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DIETARY FIBRE LIGNIN, A RESOURCE ALLOCATION WITH RELATION TO LEAF PLASTOCHRON INDEX IN AGROECOLOGICAL STUDIES OF *ALLIUM HOOKERI* THW. ENUM

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Abstract: Dietary fibre lignin, an important secondary metabolite produced by metabolic pathway in plant cell fitting to resource allocation in *Allium hookeri*, a perennial green leafy herbal spice was investigated. The potential of resource allocation with relation to relative growth rate (RGR) and leaf plastochron index (LPI) concerning with phytology, growth and development of agriculturally important and current model on advance production practices for selective cropping scheme of rabi season for two cropping years. The lignin formation begins when RGR decrease with correspondence to maturation of growth and coincidence with growth in height and size approaching to zero RGR. The LPI with relation to lignin indicating the beginning of lignification where LPI value procured equal or greater than 0.50 for 0.25 $mg.g^{-1}$ lignin, greater or equal to 1.51 for 0.249 $mg.g.^{-1}$ lignin, equal or greater 2.5 for 0.256 $mg.g.^{-1}$ lignin in 2017 and LPI greater or equal to 0.60 for 0.232 $mg.g.^{-1}$ lignin; equal or greater in 1.61 for 0.245 $mg.g.^{-1}$ lignin; equal or greater 2.58 for 0.256 $mg.g.^{-1}$ lignin. Resource allocation of lignin coincidence with LPI value greater or equal to 0.50 of plant growth and development in *Allium hookeri*. The established equation from RGR and lignin implies the absence of lignification correspond to RGR 0.251 day^{-3} i.e. 0.083 day^{-1} and 0.259 day^{-3} i.e. 0.086 day^{-1} in 2017 and 2018 cropping years respectively. Ultimately authenticate each additional $mg.g^{-1}$ of lignin in leaves RGR decreases by 0.988 and 0.99 in the 2017 and 2018 respectively. Investigation inferences the lignification is inversely proportional to relative growth rate of the test crop *Allium hookeri*.

***Key words*: Dietary fibre, Lignin, *Allium hookeri*, Perennial, Leaf plastochron index, Relative growth rate.

I.INTRODUCTION

Resource allocation in plant is fundamental to growth, development, yield formation and defenses to abiotic and biotic stress. Resources of both organic i.e. organic carbon e.g. sugars and inorganic resources i.e. mineral ions and water etc. included in respective forms. Normally organic C is initially produced in photosynthetic leaves (source) as sucrose is transported through phloem to non photosynthetic tissues (sinks) for diverse uses. Mineral ions and water are taken up by rocks from soil and transported to the aerial parts through xylem (Christrine and Nathalic Callier, 1996; Ruan et.al., 2013; Bihmidine et.al., 2013).

In plants, sugars allocate from "source" to "sink". Sugar usually produced in greenery plant parts i.e. centre of source such as green leaves and it requires to deliver to different growing parts of the plant via the phloem through translocation, the movement of sugar and other substances like amino acids etc. The point of sugar delivery after resource allocation such as roots, young shoots and developing seeds i.e. the point of centre of sink (Chlon and Bush, 1998).

Lignin, one of the important secondary metabolite produced by the phenylalanine, tyrosine metabolic pathway in plant cells, is the second most profuse biopolymers that accounts for 30% of the organic carbon content in biosphere.

Lignin and its related metabolism play important roles in the growth and development of plants. Lignin, being a complex phenolic polymer, enhances plant cell wall rigidity, hydrophobic properties and promotes mineral transport through the vascular bundles in plants (Schielz et.al., 2014; Barros, 2015). It also protects against pests and pathogens (Ithar et.al., 2007) and lodging resistance and in response to various environmental stresses (Shadle et.al., 2007; Mourn et.al., 2010, Peng et.al., 2014) and in human health effect (Lattimer and Haub, 2010; Liu 2003), in certain cases lignin has biological activity such as anti tumour and effective to colon cancer etc. (Azadfar et.al. 2015; Vinardell, 2017; Young et.al. 2005). Further lignin as component of plant cell wall, it is of great significance to plant growth and environmental adaptability. Lignin is also used as a resource for the field of energy or pharmaceutical industry.

The plastochron index (PI) permits the adjustment of plant development and metabolism for age effects. it inevitably used to demonstrate the rate of net photosynthesis, dark respiration, enzyme production, C_{14} distribution (Dickmann, 1971; Dickson, 1986). Plastochron index (PI) also extended the use of morphological indices to semi deterrent nature species (Hanson et.al., 1986). The PI and LPI values effectively elucidate the expected leaf age (leaf lifespan) and the evolutionary trait of leaf economic spectrum, the scientific variation in leaf appearance rate and other factors other than crop season (Christine et.al., 1996, Naorem et.al., 2018a; 2018b; 2018c; 2018d). Eventhough the experimental work on plastochron index and associative indices in *Allium hookeri* had started [Naorem et.al., 2018(a); (b); (c); (d)] the work on resource allocation was not yet undertaken in depth hence the advance step in resource allocation was investigated in various ways. Henceforth, the present work has been undertaken with the objectives: to determine the effectiveness of the plastochron index (PI), leaf plastochron index (LPI) on the dynamics of leaf appearness, leaf length, relative growth rate (RGR) and lignin formation with respect to growth phases codes in *Allium hookeri* cv. local type and their credible practicability of agroecological farming practices and its impact on yield and yield parameters during rabi season.

II. **MATERIAL AND METHODS**

The investigation work was conducted on farmer's experimental field at Moirangkampu Sajeb Loukol in Imphal East district, Manipur (Latitude $23^0$56'N to $25^0$44'N and $93^0$02' E to $94^0$47'E altitude 790m above the M.S.). Meteorological data were collected from Imphal International Airport, Imphal and ICAR, Lamphelpat, Imphal, the nearest meteorological stations from the experimental field. *Allium hookeri*, local variety, was planted at 1st week of June 2016 for reaching its normal growth. Plots were 1.25×1.25m and planting rate extends 25×25cm for plant to plant and row to row and arranged in a randomized block design.

Twenty (20) plants were randomly marked within each sub plot to record the main leaf number and length. Measurement was taken daily althroughout the investigation period covering leaves directly ranges of development from extremely young to fully mature lamina. The plastochron index and other requirements were computed as per formulae given below.

Consciously, the energy (or strictly power) available to the seedling in a optimal growing environment depends on leaf area. Eventually frame the novel formula following Sibly and Vincent (1997). In a simplified way the leaf area depends on leaf mass is strictly the mass of the part of the leaf that is involved in photosynthesis.

Then it comes

Energy available = A×m ---------------------------------- (1)

where 'A' is a constant of proportionality, 'm' is the leafmass. Ignoring for the moment the cost of maintenance, the energy available is allocated either to leaf growth or to lignification. A fraction 'u' of energy is allocated to leaf growth, and the remainder, 1-u' to lignification. Thus, leaf growth is measured by dm/dt and depends both on the allocable energy and on the fraction allocated to leaf growth. Thus

$dm/dt=u.Am$ --(2)

which gives the exponential growth leaf mass

$m=m_0e^{uAm}$ ---(3)

where m_0 is the leaf mass at time 0. And if m_2 represents the mass of lignin, then allocable energy, "Am" is transfer to leaf growth dm/dt and lignification.

$m_T=m+m_2$ ---(4)

and $dm_2/dt=(1-u)Am\eta_2$--------------------------------------(5)

where η_2 represents the conversion efficiency with which energy is converted to lignin mass. Equation (5) shows that lignin mass grows exponentially, since inserting Eq (3) into Eq (5) we have

$$dm_2/dt = (1-\mu)\, A\, \eta_2 m_0 e^{uAt} \text{ ------------------------------------(6)}$$

which can be integrated to give

$$m_2 = [\{\eta_2(1-u)\}/u\}\, m_0 e^{uAt} \text{ ------------------------------------(7)}$$

Hence, combining Equations (3), (4) and (7) we get

$$m_T = m_0 e^{uAt}\, [\{u{=}\eta_2(1-u)\ \}/u \text{ ---(8)}$$

Equation (8) shows that total plant mass m_T, grows exponentially Relative growth rate (RGR), measured as $1/m_T$, dm_T/dt is given by

$$RGR = 1/m_T\ dm_T/dt = uA \text{ -------------------------------------(9)}$$

Thus, RGR is proportional to the fraction 'u' of resources that are allocated to leaf growth.

Then, from equations (2) (6) (7) and (9) it comes

$$RGR = 1/m_T.dm_T/dt = 1/m\ \ dm/dt = 1/m_2.dm_2/dt \text{ ---------(10)}$$

Equation (10) shows that the relative growth rates of m_1, m_2 and m_T are all equal; that is the relative growth rates of the plant components are all equal and are equal to the relative growth rate of the whole plant.

Equation (9) can thus be rewrite (for the purpose) in terms of the fraction of energy allocated to lignification which it can now write as u_2, so that

$$u_2 = 1-u \text{ ---(11)}$$

and $RGR = A-Au_2$ --- (12)

Thus RGR is negatively proportional to the fraction u_2 of resources allocated to lignification.

Therefore, the regression equation fitted to the plotted against a crude index of lignification x assessed on growth i.e. relative growth rate (RGR) is given as follows-.

$$RGR = A-Bx \text{ --- (13)}$$

where, "x" is the lignifications value. Thus, energy cost (strictly power cost) is measured in units of lost RGR. In other words, energy cost is measured in terms of growth rate that would have resulted if the energy has been allocated to growth.

Determination of Plastochron Index (PI)

The PI was calculated using the formula of Erickson and Michelini (1957)

$$PI = n + (\ln L_n - \ln R)/\ln L_n - \ln L_{n-1} \text{ --------------------------------(14)}$$

where

L_{n+1} was the length (mm) of a leaf or organ just shorter than Rmm

n was the serial number of leaf/ organ for which is being calculated.

L_n was the length of the next leaf that was slightly longer than 'R'mm

R was the reference length of organ or leaf (30mm)

The Leaf Plastochron Index (LPI) was determined by using the approved formula

$$LPI = PI-a \text{ ---(15)}$$

where, "a" was the serial number of the choosen leaf, PI was the plastochron index

Determination of Relative Growth Rate (RGR)

$$\text{Relative Growth Rate (RGR)} = \frac{\text{Growth in given time period}}{\text{Measurement at start of time period}} \times 100$$

$$\therefore RGR = \ \{(H_2-H_1)/H_1\}\times 100 \text{ ---(16)}$$

where, H_1- height at time zero or 1^{st} time

H_2 – height at recording time or 2^{nd} time

RGR – relative growth rate

Qualitative estimation of Lignin

Lignin was quantitatively determined following Holloway et.al. (1977) method. For this Acid detergent fibre solution was prepared by measuring one gram of the dried and ground sample (Ground to pass through a fine approx 355 micron mesh sieve) in a 500 ml round flask, 100 ml of detergent solution and 2 ml of Dekalin are added. The mixture is heated to boiling in 5 or 10 minutes. Adjust the boiling to an even level and reflux for 60 minutes. The mixture is filtered on a weighed sintered glass crucible, washed with 200 ml of hot water then with minimum acetone. Dried for eight hours at 105^0C. Cool in a desiccators then reweighed. Using the acid detergent solution (made by adding 20 g Acetyl trimethylammonium bromide (citrimide) tio 1L in sulphuric acid, Dekalin (decahydronaphthaline), sulphuric acid (72%) specific gravity 1.634. Add 655.9 ml of concentrated sulphuric acid to 4270 ml of distilled water) the acid detergent fibre was determined then the crucible containing the acid detergent fibre was placed to a 50ml beaker. Cover the content of the crucible with 72% H_2SO_4 and stir with a glass rod to break up all the lumps. Fill crucible about half way with acid and stir. Then refill the crucible with 72% H_2SO_4 and stir at hourly intervals as acid drain away. After three hour filter off as much acid as possible with vaccum and wash content with hot water until free from acid. Rinse and remove stirring rod. Dry crucible for eight hours or overnight at 105^0C. Cool in a desiccators and weigh. Ignite crucible for lignin at 55^0C for two hours, cool in desiccators and weigh.

III. RESULTS AND DISCUSSIONS

Table(1) demonstrate the RGR with relation to lignin content in the leaves as the advances of their growth indicating the leaves normally emergence according to the sequences of plastochron index subsequently the lignin content comply with the distribution system of energy available in the plant as energy needed in all developmental activities. Thus in all the tested leaves (L1 to L8) the lignin content begins from 12^{th} to 18^{th} day coincidence with the decrease rate of RGR and adapted to a pattern.

The regression line made between the relative growth rate (RGR) and lignifications index, the equation substantiate to y = 0.251 – 0.988x, for crop season rabi of cropping year 2017 where, y is the relative growth rate (RGR) and x is the lignification index. Similarly the regression equation, y = 0.259 - 0.990x, was established for rabi crop season of cropping year 2018 with usual meaning of symbols. The determined regression equations so establish was presented in graphics {Figure 1(a) and Figure 1(b)}. From the regression line, it is clear that the lignifications inversely proportional to the relative growth rate of the test herbal. When the relative growth rate (RGR) comes to 0.004 the lignifications index was maximum (0.250 $mg.g^{-1}$) in cropping year 2017 and when RGR approach to 0.0016 the lignin achieved 0.26 $mg.g^{-1}$ in cropping year 2018. Further when the RGR increases to 0.053 then the lignigfication index goes to 0.200 in 2017 and RGR of 0.061 struck the lignin 0.20 only in 2018 cropping year. In other words when lignification is of 0.254 $mg.g^{-1}$ then the relative growth rate (RGR) become 0 and the relative growth rate (RGR) becomes zero when the lignifications index goes to 0.254 $mg.g^{-1}$ approximately in regression line of rabi season of cropping year 2017. In the same way in cropping year 2018 the lignification become highest (0.262 $mg.g^{-1}$) when RGR become 0. In other words the highest RGR 0.259 correspond with 0 lignin content in the leave.

Further, the determined line of equation y = 0.251-0.988x reflect the RGR value along with corresponding lignin in $mg.g^{-1}$ extend as 0.053, 0.20; 0.044, 0.21; 0.034, 0.22; 0.024, 0.23; 0.014, 0.240; 0.004, 0.25 {Figure 1(a)} for rabi season of cropping year 2017. Similarly in rabi season of cropping year 2018, the regression line of y=0.259-0.99x accord the RGR value with corresponding lignin in $mg.g^{-1}$ value as 0.061, 0.20; 0.051, 0.21; 0.041, 0.22; 0.031, 0.23; 0.021, 0.24; 0.011, 0.25; 0.0016, 0.26 {Figure 1(b)}. It is evident that the lignin formation in the plant begins when the relative growth rate decreases and attending a maturation of growth to plant which normally coincidence with maximum growth in height and approaching relative growth rate to zero. The present finding from regression line indicates the lignin formation ranged from 0.20 $mg/g/m^{-2}$ when the relative growth rate correspond to 0.053 to 0.25 $mg.g^{-1}$ with corresponding RGR of 0.004 in rabi season of 2017 cropping year {Figure 1(a)} whereas lignin formation ranged from 0.20 $mg.g^{-1}$ with RGR 0.061 to 0.26 $mg.g^{-1}$ to corresponding RGR 0.0016 {Figure 1(b)}. Thus the allocation of resources, disbursement of energy and the rate of growth and development in a plant is compensating under a principle.

Table(1): Leaf Plastochron Index (LPI), Relative Growth Rate (RGR) and Lignin of *Allium hookeri* for Rabi season of cropping year 2017 and 2018.

Sl.No.	RGR	Lignin	Days	LPI		No. of leaves	Remarks
1	0.024	0.230	12	0.50 ≤	≤ 1.50	1-2 (T_1)	Upto L8* (2017)
2	0.009	0.249	15	1.51 ≤	≤ 2.55	1-2 (T_2 +S_1)	
3	0.001	0.250	18	2.56 ≤	---	11-9 (S_1 + S_2)	
1	0.027	0.232	12	0.60 ≤	≤ 1.60	1-2 (T_1)	Upto L8* (2018)
2	0.014	0.245	15	1.61≤	≤ 2.57	1-2 (T_2 +S_1)	
3	0.003	0.256	18	2.58 ≤	---	11-9 (S_1 + S_2)	

T_1-Transition1, T_2-Transition2, S_1-Source1, S_2 – Source2,*neglecting out ranges

Moreover, the determined equation implies that each increase of 0.01 $mg.g^{-1}$ of lignin in leaves of area $1m^2$, RGR decreases by 0.016 day^{-1}. The observed values of RGR varied between 0.001 and 0.024 day^{-3} and those of Lignin between 0.250 to 0.0230 $mg.g^{-1}$ in the test crop for cropping year 2017 whereas in 2018 the observed values of RGR varied between 0.003 to 0.027 day^{-3} and those of lignin between 0.256 to 0.232 $mg.g^{-1}$ (Table 1). Consequently each additional $mg.g^{-1}$ of lignin the RGR decreases by 0.019 day^{-1}. Thus the present finding elucidates the additional $mg.g^{-1}$ of lignin the RGR value decreases by 0.016 to 0.019 day^{-1}. The finding purposively compare with other works. In this regard Grime and Hunt (1975) plotted RGR against crude index of lignifications which assessed on a five point scale. Point 0 on the scale represents no allocation to lignifications and point 5 corresponds to allocation of all resources to lignifications. Consequent to the implication of the equation it is determined that in the absence of lignifications RGR is 1.44 $week^{-1}$ or 0.205day^{-1}on average. For each one point increase in the lignifications index, RGR decreases by 0.20 $week^{-1}$ or 0.028 day^{-1}. The regression equation also shows that RGR would be 0.4/$week^{-1}$ at point 5 on the index of lignifications. Van Arendonk and Poorter (1994) support the prediction by regression analysis of data on 14 grass species. The implication of the constructed equation is that for each additional gram of lignin plus hemicelluloses in leaves of area $1m^2$, RGR decreases by 0.0225 day^{-1}. Further their observed values of RGR varied between 0.11 and 0.27 $days^{-1}$ and those of lignin between 1.93 and 8.44 gm^{-1}.

Furthermore, Table (1) revealed the Leaf Plastochron Index (LPI) with relation to RGR and Lignin indicating the beginning of lignification commence with situation where the LPI procured value equal or greater than (≤) 0.50, and extended to LPI values 1.51 to 2.55 with 0.249 $mg.g^{-1}$ lignin then 0 $mg.g^{-1}$lignin corresponds with LPI value from 2.56 to above, in cropping year 2017 rabi season whereas in rabi season of cropping year 2018, the lignifications of 0.232 $mg.g^{-1}$ lignin coincides with equal or greater than 0.60 LPI to 1.60 LPI then the lignin 0.245 $mg.g^{-1}$ concurred to LPI 1.61 to 2.57 and lignin 0.256 $mg.g^{-1}$ correspond to LPI 2.58 to above (Figure 2). The finding manifest the leaf plastochron index (LPI) legitimately correlate with the lignifications authenticating the energy distribution in the plant body of *Allium hookeri*, a perennial green spicy herbal normally harvested by plucking or removing matured leaves to whole time of the plants life at about 16-18 days old prior to senescence all throughout the year. Consequently add up the applicability of agroecological farming practices to yield and yield parameters. The finding was corroborated as leaves contain much less lignin and the lignin biosynthesis extensively contributes to plant growth tissue and organ development, lodging resistance and responses to variety of biotic and abiotic stresses (Liu, et.al. 2018; Peng, et.al. 2014; Barros, et.al 2015).

Table(2) and Table(3) exhibit the lignin content in the leaves with relation to advances of their growth indicating the leaves normally emerges according to the sequences of plastochron index consequently the lignin content follow up the distribution and allocation through system of energy available in the plant as energy needed in all developmental activities. Thus in all the tested leaves (L_1 to L_8) the lignin content accord from 12^{th} to 18^{th} day at 3 days interval coincidence with the rate of growth rate (RGR) and adopted to a pattern. All the growth phases of the crop coincidence with corresponding meteorological factors for cropping years 2017 Figure (3) and for the cropping year 2018 Figure (4). The findings authenticate the resource lignin allocation in term of energy in growth and development of *Allium hookeri*.

Table (2): Leave emergence, Relative Growth rate (RGR) and corresponding lignin estimation days in the leaves of *Allium hookeri* for Rabi crop season of cropping year 2017.

Date	Leaves							
	L1	L2	L3	L4	L5	L6	L7	L8
5/10/17	*							
12		*						
16 (12^{th} day for L1)	0.025							
19 (15^{th} day for L1)	0.007		*					
22 (18^{th} day for L1)	0.001							
23 (12^{th} day for L2)		0.025						
26 (15^{th} day for L2)		0.009		*				
29 (18^{th} day for L2)		0.002						
30 (12^{th} day for L3)			0.024					
02 (15^{th} day for L3)			0.012		*			
05 (18^{th} day for L3)			0.001					
06 (12^{th} day for L4)				0.026				
09 (15^{th} day for L4)				0.011		*		
12 (18^{th} day for L4)				0.001				
13 (12^{th} day for L5)					0.025			
16 (15^{th} day for L5)					0.014		*	
19 (18^{th} day for L5)					0.002			
20 (12^{th} day for L6)						0.021		
23 (15^{th} day for L6)						0.004		*

26 (18th day for L6)						0.001		
27 (12th day for L7)							0.024	
30 (15th day for L7)							0.012	
3 (18th day for L7)							0.002	
4 (12th day for L8)								0.023
7 (15th day for L8)								0.005
10 (18th day for L8)								0.001

*Days of leaf emergence

Table (3): Leave emergence, Relative Growth rate (RGR) and corresponding lignin estimation days in the leaves of *Allium hookeri* for Rabi crop season of cropping year 2018.

Date	Leaves							
	L1	L2	L3	L4	L5	L6	L7	L8
7/10/17	*							
14		*						
18 (12th day for L1)	0.023							
21 (15th day for L1)	0.010		*					
24 (18th day for L1)	0.002							
25 (12th day for L2)		0.021						
28 (15th day for L2)		0.009		*				
31 (18th day for L2)		0.001						
01/11 (12th day for L3)			0.026					
04 (15th day for L3)			0.010		*			
07 (18th day for L3)			0.002					
08 (12th day for L4)				0.026				
11 (15th day for L4)				0.010		*		
14 (18th day for L4)				0.003				
15 (12th day for L5)					0.025			
18 (15th day for L5)					0.014		*	
21 (18th day for L5)					0.002			
22 (12th day for L6)						0.025		
25 (15th day for L6)						0.016		*
28 (18th day for L6)						0.005		
29 (12th day for L7)							0.033	
2 (15th day for L7)							0.019	
5 (18th day for L7)							0.007	
6 (12th day for L8)								0.031
9 (15th day for L8)								0.022
12(18th day for L8)								0.005

*Days of leaf emergence

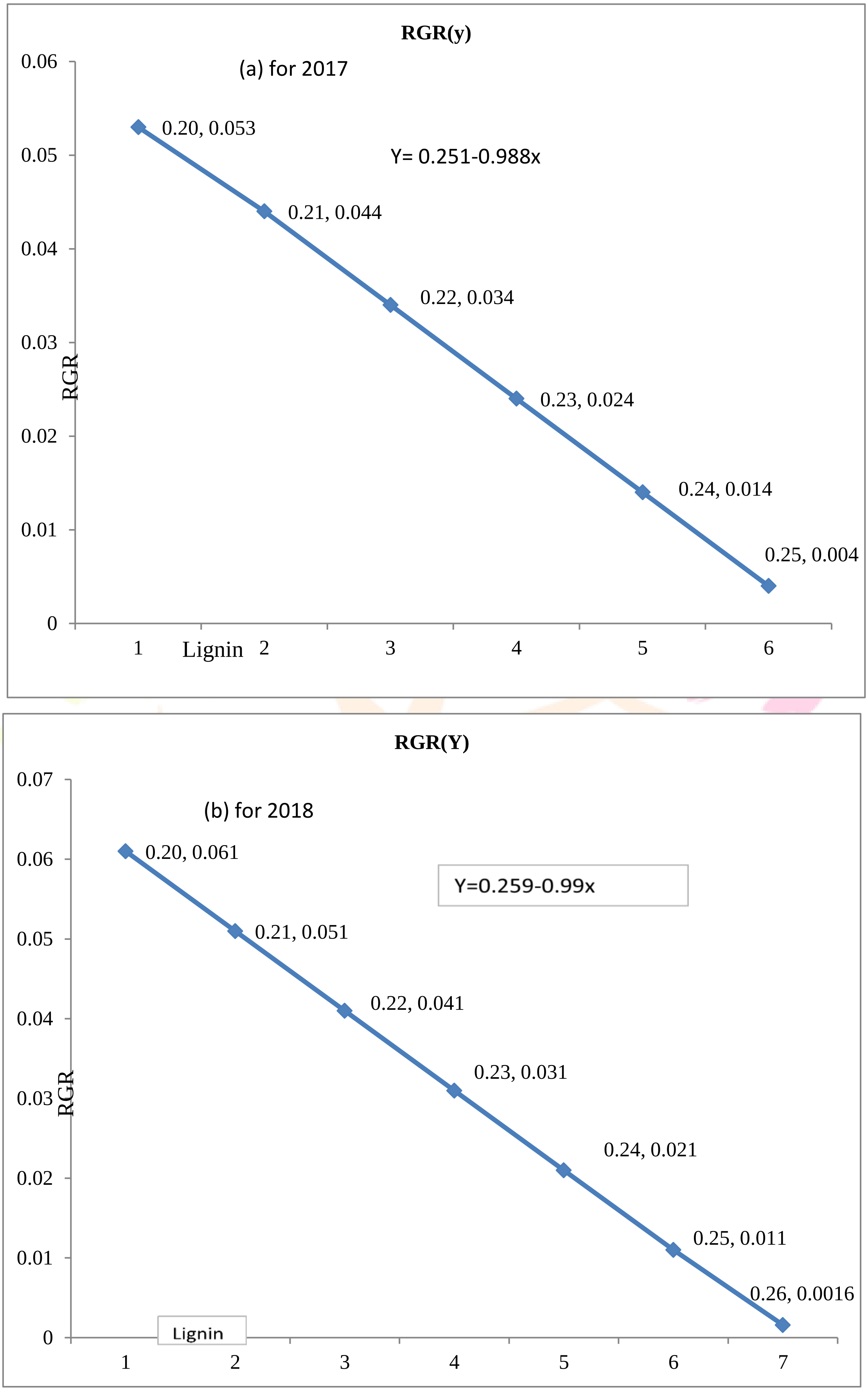

Figure 1. Predicted tradeoff between Relative growth rate (RGR) and lignifications in *Allium hookeri* for the crop season Rabi (a) for cropping year 2017 (b) for cropping year 2018.

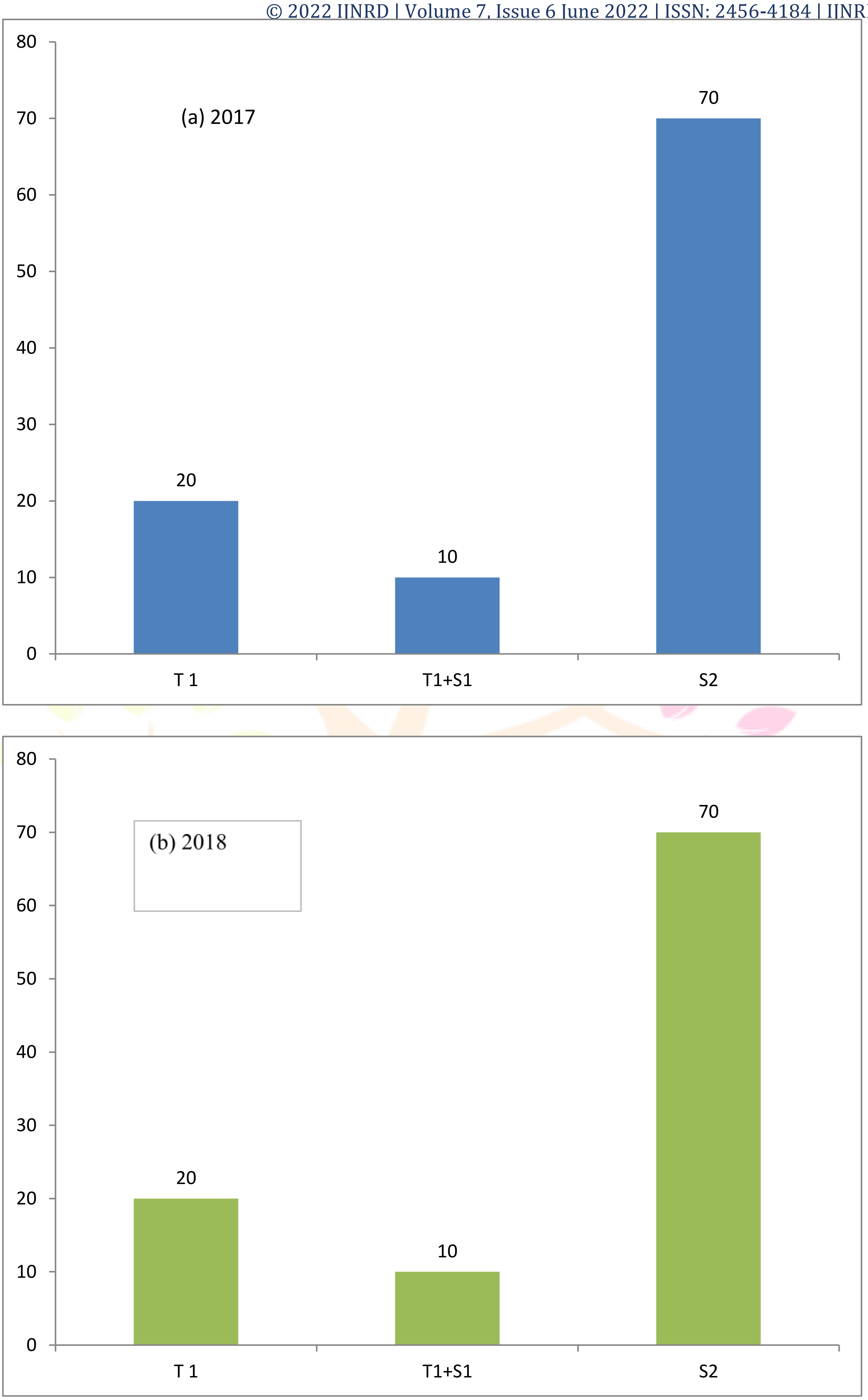

Figure 2. Graphical representation of Transition1 (T_1), Transition 2 (T_2), Source1 (S_1) and Source2 (S_2) ratio for *Allium hookeri* for Rabi season of cropping year (a) 2017 and (b) 2018.

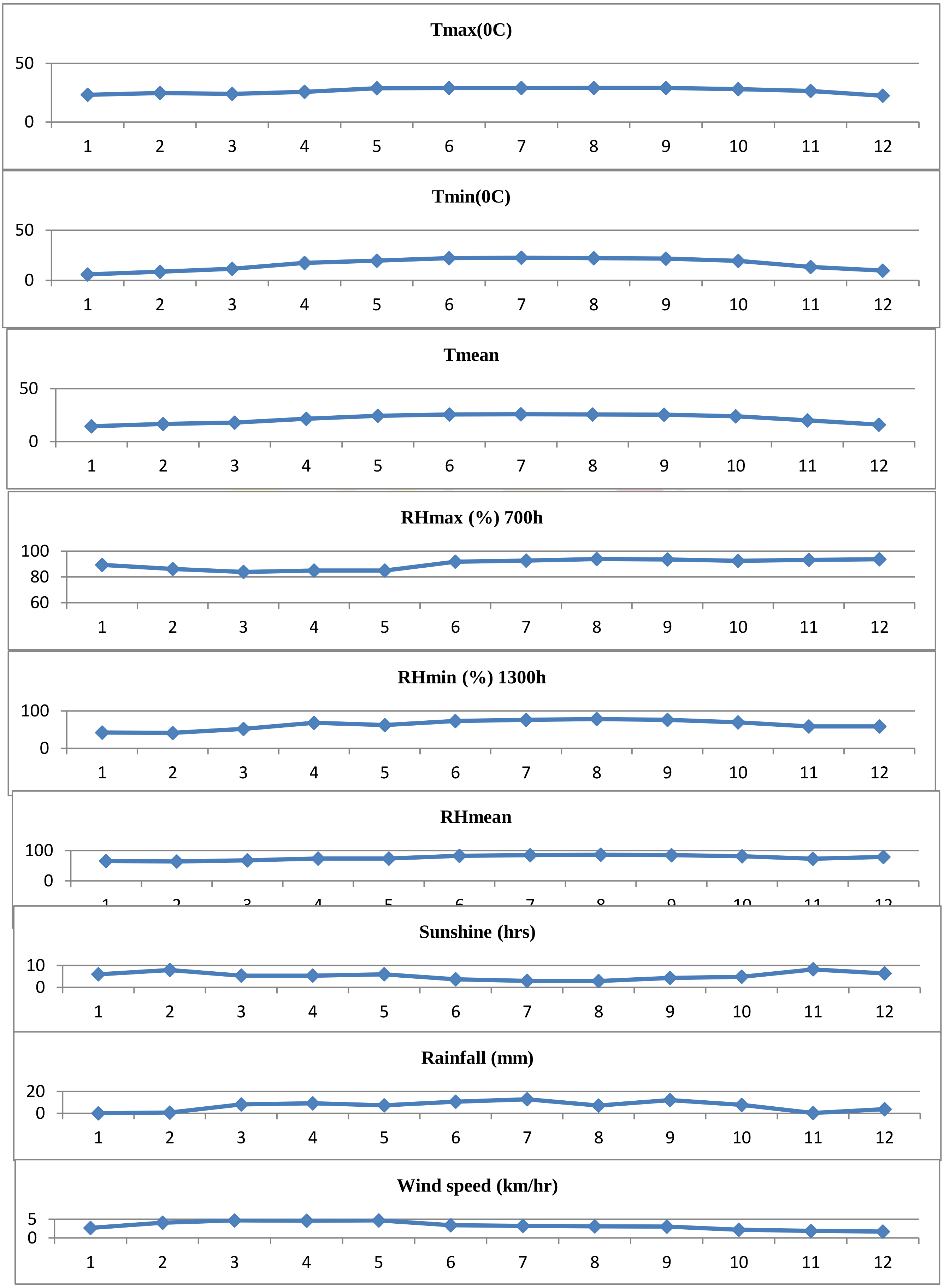

Figure 3: Graphical representation of meteorological data (average), for the year 2017
(Tmax, Tmin, Tmean, RHmax, RHmin, RHmean, Sunshine, Rainfall, Wind speed)

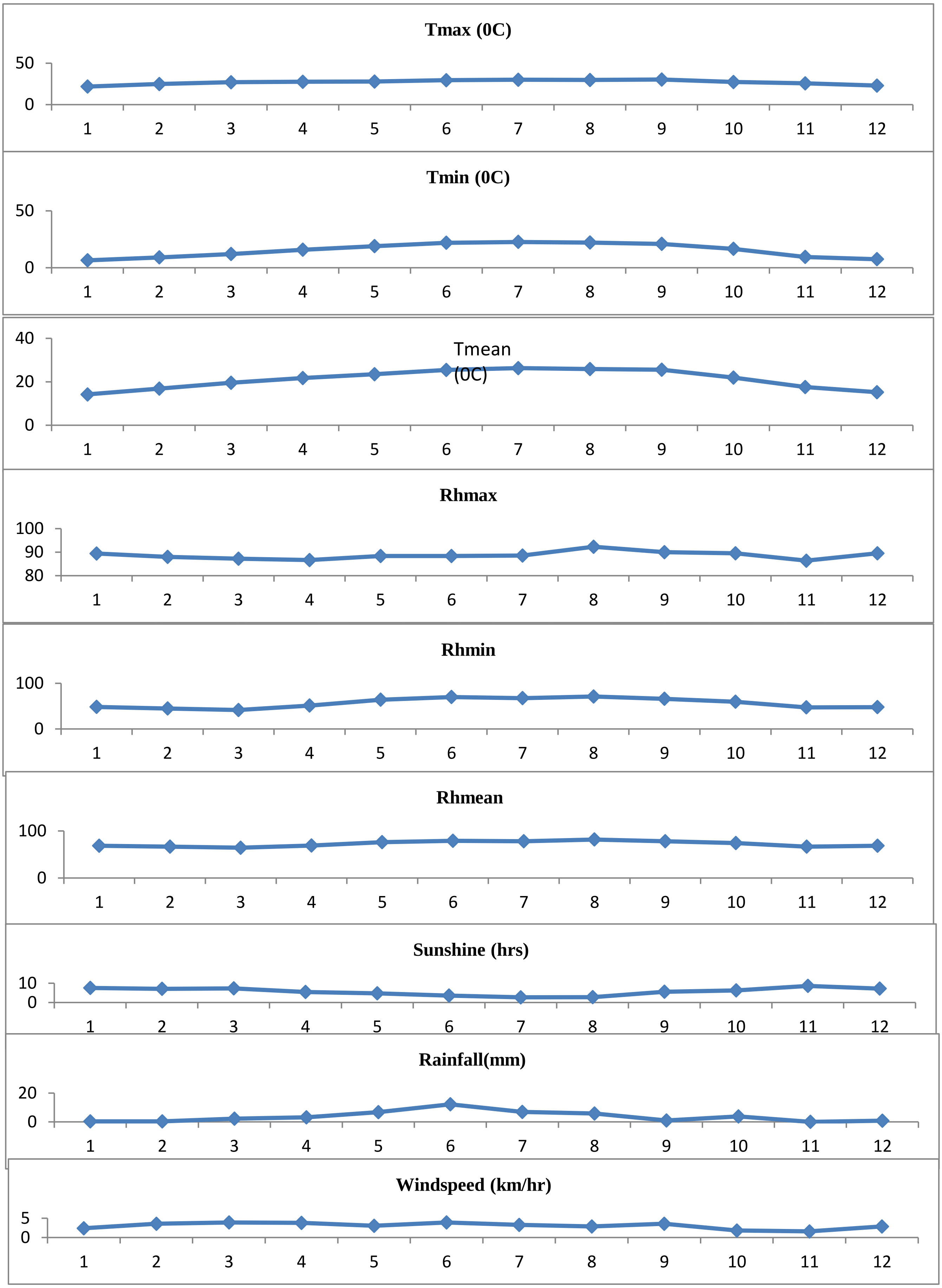

Figure 4: Graphical representation of Meteorological data (average), for the year 2018 (Tmax, Tmin, Tmean, RH max, RHmean, Sunshine, Rainfall, Windspeed).

IV. CONCLUSION

The present work out signifies the energy allocation for growth of emerging young leaves that allocate available energy either to leaf growth or to lignifications. The present findings authenticate the allocation of lignin with relation to RGR and generalized to plastochron index in *Allium hookeri*.

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