70.1	69.3	70.9	76.6	74.9	72.8	74.8
	F	D	F X D		F	D	F X D	
CD	0.8	0.8	1.3		1.2	1.2	2.1	
SED	0.4	0.4	0.6		0.6	0.6	1.0	
				IAA oxidase ($g^{-1}h^{-1}$)				
F1	18.8	19.6	20.6	19.7	31.2	32.1	32.8	32.0
F2	11.7	12.9	15.1	13.2	16.9	18.4	26.4	20.6
F3	11.6	12.0	14.8	12.8	17.6	23.6	27.5	22.9
Mean	14.0	14.8	16.8	15.2	21.9	24.7	28.9	25.2
	F	D	F X D		F	D	F X D	
CD	0.2	0.2	0.3		0.5	0.5	0.8	
SED	0.1	0.1	0.2		0.2	0.2	0.4	
				Nitrate reductase (NRASE, 1 µg NO2 $g^{-1}h^{-1}$)				
F1	90.0	100.3	102.7	97.7	92.2	104.7	106.7	101.2
F2	70.0	74.3	87.3	77.2	78.0	81.8	89.5	83.1
F3	61.3	75.3	81.3	72.6	86.0	87.3	87.3	86.9
Mean	73.8	83.3	90.4	82.5	85.4	91.3	94.5	90.4
	F	D	F X D		F	D	F X D	
CD	0.7	0.7	1.3		1.6	1.6	2.8	
SED	0.4	0.4	0.6		0.8	0.8	1.4	
				Proline content (mg g^{-1})				
F1	91.0	83.0	76.0	83.3	81.0	77.0	74.0	77.3
F2	94.0	87.0	79.0	86.7	89.0	70.0	68.0	75.7
F3	110.0	106.0	100.0	105.3	93.0	84.0	80.0	85.7
Mean	98.3	92.0	85.0	91.8	87.7	77.0	74.0	79.6
	F	D	F X D		F	D	F X D	
CD	1.7	1.7	3.0		1.0	1.0	1.8	
SED	0.8	0.8	1.5		0.5	0.5	0.9	

F1 - Regular flowering, F2 - Dormant flowering, F3 - Alternate flowering, D1 - DBH <30cm, D2 - DBH 30 -40 cm, D3 - DBH >40cm

to run for an hour at 100 °C before being stopped in an ice bath. To extract the reaction mixture, four ml of toluene was added, which was then vigorously agitated for 15-20 seconds using a test tube stirrer. The aspirated chromophore containing toluene was warmed to room temperature and read for absorbance at 520 nm, using toluene as blank. The standard curve was employed to calculate the proline concentration and expressed in mg g^{-1}.

Nitrate reductase activity

Nitrate reductase activity in physiologically active leaves was measured using the method developed by Nicholas *et al.,* (1976). The enzyme from the leaves was extracted into the assay media using 500 mg of fresh leaf samples. After 5 minutes in a desiccator, 10 ml of the assay medium (phosphate buffer + KNO3 + isopropanol) was added to the samples, which were then kept in the dark for one hour to react with the assay medium. Following, 1 ml of 1% sulphanilamide and 1 ml of 0.02% NEDH were added to 2 ml of the aliquot. The absorbance was then measured using a spectrophotometer at 540 nm. The NRASE activity was estimated using KNO_2 as the standard and expressed in g NO_2 $g^{-1}h^{-1}$.

RESULTS AND DISCUSSION

Influence of weather on the RWC, IAA oxidase activity, NRASE, and proline content of the neem trees during flowering and pre flowering phases are depicted in Table 2.

Relative water content (RWC)

In regular, dormant and alternate flowering trees, the RWC was 72.6 and 77.7 per cent, 68.4 and 76.2 per cent and 65.2 and 76.4 per cent, respectively during the pre-flowering and flowering phases. Results indicated that the RWC was significantly higher in regular flowering trees and lower in dormant flowering trees during both the pre and flowering phases. Between the stages, the flowering phase had higher RWC than pre-flowering phase invariably in all the three types of trees. There was less soil moisture due to insufficient rainfall during the pre-flowering phase, whereas 231 mm of summer

Table 3: Correlation matrix between weather parameters and physiological during the pre-flowering and flowering phase of neem tree

Parameters	Flowering phase	Tmax	Tmin	SM	Rainfall	S R
RWC	Pre-flowering	0.208	-0.251	0.218	0.271	0.073
	Flowering	0.589	-0.704*	0.391	0.365	0.350
IAA	Pre-flowering	0.217	-0.308	0.399	0.337	0.152
	Flowering	0.316	-0.360	0.589	0.451	0.321
NRASE	Pre-flowering	0.187	-0.359	0.160	0.230	0.156
	Flowering	0.283	-0.453	0.544	0.300	0.151
Proline	Pre-flowering	0.160	0.798**	-0.038	-0.580	0.380
	Flowering	0.148	0.155	-0.255	-0.131	0.103

showers was received during the flowering phase, which resulted in high soil moisture. In addition, the increasing temperature favoured higher RWC during flowering phase through absorption of more water from the soil to meet the evapotranspiration demand. These findings are in line with Sajjadinia *et al.,* (2010) in Pistachio trees, who inferred that the variation in temperature and solar radiation between the pre-flowering and flowering phases may likely to contribute the observed differences in RWC. Pugazenthi *et al.,* (2021) inferred that rainfall during the flowering would increase the RWC of neem trees.

IAA oxidase activity

The IAA oxidases activity was 19.7 and 32.0, 13.2 and 20.6 and 12.8 and 22.9 (µmoles of unoxidized IAA $g^{-1}h^{-1}$) in regular, dormant and alternate flowering trees, respectively during the pre-flowering and flowering phases. Similar to RWC, IAA oxidase activity was significantly higher in regular flowering trees and during flowering phase than other two types and pre – flowering phase. There was no significant difference between alternate flowering and Dormant flowering trees in the IAA oxidase activity. Similar observations were noted in other plants by Frenkel (1972) and Van den Ende *et al.,* (1984) where lower IAA content in pre-flowering than flowering phase. Supatra sen and Mukherji (2009) inferred that the plants that received a sufficient amount of rainfall would show higher IAA oxidase than plants under soil moisture stress. In this study there is significant variations among the treatments due to their reproductive habitats.

Nitrate reductase (NRASE)

The NRASE content was 97.7 and 101.2, 77.2 and 86.9, 72.7 and 83.1 µg NO_2 $g^{-1}h^{-1}$ in regular, dormant and alternate flowering trees, respectively during the pre-flowering and flowering phases. Similar to RWC and IAA oxidase, the NRASE was significantly higher in regular flowering trees and during flowering phase than other two types and pre – flowering phase. With presence of sufficient soil moisture due to rainfall, increasing temperature and solar radiation, the neem trees absorb more soil nitrogen along with transpired water and the NRASE activity was higher during the flowering than pre flowering stage. The increase in nitrate reductase during the flowering phase, then a gradual decline was noted by Franco *et al.,* (1979). Further the increased NRASE activity with increasing temperature was reported by Woodin and Lee, (1987) and Lina Feng *et al.,* (2021). The weather induced increase in IAA oxidase activity of the neem tree during flowering also improved the growth, development and activity of root there by increased nitrogen absorption by the plant lead to high NRASE.

Proline

In general, proline content in neem trees was just contrast to the RWC. The proline content was 83.3 and 77.3, 86.7 and 75.7, 105.3 and 85.7 mg g^{-1} in regular, dormant and alternate flowering trees, respectively during the pre-flowering and flowering phases. The moisture stress during the pre-flowering phase might be the reason for higher proline during pre-flowering phase, while the summer showers supported the flowering phase with sufficient moisture. Among the three flowering types, the regular flowering tree had observed with lowest proline content than non-flowering trees, even they had grown in the same congenial weather. Generally, trees lacking reproduction would exhibit elevated proline levels and ratios compared to those with fruits, a phenomenon also noted by Arias-Sibillotte *et al.,* (2019).

Correlation between weather and physiological responses of neem trees

Correlation analysis between physiological parameters and the weather parameters observed during the pre-flowering and flowering phase were depicted in Table 3. Most of the weather parameters had showed positive correlation with physiological parameters during pre-flowering and flowering stage, except minimum temperature (Tmin) and proline. The maximum temperature (Tmax) and solar radiation showed moderate positive correlation during the pre-flowering and flowering stage with RWC. This may be due to that the prevailed temperature during both the period were well within the cardinal temperatures (18 – 38°C) of the neem tree as found in earlier literatures. Minimum temperature showed moderately negative correlation with IAA, NRASE and significant positive correlation with proline during both pre-flowering and flowering stage. Similar results had reported in many agricultural crops that proline content was high under increasing night temperature (Guna *et al.,* 2022).

Among weather parameters, rainfall positively influences RWC and temperature influences the gas exchange activities which favourably influenced the flowering, fruit formation and yield of neem trees (Pugazenthi *et al*., 2021). Results indicated that the soil moisture (SM) favoured the positive physiological responses that favouring neem tree flowering and reduced the stress inducing proline content. In a combination of optimum maximum temperature, solar radiation and soil moisture, the trees would have positive physiological responses that in neem trees.

CONCLUSION

A variety of climatic conditions prevailed during the pre-flowering and flowering phases, causing significant variations in the physiological parameters of Neem trees with distinct flowering patterns. Regularly flowering neem trees had higher levels of IAA oxidase, relative water content, and nitrate reductase than non-flowering and alternately flowering neem trees. During the flowering phase, neem trees exhibited remarkable positive physiological responses, such as IAA oxidase, relative water content, nitrate reductase, and less proline, as a result of favourable maximum temperature, rainfall, and soil moisture. The proline content increased during the pre-flowering phase as a result of soil moisture stress and decreased during the flowering phase due to rainfall. These climate induced physiological responses can ultimately promote flowering, fruit formation and overall yield of neem trees.

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***Data availability**:* With researcher

Authors contribution: **R. Balasree**: Research work, observation, analysis and writing; **Ga. Dheebakaran**: Guidance, supervision; **A. Senthil**: Guided in physiological analysis; **N.K. Sathyamoorthy**: Guided in weather correlation studies; **P. S. Ganapati**: Statistical analysis; **K. Pugazenthi**: Observation, analysis and article writing.

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