The effects of cadmium and cow manure on nodulation and growth attributes of common bean (Phaseolus vulgaris L.)

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Abstract— *Different biotic and abiotic factors are involved in the availability of heavy metals in soil including organic matter. In order to study the effects of cow manure on cadmium availability and their interactions on common bean nodulation and growth parameters, an experiment was conducted under greenhouse conditions. The treatments included five cadmium levels (0, 2, 5, 10, 20 mg cadmium per kg soil) and four cow manure levels (0, 15, 30, 60 t ha-1) based on a completely randomized design with three replications. Results indicated that increased soil cadmium concentrations caused higher cadmium uptake by root tissues whereas, Root nodulation and total N content of shoot tissues decreased significantly at all cadmium concentration levels except for 2 mg Kg-1. In addition, the interaction of cow manure and high concentrations of cadmium caused a decrease in nodule number, nodule fresh weights and total N content of shoot in common bean.*

Keywords— cadmium, common bean, cow manure, nitrogen, nodulation.

I. INTRODUCTION

Common bean (Phaseolus vulgaris L.) is one of five cultivated species from the genus Phaseolus and it is the most important grain legume for direct human consumption (Broughton et al. 2003). This crop constitutes a traditional food for many people in Latin America, Africa, and Asia. It is high in protein, essential vitamins and minerals, carbohydrates, fiber, and it is a low fat food (Messina 1999). P. vulgaris has a unique property of symbiotically associating with Rhizobium leguminosarum and convert atmospheric nitrogen into a usable form to the plants.

Rhizobium is the most well-known species of a group of bacteria that acts as the primary symbiotic fixer of nitrogen. These bacteria can infect the roots of leguminous plants, leading to the formation of lumps or nodules where the nitrogen fixation takes place. The symbiosis between Rhizobium and legumes are a cheaper and usually more effective agronomic practice for ensuring an adequate supply of N for legume-based crop and pasture production than the application of fertilizer-N (Zahran 1999). The symbiotic relationship between rhizobium bacteria and legume contribute at least 70 million tons of N per year (Brockwell et al. 1995). Typical environmental stresses faced by the legume nodules and their symbiotic partner (Rhizobium) may include photosynthate deprivation, water stress, salinity, soil nitrate, temperature, heavy metals, and biocides (Walsh 1995).

Early studies of the effects of heavy metal on nitrogen fixation by legumes found little evidence that symbiotic N2-fixation was sensitive to heavy metal toxicity (Obbard and jones 1993; Obbard et al. 1993; Vigue et al. 1981). Cadmium toxicity is 2 to 20 times greater than any other heavy metals (Kabata-Pendias and Pendias 2001), and the limit of cadmium concentration in soil associated to biomass reduction for the majority of agricultural plants is reported to be between 5 and 15 mg Cd Kg-1 of soil (Simon 1998). The main sources of cadmium in the agricultural sector include sewage sludge, deposition from base-metal smelter emissions and uncontrolled application of Cd-rich phosphatic fertilizers (Robinson et al. 1998; Nicholson et al. 2003). Apart from the source of cadmium, the bioavailability of Cd in soils is a function of its solubility (Ernst 1996) with pH and organic matter content being the main controlling factors (Grey et al. 1998).

The toxic effects of cadmium on biological systems were reported by various authors (Mukherjee et al. 1984; Sharma et al. 1985). Presence of Cd in growth media reduces nodule formation and impairs nodule functioning in Phaseolus vulgaris (Vigue et al. 1981).There is also evidence that suggests that reduction in plant growth, nodule size and nitrogenase activity in white clover was due to Cd, Pb and Zn, when plants were grown in soils highly contaminated with these metals (Rother et al. 1983).

One of the ways to recover and maintain the soil's productivity has been shown to be the addition of organic wastes as organic amendment (Baran et al. 2001). However the use of organic wastes can lead to problems pertaining to their heavy metal content and their successive application result in heavy metal accumulation in soil. Basta et al. (2005) reported that the presence of significant quantities of humic substances in organic amendments adsorbed toxic metals temporarily, by formation of chelates (Adriano 2001) or other more complexes, which sorb them for longer periods of time (tordoff et al. 2000). However, Narwal and Singh (1998) suggested that the efficiency of the organic material in reducing Cd uptake was generally small. On the contrary, Hanc et al. (2008) found that addition of manure increased cadmium uptake by plants. The amount and type of organic matter present in the soil can also affect the metal solubility by complexation and chelation with metals. In Iran, application of cow manure is one of the most common practices used for cultivating plants. In addition, development of industrial manufactories and use of industrial waste leads to contamination of soil with heavy metals in important zones under cultivation of bean in Iran (Bahmani et al. 2012). Hence, the purposes of this experiment were to study the effects of cow manure and cadmium and their interactions on nodulation and growth Attributes of common bean (Phaseolus vulgaris L.) under greenhouse conditions.

II. MATERIAL AND METHOD

The experiment was carried out at the greenhouse of the Faculty of Agriculture, Ferdowsi University during the 2011/2012 cropping season, in a completely randomized design with factorial arrangement consisting of five levels of cadmium 0, 2, 5, 10 and 20 (mg Cd Kg-1 soil) and four levels of organic matter (cow manure) 0 t ha-1, 15 t ha-1, 30 t ha-1 and 60 t ha-1 with three replications. Some characteristics of the cow manure are shown in Table 1. Soil was collected from the field situated in the farm of Faculty of Agriculture, Ferdowsi University (latitude 36"14' N, longitude 59"40' E). For laboratory analysis, soil samples were air dried and passed through a 2-mm sieve and stored. The initial properties of the soil were: pH 7.85 in a 5:1 soil-water ratio, electrical conductivity in a 5:1 soil-water ratio, loam texture with clay 20%, silt 47% and sand 33% (Gee and Bauder 1986), organic carbon 0.5% (Nelson and Sommers 1996), total N 0.057% (Bremner and Mulvaney 1982), Olsen-P 12 (Olsen et al. 1954), DTPA extractable Cd 0.03 mg Kg-1 (Lindsay and Norvell 1978) and available K 305 mg Kg-1 (Helmek and Sparks 1996), respectively.

TABLE 1
THE CHEMICAL COMPOSITION OF THE COW MANURE (CM) USED IN THE EXPERIMENT

pH^a	EC^a (ds/m)	O.C (%)	C/N	N_t (%)	P_t (%)	Cd (mg/kg)
8.1	11	40	22.7	1.76	0.72	0.09

[a]5:1 extract
N_t: total nitrogen
P_t: total Phosphorus

For the present study, local cultivar of Phaseolus vulgaris L. (common bean) was selected to study the effect of cow manure and cadmium. For treatments, cow manure and cadmium sulfate [CdSO4.8H2O] were used. Soil, cow manure and cadmium were mixed completely. Then, with the purpose of creating a balance between the cow manure and cadmium in the soil, an incubation period of one month was spent in the greenhouse condition and during this period, the moisture of the pots was kept at about (%70±10) field capacity.

After preparing the pots and disinfecting the seeds, four common bean seeds were planted in the depth of 2 cm evenly spaced in each pot (including 2000 g of soil) and after 2 weeks thinned to 3 plants per pot. The experiment was carried out in a greenhouse at 26° C during the days with natural light, and 19° C during the nights.

Plants were harvested 10 weeks after sowing and data on the following parameters were collected: shoot and root fresh and dry weights and number and dry weight of nodules. The plant organs were oven-dried at 60°C for 48 h weighed and ground into a fine powder (0.85 mm) for the analysis of micro nutrients. The shoot and root were dried in oven at 65°C for 48 h and analyzed for cadmium (Isaac and Johnson 1975) and nitrogen content was measured by the kjeldahl procedure (Page et al. 1982). Analysis of the data was carried out through SAS software, the comparison between the means was done via Duncan's multiple range test (DMRT) at the level of %5, and the diagrams were drawn by Excel.

III. RESULTS AND DISCUSSION

3.1 The effect of cadmium, cow manure and their interaction on number of nodulation of common bean Phaseolus vulgaris L.)

The study found that different Cd levels significantly reduced number of nodule. Fig. 1 show that number of nodule was firstly increased slightly at 2 mg Cd Kg-1, but decreased significantly once the Cd concentration was above 2 mg Kg-1 soil. Similar results had been reported by Yang et al. (2005) who found that the addition of 2 or 5 mg Cd Kg-1 soil could stimulate the microbial activity in soil, while inhibitory influences induced at high Cd level of 10 or 20 mg Kg-1 soil.

The addition of low Cd pollutant increased microbial activity in rhizosphere due to the increased secretion of carbohydrate and organic acids in root exudation, which increase the carbon allocation in rhizosphere soil. Whereas a decline of the enhancement in rhizosphere was observed in high Cd treatments owing to critical inhibition on root growth and metal activation in rhizosphere, which strengthen the toxic impact of metal pollutants on soil microbes (Dong et al. 2007). The application of cow manure at 15 and 30 t ha-1 nonsignificantly affected number of nodulation in common bean, but application of cow manure at 60 t ha-1 caused a significant decrease in number of nodulation of green been in comparison to control.

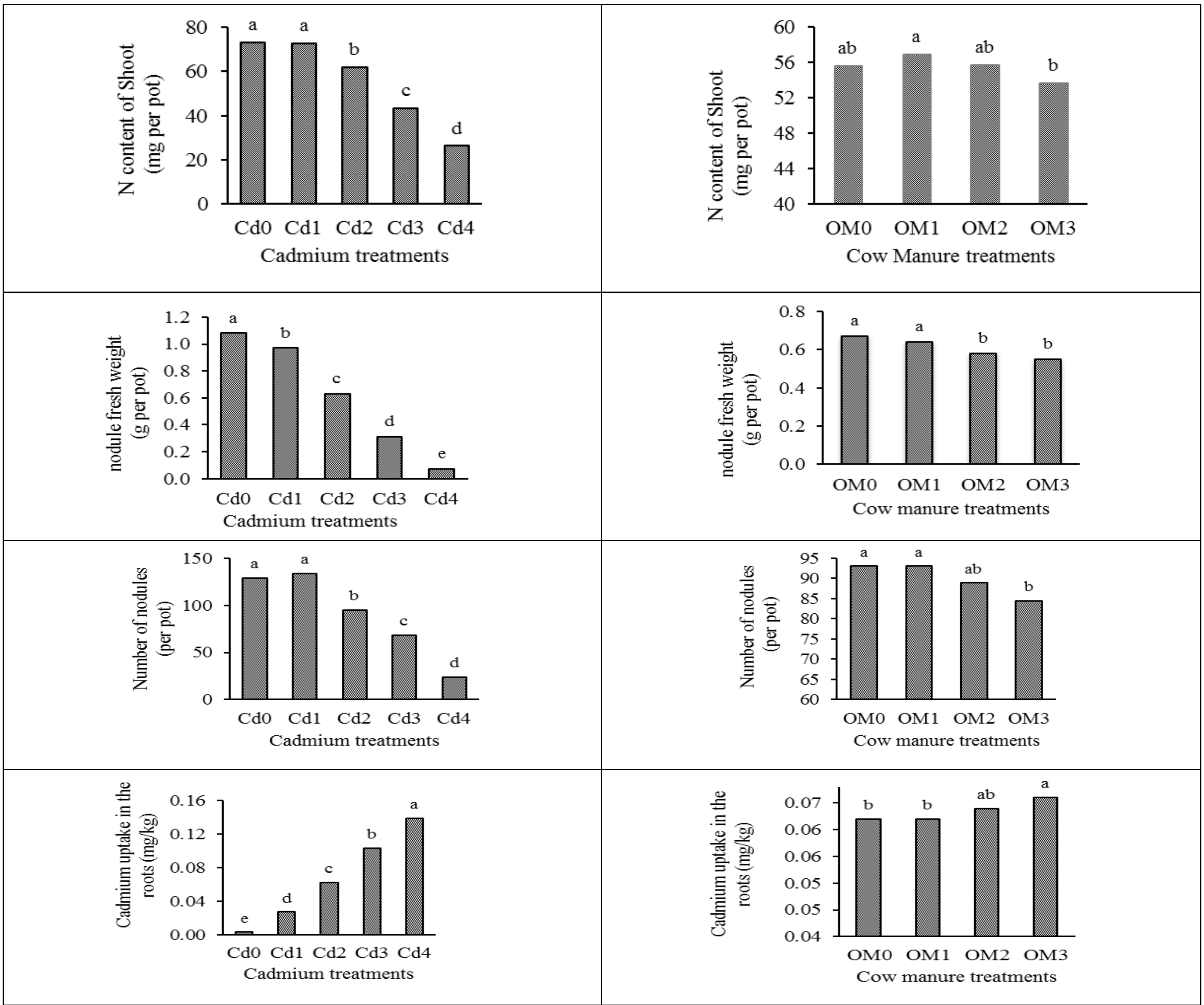

FIGURE 1. THE EFFECT OF CADMIUM AND COW MANURE ON NUMBER OF NODULES, NODULES FRESH WEIGHT, CD UPTAKE IN ROOTS AND N CONTENT IN SHOOTS OF COMMON BEAN (PHASEOLUS VULGARIS L.)

The interaction effect of Cd and CM levels are shown in Table 2. On application of 0, 2 and 5 mg Cd Kg-1 soil, the addition of all the levels of cow manure did not significantly affected number of nodulation of green been. In general, at 10 and 20 mg Cd Kg-1 soil all the levels of CM caused a decrease in number of nodulation of common bean, Moreover, at 10 and 20 mg Cd Kg-1 soil, the addition of 60 t ha-1 CM caused a decreased the number of nodulation of common bean, significantly in comparison to control. This might possibly be due to the effect of CM application on the availability of cadmium to the plants.

TABLE 2
INTERACTIVE EFFECTS OF CADMIUM AND COW MANURE ON N CONTENT, NODULATION AND CD CONCENTRATION OF COMMON BEAN.

Treatment		N content of Shoot (mg per pot)	Number of nodules	nodule fresh weight (g per pot)	Cd uptake in Root (mg per pot)
Cd0	**CM0**	69.56 [a]	129.67 [a]	1.25 [a]	0/003 [g]
	CM1	72.98 [a]	132.00 [a]	1.12 [b]	0/003 [g]
	CM2	74.81 [a]	130.00 [a]	0.98 [cd]	0/003 [g]
	CM3	75.08 [a]	125.00 [a]	0.96 [cd]	0/004 [g]
Cd1	**CM0**	68.80 [ab]	135.00 [a]	1.05 [cd]	0/027 [f]
	CM1	73.11 [a]	137.00 [a]	1.03 [c]	0/027 [f]
	CM2	74.20 [a]	133.33 [a]	0.91 [d]	0/027 [f]
	CM3	74.74 [a]	130.33 [a]	0.89 [d]	0/028 [f]
Cd2	**CM0**	61.47 [c]	95.00 [b]	0.64 [e]	0/061 [e]
	CM1	61.80 [bc]	96.00 [b]	0.65 [e]	0/061 [e]
	CM2	62.18 [bc]	95.00 [b]	0.64 [e]	0/062 [e]
	CM3	62.29 [bc]	94.33 [b]	0.61 [e]	0/062 [e]
Cd3	**CM0**	46.52 [d]	74.67 [c]	0.36 [f]	0/101 [d]
	CM1	45.63 [de]	71.67 [cd]	0.32 [fg]	0/101 [d]
	CM2	41.02 [de]	65.67 [cd]	0.29 [fg]	0/104 [d]
	CM3	39.33 [e]	59.67 [d]	0.26 [g]	0/106 [d]
Cd4	**CM0**	31.29 [f]	31.67 [e]	0.09 [h]	0/133 [c]
	CM1	30.87 [f]	29.33 [e]	0.09 [h]	0/131 [c]
	CM2	26.11 [f]	20.33 [ef]	0.08 [h]	0/141 [b]
	CM3	16.86 [g]	12.67 [f]	0.05 [h]	0/150 [a]

Each value is a mean of three replicates where each replicates constituted three plants/pot.
Mean values followed by different letters in the same column are different at P<0.05 according to Duncan's test.

3.2 The effect of cadmium, cow manure and their interaction on Nodule fresh weights of common bean (Phaseolus vulgaris L.)

The application of cadmium did significantly decrease Nodule fresh weights, with the decrease being greatest (93%) with Cd4 and least (10%) with Cd1 in comparison to control. The possible reason for this might be the deleterious effects of Cd contamination on roots, and hence inhibiting the growth of roots. The interaction of Rhizobium in the nodules of chickpea was found to be very sensitive to heavy metals resulting in a decrease in dry mass of chickpea and green gram (Woolhouse 1983; Rana and Ahmad 2002). On application of cow manure, with the exception of 15 t ha-1, did significantly decrease Nodule fresh weights in common been, with the decrease being greatest (18%) with CM3 and least (13%) with CM2 in comparison to control.

The interaction effect of Cd and CM levels were shown in Table 2. On application of 2, 5 and 20 mg Cd Kg-1 soil, the addition of all the levels of cow manure did not significantly affected number of nodulation of common. In general, at 0 mg Cd Kg-1 soil all the levels of CM caused a significant decrease in Nodule fresh weights of common been in comparison to control, Moreover, at 10 mg Cd Kg-1 soil, the addition of 60 t ha-1 CM caused a decrease in the Nodule fresh weights of common been, significantly in comparison to control.

3.3 The effect of cadmium, cow manure and their interaction on total N content in the shoots of common bean (Phaseolus vulgaris L.)

On application of cadmium, with the exception of 2 mg Cd Kg-1 soil, did significantly decrease total N content in the shoots, with the decrease being greatest (64%) with Cd4 and least (15%) with Cd2 in comparison to control. Sheirdil et al. (2012) found that growth; nodulation and N2 fixation of soybean was adversely affected with increased in Cd concentration.

The application of all the levels of cow manure nonsignificantly affected total N content in the shoots of common bean. On application of 0, 2 and 5 mg Cd Kg-1 soil, the addition of all the levels of cow manure did not significantly affected total N content in the shoots of P. vulgaris, however, at 10 mg and 20 Cd Kg-1 soil, the addition of 60 t ha-1 CM caused a decrease in total N content of shoots, significantly in comparison to control.

3.4 The effect of cadmium, cow manure and their interaction on cadmium uptake in the roots of P. vulgaris.

The study found that all the levels of cadmium significantly increased cadmium uptake in the roots. Chen and et al. (2003) found that Cd content in the plant increased as the Cd concentration in the soils increased, whereas it decreased slightly at the high Cd concentration (20 mg Kg-1 soil) and the possible reason for this might be the deleterious effects of Cd contamination on roots, and hence inhibiting the growth of roots.

The application of cow manure at 60 t ha-1 significantly increased cadmium uptake in the roots. On application of 0, 2, 5 and 10 mg Cd Kg-1 soil, the addition of all the levels of cow manure did not significantly affected cadmium uptake in the roots of common bean, however, at 20 mg Cd Kg-1 soil, the addition of 30 and 60 t ha-1 cow manure significantly increased cadmium uptake in comparison to control.

As mentioned in the previous section, organic matter in soil strongly influences the bio availability of Cd. However, the source of organic matter was a determining factor for Cd distribution in the soil and for Cd uptake by plants. The results show that addition of cow manure at 60 t ha-1 soil significantly increased cadmium uptake by plants. The results also imply that, the interaction effect of Cd at 20 mg Cd Kg-1 soil and CM at 30 and 60 t ha-1 significantly increased cadmium uptake of common been in comparison to control. The increase in cadmium uptake with the application of cow manure could be attributed to the complexing properties of cow manure, which might have increased the bio-availability of Cd. Similar results were obtained by Hanc et al. (2008) who found that addition of manure increased cadmium uptake by plants. Also, Manure application has also been linked to increasing Cd solubility in soil (Bolan et al. 2004). Again, Li et al. (2010) found that pig, dairy cow and chicken manures contained high Cd due to presence of Cd in their feeds.

In the present study, the presence of cadmium significantly affected the Rhizobium-legume symbioses in soil. The results indicate that the number of nodulation of P. vulgaris, Nodule fresh weights and total N content in the shoots might be negatively influenced by Cd uptake, and hence resulting in the reduction of N2-fixation. The presence of Cd decreased nodulation and nitrogenase activity in Phaseolus vulgaris (Dewdy and Ham 1997; Vigue et al. 1981).

The result also indicate that number of nodule was stimulated to some extent at the low level of added Cd, but decreased sharply with further increase of Cd concentrations. Further research is needed in order to study the effects of Cd pollution on nodulation, N2-fixation capabilities of root nodule, and common bean growth.

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