J. King Saud Univ., Vol. 15, *Science* (1), pp. 39-47, Riyadh (1423/2002)

Effect of Zinc Chloride and Lead Nitrate on Seed Germination and Early Seedling Growth of Rice and Alfalfa

M.N. Al-Yemeni and A.A. Al-Helal
Botany and Microbiology Department, College of Science,
King Saud Univeristy, P.O.Box: 2455, Riyadh-11451, Saudi Arabia

(Received on 00/00/0000; accepted for publication 00/00/0000)

Abstract. The effect of different concentrations of $ZnCl_2$ (0.5 to 100 mM), $Pb(NO_3)_2$ (0.5 to 50 mM) and of NaCl and KCl (25 to 300 mM) on seed germination of rice (*Oryza sativa L.*) and alfalfa (*Medicago sativa L.* Cv. Al-Ahsaa) seedlings after 4 days of incubation were investigated. Seed germination and seedling growth were inhibited significantly by $ZnCl_2$ and $Pb(NO_3)_2$ due to heavy metal toxicity, as was evidnced by the effect of NaCl and KCl. Rice seed germination was more tolerant to heavy metals than alfalfa. In rice root growth was more sensitive to heavy metals than shoot growth, while the latter was more sensitive to KCl and NaCl salt stress.

Introduction

Heavy metals include about thirty-eight elements, some of them are even necessary for the growth of higher plants, However, they act toxically at high concentrations since they have a strong affinity for numerous organic compounds [1]. The response of plant growth and metabolism to heavy metals has become a subject of great interest in recent years because of their toxicity to plants [2]. Heavy metal, contamination of soils is a worldwide problem of increasing importance and of great environmental concern [1,3]. Zinc (Zn) and lead (Pb) are often cited for their deleterious effects on both human and plant metabolisms [4]. Excessive uptake of these elements from the soil by crop plants causes several toxic effects, such as reduction in plant growth and yield, due to the inhibition of metabolic process in plants [5-8]. Lead pollution levels and its effects on plant growth and development in the Sultanate of Oman was reported [9] In addition to the inhibition of seed germination [2,6,10] contamination of the field crops is a source of heavy metals in our foods [11-13].

Lead is released to the environment by mining, smelting, refining, lead based products, pesticides, vehicular exhaust and the burning of coal and industrial waste. Of these the major release of lead to the environment is through vehicular exhaust [14,15].

In Saudi Arabia published studies to date include, the effect of nickel on plants [16]; lead, titanium and zinc in air particulate at Dhahran, during and after the Kuwait oil fires [17]; and contamination of road side soils by lead, cadmium and zinc [18].

Materials and Methods

Rice (*Oryza sativa* L. cv.-Al-Ahsaa) and alfalfa (*Medicago sativa* L. cv. Al-Ahsaa) seeds were obtained commercially from Al-Ahssa in the eastern region of Saudi Arabia, and stored at 4^{o}C.

A range of concentrations of zinc chloride (0.5 –100 mM), lead nitrate (0.1-20 mM) sodium chloride and potassium chloride (25-300 mM) were prepared in deionized distilled water. Seed germination was performed in 9 cm diameter sterilized petri-dish as having two layers of Whatman No. 1 filter paper moistened with 10 ml of the experimental solution. 25 seeds of rice and of alfalfa were then evenly distributed in the petri-dish over the surface of filter paper. Four replicates were used per treatment. Incubation was carried out at 30/20 ^{o}C day/night temperature range and 12/12 day/night cycle. The number of germinated seeds was counted after 4 days of germination and the data were expressed as a percentage of the total number of seeds. The germination percentages were subjected to an analysis of variance and LSD test at the 95% confidence level. Shoot and root lengths were measured after 5 days of germination [2].

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Results

The results presented in Table 1 shows the effect of different concentrations of $ZnCl_2$ and $Pb(NO_3)_2$, KCl and NaCl on the percentage germination of rice seeds after 4 days of incubation. Germination was high in the control after 4 days incubation (99%). Neither $ZnCl_2$ nor $Pb(NO_3)_2$ at relatively low concentrations (up to 40 mM $ZnCl_2$ and 5 mM $Pb(NO_3)_2$) had any significant effect on seed germination, since the percentage of germination was more or less the same as the control. $ZnCl_2$ at 100 mM reduced significantly seed germination to 84% after 4 days of incubation. $Pb(NO_3)_2$ at 50 mM significantly reduced germination to 85%.

To test whether the germination inhibition was caused by heavy metal cations (Zn, Pb) or chlorides, rice seeds were germinated at different KCl and NaCl concentrations. As shown in Table 1 25 and 100mM KCl reduced significantly germination to 86%. A further reduction was observed when the concentration was increased giving a threefold reduction at 300 mM. The effect of NaCl was similar to KCl at lower concentrations from 25 to 100 mM, where there was only slight effect, while at 200 mM germination was reduced significantly to 42%. No germination was observed at the highest concentration 300 mM indicating that the inhibition of seed germination by low concentrations of the heavy metal solution were due to to the cation not the anion (Cl).

Table 1. Effect of $ZnCl_2$, $Pb(NO_3)_2$, NaCl and KCl on the percentage germination of rice after 4 days incubation (mean ±s.d.).

Treatment	Concentration (mM)	Percentage germination After 4 days
H_20	-	99.0 ± 1.0 a
	0.5	99.0 ± 1.0 a
	1.0	96.0 ± 3.0 a
	5.0	97.0 ±2.0 a
	10.0	98.0 ± 2.0 a
$ZnCl_2$	20.0	99.0 ± 2 a
	40.0	97.0 ± 3.0 a
	60.0	91.0 ± 3.0 b
	80.0	91.0 ± 5.0 b
	100.0	84.0 ± 4.0 c
	0.1	95.0 ± 2.0 a
	0.5	96.0 ± 3.0 a
	1.0	97.0 ± 3.0 a
$Pb(NO_3)_2$	5.0	96.0 ± 4.0 a
	10.0	92.0 ± 2.0 b
	25.0	94.0 ± 2 ab
	50.0	85.0 ± 4.0 b
	0.0	93.0 ± 3.0 a
	25.0	89.0 ± 2.0 b
NaCl	50.0	87.0 ± 3.0 b
	100.0	87.0 ± 3.0 b
	200.0	42.0 ± 5.0 c
	300.0	0.0
	0.0	93.0 ± 4.0 a
	25.0	88.0 ± 2.0 b
KCl	50.0	86.0 ± 3.0 b
	100.0	86.0 ± 4.0 b
	200.0	77.0 ± 6.0 c
	300.0	24.0 ± 5.0 d

Note: Values without a common letter are significantly different at 95% level.

Table 2. presents the effect of $ZnCl_2$, $Pb(N0_3)_2$, KCl and NaCl on the percentage germination of alfalfa seeds after 4 days incubation. Germination by the control was 94%. Both $ZnCl_2$ and $Pb(NO_3)_2$ significantly inhibited seed germination at low concentrations. The inhibition increased at higher concentrations reaching 1 % at 60 mM after 4 days of incubation. $Pb(NO_3)_2$ at higher concentration reduced germination percentage significantly. No germination was observed at concentrations higher than 20.0 mM after 4 days incubation.

Table 2. Effect of $ZnCl_2$, $Pb(NO_3)_2$, NaCl and KCl on percentage germination of alfalfa after 4 days incubation (mean ±s.d.).

Treatment	Concentration	Percentage germination after 4 days
(H_20)	-	94.0 ± 3.0 a
	0.05	87.0 ± 3.0ab
	0.10	90.0 ± 2.0 a
	0.50	86.0 ± 5.0 ab
	1.00	88.0 ± 2 ab
$ZnCl_2$	5.00	70.0 ± 4.0 c
	10.0	42.0 ± 4.0 d
	20.0	8.0 ± 2.0 e
	40.0	11.0 ± 3.0 f
	60.0	1.0 ± .30 g
	0.1	93.0 ± 2.0 a
	0.5	94.0 ± 3.0 a
$Pb(NO_3)_2$	1.0	88.0 ± 2.0 b
	5.0	14.0 ± 2.0 c
	10.0	15.0 ± 3.0 c
	20.0	0.0
	25.0	89.0 ± 2.0 a
	50.0	88.0 ± 5.0 a
NaCl	100.0	87.0 ± 4.0 a
	200.0	48.0 ± 6.0 b
	300.0	0.0
	25.0	83.0 ± 5.0 a
	50.0	87.0 ± 6.0 a
KCl	100.0	86.0 ± 4.0 a
	200.0	73.0 ± 5.0 c
	300.0	27.0 ± 4.0d

Note: Values without a common letter are significantly different at 95% level.

KCl and NaCl at 100 mM had no significant effect on germination, but at 200 mM reduced germination to 77% for KCl and 42% for NaCl. At 300 mM KCl a higher inhibition 27% was observed compared with 0% for NaCl. This indicates that the inhibitory effects of $ZnCl_2$ and $Pb(NO_3)_2$ at low concentrations was due to heavy metal toxicity.

Root and shoot elongation of rice seedlings were greatly inhibited by $ZnCl_2$ and $Pb(NO_3)_2$ and the degree of inhibition increased as the concentration increased Table 3. Radicle elongation was more adversely affected than shoot elongation. At 20mM of $ZnCl_2$ and 10 and 25 mM $Pb(NO_3)_2$ the radicle of a number of seeds emerged through the seed coat, but failed to elongate and the radicle failed to emerge at higher concentrations, while the shoots were relatively longer at these concentrations.

Table 3. Effect of $ZnCl_2$ and $Pb(NO_3)_2$ on the root and shoot length of rice after 5 days incubation. (mean ±s.d.).

Treatment	Concentration (mM)	Root Length (mm)	Shoot Length (mm)
H_20	-	18.0 ± 2.0 a	46 ± 3.0 a
	0.5	16.3 ± 2.5 a	32.6 ± 3.5 a
	1.0	16.0 ± 2.0 a	20.0 ±2.5 b
	5.0	9.1 ± 0.7 b	15.0 ± 1.7 c
	10.0	3.0 ± 0.6 c	10.0 ± 0.5 d
$ZnCl_2$	20.0	0.0d	9.1 ± 0.4 d
	40.0	0.0	5.9 ± 0.5 f
	60.0	0.0	4.0 ± 0.4 f
	80.0	0.0	2.0 ± 0.1 g
	100.0	0.0	1.5 ± 0.2 I
	0.1	17.3 ± 2.0a	42.0 ±2.5 a
	0.5	13.4 ± 2.0 b	34.6 ± 3.0 b
	1.0	12.9 ± 1.5 b	2.3 ± 0.3c
$Pb(NO_3)_2$	5.0	8.2 ± 1.0 c	0.0
	10.0	4.1 ± 0.5 d	0.0
	25.0	1.1 ± 0.2 f	0.0
	50.0	0.0f	0.0
KCl	50.0	29 ±4.0 a	30.0 ±2.5 a
	100	11 ±1.5 b	7.0 ±1.4 b

Note: Values without a common letter are significantly different at 95% level.

KCl at 50mM reduced the elongation of both root and shoot, but shoot elongation was more affected. The effect of 50mM KCl was almost the same as 0.1mM ZnCl2 and $Pb(NO_3)_2$, which indicates the effects of $ZnCl_2$ and $Pb(NO_3)_2$ at low concentration are due to heavy metal toxicity.

The elongation of both the root and the hypocotyl of alfalfa seedlings was inhibited markedly by the $ZnCl_2$ and $Pb(NO_3)_2$ Table 4. $Pb(NO_3)_2$ was less effective in reducing seedling growth than ZnCl2.

Table 4. **Effect of $ZnCl_2$ and $Pb(NO_3)_2$ on the root and shoot length of alfalfa seedlings after 5 days incubation (mean ±s.d.).**

Treatment	Concentration (mM)	Root Length (mm)	Shoot Length (mm)
H_20	-	5.2 ± 0.4 a	9.5 ± 0.3 a
	0.05	0.0	9.1 ± 0.4 a
	0.1	0.0	7.1 ± 0.2 a
$ZnCl_2$	0.5	0.0	4.7 ± 0.3 b
	1.0	0.0	3.2 ± 0.2 c
	5.0	0.0	0.4 ± 0.1 e
	1.0	4.8 ± 0.2 a	8.4 ± 0.5 a
$Pb(NO_3)_2$	5.0	0.0	3.0 ±0.2 b
	10.0	0.0	3.0 ± 0.1b
KCl	50	5.1 ± 0.3 a	
	100	2.0 ± 0.1 b	

Note: Values without a common letter are significantly different at 95% level.

The effect of 50mM KCl on seedling growth was almost the same as the effect of 0.05 mM $Pb(NO_3)_2$ and it is less inhibitory to growth than 1.0mM $ZnCl_2$. This suggests that the inhibitory effects of $ZnCl_2$ and $Pb(NO_3)_2$ at low concentrations was due to heavy metal toxicity.

Discussion

It is clear from the results that $ZnCl_2$ and $Pb(NO_3)_2$ had inhibitory effect on seed germination of rice and alfalfa. The inhibition appeared to be due to the toxicity of the heavy metals zinc and lead, since 50mM KCl caused only a slight reduction in the final germination percentage. Seed germination inhibition by heavy metalshas been reported [6,19].

The results indicated that germination of rice seeds was more tolerant to heavy metals than alfalfa seeds. However, the germination of rice and alfalfa seeds appeared to react similarly to salt stress, since 200mM KCl and NaCl caused 77 and 42 % inhibition respectively. Seed germination at high salt concentrations 300 mM gave a different response, NaCl decreasing seed germination more than KCl. This inhibitory effect of salt on seed germination may be due to both ionic toxicity and osmotic effects.

The results demonstrated that the heavy metals Zn and Pb had an inhibitory effect on the growth of rice and alfalfa seedlings. Inhibition of plant growth due to heavy metal toxicity has been previously reported by a number of investigators [2,6,10,19].

The results also indicated that the elongation of the radicle of rice seedlings was affected more adversely by heavy metals than shoot growth, similar results have been reported by other workers [2,4,19,20]. The causes of growth inhibition by heavy metals might be due to rapid effects on loss of cell turgor as a result of plasmamembrane damage by these heavy metals; which in turn lead to leakage of soluble constituents of the cells [21]. Also heavy metals effect plant metabolism e.g. cadmium can inhibit Calvin cycle in *Phaseeolus vulgaris* [22]. Pb also found to effect the activity of δ-amino laevulinic acid dehydratase and porphobilinogenase in isolated chloroplasts of spinach [21]. Zn has been shown to impair water uptake capacity, to promote leakage of metabolites, to depress chlorophyll development and Hill reaction activity of chloroplasts in rice seedling [23].

The reason for the different responses of root and shoot growth to heavy metals is not known, but might be due in part to a more rapid accumulation of heavy metals in the roots than in the shoots or to a faster rate of detoxification in the shoot than the root [2] Shulz-Baldes and Lewin [24] suggested that lead arrests cell division and uptake of essential elements as it deposits on the cell membrane.

Most growth inhibition by $ZnCl_2$ and $Pb(NO_3)_2$ appeared to be due to the toxicity of heavy metals, since $ZnCl_2$ and $Pb(NO_3)_2$ were more effective in inhibition than either

KCl or NaCl. However, growth inhibition at high concentrations of KCl and NaCl might be due to an osmotic effects and / or ionic toxicity of Cl, K, and Na.

Root and shoot growth of rice responded differently to salt and heavy metal, while 50mM KCl and NaCl were more inhibitory to shoot than root growth. The reason for this differences is not known, but it might be due to the different mechanism of resistance to salt stress and heavy metals. Shaukat *et al.*, [19] suggested that heavy metal concentrations in the shoot lead to elevated phenolic compound which could be responsible for germination and growth inhibition. Phenolic acids have been shown to exert dramatic effect on membrane permeability and membrane electrical potentials [25, 26].

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تأثير كلوريد الزنك ونترات الرصاص على الإنبات والنمو المبكر لنباتي الأرز والبرسيم الحجازي

محمد بن ناصر اليمني ، علي بن عبد المحسن الهلال

قسم النبات والأحياء الدقيقة ، كلية العلوم، جامعة الملك سعود ، ص.ب. ٢٤٥٥ ،

الرياض ١١٤٥١ ، المملكة العربية السعودية

(قدّم للنشر في ١/٥/١٤٢١هـ ؛ وقبل للنشر في ١٣/٨/١٤٢٢هـ)

ملخص البحث. تم في هذا البحث دراسة أثر تراكيز مختلفة (٠,٥ إلى ١٠٠ ملمـول) من كلوريد الزنك و (٠,٥ إلى ٥٠ ملمـول) ، نـترات الرصاص و (٠,٢٥– ٣٠٠ ملمول) لكل مـن كلوريد الصوديوم وكلوريد البوتاسيوم على الإنبات والنمو المبكر لنباتي الأرز والبرسيم الحجازي بعد تحضين بذورهما لمدة أربعة أيام .

أوضحت النتائج أن كلوريد الزنك ونترات الرصاص عملت على تثبيط الإنبات والنمو كما أوضحت أن تثبيط الإنبات يرجع كُليًّا إلى سُمِّيّة المعادن الثقيلة المستخدمة بينما يرجع تثبيط النمو جزئيًّا إلى كل من السُمّية والإجهاد الملحي كما هو ظاهر من التأثير السلبي للتراكيز العالية لكل من كلوريد الصوديوم والبوتاسيوم . وأظهرت النتائج أيضًا أن بادرات نبات البرسيم الحجازي أكثر مقاومة للمعادن الثقيلة من بادرات نبات البرسيم الحجازي، و جذور نبات الأرز أكثر حساسية من المجموع الخضري للأجهاد الملحي لكل من كلوريد الصوديوم والبوتاسيوم .