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Research Application Summary

Effect of soil copper accumulation on proliferation and survival of rhizobia

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Abstract

Two strains of the fast-growing bean rhizobia- PV_1 and PV_2 and two others of the slow-growing cowpea and soybean bradyrhizobia- CP_1 and GM_8 respectively, were used to test their *in vitro* proliferation and *in vivo* survival. Known amounts of each of the four strains studied were exposed to Cu concentrations in the range:- 0, 20, 40, 60, 80 and 100 ppm both *in vitro* and *in vivo*. It was observed that slow-growing rhizobia are more susceptible to Cu toxicity both *in vitro* and *in vivo* than the fast-growing rhizobia.It is important however to assess the effect of copper on nodulation and nitrogen fixing.

Key words: N_2 fixing, Bradyrhizobia, copper toxicity, cowpea, pesticides

Résumé

Deux souches de haricots rhizobia-PV_1 et PV_2 de croissance rapide et respectivement deux autres du niébé et de soja bradyrhizobiums-CP_1 et GM_8 de croissance lente, ont été utilisés pour tester leur prolifération *in vitro* et leur survie *in vivo*.Des quantités connues de chacune des quatre souches étudiées ont été exposées à des concentrations en Cu dans la fourchette suivante: - 0, 20, 40, 60, 80 et 100 ppm à la fois *in vitro* et *in vivo*. Il a été observé que les rhizobiums à croissance lente sont plus sensibles à la toxicité du Cu *in vitro* et *in vivo* que les rhizobiums à croissance rapide.Il est toutefois important d'évaluer l'effet du cuivre sur la nodulation et la fixation de l'azote.

Mots clés: Fixation de N_2, Bradyrhizobia, la toxicité du cuivre, niébé, pesticides

Background

There is evidence that rhizobia and their legume associations can suffer from stress due to copper-based fungicide toxicity. Fisher and Hayes (1981) found that N_2 fixation was reduced mainly where the vigour and growth of the plant was inhibited by fungicides. Baijukya (1996) showed that nodulation and N_2 fixation was impaired when Kocide 101 (77% cupric hydroxide) was used at concentrations higher than the recommended rate. He demonstrated that this was due to its adverse effects on

the bean plant rather than on rhizobia. However, the role of copper on survival and occurrence of rhizobia in soil has not been unequivocally established.

In the current study, two strains of the fast – growing bean rhizobia- PV_1 & PV_2 and two slow – growing strains, one each from cowpeas and soybean - CP_1 & GM_8 respectively, were used to test their ability to proliferate and survive in copper contaminated environments.

Literature Summary

Rhizobia show varied *in vitro* growth under pesticide treatment. Some pesticides are not detrimental to the growth of rhizobia when applied at field rates, whereas other pesticides were found to be toxic to rhizobia when applied at low as well as high rates (Martensson, 1992). Certain strains of rhizobia could resist high levels of pesticides by adaptation (Kanta *et al.*, 1987).

Study Description

Known amounts of each of the four strains under study were exposed to copper concentrations in the range:- 0, 20, 40 60, 80 and 100 ppm in Yeast - extract Mannitol Broth. After incubation, the popuplation of each strain was determined. These strains were also exposed to copper at the same concentrations as above in modified Leonard Jar assemblies potted with soil. Three parameters, i.e., fresh nodule volume, fresh nodule mass and total plant nitrogen were monitored. Medical flat bottles were used to determine cell survival. Most Probable Number (MPN) studies were also done to estimate populations of the native strains of the fast - and slow - growing rhizobia in Cu - contaminated soil.

Research Application

From this study it was observed that slow-growing rhizobia (i.e. *Bradyrhizobium* spp.) were more susceptible to Cu toxicity both *in vitro* and *in vivo* than the fast-growing rhizobia (i.e. *Rhizobium* spp.). It was noted further, that, while strains of the fast growing *Rhizobium* did not differ much in their survival response to copper toxicity, there was differential survival response to copper toxicity stress between strains of *Bradyrhizobium*. *S*train CP_1 was less affected by copper toxicity than strain GM_8 (Table 1).

MPN counts however, indicate that, except for the native cowpea bradyrhizobial strain, did not significantly ($p=0.05$) affect the numbers of native strains of the other (brady) rhizobial strains (Figs. 1 - 4). There is however, need to examine whether the above phenomena is true when more strains are involved and

Table 1. Effects of copper on *in vitro* proliferation of rhizobia/bradyrhizobia.

Copper concentration (ppm)	Mean microbial population (Log_{10} CFU/ml)			
	PV_1	PV_2	CP_1	GM_8
0	>9.979	>10.115	>9.822	>14.739
20	9.979a	10.115a	9.822a	14.739a
40	9.053b	9.301b	8.418b	12.778b
60	9.204b	9.587c	8.000c	12.301c
80	8.667c	9.038d	0.000d	0.000d
100	8.519c	8.560e	0.000d	0.000d
**C.V (%)	3.10	0.50	1.00	1.60

Means in the same column followed by the same letters are not significantly different. (p = 0.05) according to the Least Significant Difference (LSD) Test. CFU = Colony Forming Units **CV = Coefficient of variation.

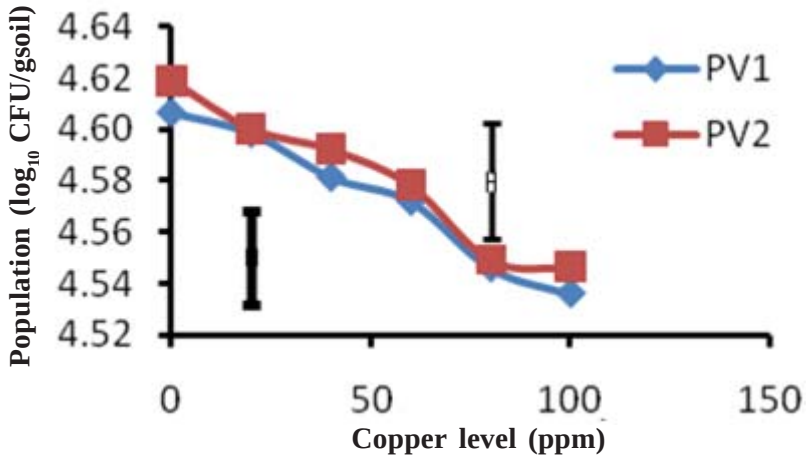

Figure 1. Effect of copper on soil populations of rhizobial strains-PV_1 & PV_2. Bar indicates LSD (0.05)-

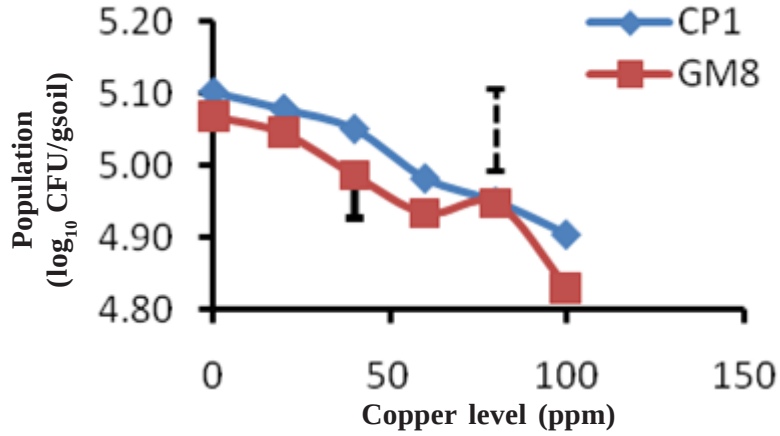

Figure 2. Effect of copper on soil populations of bradyrhizobial strains-CP_1 and GM_8. Bar indicates LSD (0.05) – Solid line for CP_1 and dashed line for GM_8.

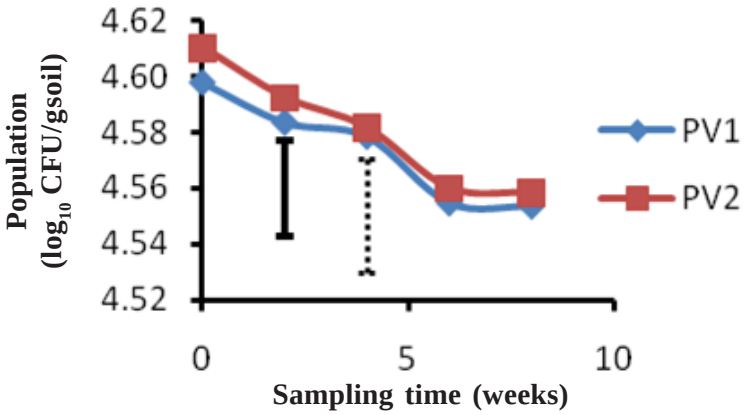

Figure 3. Effect of sampling time on survival of rhizobial strain-PV_1 and PV_2. Bar indicates LSD (0.05)- Solid line for PV_1 and dashed line for PV_2.

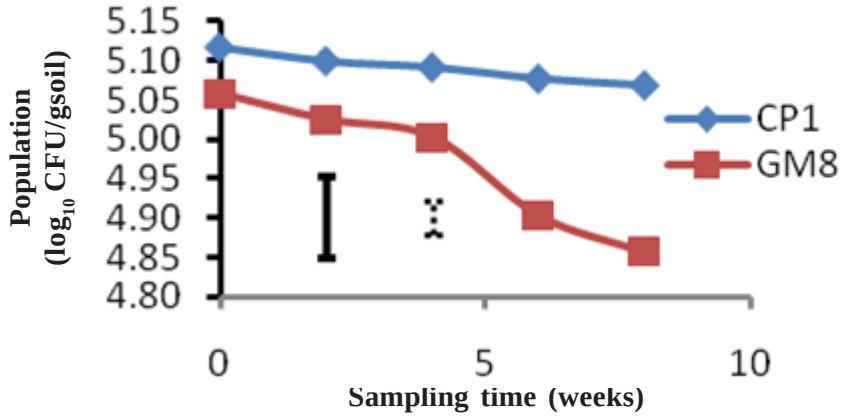

Figure 4. Effect of sampling time on survival of bradyrhizobial strains- CP_1 and GM_8. Bar indicates LSD (0.05) – Solid line for CP1 and dashed line for GM8.

in a wider range of Cu - contaminated soils. It is then that reliable conclusions can be made regarding the differential response of slow – and fast – growing rhizobia to Cu contamination.

Recommendation

There is clearly a negative effect of copper concentration on the survival of rhizobia. There is therefore need to judiciously use agro-inputs that contain copper to ensure that soils are not contaminated with copper. However the effect of copper on nodulation and nitrogen fixation needs to be determined.

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