

# Effect of Plant Population Densities and Application of Growth Retardants on Cottonseed Yield and Quality

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In field trials at Giza in 1986–1987, cotton cv. Giza 75 was sown at 166,000, 222,000 and 333,000 plants/ha and given foliar applications of 0, 250, 500 and 750 ppm Cycocel (chlormequat) or Alar (daminozide). As plant density increased, there was a decrease in cottonseed yield/ha, seed index, seed protein content, oil and protein yields/ha, oil refractive index, iodine value, unsaponifiable matter and unsaturated fatty acids (myristoleic, oleic and linoleic). In contrast, as plant density increased, there was an increase in oil acid value, saponification value and saturated fatty acids (caprylic, capric, lauric, tridecyllic, myristic, palmitic and stearic). Application of Cycocel or Alar increased cottonseed yield/ha, seed index, seed protein content and oil and protein yield/ha, oil refractive index, iodine value, unsaponifiable matter and unsaturated fatty acids. However, there was a decrease in oil acid value and saponification value. There were no differences among application rates of either chemical on cottonseed yield/ha. The highest oil and protein yield/ha was observed with Cycocel applied at 750 ppm, followed by Alar at 250 ppm. Applying Cycocel at 250 ppm gave the highest oil refractive index and unsaponifiable matter, and the lowest acid value. Application of Alar at 250 ppm gave the highest oil iodine value and the lowest saponification value, and also at 250 or 500 ppm gave the highest oil unsaturated fatty acid composition. Interaction was positive between plant density, Cycocel and Alar and affected cottonseed yield/ha. The 166,000 plants/ha and application of Cycocel at 750 and Alar at 250 ppm are recommended for the improvement of cottonseed yield and quality.

**KEY WORDS:** Cottonseed yield, growth retardants, oil fatty acid composition, plant density, seed index, seed oil content, seed oil properties, seed protein content.

Cultivated cotton is the most important fiber crop in Egypt and is becoming a popular food crop because of its seed oil and high-quality protein. In recent years, it has become apparent that oil and protein play extremely important roles in the normal function of all living systems. Great efforts must be directed toward the improvement of all oil and protein resources to fill the gap between oil and protein production and consumption. Approaches considered to achieve this goal are: (i) developing high-yielding varieties through selection programs, interspecific crossing and induced mutations; (ii) insect and pest control; (iii) application of appropriate agronomic practices; and (iv) plant growth regulators (PGR). The latter, PGRs, are expected to play an increasingly important role in meeting these challenges because plant hormones and growth regulators influence virtually every aspect of plant growth and development.

Most previous research has focussed on studying the effect of plant density and Cycocel (PGR) application on cotton yield and fiber quality (1–4). However, there have been

few studies addressing plant density and Cycocel effects on the quality and quantity of protein and oil from cottonseed (5). In available literature, only one reference exists relating the effect of Alar (PGR) on the cotton plant (6).

The main objective of this study was to evaluate the effects of Cycocel and Alar on the cottonseed, protein and oil yields and oil properties of Egyptian cotton at different hill spacings under field conditions.

## MATERIALS AND METHODS

Two field experiments were conducted at Giza Agricultural Research Station, Agricultural Research Center, Ministry of Agriculture, Egypt during the 1986 and 1987 seasons, with the Egyptian cotton cv. Giza 75 (*Gossypium barbadense*, L.) on a clay loam soil. Treatments were arranged in a randomized complete block design with four replications. Each experiment included 21 treatments, i.e., 3 plant densities × 6 growth retardant concentrations and a control.

Plant densities of 166,000, 222,000 and 333,000 plants/ha were foliar-sprayed with 250, 500 and 750 ppm of either 2-chloroethyl trimethyl ammonium chloride (chlormequat chloride, Cycocel or CCC) or succinic acid 2,2-dimethyl hydrazide (daminozide, SADH, B-Nine, Kylar or Alar). Water alone was sprayed as a control and 166,000 plants/ha was the control for density. This concept is based on the fact that the plant density of 166,000 plant/ha is the recommended density in Egypt. Treatments were foliar-sprayed 105 d after planting, which was during the square-and-bolling stage. Plot size was 3 rows, 4 m long × 0.6 m wide. Plots were planted on April 8, 1986 and April 15, 1987. Seeds were planted in hills, 20, 15 and 10 cm apart within the row and thinned to 2 plants/hill six weeks after planting to give plant densities of 166,000, 222,000 and 333,000 plants/ha. Standard cultural practices of the Research Station were used.

At harvest, total cotton yield/plot was determined. Following ginning, the cottonseed yields were determined in kg/ha and seed index weight in grams per 100 seeds. Laboratory tests were conducted on a 200-g random sample of seed representative of each plot. A composite seed sample of the four replicates of each treatment was used for the chemical analysis. The following chemical analyses were conducted: (i) seed crude protein content according to AOAC (7); (ii) seed oil content in which oil was extracted three times with chloroform/methanol (2:1, vol/vol) mixture according to the method outlined by Kates (8); (iii) oil quality traits, i.e., refractive index, acid value, saponification value, iodine value and unsaponifiable matter were measured according to the methods described by AOCS (9); and (iv) identification and determination of oil fatty acids by gas-liquid chromatography. The lipid materials were saponified, unsaponifiable matter was removed and the fatty acids were separated. The free fatty acids were methylated with diazomethane (10). The fatty acid methyl esters obtained from cottonseed oil were analyzed by a Sigma 3B gas-liquid chromatograph equip-

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ped with dual flame ionization detectors (St. Louis, MO). The separation conditions were similar to those reported by Ashoub *et al.* (11). Results were expressed as an area percentage of the chromatograms. Data obtained for the cottonseed yield and the seed index were statistically analyzed factorially according to Snedecor and Cochran methods (12).

## RESULTS AND DISCUSSION

**Cottonseed yield.** Cottonseed yield/ha significantly decreased as plant density increased (Table 1). Thiagarajan and Ramaswamy (13) found that retention rate of fruiting forms and boll size were much lower in the high plant densities, and percentage boll shedding was lower with wider than with narrower spacing. Nikolov (14) observed that the percentage of plants without fruits was increased at higher plant densities. Photosynthesis by plants at high density is lower than its potential maximum due to mutual shading of leaves (15). Similar results were obtained by Bruyn *et al.* (16).

Both Cycocel and Alar significantly increased cottonseed yield/ha, with no difference among application rates of either compared with untreated plants. Cycocel apparently blocks the gibberellin biosynthesis pathway, promoting flowering (17). Cycocel increased chlorophyll content in all stages of growth. This may be due to the assumption that Cycocel stimulates the formation of phytol, which is an essential compound for chlorophyll formation (18). Gardner (19) found that Alar increased the photosynthetic pigments. Pinkhasov *et al.* (20), studying the C (carbon) balance during different stages of fruiting, found that the outflow of C<sub>14</sub>-assimilates from leaves and their influx into maturing bolls were accelerated by Cycocel. Hsi and Davidson (21) found that Alar increased assimilate partitioning to pods in peanut. Agakishiev and Kuzier

(22) found that application of Cycocel to cotton during fruit formation inhibited stem growth, accelerated boll formation and increased seed cotton yields. For these reasons the increase in cottonseed yield by applied Cycocel and Alar appears to be attributed to the increase in fruiting bodies and accumulation of dry matter in the reproductive parts. These results agree with Azab *et al.* (23), Gadakh *et al.* (24) and Kler *et al.* (4) for Cycocel and with Abdel-Al and Eid (6) for Alar. Interaction was noted between plant density and both Cycocel and Alar in their effect on cottonseed yield/ha, but not on other measured characters (Table 2). The favorable effect of low plant density on cottonseed yield was more obvious when treating cotton plants with Cycocel or Alar, especially Cycocel at 500, 750 ppm and Alar at 250 ppm. This implies that the effect of growth retardant on cottonseed yield depends especially on the number of plants per unit area. It was obvious, however, that the lowest plant density of 166,000 plants/ha produced higher cottonseed yield when either Cycocel or Alar was applied, as compared with the other two densities.

**Seed index.** Widening the distance between hills significantly increased the seed index (Table 1) Terbea *et al.* (25), in sunflower, found that chlorophyll a and b, carotenoid content and total biomass accumulation decrease with increase in plant density. Similar results were obtained by Singh and Warsi (26). Applied Cycocel or Alar at different concentrations significantly increased seed index compared with the control treatment; using Alar either at 500 or 750 ppm gave the greatest differences. This increase in seed index from applied Cycocel and Alar may be due to increased photosynthetic pigments (18,19). In turn, there is a stimulation of photosynthetic activity and dry matter accumulation per plant (27), which may enhance the formation of fully matured seeds and consequently of increased seed weight. These results agree with

TABLE 1

Effect of Plant Density and Growth Retardants on Cottonseed Yield, Seed Index, Seed Oil, Seed Protein, Oil and Protein Yields

Treatments	Cottonseed yield (kg/ha) <sup>a</sup>	Seed index (g) <sup>a</sup>	Seed oil (%) <sup>b</sup>	Oil yield (kg/ha) <sup>b</sup>	Seed protein (%) <sup>b</sup>	Protein yield (kg/ha) <sup>b</sup>
Plant density (plants/ha)						
166,000	1922 <sup>c</sup>	10.91 <sup>c</sup>	19.30	371.0	21.98	423.1
222,000	1874 <sup>c</sup>	10.79 <sup>c</sup>	19.42	363.8	21.78	408.5
333,000	1765	10.67	19.50	343.9	21.52	380.2
L.S.D. 0.05 <sup>d</sup>	56	0.07				
S.E. <sup>d</sup>			0.058	8.10	0.13	12.59
Cycocel and Alar, concentration (ppm) <sup>e</sup>						
Control, 0	1679	10.52	19.37	325.0	21.22	356.4
Cycocel, 250	1830 <sup>c</sup>	10.64 <sup>c</sup>	19.39	355.0	22.08	404.6
Cycocel, 500	1851 <sup>c</sup>	10.63 <sup>c</sup>	19.44	359.8	21.76	403.1
Cycocel, 750	1955 <sup>c</sup>	10.75 <sup>c</sup>	19.45	379.6	21.54	421.5
Alar, 250	1935 <sup>c</sup>	10.92 <sup>c</sup>	19.46	376.9	21.72	420.7
Alar, 500	1869 <sup>c</sup>	11.03 <sup>c</sup>	19.35	361.3	21.86	409.0
Alar, 750	1859 <sup>c</sup>	11.04 <sup>c</sup>	19.37	359.5	22.16	412.1
L.S.D. 0.05	85	0.10				
S.E.			0.017	6.76	0.12	8.37

<sup>a</sup>Combined statistical analysis from 1986 and 1987 seasons.

<sup>b</sup>Mean data from a four replicate composite for 1986 and 1987 seasons.

<sup>c</sup>Significant at 5% level.

<sup>d</sup>L.S.D. = least significant difference, S.E. = standard error.

<sup>e</sup>Data represent the mean of all three densities.

## EFFECT OF PLANT POPULATION DENSITIES/APPLICATION OF GROWTH RETARDANTS

TABLE 2

Effect of Interaction Between Plant Density and Growth Retardants on Cottonseed Yield (kg/ha)

Cycocel and Alar, concentration (ppm)	Plant density (plants/ha)		
	166,000	222,000	333,000
Control, 0	1742	1675	1620
Cycocel, 250	1941	1756	1795
Cycocel, 500	2054	1839	1659
Cycocel, 750	1981	2021	1862
Alar, 250	1997	2023	1784
Alar, 500	1879	1947	1781
Alar, 750	1862	1858	1856
L.S.D. 0.05 <sup>a</sup>	148		

<sup>a</sup>L.S.D. = least significant difference.

those of Azab *et al.* (23), and Kler *et al.* (4) for Cycocel and with Abdel-Al and Eid (6) for Alar.

**Seed oil concentration and yield.** Seed oil concentration increased slightly as plant density increased but oil yield/ha decreased, which is attributed to the reduction in cottonseed yield (Table 1). Similar results were obtained by El-Kholany and Sawan (28) and Kotecki and Malarz (29) in sunflower. Gubanova *et al.* (5) found that the oil content at 140,000–210,000 plants/ha was similar, and higher than at 70,000 plants/ha.

In most cases, application of Cycocel and Alar slightly increased the oil concentration above the control. Also, both Cycocel and Alar increased the seed oil yield/ha over the control, with the clearest effect from Cycocel at the highest concentration, followed by Alar at the lowest concentration. Kariev (30) observed that applied Cycocel to cotton increased seed oil content. Al-Gharbi and Yousif (31) found that Cycocel increased the sunflower seed oil content. Zafirova *et al.* (32) reported that Alar increased seed and oil yields of sunflower.

**Seed protein content and yield.** Wide hill spacing increased the seed protein content and yield/ha (Table 1). Seed protein content and protein yield/ha were increased in plants treated with Cycocel and Alar compared to the

untreated control. Highest protein content was produced by Alar applied at 750 ppm, followed by Cycocel at 250 ppm, while the highest seed protein yield was observed when Cycocel was applied at 750 ppm, followed by Alar at 250 ppm. Hedin *et al.* (33) found that Cycocel increased protein content by 17–50% in leaves and squares harvested 4 wk after first application. Kler *et al.* (4) observed that Cycocel increased seed protein content. Kar *et al.* (34) in safflower showed that Cycocel and Alar maintained the level of chlorophyll, protein and RNA contents.

**Seed oil properties.** Increasing plant density was accompanied by an increase in oil acid value and saponification value, and by a decrease in oil refractive index, iodine value and unsaponifiable matter (Table 3). Both Cycocel and Alar tended to increase the oil refractive index, iodine value and unsaponifiable matter, and to decrease acid value and saponification value compared with the untreated control. Applied Cycocel at 250 ppm gave the highest refractive index and unsaponifiable matter and the lowest acid value, while Alar at 250 ppm give the highest iodine value and the lowest saponification value. Osman and Abu-Lila (35) found a negligible variation in refractive index and decrease in iodine value of flax oil when the plants were treated with Cycocel at the application rates of 25 and 50 ppm.

**Oil fatty acid composition.** Results showed trace amounts of the saturated fatty acids caprylic and capric at 333,000 plants/ha, while tridecylic was found at 166,000 plants/ha. Lauric acid was found at all plant densities, but increased fourfold as the plant density increased from 166,000 to 333,000 plants/ha. Because these four fatty acids were found only in trace amounts, they were not tabulated. Myristic acid increased gradually by increasing the plant densities (Table 4). Palmitic acid was the predominant saturated fatty acid, and the high content was at the intermediate plant density. As plant population increased from 166,000 plants/ha, the percentage of stearic acid increased by 2.32- and 4.91-fold for 222,000 and 333,000 plants/ha, respectively. These results indicate that increasing plant density encourages the synthesis of most saturated fatty acids.

Application of the growth retardant Cycocel resulted

TABLE 3

Effect of Plant Density and Growth Retardants on Seed Oil Properties<sup>a</sup>

Treatments	Refractive index	Acid value	Saponification value	Iodine value	Unsaponifiable matter (%)
Plant density (plants/ha)					
166,000	1.4686	0.333	192.8	109.1	0.354
222,000	1.4683	0.338	193.7	108.7	0.353
333,000	1.4683	0.343	193.9	108.6	0.349
S.E.	0.0001	0.0029	0.338	0.153	0.0015
Cycocel and Alar, concentration (ppm)					
Control, 0	1.4681	0.353	194.3	107.3	0.337
Cycocel, 250	1.4688	0.320	194.0	109.0	0.367
Cycocel, 500	1.4686	0.350	193.0	107.7	0.360
Cycocel, 750	1.4683	0.350	193.5	107.3	0.363
Alar, 250	1.4684	0.337	192.8	110.7	0.350
Alar, 500	1.4682	0.330	193.2	110.0	0.347
Alar, 750	1.4682	0.327	193.7	109.7	0.340
S.E.	0.000095	0.0049	0.20	0.52	0.0044

<sup>a</sup>Mean data from a four replicate composite for 1986 and 1987 seasons. S.E. = standard error.

TABLE 4

Effect of Plant Density and Growth Retardants on the Relative Percentage of Saturated Fatty Acids<sup>a</sup>

Treatments	Relative % of saturated fatty acids			
	Myristic	Palmitic	Stearic	Total <sup>a</sup>
Plant density (plants/ha)				
166,000	0.246	28.43	0.318	29.12
222,000	0.413	31.20	0.737	32.40
333,000	0.651	30.17	1.560	32.98
S.E.	0.118	0.809	0.365	1.202
Cycocel and Alar, concentration (ppm)				
Control, 0	0.257	39.11	0.613	39.98
Cycocel, 250	0.527	30.62	1.037	32.30
Cycocel, 500	0.297	30.06	0.970	31.33
Cycocel, 750	0.333	30.35	0.647	31.48
Alar, 250	0.130	27.65	0.560	28.34
Alar, 500	0.393	25.45	1.140	28.35
Alar, 750	0.120	27.31	1.137	28.73
S.E.	0.168	1.739	0.097	1.544

<sup>a</sup>Mean data from a four replicate composite for 1986 and 1987 seasons. S.E. = standard error.

<sup>b</sup>Total includes trace amounts of caprylic, capric, lauric and tridecylic saturated fatty acids.

TABLE 5

Effect of Plant Density and Growth Retardants on the Relative Percentage of Unsaturated Fatty Acids<sup>a</sup>

Treatments	Relative % of unsaturated fatty acids				TU/US <sup>c</sup> ratio
	Palmitoleic	Oleic	Linoleic	Total <sup>b</sup>	
Plant density (plants/ha)					
166,000	2.80	20.23	47.83	70.88	2.43
222,000	4.29	19.67	43.64	67.59	2.08
333,000	5.96	19.40	41.54	67.02	2.03
S.E.	0.91	0.24	1.85	1.20	0.126
Cycocel and alar, concentration (ppm)					
Control, 0	5.32	15.280	39.41	60.02	1.50
Cycocel, 250	4.95	21.86	40.88	67.69	2.09
Cycocel, 500	4.00	16.59	48.07	68.67	2.19
Cycocel, 750	1.41	21.28	45.81	68.51	2.17
Alar, 250	5.28	16.61	49.76	71.65	2.52
Alar, 500	6.25	20.83	44.26	71.64	2.52
Alar, 750	3.22	25.90	42.14	71.27	2.48
S.E.	0.61	1.43	1.44	1.54	0.137

<sup>a</sup>Mean data from a four replicate composite for 1986 and 1987 seasons. S.E. = standard error.

<sup>b</sup>Total includes myristoleic acid.

<sup>c</sup>TU/TS ratio = (total unsaturated fatty acids) ÷ (total saturated fatty acids).

in trace amounts of lauric and tridecylic acid. Myristic acid content increased due to Cycocel at 250 ppm. The abundant saturated fatty acid palmitic decreased by the application of Cycocel compared to the untreated control. Stearic acid increased when Cycocel was applied at 250 and 500 ppm. Alar applied at 500 ppm resulted in trace amounts of caprylic, capric, lauric and tridecylic acid. Alar applied at 750 ppm produced trace amounts of caprylic and lauric acid. Palmitic acid decreased by the application of Alar compared with control. Stearic acid showed a slight decrease when Alar was applied at 250 ppm and increased twofold when applied at 750 ppm, compared to the control. Myristic acid tended to decrease when Alar

was applied at 250 and 750 ppm, while it tended to increase when applied at 500 compared to the control.

The unsaturated fatty acid palmitoleic increased with higher plant densities (Table 5). Linoleic acid was the most abundant unsaturated fatty acid followed by oleic acid. A slight decrease in both oleic and linoleic acids was observed as plant densities increased. The ratio between total unsaturated fatty acids and total saturated fatty acids (TU/TS) decreased as plant densities increased.

Applied Cycocel or Alar produced an oil characterized by absence of myristoleic acid except for a trace at 500 ppm Alar. Levels of palmitoleic acid were low, variable and equal to the control only at 250 and 500 ppm Alar. Both

Cycocel and Alar increased oleic acid compared with the control. The highest increases were found with applications of 750 ppm Alar, followed by Cycocel at 250 and 750 ppm. Applied Cycocel and Alar produced an oil characterized with high linoleic acid content compared with the control. Alar at 250 ppm, followed by Cycocel at 500 ppm, caused the highest increase in linoleic acid. The ratio between total unsaturated and saturated fatty acids revealed that Cycocel and Alar were consistently higher than the control. These data are in agreement with those obtained by Sawan *et al.* (36) with Pix (plant growth retardant). Osman and Ahmed (37) found that spraying sesame plants with Alar caused an increase in the unsaturated fatty acids. Osman and Abu-Lila (35) indicated that spraying flax plants with Cycocel increased the levels of unsaturated fatty acids. The increase in TU/TS ratio may be attributed to Cycocel and Alar growth regulators encouraging enzymes that catalyzed the biosynthesis of the unsaturated fatty acids. The PGRs promote or inhibit enzyme activities intervening in the sequence of chemical events in which genes control the synthesis of enzymes.

Based on the conditions prevalent during this study, the low plant density (166,000 plants/ha) and application of Cycocel at 750 and Alar at 250 ppm are recommended for improvement of the characters evaluated. Those characters were cottonseed yield/ha, seed index, seed protein content, oil and protein yield/ha, oil refractive index, iodine value, unsaponifiable matter and unsaturated fatty acids. There was a decrease in oil acid value and saponification value.

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