J. King Saud Univ., Vol. 5, *Science* (2), pp. 107–117 (A.H. 1413/1993)

Senescence of Date Palm Leaf and Copper Salts

M.H. Al-Whaibi
Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

(Received 28 June 1992; Accepted for publication 19 December 1992)

Abstract. The changes in chlorophyll (chl) content of detached segments from four cultivars of date palm *(Phoenix dactylifera* L.) incubated in the dark for four days were investigated as affected by mono and divalent ions. Copper (Cu^{++}) and Zinc (Zn^{++}) salts (acetate, chloride, nitrate and sulphate) as well as KCl were used in three concentrations (10^{-6}, 10^{-4}, and 10^{-2} M). This study reveals that there is a specific ion effect on the retardation of Chl *b* senescence and enhance diminution of Chl *a*. The most effective ion in this system was Cu^{++}, where at 10^{-2} M it enhances the degradation of Chl *a* and increases the relative amount of Chl *b* in all cultivars. Chl content in the four cultivars was also found to be different. It seems that Cu^{++} interacts with the protective mechanism of Chl *a* and causes the shift metabolism resulting in an increase of Chl *b* content relative to the control level.

Introduction

The changes in pigment content during leaf development is a result of the balance between biosynthesis and degradation of these pigments [1, pp. 691-696]. On the other hand, the loss or deceleration of chlorophyll pigments at the end of development (chlorosis) is commonly observed symptoms that result from different environmental conditions such as etiolation, iron deficiency and heavy metal toxicity. For example, when green seedlings or detached leaves are placed into darkness, an enzymatic activity that destroys magnesium porphyrins increases in parallel with chlorophyll destruction [2, pp. 115-123]. As for heavy metal toxicity, plants differ in their tolerance. Depending on the concentrations of heavy metals (10^{-4} M Zn^{++}) in the root media of different ecotypes of *Agrostis tenuis*, susceptible plants lost 50% of their chlorophyll content while tolerant ecotypes were unaffected. Likewise, when leaf segments of these plants were floated on solutions containing zinc or copper ions in the dark for four days, there was a greater loss of chlorophyll content in leaf segments of susceptible ecotypes but not in those of tolerant ones [3, pp. 231-259].

Furthermore, few other reports indicate that lower concentrations of some heavy metals retarded the destruction of chlorophyll. Higher concentrations of these ions enhanced the chlorophyll destruction [4].

It has been observed that some cultivars of date palm trees (*Phoenix dactylifera* L.) growing together at certain locations in central Saudi Arabia show earlier yellowing of older leaves than other trees at the same location. It is, therefore, of interest to test the effect of some heavy metals that are considered as micronutrients to higher plants on the degradation of chlorophyll in detached leaflets of some date palm cultivars.

Materials and Methods

Segments (1 cm) of date palm leaflets were washed thoroughly with distilled water several times to remove dust. The leaves were chosen to be of the same age as much as possible. These segments were then blotted, weighed and put in test tubes containing the treatment solutions. These solutions consisted of different concentrations ($0, 10^{-6}$ 10^{-4}, or 10^{-2} M) of Zn^{++} or Cu^{++} salts. The salts were acetate, chloride, nitrate and sulphate. Leaflets were taken from cultivars (one tree each) growing on the same location in the Riyadh area. The cultivars were Barhi, Sekkeri and Osaila female trees while the fourth was a male grown from Barhi seed, which will be designated as Barhi male. All the trees were about 8 years old, of the same vigour and without any obvious disease. Potassium chloride was used to check the specific ion effects. The segments were left in the dark (23°C) for four days then transferred to N,N- dimethylformamide for one day and their chlorophyll content determined spectrophotometrically using a Pye Unicam SP7-500 UV-Visible spectrophotometer according to the method of Moran and Porath [5]. All data are the mean ± SD of four replicates or as % of distilled water as control.

Results

Chlorophyll (Chl) content of the four cultivars of date palm is shown in Table 1 for zero time and then control (100%) when the segments of leaflets were incubated in distilld water for four days in darkness at 23°C. As calculated, female Barhi contained about 26% more Chl *b* than the male. Reduction in Chl content after four days in the dark was 28,33,21 and 60% of the control for female Barhi, male Barhi, Sekkeri and Osaila cultivars, respectively. In male Barhi and Osaila the reduction in Chl *a* was greater than that of Chl *b* by about 12%.

The effect of incubating leaflet segments in different concentrations of four copper salts on the degradation of Chl *a* in female Barhi cultivar is illustrated in Fig. 1A. It is clear that there were slight changes of Chl *a* content over the control when both 10^{-6} and 10^{-4} M copper salts (acetate, chloride, nitrate and sulphate) were used. A

Table 1. Chlorophyll content (mg/g Fresh wt.) of the four cultivars at zero time after 4 days in distilled water incubated in the dark

Cultivar	chl a		chl b		Total	
	Zero	H_2O	Zero	H_2O	Zero	H_2O
Barhi ♀	1.39±0.16	0.94±0.05	0.93±0.08	0.63±0.01	2.22±0.15	1.58±0.10
Barhi ♂	1.34±0.09	0.81±0.05	0.68±0.04	0.49±0.01	1.97±0.12	1.31±0.05
Sekkeri	1.35±0.12	1.07±0.06	0.83±0.06	0.64±0.05	2.20±0.15	1.73±0.10
Osaila	1.49±0.22	0.54±0.03	0.79±0.08	0.38±0.10	2.29±0.32	0.91±0.04

significant decrease of Chl *a* occurred at the high concentration (10^{-2} M) of all salts used. Similar indications of the same treatments on Chl *a* of male Barhi could be perceived from Fig. 2A. As for Chl *b*, low contentration (10^{-6} M) of these salts had little effect on either male or female Barhi but, as the concentrations increased, the relative Chl *b* content increased (Fig. 1B and Fig. 2B). Interestingly, copper nitrate or sulphate at 10^{-2} M showed a remarkable percentage increase of Chl *b* (almost double the value of control) in male Barhi (Fig. 2B) compared to the female.

Chlorophyll *a* content of Sekkeri cultivar was affected similarly to that of Barhi (Fig. 3A). The relative chlorophyll *b* content of Sekkeri increased proportionally to the concentration of copper salts (Fig. 3B). The higher concentration (10^{-2}) of almost all salts resulted in almost double the relative content of Chl *b*. However, the salts showed somewhat different effects where chloride caused the lowest value (192%) while nitrate caused the highest value (226%) of all salts in this cultivar.

Figure 4A shows the effect of copper salts on the Chl *a* content of the cultivar Osaila. A slight increase (more than 35% of the control when sulphate was used) is noticed at low concentrations of copper but at 10^{-2} M, copper caused a decrease similar to that in other cultivars. Chlorophyll *b* content of this cultivar was not changed at 10^{-6} M of all salts but at 10^{-4} M a slight increase (up to 20% in the case of nitrate) was observed. At the higher concentration (10^{-2} M) a significant percentage increase of Chl *b* content (double the amount of the control when sulphate was used) is clear (Fig. 4B).

When similar salts of zinc were checked in three cultivars (female and male Barhi and Sekkeri) Chl *a* and *b* of both male and female Barhi decreased or increased slightly, depending on the salt, as shown in Table 2. The Table contains also the values of Chl *a* and *b* content of Sekkeri cultivar as affected by zinc salts treatment. Both Chl *a* and *b* either did not change or increased slightly (not more than 21% when 10^{-2} M zinc acetate was used).

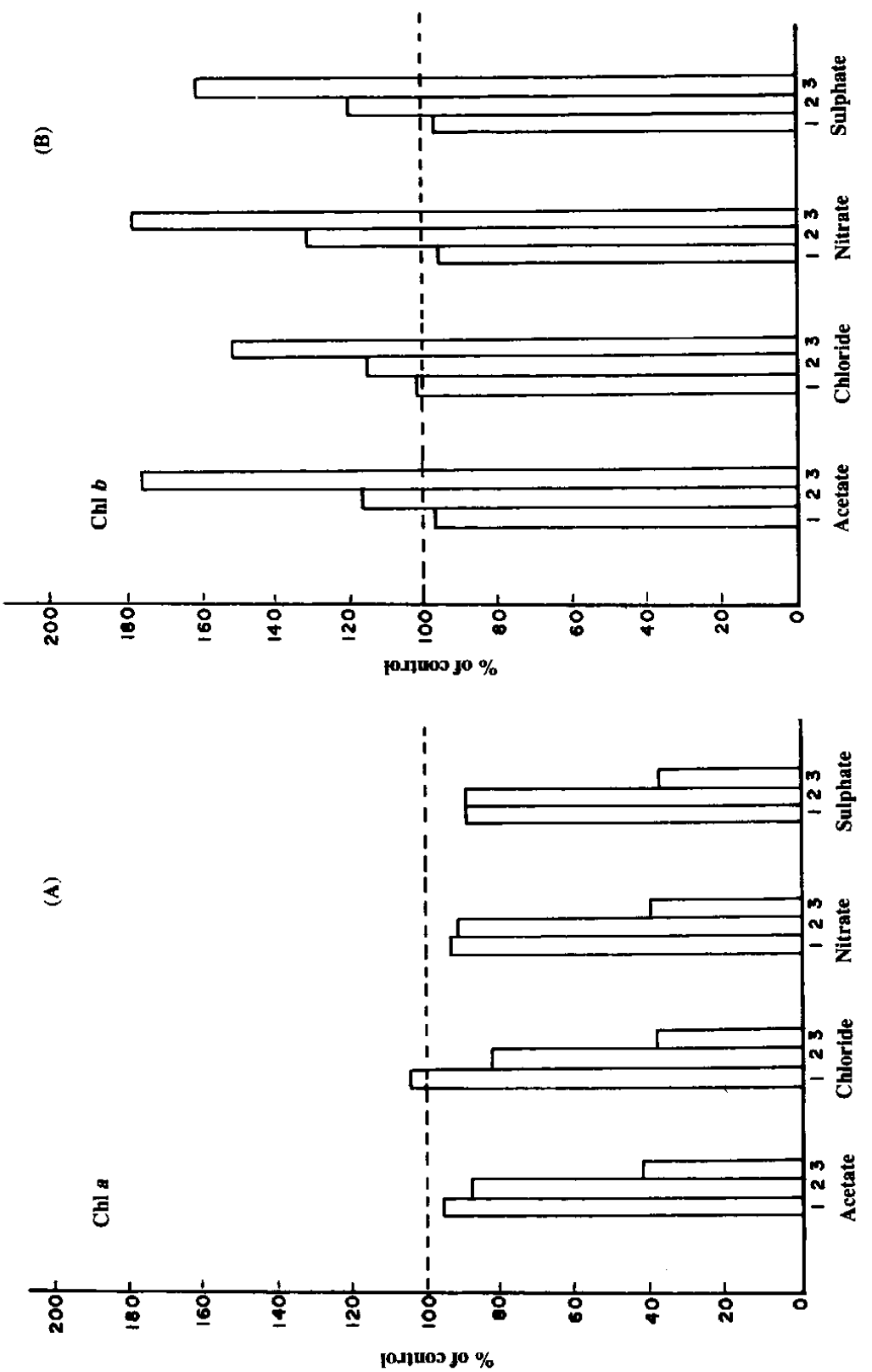

Fig. 1. Effect of copper salts at three concentrations (1 = 10^{-6}, 2 = 10^{-4} and 3 = 10^{-2} M) on Chl *a* and Chl *b* content (% of control of date palm leaflet segments (cv. Female Barhi) after 4 days in the dark.

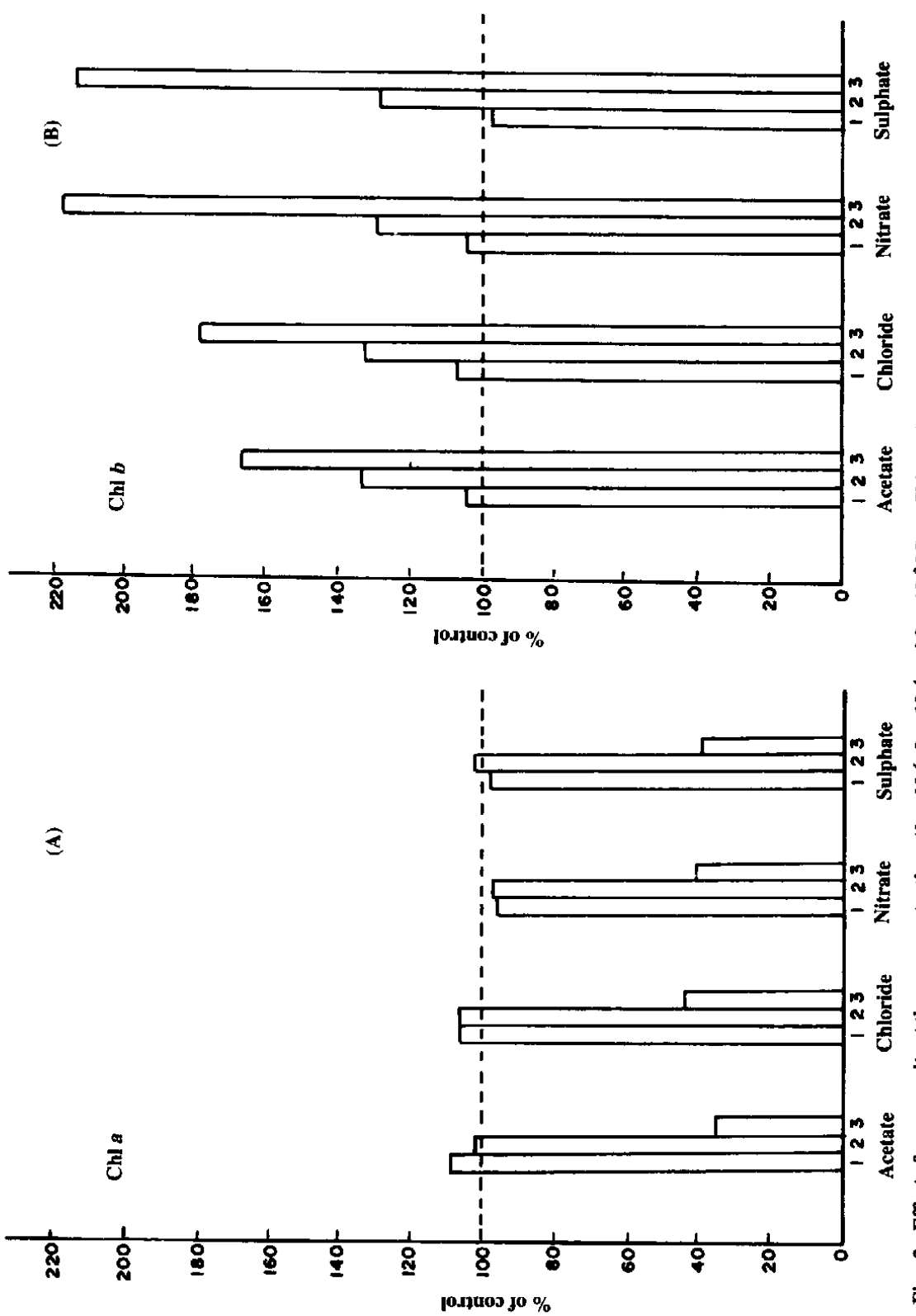

Fig. 2. Effect of copper salts at three concentrations (1 = 10^{-6}, 2 = 10^{-4} and 3 = 10^{-2} M) on Chl *a* and Chl *b* content (% of control) of date palm leaflet segments (cv. male Barhi) after 4 days in the dark.

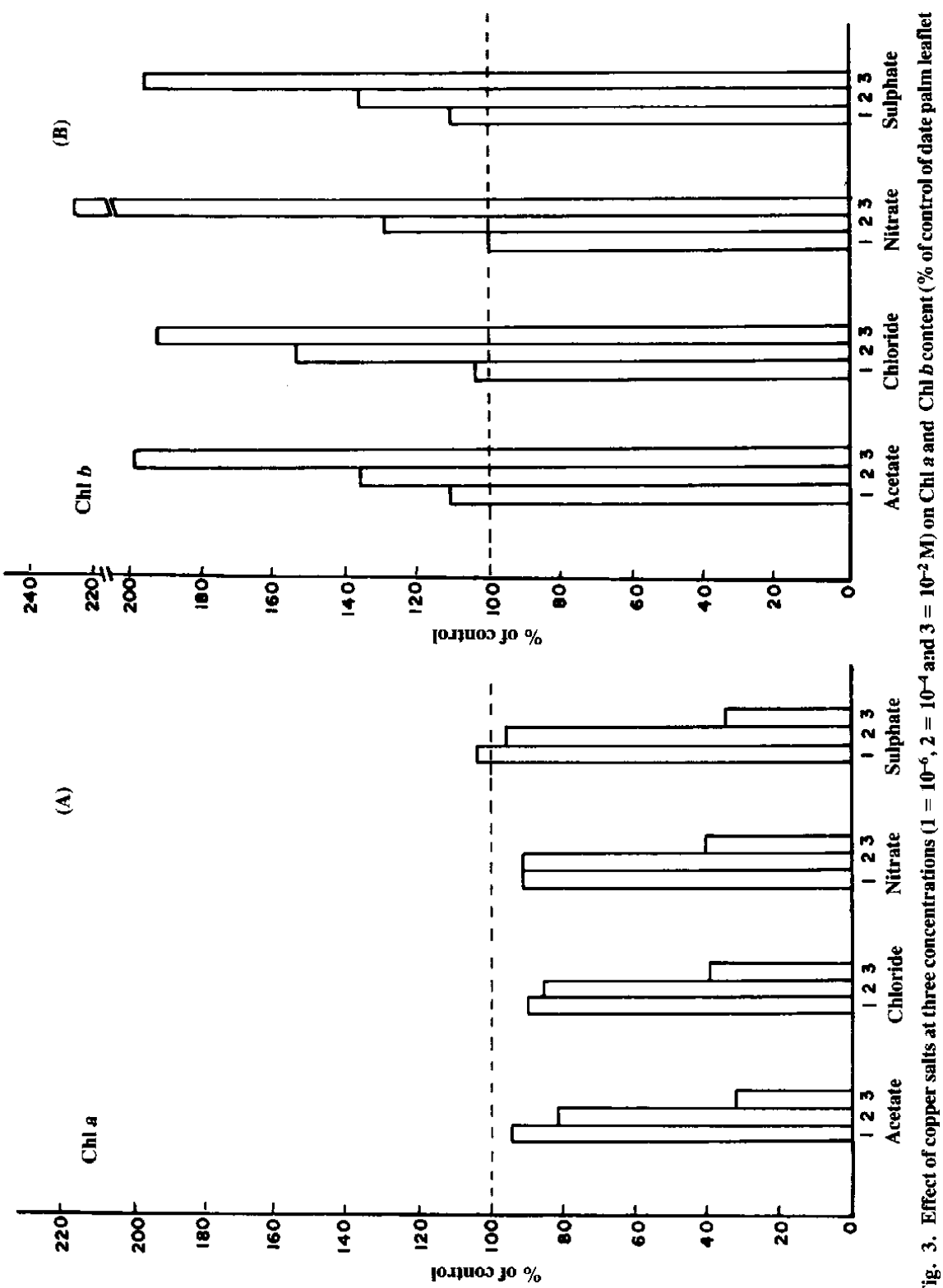

Fig. 3. Effect of copper salts at three concentrations (1 = 10^{-6}, 2 = 10^{-4} and 3 = 10^{-2} M) on Chl *a* and Chl *b* content (% of control of date palm leaflet segments (cv. Sekkeri) after 4 days in the dark.

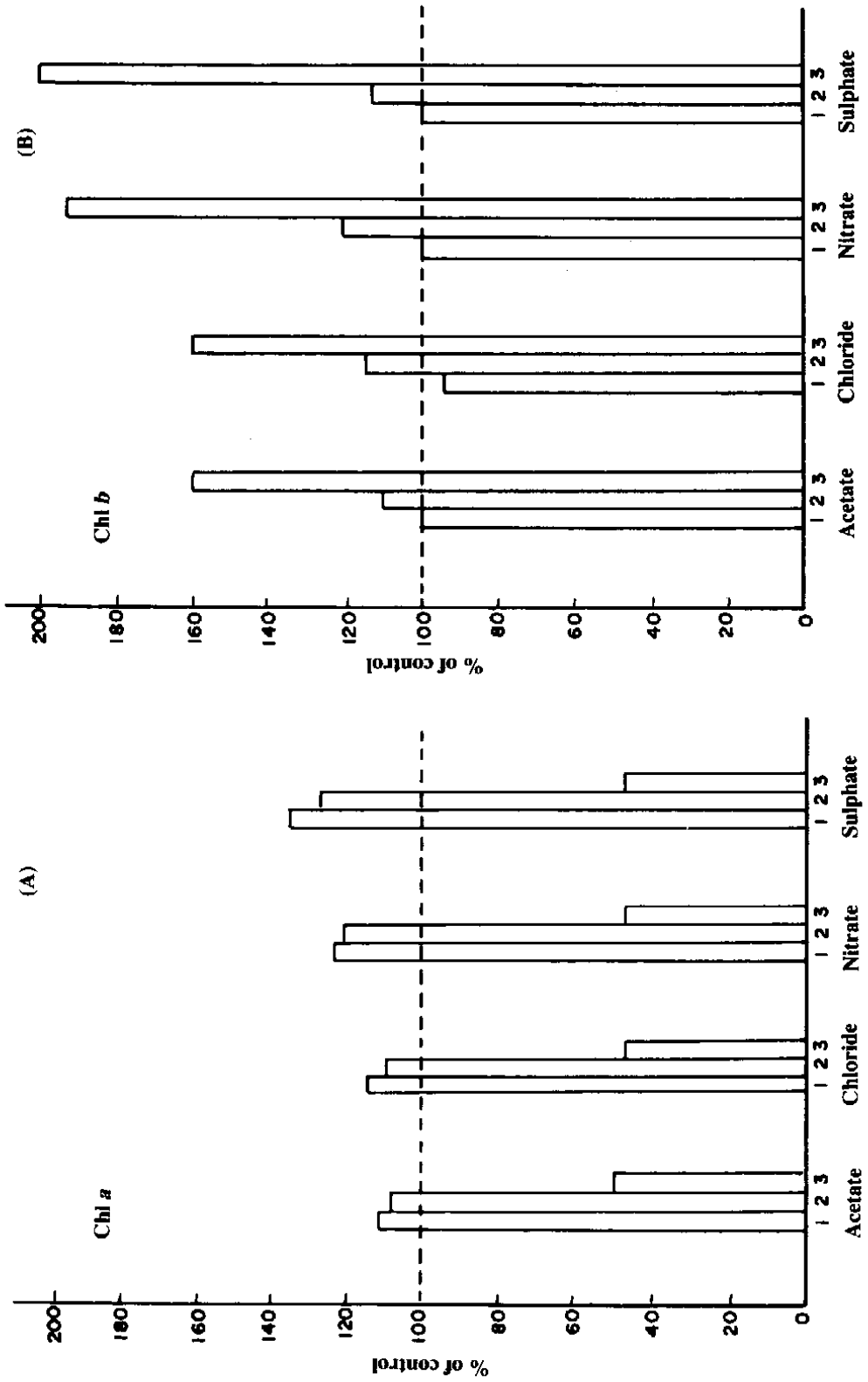

Fig. 4. Effect of copper salts at three concentrations (1 = 10^{-6}, 2 = 10^{-4} and 3 = 10^{-2} M) on Chl *a* and Chl *b* content (% of control of date palm leaflet segments (cv. Osaila.) after 4 days in the dark.

Table 2. Chlorophyll content (as % of control) of the three cultivars as affected by three concentrations of zinc salt after 4 days of incubation in the dark

Treatment	Conc. m	Cultivar					
		Barhi ♀		Barhi ♂		Sekkeri	
		chl *a*	chl *b*	chl *a*	chl b	chl *a*	chl *b*
Zinc acetate	10^{-6}	94±01	96±03	100±11	95±10	105±05	114±04
	10^{-4}	93±05	95±01	91±17	87±16	101±04	106±08
	10^{-2}	96±05	98±06	103±08	89±09	117±07	121±06
Zinc chloride	10^{-6}	81±09	87±03	93±09	116±29	116±02	118±06
	10^{-4}	87±07	92±12	100±06	97±06	113±06	118±06
	10^{-2}	95±03	100±06	108±12	97±12	113±11	118±09
Zinc nitrate	10^{-6}	93±10	96±06	97±11	97±10	100±08	101±07
	10^{-4}	84±03	88±03	95±16	95±04	116±02	118±01
	10^{-2}	91±07	95±01	104±06	110±33	113±06	118±06
Zinc sulphate	10^{-6}	89±03	92±03	97±10	102±16	100±02	107±02
	10^{-4}	91±10	93±05	103±10	106±09	100±06	106±05
	10^{-2}	91±03	96±01	107±04	112±18	100±08	114±06

Table 3 shows the chlorophyll content of two cultivars (female Barhi and Sekkeri) as affected by three concentrations of potassium chloride. Comparing these values with that of the control (Table 1), no distinct differences between them were found. One exception is that 10^{-4} and 10^{-2} M of KCl caused a slight increase in Chl *b* content of Sekkeri cultivar in the range of 10%.

Table 3. Chlorophyll content (mg/g Fresh wt.) of the two cultivars as affected by three concentrations of potassium chloride after incubation for 4 days in the dark

Cultivar	Conc. m	chl *a*	chl *b*	Total
Barhi ♀	10^{-6}	0.90±0.04	0.63±0.02	1.52±0.06
	10^{-4}	0.92±0.05	0.61±0.02	1.53±0.07
	10^{-2}	0.90±0.01	0.60±0.01	1.50±0.01
Sekkeri	10^{-6}	1.01±0.04	0.64±0.03	1.62±0.08
	10^{-4}	1.10±0.13	0.70±0.06	1.79±0.19
	10^{-2}	1.06±0.17	0.72±0.04	1.86±0.15

Discussion

Cultivar differences between date palm trees in chlorophyll content have been reported [6, pp. 591-605]. It was concluded that cultivars originating from the same region were similar in chlorophyll content. El-Hassan *et al.* [7] found significant difference between four cultivars growing in the Al-Hassa region. The results of the present study indicate that there are differences between cultivars in their chlorophyll content (Table 1). Further differences exist between male and female, where 20% increase in Ch *b* in female Barhi over that in the male is noticed. Although Sekkeri and Osaila cultivars were originally from one region (Al-Qassim), they are different in their chlorophyll content. This could be due to their growing region (Riyadh). Other cultivar differences of date palm could be perceived by the quantity of chlorophyll lost during incubation in the dark for four days. The data show that the diminution of chlorophyll content from leaflet segments of the four cultivars is not equal (Table 1). It is in the the order of 50, 33, 38 and 21% for Osaila, male Barhi, female Barhi and Sekkeri, respectively.

When excised segments of the leaflet of date palm were left in the dark for four days in solutions containing 10^{-2} M copper salt, there was a noticeable relative decrease in Chl *a* with a noticeable relative increase in Chl *b*. This phenomenon of decrease and increase in chlorophyll content, however, has been reported in other plants subjected to heavy metals [4;8;9]. Wang and Waygood [9], studying the effect of benzimidazole and nickel on detached leaves of Khapli wheat, suggested that both benzimidazole and nickel protect the chlorophyll from destruction. Agarwala and Kumar [8] suggested that the loss of chlorophyll could be a result of the depression of cytochrome oxidase that might regulate chlorophyll synthesis. However, another suggestion is that the decrease in Chl *a* is due to an enzyme that destroys Mg protoporphyrin, a glycoprotein with an apparent molecular weight of 49 Kd [2]. The author's argument was that this enzyme is synthesized when green leaves of barley were placed in the dark which coincides with the chlorophyll decrease.

From this study, it may be noticed that there was no distinct effect of the anions whether acetate, chloride, nitrate or sulphate. Another point is that Zn^{++} ions do not show a significant effect on Chl *a*. All this would suggest a specific ion effect of copper on the changes in the chlorophyll content. This is further borne out by the data (Table 3) of the effect of KCl where no significant change in chlorophyll content was noticed in this system. However, the results of Zn^{++} treatment could be misleading if the conclusion of Rosko and Rachlin [10] holds in this system. They found that Zn^{++} caused a downward shift in the absorption spectra in the blue region with maximum absorption at 340 nm for Chl *a* of *Chlorella vulgaris*. Further investigation of this point is under study.

References

[1] Siffel, P. and Lebeder, N.N. "Changes in Shape of Chloroplast Spectra during Ontogeny of Primary Leaves of French Bean Correlate with Rate of Chlorophyll Accumulation." In: G. Akoyunoglou and H. Senger (Eds). *Regulation of Chloroplast Differentiation.* New York: Alan R. Liss Inc., 1986.

[2] Gassman, M. and Ramanujan, P. "Relation between Enzymatic Destruction of Magnesium Porphyrins and Chloroplast Development. "In: G. Akoyunoglou and H. Senger (Eds). *Regulation of Chloroplast Differentiation.* New York: Alan R. Liss Inc., 1986.

[3] Wainwright, S.J. and Woolhouse, H.W. "Physiological Mechanisms of Heavy Metal Tolerance in Plants." In: M.J. Chadwick and G.T. Goodman (Eds.) *The Ecology Resources Degradation and Renewal.* British Ecological Society Symposium No. 15. Oxford: Blackwell Scientific Publications. 1975.

[4] Sing, S.N. "Effect of Nickel on Chlorophyll in Excised and Intact *(Luffa aegyptiaca)* Cotyledons." *Indian J. Plant Physiology.* 26 (1983), 421-424.

[5] Moran, R. and Porath, D. "Chlorophyll Determination in Intact Tissues Using N,N-dimethylformamide." *Plant Physiol.*, 65 (1980), 478-479.

[6] Khalil, M.M.; AboEl-Nil, M.M. and Al-Gamdi, A.S. "Leaf Chlorophyll Content in Different Date Palm Cultivars as Affected by Leaf Aging." *Proc. of the Second Symposium on the Date Palm in Saudi Arabia.* March 3-6, 1986. King Faisal University, Al-Hassa, 1989.

[7] El-Hassan, G.M.; Qassim, A.A. and Karrar, K.A. "Chlorophyll Content of Date Palm Leaves as Affected by Cultivar, Tree Age and Leaf Positional Direction." *Proc. of the Second Symposium on the Date Palm in Saudi Arabia.* March 3-6, 1986. King Faisal University, Al-Hassa, 1989, 601-605.

[8] Agarwala, S.C. and Kumar, A. "The Effect of Heavy Metals and Bicarbonate on Sun Flower Plants Grown in Sand Culture with Special Reference to Catalase and Peroxidase." *J. Indian Botan. Soc.*, 41 (1962), 77-92.

[9] Wang, D. and Waygood, E.R. "Effect of Benzimidazole and Nickel on the Chlorophyll Metabolism of Detached Leaves of Khapli Wheat." *Can. J. Botany,* 37 (1959), 743-749.

[10] Rosko, J.J. and Rachlin, J.W. "The Effect of Cadmium, Copper, Mercury, Zinc and Lead on Cell Division, Growth, and Chlorophyll a Content of the Chlorophyte *Chlorella vulgaris." Bull. Torry Bot. Club,* 104 (1977), 226-233.

شيخوخة أوراق نخيل البلح وأملاح النحاس

محمد حمد الوهيبي

قسم النبات والأحياء الدقيقة، كلية العلوم، جامعة الملك سعود، ص. ب ٢٤٥٥، الرياض ١١٤٥١، المملكة العربية السعودية

(سُلِّم في ٢٧ ذو الحجة ١٤١٢هـ، وقُبل للنشر في ٢٥ جمادى الآخرة ١٤١٣هـ).

ملخص البحث. جرت دراسة تأثير الأيونات الأحادية والثنائية على المحتوى الكلوروفيلي لقطع أوراق مفصولة من أربعة أصناف من نخيل البلح المحضّنة في الظلام لمدة أربعة أيام. وقد تم استخدام أملاح النحاس والزنك والبوتاسيوم (خلات، كلوريد، نترات وكبريتات) بثلاثة تراكيز هي 10^{-6} و10^{-4} و10^{-2} جزيء. تبين من الدراسة أن هناك تأثيراً معيّناً للأيون على تأخير شيخوخة الكلوروفيلات حيث إن أكثر الأيونات تأثيراً هو النحاس، إذ أنه عند تركيز 10^{-2} جزيء، وجد تعجيل لتكسير كلوروفيل ا وزيادة في كمية كلوروفيل ب في جميع الأصناف. يبدو من الدراسة أن أيون النحاس يتفاعل مع آلية حماية كلوروفيل ا ويتسبب في تغيير أيض يؤدي إلى زيادة كلوروفيل ب.