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RESEARCH ARTICLE

DECLINED MORPHOLOGY IN TOBACCO (*Nicotiana glauca* L.) DUE TO CADMIUM STRESS.

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Abstract

Cadmium is one of the most toxic heavy metal in the environment. Cadmium presence in soil can cause serious problem to all organisms through food chain. The present study was conducted through pot culture experiment to estimate the effect of cadmium (10, 25, 50, 75, 100, & 200mg kg⁻¹ soil) on the morphological parameters (root length, shoot length, number of leaves, total leaf area, fresh and dry weight of root, stem, leaf) of tobacco. The values were recorded on 30th, 60th, 90th, 120th, 150th and 180th days of tobacco. For all the morphological parameters, the highest values were recorded in control. Due to increase in cadmium concentrations all the morphological parameters were decreased from 10mg kg⁻¹ to 200mg kg⁻¹ of soil.

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Introduction:-

Due to globalization and industrialization, heavy metal (HM) pollution is one of the most important and widespread environmental problem of the modern world, due to its hazardous and toxic effects on all living organisms. Heavy metal pollution is a serious environmental problem because heavy metals are non-biodegradable and persistent in nature [10,11].

Heavy metals are defined as a group of metals and metalloids with atomic density higher than 5 g cm⁻³. The remediation of heavy metal contaminated soil requires the removal of toxic metal from contaminated areas. Heavy metals were important environmental pollutants particularly in areas where there was a high anthropogenic pressure, but they also occur naturally (Sanità and Gabbrielli, 1999). Anthropogenic cadmium contamination often results from mining or smelting of metal ores, but cadmium is also released into the environment by power stations, heating systems, waste incinerators, urban traffic, and cement factories and as a by-product of phosphate fertilizers. Use of sewage sludges as fertilizers has further contributed to a significant contamination of agricultural soils. Cadmium presence in the soil can cause serious problems to all organisms. Although non-essential for plant growth, when bio available, cadmium is readily taken up by roots and translocated into aerial organs where it can accumulate to high levels. The most apparent visible symptoms of cadmium toxicity in plants are retardation of plant growth, chlorosis and stunting (Das *et al.*, 1997). The present investigations was carried out to analyse the changes in growth parameters such as, root and shoot length, number of leaves and total leaf area, fresh and dry weight of root, stem, leaf of tobacco plants due to cadmium toxicity.

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Materials and Methods:-

Seed Materials:-

The seeds of tobacco were procured from Central Tobacco Research Institute, Research Station, Vadasandur, Dindugal Dt, Tamilnadu. The duration of the crop was 180 days. Seeds of uniform size, colour and weight were chosen for the experiments. Cadmium and other chemicals used in this investigation were analytical grade. Cadmium was used in the form of chloride.

Pot Culture Experiments:-

The experiments were performed during the months of December 2011 to May 2012. Tobacco plants were grown in pots in untreated soil (control) and in soil to which cadmium had been applied (0, 10, 25, 50, 75, 100 and 200 mg kg⁻¹ of soil). The inner surface of pots was lined with a polythene sheet. Each pot contained 3.00 kg of air dried soil. Cadmium as chloride (CdCl₂ 2½ H₂O) were finely powdered and applied to the surface soil. This was then thoroughly mixed with the soil and 1 gram of seeds was sown in each pot. All pots were watered to field capacity twice a day. Plants were thinned to a maximum of three per pot after a week of germination. The treatments were replicated five times in a completely randomized block design.

Sampling:-

Plant samples were collected at random, at regular intervals (30, 60, 90, 120, 150 and 180th days) and used for morphological growth parameters. Three plants from each replicate of a pot were analysed for its various parameters and the average was calculated. These mean values of the replicates were used for statistical analysis.

Growth:-

Root and Shoot Length:-

The root and shoot length and elongation rate are essential for plants exploring for water and mineral nutrients. In the pot culture experiments there was a gradual decrease in the root and shoot length with an increase in cadmium level 10, 25, 50, 75, 100 and 200 mg kg⁻¹ in the soil in all the sampling days. The inhibitory action of excess of cadmium in root and shoot length might be due to reduction in cell division, toxic effect of heavy metals on photosynthesis, respiration and protein synthesis. These obviously contributed to the retardation of normal growth Kupper, et al. (1996). The above results were in agreement with the findings of Chen, et al. (2003) in soybean, Rai, et al. (2005) in *Phyllanthus amarus* and Xu, et al. (2008) in garlic. Hagemeyer et al. (2002.) and Marciano et al. (2002) also suggested that the morphological and structural effects caused by metal toxicity in plants was due to decrease in root elongation, root tip damage, decrease in root formation, suppression of elongation growth rate of cells, affecting the ultra cellular structure of meristematic cells and inhibition of the size of plant cells and inter cellular spaces.

Number of Leaves and Total Leaf Area:-

Number of leaves and total leaf area were decreased due to the increase of cadmium concentrations in all the sampling days. Number of leaves, branches and leaf area decreased as the concentration of metal increased. Strong decrease in leaf area was correlated to accumulation of chlorophyll pigments as disturb integration of chlorophyll molecules into stable complex (Skkorzynska Polit and Baszynski, 1997). Similar findings have been reported by Mehendirata et al. (2000)

Fresh and Dry weight:-

Fresh and dry weight of various parts of the tobacco plants showed a marked decline with the increase in cadmium level in the soil in all the sampling days. Hédiji et al. (2010) reported fresh weight reduction of *Solanum lycopersicum* under high Cd levels. There was a progressive fall in the dry matter yield of root, shoot and leaves with the corresponding increase in cadmium (10-200 mg kg⁻¹) level in the soil, in all the sampling days. Similar results were obtained by several authors in a number of plants such as Vitoria et al., (2001) in radish, Kim et al., (2002) in cabbage and lettuce, Zhang et al., (2002) in wheat, Shukla et al., (2003) in wheat, Rai et al., (2005) in *Phyllanthus amarus*. Abdo, Fatma (2008) in soybean.

Table 1:- Effect of various concentration of cadmium on root length (cm plant⁻¹) of tobacco (*Nicotiana tabacum* (L.).

Cadmium added in the soil (mg kg ⁻¹)	Sampling days					
	30	60	90	120	150	180
Control	3.20	49.30	65.72	76.34	82.54	85.69
10	3.00 (-6.25)	46.32 (-6.04)	62.35 (-5.13)	72.58 (-4.93)	77.25 (-6.41)	80.65 (-5.88)
25	2.80 (-12.50)	43.67 (-11.44)	58.46 (-11.05)	70.32 (-7.89)	74.36 (-9.87)	76.39 (-10.85)
50	2.40 (-25.00)	35.24 (-28.52)	52.40 (-20.27)	67.42 (-11.68)	72.32 (-12.38)	75.42 (-11.99)
75	2.10 (-34.38)	32.45 (-34.18)	49.72 (-24.35)	62.35 (-18.33)	66.54 (-19.38)	69.24 (-19.20)
100	2.00 (-37.50)	24.65 (-50.00)	42.65 (-35.10)	56.49 (-23.00)	59.22 (-28.25)	63.25 (-26.19)
200	1.80 (-43.75)	21.44 (-56.51)	36.75 (-44.08)	52.75 (-30.90)	56.33 (-31.75)	59.35 (-30.74)

Table 2:- Effect of different treatments of cadmium on shoot length (cm plant⁻¹) of tobacco (*Nicotiana tabacum* (L.).

Cadmium added in the soil (mg kg ⁻¹)	Sampling days					
	30	60	90	120	150	180
Control	5.40	28.00	39.67	54.23	58.13	62.56
10	5.10 (-5.56)	27.78 (-0.79)	37.63 (-5.14)	50.28 (-7.28)	56.73 (-2.41)	58.84 (-5.95)
25	4.80 (-11.11)	25.18 (-10.07)	34.56 (-12.88)	46.80 (-13.70)	52.19 (-10.22)	55.80 (-10.81)
50	4.20 (-22.22)	22.65 (-19.11)	31.23 (-21.28)	43.39 (-19.99)	48.49 (-19.58)	52.50 (-16.08)
75	4.00 (-25.93)	17.00 (-39.29)	26.54 (-33.10)	34.69 (-36.03)	38.67 (-33.48)	45.39 (-27.45)
100	2.60 (-51.85)	13.32 (-52.43)	23.45 (-40.89)	31.36 (-42.17)	36.52 (-37.18)	39.24 (-37.28)
200	2.80 (-48.15)	11.34 (-59.50)	19.32 (-51.30)	28.65 (-47.17)	33.56 (-42.27)	34.69 (-44.55)

Table 3:- Effect of various cadmium concentration of leaf number (plant⁻¹) of tobacco (*Nicotiana tabacum* (L.).

Cadmium added in the soil (mg kg ⁻¹)	Sampling days					
	30	60	90	120	150	180
Control	5.00	9.32	13.58	19.34	18.44	17.45
10	4.00 (-20.00)	8.65 (-7.19)	12.62 (-7.07)	18.66 (-3.52)	17.24 (-6.51)	16.58 (-4.99)
25	4.00 (-20.00)	8.00 (-14.19)	12.24 (-9.87)	18.00 (-6.93)	16.63 (-9.82)	16.30 (-6.59)
50	3.00 (-40.00)	7.34 (-21.24)	11.24 (-17.23)	16.45 (-14.94)	15.84 (-14.10)	15.08 (-13.58)
75	2.00 (-60.00)	7.00 (-34.89)	9.52 (-29.90)	15.76 (-18.51)	15.22 (-17.46)	13.34 (-23.55)
100	2.00 (-60.00)	6.33 (-32.08)	8.80 (-35.20)	15.49 (-19.91)	13.39 (-27.39)	12.66 (-27.45)
200	2.00 (-60.00)	5.82 (-37.55)	8.24 (-39.32)	13.62 (-29.58)	12.54 (-32.00)	12.09 (-30.72)

Table 4:- Effect of cadmium on total leaf area (cm²) of tobacco (*Nicotiana tabacum* (L.)).

Cadmium added in the soil (mg kg ⁻¹)	Sampling days					
	30	60	90	120	150	180
Control	62.14	657.49	936.05	1056.45	865.32	788.90
10	60.01 (-3.43)	624.52 (-5.01)	924.69 (-1.21)	988.40 (-6.44)	846.56 (-2.17)	727.13 (-7.83)
25	57.57 (-7.35)	595.43 (-9.44)	861.13 (-8.00)	934.25 (-11.57)	812.30 (-6.13)	677.29 (-14.15)
50	57.38 (-7.66)	546.80 (-16.84)	836.72 (-10.61)	908.57 (-14.00)	768.27 (-11.22)	643.15 (-18.48)
75	43.82 (-29.48)	510.87 (-22.30)	741.98 (-20.73)	897.23 (-15.07)	754.35 (-12.82)	609.24 (-22.77)
100	37.11 (-40.28)	489.64 (-25.53)	683.76 (-26.95)	765.27 (-27.56)	723.40 (-16.40)	570.12 (-27.73)
200	28.72 (-53.78)	453.16 (-31.08)	548.97 (-41.35)	738.67 (-30.08)	698.24 (-19.31)	534.25 (-32.28)

Table 5:- Effect of cadmium on root fresh weight (g plant⁻¹) of tobacco (*Nicotiana tabacum* (L.)).

Cadmium added in the soil (mg kg ⁻¹)	Sampling days					
	30	60	90	120	150	180
Control	3.380	5.628	8.212	10.504	12.720	14.792
10	3.093 (-8.49)	5.231 (-7.05)	8.120 (-1.12)	10.345 (-1.51)	12.546 (-1.37)	14.025 (-5.19)
25	2.856 (-15.50)	4.674 (-16.95)	7.459 (-9.17)	10.256 (-2.36)	12.190 (-4.17)	13.564 (-8.30)
50	2.530 (-25.15)	4.267 (-24.18)	7.235 (-11.90)	10.178 (-3.10)	11.589 (-8.89)	12.785 (-13.57)
75	2.324 (-31.24)	4.067 (-27.74)	7.189 (-12.46)	9.467 (-9.87)	11.280 (-11.32)	11.568 (-21.80)
100	2.102 (-37.81)	3.780 (-32.84)	6.984 (-14.95)	8.432 (-19.73)	10.453 (-17.82)	10.482 (-29.17)
200	1.345 (-60.21)	3.455 (-38.61)	6.745 (-17.86)	8.345 (-20.55)	10.260 (-19.34)	9.680 (-34.56)

Table 6:- Impact of cadmium on stem fresh weight (g plant⁻¹) of tobacco (*Nicotiana tabacum* (L.)).

Cadmium added in the soil (mg kg ⁻¹)	Sampling days					
	30	60	90	120	150	180
Control	5.435	17.028	35.635	66.849	73.760	80.534
10	5.178 (-4.73)	16.324 (-4.13)	35.097 (-1.51)	63.765 (-4.61)	71.005 (-3.74)	78.546 (-2.47)
25	4.325 (-20.42)	14.455 (-15.11)	32.678 (-8.30)	60.324 (-9.76)	69.425 (-5.88)	74.750 (-7.18)
50	3.754 (-30.93)	13.769 (-19.14)	30.537 (-14.31)	57.920 (-13.36)	66.439 (-9.93)	72.435 (-10.06)
75	3.432 (-36.85)	13.769 (-19.14)	28.980 (-18.68)	55.345 (-17.21)	63.761 (-13.56)	70.523 (-12.43)
100	3.098 (-43.00)	11.560 (-32.11)	25.008 (-29.82)	52.894 (-20.88)	62.835 (-14.81)	69.425 (-13.79)
200	2.270 (-58.23)	10.426 (-38.77)	23.349 (-34.48)	49.670 (-25.70)	60.033 (-18.61)	67.213 (-16.54)

Table 7:- Influence of different treatments of cadmium on leaf fresh weight (g plant⁻¹) of tobacco (*Nicotiana tabacum* (L.)).

Cadmium added in the soil (mg kg ⁻¹)	Sampling days					
	30	60	90	120	150	180
Control	7.692	19.296	34.938	53.342	44.890	40.453
10	7.420 (-3.54)	17.650 (-8.53)	32.540 (-6.86)	49.430 (-7.33)	40.563 (-9.64)	38.546 (-4.71)
25	6.765 (-12.05)	16.548 (-14.24)	30.123 (-13.78)	45.367 (-14.95)	38.425 (-14.40)	35.638 (-11.90)
50	6.324 (-17.78)	14.670 (-23.97)	28.324 (-18.93)	44.215 (-17.11)	36.430 (18.85)	32.879 (-18.72)
75	5.798 (-24.62)	13.567 (-29.69)	26.365 (-24.54)	40.652 (-23.79)	34.630 (-22.86)	30.098 (-25.60)
100	4.430 (-42.41)	12.646 (-34.46)	24.310 (-30.44)	37.638 (-29.44)	30.768 (-31.46)	27.432 (-32.19)
200	3.544 (-53.93)	10.780 (-44.13)	22.645 (-35.19)	35.319 (-33.79)	28.429 (-36.67)	25.560 (-36.82)

Table 8:- Various treatment of cadmium on root dry weight (g plant⁻¹) of tobacco (*Nicotiana tabacum* (L.) .

Cadmium added in the soil (mg kg ⁻¹)	Sampling days					
	30	60	90	120	150	180
Control	0.845	1.657	2.053	2.876	3.180	3.698
10	0.765 (-9.47)	1.465 (-11.59)	1.596 (-22.26)	2.563 (-10.88)	2.897 (-8.90)	3.182 (-13.95)
25	0.709 (-16.09)	1.089 (-34.28)	1.256 (-38.82)	2.239 (-22.15)	2.569 (-19.21)	2.845 (-23.07)
50	0.675 (-20.12)	0.960 (-42.06)	1.189 (-42.08)	1.980 (-31.15)	2.165 (-31.92)	2.654 (-28.23)
75	0.496 (-41.30)	0.827 (-50.09)	1.025 (-50.07)	1.635 (-43.15)	1.988 (-37.48)	2.267 (-38.70)
100	0.463 (-45.21)	0.724 (-56.31)	0.898 (-56.26)	1.298 (-54.87)	1.760 (-44.65)	1.918 (-48.13)
200	0.375 (-55.62)	0.549 (-66.87)	0.617 (-69.95)	1.056 (-63.28)	1.562 (-50.88)	1.875 (-49.30)

Table 9:- Different treatment of cadmium on stem dry weight (g plant⁻¹) of tobacco (*Nicotiana tabacum* (L.)).

Cadmium added in the soil (mg kg ⁻¹)	Sampling days					
	30	60	90	120	150	180
Control	2.860	7.684	12.456	15.675	18.467	21.019
10	2.389 (-16.47)	7.398 (-3.72)	12.180 (-2.22)	15.270 (-2.58)	18.167 (-1.62)	20.867 (-0.72)
25	2.160 (-24.48)	7.212 (-6.14)	11.678 (-6.25)	15.055 (-3.96)	17.985 (-2.61)	19.539 (-7.04)
50	1.989 (-30.45)	6.389 (-16.85)	11.398 (-8.49)	14.784 (-5.68)	17.580 (-4.80)	18.480 (-12.08)
75	1.670 (-41.61)	6.318 (-17.78)	11.267 (-9.55)	14.470 (-7.69)	17.360 (-5.99)	18.384 (-12.54)
100	1.467 (-48.71)	6.265 (-18.47)	11.098 (-10.90)	14.354 (-8.43)	16.936 (-8.29)	17.934 (-14.68)
200	1.082 (-62.17)	5.545 (-27.84)	10.865 (-12.77)	13.088 (-16.50)	16.680 (-9.68)	17.685 (-15.86)

Table 10:- Efficacy of cadmium treatment on leaf dry weight (g plant⁻¹) of tobacco (*Nicotiana tabacum* (L.).

Cadmium added in the soil (mg kg ⁻¹)	Sampling days					
	30	60	90	120	150	180
Control	2.564	6.435	11.546	17.756	15.762	13.453
10	2.380 (-7.18)	6.319 (-1.80)	11.345 (-1.74)	17.534 (-1.25)	15.564 (-1.26)	13.235 (-1.62)
25	2.245 (-12.44)	6.212 (-3.47)	10.689 (-7.42)	17.231 (-2.96)	14.432 (-8.44)	13.154 (-2.22)
50	1.987 (-22.50)	5.289 (-17.81)	10.453 (-9.47)	16.564 (-6.71)	14.245 (-9.62)	13.058 (-2.94)
75	1.589 (-38.03)	5.167 (-19.70)	10.156 (-12.04)	15.780 (-11.13)	14.180 (-10.04)	11.890 (-11.62)
100	1.420 (-44.62)	5.098 (-20.78)	10.098 (-12.54)	15.324 (-13.70)	13.657 (-13.35)	11.134 (-17.24)
200	0.982 (-61.70)	4.545 (-29.37)	09.437 (-18.27)	15.160 (-14.62)	13.423 (-14.84)	10.985 (-18.35)

Conclusion:-

In the present study tobacco plants were grown in control and different levels of cadmium were assessed. Results showed that all the morphological parameters of tobacco were decreased as cadmium level increased. The results indicated that all the morphological parameters dependent on both the different levels of cadmium accumulation and biomass of the plants, when the tobacco plants were grown in cadmium contaminated soil.

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