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Effect of 2, 4-Dichlorophenoxy acetic acid on nutrients and heavy metals accumulation in wheat (*Triticum durum*) root.

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ABSTRACT: The experiment was designed to determine the role played by the herbicidal auxin 2, 4-D on accumulation of two micro, two macro-nutrients and two heavy metals in root of tetraploid wheat (T. durum). Three different doses of 2, 4-D 48, 96 and 240ppm were applied on wheat seed and allowed to germinate. Iron, Manganese, Magnesium, Calcium, Chromium and Lead content were analyzed by Flame Atomic Absorption Spectrometry (FAAS) in samples from different time interval (3, 5 and 7 days). Abnormal protuberance growth was observed at root of wheat plant in each concentration of the applied herbicide after three days of treatment. The herbicidal dose had significant inhibitory effect on uptake of micro nutrients, Iron and Manganese and macro nutrients, Calcium and Magnesium by the root of the tested plant. However, no inhibitory effect was observed on the uptake of heavy metals like Chromium and Lead. Incubation time had no effect on Chromium content but both dose and time had positive association with Lead content. It may be concluded that 2, 4–Dichlorophenoxy acetic acid impairs the nutrient uptake and increase the content of heavy metal in root of wheat.

Key Words: 2, 4-Dichlorophenoxy acetic acid, Heavy metal, Herbicide, Protuberance, and Tetraploid wheat.

INTRODUCTION

The food crisis and nutrition deficiency of increasing population in current century demands huge production of wheat. To increase the grain yield using chemical herbicide is recently common scenario in cultivation practice because of its easy application and satisfactory residual action. The presence of pesticide residues distress the water potential which reduces uptake of nutrients from the surrounding soil (Rengel & Wheal 1997) and depolarizes the plasma membrane of root cells (Hausler et al. 1990; Shimabukuro, 1990; Shimabukuro & Hoffer, 1994; Wright, 1994) that would affect the uptake of nutrients (Tester, 1990). Thus the inability of plants to take up the essential nutrients can create deficiency which might be reflected in the abnormality in the different growth parameters. In wheat field application of chemical herbicide like 2, 4-D had significant effect on non-targeted host plant where all the morphological and cytological parameters were decreased with the increase of doses (Razu et al. 2012). As 2, 4-D is a growth regulator herbicide belongs to phenoxy group can leads to uncontrolled growth and eventual collapse of vascular tissues may hamper the nutrient uptake. In this regards a field observation in Brazil and the US showed that frequent applications of glyphosate like herbicide may directly or indirectly induce iron (Fe), zinc (Zn), and manganese (Mn) deficiencies in glyphosate resistant (GR) as well as non-GR plants (Huber 2006; Jolley et al. 2004; Huber and McCay-Buys 1993). On the other hand, Another investigation was done by Chiroma et al. (2007) on accumulation of heavy metal by spinach with $Delvap_{100EC}$ pesticide treatment where he stated that lead concentration was higher than untreated plant. Herbicide may also alter the enhancing substances (e.g. ascorbic acid, S-containing amino acids etc.) that promote micronutrient bioavailability (Welch 2002; Welch and Graham 2004; Welch 2005). Therefore, extensive use of herbicides in this second most widely cultivated cereal crop (Shamsi et al. 2006) field may exerts a threat on plant growth and development by the impairment with nutrient and heavy metal. Thus, herbicide can create health hazards by accumulating heavy metals and allowing it to enter in our food chain through the food made by tetraploid wheat (Kulp et al. 2000). Considering all this viewpoints, the present communication was aimed to study the effects of 2, 4-D on nutrient and heavy metal concentration in root of tetraploid wheat (Triticum durum L.)

MATERIALS AND METHODS

The seeds of wheat (T. durum) were collected from Regional Wheat Research Station, Rajshahi and surface sterilized with 0.5% of $HgCl_2$ (w/v) for 5 min followed by washing with distilled water thrice to remove the traces. The filter papers were cut into round strips (90mm) and kept in petri dishes (90mm). Then the different concentrations like 0ppm (D_0), 48ppm (D_1), 96ppm (D_2) and 240ppm (D_3) ppm of 2, 4-Dichlorophenoxyacetic acid ($C_8H_6C_{12}O_3$) were prepared by mixing with tap water. The metal contents of tap water are given in Table 1. Later about 70-80 seeds were kept in filter papers which were soaked with each concentrations of herbicide and maintained under normal laboratory temperature (25±2°C) and light condition. After 3, 5 and 7 days of incubation root of tested plant was fixed in aceto-alcohol (1:3) solution. Seed under the same treatment except addition of 2, 4-D served as control. Then, roots were transferred from fixative to 70% ethanol and stored in the refrigerator until they were used in the further analysis. For nutrient and heavy metal analysis 0.5g of air dried samples were taken in digestion vessels along with 5ml of 65% HNO_3 and 2ml of H_2O_2. Vessels were put it into microwave oven (Milestone, Start-D) and digested through the program (Table 2). After 55min, vessels were cooled for 20min under fume hood and vessels cap was opened. Then the solutions were transferred into 50ml volumetric flask and evaporated at 120°C for few min. Once filtrated, solutions were transferred to another 50ml volumetric flask. For dissolution of the sample, 3ml of 5% HNO_3 was added and made upto the mark by deionized water (produced by LABCONCO). Finally, all the samples were run in FAAS (Shimadzu, AA-7000) against standard curve for two micro nutrients (Iron and Manganese), two macro nutrients (Magnesium and Calcium) and two heavy metals (Chromium and Lead) which was just prepared before by the specific standard solutions. Both micronutrient, macronutrient and heavy metal concentrations were measured automatically through the FAAS operating software. The experimental design was completely randomized and all the experiments were repeated thrice. Statistical analysis such as mean, standard deviation, ANOVA and correlation was done by SPSS package program V13.

RESULTS AND DISCUSSION

In the present investigation durum wheat seeds were treated with different concentrations of 2, 4-D herbicides and kept in petri dishes for germination (Figure 1). After 3, 5 and 7 days of germination roots were collected in 70% ethanol fixative (Figure 2) for further nutrient and heavy metal analysis. During the root collection an abnormal protuberances growth was observed at root due to all doses of 2, 4-Dherbicide in laboratory (Table 3) and total hindrance for further growth of root tip occurred at 240 ppm concentration (Figure 3).

In the present investigation an abnormal protuberances growth was found at root in all doses of 2, 4-D treatment. This abnormal mass protuberance might be a sign of increased root tip width and probably persuade for increase in cell size. Sanjoy Kumar (2010) also reported the root tip protuberances in the application of herbicides 2, 4-D in Triticum aestivum at 200 ppm. Protuberance was formed may be due to abnormal growth responses a consequence of hormonal imbalance (Over beek, 1964).

Impact of dose on micro and macro nutrient and heavy metal absorption in different days are shown in Table 4 whereas Table 5 presents the p-value between the treated groups. At day 3 the micronutrients iron (Fe) and manganese (Mn) concentrations range were 0.205 to 0.229 and 0.615 to 0.765, macronutrients magnesium (Mg) and calcium (Ca) concentrations range were 0.919 to 2.314 and 1.569 to 2.216 and heavy metal chromium (Cr) and lead (Pb) concentrations range were 0.104 to 0.228 and 0.034 to 0.194 ppm respectively. At day 5 the Fe , Mn, Mg and Ca concentrations range were 0.058 to 0.399 and 0.221 to 0.636, 0.919 to 2.314 and 1.569 to 2.216 ppm respectively. Whereas the range for Cr and Pb were 0.116 to 0.244, and 0.034 to 0.194 ppm, respectively. At day 7 the highest Fe, Mn, Mg and Ca concentrations were 0.473, 2.665, 4.081 and 6.13ppm and heavy metal Cr and Pb content were 0.253 and 0.503 ppm, respectively (Table 4).

This experiment demonstrated that concentration of Fe was inhibited by the all doses of 2, 4-D after 5 days and 7 days whereas in D_1 and D_3 dose after 3 days had non-significant difference except D_1 than control. Besides, concentration of Fe was inversely related to herbicide dose after waiting period of day 5 and 7 whereas positive relation was observed at control and D_1 of herbicide and decreased at dose D_3 with time which was not in agreement with Reddy et al. (1997). However, absorption of Mn was significantly higher than control in all doses of herbicide after all the incubating days except dose D_1 in day 5. Mn absorption was significantly decreased after day 5 with the increase of herbicidal dose and increased positively with incubation time at D_1 and D_2 of herbicide. The results of this present study was in agreement with Neumann et al. (2006) who demonstrated that glyphosate herbicide applied exclusively to glyphosate-resistance (GR) soybean leaves; impaired Mn uptake of non-GR sunflower seedlings cultivated simultaneously suggested an inhibition of micronutrient uptake by root. Similarly, hydroponic experiments demonstrated that even low levels of

glyphosate caused a pronounced decline in acquisition, root uptake and root-to-shoot translocation of radio-labeled Fe and Mn in non-GR sunflower (Ozturk et al. 2008; Eker et al. 2006).

In case of macronutrient Ca and Mg significantly lower difference was observed in all doses after all days than control. The macro nutrients amounts of Ca and Mg in root of tetraploid wheat revealed significantly lower than control in all doses after in incubating days. Ca and Mg content were positively correlated with duration in absence of 2, 4-D but vice versa with the herbicidal dose. This result was in accordance with Reddy et al. (1997) for Ca who stated that at varying waiting periods (0, 7, 14 and 21 days) after the spraying pesticides calcium contents of cabbage were found to be decreased remarkably. The present observation was in accordance with Patrick (2013) who stated that pre-emergence herbicide indaziflam reduced magnesium (Mg) and manganese (Mn) in Bermuda grass.

For heavy metal Cr and Pb significant higher results were found in all cases of herbicide treatment after all the 3 days. Moreover, among the doses micronutrients significantly decreased in D_2 and D_3 dose than D_1 after 5 and 7 days whereas similar results also found in macronutrients after all the three days. However, concentration of heavy metal was significantly increased in D_2 and D_3 than D_1. From the Table 6 showed that after day 3, 5 and 7 the heavy metal Cr and Pb content was found positively correlated whereas a reverse condition was observed in case of micro nutrients and macro nutrients with the dose of 2, 4-D. On the other hand, micronutrient was increased at D_1 and decreased at D_3 with time. Incubation time had no consistent pattern on macronutrients whereas heavy metal content increased with the increasing of it. But when 96 and 240 ppm of 2, 4-D was applied the pattern changed in case of macronutrients (Table 7). Amount of heavy metal Cr was significantly higher than control after 5 and 7 days except dose D_1 and D_2 in day 3. Moreover, Cr content had positive relation with herbicidal dose and had no significant relation with waiting days except control. On the other hand, concentration of Pb revealed significantly high than control in all cases and even had positive relation to the increasing of herbicide dose and incubation time. However, it is well-documented that ability of glyphosate to form stable complexes with metal cations such as Al, Fe, Zn, Mn, Ca and Pb (Sprankle et al. 1975) may thereby induce internal micronutrient deficiencies. The nutrients uptake was imbalanced may be due to loss of roots its ability to absorb water and salts (Shaddad et al. 1990)

CONCLUSION

This experiment illustrated that content of micronutrients Fe and Mn, macronutrients Mg and Ca in germinated wheat roots were reduced with the increase of 2, 4-D dose. However, heavy metal Cr and Pd uptake were enhanced by the 2,4-D. It may be concluded that 2, 4–Dichlorophenoxy acetic acid hamper or collapse the function of vascular tissue which make disturbance in root of wheat for nutrient uptake and enhance the accumulation of heavy metal. However, these results show that the durum wheat have ability to remove and accumulate heavy metals.

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Figure 1. Durum wheat seed treatment by different herbicidal concentration in petri dishes.

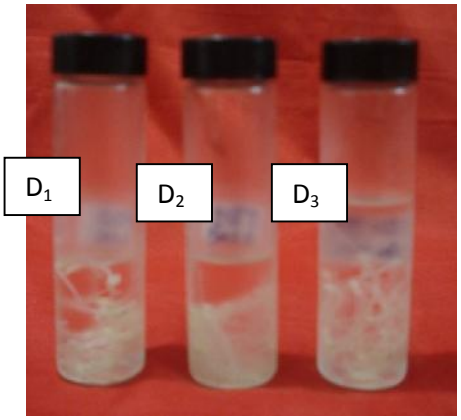

Figure 2. Collection of wheat root in fixative prior to nutrient concentration analysis (D_1= 48ppm, D_2= 96ppm and D_3= 240ppm)

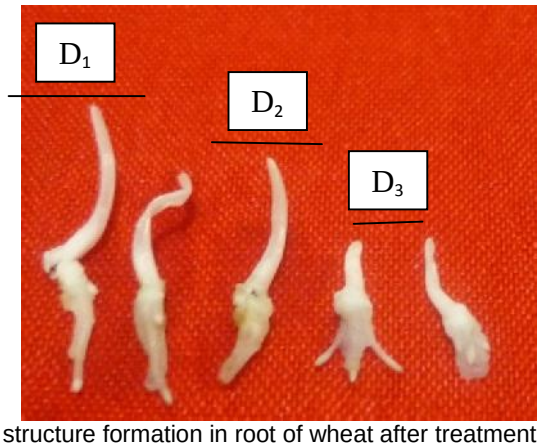

Figure 3. Bulbous structure formation in root of wheat after treatment of different dose of 2, 4-D.

Table 1. Mineral in Tap water

Mineral in Tap water	Concentration (ppm)
Fe	0.80
Mn	0.19
Cr	0.10
Mg	6.84
Ca	17.00
Pb	0.52

Table 2. Digestion programme of sample by Milestone, Start-D

	Parameter of MW	
<12 vessels were used along with reagent blank	250W	6min
	630W	12min
	500W	22min
	0W	15min

Table 3. Protuberances presence in root of tetraploid wheat at different doses of 2, 4-D

	0 ppm	48 ppm	96 ppm	240 ppm
Day 3	-	+	+	+
Day 5	-	+	+	+
Day 7	-	+	+	+

Table 4. Impact of herbicidal dose on nutrient and heavy metals concentration in different days.

		Micronutrients (ppm)		Macronutrients (ppm)		Heavy metals (ppm)	
Day	Doses	Fe	Mn	Ca	Mg	Cr	Pb
3	D_0	0.205±0.007	0.615±0.023	2.216±0.013	2.341±0.373	0.104±0.003	0.034±0.009
	D_1	0.214±0.011	0.758±0.005[a]	1.589±0.040[a]	1.102±0.105[a]	0.125±0.006	0.078±0.010[a]
	D_2	0.229±0.016	0.687±0.008[a]	1.589±0.012[a]	1.049±0.049[a]	0.134±0.010	0.119±0.004[a]
	D_3	0.213±0.010	0.756±0.050[a]	1.361±0.091[a]	0.919±0.016[a]	0.228±0.089[a]	0.194±0.014[a]
5	D_0	0.399±0.002	0.605±0.052	5.684±0.359	3.954±0.168	0.116±0.003	0.268±0.010
	D_1	0.206±0.002[a]	0.636±0.033[a]	1.171±0.052[a]	1.780±0.135	0.145±0.010[a]	0.322±0.017[a]
	D_2	0.105±0.004[a]	0.366±0.018[a]	0.983±0.001[a]	0.822±0.001[a]	0.232±0.004[a]	0.308±0.004[a]
	D_3	0.058±0.005[a]	0.221±0.010[a]	0.130±0.001[a]	0.820±0.032[a]	0.244±0.026[a]	0.335±0.003[a]
7	D_0	0.473±0.004	0.553±0.008	6.130±0.122	4.081±0.061	0.127±0.002	0.406±0.003
	D_1	0.440±0.019[a]	2.665±0.306[a]	1.613±0.001[a]	3.179±0.062[a]	0.137±0.003[b]	0.434±0.010[a]
	D_2	0.188±0.012[a]	2.263±0.144[a]	0.527±0.013[a]	0.901±0.001[a]	0.152±0.010[a]	0.474±0.001[a]
	D_3	0.093±0.004[a]	1.785±0.284[a]	0.603±0.004[a]	0.142±0.001[a]	0.253±0.001[a]	0.503±0.005[a]

a = Significant at the level P<0.01, b= Significant at the level P<0.05, D_0=Control, D_1=48 ppm, D_2=96 ppm and D_3=240 ppm

Table 5. ANOVA analysis of nutrient and heavy metals concentration in different days at different herbicidal dose.

			P value					
			Micro nutrient		Macro nutrient		Heavy metal	
Day	Dose V Dose		Fe	Mn	Mg	Ca	Cr	Pb
3	D_1	D_2	0.13	0.02	0.75	1.00	0.81	<0.001
		D_3	0.94	0.94	0.29	<0.001	0.02	<0.001
	D_2	D_3	0.12	0.02	0.44	<0.001	0.03	<0.001
5	D_1	D_2	<0.001	<0.001	<0.001	0.24	0.00	0.14
		D_3	<0.001	<0.001	<0.001	<0.001	0.00	0.16
	D_2	D_3	<0.001	<0.001	0.99	<0.001	0.33	0.01
7	D_1	D_2	<0.001	0.06	<0.001	<0.001	0.01	<0.001
		D_3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	D_2	D_3	<0.001	0.03	<0.001	0.17	<0.001	<0.001

Significant at the level P<0.05

Table 6. Correlation between herbicide dose with nutrient and heavy metal concentration in different days.

		Micro nutrients		Macro nutrients		Heavy metals	
Group		Fe	Mn	Ca	Mg	Cr	Pb
Day 3	r	0.15	0.59 [b]	-0.81 [a]	-0.68 [b]	0.78 [a]	0.98 [a]
Day 5	r	-0.85[a]	-0.910 [a]	-0.75 [a]	-0.75 [a]	0.85 [a]	0.74 [a]
Day 7	r	-0.91 [a]	0.26	-0.70 [a]	-0.91 [a]	0.98 [a]	0.94 [a]

a Correlation is significant at the 0.01 level.
b Correlation is significant at the 0.05 level.

Table 7. Correlation between incubation time with nutrient and heavy metal concentration in different herbicidal doses.

		Micro nutrients		Macro nutrients		Heavy metals	
Group		Fe	Mn	Cr	Mg	Ca	Pb
Control	r	0.97 [a]	-0.654	0.91 [a]	0.87 [a]	0.98 [a]	0.99 [a]
D_1 (48ppm)	r	0.85 [a]	0.83 [a]	0.05	0.98 [a]	0.52	0.98 [a]
D_2(96ppm)	r	-0.32	0.77 [b]	-0.99 [a]	-0.62	0.18	0.99 [a]
D_3(240ppm)	r	-0.74 [b]	0.63	-0.61	-0.92 [a]	0.23	0.99 [a]

a Correlation is significant at the 0.01 level.
b Correlation is significant at the 0.05 level.