III. MATERIALS AND METHODS

Two filed experiments were conducted at the Experimental Farm, Faculty of Agriculture (Saba Basha), Alexandria University, Egypt, during the summer growing seasons of (2013 and 2014) to study the effect of water stress, humic acid application and their interaction on growth and yield of three maize hybrids.

3.1. Treatments

3.1.1. Water stress treatments (main plot)

- Irrigation every 10 days.
- Irrigation every 15 days.
- Irrigation every 20 days.

3.1.2. Maize hybrid (sub plot)

- SC.166.
- SC.168.
- TWC352.

3.1.3. Humic acid application (sub sub plot)

- Without addition.
- 7.2 kg humic acid/ha.
- 14.4 kg humic acid/ha.

3.2.Field experimental design

Treatments were arranged in a split-split plot design with three replications during both seasons of the study. Whereas, the main plots were designated for water stress, while subplot were located for three maize hybrids and the three humic acid applications were occupied the sub- sub plot.

Each sub plot consisted of 6 ridges, 3 meters in length, 70 cm width and 20 cm between hills. The two sowing dates were 5th June and 8th June in 2013 and 2014 seasons, respectively.

3.3.Soil analysis

Representative soil samples were taken from experimental soil before starting experimental work.

The soil samples were air dried, passed through a 2 mm sieve, and then analyzed according to the method described by **Rhoades** *et al.*, **(1982)** and **Page** *et al.* **(1982)**. The soil type of experimental site was clay loamy. The mechanical and chemical analysis of the experimental site is presented in Table (1).

The proceeding crop in the experimental site was Egyptian clover (*Trifolium alexandrinum*) in the first season while wheat (*Triticum aestivum*, L.) was in the second season.

Table (1): Some physical and chemical properties of the experimental soil in 2013 and 2014 season

Soil parameter	Season	
	2013	2014
Particle size distribution %		
Sand	37	39
Silt	34	32
Clay	30	30
Textural class	Clay loam	Clay loam
Chemical properties:		
pH (1:1) (soil: water suspension)	8.30	8.29
EC (1:1) (soil: water extract), dS/m	3.75	3.81
Soluble cations (1:2) (cmo1/kg soil)		
K^{+}	1.60	1.57
Ca^{++}	9.3	8.8
Mg^{++}	18.3	18.5
Na^{++}	13.50	13.8
2- Soluble anions (1:2) (cmo1/kg soil)		
CO^{-}_{3}+ HCO^{-}_{3}	2.90	2.80
CL	20.4	19.80
SO^{-}_{4}	12.50	12.60
Calcium carbonate, %	6.50	7.00
Total nitrogen, %	1.10	1.11
Available P (mg/kg)	3.70	3.55
Organic matter, %	1.47	1.45

3.4. Application of fertilizer

The field experiment was ploughed twice then it was fertilizer by 240 kg calcium super phosphate ha^{-1} (15.5 % P_2O_5) at the soil preparation before sowing in each season and 120 kg K_2O/ha., potassium sulfate (48% K_2O). Nitrogen fertilizer (480 kg ha^{-1}) in the form of urea (46.5 % N) was applied at two doses, the first one was applied 21 days from sowing and the second one was applied before the second irrigation. The hills were thinned to one plant per hill and irrigation was applied according to irrigation intervals under this study. Other agricultural practices for growing maize plants were done as recommended.

Humic acid treatments (0.00, 7.20, and 14.40 kg/ha.) were applied as soil application with sowing. The product (Techno potas- humic acid) analysis is presented in Table (2).

Table (2). Humic acid analysis

Product analysis	
Product name	Techno Potas- Humic acid
Formula (W/W)	12% K_2O – HA 75 %
Potassium K_2O (on dry basis)	12 % (W/W)
Humic acid (on dry basis)	75 % (W/W)
Moisture	15 (Max.)
P^H (1% solution)	9 -10 (Max.)
Water solubility	95 % (Min.)

3.5. Studied characters

3.5.1. Growth characters

3.5.1.1. Plant height (cm), it was measured as the mean of five plants measured from the soil surface to the top of the plant.

3.5.1.2. Leaf area index (LAI), LAI expresses the ratio of leaf surface to the ground area occupied by the plant as determined by Radford (1967).

3.5.1.3. Dry matter accumulation (g/plant).

3.5.1.4. Crop growth rate (CGR) (g/m^2/week) was calculated according to the formula suggested by **Brown (1984)**

$CGR = W_2\text{-}W_1/SA/T_2\text{-}T_1$

Where W_1 and W_2 are plant dry weights at time (T_1) and at time (T_2) corresponding days.

SA= the soil area occupied by the plant at each sampling (m^2).

3.5.1.5. Relative growth rate (RGR) (g/g/week) was calculated according to the formula suggested by **Brown (1984)**

$RGR= \log_e W_2 - \log_e W_1 / T_2\text{-}T_1$ Where

$\log_e$= Nabrain $\log_e$

(W_1) and (W_2) are plant dry weights

T_1 and T_2 corresponding days.

3.5.1.6. Total chlorophyll content

The Chlorophyll pigments were measured by direct digital reading on chlorophyll meter SPAD-502, where the value measured by the chlorophyll present in the plant leaf. The values are calculated based on the amount of light transmitted by the leaf in two wave length regions in which the absorbance of chlorophyll is different. Total chlorophyll was determined by digital apparatus (SPAD-502) according to Monje and Bugbee (1992) and Coste et al. (2010) who suggested the following equation to transfer SPAD units to mg m^{-2}.

$Y = 1.034 + 0.308 * X + 0.11 * X^2$

Where; X= SPAD units

3.5.2 Yield and yield components

At harvest time, the ears were harvested from the two middle rows of each plot to determine the following characters:

3.5.2.1 Cob length (cm)
3.5.2.2 Number of kernels/row.
3.5.2.3 Number of rows/ear.
3.5.2.4 Number of kernels/ear.
3.5.2.5 100 kernel weight (g)
3.5.2.6 Straw yield (tons/ha.)
3.5.2.7 Grain yield (tons/ha.)
3.5.2.8 Biological yield (tons/ha.).
3.5.2.9 Harvest index (HI).

3.5.3 Protein percentage was determined according to the improved Kyledahl methods of Association of official Agricultural chemists **(A.O.A.C., 1980),** crude protein percentage was calculated by multiplying the total nitrogen for each sample by 6.25.

3.5.4. Water Content Analysis

3.5.4.1.Relative water content

Vegetation status is measured either by the Relative Drought Index (RDI) (**Hofler** *et al*., **1941**) or the Relative Water Content Index (RWC) (**Inoue** et al., **1993**).

RWC compares the water content of a leaf with the maximum water content at full turgor.

$$RWC = \frac{FW - DW}{TW - DW}$$

where FW is the field weight, DW the oven dry weight, and TW the turgid weight. Limitations of retrieving RWC using reflectance in the optical domain have been discussed by **Bowman (1989)** and **Ripple (1986).** Unlike the vegetation stress indices, RWC do take into consideration the quantity of water in the plant. However, two different species may have the same RWC values with different amounts of water in their leaves. For the purpose of assessing burning efficiency and risk of fire occurrence, these indices might not be suitable because they do not provide an absolute measure of plant water content.

3.5.4.2.Water use efficiency (WUE).

Water use efficiency was calculated according to the formula suggested by **Stanhill, 1987**

Water use efficiency = Grain yield (kg) / Total water used (m^3)

3.6. Statistical analysis

Data were statistically analyzed as split-split plot design according to **Gomez and Gomez (1984)**, using the split- split model obtained by **CoStat 6.311 (1998-2005)** as statistical program. Treatment means were compared according to LSD test at 0.05 level of probability to estimate the significant differences among treatments.