Direct and Residual Effect of Foliar Applications of Copper and Manganese on Cottonseed Yield and Quality

Zakaria M. Sawan,*,† Ahmad E. Basyony,‡ Willis L. McCuistion,§ and Abdel Hamid A. El Farra‖

Cotton Research Institute, Field Crops Research Institute, National Agricultural Research Project, and Central Laboratory of Food and Feed, Agricultural Research Center, Giza, Egypt

In 1986–1987 field experiments at Giza, Egypt, cotton cv. Giza 75 was given foliar application of the micronutrients copper and manganese (Cu at 0.0, 12.5, 25.0, and 37.5 ppm and Mn at 0.0, 25.0, and 50.0 ppm) applied at 85 and 105 days after sowing. Individual Cu or Mn applications increased cottonseed yield and seed index above those of the control (0.0 Cu, 0.0 Mn). Cu at 37.5 ppm with Mn at 50 ppm gave the highest seed oil content. Highest oil and protein yields resulted from Cu and Mn at 25 ppm, when applied separately, while oil refractive index, and iodine value increased from Cu and Mn added separately or in combination. Unsaponifiable matter increased as a result of almost all treatments. All Cu or Mn treatments, applied either separately or in combination, generally decreased saturated fatty acids (myristic, palmitic, and stearic) and increased unsaturated fatty acids (palmitoleic, oleic, and linoleic). Cu at 12.5 ppm or Mn at 25 ppm gave the lowest levels of saturated fatty acids.

INTRODUCTION

Cotton is one of the most important crops in Egypt, where it is consumed locally or exported. Fertilization is one of the main practices contributing to increases in the productivity and quality of cotton. The use of chemically pure fertilizers necessitates the supply of micronutrients in intensive agriculture. The demand for micronutrients in plants is rather low; however, they are essential to prevent morphological, physiological, and biochemical deficiencies and assure optimum yield and quality. Monitoring the availability of micronutrients is important in Egypt due to the marked decrease of these elements in Nile River water following the construction of the Aswan High Dam. Increasing soil pH decreases the availability of Cu and Mn in soils. Most Egyptian soils are alkaline, with pH values between 7.5 and 8.5. Under conditions of calcareous soil or high percentages of calcium carbonate, available micronutrients will be low (Buchner and Sturm, 1980).

Cottonseed is considered an essential industrial source of edible oil and meal. Many changes have occurred in Egyptian cotton production over the past four decades. The land used for cotton production declined from a high point of 840 000 ha in the early 1950s (Wally, 1982) to a low level of 360 000 ha in 1991. Much of this decline is attributed to the increased demand of cereal crops required for the rapidly increasing population in Egypt. For this reason, an increase in cotton production per unit area is necessary. The effect of Cu and Mn nutrition on seed yield and fiber quality has been extensively studied (Isaev and Mamanov, 1980; Mamanov et al., 1982; Khuzhanazarov et al., 1983; Sawan, 1985). Few studies have documented the effect of Cu and Mn on seed protein, oil content, and oil properties (Tailakov and Ataev, 1973; Khalileva and Yusupov, 1985; Sawan et al., 1988).

This investigation was designed to test the hypothesis that foliar application of Cu- or Mn-EDTA and their combination at different concentrations, during square initiation and bolling stage, will have positive effects on seed yield and quality characters of Egyptian cotton.

MATERIALS AND METHODS

Two field experiments were carried out during 1986 and 1987 seasons at Giza Agricultural Research Station, Agricultural Research Center, Ministry of Agriculture, Egypt, using the cotton cv. Giza 75 (*Gossypium barbadense* L.). Each experiment included 12 treatments, which were the combination of the chelated form [ethylenediaminetetraacetic acid (EDTA)] of the microelements Cu (at 0.0, 12.5, 25.0, and 37.5 ppm) and Mn (at 0.0, 25, and 50.0 ppm). Each treatment was sprayed twice, first at 85 and then at 105 days after the sowing date (during square initiation and bolling stages). The volume of solution used for each treatment was 960 L/ha. The average mechanical and chemical analyses of the soil (Jackson, 1973) are shown in Table I. Soil micronutrients were extracted by DTPA (Lindsay and Norvell, 1969), and available soil phosphorus was extracted by sodium bicarbonate (Olsen et al., 1954); pH 8.5 is considered medium. Total soluble nitrogen was extracted by 1% potassium sulfate solution, as described by Allam (1951).

The experimental design used was a randomized complete block with four replications. Sowing dates were April 8 and 15 for 1986 and 1987, respectively. Plot size was 1.8 × 4 m including three ridges. Cultural practices were standard for cotton production at the Giza Agricultural Research Station. Total cotton yield per plot was determined at harvest. Cottonseed yield (kilograms per hectare) and seed index (weight in grams of 100 seeds) were determined following ginning. Laboratory tests were conducted on a 200-g random sample of seed representative of each plot. A composite seed sample representing the four replications of each treatment was used for the following chemical analyses: (a) the seed crude protein content according to AOAC (1985); (b) the seed oil content from which oil was extracted three times with a chloroform–methanol (2:1 v/v) mixture according to the method outlined by Kates (1972); (c) the oil quality traits, i.e., refractive index, acid value, saponification value, iodine value, and unsaponifiable matter, which were measured according to the methods described by AOCS (1985); and (d) the identification and determination of oil fatty acids by gas–liquid chromatography (GLC). The lipid materials were saponified, unsaponifiable matter was removed, and the fatty acids were collected. The free fatty acids were methylated with diazomethan (Vogel, 1975). The fatty acids, methyl esters, obtained from cottonseed oil were analyzed by a Sigma 3B gas–liquid chromatograph equipped with dual flame ionization detector. The separation conditions were similar to those

* To whom correspondence should be addressed at Agricultural Research Center, 9 Gamaa Street, Giza, Egypt.
† Cotton Research Institute.
‡ Field Crops Research Institute.
§ National Agricultural Research Project.
‖ Central Laboratory of Food and Feed.

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Table I. Mechanical and Chemical Analysis of the Soil

mechanical analysis		chemical analysis	
clay, %	43.00	pH (1:2.5)	8.13
silt, %	28.40	total nitrogen, %	0.12
fine sand, %	19.33	soluble nitrogen, ppm	27.50
coarse sand, %	4.31	available phosphorus, ppm	15.00
organic matter, %	1.83	available potassium, ppm	270.00
calcium carbonate, %	3.00	available zinc, ppm	1.33
total soluble salts, %	0.13	available iron, ppm	4.35
texture: clay loam		available manganese, ppm	3.57
		available copper, ppm	0.45

Table II. Effect of Foliar Application of Cu and Mn on Cottonseed Yield, Seed Index, Seed Oil, Seed Protein, and Oil and Protein Yields

treatment, ppm		cottonseed yield,[a] kg/ha	seed index,[a] g	seed oil,[b] %	oil yield,[b] kg/ha	seed protein,[b] %	protein yield,[b] kg/ha
Cu	Mn						
0.0	0.0	1886.3	10.60	18.74	352.1	22.10	415.4
	25.0	2174.5[c]	10.78[c]	19.11	415.8	21.80	474.3
	50.0	2117.6[c]	10.72[c]	19.22	406.2	21.72	459.4
12.5	0.0	2088.5[c]	10.75[c]	19.21	401.1	22.03	460.0
	25.0	1966.2	10.70[c]	19.14	374.3	22.13	432.7
	50.0	1926.8	10.68	19.18	367.6	22.10	423.4
25.0	0.0	2175.2[c]	10.84[c]	19.20	416.1	21.97	476.0
	25.0	1904.2	10.66	19.32	365.8	21.75	411.6
	50.0	1894.3	10.65	19.36	364.8	21.68	408.6
37.5	0.0	2116.7[c]	10.74[c]	19.26	406.8	21.77	459.8
	25.0	1891.3	10.59	19.26	362.6	21.75	409.2
	50.0	1872.4	10.59	19.38	361.2	21.65	403.2
LSD for Cu × Mn	0.05	135.50	0.098				
SE[d]				0.048	6.96	0.052	8.02

[a] Mean values from combined data of 1986 and 1987 seasons. [b] Mean data of two samples from a four-replicate composite for 1986 and 1987 seasons. [c] Significant at 5% level. [d] SE, standard error.

reported by Ashoub et al. (1989). Results are expressed as an area percentage of the chromatograms. Data obtained for the cottonseed yield and the seed index were statistically analyzed factorially according to the method of Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Cottonseed Yield. Copper significantly increased cottonseed yield per hectare as treatment rate increased to 37.5 ppm, with 25 ppm being the highest, compared to the control (Table II). No significant differences occurred among combined Cu and Mn rates. Similarly, Mn at rates up to 50 ppm significantly increased yield, with the highest yield at 25 ppm. Such results could be attributed to the connection of Mn photosynthesis and NO_3^- reduction (Anderson and Pyliotis, 1969). Also, Cu in protein DNA and RNA synthesis (Ozolina and Lapina, 1965) and carbohydrate metabolism (Brown and Jones, 1975) in plants can cause an activation of photosynthesis and enhance the growth and development. Graham (1975) found a corresponding high localized demand for Cu and the synchronous meiotic division of pollen mother cells. All of these may have a favorable effect on yield components, i.e., number of opened bolls per plant, boll weight, or both, reflecting better yield. It is likely that a high rate of Cu or Mn (more than 25 ppm) may produce some physiological imbalances in the nutrition of the plants. From the data obtained, it is worth considering why the addition of Cu and Mn in combination had no effect on the cottonseed yield. This may be due to the antagonism between the two elements in the plant. The soil levels were clearly below the response threshold for both nutrients. This can be explained only in terms of the balance between supply and demand. Under conditions of high potential fruit set, the cotton plant may need additional readily available amounts of Cu and Mn at critical times. Foliar spray thus seems to be an efficient mode of application because of rapid availability at the critical period of demand and lack of interference. The favorable effects of these elements on cottonseed yield have also been observed by Mamanov et al. (1982), Khuzhanazarov et al. (1983), Sawan (1985), and Hegab et al. (1986).

Seed Index. Seed index (grams per 100 seeds) significantly increased with Cu or Mn application at different rates compared to that of the control (Table II). The 25 ppm treatment of either Cu or Mn alone gave the highest seed index. Only one combined application, 12.5 ppm of Cu with 25 ppm of Mn, significantly increased the seed index over that of the control; other combinations had no significant effect. Copper plays a role in photosynthesis, as it is a part of the chloroplast enzyme plastocyanin in the electron transport system between photosystems I and II (Gardner et al., 1985). Agarwala et al. (1989) found in maize that Cu deficiency decreased dry weight, chlorophyll, leaf Cu and protein N contents, and cytochrome oxidase and nitrate reductase activities. Manganese is capable of bridging with certain enzymes e.g., phosphokinase and phosphotransferase (Gardner et al., 1985). Thus, applied Cu and Mn may increase the dry matter accumulation in reproductive parts of cotton plants, which may have a direct impact on seed weight. Isaev and Mamanov (1980), applying Cu alone, and Sawan (1985), using Cu or Mn, also reported that seed index was increased.

Seed Oil Content and Yield. Applying Cu or Mn at different rates, alone or in combination, resulted in an increase in seed oil content over that of the control (Table II). The seed oil content tended to increase by increasing the combined concentration of Cu and Mn with the highest value obtained from Cu at 37.5 ppm and Mn at 50 ppm. Oil yields from treatments compared to the control were 1.14-, 1.18-, and 1.16-fold when cotton plants were treated with Cu at 12.5, 25.0, or 37.5 ppm, respectively. Oil yields were 1.18- and 1.15-fold higher than the control with Mn treatments at 25 or 50 ppm. The highest oil yields were obtained from Cu or Mn alone applied at 25 ppm. Sharafutdinova et al. (1974) found that seed treatment with $CuSO_4$ accelerated lipid synthesis and increased the oil content of the seeds. These results were supported by Tailakov and Ataev (1973) and Sawan et al. (1988) for Mn and Cu and by Khalileva and Yusupov (1985) with Cu.

Seed Protein Content and Yield. Copper or manganese applied at different rates, alone or in combination, did not affect the seed protein content as compared to that of the untreated control (Table II). Copper increased protein yield as treatment rate increased to 37.5 ppm, compared to untreated plants; Cu at 25 ppm gave the best result. Similarly, Mn at rates up to 50 ppm increased protein yield, with highest yield from 25 ppm. The combination of Cu and Mn had no effect on protein yield. The data obtained were in line with the findings of Tailakov (1976) and Sawan et al. (1988).

Seed Oil Properties. Application of Cu and Mn produced a slight increase in the oil refractive index and iodine value over those of the control (Table III). Neither Cu nor Mn caused differences on the oil acid value. Oil saponification value tended to decrease by application of the higher rates of Cu and Mn. Unsaponifiable matter increased as a result of all treatments, except that of the

Table III. Effect of Foliar Application of Cu and Mn on Seed Oil Properties[a]

treatment, ppm		refractive index	acid value	saponification value	iodine value	unsaponifiable matter
Cu	Mn					
0.0	0.0	1.4665	0.30	195	100	0.38
	25.0	1.4672	0.29	195	103	0.44
	50.0	1.4670	0.30	193	102	0.51
12.5	0.0	1.4674	0.30	195	106	0.42
	25.0	1.4681	0.30	194	108	0.45
	50.0	1.4686	0.30	194	111	0.38
25.0	0.0	1.4687	0.31	193	105	0.47
	25.0	1.4682	0.31	193	109	0.41
	50.0	1.4693	0.30	192	113	0.62
37.5	0.0	1.4688	0.30	191	115	0.55
	25.0	1.4710	0.31	194	118	0.58
	50.0	1.4700	0.29	193	114	0.63
SE		0.00037	0.002	0.36	1.63	0.026

[a] Mean data for 1986 and 1987 seasons.

Table IV. Effect of Foliar Application of Cu and Mn on the Relative Percentage of Saturated Fatty Acids[a]

treatment, ppm		relative % of saturated fatty acids			
Cu	Mn	myristic	palmitic	stearic	total
0.0	0.0	0.24	34.00	2.31	36.55
	25.0	0.32	27.73	0.38	28.43
	50.0	0.55	32.70	0.10	33.35
12.5	0.0	0.73	25.00	0.78	26.51
	25.0	1.68	29.28	2.52	33.88
	50.0	0.37	34.46	1.22	36.05
25.0	0.0	0.42	32.84	0.18	33.44
	25.0	0.36	32.47	1.00	33.83
	50.0	1.00	32.05	1.00	34.05
37.5	0.0	0.94	30.68	0.47	32.09
	25.0	0.30	34.27	0.43	35.00
	50.0	1.70	33.00	1.65	36.35
SE		0.1494	0.8323	0.2305	0.9482

[a] Mean data for 1986 and 1987 seasons.

combination between Cu at 12.5 ppm and Mn at 50 ppm, which was the same as the control. The increment of the unsaponifiable matter is known to be beneficial for its role in oil stability; thus, its components need further study. Sawan et al. (1988) found that oil unsaponifiable matter tended to increase, while iodine and saponification values tended to decrease, by the application of Cu and Mn.

Oil Fatty Acid Composition. The effects of Cu and Mn as foliar application to cotton plants on the relative percentage of fatty acid are shown in Tables IV and V. Stearic acid content of cottonseed oil from the untreated control was 2.31%, while palmitic acid was found to be the major saturated fatty acid (34%). Applying Cu alone at 12.5 ppm increased myristic acid, while a marked decrease was found in palmitic and stearic acids compared to control. Copper at 25 ppm caused a sudden decrease in stearic acid. Myristic acid increased 4-fold for the Cu (37.5 ppm) above the control; however, there was a slight decrease in the amount of palmitic acid and a marked decrease in stearic acid.

Applying Mn at 25 ppm markedly decreased the palmitic and stearic acids. Manganese at 50 ppm increased myristic acid 2-fold and decreased palmitic acid compared to the control. In general, the combined treatments of Cu and Mn showed an increase in the three saturated fatty acids compared to each element alone.

Applied Cu treatments alone resulted in reduced percentages of palmitoleic and oleic acids and increased

Table V. Effect of Foliar Application of Cu and Mn on the Relative Percentage of Unsaturated Fatty Acids[a]

treatment, ppm		relative % of unsaturated fatty acids				TU/TS
Cu	Mn	palmitoleic	oleic	linoleic	total	ratio[b]
0.0	0.0	2.50	22.31	37.84	62.65	1.677
	25.0	3.51	16.34	51.42	71.27	2.481
	50.0	1.37	16.28	49.00	66.65	1.999
12.5	0.0	0.50	19.21	53.10	72.81	2.679
	25.0	0.70	20.76	44.46	65.92	1.946
	50.0	5.08	19.07	38.87	63.02	1.704
25.0	0.0	1.80	19.00	45.27	66.07	1.948
	25.0	5.23	20.13	39.30	64.66	1.830
	50.0	3.12	19.54	40.42	63.08	1.709
37.5	0.0	1.50	22.55	41.81	65.86	1.929
	25.0	1.87	21.10	41.80	64.77	1.839
	50.0	2.38	19.83	40.24	62.45	1.663
SE		0.443	0.566	1.472	0.947	0.0920

[a] Mean data for 1986 and 1987 seasons. [b] TU/TS ratio = (total unsaturated fatty acids)/(total saturated fatty acids).

linoleic acid compared to control. Manganese treatment alone at 25 ppm caused an increase in palmitoleic and linoleic acids, while oleic acid decreased compared with control. Manganese at 50 ppm, in contrast to 25 ppm, decreased palmitoleic acid but had no effect on oleic and linoleic acids. In general, the lowest concentration of either Cu or Mn applied separately produced an oil characterized by high levels of unsaturated fatty acids. The combined treatments of Cu and Mn at different levels decreased all of the unsaturated fatty acids compared to the single applications, but the levels were still higher than those of the control, especially in linoleic acid content.

The ratio between the total unsaturated and saturated fatty acids showed that all treatments gave higher ratios than the control, except for the treatment of Cu at 37.5 ppm with Mn at 50 ppm. Low content of saturated fatty acids is desirable for edible uses. In seeds, all of the fatty acids produced can be esterified with glycerol to produce fats that develop into oleosomes directly in the endoplasmic reticulum (Salisbury and Ross, 1986). Copper and manganese may be affected in these steps because Cu is present in several enzymes and proteins involved in oxidation and reduction. Two notable examples are cytochrome oxidase, a respiratory enzyme in mitochondria, and plastocyanin, a chloroplast protein. Biochemical work shows that Mn plays a structural role in the photosynthetic split of H_2O. The Mn^{2+} ion activates numerous enzymes (Salisbury and Ross, 1986). These data are in agreement with those of Sharafutdinova and Mutalov (1978) and Wilson et al. (1982).

Conclusion. Under the conditions of this study, it is concluded that applying Cu or Mn individually at the concentration of 25 ppm gave the highest cottonseed yield, seed index, and oil and protein yields. This concentration also generally increased oil refractive index, iodine value, and unsaponifiable matter over the control. It also generally decreased the saturated fatty acids with an increase in the unsaturated fatty acids compared to the untreated control.

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