Yield and nutritive value of maize (*Zea mays* L.) forage as affected by plant density, sowing date and age at harvest

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Abstract

The current study was carried out in Alexandria, Egypt and investigated the effect of sowing date; 1st May, 1st July, and 1st September 2017 and 2018, and age at harvest; 45, 55 and 65 days after sowing (DAS) on yield and quality of maize green forage grown with three plant densities (120, 160, and 200 kg ha^{-1}).

Sowing at 1st of May produced the highest significant amount of fresh yield (41.51 t ha^{-1} in average). Sowing at 1st of July resulted in the production of significantly lower yield (24.54 t ha^{-1} in average), however, higher dry matter content (175.99 g kg^{-1} in average), compared to sowing at 1st of May (143.62 g kg^{-1} in average). A pronounced increase in fresh yield was observed when maize was harvested at 55 DAS (30.89 t ha^{-1} in average) compared to harvesting at 45 DAS (22.92 t ha^{-1} in average). Meanwhile, the increase in fresh yield from 55 DAS to 65 DAS was non-significant. The effect of sowing date on quality parameters was greatly dependent on the age of plant at harvest. Harvesting maize green forage at 65 DAS, reflecting an advanced stage of maturity, caused a significant reduction in the crude protein (CP), and a significant increase in the neutral and acid detergent fibre fractions (NDF and ADF), resulting in a decline in the digestible organic matter (DOM). Plant density exerted non-significant influence on the fresh yield and DM content, minimal effect on the CP content, while the effect on the NDF and ADF contents was dependent on the age at harvest. The lignin content (ADL) of the herbage significantly decreased with increasing the plant density. Variations in the DOM were most dependent on the variations in CP content, followed by the variations in ADF and ADL contents. In conclusion, it is recommended to grow green forage maize twice a season on the 1st of May and 1st of July, with intermediate plant density (160 kg ha^{-1}), and harvest it not later than 55 DAS to achieve the optimum balance between herbage productivity and nutritive value.

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Introduction

One of the major challenges facing the forage production sector in Egypt is how to provide adequate forage with satisfactory nutritive value that could support the livestock production especially in the summer season, where the feed shortage reach its maximum (Salama and Zeid, 2016).

Green forage is a very important component in the forage and animal production sectors. Green forages are rich, yet cheap sources of all the nutritious compounds important for animal husbandry. Feeding of green forages reduces the costs of the produced milk and meat substantially. In addition, it reduces the micronutrients deficiencies, and, thus, helps keeping the animals healthy, which, consequently, reflects on increasing milk and meat yields (Chaudhary *et al*., 2014). More investment should, thus, be put into improving the yielding performance and nutritive value of the common summer green forages grown in Egypt.

Maize (*Zea mays* L.) is an important multipurpose crop in Egypt and the world, used as human food and animal feed. Based on the cultivated area and production, it occupies the third rank after wheat and rice. The world production of maize has increased in the last decades, and it is expected to increase further in response to the climate change especially in the southern parts of Europe exhibiting a Mediterranean climate (Elsgaard *et al.,* 2012). In Egypt, it is one of the main summer annual green forage crops, upon which the feeding of livestock and poultry is greatly dependent. Maize is one of the most convenient non-legume green forages. It is a quick growing crop, with high biomass production that may reach 40-50 t ha^{-1} (Chaudhary *et al.,* 2014). When grown as green forage, it is advantaged by the high production of succulent vegetative parts in a comparatively short time. Compared to other forage grasses, maize is characterized by its high-energy content, considerable protein content (Safari *et al*., 2014), high palatability and digestibility (Cusicanqui and Lauer, 1999). In addition, it is free from any anti-nutritious compounds, which gives it a great advantage over sorghum and pearl millet, which are disadvantaged by the presence of hydrocyanic acid and oxalate, respectively (Chaudhary *et al.,* 2014). Moreover, green forage maize possesses sufficient quantities of non-structural carbohydrates, especially soluble sugars, which make it suitable for ensiling (Mandić *et al.* 2013).

In Egypt, green forage maize is a single cut crop, commonly cultivated from May to August, and could be cultivated twice a season (Shimizu *et al.,* 2015), which gives it a special importance over the other existing summer forages. While crop management practices for maize grown for grain production are well established, management practices for forage maize are more controversial among farmers and different locations. Yield and quality of forage maize is a function of numerous interacting environmental, genetic, and management factors. Among the environmental factors, temperature, available ground water, day length and light are major factors affecting maize development and productivity (Struik, 1983; Dwyer and Stewart, 1986; Meisser and Wyss, 1998). Since sowing date is the main determinant to the temperature and day length to which the crop is exposed during its growth and development, it has to be accurately adjusted.

The significant variable response of the crop's yield and quality to plant maturity (adjusted in terms of age at harvest) is well documented. As the plant matures, the forage yield, dry matter content, starch and energy contents usually increase (Seleiman *et al.,* 2017). On the contrary, crude protein and fibre fractions are reported to decrease with advanced maturity (Darby and Lauer, 2002; Lewis *et al.*, 2004; Keady, 2005). Variable observations were reported for the effect of crop maturation on digestibility of forage maize. While a slight decrease in the whole plant apparent digestibility with maturation was reported by Browne *et al.* (1999), Cone *et al.* (2008) stated that the organic matter digestibility did not change. Therefore, it is crucial to determine the best age at which the green crop should be harvested to achieve optimum balance between yield and dry matter content, on one hand, and quality and digestibility, on the other hand.

In addition to sowing date and age at harvest, plant density is of particular importance in maize cultivation, because it lacks the tillering capacity to adjust to variation in plant population (Safari *et al.,* 2014). Plant density is also known to exert variable effects on forage maize under different environmental conditions and management practices (Bavec and Bavec, 2002). However, the relationship between green forage maize yield and quality, and plant density is not well established (Carpici *et al.,* 2010; Mandic *et al.,* 2015).

The current study was designed to investigate the effect of the three sowing dates; 1st May, 1st July, and 1st September, and three ages at harvest; 45, 55 and 65 days after sowing (DAS) on yield and quality of maize green forage grown with three different plant densities (120, 160, and 200 kg ha^{-1}). The studied parameters included fresh yield (t ha^{-1}), dry matter content (g kg^{-1}), and the following quality attributes: crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), ash, and organic matter digestibility (g kg^{-1}). In addition, analyse the amount of contribution of each of the studied yield and quality parameters in determining the crop digestibility.

Materials and methods

Site description

The field experiments were carried out at the experimental station of the Faculty of Agriculture, Alexandria University, Alexandria, Egypt during the summer seasons of 2017 and 2018. Soil of the experimental location was moderately alkaline (pH 8.2), sandy loam in texture, with 1.55% organic matter content and electrical conductivity 1.35 dS m^{-1}.

Design, treatments, and sampling

A split-split-plot experimental design with three replicates was used to investigate the effect of three sowing dates (SD) - assigned to the main plots, three plant densities (PD), in the sub-plots and three ages at harvest (AH) in the sub-sub-plots on the yield and some quality attributes of fodder maize (*Zea mays* L.). The three investigated sowing dates were the 1st of each of the months of May, July and September, in both growing seasons (2017 and 2018). The three tested plant densities were, 120, 160, and 200 kg ha^{-1}. Experimental plots were harvested either after 45, 55, or 65 DAS for each sowing date, representing the three tested ages at harvest.

Seed beds were prepared by dividing each plot (7.2 m^2) into four ridges (60 cm apart and 3 m long), the amount of seeds representing each of the tested plant densities was drilled in a row on the upper half of one side of each ridge. Crop management was identical for all experimental plots concerning fertilisation and irrigation schemes. Nitrogen, in the form of ammonium nitrate (33.5%N), was applied at the rate of 40 kg N ha^{-1}, two weeks after sowing, while, phosphorous was added once with seed bed preparation at the rate of 100 kg P_2O_5 ha^{-1}, in the form of calcium mono phosphate (15.5% P_2O_5). At the time of harvesting, plants were cut with a sickle directly above ground level, and fresh yield per plot was determined. A representative sub sample of approximately 1 kg from each plot was dried at 60°C until constant weight was reached to determine the dry matter (DM) concentration per plot.

Analytical procedures

The dried sub-samples of the whole plants were uniformly ground over a 1-mm screen. The nitrogen (N) content was analysed by the Kjeldahl procedure (AOAC, 2012), and CP content was calculated from the N content (CP=N×6.25). The concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined sequentially using the semiautomatic ANKOM^{220} Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) after Van Soest *et al.* (1991). NDF and ADF were analyzed without a heat stable amylase and expressed inclusive of residual ash, while ADL content was corrected after the residual ash content. Ash was determined by incineration of the sub-sample in a muffle furnace at 550°C for 3h (AOAC, 2012). The *in vitro* cellulase technique developed by De Boever *et al.* (1988) was used to determine the digestibility of herbage samples. The percentage of digestible organic matter (DOM) was then calculated by applying the following equation of Weißbach *et al.* (1999):

$$DOM\ (\%) = 100 \times (940 - CA - 0.62 \times EULOS - 0.000221 \times EULOS^2) / (1000 - CA) \quad (1)$$

where CA = crude ash and EULOS = enzyme insoluble organic matter; CA and EULOS are expressed in g kg^{-1} DM.

Statistical procedures

The sowing date, plant density, and age at harvest were tested for significance using Proc Mixed of SAS 9.4 (SAS Institute, Inc., 2012). Data of yield and quality parameters were presented in a combined analysis for the two growing seasons due to homogeneity of variance's error between the two experimental seasons (Winer, 1971). Only replicates were considered random. The investigated response variables (*V*) were analysed according to the following model:

$$V_{ijk} = \mu + R_l + SD_i + e_i + PD_j + (SD \times PD)_{ij} + s_{ij} + AH_k + (SD \times AH)_{ik} + (PD \times AH)_{jk} + (SD \times PD \times AH)_{ijk} + t_{ijk} \quad (2)$$

where: μ is the overall mean, R_l is the replication (l = 1,2,3), SD_i is the sowing date effect (i = 1,2,3), PD_j is the plant density effect (j = 1,2,3), AH_k is the age at harvest effect (k = 1,2,3), $(SD \times PD)_{ij}$ is the effect of the interaction between the sowing date and plant density, $(SD \times AH)_{ik}$ is the effect of the interaction between the sowing date and age at harvest, $(PD \times AH)_{jk}$ is the effect of the interaction between plant density and age at harvest, and $(SD \times PD \times AH)_{ijk}$ is the effect of the interaction between sowing date, plant density and age at harvest. In addition, e_i is the effect of main plot, and s_{ij} is the effect of sub-plot, and tijk is the effect of the sub-sub-plot.

Significance was declared at $P<0.05$, and means were compared with the least significant difference procedure.

A stepwise regression analysis with a forward selection procedure was adopted to test how much the variable *Digestible organic matter* is dependent on the other candidate variables in the experiment. The forward procedure depends on selecting the variable that has the highest R-Squared, then at each step, select the candidate variable that increases R-Squared the most. Stop adding variables when none of the remaining variables are significant. After each step in which a variable was added, all candidate variables in the model are checked to see if their significance has been reduced below the specified tolerance level (0.2000). If a non-significant variable is found, it is removed from the model.

Results

Analysis of variance presented in Table 1 reveals that the different investigated sowing dates exerted a significant influence on all the studied parameters except the CP content. While, the significant effect of the plant density was limited to the CP, ADL and DOM. Moreover, all the investigated yield and quality parameters were significantly affected by the ages at harvest. The sowing date × age at harvest interaction was significant in case of the fresh yield, DM, NDF, and ADF contents. In addition, significant variations were declared for CP, NDF and ADF contents as affected by the plant density × age at harvest interaction. Only in case of ADL content, was the three way interaction significant. Main effects of the three studied factors will be presented and discussed only when their interactions are not significant.

Fresh yield and dry matter content

Means of fresh yield (t ha^{-1}) as affected by the two way interaction between the sowing date and age at harvest reveal that, the highest significant amount of fresh yield was always produced for sowing at 1st of May (Table 2). The percentage decrease in fresh yield between sowing at 1st of May and at 1st of July amounted to 37, 44, and 41% for harvesting at 45, 55, and 65 DAS, respectively. While, significantly similar yields were achieved when sowing 1st of July and 1st of September. Expectedly, the older the crop at harvesting, the more fresh yield was produced. Where, the highest significant amount of fresh yield was achieved when harvesting at 65 DAS, followed by 55 DAS, then finally 45 DAS. This was true for all the tested sowing dates. A pronounced increase in fresh yield

Table 1. Mean squares and levels of significance of the fresh yield (t ha^{-1}), dry matter concentration (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), ash and digestible organic matter (DOM) contents (g kg^{-1}), of forage maize as affected by the sowing dates (SD), plant density (PD), and age at harvest (AH), and their interactions, combined over 2016 and 2017 growing seasons.

Effect	D.F.	Mean square							
		Fresh yield	DM	CP	NDF	ADF	ADL	Ash	DOM
SD	2	3177.09**	8973.66**	18.25^{ns}	21016.37**	13226.52**	544.06**	4244.02**	12821.46**
PD	2	215.64^{ns}	36.52^{ns}	50.39**	1741.55^{ns}	1663.22^{ns}	1123.70**	168.64^{ns}	562.10*
AH	2	829.45**	22059.05**	2483.05**	10652.91**	7483.14**	18137.49**	5332.44**	103320.29**
SD×PD	4	42.32^{ns}	219.88^{ns}	14.33^{ns}	651.97^{ns}	221.97^{ns}	656.09**	280.07^{ns}	123.49^{ns}
SD×AH	4	108.19*	1079.51**	1.45^{ns}	1469.04**	1126.611**	201.05*	186.99^{ns}	40.16^{ns}
PD×AH	4	28.33^{ns}	167.33^{ns}	27.52*	480.71*	638.89*	394.12**	152.79^{ns}	541.94^{ns}
SD×PD×AH	8	15.50^{ns}	302.83^{ns}	1.06^{ns}	182.50^{ns}	117.92^{ns}	446.25*	319.54^{ns}	34.22^{ns}
C.V.	19.08	8.65	3.52	1.96	4.00	13.03	14.19	2.34	

*Significant at 0.05 level of probability; **Significant at 0.01 level of probability; ns, non-significant; D.F., degrees of freedom; C.V., coefficient of variation.

Table 2. Variations in fresh yield (t ha^{-1}), and dry matter concentration (g kg^{-1}) as affected by the interaction between the sowing date and age at harvest, combined for both growing seasons.

Age at harvest	Fresh yield			Dry matter		
	Sowing date					
	1st May	1st July	1st Sept.	1st May	1st July	1st Sept.
45 DAS	30.86^{aB}	19.45^{bB}	18.44^{bA}	118.47^{bC}	149.74^{aB}	107.88^{bC}
55 DAS	45.68^{aA}	25.62^{bAB}	21.37^{bA}	144.60^{bB}	185.16^{aA}	151.59^{bB}
65 DAS	47.99^{aA}	28.55^{bA}	24.17^{bA}	167.80^{bA}	193.08^{aA}	186.34^{abA}

*Means followed by different small letter(s) within the same row, and different capital letter(s) within the same column, for each studied parameter, are significantly different according to the L.S.D. test at 0.05 level of probability.

was observed when the crop was harvested at 55 DAS compared to harvesting at 45 DAS. This percentage increase reached 48, 32, and 16%, for the three respective sowing dates. Meanwhile, the increase in fresh yield from 55 DAS to 65 DAS was non-significant. Among the three tested sowing dates, sowing at 1st of July resulted in the highest significant DM accumulation, followed by the other two sowing dates (Table 2). Concerning the variations in DM content among the three investigated ages at harvest, obviously, harvesting at 65 DAS gave the crop a better chance to accumulate high amounts of DM, followed by harvesting at 55 DAS and then harvesting at 45 DAS, which resulted in herbage with least significant amount of DM. Similar to the fresh yield results, the percentage increase in DM accumulation between harvesting at 45 DAS and 55 DAS reached, 22, 24, and 41%, for sowing at 1st May, 1st July and 1st September, respectively. A less percentage of increase was observed between harvesting at 55 DAS and 65 DAS, and amounted to 16, 4, and 23%, for the three respective sowing dates.

Crude protein and fibre fractions

The two-way interaction between plant density and age at harvest caused significant variation in the CP content of green forage maize. Means presented in Table 3, demonstrate that, only with the late harvesting (65 DAS), the low plant density (120 kg ha^{-1}) produced plants with superior CP content than the higher plants densities (160 and 200 kg ha^{-1}). However, despite the statistical significance, the difference in CP content among the three plant densities was negligible, and was around 0.30%. A more pronounced variation in the CP content was observed among the three ages at harvest. The early harvest (45 DAS) produced forage with the highest significant CP content, amounting to 94.19, 92.24, and 94.26 g kg^{-1}, for the respective plant densities 120, 160, and 200 kg ha^{-1}. The percentage decreases in the CP content when delaying the harvesting till 55 DAS reached 0.95, 0.78, and 1.18% for the three respective plant densities. Moreover, when delaying the harvesting till 65 DAS, the decreases in CP content, from the initial CP content at 45 DAS, reached 1.69, 1.77, and 2.29%, for the three respective plant densities. The same two-way interaction between plant density and age at harvest significantly influenced the two major fibre fractions (NDF and ADF). Concerning the NDF content (Table 3), no significant variations were observed among the three tested plant densities for all ages at harvest. An inconsistent direction of variation was, however, observed, which might have contributed to the significant interaction. Where, with the early (45 DAS), and late (65 DAS) harvests, NDF content increased by increasing the plant density, while when harvesting at 55 DAS, the intermediate plant density (160 kg ha^{-1}) resulted in the lowest NDF content. Nonetheless, a clear significant increase in the NDF content was detected with advancement in harvesting under all tested plant densities. The significantly highest NDF content was achieved when harvesting at 65 DAS and reached 668.35, 672.76, and 681.68 g kg^{-1}, for the three respective plant densities. No significant difference in the NDF content was detected between harvesting at 45 DAS and at 55 DAS. Similar results were reported for the ADF content (Table 3), where the different plant densities caused slightly significant vari-

Table 3. Variations in crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) contents (g kg^{-1}) as affected by the interaction between the plant density and age at harvest, combined for both growing seasons.

Age at harvest	CP			NDF			ADF		
	Plant density (kg ha^{-1})								
	120	160	200	120	160	200	120	160	200
45 DAS	94.19^{aA}	92.24^{aA}	94.26^{aA}	630.29^{aB}	636.91^{aB}	645.76^{aB}	322.63^{aB}	311.73^{aB}	323.03^{aB}
55 DAS	84.68^{aB}	84.48^{aB}	82.44^{aB}	645.94^{aB}	627.30^{aB}	654.55^{aB}	329.12^{abAB}	311.81^{bB}	342.75^{aAB}
65 DAS	77.30^{aC}	74.51a^{bC}	71.35^{bC}	668.35^{aA}	672.76^{aA}	681.68^{aA}	344.05^{aA}	352.65^{aA}	357.29^{aA}

*Means followed by different small letter(s) within the same row, and different capital letter(s) within the same column, for each studied parameter, are significantly different according to the L.S.D. test at 0.05 level of probability.

Table 4. Variations in neutral detergent fiber (NDF), and acid detergent fiber (ADF) contents (g kg^{-1}) as affected by the interaction between the sowing date and age at harvest, combined for both growing seasons.

Age at harvest	NDF			ADF		
	Sowing date					
	1st May	1st July	1st Sept.	1stMay	1st July	1st Sept.
45 DAS	593.38^{bB}	654.29^{aB}	665.29^{aB}	284.55^{cB}	323.32^{bA}	349.52^{aB}
55 DAS	626.99^{bA}	647.14^{aB}	653.65^{aB}	317.24^{bA}	322.64^{abA}	343.80^{aB}
65 DAS	638.91^{bA}	686.40^{aA}	697.49^{aA}	335.14^{bA}	343.37^{bA}	375.48^{aA}

*Means followed by different small letter(s) within the same row, and different capital letter(s) within the same column, for each studied parameter, are significantly different according to the L.S.D. test at 0.05 level of probability.

Table 5. Variations in acid detergent lignin (ADL) content (g kg^{-1}) as affected by the interaction between the sowing date, plant density, and age at harvest, combined for both growing seasons.

Sowing date	Plant density (kg ha^{-1})								
	120			160			200		
	45 DAS	55 DAS	65 DAS	45 DAS	55 DAS	65 DAS	45 DAS	55 DAS	65 DAS
1st May	34.43	74.96	115.91	39.74	63.99	85.42	42.08	45.31	59.97
1st July	48.46	62.61	112.03	61.88	67.48	94.32	39.75	44.99	96.35
1st Sept.	38.73	49.00	78.76	40.31	66.93	94.91	40.19	43.89	101.82
L.S.D.$_{0.05}$					13.95				

ations in the ADF content. On the other hand, the variations due to the different ages at harvest were very clear. Similar to the NDF content, the older the crop at harvesting, the more the ADF content. Maximum amounts of ADF were reached when the crop was harvested at 65 DAS and amounted to, 344.05, 352.65, and 357.29 g kg^{-1}, for the three respective plant densities.

Means presented in Table 4, illustrate a significant influence for the interaction between sowing date and age at harvest on the NDF and ADF contents. Sowing at 1st of May resulted in the production of herbage with the lowest significant NDF content, amounting to 593.38, 626.99, and 638.91 g kg^{-1}, for harvesting at 45, 55, and 65 DAS, respectively. Meanwhile, as previously mentioned, the NDF content, significantly increased with increasing age at harvest. Similar trend was observed for the variations in ADF content under the effect of the two-way interaction between the sowing date and age at harvest. Despite the consistent direction of variation, its magnitude was variable, which might have caused the significant interaction. The percentage increase in NDF from sowing at 1st of May to sowing at 1st of September amounted to 7.19, 2.67, and 5.86%, for the three respective ages at harvest. Similar percentages of increase were reported for the ADF content and reached 6.50, 2.66, and 4.03%, for the three ages at harvest, respectively.

Analysis of variance for ADL content revealed a significant three-way interaction among sowing date, plant density and age at harvest. Means of ADL content, presented in Table 5, were highly variable as affected by the three-way interaction. Results indicated that the highest significant ADL content was achieved when plants were sown 1st of May or 1st of July, harvested 65 DAS under the low plant density (120 kg ha^{-1}). Generally, the difference between the highest (115.91 g kg^{-1}) and the lowest (34.43 g kg^{-1}) ADL values among all treatments was 8.15%. Although the direction of response was consistent among all treatments, the highly variable magnitude of response might have greatly contributed to the significant three-way interaction.

Ash and digestible organic matter

Main effects of the tested factors on the ash and DOM are presented in Table 6. The significantly highest ash content was achieved with the latest sowing date (1st of September), and amounted to 109.07 g kg^{-1}. Similarly, harvesting 45 DAS produced the significantly highest ash content (111.37 g kg^{-1}) compared to the other two ages at harvest. Non-significant variations were reported for the ash content among the different plant densities. DOM was the highest with sowing at 1st of May and gradually decreased with later sowing dates. In addition, the DOM values for the younger plants were significantly higher than the older plants. The DOM value when harvesting 45 DAS was 754.83 g kg^{-1}, while when harvesting 20 days later, the DOM decreased to 631.11 g kg^{-1}. Negligible variations in the DOM among the three tested plant densities were observed.

Stepwise regression analysis

Results of the stepwise regression analysis presented in Table 7, reveal that the variations in the DOM was most dependent on the variations in CP content (r^2 = 0.8279), followed by the variations in ADF and ADL contents, with r^2 values equal 0.0836 and 0.0174, respectively. The contribution of fresh yield and NDF content in determining the DOM values was non-significant with P<0.1018, and P<0.0997, respectively. No other variable met the 0.2000 significance level for entry into the model, therefore, DM and ash variables were excluded.

Table 6. Variations in the ash content, and digestible organic matter (g kg^{-1}) as affected by the sowing dates, plant density, and age at harvest combined for both growing seasons.

Effect	Ash	DOM
Sowing date		
1st May	95.68^{b}*	715.37^{a}
1st July	84.02^{c}	691.33^{b}
1st Sept.	109.07^{a}	671.87^{c}
Plant density (kg ha^{-1})		
120	96.94^{a}	693.66^{a}
160	93.49^{a}	687.95^{b}
200	98.35^{a}	696.97^{a}
Age at harvest		
45 DAS	111.37^{a}	754.83^{a}
55 DAS	93.83^{b}	692.62^{b}
65 DAS	83.58^{c}	631.11^{c}

*Means followed by different small letter within the same column, for each studied parameter, are significantly different according to the L.S.D. test at 0.05 level of probability.

Discussion

Effect of age at harvest

Among the studied factors in the current study, age at harvest exerted the strongest influence on the investigated yield and quality parameters of green forage maize, either as main effect or in combination with sowing date and/or plant density. It is evident that crucial changes in yield and nutritive value of green forage maize occur with advanced maturity.

Results of the current study confirmed that the delayed harvesting led to a significant increase in maize yield, this was in agreement with the findings of several researchers worldwide, *e.g.*

Table 7. F-values and levels of significance of the forward selection of the stepwise regression procedure between the digestible organic matter (DOM) as dependent variable and the other tested variables. Significance was declared at 0.2000 significance level.

Step	Variable	Partial R-square	Model R-square	F-value	*Pr* > F
1	CP	0.8279	0.8279	120.29**	>0.0001
2	ADF	0.0836	0.9115	22.69**	>0.0001
3	ADL	0.0174	0.9290	5.64*	0.0263
4	Fresh Yield	0.0083	0.9373	2.92^{ns}	0.1018
5	NDF	0.0078	0.9450	2.97^{ns}	0.0997

*Significant at 0.05 level of probability; **Significant at 0.01 level of probability; ns, non-significant.

Lewis *et al.* (2004), Little *et al.* (2005), and Gaile (2008). A pronounced increase in fresh yield was observed when the crop was harvested at 55 DAS compared to harvesting at 45 DAS. Meanwhile, the increase in fresh yield from 55 DAS to 65 DAS was non-significant. Similar trend was observed for the DM accumulation, where, obviously, the older plants had longer chance to accumulate higher amounts of DM than the younger plants. This observation was in line with the findings of Cone *et al.* (2008). According to the description of Bell (2017) to maize growth and developmental stages, plants harvested at 65 DAS were in the late vegetative stage (V16-V17). In their study to the DM accumulation with different phenological stages of maize, Koca and Erekul (2016) reported a progressive increase in the DM accumulation with advanced maturity till the late vegetative stage (V16). They added that, under favourable environmental conditions, the accumulated DM in leaves and stems of maize will be translocated to the ear and seeds during the generative growth stages.

Crude protein content of the green forage maize was, generally, low, and ranged from 7.13 to 9.43%. Similar values were reported by Gunn (1978), who recommended that when maize is the main forage in a ration it is important to balance it with an additional high protein source. The current results clarified that, advanced maturation of green forage maize caused a clear decline in the CP content. This was confirmed by the findings of Gaile (2008). On the other hand, NDF and ADF contents increased as plants advanced in maturity. Contrarily to the present results, Cone *et al.* (2008) reported a decrease in the investigated fibre fractions (NDF and ADF) with advanced maturity of forage maize. In their investigation, maize was harvested at ripening growth stages, characterized with very high starch contents. They, therefore, attributed the decrease in all the other chemical components (especially the fibre fractions) to the dilution effect of the increasing amounts of starch. High variability was observed among the ADL values, in the current study, denoted by the significant three-way interaction. As the three fibre fractions were sequentially determined in the same sample, after Van Soest *et al.* (1991), it is expected that the last determined fraction (ADL) would accumulate all the error generated from the analysis, resulting in the detected high variability in the results. As expected, significant increase in the lignin content was reported with advanced maturity due to the lignification taking in place in the stems and leaves, which was also linked to the increased DM content with maturation (AHDB Dairy, 2014). In close agreement with the current results, Russell (1986) reported a decrease in the *in vitro* digestibility of green forage maize over a maturation period of 60 days. Generally, the proportion of cell wall components (cellulose, hemicellulose and lignin) of forage grasses increases with advanced maturity, whereas the proportion of cell contents decreases (Osbourn, 1980; Bosch *et al.*, 1992). In addition, with increasing maturity, the ratio of stems to leaves increase (Terry and Tilley, 1964) , and given that the digestibility of the stem component is already lower than the digestibility of the leaf component and undergoes faster decline over time, this leads to decline in the digestibility with advanced maturity. Nonetheless, temperature plays an important role in determining the rate of decline in digestibility with increasing maturity (Struik *et al.*, 1985; Wilson *et al.*, 1991). Although it is believed that the digestibility of grasses is highest in the vegetative stage (Terry and Tilley, 1964; Groot, 1999), then it starts to decline, it is suggested that when maize is in the reproductive stage, its digestibility remains almost consistent, this is probably because the declining quality of stem and leaf fractions, as the crop matures, is counterbalanced by the increasing growth of the highly digestible grain in the ear (Phipps and Wilkinson, 1985).

Effect of sowing date

Variation in the sowing date of maize has more influence on grain yield than it has on forage yield (Bunting, 1978; Fairey, 1980). In the current study, high variability in the total fresh yield of green forage maize was detected among the three investigated sowing dates. Where, the highest significant amount of fresh yield was produced when sowing at 1st of May, accompanied with late harvesting. While, sowing at 1st of July and at 1st of September resulted in the production of similar amounts of fresh yield. Nonetheless, sowing at 1st of July was superior to the other two sowing dates in the DM accumulation of the crop. Considering the variations in yield and DM content with the sowing date, early planting has been shown to particularly enhance yield (Fairey, 1983), and quality of maize, given that the quality is determined on DM basis and, thus, closely linked to the DM content (Bunting, 1978; Fairey, 1980).

The effect of sowing date on the investigated fibre fractions was greatly dependent on the age at harvest. Generally, the NDF and ADF contents of green forage maize significantly increased with delayed sowing, however, the percentage increase was more noticeable in case of the early harvesting (45 DAS), followed by the late harvesting (65 DAS), while, harvesting at 55 DAS caused relatively slight change in the two fibre fractions. Similar to the present results, close linkage between sowing and harvesting dates in determining quality of maize was reported by (Fairey, 1983). Sowing date of maize is closely associated with the atmospheric and soil temperatures, which are major determinants of maize emergence and growth (Meisser and Wyss, 1998). Beauchamp and Lathwell (1967) reported a reduction in the emergence time of maize from 16 to 9 days with increasing the root-zone temperature from 12.5 to 17.5°C. Given that delayed sowing reduces the number of days required for crop emergence (Fairey, 1983), late sowing accompanied with late harvesting, in the current study, led to prolonged exposure of the growing maize crop to the continuous increasing temperature in Egypt during the summer season, which resulted in an increase in the NDF and ADF contents (Salama and Nawar, 2016). Deinum and Dirven (1972, 1975) associated the increases in temperature from 24 to between 28 and 33°C with greater stem weight and increased crude fiber of both C3 and C4 forage grasses. The increase in fibre fractions with delayed sowing was, obviously, accompanied with reduction in the digestible organic matter. Similar observation was reported by (Fairey, 1983) for dry matter digestibility.

Effect of plant density

It is well documented that optimum plant density may differ according to the purpose of maize planting, whether for grain or forage production, with higher plant densities favouring forage rather than grain yield (Olson and Sander, 1988).

Contrary to our expectations, the tested plant densities in the current study exerted non-significant influence on the fresh yield and DM content. Similar results were documented by Ferraira *et al.* (2014), and was attributed to the abundant precipitation during the growing season. On the other hand, many researchers reported significant influence of the plant density on forage maize yield, DM content and quality (*e.g.* Carpici *et al.,* 2010; Mashreghi *et al.,* 2014; Mandic *et al.,* 2015), however results were highly controversial. The significant effect of the increasing plant density on the crop's productivity is mainly attributed to the increased inter-plant competition for the different environmental parameters; mainly, light, water, and available soil nutrients (Mandic *et al.*, 2015), with light being the most limiting factor (Prine and Schrode, 1964).

Thus, in the present study, the high availability of soil water and nutrients at the experimental location, as well as the sunny summer season, might have contributed to limiting the effect of the tested plant densities on forage maize yield.

The interaction between the plant density and age at harvest was significant in case of CP and fiber fractions. Only at late harvesting (65 DAS) a slight significant decrease in CP content was reported with increasing plant density. This outcome was in agreement with the results reported by Widdicombe and Thelen (2002), who stated that CP content of forage maize was negatively correlated to plant densities. On the contrary, Jiwang *et al.* (2004) reported an increase in the CP content of forage maize with increased plant density. Meanwhile, Patricio Soto *et al.* (2002), and Carpici *et al.* (2010), found no statistical relation between CP content and plant density.

Results of previous researches investigating the relationship between plant density and fiber fractions were not less controversial. While, Iptas and Acar, (2006) reported significant variation in NDF and ADF contents among different plant densities, an insignificant effect was reported by Carpici *et al.* (2010). In the current study, little however significant effect of plant density on the NDF and ADF contents, was detected. In addition, it was observed that the lignin content tended to significantly increase with decreasing plant density. The increased lignin content associated with decreasing plant density was reported for several crop members of the grass family, *e.g.* wheat (Zheng *et al.*, 2017), buckwheat (Wang *et al.*, 2015), and maize (Shi *et al.,* 2016). Results of previous researches concluded that at low plant densities, stem diameter, wall thickness and dry weight per unit length will be increased, and, in addition to the improved structure of sclerenchyma and vascular bundles, the stem lignification will significantly increase.

Stepwise regression analysis

Digestibility is a basic determinant to the nutritive value of forages. In their review to the factors affecting *in vitro* digestibility of forages, Bruinenberg *et al.* (2002) mentioned that digestibility of different forage species is generally dependent on stage of maturity, ontogeny, plant characteristic, forage conservation, and chemical composition. Especially the chemical composition of the feed directly reflects its nutritive value, and is closely correlated to its digestibility and, to the expected performance of the ruminant receiving the feed. Digestibility of organic matter comprises digestibility of cell components, which are almost 100% digestible, as well as, digestibility of the cell wall components, which is variable. Dry matter content was believed to be practical and reliable measure for determining the suitable maturity stage at which maize should be harvested for high digestibility, until Givens and Deaville (2001) suggested that DM is not the most accurate measurement of plant maturity, and thus, digestibility, and proposed NDF content to be a better indicator. Whereas, Castillo-Jiménez *et al.* (2009) stated that the ADF content is the component that is most related to digestibility because it is constituted from cellulose, lignin and usually used to estimate energetic value of forage maize (INIFAP, 2006). Nevertheless, both NDF and ADF contents are negatively correlated to digestibility (Bruinenberg *et al.,* 2002).

In the current study an attempt was made to analyse the amount of contribution of each of the studied yield and quality parameters in determining the digestible organic matter of green forage maize. Results revealed that the highest significant contribution was reported for the CP content. It was hypothesized that, as a cell component positively correlated with digestibility, the bundle of treatments that would significantly increase CP content would, in turn, increase the forage digestibility. The homogeneous trend of the CP and DOM in response to the investigated treatments, in addition to the results of the stepwise regression analysis confirmed this hypothesis. After CP content, ADF and lignin contents significantly affected the organic matter digestibility. Dry matter content proved to have no contribution in determining the digestibility of the green forage maize, therefore it was excluded from the analysis.

Conclusions

Results of the current study have major practical relevance, considering the green forage maize management in Northern Egypt as well as any other Mediterranean country. Despite that the late harvest (65 DAS) was significantly superior in fresh fodder yield production and dry matter content, it was characterized by the significantly lowest CP and highest fiber fractions, which were reflected on the very low digestible organic matter values. Early sowing at 1st May, gave significantly highest green forage yield than the later sowing dates, meanwhile, the low yield produced when sowing at 1st of July was compensated with the significantly highest DM accumulation. Whereas, sowing at 1st of September produced forage with relatively high fiber content and, thus, low digestibility. The tested plant densities exerted minimal effect on the CP content, while their effect on the NDF and ADF contents was dependent on the age at harvest, with the intermediate plant density (160 kg ha^{-1}) resulting in the lowest NDF content when harvesting at 55 DAS. The lignin content of the herbage significantly decreased with increasing the plant density. The variations in the DOM were most dependent on the variations in CP content, followed by the variations in ADF and ADL contents. The contribution of fresh yield and NDF content in determining the DOM values was non-significant. Under similar conditions to the current study, it is recommended to grow green forage maize twice a season on the 1st of May and 1st of July, with intermediate plant density (160 kg ha^{-1}), and harvest it not later than 55 DAS to achieve the optimum balance between herbage productivity and nutritive value.

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