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Copper toxicity of clove [*Syzygium aromaticum* (L.) Merr. and Perry] seedlings

(Keracunan kuprum pada anak benih cengkih [*Syzygium aromaticum* (L.) Merr. dan Perry])

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Key words : copper, toxicity symptoms, toxicity level

Abstrak

Anak pokok cengkih yang berumur setahun dan ditanam di dalam medium pasir telah diberi larutan nutrien lengkap dengan kadar larutan kuprum (Cu) yang berbeza (0.25 hingga 250 bpj Cu/L) dan diteliti untuk gejala keracunan Cu. Pokok yang disiram dengan larutan Cu pada kadar 0.25 dan 2.5 bpj tumbuh dengan baik dan subur. Akan tetapi, gejala keracunan Cu kelihatan di daun pada pokok yang disiram dengan 25 dan 250 bpj Cu. Kandungan Cu di dalam daun dan akar pokok subur ialah masing-masing antara 13.7 hingga 19.5 bpj dan 77.0 hingga 92.3 bpj. Pokok yang terkena keracunan Cu pula menunjukkan peningkatan kandungan Cu di dalam daun, 3–10 kali ganda berbanding dengan pokok yang subur. Kandungan Cu di dalam akar meningkat 8-50 kali ganda. Gejala keracunan Cu pada pokok cengkih juga dibincangkan.

Abstract

One-year-old clove seedlings grown in sand culture were given complete nutrient solutions with varying levels of copper (Cu) concentrations (0.25–250 ppm Cu/L) and observed for symptoms of Cu toxicity. The plants grew normally with applications of 0.25 and 2.5 ppm Cu solution. However, Cu toxicity symptoms were observed in the leaves at 25 and 250 ppm Cu concentrations. The leaf and root Cu content of healthy plants ranged from 13.7 to 19.5 ppm and 77.0 to 92.3 ppm, respectively. The affected plants showed 3 to 10-fold increase in leaf Cu content compared with the healthy plants. The root Cu content of affected plants increased by 8 to 50-folds. The Cu toxicity symptoms on clove are also discussed.

Introduction

Copper (Cu) toxicity in plants seldom occur as copper is very strongly bound in soils (Mengel and Kirkby 1982). However, cases of high accumulation of Cu in soils which can lead to toxicity problems do occur occasionally in soils which have been regularly treated with copper salts (Nag Raj and George 1960; Delas 1963). One suspected case of copper toxicity incidence was recorded in a clove farm in Kota Tinggi, Johore, Malaysia. Trees at this farm were generally growing very well but they deteriorated slowly after the severe drought in 1983. This resulted in the development of stag-head (branches with little or no leaves) canopies. New shoots formed on some trees after rain but these trees never regained their

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former healthy condition.

Suspicion of disease problems led to pathological work being done. Isolations from dying twigs or branches showed *Phomopsis* sp., *Botryodiplodia* sp. and *Phoma* sp. with *Fusarium* sp. and *Phytopthora cinnamonii* isolated from the roots. All these fungi had been found to be pathogenic on wounded (pricked) young clove leaves in the laboratory, but infections could not be obtained on stems/branches nor was the decline symptoms reproducible in the field. From the uniformity of declining plants field-wide, a primarily pathogenic origin for the disease appeared unlikely. In addition, plant die-back with root deterioration commonly occurred in the new plantings and resupply trees. Fungicide drenching with benomyl, fesothyl-aluminium and nematicide treatment with fenamiphos had no positive effects on these plants. Thus, other factors were looked into, especially that of copper toxicity as routine sprayings of copper-based fungicides had been carried out since field planting, for a period of approximately 5 years. Results of plant and soil analyses from this farm showed significantly higher copper levels, 20-30 times, than in normal trees of similar age and size from a farm in Kluang (*Table 1*). In view of all these findings, an investigation was carried out at MARDI Kluang to examine the effects of copper on clove and to record its toxicity symptoms.

Materials and methods

Sixteen one-year-old clove seedlings of relatively uniform size were grown in sand culture in plastic pails. The sand had previously been treated for a week with 3.0% hydrochloric acid and subsequently rinsed three times with distilled water to get rid of the acid traces. Sixteen 18-L plastic pails, each with a 25-cm width and a 1-cm hole bored at the base, were filled up with 18 kg of the cleaned sand. After transplanting, the clove plants were 'soil'-drenched daily with 200 mL of complete nutrient solution for 3 months in the glasshouse to allow the plants to stabilize. This was then followed by daily application of treatment solutions. Copper was applied in the form of $CuSO_4$ and the concentrations ranged from 0.25 to 250 ppm/L *(Table 2)*. The composition of complete nutrient solution followed that described by Vimala and Ravoof (1981). Each treatment consisted of four plants with four replications. The plants were arranged in a completely randomised design.

Leaf toxicity symptoms were recorded weekly. Copper in leaves and roots of all the plants were analysed at the end of 6 weeks by an inductively coupled plasma emission spectrometer. Leaf and root iron (Fe) contents were also analysed from healthy and affected plants as Cu often interfered with the uptake of Fe (Reuther and Smith 1953). Plant growth parameters were not taken as the plants were deliberately chosen on their basis of uniform height and size. In addition, the duration of the study was too short to cause any appreciable increase in plant height and dry matter content.

Table 1. Plant and soil analysis of two clove farms in Johore

Farm location	Soil type	Plant description		Mean Cu content (ppm)	
		Age	Conditions	Leaf	Soil
Kota Tinggi	Harimau series	5 years	Juvenile decline and die-back	91.6	20.0
Kluang	Rengam series	5 years	Healthy	4.5	0.6
t-test (p = 0.05)		-	-	*	*

Table 2. Composition of nutrient solutions for sand-culture plants

Compositions	Concentration of treatment solutions (ppm/L)			
	T1*	T2	T3	T4
KNO_3	303	303	303	303
$Ca(NO_3)_2 . 4H_2O$	590	590	590	590
$(NH_4)_2SO_4$	132	132	132	132
$NaH_2PO_4 . 2H_2O$	156	156	156	156
$MgSO_4 . 7H_2O$	368	368	368	368
$CaSO_4 . 2H_2O$	258	258	258	258
$MnSO_4 . 4H_2O$	2.23	2.23	2.23	2.23
$ZnSO_4 . 7H_2O$	0.58	0.58	0.58	0.58
H_3BO_3	1.86	1.86	1.86	1.86
$NaMoO_4 . 2H_2O$	0.06	0.06	0.06	0.06
$FeC_6H_5O_7 . 5H_2O$	6.7	6.7	6.7	6.7
$CuSO_4 . 6H_2O$	0.25	2.5	25	250

* After Vimala and Ravoof (1981)

Results and discussion

Leaf toxicity symptoms

Clove growth was very good and the plants remained healthy with the application of 0.25 and 2.5 ppm Cu solutions. However, growth suppression was visible in plants which had been 'soil'-drenched with higher concentrations of Cu solution (25 and 250 ppm Cu). At 25 ppm, a mild form of Cu toxicity was observed, as shown by the stunting and scorching of leaf tips and margins 4 weeks after Cu application. The affected plants also showed some degree of chlorosis in the mature leaves. Before chlorosis and scorching set in, some vegetative growth was also observed *(Plate 1)*. With the 250 ppm Cu, there was no new vegetative growth, the branches drooped, leaves wilted and became flaccid in the afternoon just 2 weeks of solution application. However, these affected plants were able to recover the following morning. These wilting symptoms lasted for another 2 weeks before the leaves finally dried up and dropped off at the end of the fourth week. The symptoms described may be common to many types of plants as similar acute toxicity symptoms had also been reported in pepper by Kueh (1979).

Plant copper content

There were significant differences in leaf and root Cu contents between the various concentrations of Cu applications *(Table 3)*. The logarithms transformation was used to stabilize the variance. The normal healthy plants showed no significant difference in Cu contents both in the leaves and roots when 'soil'- drenched with 0.25 to 2.5 ppm Cu solution but were significantly different for plants applied with 25 and 250 ppm Cu solution. The Cu content in the leaf and root of healthy plants ranged from 13.7 to 19.5 ppm and 77.0 to 92.3 ppm. The leaf Cu content was found to be between the normal limits of most non-Cu-tolerant plants, i.e. 2–20 ppm (Robson and Reuter 1981). When the plants were 'soil'-drenched with 25 ppm Cu, the Cu content in the leaf and roots was 41 ppm and 631.7 ppm respectively. Cu levels were significantly higher than those in plants applied with 0.25 and 2.5 ppm Cu. The affected plants showed mild toxicity symptoms through slight chlorosis and leaf scorching. At 250 ppm Cu solution, the leaf and root Cu contents was 154 and 3 882.7 ppm respectively and these levels of Cu were significantly higher than those of the other treatments. This high concentration of Cu application was found to be toxic and lethal to plant growth as the plant dried up

Plate 1. Clove seedlings drenched with 25 ppm (T3) and 250 ppm Cu (T4) showing scorching, chlorosis and wilting appearance 4 weeks after Cu application

Table 3. Effects of $CuSO_4$ solutions on the Cu content in leaves and roots of clove seedlings in sand culture

Cu concentration (ppm/L)	Log mean copper content (ppm)	
	Leaf	Root
0.25	1.12a (13.7)	1.87a (77.0)
2.50	1.29a (19.5)	1.98a (92.3)
25.00	1.61b (41.0)	2.86b (631.7)
250.00	2.14c (154.0)	3.58c (3 882.7)

*Figures with the same letters are not significant at $p = 0.05$ according to DMRT
Figures in brackets are actual means

and died just 4 weeks after treatment.

Generally, the root Cu content was higher than those in the leaves. Keller and Deuel (1958) explained that Cu was able to displace most other ions from ion exchange site and was very strongly bound in the root free space. As such, higher Cu content was frequently found in the root compared with other plant tissues. Smith and Specht (1953) also reported high accumulation of Cu in citrus roots, to the point of toxicity, without much being transported to the leaves.

Copper–iron interaction

Leaves and roots of healthy and Cu-injured plants were analysed for Fe content. The result showed higher Fe content in the healthy plants than injured plants. The Fe content in leaves and roots of healthy plants was 258.3 and 1 057.0 ppm respectively. However, the chlorotic plants had a slightly lower Fe in the leaves and roots, 194.0 and 686.0 ppm respectively *(Table 4)*. This probably explained the slight chlorosis due to lower Fe in the leaves of Cu toxicity plants. Similar chlorosis symptoms for Cu toxicity plants were also reported on citrus and corn (Reuther and Smith 1953; Smith and Specht 1953; Baker 1974). Smith and Specht (1953) explained that Cu blocked the upward translocation of Fe and this induced

Table 4. Iron content of various plant parts of healthy and chlorotic clove plant

Plant part	Iron content in plant (ppm)	
	Healthy & green plants	Chlorotic plants induced by excessive Cu
Leaves	258.3	194.0
Roots	1 057.0	686.0

Fe chlorosis in citrus leaves. Thus, diagnosis of Cu toxicity through leaf symptoms alone would not be reliable as it could superficially resemble those of Fe deficiency. As such, Robson and Reuter (1981) suggested that for the diagnosis of Cu toxicity, plant analysis would be a more reliable method.

Conclusion

The study provided some basic information on the range within which Cu can be tolerated by clove, especially at the seedling stage. The plants grew normally with application of 0.25 and 2.5 ppm Cu solution but at 25 and 250 ppm, Cu toxicity symptoms were observed. The branches of affected plants drooped, the leaves wilted and dried up before dropping off. The leaf Cu content of healthy plants ranged from 13.7 to 19.5 ppm compared with 154 ppm in the affected plants. However, it should be emphasized that the study is preliminary and limited in nature, and the findings on Cu tolerance level of plants growing in sand culture in glasshouse may not necessarily apply to plants growing in the field.

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