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Growth Activities of *Vigna sinesis* as Influenced by Zn and Microsymbiont of Mycorrhizae and *Rhizobium*

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The influence of different concentrations of Zn and microsymbiont of VAM fungi and *Rhizobium* bacterium on some growth parameters of *Vigna sinesis* (cowpea) indicated that Zn concentration of 100-200mg/ kg air dry soil was optimum for attaining the best growth parameters of cowpea of leaf number, leaf area, fresh and dry weights of shoot and root, as well as, stem and root lengths, either microsymbiont inoculated or non. Microsymbiont inoculations were needed for higher growth values, at the different tested concentrations of Zn as compared with microsymbiont free treatments, i.e. microsymiont improving the tolerance of the plant against the toxicity of higher concentrations of Zn.

Chlorophyll a,b and carotenoids formation by the plant leaves were reduced markedly by Zn levels more than 50mg/ kg soil. While 100mg of the metal was responsible for maximum plant contents of carbohydrates. However, the best total nitrogen and phosphorus accumulation in leaves occurred at 200mg Zn/ kg soil. Inspite of the toxicity of Zn(>200mg), microsymbiont inoculation was accompanied by noticeable higher values of the tested plant metabolites at the different tested heavy metal concentrations.

Zn was mainly accumulated in the plant roots than in leaves and microsymbiont inoculation clearly increased its accumulation than non-inoculated soils, i.e. microsymbiont improving the tolerance of the plant against Zn toxicity. Zn has detectable influences on rhizobial nodulation and VAM root colonization. Zn level of 200 mg/kg soil was responsible for the best nodulation and VAM colonization.

Keywords: *Vigna sinesis*, Mycorrhizae and *Rhizobium*.

Enhanced symbiotic nitrogen fixation or colonization by vesicular-arbuscular mycorrhizal (VAM) fungi often results in improved host growth and nutrient assimilation (Barea and Azcon - Aguilar, 1983; Pacovsky et al, 1991). The relationship between the VAM fungi and rhizobia in terms of effects on plant yield is influenced by differences among species, strains and cultivars of the symbionts (Carling and Brown, 1980). Both rhizobia and VAM fungi are active in root cortical cells and, hence, it can be assumed that the presence of microsymbiont will affect the activity of the others (Vejsadova et al, 1992). Most studies have concentrated on indirect relationships between VAM fungi and rhizobia, in which a successful symbiosis has been measured as an increased uptake of nutrient elements by the plant (Ames and Bethlenfalvay, 1987) and increased N_2- fixation and nodule mass (Fredeen and Terry, 1988), but more direct non-nutritional effects of VAM fungus – rhizobia interactions have also been reported (Ross and Harper, 1970; Bethlenfalvay et al, 1985).

The toxicity of higher Zn concentrations to vascular plants is well documented. Zinc is well known as an essential nutrient mineral for normal growth of plants (Jyung et al, 1975). However, higher concentrations of Zn, as a result of soil pollution, reduce photosynthesis, respiration, accumulation of sugars, starch and protein, as well as several physiological processes (El-Kherbawy et al, 1989; Xian, 1989; Turnau et al, 1996).

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VAM fungi are wide- spread in soils and their interaction with minerals other than P, particularly heavy metals, has been the subject of many studies because of the possibility of a beneficial effect of mycorrhizae in improving the tolerance of plants against toxicity (Haselwandter et al, 1994; Diaz et al, 1996; Ahonen-Jonnarth and Finlay, 2001; Liao et al, 2003; Vivas et al, 2003). The uptake of metals by mycorrhizal plants depends on several factors such as the physico-chemical properties of the soil (Wang and Chao, 1992), pH (El-Kherbawy et al, 1989), the host plants (Griffioen and Ernst, 1989), the fungi involved (Gildon and Tinker, 1981), and above all , the concentration of the metals in the soil . Under deficiency conditions, most studies point to an increase in metal uptake by mycorrhizal plants (Manjunath and Habte, 1988; Kothari et al, 1990). When the soils contain high potentially toxic amount of heavy metals, mycorrhizal formation usually induces lower concentrations of these metals in the aerial part of the plant and consequently a beneficial effect on plant growth (Guo et al 1996; Jurkiewicz et al, 2001).

In the present work, the effect of different concentrations of Zn and microsymbiont of VAM fungi and *Rhizobium* on some growth parameters and metabolic activities of cowpea (*Vigna sinesis*) were carried out . The rhizobial nodulation and VAM root colonization were also investigated.

MATERIALS AND METHODS

The soil

Sandy loam soil (1:1) was air dried, passed through 2 mm sieve, mixed thoroughly for homogênicity and sterilized by autoclaving at 121° C for 20 min to kill soil microflora. The soil is non-saline, with pH 7.9, field capacity 653 ml/ kg air dry soil and has 1.35 % organic matters. The total soluble salts were 1.17 %, with total nitrogen content of about 0.89 mg/kg and phosphorus content of 0.042 mg/kg. It contains (µg/g air dry soil) 13.4 Mn, 94 K, 138 Mg, 10.7 Zn, 0.83 Cd, 3.11 Cu and 0.49 Pb.

Test plant

Seeds of cowpea (*Vigna sinesis*) were kindly provided from Hada Al-Sham farm, Faculty of Metrology, King Abdulaziz University, Jeddah, Saudi Arabia. The seeds sizes and weights were homogenous. They were surface sterilized (0.1% $HgCl_2$ + 0.2% HCl for 5 min), followed by repeated washes with sterile distilled water (Vincent, 1976). Seeds were planted in plastic pots (18 cm in diameter and 13 cm in depth) loaded with 2 kg air dried sterilized sandy loam soil, received 60 ml weekly of Hoagland's solution, minus phosphate, (Hoagland and Arnon, 1950, Downs and Hellmers, 1975). This solution consists of two liquids, A and B. Before they were used, 2 ml of each solution were added to one litre of tap water. Soil was mixed with 0.2 g rock phosphate / kg soil , which consists of (%) : P_2O_5, 29; CaO, 45.9; Fe_2O_3, 1.9; Al_2O_3, 0.39; MgO, 0.23; Na_2O,0.3 ; K_2O , 0.01 ; Fe, 2.9; SiO_2, 13.5 ; SO_3, 0.56; Co_2, 4.25; Cl 0.05 and organic matter 0.25 . Ten seeds were planted in each pot at equal intervals. Field capacity of tap water was applied per pot for irrigation. The seeds were irrigated three times a week, until the plant seedlings emerge (about 5cm height) and thinned to five per pot.

The pots are classified into six groups, each one have 5 replicas (pots). The first group has no additives (control treatment) of heavy metal (Zn) and microsymbiont (*Rhizobium* cells and VAM spores), the second receives the microsymbiont only. The third treatment receives 50 mg Zn (about 0.22 g of $ZnSO_4.7H_2O$) per kg air dry soil and the fourth amended with microsymbiont and 50 mg Zn/kg soil.

Cowpea (*Vigna sinesis*) seedlings were allowed to grow under greenhouse conditions (30° C and illumination period of 13 h/day) for seven weeks. At the end of the tested growth period, plant samples were carefully uprooted, washed thoroughly with tap water and rinsed twice with distilled water. After washing, the root and leaves were separated, thereafter the necessary analyses are carried out.

Microsymbiont inoculations

Mycorrhizal spores

The spores of VAM fungi were extracted from their cultivation and propagation pots, planted by *Zea mays*, using wet sieving and decanting method (Daniels and Skipper, 1982). The spore suspension was diluted with water, so that each ml has 55 spores. For soil inoculation, the surface soil crushed and mixed thoroughly with 15 ml spore suspension and return back to its pot.

Rhizobium strain (ARC 610)

It was kindly provided by biofertilization unit of Faculty of Agriculture, Ain-Shams University. Cairo, Egypt. The Culture was cultivated, propagated and maintained in mannitol yeast extract medium (Allen, 1961), with regularly sub-culturing every two weeks. The inoculum (bacterial cells) was prepared from liquid mannitol yeast extract medium after 7 days of incubation at 37°C. The inoculum was 10 ml / pot (2 kg soil) having about 17.5×10^6 cells.

Growth parameters of cowpea

The plant height (cm), root system length (cm), leaf number and leaf area (cm^2) were measured. The fresh and dry weights of the root and shoot systems were also determined.

Total carbohydrates

Total carbohydrates were determined, after hydrolysis, colourimetrically using anthron reagent (Fales, 1951).

Plant pigments

Chlorophyll a, b and carotenoids were estimated spectrophotometrically (Metzner et al, 1965), after acetone extraction of the pigments from fresh leaves.

Total nitrogen content

It was estimated colourimetrically using Nessler reagent (Delory, 1949; Humphries, 1956).

Determination of phosphorus and Zn

The plant material digested in nitric-perchloric acid mixture (5: 3) and analyzed colourimetrically with malachite green reagent (Fernandez et al, 1985) to determine P concentration, and by atomic absorption spectrophotometry in Perkin – Elmer 500 instrument for Zn.

Mycorrhizal root infection

Mycorrhizal colonization was assessed using the grid – line intersect method (Giovannetti and Mosse, 1980) for examination of cleared and stained (Phillips and Hayman, 1970) root samples.

Rhizobial root nodules

The root nodules were estimated for each plant, separated and collected to determine their fresh and dry mass (Vejsadova, et al, 1992).

Each treatment was carried out in five replicates and the recorded results were the arithmetic mean. Data were statistically analyzed using one way analysis of variance ANOVA on the basis of which LSD values ($P \leq 0.05$ for $N=5$) for any two compared means were calculated.

RESULTS AND DISCUSSION

Effect of Zn and microsymbiont on some growth parameters of cowpea

The effect of different concentrations of Zn and microsymbiont of VAM fungi and *Rhizobium* on some growth parameters of *Vigna sinesis* (Figs. 1-3) indicated that Zn concentration of 200 mg/kg air dry soil was optimum for stem and root lengths either with microsymbiont inoculation or non, and lower or higher concentrations were concomitant with lower root and stem lengths. However, Zn level of 100 mg / kg soil was the best to attain higher values of the other tested growth parameters of cowpea (leaf number, leaf area, fresh and dry weights of shoot and root systems), either the soil has no microsymbiont of VAM fungi and *Rhizobium* or it inoculated with them. The results, revealed that inoculation with the microsymbiont was responsible for higher growth values at different Zn concentrations as compared with non-inoculated soil. Generally, it is safe to conclude that Zn at 100-200 mg/kg soil was necessary to attain the best growth parameters, under the tested condition by cowpea plant. However, higher levels of Zn are conducive to the tested parameters. The results fairly indicated that the presence of VAM fungi and *Rhizobium* bacterium noticeably improving the tolerance of *Vigna sinesis* plants against toxicity of Zn. Thus at the more toxic Zn concentration (1g Zn / kg air dry soil) microsymbiont inoculation increases stem and root lengths, leaf number and area, and shoot system fresh and dry weights, as well as, root fresh and dry weights by about 40, 38, 28, 40, 50, 63, 35 and 75 %, respectively, as compared to non-inoculated soils.

The previous results assessed that Zn is an essential mineral nutrient, within certain concentrations, for normal plant growth (Jyung et al, 1975).

In accordance with these findings, it was reported that microsymbiont of nitrogen fixer and VAM improved host plant growth and nutrient

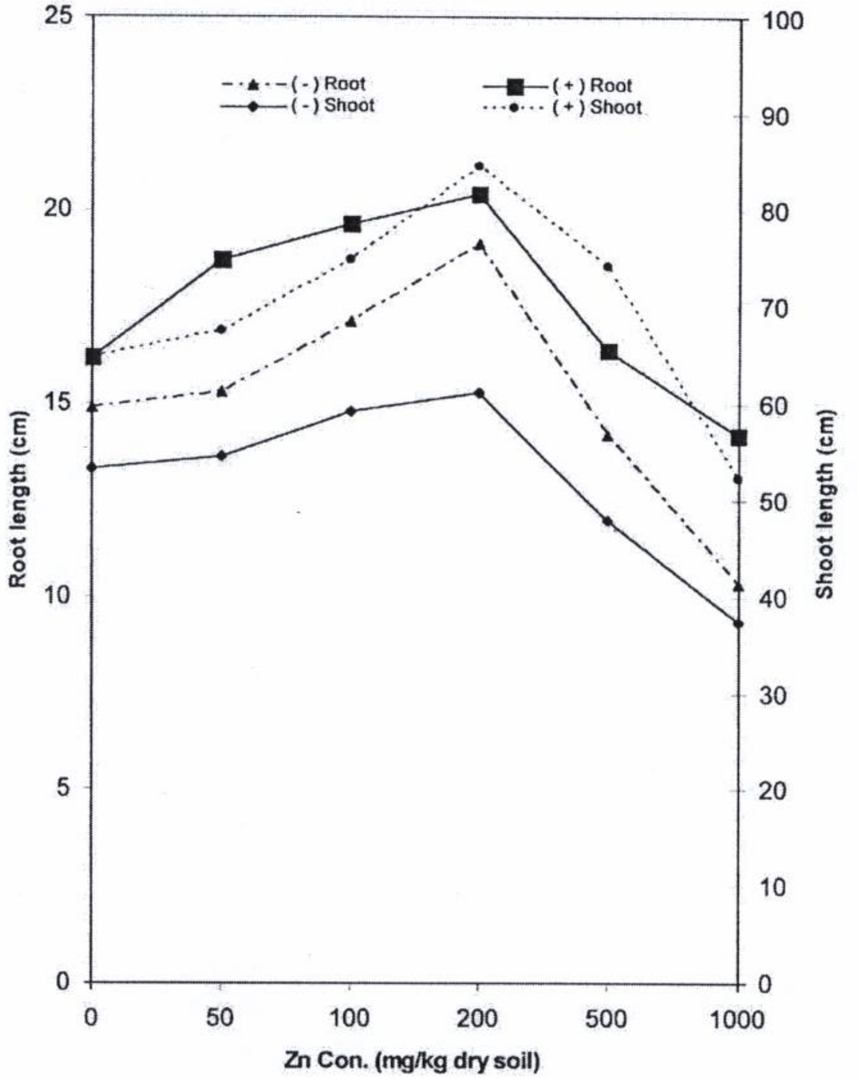

Fig. 1. Effect of different concentrations of Zn on shoot and root lengths of cowpea (*Vigna sinesis*) inoculated with microsymbiont (+), or non-inoculated (-), after 7 weeks of planting

assimilation (Pacovsky et al, 1991) and VAM fungi positively affected soybean biomass production and dry matter yield (Vejsadova et al, 1992; Andrade et al, 2003). The uptake of heavy metals by some plants was affected by the colonization of roots with VAM fungi (Abou-Shanb et al , 2004). Generally, VAM fungi improving the tolerance of plants against toxicity of heavy metals (Ahonen-Jonnarth and Finlay, 2001; Liao, et al, 2003; Vivas, et al, 2003). It was also reported that heavy metals addition to the soil induced a reduction in plant growth (Diaz et al, 1996).

Effect of Zn and microsymbiont on some metabolic activities of cowpea

The influence of different concentrations of Zn on some metabolites (leaf pigments, total carbohydrates and nitrogen) and mineral content of P and Zn of *Vigna sinesis* inoculated with microsymbiont of *Rhizobium* cells and VAM spores or non-inoculated (Figs. 4,5) indicated that inoculation of microsymbiont to soil, in presence

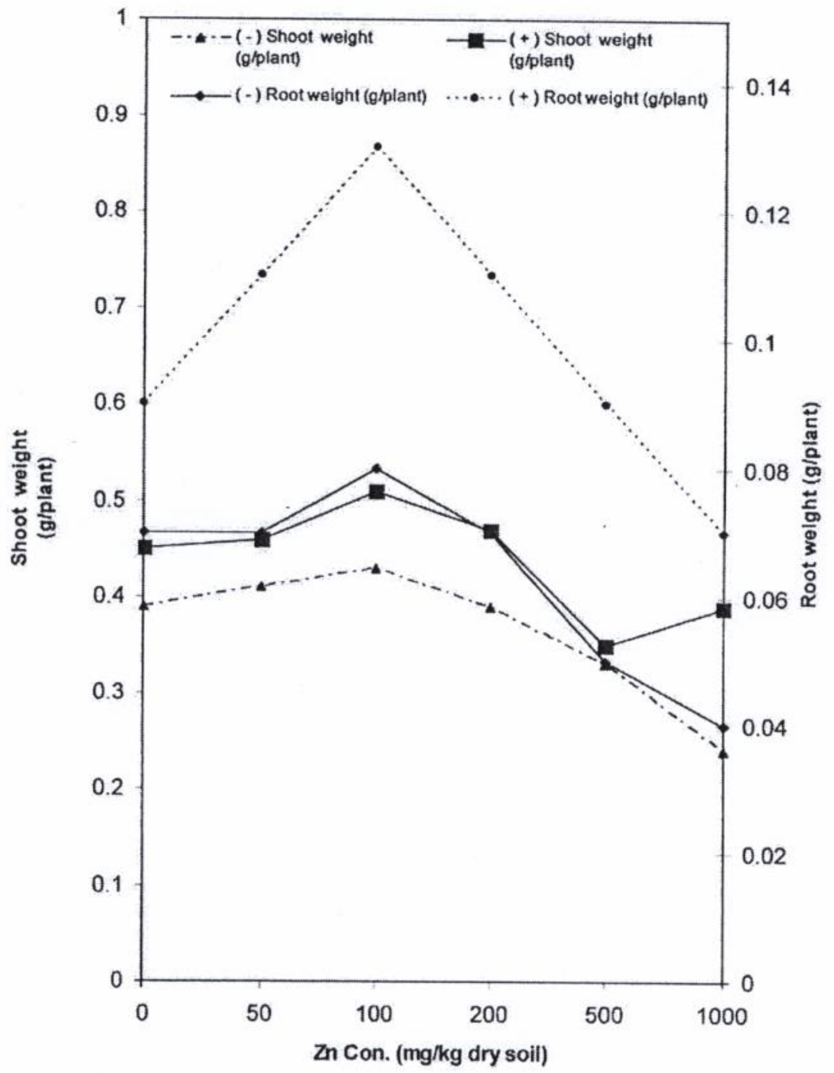

Fig. 2. Effect of different concentrations of Zn on dry weight of cowpea (*Vigna sinesis*) inoculated with microsymbiont (+), or non-inoculated (-), after 7 weeks of planting.

of different concentrations of Zn improves cowpea growth and nutrient assimilation (Pacovsky et al, 1991). Zn concentrations more than 50 mg/kg air dry soil were conducive (inhibitory) to pigments (chlorophyll a, b and carotenoids) formation by *Vigna sinesis* leaves. Chlorophyll a, b and carotenoids showed about 2.5, 1.5 and 2.0, fold decrease, respectively, as Zn concentration increased from 50 to 1000 mg/kg soil, in non-microsymbiont inoculation. However, microsymbiont inoculation at lg Zn / kg soil lead to about 63, 100 and 32 % increase in formation of chlorophyll a, b and carotenoids, respectively, as compared with non – inoculated soil at the same Zn concentration. It is also indicated that carbohydrates biosynthesis by cowpea stimulated by microsymbiont inoculation more than non-microsymbiont and Zn concentration of 100 mg/kg soil was necessary for maximal carbohydrates formation. Higher Zn levels more than 200 mg/kg soil were of inhibitory action on carbohydrates biosynthesis.

As for the total nitrogen content, the plant leaves accumulate nitrogen compounds

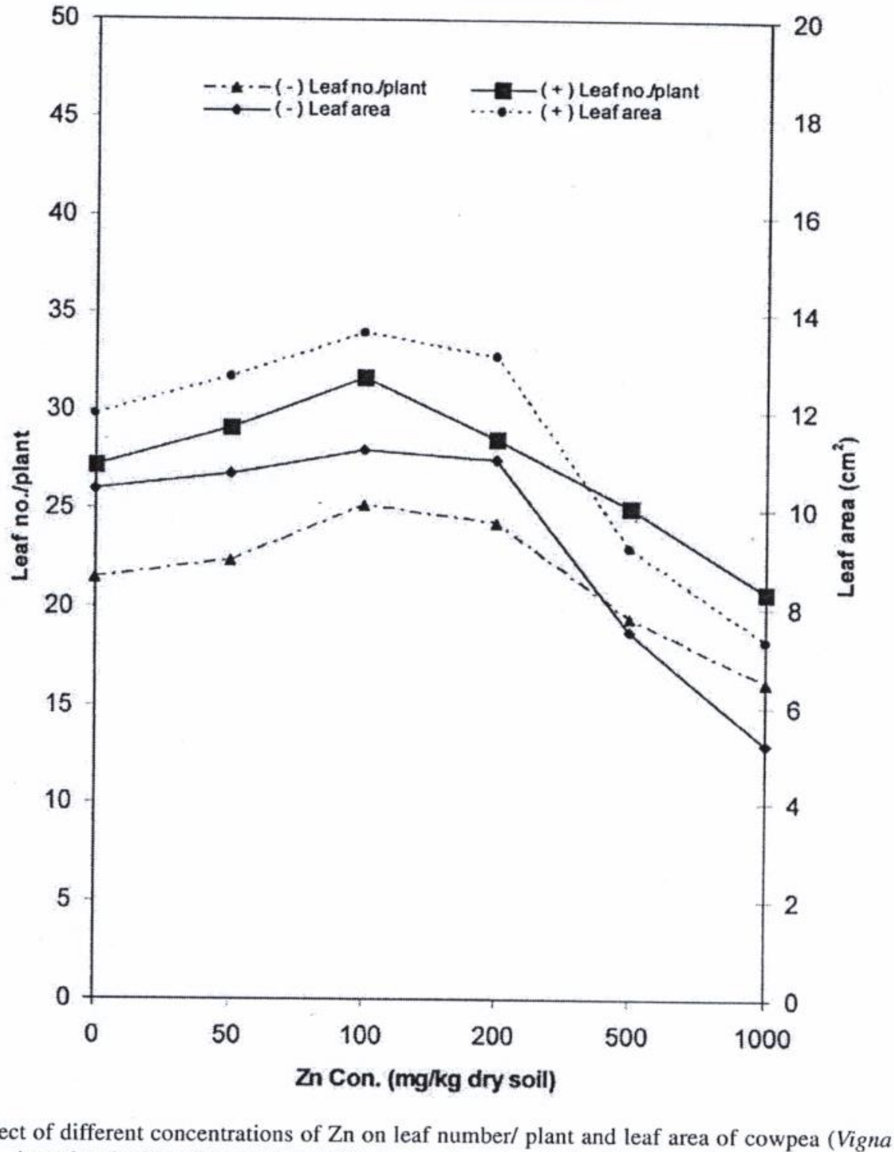

Fig. 3. Effect of different concentrations of Zn on leaf number/ plant and leaf area of cowpea (*Vigna sinesis*) inoculated with microsymbiont (+), or non-inoculated (-), after 7 weeks of planting.

more than do the root system (generally, about 7 fold increase) and Zn level (100 – 200 mg/kg soil) was responsible for maximal total nitrogen accumulation in the root system, while 200 mg Zn/kg soil was inductive to maximum accumulation in leaves , either in absence or presence of microsymbiont. Microsymbiont inoculation stimulated total nitrogen content of root and leaves, at the different Zn concentrations higher than non-inoculated plants.

Microsymbiont inoculated cowpea was able to accumulate phosphorus in root and leaves higher than non-inoculated ones. Phosphorus mainly translocated from root to leaves, so that it is accumulated properly in leaves by about 5 folds increase at different Zn levels, in both inoculated and non-inoculated soils. Zn concentration of 100 mg / kg in microsymbiont soil was responsible for the highest accumulated P in roots, whereas, 200 mg Zn was needed for maximum P in leaves, under the same conditions.

Vigna sinesis grown in microsymbiont inoculated soils accumulated Zn mainly in the root system by about 4 fold increase (at higher Zn

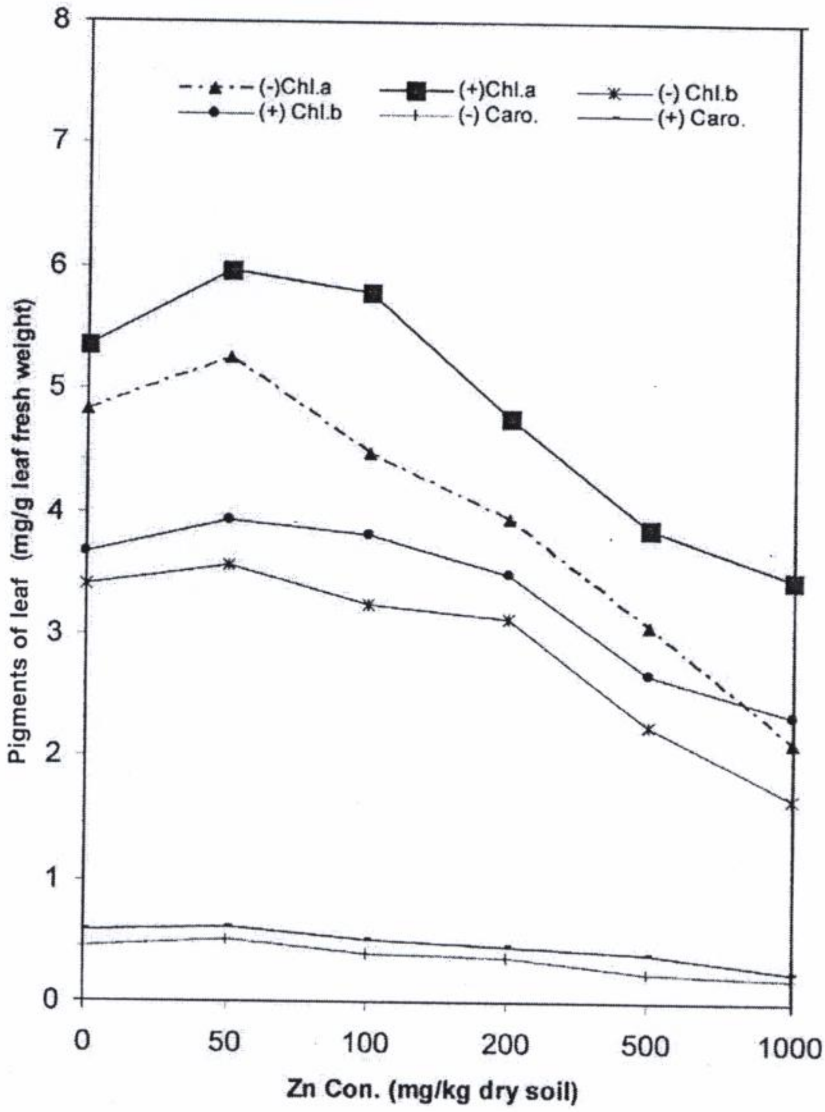

Fig. 4. Effect of different concentrations of Zn on pigments of leaf (mg/g^{-1} leaf fresh weight) of cowpea (*Vigna sinesis*) inoculated with microsymbiont (+), or non-inoculated (-), after 7 weeks of planting.

concentrations, 0.5, 1 g / kg soil) than in leaves, where in absence of microsymbiont not exceeded 2 folds. And as the Zn concentration increase it is mainly accumulated in roots.

It was reported as the soil contain high toxic amounts of heavy metals, VAM fungi usually induces lower concentrations of these metals in the aerial part of the plant (Guo et al, 1996 ; Jurkiewicz et al, 2001). In accordance with these findings , it was reported that chlorophyll contents and organic compounds in the leaves and stems of crowberry (*Empetrum nigrum* L.) were lower as a result to heavy metal pollution (Monni et al, 2001). VAM fungi contributed substantially to Zn uptake (Guo et al, 1996). It was indicated that shoots and roots of mycorrhizal plants had higher P and Zn concentrations compared to non-mycorrhizal plants and these could be attributed to a substantial translocation of P and Zn from hyphal compartments to the plant via the mycorrhizal hyphae (Kothari et al, 1991). Also, VAM increased the tolerance to heavy metals and this coupled with a reduction in their translocation to the shoot system (Brown and Wilkins , 1985)

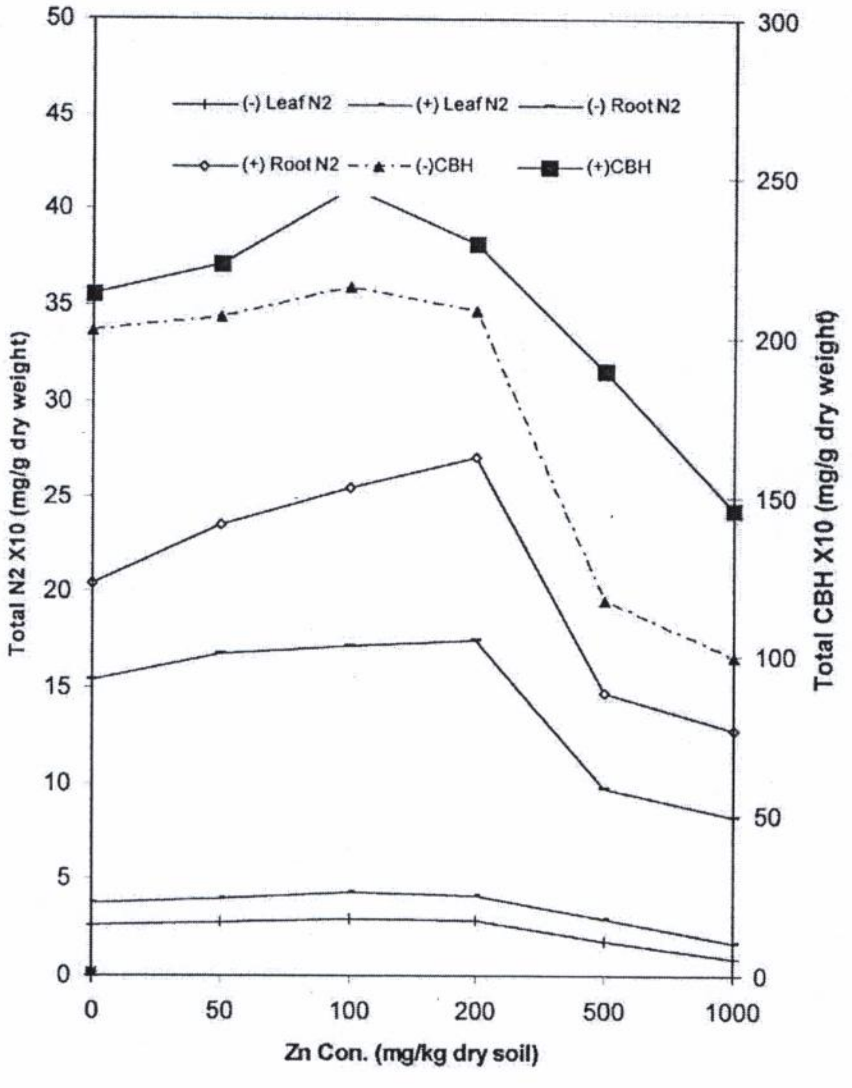

Fig. 5. Effect of different concentrations of Zn on total N_2 and CBH contents of leaves and roots of cowpea (*Vigna sinesis*) inoculated with microsymbiont (+), or non-inoculated (-), after 7 weeks of planting.

and at excessive concentration of soil metals, mycorrhizal roots decrease the bioavailability of toxic heavy metals by changing their forms (Huang et al, 2000) . It was found that VAM inoculation improved soybean dry matter, P concentration and Zn shoot contents (Andrade et al, 2003).

Effect of different concentrations of Zn on rhizobial root nodulation and VAM fungal infection of cowpea

The results (Figs. 6,7) showed concomitant increase of rhizobial nodulation parameters (number, fresh and dry weight / plant) as the concentration of Zn increased up to 200 mg / kg soil. Thus, under these conditions about 23% increase in both nodules number and their fresh weight, and about 10% increase in nodules dry weight. However, 0.5g Zn /kg soil was drastic to rhizobial nodulation, that failed to be formed at 1 g Zn/ kg soil. While , VAM successively colonize *Vigna sinesis* roots as Zn level increases up to 200 mg/kg (87% infection) , then dropped

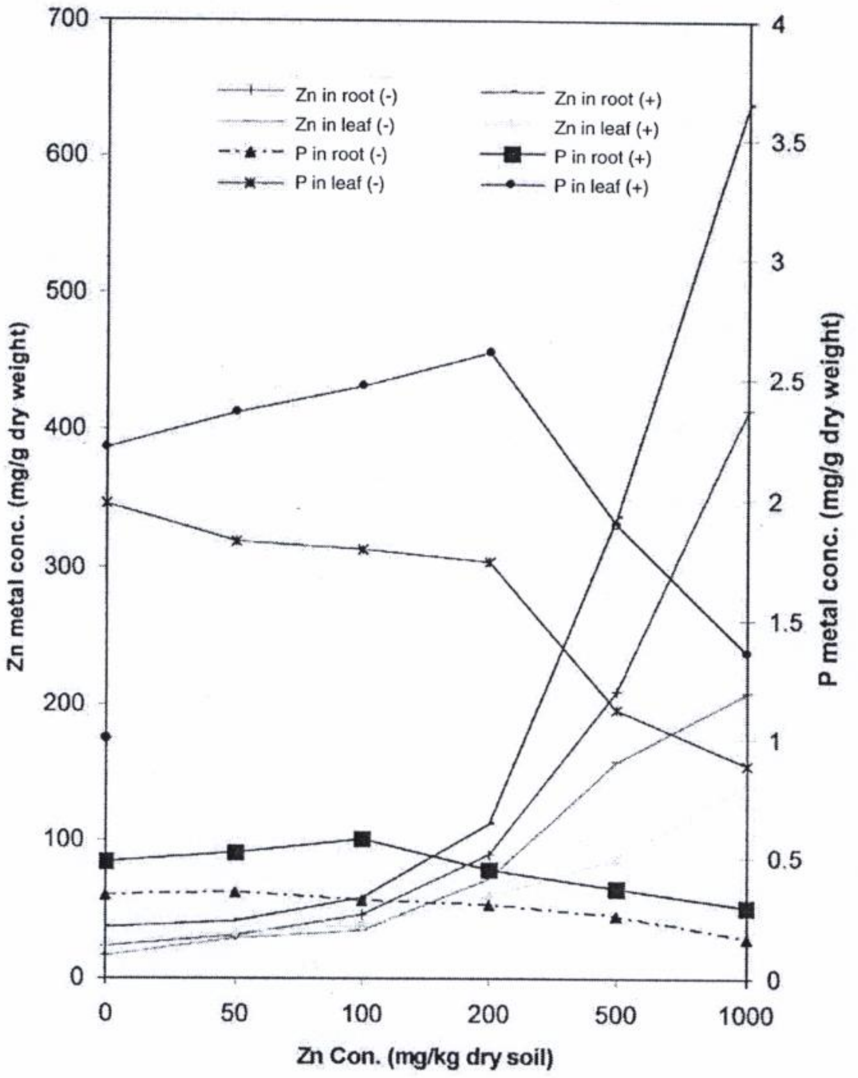

Fig. 6. Effect of different concentrations of Zn on P, Zn contents of leaf and root of cowpea (*Vigna sinesis*) inoculated with microsymbiont (+), or non-inoculated (-), after 7 weeks of planting.

suddenly to be 24% infection at 0.5 g Zn concentration and weak infection (8%) was recorded at 1 g Zn.

Positive effects of dual colonization of soybean roots by VAM fungi and rhizobia was recorded (Pacovsky et al, 1986) and successful symbiosis between them measured as an increase of nutrients by the plant (Ames and Bethlenfalvay, 1987) and increased N_2-fixation and nodule mass (Fredeen and Terry, 1988). It was also reported that *Phaseolus vulgaris* L. maintained a higher growth rate, supported with more nodules and assimilated more N or P when colonized by microsymbiont of nitrogen- fixing bacteria and VAM fungi (Pacovsky et al, 1991). The development of VAM fungi themselves might be affected by high soil heavy metals concentrations, since they are probably more exposed to the toxic environment than the roots of vascular plants (Tyler et al, 1989).

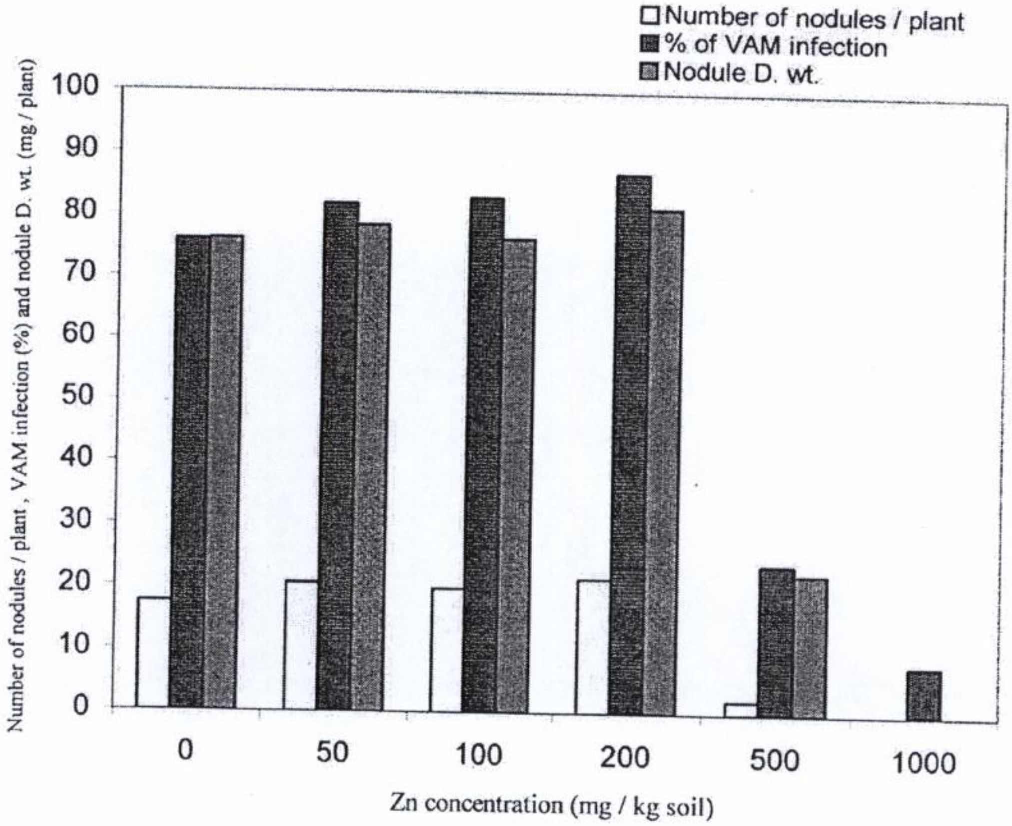

Fig.7. Effect of different concentrations of Zn on root nodulation and VAM infection of cowpea (*Vigna sinesis*) inoculated with microsymbiont (+), or non-inoculated (-), after 7 weeks of planting.

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