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# Phytoremediation Potential of Induced Cd Toxicity in *Trigonella Foenum-Graecum* L. and *Vigna Mungo* L. by Neem Plants parts

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## Abstract

*Plants can be used to remove heavy metal from the environment and hence reduce the toxic effect of heavy metal on health of all living organism. Cadmium is a non-essential heavy metal that does not have any metabolic use and can be harmful even at low concentrations. Fenugreek (Trigonella foenum-graecum L.) and Blackgram (Vigna mungo L.) are two important pulse crops grown in India to eradicate the malnutrition and hunger from developing country like India. Plants were grown in pots treated with 60 mg Cd kg$^{-1}$ soil as $CdCl_2$ caused significant damage to both the crops. The toxicity was significantly reduced when these plants earlier treated with Cd with different plants parts of neem. The changes in plant height, fresh as well as dry weights, percent pollen fertility, number of pods per plant, total chlorophyll content and nitrate reductase activity were observed. The highest improvement was noted in those plants treated with neem fruits alone while the minimum in those plants treated with Cd, neem leaf respectively. The effect was more pronounced in fenugreek than blackgram.*

## Introduction

Fenugreek (*Trigonella foenum graecum* L.) and Blackgram (*Vigna mungo* L.) are important pulse crops. These have immense value and a good source of vitamins, proteins and essential oils. These crops are very important for agricultural economy and ability to increase soil fertility. Plant pathogens cause serve losses in productivity of fenugreek and blackgram [1, 2]. Cadmium (Cd) is a common metal pollutant introduced into the environment through industrial activities, sewage sludge application and commercial phosphorus fertilizers and subsequently become a part of the food chain [3]. Therefore, it is very essential to manage pathogens and reduced phytotoxicity of Cd in order to produce more plant biomass and grain of improved quality. This management objective can be achieved with the help of chemical fertilizers, broad spectrum pesticides and organic amendment of plant origin [4]. Bioorganic organic farming is a new concept that recently introduced in agriculture with an eye to stop applying the enormous amounts of agro chemicals that led to serve environmental and health troubles. The conjoint application of botanicals as organic manures is a suggested alternative way to replace the chemical fertilizations and obtain reasonable yield quality and high quality. This not only to reduce the phytotoxicity of Cd but increased the soil fertility. The present investigation aims at studying the different parts of neem plant and Cd individually as well as concomitantly in relation to growth parameters of fenugreek and blackgram.

## Materials and Methods

The pot experiments were performed in randomized block during Rabi and Kharif season of (2010) in the net house of Department of Botany, Aligarh Muslim University, to investigate the efficacy of some botanicals such as Fenugreek (*Trigonella foenum graecum* L.) and Blackgram (*Vigna mungo* L.) respectively. The aim of these experiments were to study the toxicity of Cd alone and in combination with neem plant parts such as neem leaves and neem fruits.

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The seeds of *Trigonella foenum- graecum* L., and *Vigna mungo* L., were obtained from Indian Agricultural Research Institute, New Delhi. The seeds were surface sterilized with dilute solution of sodium hypochloride to prevent any fungal contamination. The 10 seeds of *Trigonella foenum-graecum* L. and *Vigna mungo* L., were sown is earthen pots containing 4 kg steam sterilized soil – manure mixture. Thinning was done after one week of seed germination and retains five plants per pot. The concentrations of Cd in the form of (60 mg $Kg^{-1}$ of soil) $CdCl_2$, Cd + neem leaves, Cd+ neem fruits @ 110 kg N/ha alone and in combination were added before sowing of seeds as per inoculation scheme in Table 1 and 2. The plants with no added heavy metals served as control. The treatments were given at a temperature of 22±2 °C before 1 day of sowing. There were five replicates of each treatment. Necessary watering and weeding were done whenever required. The plants were kept at glasshouse benches in the randomized manner.

The experiments were terminated 120 days after seed germinations. Plant height, fresh as well as dry weights, percent pollen fertility, number of pods per plant, total chlorophyll content and nitrate reductase activity were measured. Pollen fertility (%) was estimated by the method of Brown [5], using stainability of pollen grains in 1 % acetocarmine solution.

**Chlorophyll Estimation**

The chlorophyll content in the fresh leaf was measured by Mackinney [6], 1 g of finely cut fresh leaves was ground to fine pulp using mortar and pestle after pouring 20 $cm^3$ of 80 % acetone. The mixture was centrifuged at 5,000 rpm for 5 minutes. The supernatant was collected in 100 $cm^3$ volumetric flask. The residue was washed three times, using 80 % acetone. Each washing was collected in the same volumetric flask and volume was made up to the mark, using 80 % acetone. The absorbance was read at 645 and 663 nm on spectrophotometer. The chlorophyll content present in the extract (mg $kg^{-1}$ tissue) was calculated by following equation:

$$\text{Chlorophyll a kg}^{-1}\text{ tissue} = 12.7\,(A_{663}) - 4.68\,(A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b kg}^{-1}\text{ tissue} = 20.2\,(A_{645}) - 18.02\,(A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll in fresh tissue} = 20.2\,(A_{645}) + 8.02\,(A_{663}) \times \frac{V}{1000 \times W}$$

A = absorbance at specific wavelength
V = final volume of chlorophyll extract in 80 % acetone
W = fresh mass of tissue, used for extraction

**NR Activity Estimation**

The activity of nitrate reductase was measured by Jaworski [7]. The leaves were cut into small pieces (1 $cm^2$). 0.2 g of these chopped leaves weighed and transferred to plastic vials. To each vial 2.5$cm^3$ of phosphate buffer pH 7.5 and 0.5 cm of potassium nitrate solution was added followed by the addition of 2.5 $cm^3$ of 5 % isopropanol. These vials were incubated in BOD incubator ± 2°C in dark 0.4 $cm^3$ of incubated mixture was taken in a test tube to which 0.3 $cm^3$ each of sulfanilamide solution and NED-HCl was added. The test tube was left for 20 minutes, for maximum colour development. The mixture was diluted to 5 $cm^3$ with DDW. The absorbance was read at 450 nm on spectrophotometer. A blank was run simultaneously with each sample. Standard curve was plotted by using known graded concentration of $NaNO_2$ (Sodium nitrite) solution. The absorbance of each sample was compared with that on the calibration curve and nitrate reductase activity (n $mg^{-1}$ $h^{-1}$) was noted on fresh mass basis.

## Results and Discussion

The data presented in Table 1 and 2 clearly revealed that soil application of some botanicals and Cd alone as well as in combination with neem leaves and neem fruits significantly improved the plant growth, percent pollen fertility, and chlorophyll content in all treatments as compared to the untreated control. The highest improvement was noted with neem fruits and lowest was recorded with Cd + neem leaves and Cd alone respectively. The application of neem plant parts to soil is beneficial effects on soil nutrient, soil physical condition, soil biological activities and improvement of crop.

The improvement was more prominent in growth parameters of *Trigonella foenum –graecum* than *Vigna mungo*. The improvement may be due to reduction in plant pathogens and due to their manurial effect. Incorporation of botanicals increased microbial activity are known to bring about increased conversion of N to nitrate form [8]. Which in turn appears to be responsible for stimulation of nitrate reductase activity. Similarly chlorophyll content was also increased by amendments with their botanicals. Ahmad *et al*., [9] also observed increased chlorophyll content due to application of organic matter. The application of Cd caused much reduction in all the growth parameters including NR activity and chlorophyll content of both the crop plants. Our results are in conformity with those of Mobin and Khan [10], who observed reduction in phytosynthetic content even at low concentration. Arduini *et al*., [11] and Khan *et al*., [12] also noticed reduction in plant growth and enzymes activity in the presence of Cd inoculated plants. However, addition of neem plants parts reduced the toxicity of Cd when inoculated concomitantly. This is an agreement with those of Braek *et al*., [13] and Wang and Wood [14] when incorporated with algae and *Azolla* with heavy metals respectively.

The researcher outcome suggests the aptness of botanicals for management of diseases caused by fungi, insects and plant- parasitic nematodes along with affluence of soil health if the crop is managed under the umbrella of organic farming. The economics of using botanicals also works out to be adequate in high value commodity in pulse crops. Thus, the present finding strongly advocate the array of botanicals in general and neem in particular for pest management as well as

**Table 1:** Impact of Neem plant parts and Cd Inoculation on growth of *Trigonella foenum graecum* L (Each value is an average of 5 replicates)

| Treatments | Plant height (cm) | Plant fresh weight (gm) | Plant dry weight (gm) | Pollen fertility (%) | Number of fruits/plants | Chlorophyll content (mgg$^{-1}$) | NR Activity (μ mole $NO_2$-h$^{-1}$g$^{1}$fersh weight) |
|---|---|---|---|---|---|---|---|
| **Control** | 24.84 | 10.63 | 3.54 | 96.74 | 24.08 | 4.327 | 0.373 |
| **Neem leaf** | 31.67 | 18.18 | 6.06 | 98.02 | 30.02 | 7.460 | 0.643 |
| **Neem fruit** | **34.93** | **19.74** | **6.58** | **99.01** | **33.90** | **8.164** | **0.703** |
| **Cd** | **6.16** | **3.51** | **1.17** | **31.92** | **9.90** | **1.43** | **0.113** |
| **Cd+ Neem leaf** | 18.67 | 7.86 | 4.16 | 55.34 | 14.68 | 2.50 | 0.403 |
| **Cd+ Neem fruit** | 21.62 | 8.50 | 4.75 | 59.24 | 16.08 | 2.14 | 0.470 |
| **C.D. (*P*=0.05)** | 2.39 | 1.27 | 0.59 | 5.44 | 2.23 | 0.128 | 0.037 |

**Table 2:** Impact of Neem plant parts and Cd Inoculation on growth of *Vigna mungo* L (Each value is an average of 5 replicates)

| Treatments | Plant height (cm) | Plant fresh weight (gm) | Plant dry weight (gm) | Pollen fertility (%) | Number of fruits/plants | Chlorophyll content (mgg$^{-1}$) | NR Activity (μ mole $NO_2$-h$^{-1}$g$^{1}$fersh weight) |
|---|---|---|---|---|---|---|---|
| **Control** | 45.00 | 4.57 | 1.52 | 93.07 | 18.98 | 3.567 | 0.342 |
| **Neem leaf** | 63.34 | 8.39 | 2.80 | 95.09 | 24.67 | 4.386 | 0.538 |
| **Neem fruit** | **70.85** | **8.46** | **2.82** | **96.70** | **27.05** | **4.793** | **0.647** |
| **Cd** | **30.04** | **1.51** | **0.50** | **40.20** | **07.09** | **1.660** | **0.114** |
| **Cd+ Neem leaf** | 34.14 | 5.39 | 1.80 | 73.06 | 13.87 | 2.197 | 0.260 |
| **Cd+ Neem fruit** | 37.60 | 6.26 | 2.09 | 77.80 | 14.98 | 2.980 | 0.300 |
| **C.D. (*P*= 0.05)** | 3.83 | 0.54 | 0.26 | 4.37 | 1.77 | 0.136 | 0.040 |

reduction in phytotoxicity in an organic agriculture. Organic pulses are considered valuable and it is also believed that post- harvest losses of such vegetables are usually lower as to conventionally grown pulses. Such types of produce are free from any health hazard and also safe for environment.

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