Saudi Journal of Biological Sciences 14 (1) 109- 114, June 2007
ISSN 1319-562 X
The Official Journal of the Saudi Biological Society
htt:www.saudibiosoc.com

Responses of *Cassia Cenna* (L.) and *Cassia Italica* (Mill.) to Different Levels of Manganese

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Abstract

The purpose of this investigation was to examine the responses of *C. senna* (L.) and *C. italica* (Mill.) to different levels of manganese, and to highlight the significant ecological aspects related to the performance of these two plant species in response to manganese additions. The study site selected for this study was the enclosed reserved area at the northern location of the Faculty of Education, University of Khartoum, Sudan (Lat. 15°30/ N ; Long. 32°33/ E). The study was carried out by soil chemical analysis and a soil - culture experiment. The soil samples for chemical analysis were collected randomly from 25 sampling points at the selected site. Seven levels of Mn were used for the growth experiment, together with control, making total of eight treatments. Three replicates corresponding to each treatment were set up for each of the two investigated species. The soil chemical analysis data showed that the investigated site was slightly alkaline (pH = 7.74 ± 0.65) and characterized with high levels of Ca and adequate quantities of Mg, K, and Na. The levels of Mn were low as expected due to the soil alkalinity. The results of the soil - culture experiment showed that the growth of *Cassia senna* (L.) increased with an increase of manganese concentration from 3 – 6 mg Mn; whereas, that of *Cassia italica* (Mill.) continued to increase till 9 mg Mn. Beyond these levels a gradual inhibition occurred. The results also showed that the yield of *C. italica* (Mill.) at each particular Mn-level (except the control) was significantly higher compared to that of *C. senna* (L.). The increased growth by the two investigated species to manganese additions between 3 - 6 mg Mn for *C. senna* and 3 – 9 mg Mn for *C. italica* indicated that manganese was a limiting factor of growth for the two species in their natural habitats. The greater yield of *C. italica* (Mill.) at high Mn-levels compared to that of *C. senna* (L.) indicated that *C. italica* (Mill.) is more tolerant to high levels of manganese concentrations than *C. senna* (L.).

Key words: *Cassia senna* (L) -*Cassia italica* (Mill) –Manganese –Responses –Levels.

Introduction

Cassia senna (L.) and *Cassia italica* (Mill.) are widely distributed in central Sudan. They are usually found in mixed stands with *Calotropis procera* (Ait.), and they seem to replace plant communities in consequence of destruction (Obied and Mahmoud, 1971). Both *C. senna* (L.) and *C. italica* (Mill.) are undershrubs, nearly of the same size, and they mainly differ in the shape of the pod. In *C. senna* (L.) the pod is straight, smooth and not crested; while in *C. italica* (Mill.) the pod is transversely nerved, slightly curved, with a crest of elevated projections along the middle of both sides (Migahid, 1978).

Manganese is required for healthy plant growth (Bidwell,1979). It is an essential factor in respiration and nitrogen metabolism, and in both processes it functions as an enzyme activator (Delvin, 1975; Barber, 1995). It is required for the operation of nitrate reductase; manganese- deficient plants usually require NH_3 for this reason. It is also required for the operation of some enzymes in the metabolism of the hormone indoleacetic acid. The most important role of manganese in photosynthesis is in the sequence of reactions by which electrons are derived from water and oxygen is liberated. Manganese may also have a structural role in chloroplasts, which become light sensitive in its absence and ultimately lose their structure and disintegrate under conditions of extreme manganese shortage. Manganese is absorbed by plants as the manganous ion (Mn^{2+}) and in molecular combination with certain complexing agents

such as EDTA (Nelson and Tisdale, 1966; Laurie *et al*., 1995). Soil microorganisms appear to play major role in determining soil levels of reduced Mn^{2+} especially at the high soil pH values (Alloway, 1990). As a micronutrient, manganese is required in relatively small amounts by plants, and high levels of manganese were found to be distinctly toxic to plants (Etherington, 1982; Bennett, 1993). The common symptom of manganese toxicity is the formation of chlorotic grading to dead spots on the leaf. These spots are frequently near the ends of xylem vessels, so tend to be near the leaf margin and in interveinal positions. Usually the symptoms are more severe in the older leaves that have had the longest time to accumulate manganese. The high concentration levels of manganese in the cytoplasm would upset the control of the manganese activated enzymes and thus causing chlorosis, and slowing the growth (Chapin, 1980; Pandya *et al*., 2004).

The purpose of this study was to examine the responses of *C. senna* (L.) and *C. italica* (Mill.) to different levels of manganese, and to highlight the significant ecological aspects related to the performance of these two plant species in response to manganese additions, that can be used for subsequent investigations.

Materials and Methods

Study Site Description

The site chosen for this study was the enclosed reserved area at the northern location of the Faculty of Education, University of Khartoum, Sudan (Lat. 15°30/ N ; Long. 32°33/ E). It lies on the Nubian sandstone, about 380 m above sea level. The mean annual temperature in summer is about 37°C. The site is more or less flat with very gentle undulations. In addition to *C. senna* (L.) and *C. italica* (Mill.) the site is also dominated by perennials and annuals; and occupied by an upper layer of scattered trees and shrubs mainly *Acacia nilotica* (Benth.), *A. mellifera* (Val.) and *Ziziphus spina-christi* (L.).

Soil Collection and Primary Investigation

The investigation was carried out by soil chemical analysis and a soil – culture experiment. Twenty five random soil samples were collected from the investigated site. The samples were taken from the plough depth (15-20 cm), because the plough layer is thought to be the most useful part of the soil and will best represent its ability to supply nutrients. The samples were placed in plastic bags and stored in the laboratory at temperature of about 25°C. Cations (expressed in mg 100 g^{-1} soil) were extracted from a measured volume of soil (5 grams from each sample) with ammonium acetate at pH = 7. The pH of the collected soil samples was determined on a 1:2 suspension of a soil sample in deionized water, using a glass electrod. K and Na were estimated by flame emissions, and other metallic cations by atomic absorption spectrophotometer. The analytical procedures, in general, were described by Allen *et al.*, (1974) and Chapman, (1976).

Experimental Design and Treatments

The soil for the experiment was collected from the site on August 1991. The soil was well mixed together and the pots were filled equally with 2 Kg of soil. The seeds of the two investigated species were sown on pots. The pots were irrigated on daily basis. $MnCl_2$ was used as a source of Mn. Seven levels of Mn were used, together with control, making total of eight treatments as follows: 0.0, 3.0, 6.0, 9.0, 12.0, 15.0, 18.0 and 21.0 mg Mn /pot. These weights are equivalent to 0.0000, 0.0069, 0.0138, 0.0207, 0.0276, 0.0345, 0.0414, 0.0483 grams of $MnCl_2$. Three replicates corresponding to each treatment were set up for each of the two investigated species. The pots were completely randomized before application of the treatments. The nutrients were applied ten days after sowing as dilute solutions around the roots in two treatments one at the start of the experiment and the other after 10 days. The plants were harvested after 6 weeks from applying the treatments. The fresh and dry weights of the whole plants (the root and shoot systems) were measured.

Results

Primary Investigation

The results of pH and extractable ions of the investigated

Table 1: Summary of the soil chemical analysis data: pH and extractable ions expressed in mg / 100 g soil of the investigated site. Figures are means ± S.D. calculated from 25 soil samples.

pH	7.74 ± 0.65	K	45.48 ± 17.58
Ca	436.90 ± 72.2	Na	38.76 ± 14.80
Mg	64.32 ± 21.80	Mn	0.21 ± 0.09

site expressed in mg / 100g soil were summarized in Table 1. The results showed that the site was slightly alkaline (pH = 7.74 ± 0.65) and characterized with high levels of Ca and adequate quantities of Mg, K, and Na. The levels of Mn, however, were low and associated with the soil alkalinity. It was reported that Mn precipitates in forms of dark-coloured nodules in the lower horizons of soils of semi-arid regions due to the high pH at those horizons (Thompson and Troeh, 1978).

Manganese Treated Soils

The results of *Cassia senna* (L.) and *Cassia italica* (Mill.) growing on a soil culture experiment in response to additions of different levels of manganese showed that the growth of *Cassia senna* (L.) increased with an increase of manganese concentration from 3 – 6 mg Mn; whereas, that of *Cassia italica* (Mill.) continued to increase till 9 mg Mn. Beyond these levels, however, a gradual inhibition occurred (Table 2). The results also showed that the yield of *C. italica* (Mill.) at each particular Mn treatment was higher compared to that of *C. senna* (L.). Between 3- 6 mg Mn, for instance, the difference between the yield of the two investigated species was significant at $p < 0.05$. From 9 – 21 mg Mn, however, the differences were significant at $p< 0.01$.

Discussion

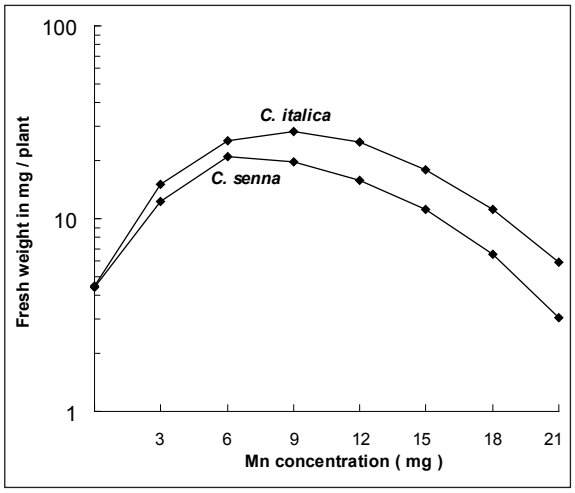
Various forms of manganese may be present in the soil, but the reduced manganous ion (Mn^{2+}) is the form in which it is largely absorbed (Bidwell,1979), and this is why manganese was used in this study in the form of $MnCl_2$. Manganese is required for healthy plant growth because it is widely involved in catalytic roles in plants, being the enzyme-activating metal of some respiratory enzymes and in reactions of nitrogen metabolism and photosynthesis (Delvin, 1975; Barber, 1995). In this study manganese additions increased the growth of *C. senna* in the range of 3-6 mg Mn / pot, and that of *C. italica* in the range of 3 - 9 Mn / pot. This was an indication that manganese was a limiting factor of growth for the two investigated species at that particular range. Actually this was not surprising because the soil of the investigated site had low levels of Mn (Table 1). These low levels were associated with the slightly alkaline soil of the investigated site which was characterized with more or less high pH and high levels of Ca. Under such conditions manganese deficiency is caused by the conversion of manganous compounds to the manganic form which is unavailable to plants (Brady and Well, 1999). But it can be to a lesser extent be induced by an imbalance with other elements such as Ca^{2+}, Mg^{2+}, and Fe^{2+} (Hayati and Proctor, 1991).

High manganese levels were found to reduce the growth of the two investigated species. The reduction in the growth of *C. senna* (L.), however, was more pronounced than that of *C. italica* (Mill.) (Figure, 1).

Table 2: Fresh and dry weight yield of *Cassia senna*(L.) and *Cassia italica* (Mill.) growing on a soil culture experiment in response to additions of different levels of manganese. The fresh and dry weight readings represent the average of three replicates ± S. D. The status of statistical significance between the yields (means) of the two investigated species, calculated by using t-test, at each particular level of Mn is given in the last column of the table. The data was analyzed statistically after logarithmic transformation.

Levels of Mn (mg / pot)	Fresh-weight (gm /plant)		Dry-weight (gm /plant)		Status of Statistical Significance
	C. senna	*C. italica*	*C. senna*	*C. italica*	
0.00	04.40 ± 0.02	04.50 ± 0.23	1.54 ± 0.70	1.57 ± 0.11	Not Significant
3.00	12.30 ± 0.14	15.10 ± 0.87	3.20 ± 0.11	3.54 ± 0.35	p<0.05
6.00	21.01 ± 0.06	25.50 ± 0.37	4.50 ± 0.08	5.10 ± 0.13	p<0.05
9.00	19.60 ± 0.64	28.50 ± 1.09	4.30 ± 0.15	5.42 ± 0.09	p<0. 01
12.00	15.80 ± 0.84	25.09 ± 0.60	3.70 ± 0.18	4.65 ± 0.15	p<0. 01
15.00	11.20 ± 0.29	17.80 ± 0.35	2.90 ± 0.05	3.75 ± 0.10	p<0. 01
18.00	06.50 ± 0.15	11.10 ± 1.09	2.10 ± 0.11	2.80 ± 0.08	p<0. 01
21.00	03.08 ± 0.04	05.98 ± 0.32	1.35 ± 0.07	1.85 ± 0.07	p<0. 01

For instance, *C. senna* (L.) receiving 21 mg Mn /pot exhibited lower yield than those receiving zero mg Mn /pot (the control); while the yield of *C. italica* (Mill.) receiving 21 mg Mn /pot was greater than that of the control (Table 2). This greater yield of *C. italica* (Mill.) at high Mn-levels compared to that of *C. senna* (L.) indicated that *C. italica* (Mill.) is more tolerant to high manganese concentration than *C. senna* (L.). Although high manganese levels did reduce the growth of the two investigated species, they did not induce clear toxicity symptoms. It seems that toxicity symptoms would have appeared if Mn levels higher than those used in this experiment were applied. Actually manganese toxicity is a problem in some strongly acid soils, and it can also occur at high pH levels in soil under reducing conditions created by flooding or organic matter accumulation (Bennett, 1993). It has been found that *C. senna* (L.) was absent in slightly acidic wet soils characterized by high levels of Mn (Hayati and Yahia, 2002). Moreover, manganese tolerance in certain plants has been attributed to reduced absorption, less translocation of excess manganese to plant tops and / or greater tolerance to high manganese levels within plant tissues (Etherington, 1982; Bennett, 1993).



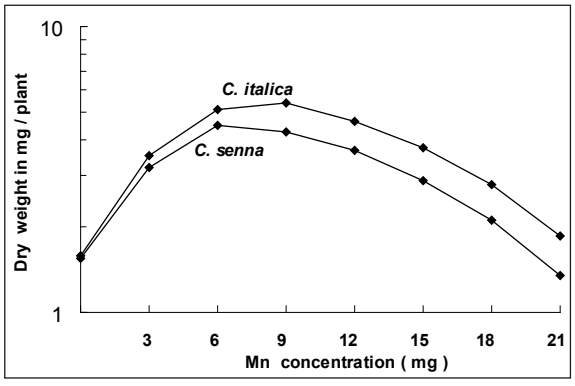


Figure1: Fresh and dry-weight yield of *Cassia senna* (L.) and *Cassia italica* (Mill) growing on a soil culture experiment in response toadditions of different levels of manganese. Values of Y axis were plotted on a logarithmic scale.

In conclusion, the two closely related investigated species *Cassia senna* (L.) and *Cassia italica* (Mill.), which are nearly of the same size, showed significant differences in their response to added manganese. This result confirmed the fact that plant species and even cultivars within the same species differ widely in their response to soluble or exchangeable manganese (Bennett 1993; Park *et al.*, 1999). These differences in response to mineral nutritional factors are genetically controlled by the different plant species (Epstein, 1972; Yan *et al*., 1996).

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استجابة نبات *Cassia senna* (L.) و نبات *Cassia italica* (Mill.) لمستويات مختلفة من المنجنيز

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الملخص

هدفت هذه الدراسة إلى معرفة استجابة نبات *Cassia senna* (L.) و *Cassia italica* (Mill.) لمستويات مختلفة من أيون المنجنيز، ولإلقاء الضوء على الجوانب الإيكولوجية ذات المعنوية الإحصائية فيما يتعلق بتأثير أيون المنجنيز على أداء هذين النباتين. أختير موقع هذه الدراسة بالجزء الطبيعي المحاط بالسور الشمالي لكلية التربية بجامعة الخرطوم (خط عرض 15°30/ شمالا، و خط طول 32°33/ شرقا). أجريت الدراسة عن طريق التحليل الكيميائي لعينات من التربة وزراعة النباتين في مزرعة ترابية (soil–culture) تراوحت تركيزات أيون المنجنيز فيها من صفر إلى 21 ملجم. جمعت عينات التربة لأغراض التحليل الكيميائي عشوائيا من 25 نقطة من موقع الدراسة. أوضحت نتائج التحليل الكيميائي لعينات التربة أن موقع الدراسة خفيف القلوية (pH = 7.74 ± 0.65) و يمتاز بمستويات عالية من الكالسيوم و مستويات كافية (adequate) من الماغنسيوم و البوتاسيوم و الصوديوم، أما مستويات أيون المنجنيز فكانت قليلة وهذا أمر متوقع نسبة لقلوية تربة الموقع. أوضحت نتائج تجربة المزرعة الترابية (soil – culture experiment) أن نمو *Cassia senna* (L.) قد زاد بزيادة أيون المنجنيز في المدى من 3 إلى 6 ملجم منجنيز، بينما استمر نمو *Cassia italica* (Mill.) في الزيادة حتى 9 ملجم منجنيز. أما نمو النباتين في المستويات العليا من أيون المنجنيز فقد تناقص بصورة ملحوظة. أوضحت التجربة كذلك أن إنتاجية نبات *Cassia italica* (Mill.) في المدى من 3 إلى 21 ملجم منجنيز أكبر من إنتاجية نبات *Cassia senna* (L.) في المدى نفسه، و أن الفرق في الإنتاجية ذو معنوية إحصائية. إن الزيادة في نمو نبات *Cassia senna* (L.) في المدى من 3 إلى 6 ملجم منجنيز و نبات *Cassia italica* (Mill.) في المدى من 3 إلى 9 ملجم منجنيز يشير إلى أن أيون المنجنيز يعتبر من العوامل المحددة لنمو كل من النباتين في بيئاتهما الطبيعية. كما أن الإنتاجية العالية لنبات *Cassia italica* (Mill.) في المستويات العليا لأيون المنجنيز مقارنة مع إنتاجية نبات *Cassia senna* (L.) تشير إلى أن نبات *Cassia italica* (Mill.) أكثر تحملا للمستويات العالية من أيون المنجنيز مقارنة مع نبات *Cassia senna* (L.).