IOSR Journal of Polymer and Textile Engineering (IOSR-JPTE)
e-ISSN: 2348-019X, p-ISSN: 2348-0181, Volume 9, Issue 3 (May. – June. 2022), PP 01-09
www.iosrjournals.org

Comparative Study on Egyptian cotton Fiber Maturity Measurements Using Direct and Indirect Methods

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Abstract
The present study was carried out in Cotton Technology Res. Department Cotton Res. Institute, Agric. Res. Center at Giza in 2017 season. Aiming to conduct a comparative study on fiber maturity measurements of the Egyptian cotton using direct and indirect methods. Lint cotton samples of three micronair levels (Mic levels) from six Egyptian cotton genotypes representing ELS, Delta and Upper Egypt LS genotypes were tested by HVI, cutter & causticare and Cross section-Image analysis mothds. The results indicated that mature fibers have bigger hair weight (HW), Perimeter (P), area of cross section (ACW), area of secondary wall(ASCW) and degree of thickening(Ɵ) than low mature fibers; HVI MR was nearly similar to caustic soda MR. HW1, HW2 and HW3 ranked the studied genotypes in the same order. Calculated ASCW and degree of thickening values using1.52 cellulose density was closer to what obtained from Image analysis direct reference method than those calculated using 1.14 cell wall density. Correlation coefficients between cotton fiber maturity and between calculated and measured degree of thickening were highly significant. Regression equations were developed to make corrections to the degree of thickening calculated from Lord equation, Hequet equation, cutter and causticsoda methods, HVI MR and caustic soda MR.

Keyword: *cotton – fiber maturity – comparative study*

Date of Submission: 20-05-2022 Date of Acceptance: 03-06-2022

I. Introduction

Maturity of cotton fibers is associated with the deposition of cellulose in the secondary wall during the end of elongation phase and continues to another 12 to 22 days[1]. Cotton maturity refers to the degree of thickening of the fiber cell wall relative to the perimeter or effective diameter of the fibers[2,3]. It is considered as one of the determinants in evaluating the quality of cotton raw material that directly or indirectly affects physical and mechanical fiber properties, which is desirable and important essential element for cotton growers, breeder and processors to improve their quality control[1,3] .Furthermore,[4,5] added that mature fibers usually possess greater strength and better resilience, while immature cottons result in large processing wastes, lower yarn strength and increase ends down in spinning, besides causing neps that badly affect dye uniformity and show up as white specks in dyed fabrics.

There are a lot of methods to asses' cotton maturity; it can be classified into direct and indirect approaches. Direct methods are relatively reliable but not fast enough while indirect methods are fast but not accurate enough[5]. The indirect methods are based on measurements or observations of some related secondary characteristics, such as the Micronaire instrument[6], differential dye ability[7], causticare test[8], Advanced Fiber Information System (AFIS)[9], double compression air flow test[10], Near Infrared Methods that have been developed and discussed by[11]. However,[3] reported that the lack of standards of reference for maturity has made it impossible to calibrate the existing instruments of air flow instruments with double compression, AFIS and Maturity module in High volume instrument (HVI) that provides an indication of maturity but with certain limitations.[12] Moreover, Micronaire and hair weight measurements alone are not good predictors for fiber maturity as well as its results are a combination of maturity (degree of secondary cell wall thickness) and intrinsic fineness of the fibers which is not practically sufficient and useful for critical evaluations of cotton maturity[13,14] , in addition low micronaire cotton could result from immature fibers or genetically fine fibers and may also indicate fine fibers with adequate maturity, while, a higher micronaire value indicates either coarser fibers or thinner fibers with thick cell walls[15,16], although Micronaire (mic) is being used as the official cotton standard for fiber fineness (H) and maturity (M)[17].

[18]developed the following empirical relationship between micronaire and the product MH, $MH=3.86\times mic^2+18.16\times mic+13$, where H is the linear density, M is the maturity ratio, mic is the micronaire value, thus given that any two of the parameters (fineness, maturity, or micronaire) are known, the third can be determined. Recent work by[19] has suggested that some minor adjustment to this relationship is needed, they proposed the following relationship between the micronaire and the product MH, $MH=39.38$ micronaire-22.67,

where M is the maturity ratio, H is the fineness. [20]proposed that since HW is the amount of cellulose in 1cm = area of cellulose x cellulose density; then area of cellulose (ASCW) = HW / cellulose density. [3]added that the "cut and weigh" method for determining HW is not independent of fiber length because only fibers longer than 1.5 cm are evaluated. Furthermore, [13,16,21]adopted that the degree of wall thickening theta (θ) is defined as the ratio of the cell wall area (Ac) to that of a circle having the same perimeter of the fiber cross section, where A_C is the area of secondary cell wall thickening that calculated from A_C (μm^2) = HW/1.52 g/cm^3 (cellulose density), while[22], indicated that A_C (μm^2) obtained from the previous relationship assumed that the cellulose cell wall density is constant at 1.52 g/cm^3. But in practice the cell wall area is generally the combination of both the primary cell wall and the secondary cell wall), nevertheless the density of the cell wall is only 1.14 g/cm^3. [23,24] mentioned that the effective density required for this equation [A_C (μm^2) = HW/1.52] may be less than 1.52 g/cm^3 particularly for immature fibers. More equations were developed by[3,13,19,20,22,25,26] to be used in the determination of fiber maturity parameters as follows:

HS (hair weight standard) in mtex = (HW*MR)/MR^2 = HW/ MR

ASCW (area of cell wall) (μm^2) = HW /1.52 g/cm^3 (cellulose density)

P (perimeter μm) = 3.7853√ (HS)

D (diameter μm) = P/3.1416, or 1.2047√ (HS)

Degree of thickening Ө = MR* 0.577

Ө = MR* 0.577 + 0.079

Applying the mentioned equations to HVI micronaire value and maturity ratio (MR) can provide any of the needed fiber fineness and maturity parameters separately, which is very important in cotton breeding programs and research when dealing with high number of samples from different cotton genotypes, crosses and varieties since the direct methods are slow and time consuming as aforementioned.

The direct methods are based on microscopic evaluations of some geometric parameters in cross sectional or longitudinal views. [27]reported three of the most significant direct methods include:

1- The caustic soda swelling test, for determining three parameters of cotton fiber maturity coefficient of maturity: percentage of mature fiber and maturity ratio. [28,29,30] stated that the maturity obtained after swelling the fibers in alkali may not represent the true botanical maturity, also[31] have demonstrated that swelling technique will grade the varieties maturity in an order different from what exists before treating with NaoH.

2- Microscopic observation of longitudinal fiber views in polarized light[33].

3- Image analysis and cross-section technique which is acknowledged as a direct reference method for fineness and maturity measurements on cotton. [1,34]stated that the cross section by image analysis can provide accurate and reliable data with a minimum of problems for determining the cotton fiber maturity. [13,35,36] reported that the cross-sectional measurement of cotton fibers is the most accurate technique to determine cotton fibers maturity and can be used as a reference method in order to calibrate other methods, on the other hand [1]stated that in cross sectional approach, the preparation of samples is difficult and need long time to perform microscopic observations, and it is not robust when evaluating extremely immature cottons because the cross-sectioning often breaks the thin walls of dead fibers. Furthermore, [22]added that although the cross-section analysis can provide specific and accurate measurements for maturity, the method is not suitable for routine, high volume testing because it is tedious, time-consuming and costly operations, [3]reported that these direct methods are tedious and too slow to be of practical use in commercial operations or cotton breeding programs. On the other hand, airflow methods are most popular to measure fineness and maturity of cotton fibers, due to the testing speed and the acceptable accuracy level of their measurements in cotton marketing purposes.

The most important maturity measurements in microscopic cross sectional viewing parameter is:

1- The degree of thickening theta (θ) which obtained by the ratio of the cross sectional area of the total fiber wall by the area of a circle of the same perimeter [A (cell wall area) / $(1/2D)^2 \times 3.14$],). [25,37,38] found that mature cottons have average θ excess 0.60 whilst immature cottons have average θ values of less than 0.30.

2- [2,32,38]define circularity as an approximation of the degree of thickening, to show the importance of the wall thickness (cellulose amount), $C= 4\pi A / P^2$, where: P = perimeter of a given fiber cross-section, A = area of the cross-section secondary fiber wall defined by equation, $A=T(P-\pi T) = \pi R^2[1- (r/R)^2]$, where: T = thickness of the secondary fiber wall, R = maximum fiber radius and r = lumen radius,

3- Maturity ratio' is directly proportional to the degree of wall thickening and serves for everyday practical purposes [38,39] ,it is calculated as follows, Maturity ratio (MR) = [N%−D% /200] + 0.7, where N is the percentage of normal fibers (Ө ≥ 0.5); D is the percentage of dead fibers (Ө ≤ 0.25). Cotton with maturity ratio MR closer to unit value is considered mature, whereas cotton with maturity ratio MR in the interval 0.7 to 0.8 are immature, fibers of a MR in the interval 0.8 to 1 are mature, and fibers of a MR > 1 have been very rarely met.

[40]stated that the direct methods for fiber maturity are in general more accurate and precise, and in practice they are used to calibrate or standardize the indirect methods, but are much slower, not practicable routine test and more tedious than indirect methods.

The objective of this research work is studying the effect of micronaire levels (maturity levels), measuring and calculating methods on measured and calculated fiber maturity parameters.

To compare some of maturity parameters obtained and calculated from indirect methods (HVI micronaire and MR data, cellulose density, cell wall density and different modifications in calculating degree of thickening) and direct methods (caustic soda and cutter method) with those corresponding ones obtained from the reference method cross section image analysis technique to clarify their accuracy and reliability to be used in breading programs since they are fast enough, easy to perform and of low cost.

To study the relationships between the measured and calculated parameters of cotton fiber maturity.

II. Material and Methods

The present study was carried out in Cotton Technology Res. Department Cotton Res. Institute, Agric. Res. Center at Giza to conduct comparative study on cotton fiber maturity measurements. Six Egyptian cotton genotypes (five Egyptian cotton varieties and one promising cross), namely Giza 92 and Giza 93 Extra Long Staple cottons (ELS), Giza 94 and Giza97 Delta Long Staple cottons (Delta LS), Giza 95 and the promising cross (G.90 X CB58) Upper Egypt Long Stable cottons (Upper Egypt LS) were used in this study. The lint cotton samples of these cottons were selected from the yield trials included in the breeding and maintenance of varieties genetic purity programs of Cotton Research Institute delivered to High Volume Instrument (HVI) lab, Cotton Fiber Res. Section, Cotton Res. Institute in 2017 season. All the cotton samples were homogenized, conditioned and tested under standard temperature 20±2ºC and relative humidity 65 ± 2% RH, as specified by[41]. HVI Spectrum II was employed for testing these samples according to[42]. Based on HVI micronaire values, the different samples of each variety were divided into three levels of micronaire (three levels of maturity), each level of maturity within each genotype was represented by three repetitions. The obtained data of HVI micronaire and maturity ratio (MR) was computed to calculate the product of fineness and maturity (HW*MR) from the relationship: $HW*MR_1 = 3.86(Mic)^2 + 18.16(Mic) + 13.0$. Caustic soda (NaoH 18%) and cut and weight methods (direct methods) were used to determine direct values of MR and HW according to British Standard Methods,[29,43]. All the obtained HW and MR values were used to calculate hair weight standard (HS). Applying the following equations to these parameters provided calculated fiber perimeter (P), diameter (D), area of a circle having the same fiber perimeter (ACW) and area of secondary wall (ASCW) to be used to calculate the degree of thickening as accurate parameters of fiber maturity:

a- Degree of thickening (ϴ) = ASCW/ACW

To calculate ASCW, the formula proposed by[20] was applied as follows:

HW (hair weight) = weight of cellulose in 1cm = ASCW x 1 x Cellulose density

ASCW = HW / cellulose density

HW was divided once by 1.52 cellulose density according to Hequet et al. and second by 1.14 (fiber wall density) according to the modification made by[23,44]

HS (hair weight standard) in mtex = $(H*MR)/MR^2 = HW/MR$

ASCW (area of cell wall) (μm^2) = HW/1.52 g/cm^3 (cellulose density)

ASCW (area of cell wall) (μm^2) = HW/1.14 g/cm^3 (cell wall density)

P (perimeter µm) = $3.7853\sqrt{(HS)}$

D (diameter µm) = P/3.1416, or $1.2047\sqrt{(HS)}$

ACW = area of a circle having the cross section perimeter = $1/2\ D^2 \times 3.14 = (1/2 \times 1.2047\sqrt{HS})^2 \times 3.14$

The direct methods for measuring fiber maturity included caustic soda method in which a sample of cotton is swelled in 18% caustic soda (NaoH) and then examined under the microscope. The fibers were classified into three groups according to their appearance depending on cell wall degree of thickening:

a- Normal fibers that appear as solid and shown no continue lumen after swelling.
b- Dead fibers that have continue lumen and the wall thickness is a fifth of ribbon width.
c- Thin walled fibers that are not classed as normal or dead.

According to[29], cotton fiber maturity ratio (MR) is expressed as the average percentage of normal (N %) and dead (D %) and calculated from the equation: MR=[N% -D%/200]+0.7.

Maturity ratio MR was converted to the average degree of thickening (ϴ) = MR× 0.577). Moreover maturity ratio (MR) and gravimetric fineness (HW) by cut and weight method[43], were used to demonstrate perimeter, area of secondary wall ($ASCW_3$), to calculate the degree of thickening.

According to the American way of measuring maturity[33] cotton fibers are assigned to just two classes, mature and immature, and the results is expressed as percentage of mature fibers or maturity percent (M%)

M%= [number of mature fibers/ total number of fibers] ×100

Cross sections were prepared from the three samples of each micronaire level within each genotype to be tested by image analysis direct method. The procedure was conducted as explained and used by[45] at the lab of Fiber Chemical and Structural Properties, Fiber Res. Section Cotton Res Institute to determine fiber perimeter

(P), diameter (D), area of the circle having the same fiber perimeter ACW, area of fiber cross section (ACS), Lumen area (AL) and area of secondary cell wall (ASCW) which equal: ACS - AL.

The experimental design used was complete randomized design with three replicates the obtained data were computed using SAS program. Analysis of variance and LSD 5 % test, outlined by[46] were employed to study the effect of Mic levels, cotton genotypes and measuring and calculating methods on the measured and calculated values of fiber maturity parameters. Regression and correlation analysis was used to study the relationship between the calculated and measured parameters of maturity. Each replicate was represented by two sub samples for testing. The results of the two sub samples were averaged to be used in the Analysis of variance the number of observation used is 54. Aiming to the calculation of correlation coefficients and regression equations according to[46]. The values of the six samples of each replicate for the three levels of Mic (maturity) within each cotton genotype were averaged to be used to calculate correlation coefficients and regression equations. The number of observations used is 18 for each trait (3 mic levels * 6 genotypes).

III. Results and Discussion

Effect of micronaire levels, cotton genotypes, measuring and calculating methods on MR, M%, HW, Hs and ACW values:

Data in Table `1 indicated that cotton varieties, micronaire levels, measuring and calculating methods exhibited significant differences in the measured and calculated MR, M%, HW, Hs and ACW in most cases. The cotton genotypes showed insignificant differences in their means of maturity ratio whether obtained from HVI or measured by caustic soda direct method. Both methods showed nearly equal values of MR in the three levels of maturity to be low in low Mic level and goes up in the normal and high Mic levels being 0.76, 0.89 and 0.97 for HVI MR and 0.75, 0.90 and 0.98 for caustic soda MR. Furthermore, maturity % (M%) measured by caustic soda 18% direct method showed the same trend of MR to be of insignificant differences between cotton genotypes and averaged 75.7%, 81.1% and 87.2% for the three levels of Mic respectively, while the corresponding values of micronaire averaged 2.94, 4.01 and 4.59. It is clear that HVI MR values match well Caustic soda MR values and M% showed the same trend in most of the studied cotton genotypes.

Hair weight (HW) which is the weight of cellulose deposited in the fiber wall. It is a measure of fiber gravimetric fineness affected so much by maturity. HW showed very big differences between both of HW1 values calculated from applying[18] equation and (HW2) calculated from[19] equation to the HVI Mic and MR values and (HW3) obtained from cut and weight direct method. HW1 values were very high compared to HW2 and HW3 measured by the cut and weight method, while the values of HW2 and HW3 are generally closer especially in the high maturity level.

Table (1): Mic values, MR, HW, Hs, P and ACW of some Egyptian cotton Genotypes measured and calculated from HVI, cutter and caustic soda methods

		Indirect method (HVI MIC & MR)										Direct method							
		HVI data		Data calculated from Lord equation				Data calculated from Hequet equation				Caustic soda & Cutter method						Cross section data	
COTTONS	Mic levels	Mic levels	MR	HW1	HS1	P1 μ	ACW1 μ²	HW2	HS2	P2 μ	ACW2 μ²	MR	M%	HW3	HS3	P3 μ	ACW3 μ²	P4 μ	ACW4 μ²
Giza 92	Low	2.80	0.78	120.7	154.7	47.1	176.3	112.3	144.0	45.4	164.1	0.77	76.6	110.7	143.7	43.0	164.2	44.6	158.4
	Normal	3.36	0.88	133.8	152.1	46.7	173.4	124.7	141.8	45.1	161.6	0.90	80.7	130.6	145.1	45.6	165.5	45.3	163.4
	High	4.18	0.96	163.1	169.9	49.3	193.6	147.9	154.0	47.0	175.6	0.97	87.6	143.3	147.7	46.0	168.5	46.1	169.2
mean		3.45	0.87	140.6	161.0	47.7	181.1	129.5	148.3	45.8	167.1	0.88	81.8	130.9	148.7	46.2	169.7	45.3	163.7
Giza 93	Low	2.17	0.76	92.9	122.2	41.8	139.3	82.6	108.7	39.5	123.9	0.79	75.3	91.8	116.2	40.8	132.6	43.1	147.9
	Normal	2.93	0.88	112.9	128.3	42.9	146.3	105.4	119.7	41.4	136.5	0.89	81.5	115.0	129.2	43.0	147.4	43.6	151.4
	High	3.30	0.93	122.5	131.7	43.4	150.2	115.4	124.0	42.2	141.4	0.95	87.2	128.5	135.3	44.0	154.3	43.8	152.7
. mean		2.80	0.86	110.5	128.9	42.7	145.3	102.2	119.4	41.0	133.9	0.88	81.5	111.8	127.5	42.7	145.4	43.5	150.7
Giza 94	Low	2.97	0.78	129.3	165.8	48.7	189.0	120.7	154.8	47.1	176.4	0.74	74.9	129.1	174.5	50.0	199.0	46.1	169.2
	Normal	4.29	0.91	178.0	195.6	52.9	222.9	160.7	176.6	50.3	201.4	0.90	80.9	152.3	169.2	49.2	193.0	46.3	170.7
	High	4.55	0.96	183.0	190.7	52.3	217.4	163.2	170.0	49.3	193.8	0.95	86.5	165.4	174.1	49.9	198.6	46.6	172.9
mean		3.94	0.88	165.5	187.3	51.3	209.8	149.8	169.6	48.9	190.5	0.86	81.3	148.9	172.5	49.7	196.8	46.3	170.9
Giza 97	Low	3.47	0.78	156.9	201.1	53.7	229.2	146.0	187.1	51.8	213.3	0.77	76.2	134.3	174.4	50.0	199.0	51.6	212.0
	Normal	4.34	0.89	184.9	207.7	54.6	236.8	166.6	187.1	51.8	213.4	0.91	80.7	159.2	174.9	50.1	199.6	52.4	218.6
	High	4.92	0.97	201.8	208.1	54.6	237.2	176.4	181.8	51.0	207.3	0.98	87.2	172.0	175.5	50.1	200.2	52.6	220.3
. mean		4.24	0.88	182.8	207.8	54.3	234.4	164.1	186.5	51.5	211.3	0.89	81.5	155.2	175.0	50.1	199.6	52.2	217.0
Giza 95	Low	2.85	0.70	137.3	196.1	53.0	223.6	127.9	182.8	51.2	208.4	0.74	74.9	130.4	176.2	50.2	201.0	52.5	219.4
	Normal	4.55	0.88	199.5	226.7	57.0	258.4	177.9	202.1	53.8	230.4	0.92	81.8	164.6	178.9	50.6	204.1	52.8	222.2
	High	5.16	0.98	213.8	218.1	55.9	248.7	184.2	188.0	51.9	214.3	1.00	87.2	178.3	178.3	50.5	203.4	53.1	224.5
. mean		4.19	0.85	187.9	220.2	55.3	243.6	166.6	195.3	52.3	217.7	0.89	81.3	157.8	177.9	50.5	203.0	52.8	222.0
G.90 X CB58	Low	3.38	0.78	151.7	194.5	52.8	221.7	141.4	181.3	51.0	206.7	0.70	76.2	136.3	194.7	52.8	222.1	53.2	225.3
	Normal	4.60	0.89	200.4	225.2	56.8	256.8	178.2	200.2	53.6	228.3	0.93	81.1	164.0	176.3	50.3	201.2	53.6	228.7
	High	5.43	1.00	225.6	225.6	56.9	257.2	191.3	191.3	52.4	218.1	1.01	87.2	178.6	176.8	50.3	201.7	54.1	233.0
. mean		4.47	0.89	195.6	219.8	55.5	245.2	172.4	193.7	52.7	220.8	0.88	81.7	159.6	181.4	51.0	206.9	53.7	229.0
Mic levels (Maturity)	Low	2.9	0.76	131.5	172.4	49.5	196.5	121.8	159.8	47.7	182.1	0.75	75.7	122.1	163.3	47.8	186.3	48.5	188.7
	Normal	4.0	0.89	168.3	189.3	51.8	215.8	152.3	171.3	49.3	195.3	0.91	81.1	147.6	162.3	48.1	185.1	49.0	192.5
	High	4.6	0.97	185.0	190.7	52.1	217.4	163.1	168.2	49.0	191.8	0.98	87.2	161.0	164.6	48.5	187.8	49.4	195.4
Genotypes mean		3.8	0.87	163.8	187.5	51.1	209.9	147.4	168.8	48.7	190.2	0.88	81.5	144.0	163.8	48.4	186.9	49.0	192.2

L**.S.D. at 5% level of significance**

	MR	HW	M%	Hs	P	ACS
L.S.D Mic level (m)	0.03	4.1	2.0	4.3	0.7	1.9
L.S.D. variety (v)	0.03	4.3	2.3	4.4	0.7	2.1
L.S.D. measuring methods (th)	0.04	4.8	2.5	4.7	0.8	2.3
L.S.D. m × v	0.05	5.1	2.8	5.3	0.9	2.7
L.S.D. m × th	0.05	5.3	2.8	5.5	1.2	2.9
L.S.D. v × th	0.05	5.5	3.0	5.7	1.3	2.9
L.S.D. m × v × th	0.06	6.2	3.5	6.5	I.5	3.6

The recorded averages were; 163.8, 147.4, 144.0 m/tex for HW1, HW2 and HW3 respectively. Moreover, HW1 averaged 131.8, 168.2 and 185.0 m/tex in the low, normal and high mic (maturity) levels, while HW2 averaged 121.8, 152.3 and 163.2 m/tex in the mentioned three Mic levels and HW3 averaged 122.1, 147.6 and 161.0 m/tex for the three maturity levels respectively. It is clear from these results that HW2 and HW3 averages did not differ significantly and nearly similar in the low and high maturity levels. It is worthy to report that the values of HW1, HW2 and HW3 ranked the studied genotypes in the same order according to their means of HW being Giza 93, Giza 92, Giza 94, G.97, G95 and G.90xCB58.

Regarding the standard hair weight (Hs) which expresses the hair weight without the effect of maturity. Data in Table 1 indicated that Hs exhibited the same trend of HW being higher in Hs1 than Hs2 and Hs3; moreover, the calculated Hs values showed more fluctuation between maturity levels when calculated from the two equations than noticed in Hs3 obtained from cut and weight and caustic soda direct methods. The recorded averages were; 187.3, 168.8, and 163.8 m/tex for Hs1, Hs2 and Hs3 respectively. The differences and fluctuations in HW and Hs values will be reflected on and affect the calculated values of fiber perimeter (P), diameter (D) and area of the circle having the same perimeter of the fiber (ACW), subsequently the parameters calculated from them.

Using Hs values to calculate fiber perimeter μm $P = 3.7853\sqrt{(HS)}$ to calculate the diameter $D = P / 3.14$ and the area of a circle having the same perimeter of the fiber cross section $ACW = 3.14 \times (1/2\ D)^2$, from the two equations that used HVI Mic and MR (ACW1 And ACW2) in addition to that obtained from the cutter & caustic soda direct method (ACW3) compared to ACW4 calculated from the actual perimeter measured by the cross section image analysis direct reference method. The results in Table 1 cleared that the average of fiber perimeter obtained from Lord equation (P1) was higher than P2 from Hequet equation and P3 from cutter and caustic soda method and P4 measured by cross section image analysis direct reference method. P2, P3 and P4 showed practically closer means in all the studied genotypes. The recorded means were; 51.4, 48.7, 48.4 and 48.9 **μ** for P1, P2, P3 and P4 respectively, furthermore, the low maturity level showed lower values of perimeter compared to those of the middle (normal) and high maturity levels. The increase in fiber perimeter due to maturity could be explained by the pressure of the deposited cellulose layers during fiber maturity on the fiber primary wall forcing it to expand and increasing fiber perimeter. P1, P2, P3 and P4 ranked the studied genotypes in the same order to be Giza 93, Giza 92, Giza 94,G.97, G95 and G.90xCB58 This rank is controlled by the intrinsic fineness of these genotypes which controlled mainly by its genetic structure. These results agreed with[30,47,48,49].

Concerning ACW [$3.14 \times (1/2\ D)^2$], which is the area of the circle having the same perimeter of the fiber, the results in Table 1 indicated that means of ACW2, ACW3 and ACW4 were nearly close while ACW1 was significantly of higher mean than them, the recorded means were: 209.9, 190.2, 186.9 and 192.2 μ^2 respectively. This trend is expected since these areas are calculated from different Hs values and Hs1was higher than Hs2 and Hs3 while ACW4 was calculated from the perimeter of the cross section measured by image analysis direct method. All the calculated and measured ACW means ranked the studied genotypes similarly and similar to the rank according to HW and perimeter. However, the differences between measured and calculated ACW values differed from one genotype to another as well as from micronaire level to another. It is worthy report that ACW1, ACW2, ACW3 and ACW4 are expected to be larger than the actual fiber cross section area, which is not a circle, and its circularity is affected so much by cellulose deposition (maturity). [47,48,49]came to similar conclusions.

Effect of micronaire levels, genotypes, measuring and calculating methods on ASCW and degree of thickening:

In regard to Area of secondary cell wall ASCW calculated from dividing HW by cellulose density 1.52 g/cm^3 and by cell wall density 1.14 g/cm^3 the results in Table 2 showed that ASCW differed significantly between the different genotypes according to their maturity and differed between the three levels of maturity as well. ASCW ranked the genotypes by the same order of maturity ratio and HW which is logic since it is calculated from these parameters. The low Mic level (low maturity level) showed the lowest ASCW mean followed by the middle level whilst the high maturity level showed the highest ASCW regardless the cotton genotype and the calculation and determination method, for instance, the recorded means of ASCW4 were 89.7, 107.6 and 114.1 μ^2 for the three levels of maturity respectively.

Table(2): ASCW, and degree of thickening of some Egyptian cotton genotypes calculated from indirect methods and measured by direct methods.

		Indirect method (HVI MIC & MR)														Direct method	
		Data calculated from Lord equation				Data calculated from Hequet equation				Caustic soda & Cutter method						Cross section data	
COTTONS	Mic levels	ASCW 1a μ²	Degree of thickening 1a	ASCW 1b μ²	Degree of thickening 1b	ASCW 2a μ²	Degree of thickening 2a	ASCW 2b μ²	Degree of thickening 2b	ASCW 3a μ²	Degree of thickening 3a	ASCW 3b μ²	Degree of thickening 3b	Θ = MR*0.577 From HVI	Θ = MR*0.577 Caustic soda method	ASCW4 μ²	Degree of thickening4
Giza 92	Low	79.4	0.45	105.8	0.60	73.9	0.45	98.5	0.60	78.1	0.44	104.1	0.59	0.45	0.44	80.1	0.51
	Normal	88.0	0.51	117.4	0.68	82.1	0.51	109.4	0.68	85.9	0.52	114.6	0.69	0.51	0.52	91.6	0.56
	High	107.3	0.55	143.0	0.74	97.3	0.55	129.7	0.74	94.3	0.56	125.7	0.75	0.55	0.56	99.1	0.59
mean		91.6	0.50	122.1	0.67	84.4	0.50	112.5	0.67	86.1	0.51	114.8	0.68	0.50	0.51	90.3	0.55
Giza 93	Low	61.1	0.44	81.5	0.58	54.3	0.44	72.5	0.58	60.4	0.46	80.5	0.61	0.44	0.46	70.0	0.47
	Normal	74.3	0.51	99.0	0.68	69.3	0.51	92.4	0.68	75.7	0.51	100.9	0.68	0.51	0.51	83.1	0.55
	High	80.6	0.54	107.5	0.72	75.9	0.54	101.2	0.72	84.5	0.55	112.7	0.73	0.54	0.55	86.9	0.57
. mean		72.0	0.49	96.0	0.66	66.5	0.49	88.7	0.66	73.5	0.51	98.0	0.67	0.49	0.51	80.0	0.53
Giza 94	Low	85.1	0.45	113.4	0.60	79.4	0.45	105.9	0.60	84.9	0.43	113.2	0.57	0.45	0.43	82.2	0.49
	Normal	117.1	0.53	156.1	0.70	105.7	0.53	141.0	0.70	100.2	0.52	133.6	0.69	0.53	0.52	99.5	0.58
	High	120.4	0.55	160.6	0.74	107.3	0.55	143.1	0.74	108.8	0.55	145.1	0.73	0.55	0.55	106.1	0.61
mean		107.5	0.51	143.4	0.68	97.5	0.51	130.0	0.68	98.0	0.50	130.6	0.66	0.51	0.50	95.9	0.56
Giza 97	Low	103.2	0.45	137.6	0.60	96.0	0.45	128.0	0.60	88.4	0.44	117.8	0.59	0.45	0.44	98.3	0.46
	Normal	121.6	0.51	162.2	0.68	109.6	0.51	146.1	0.68	104.7	0.52	139.6	0.70	0.51	0.53	120.7	0.55
	High	132.8	0.56	177.1	0.75	116.0	0.56	154.7	0.75	113.2	0.57	150.9	0.75	0.56	0.57	131.9	0.60
. mean		119.2	0.51	158.9	0.68	107.2	0.51	143.0	0.68	102.1	0.51	136.1	0.68	0.51	0.51	117.0	0.54
Giza 95	Low	90.3	0.40	120.4	0.54	84.2	0.40	112.2	0.54	85.8	0.43	114.4	0.57	0.40	0.43	104.1	0.47
	Normal	131.2	0.51	175.0	0.68	117.0	0.51	156.0	0.68	108.3	0.53	144.4	0.71	0.51	0.53	125.6	0.57
	High	140.6	0.57	187.5	0.75	121.2	0.57	161.6	0.75	117.3	0.58	156.4	0.77	0.57	0.58	129.6	0.58
. mean		120.7	0.49	161.0	0.66	107.5	0.49	143.3	0.66	103.8	0.51	138.4	0.68	0.49	0.51	119.8	0.54
G.90xCB58	Low	99.8	0.45	133.1	0.60	93.0	0.45	124.0	0.60	89.7	0.40	119.6	0.54	0.45	0.40	103.4	0.46
	Normal	131.9	0.51	175.8	0.68	117.2	0.51	156.3	0.68	107.9	0.54	143.9	0.72	0.51	0.54	124.8	0.55
	High	148.4	0.58	197.9	0.77	125.9	0.58	167.8	0.77	117.5	0.58	156.7	0.78	0.58	0.58	130.7	0.56
. mean		126.7	0.51	168.9	0.68	113.4	0.51	151.2	0.68	105.0	0.51	140.0	0.68	0.51	0.51	119.6	0.52
Mic levels (Maturity)	Low	86.5	0.44	115.3	0.59	80.1	0.44	106.9	0.59	81.2	0.43	108.3	0.58	0.44	0.43	89.7	0.48
	Normal	110.7	0.51	147.6	0.68	100.2	0.51	133.5	0.68	97.1	0.52	129.5	0.70	0.51	0.53	107.6	0.56
	High	121.7	0.56	162.3	0.75	107.3	0.56	143.0	0.75	105.9	0.57	141.3	0.75	0.56	0.57	114.1	0.59
Genotypes mean		106.3	0.50	141.7	0.67	96.1	0.50	128.1	0.67	94.8	0.51	126.3	0.68	0.50	0.51	103.8	0.54

L**.S.D. at 5% level of significance**

	ASCW	**degree of thickening.**
L.S.D Mic level (m)	1.4	0.02
L.S.D. variety (v)	1.8	0.02
L.S.D. measuring methods (th)	1.9	0.02
L.S.D. m × v	2.3	0.03
L.S.D. m × th	2.7	0.03
L.S.D. v × th	2.8	0.03
L.S.D. m × gt × th	3.4	0.04

ASCW1a calculated from Lord equation and 1.52 g/cm^2 cellulose density very close to ASCW4 measured by image analysis cross section direct method while ASCW2a and ASCW3a were slightly lower than ASCW4, the recorded means were106.3, 96.1, 94.8 and 103.8 **μ**^{2} for ASCW1, ASCW2, ASCW3 and ASCW4 respectively. On the other hand ASCWb calculated by the different methods considering the wall density is 1.14 g/cm^2 showed very high means of ASCW than ASCW4 measured by Image analysis (the reference method), the recorded means were 141.7, 128.1, 126.3 and 103.8 **μ**^{2} for ASCW1b, ASCW2b, ASCW3b and ASCW4 respectively. These results indicated that considering the cell wall density 1.14 g/cm^2 led to big increase in the values of calculated area of cellulose when compared with the actual one measured by the image analysis direct method, this will be reflected in and led to high biased calculated degree of thickening which reached 0.67 and 0.68 compared to 0.54 in the degree of thickening measured by image analysis direct method, while all the other methods used 1.52 cellulose densities led to slightly lower values of degree of thickening but closer to those measured by image analysis. The recorded means were 0.50, 0.50, 0.51 and 0.54 for degree of thickening 1a, 2a, 3a and 4. Furthermore, the low maturity level showed lower means of degree of thickening than the normal level while the high level showed the highest ones regardless the calculating methods and genotypes, being; 0.48, 0.56 and 0.59 for the degree of thickening obtained from image analysis reference method for the three levels of maturity respectively.

In the three levels of maturity the Extra fine ELS cotton variety G 93 and the ELS Giza 92 showed lower ASCW and slightly lower degree of thickening than the Delta LS Giza 94 and Giza 97 while, Upper Egypt LS cottons Giza 95 and the promising cross G.90xCB58 showed the highest ones regardless the calculating and measuring methods.

Concerning Degree of thickening (Ɵ) = MR x 0.577 according to[13], the results in Table 2 indicated that both of MR from HVI and MR from caustic soda direct method showed closer means of Ɵ but also slightly lower than those obtained from image analysis data to be; 0.44 and 0.43 in the low maturity level, 0.51 and 0.53 in the normal level and 0.56 and 0.57 in the high maturity level compared to 0.48, 0.56 and 0.59 for the degree of thickening obtained from the image analysis of the three maturity levels respectively.

It is clear from these results that the differences in the Degree of thickening calculated using 1.52 cellulose density obtained from Lord equation, Hequet equation, cutter & caustic soda and image analysis are of low magnitude despite of their significance in some cases, however, the degree of thickening values obtained from image analysis method showed slightly higher values of degree of thickening Ɵ than those obtained from the indirect and cutter& caustic soda methods. On contrary using 1.14 cell wall densities according to the modification made by[23,44] led to biased high values of degree of thickening in the three maturity levels of the studied genotypes. Using HVI MR and caustic soda MR provided values of degree of thickening Ɵ slightly lower than those obtained from image analysis measurements. [13.21,24,25,36] found similar conclusions

The relationships between the different fiber maturity Parameters of some Egyptian cotton genotypes:

Simple correlation coefficients between fiber maturity parameters obtained from direct and indirect methods for the Egyptian cotton genotypes illustrated in Table 3 were positive and highly significant. The correlation coefficients ranged from 0.515 to 1.000.indicating that all of these parameters are better measures for fiber maturity. These results are in line with [27,48,50].

Table (3): Simple correlation coefficients between some fiber maturity parameters of some Egyptian cotton Genotypes

	MR	HW1	ASCW1a	ASCW1b	HW2	ASCW2a	ASCW2b	MR3	NOH18 % M% 3	HW3	ASCW3a	ASCW3b	ASCW4
Mic	0.808**	0.972**	0.972**	0.972**	0.962**	0.962**	0.962**	0.770**	0.737**	0.810**	0.810**	0.810**	0.622**
HVI MR	1.000	0.657**	0.657**	0.657**	0.624**	0.624**	0.624**	0.949**	0.954**	0.546**	0.547**	0.546**	0.189
HW1		1.000	0.657 **	0.657**	0.992**	0.992**	0.992**	0.629**	0.579**	0.838**	0.838**	0.838**	0.738**
ASCW1a			1.000	0.908**	0.992**	0.992**	0.992**	0.629**	0.579**	0.828**	0.838**	0.838**	0.738**
ASCW1b				1.000	0.992**	0.992**	0.992**	0.629**	0.579**	0.838**	0.838**	0.838**	0.734**
HW2					1.000	0.999**	0.999**	0.593**	0.550**	0.684**	0.838**	0.828**	0.733**
ASCW2a						1.000	0.999**	0.593**	0.550**	0.828**	0.838**	0.828**	0.734**
ASCW2b							1.000	0.593**	0.550**	0.828**	0.838**	0.828**	0.734**
NaOH 18% (MR3)								1.000	0.937**	0.515**	0.515**	0.515**	0.168
NaOH 18% (M%)									1.000	0.525**	0.525**	0.515**	0.155
HW3										1.000	1.000**	1.000**	0.674**
ASCW3a											1.000	1.000**	0.674**
ASCW3b												1.000	0.674**

R 0.05 = 0.267 R 0.01 = 0.321 n=18

Simple correlation regression coefficients between measured and calculated degree of thickening of the Egyptian cotton genotypes illustrated in Table 4 and Figure 1 were positive and highly significant. Correlation coefficients ranged from 0.867 to 1.000 indicating that the relationships between the different calculated and measured degree of thickening were very strong, moreover degree of thickening whether calculated directly from HVI MR(Θ = MR x 0.577) and caustic soda MR or by applying Lord and Hequet equations using 1.52 cellulose density were accurate and reliable enough to be used for measuring degree of thickening instead of the sophisticated slow image analysis method.

Table (4): Simple correlation coefficients between degree of thickening of some Egyptian cotton genotypes calculated and measured by direct and indirect methods:

	Degree of thickening 1a	Degree of thickening 1b	Degree of thickening 2a	Degree of thickening 2b	Degree of thickening 3a	Degree of thickening 3b	Degree of thickening 4	ϴ = HVI MR x 0.577	ϴ = MR3 x 0.577
Degree of thickening 1a	1.000	0.995**	0.990**	0.996**	0.940**	0.944**	0.898**	0.940**	0.941**
Degree of thickening 1b		1.000	0.981**	1.000**	0.940**	0.941**	0.896**	0.950**	0.945**
Degree of thickening 2a			1.000	0.996**	0.942**	0.942**	0.890**	0.948**	0.941**
Degree of thickening 2b				1.000	0.944**	0.946**	0.905**	0.946**	0.951**
Degree of thickening 3a					1.000	0.946**	0.905**	0.991**	0.921**
Degree of thickening 3b						1.000	0.900**	0.994**	0.922**
Degree of thickening 4							1.000	0.901**	0.876**
ϴ = HVI MR x 0.577								1.000	0.921**
ϴ = MR3 x 0.577									1.000

R 0.05 = 0.267 R 0.01 = 0.321 n=18

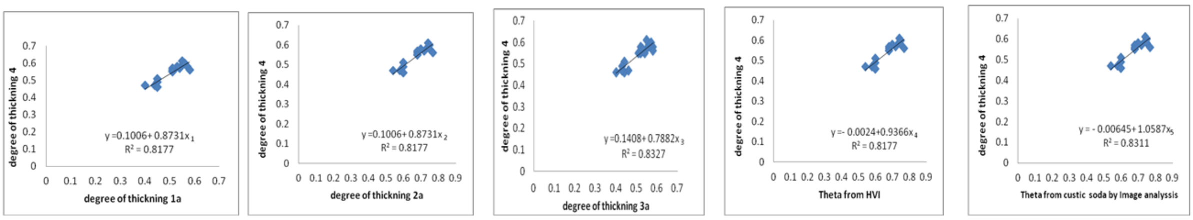

Fig (1): Simple regression between degree of thickening of some Egyptian cotton genotypes calculated and measured by direct and indirect methods

Aiming to obtain calculated degree of thickening values very close or even equal to those measured by Image analysis method, the following Regression equations were developed:

Corrected degree of thickening:-

$Y = 0.1006 + 0.8731x_1$ where x_1 is degree of thickening1a and $R^2 = 0.8177$ (from Lord equation)

$Y = 0.1006 + 0.8731x_2$ where x_2 is degree of thickening2a and $R^2 = 0.8177$ (from Hequet equation)

$Y = 0.1408 + 0.7882x_3$ where x_3 is degree of thickening3 and $R^2 = 0.8327$ (from Caustic soda&Cutter method)

$Y = -0.0024 + 0.9366x_4$ where x_4 is degree of thickening4 and $R^2 = 0.8177$ (from Θ =HVI MR x 0.577)

$Y = -0.0645 + 1.0587x_5$ here x_5 is degree of thickening5 and $R^2 = 0.8311$ (from Θ=Caustic soda MR x 0.577)

IV. Conclusion:

Summing up the results obtained from this study it could be concluded that:

Mature fibers have bigger hair weight (HW), Perimeter (P), cross section area (ACW), area of cell wall (ASCW) and degree of thickening (Ө) than low mature fibers.

Calculated and measured HW ranked the studied genotypes in the same order.

Calculated ASCW and degree of thickening values using 1.52 cellulose densities was closer to what obtained from Image analysis direct reference method than those calculated using 1.14 cell wall density which resulted in biased high ASCW and degree of thickening values.

The relationships between cotton fiber maturity parameters and between calculated and measured degree of thickening were positive and highly significant.

Degree of thickening (Ө) calculated from Lord equation, Hequet equation and cutter & caustic soda method slightly lower than obtained from Image analysis direct reference method furthermore degree of thickening (Ө) values calculated from HVI MR using the equation degree of thickening (Ө) = MR× 0.577) were very close to those measured by Image analysis method indicating the possibility of using HVI micronaire and MR to calculate accurate and reliable estimates of degree of thickening instead of the direct sophisticated and slow methods.

Regression equations could be used to make corrections to the degree of thickening calculated from Lord equation, Hequet equation, cutter and caustic soda methods, HVI MR and caustic soda MR.

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Sief M. G, et. al. “ Comparative Study on Egyptian Cotton Fiber Maturity Measurements Using Direct And Indirect Methods.” *IOSR Journal of Polymer and Textile Engineering (IOSR-JPTE)*, 09(03), 2022, pp. 01-09.