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Effects of soil treated glyphosate on growth parameters and chlorophyll content of maize *Zea mays* L. and bean *Phaseolus vulgaris* L. plants

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Abstract. Extended use of glyphosate poses the problem of its accumulation into soil and further on crops. The objective of this study was to evaluate the effects of different doses of soil glyphosate on growth parameters and chlorophyll content of maize *Zea mays* L. and bean *Phaseolus vulgaris* L. plants. An experiment was arranged in randomized complete blocks design with ten replications. Glyphosate solutions were applied to the soil in six concentrations, two subdoses of 0.1 and 0.2 g, the recommended dose of 0.4 g and three overdoses of 0.6, 0.8 and 1.0 g kg^{-1} of soil. An uncontaminated soil control was used. At 21, 28, 35 and 42 days after sowing (DAS), evaluations of plant height, number of leaves, stem diameter, leaf surface and plant injury were conducted. At 42 DAS, nodulation (in bean), root length, chlorophyll and dry matter yield were assessed. Already at the recommended dose and beyond, glyphosate caused significant damage to growth characteristics, nodulation and chlorophyll content in both plants. Some similar effects of the product were observed on the both plants at each time period. Therefore, there was an evidence that soil glyphosate already at the recommended dose had an inhibitory and even a phytocide effects on non-target plants.

Keywords: Common bean; Maize; Glyphosate doses; Plant growth; Chlorophyll.

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Introduction

In most countries of the world, increased population growth leads to increase the agricultural areas, characteristic of continuous and intensive agriculture of recent years (Gomez et al., 2008). This continuous and intensive agriculture has generated changes at an ecosystem level by incorporating different management practices among which the growing use of agrochemicals is remarkable. The application of these synthetic compounds generates environmental concern by the potential for unwanted side effects, as large amounts of substances with different degradation rates are released into the environment (Gomez et al., 2008). Thus, agrochemicals or pesticides are found as common contaminants of soil, air, and water, and on non-target vegetation in our urban landscapes (Sarmamy et al., 2013). Once there, they can harm plants, soil microorganisms, non- target plants, animals and human beings. So, the use of large amount of pesticide is the main reason for agricultural soil pollution (Jiang and Wan, 2009). All these constraints create disequilibrium between the available quantity of crops produced and the population needs on one hand, and the environment on the other, suggesting that this population is not eating or living safely (Cooke, 1998).

An indispensable way for crop production is weed management. During several years, glyphosate has been widely applied to genetically modified glyphosate resistant (GR) crops as an herbicide aiming to control weeds (Huang et al., 2012). It is a broad-spectrum herbicide, non-selective highly toxic to sensitive species, it may cause severe damage if it drifts onto non-GR crops, and farmers could, therefore, happen significant losses (Yao et al., 2012). This problem has become more severe recently due to the increased use of glyphosate, which makes the early detection of crop injury caused by glyphosate drift a crucial problem for field managers (Ding et al., 2011b).

Indeed, in the agricultural systems, the rates of glyphosate generally recommended for herbicide use are far in excess of the amount required to kill most weeds. Excess application has occurred primarily as a result of advertising promotions, ease of application, increasing weed resistance, low cost of the product, and apathy towards the extensive non-target environmental effects of glyphosate (Johal and Huber, 2009). Usage of glyphosate is also increasing with the widespread cultivation of transgenic plants and the adoption of no-tillage cropping systems (Cerdeira and Duke, 2006).

In cropping systems, the application of glyphosate can improve crop yields (Anderson and Kolmer, 2005), but soil adsorption may occur as well, and these chemicals accumulate in the soil, hence the glyphosate residues may be toxic and may disturb the biotic and abiotic components of soil ecosystem and thus the fertility of the soil decrease (Nollet and Rathore, 2010). Indeed, glyphosate spray drift and residues can cause severe damage to non-target plants (Cornish and Burgin, 2005), also repeated applications year after year favour the accumulation of the product in glyphosate-treated soils. This situation has influenced the growth and development of agricultural plants (Bellaloui et al., 2006), which exhibit in general the chlorosis (Stenersen, 2004).

Despite the importance of this threat, the environmental consequences of the widespread use of this compound are not clearly elucidated (Gomez et al., 2008). Numerous studies have been done to show the effects of foliar application of different glyphosate doses on vegetative growth (Meier et al., 2006; Carvalho et al., 2013), photosynthetic activity and chlorophyll content (Zobiole et al., 2009), and soil glyphosate on plant growth characteristics (Sarmamy et al., 2013). These previous data did not clearly state whether accumulated soil glyphosate affects directly or modifies crop development. For this reason, we aimed to study the effects of different doses of soil glyphosate on growth parameters and chlorophyll content of maize *Zea mays* L. and bean *Phaseolus vulgaris* L. plants.

Materials and methods

Soil, sand and compost mixture

We selected the soil based on the fact that this one had no previous history of agrochemicals used. Soil samples were collected in one location site within the campus of the University of Ngaoundere according to method of Swift et al. (2001) with few modifications. A total of 96 soil samples were collected and well mixed to get a composite soil sample. Before sampling, un-decomposed surface debris such as plant residues and stones were removed from the soil and let to drying under laboratory conditions. Samples were taken from different depths until 20 cm. After drying, samples were passed into

2 mm stainless steel sieve. A sample was analyzed in order to determine the physical and chemical properties.

The compost was prepared from cow excrements, herbs and inoculum from rubbish bin (from November to February 2014) to fertilize and enable plant growth. The sand was carried from a drain in the rainy season (middle may).

Glyphosate application and plants

The experiment was performed in pots using substrate in a ratio 2:1:1 containing 500 g of soil, 250 g of compost and 250 g of sand well mixed. Treatments consisted of glyphosate solutions in concentrations: 0.1 and 0.2 g (subdoses), 0.4 g (recommended dose by the manufacturer) and 0.6, 0.8 and 1.0 g (overdoses). An uncontaminated soil control was used.

The glyphosate solutions were prepared using a commercial formulation of glyphosate Roundup (granule form, SuperMachette, Monsanto). The application was accomplished using a hand held sprayer with an application volume of 200 mL/dose into corresponding pots. The application was performed in the morning, when environmental conditions were appropriate such as air temperature, low wind speed and open sky without clouds (Zobiole et al., 2009).

The *Phaseolus vulgaris* L. and *Zea mays* L. seeds used for this experiment were the “GLP 290” (big grain) for bean and “CMS 8704” for maize cultivar purchased from a food store in the local market. Glyphosate was obtained from a local shop supplier of agricultural products in Ngaoundere. Seeds of a similar size were selected for the experiments and directly sown in plastic pots.

Experiment 1. Seed germination, plant height, number of leaves, stem diameter, leaf surface and plant mortality

Three seeds were sown in each pot at about 5 cm depth and 10 cm for bean and maize seeds respectively. After 14 days, seedlings were thinned to one seedling per pot. The pots were exposed in open air and irrigated whenever needed to maintain soil moisture. Seed germination was counted from 7 till 14 DAS. Plant height, number of leaves, stem diameter and leaf surface were measured at 21, 28, 35 and 42 days after sowing (DAS). Maize and bean injury were visually estimated at 21, 28, 35, 42 days after growing on a relative scale of 0 (no plant injury) to 100% (plant death) (Ding et al., 2011a).

Experiment 2. Nodulation, root length, chlorophyll, shoot and root dry weight

Bean and maize plants were sampled from three experimental units (pot) at 42 DAS. Shoots were harvested by means of hand-cutting using scissors at the soil surface, and placed into paper bags. Plants were oven dried at 60 °C for 72 h and dry weights recorded (Ding et al., 2011a). The root system was dipped in water to remove all the soil and to enable better visualization of nodules (Ngakou et al., 2009), which were picked and counted. All root nodules collected from each plant were dried in a hot air oven for 12 h at 60 °C and weighed separately (Ngakou et al., 2009) using a Mettler Toledo balance at 0.01 g sensibility. The photosynthetic pigments were extracted from fresh leaves in 80% acetone by the mean of sterilized sand in a mortar. The extract was centrifuged at 12,000 rpm for 10 min. The volume of clear extract was made up in 10 mL of 80% acetone, and then centrifuged again in order to extract the total chlorophyll (Priso et al., 2010). Absorbance was read at 645 and 663 nm measured with a spectrophotometer (Spectroquant® Pharo 100 M). The concentrations in mg/L of photosynthetic pigments were determined by the following formulae (Arnon, 1949):

$$\text{Chlorophyll a} = 12.7\ Y - 2.69\ X$$
$$\text{Chlorophyll b} = 22.9\ X - 4.68\ Y$$
$$\text{Chlorophyll (a+b)} = 20.21\ X + 8.02\ Y$$

where X is the absorbance of chlorophyll at 645 nm and Y absorbance at 663 nm.

Statistical analysis

Glyphosate treatments were arranged in randomized complete blocks design. There were ten replications of all treatments. Separate experiments were conducted for maize and bean plants. ANOVA was used for testing the effects of glyphosate concentration, sampling time and interactions between these. For differences between single concentration level and the control the Dunnett's test was used. Mean values are considered significantly different from $p \leq 0.05$.

Results and discussion

Experiment 1. Seed germination, plant height, number of leaves, stem diameter, leaf surface and plant injury

The results of six soil glyphosate doses investigation on two growing plants commonly consumed by people namely: *Zea mays* L. and *Phaseolus vulgaris* L. are shown in Tables 1 and 2.

Table 1. Effect of glyphosate on plant height and number of leaves in maize and bean plants overtime.

Glyphosate doses (g kg^{-1})	Plant height (cm)				Number of leaves (no plant $^{-1}$)			
	21 DAS	28 DAS	35 DAS	42 DAS	21 DAS	28 DAS	35 DAS	42 DAS
Maize plants								
0.0	28.24	35.5	42.40	45.28	5.90	6	5.70	5.70
0.1	29.17	36.15	40.32	44.69	6.10	5.50	5.50	5.30
0.2	23.58	31.94	40.01	44.52	5.70	6.30	6.30	5.80
0.4	17.07**	24.79*	30.14*	36.25	5.90	4.70	4.70	5.62
0.6	11.64***	19.26***	24.34**	30.38*	4*	4.75	4.25	4.28
0.8	11.22***	20.56**	31.37*	35.19*	4*	5.12	6.17	6
1.0	8.12***	9.60***	13.50***	11.85***	3.37*	3.75*	3.83*	3*
Bean plants								
0.0	23.39	25.79	27.07	29.50	8.50	12.80	17.90	22.30
0.1	16.25*	20.48	23.21	27.80	5.67	10.44	13.67	17.62
0.2	15.11**	20.12	21.42	23.61	3.50**	6.70**	9.60**	15.80*
0.4	9.06***	9.41***	10.70***	10.75***	1.60***	1.55***	3***	5.17***
0.6	6.48***	6.76***	7.16***	8***	2***	1.37***	1.43***	1.67***
0.8	6.07***	6.20***	8.05***	9.27***	1.71***	1.43***	***	***
1.0	4.17***	3.40***	-	-	2***	0***	-	-

Each value is a mean of three values for the same treatment group. The separation results are based on the Dunnett's test for differences between single concentration level and the control. Means with 'star' are significantly different with the control at *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ levels of significance.

Table 2 shows the glyphosate effects on stem diameter, leaf area and seed germination in maize and bean plants at each time period. It can be seen that the increasing glyphosate concentrations had reduced the seed germination rate in maize significantly while in bean only the lowest and highest concentrations showed significant decreased. Besides here the high percentages of seed germination for glyphosate treatments were observed at 0.1 and 0.2 g in maize (when compared to 0; alike percentage can be seen in bean at 0.2, 0.4, 0.6 and 0.8 g). The stem diameter of the treated soils, however, was negatively affected at the highest concentration of glyphosate in maize plants, and even beans all had died by the end of the experiment. Leaf area tended to be significantly delayed in glyphosate doses of 0.4, 0.6, 0.8 and 1.0 g in bean pots while maize leaf area hardly increases at 0.8 and 1.0 g. The interaction is clearly visible by the leaf area increase over time at 0.1 and 0.2 g (when compared to 0; alike effect can be seen in maize at 0.1, 0.2, 0.4 and 0.6 g).

Table 1 summarizes the variations in plant height and number of leaves for different treatments at each time period. The separation analysis of these averages with control showed that in general, glyphosate doses of 0.4, 0.6, 0.8 and 1.0 g had negative effects on plant height and that at the highest dose of 1.0 g maize hardly grew at all whereas beans all had died by end of the experiment. Interesting here is also that bean stopped growing already at 0.4 g glyphosate, unlike effect can be seen in maize at 1.0 g. However, mean values of number of leaves showed that already at the low dose of 0.2 g glyphosate had influence negatively on number of leaves of bean while number of leaves of maize hardly increases at the highest concentration. On the other hand, leaf fall was predominant in high glyphosate concentrations in both plants.

Table 2. Glyphosate effect on stem diameter, leaf area and seed germination in maize and bean plants overtime.

Glyphosate dose (g kg-¹)	**Stem diameter (mm)**				**Leaf area (cm²)**				**Seed germ (% pot ⁻¹)**
	21 DAS	**28 DAS**	**35 DAS**	**42 DAS**	**21 DAS**	**28 DAS**	**35 DAS**	**42 DAS**	**14 DAS**
Maize plants									
0.0	4.50	5.38	6.53	6.96	33.03	53.35	79.33	86.94	55
0.1	5.16	5.83	6.48	7.05	29.85	53.99	70.74	78.07	52.5
0.2	4.31	5.31	6.36	7.14	24.53	45.04	71.43	83.09	55
0.4	4.00	4.91	5.57	6.70	19.59*	36.08	55.96	71.37	40
0.6	3.14*	4.44	5.14	5.61	12.42**	32.57	51.83*	80.15	35*
0.8	3.81	5.19	6.35	6.70	13.26**	25.09**	47.34*	60.88*	35*
1.0	3.43*	4*	4.13*	4.50*	6.99**	8.24***	10.19***	10.37***	27.5**
Bean plants									
0.0	3.91	3.90	4.31	4.45	59.15	70.49	83.77	100.52	62.67
0.1	4.20	4.07	4.41	4.95	47.39	57.57	87.51	90.29	38*
0.2	4.07	4.18	4.39	4.56	54.47	66.56	84.24	90.77	56
0.4	4.02	3.62	4.17	4.32	25.79*	27.55*	27.74*	34.20**	56
0.6	3.92	3.94	4.07	4.22	9.50***	17.22**	31.01*	34.20**	50
0.8	3.97	3.96	4.57	4.87	12.21*	13.31**	11.55***	11.58***	52
1.0	3.93	3.50	-	-	2.88***	1.58***	-	-	40*

Each value is a mean of three values for the same treatment group. The separation results are based on the Dunnett's test for differences between single concentration level and the control. Means with 'star' are significantly different with the control at *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ levels of significance.

The plant injury rate showed an increasing trend with time. The percentages revealed that in bean plants, glyphosate doses of 0.4, 0.6, 0.8 and 1.0 g caused plant mortality with 50, 80, 90 and 100% respectively, after 42 days. Whereas in maize plants, only high doses of 0.6, 0.8 and 1.0 g caused marked percentage of 50% death plants after 42 days (Figure 1).

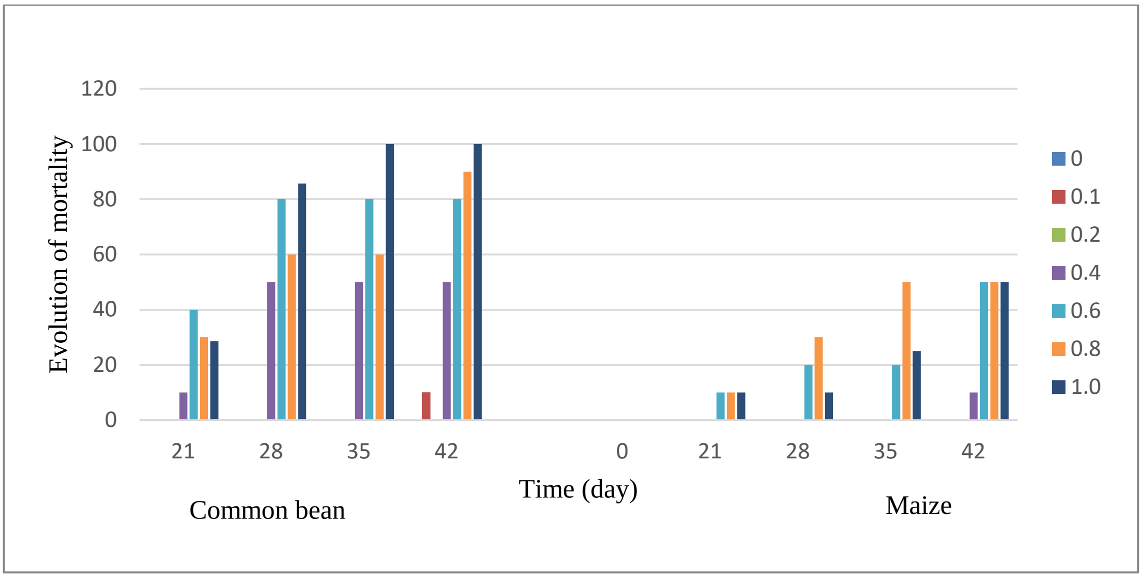

Figure 1. Evolution of the two plants mortality (common bean and maize) according to glyphosate doses and time.

The maize plants results disagree with Sarmamy et al. (2013) who observed no significant decrease in wheat seed germination after glyphosate application, but results on bean agreed with their results on *Vicia faba* seed germination. Mitra and Raghu (1998) reported glyphosate toxic symptoms in wheat seeds. Also Fox et al. (2007) found that germination of alfalfa seeds were reduced significantly when treated with herbicide. The stem diameter results disagree with those found by Carvalho et al. (2013) on coffee plants after glyphosate application at different growth stages. The results on leaf number were in disagreement with previous studies, which concluded that glyphosate at high levels increase number of branches per plant in faba bean (Shaban et al., 1987), while agreeing with Mitra and Raghu (1998), regarding the phytotoxic effects of pesticides on different physiological processes such as distortion of leaves and growing points. Nilsson (1977) has also suggested that glyphosate enhances senescence. Also reduction in number of heads was reported as disadvantageous side effects of glyphosate applied to wheat plants (Petróczi et al., 2002). Sarmamy et al. (2013) observed significant reduction on number of tillers per plant in soil treated with glyphosate.

Carvalho et al. (2013) observed that the leaf area at 45 days after foliar application of glyphosate on coffee plants decreased with the increased dose, resulting in a 44% reduction in leaf area. Moreover they also observed that the leaf area evaluated at the same time showed no effect from the application of the product. Other studies on the following crops have visually assessed the intoxication few days after glyphosate treatment and the effect on leaf area only after 40 days of application: coffee plants (França et al., 2010), physic nut plants (Costa et al., 2009) and eucalyptus plants (Machado et al., 2010).

Previous reports showed that glyphosate decreased the plant height of faba bean (Shaban et al., 1987) and winter wheat (Petróczi et al., 2002). Shaban et al. (1987) suggested that glyphosate may increase the level of ethylene, while others as Stanley and Burg (1973) reported that ethylene inhibited cell division of meristematic tissues and noticed that plants exposed to ethylene induced inhibition of stem height, so as a result, plant height may be decreased when treated with glyphosate. Crozier et al. (2000) hypothesized that the morphological responses observed at low glyphosate doses were due to an increase in activation of auxin, because glyphosate interferes with the auxin pathway by blocking some of the precursors of auxin synthesis. These results of glyphosate action on plant growth may be due to that glyphosate is a wide spectrum herbicide (non-selective) (Gravena et al., 2009), and the application of highest rates may disturb or even interrupt plant growth. Recently, Bellaloui et al. (2006) showed that a simulated glyphosate drift at 12.5% of the usually applied rate impaired shoot growth in a non glyphosate resistant soybean, especially during early vegetative growth.

About plant mortality, we can speculate on the age dependency for the two plants associated to glyphosate concentrations that we have observed. In general, the death of plants appears earlier in bean than in maize plants after inhibition of plant growth during a certain time. One explanation to this may be due to the fact that plants exposed to the high glyphosate doses had less time to establish their roots in the substrate to catch a significant amount of nutrients (specifically micronutrients Zobiole et al., 2009) indispensable for its growth and its development. Since glyphosate is known to reduce the root uptake and translocation of essential micronutrients in plants (Eker et al., 2006; Ozturk et al., 2008). It is clear that these two crops suffer from a range of nutrient deficiencies induce by the application of glyphosate. This was observed in the field by chlorosis, necrosis, wilting and not fully expanded young leaves, just after emergence of seedlings.

On the other hand, as the experimental substrate (soil + compost) has a high amount of organic matter (including the phosphate particularly), this may raise the bioavailable concentrations of glyphosate, since phosphate competes with glyphosate to binding soil particles, thus making it mobile in the soil (Simonsen et al., 2008). Because of this, glyphosate can increase its penetration efficiency through the roots and enable more substance reaching 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) of the shikimate pathway.

Experiment 2. Nodulation, root length, chlorophyll, shoot and root dry weight

Mean values of glyphosate effect on nodule number, fresh and dry weights of nodules as well as root length, shoot and root dry weight at 42 DAS are shown in Table 3. It can be seen that the glyphosate doses affected negatively the number of nodules per plant, with control that stimulating formation of more root nodules than the pots treated with glyphosate. The mean values of nodules number were 233, 144.67 and 0 for glyphosate doses of 0.1, 0.2 and 0.4 g respectively compared with 289 in control. Interesting here is that already at 0.4 g, no nodules were formed. Meanwhile, other two parameters, fresh and dry weights tended to decrease with increasing glyphosate dose. With fresh weights of 0.89, 0.85 and 0 g for glyphosate doses of 0.1, 0.2 and 0.4, respectively, compared with 1.57 g for the control. As indicated in Table 3 shoot and root dry weight in both plants was negatively affected already at the recommended dose of 0.4 g. Data analyzed using Dunnett's test showed that root length was significantly delayed in high doses of 0.8 and 1.0 g in maize while in bean significantly reduced already at 0.4 g. It is evident from that Table 4 that there was a gradual decrease in total chlorophyll content as the doses of glyphosate increase.

Table 3. Glyphosate effect on shoot and root dry weight, root length, nodule number, fresh and dry weight of nodules at 42 DAS.

Glyphosate doses (g kg^{-1})	Shoot dw (g plant $^{-1}$)	Root dw (g plant $^{-1}$)	Root length (cm plant $^{-1}$)	Nodule ber (no.plant^{-1})	Nodule fw (g plant $^{-1}$)	Nodule dw (g plant $^{-1}$)
Maize plants						
0.0	14.82	18.17	47.50			
0.1	17.21	21.03	56.50			
0.2	17.22	17.17	48.17			
0.4	10.45*	13.35*	45.83			
0.6	11.59*	10.73*	39.25			
0.8	11.62*	10.67*	35.67*			
1.0	7.73**	8.04**	4.20***			
Bean plants						
0.0	20.29	16.44	42.40	289	1.57	0.28
0.1	22.09	18.27	44.37	233	0.89*	0.25
0.2	22.80	18.82	39.92	144.67**	0.85*	0.18
0.4	14.93*	12.24*	9.40***	0***	0**	0*
0.6	14.94*	12.12*	4.20***	0***	0**	0*
0.8	12.40**	7.89**	2.50***	0***	0**	0*
1.0	-	-	-	-	-	-

Each value is a mean of three values for the same treatment group. The separation results are based on the Dunnett's test for differences between single concentration level and the control. Means with 'star' are significantly different with the control at $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$ levels of significance.
dw= dry weight, fw= fresh weight, ber= number.

The results corroborate those found by Sarmamy et al. (2013) who showed that soil treated glyphosate decreased the nodulation. Others authors (Zablotowicz and Reddy, 2004; Fox et al., 2007; Kremer and Means, 2009) showed that glyphosate cause decrease in legume nodulation. *Rhizobium* infects plant roots through root hairs and thus they hypothesized that glyphosate affecting root hair development might interfere with nodulation. King et al. (2001) attributed delayed nodulation to inhibited infection of the symbiotic N_2-fixing bacterium *Bradyrhizobium japonicum.* According to Mårtensson (1992), glyphosate caused root hair deformations that apparently resulted in fewer nodules being formed. Further studies (Mårtensson, 1992; Koopman et al., 1995) suggested that herbicide induced reduction in nodulation are due to effects upon the plant such as reduction in photosynthate transport to the roots and disruption of root hair infection by *Rhizobium* which are often accompanied by root stunting or other damage caused by the herbicide. In most cases the Rhizobia remain viable, but they are not capable to make nodules in the host plants or they cannot efficiently fix biological nitrogen (Sarmamy et al., 2013)

Table 4. Influence of glyphosate on total chlorophyll of bean and maize plants.

Species	Glyphosate doses (g kg^{-1})	Chlorophyll a (mg/L)	Chlorophyll b (mg/L)	Chlorophyll a+b (mg/L)
Phaseolus vulgaris L.	0.0	33.37	28.06	61.41
	0.1	31.86*	28.72	60.56
	0.2	29.86*	23.73*	53.57*
	0.4	31.12*	24.48*	55.58*
	0.6	25.29*	20.10*	45.37*
	0.8	24.69*	20.53*	45.20*
	1.0	-	-	-
Zea mays L.	0.0	11.83	8.68	20.50
	0.1	12.73	8.18	20.90
	0.2	10.60	6.68*	17.27*
	0.4	10.79	5.57*	16.36*
	0.6	11.28	5.27*	16.54*
	0.8	9.97*	6.69*	16.66*
	1.0	7.79**	3.19**	10.97**

Each value is a mean of three values for the same treatment group. The separation results are based on the Dunnett's test for differences between single concentration level and the control. Means with 'star' are significantly different with the control at $*p \leq 0.05$, $**p \leq 0.01$ levels of significance.

or improve plant nutrients uptake. As documented by Bellaloui et al. (2006) that a few amount of glyphosate more than usually applied rate impaired nodule activity of both nitrate reductase and nitrogenase in a non glyphosate resistant soybean, especially during early vegetative growth.

Additionally, the studied treatments have shown different patterns in nodule distribution. In control plants the majority of nodules were located on the upper of 7 cm of the primary root (Figure 2), whereas in pots treated with 0.1 g the nodules were located at approximately 10 cm on the upper of the primary root (Figure 3). Already at 0.4 g of glyphosate, no nodules were found on the roots of bean plants. The nodules size varied between 1 and 3 mm for 0 g (control) and between 1 and 2 mm for 0.1 and 0.2 g, respectively (Figure 4 and 5). According to Eberbach and Douglas (1991), some herbicides interfere with the process of nodule initiation or establishment of the nodules.

In a previous study it was observed that in sugarcane the accumulation time of dry matter is from the time of plant emergence until the time of assessment (Galon et al., 2010). This may confirm the report of Andy et al. (2001) who showed that glyphosate decreased shoot growth proportionately the same for plants supplemented with Nitrogen fertilizer. Carvalho et al. (2013) showed that decrease of plant biomass in coffee plants is due to the glyphosate effect on the shikimate metabolic pathway, since this pathway is responsible for the formation of phenolic compounds, which can represent up to 35% of plant biomass (Boudet et al., 1985).

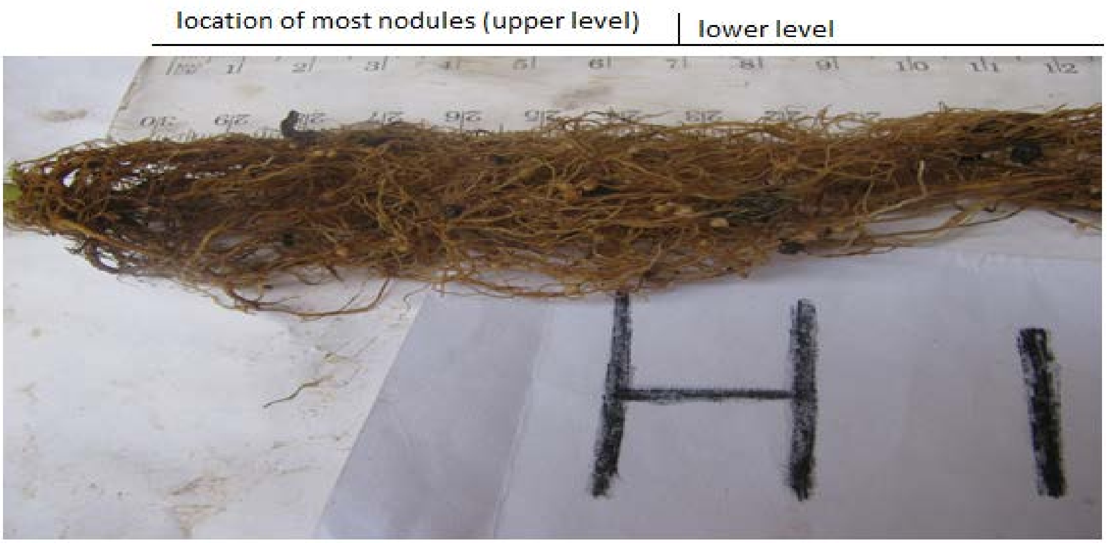

Figure 2. Root nodules (control) after fourty-two days of glyphosate application.

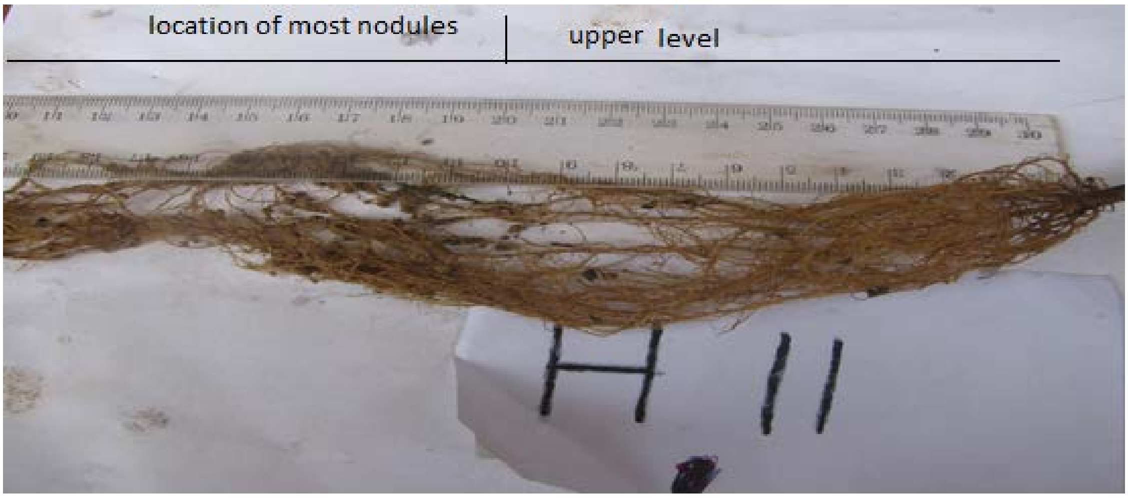

Figure 3. Root nodules after fourty-two days of glyphosate application of 0.1 g.

Figure 4. Nodule samples (control) after fourty-two days.

Figure 5. Nodule samples after fourty-two days of glyphosate application of 0.1 g.

According to Eberbach and Douglas (1991), some herbicides affect nodulation, by reducing growth of root system. Johal and Huber (2009) documented that root growth reduced from the accumulation of glyphosate in root tips results in less contact of the roots with dispersed nutrients in the soil profile. This corroborates results of this study. Zobiole et al. (2009) showed that glyphosate reduce root biomass production. Reports done on *Vicia faba* showed that ethylene and ammonia accumulated by glyphosate in faba bean resulted in reduction in total dry weight per plant (Shaban et al., 1987), and roots were reduced as a result of pesticide treatments (Al-Abdulsalam and Abdulsalam, 1995). Others found that dry weight was adversely affected as the exposure time and concentration of glyphosate increased (El-Tayeb and Zaki, 2009).

The most common symptoms of glyphosate when applied on plant foliage are chloroses which appear after 7-14 days of application, followed by necrosis (Stenersen, 2004). These results are in agreement with Zobiole et al. (2009) who showed that glyphosate application decreased the chlorophyll content of GR soybeans. Also in agreement with reports that herbicide application to the soil adversely affected physiological characteristics in crop plants (Mitra and Raghu, 1998; Petróczi et al., 2002). These were examples of evidence concerning the detrimental effects of the active ingredient of Roundup at higher doses disrupt or even inhibited the synthesis of chlorophyll by reducing the availability of plant nutrients, which are responsible for the synthesis of this one.

Various studies and field observations have reported that glyphosate affects micronutrient nutrition of plants, which has been correlated with its ability to form insoluble glyphosate-metal complexes (Coutinho and Mazo, 2005). According to Eker et al. (2006), after absorption of glyphosate into the plant, the uptake and transport of cationic micronutrients may be inhibit by the formation of poorly soluble glyphosate-metal complexes within plant tissues. Very low rates of glyphosate also reduce the root uptake and translocation of Mn and other essential micronutrients in plants (Eker et al., 2006; Ozturk et al., 2008). Moreover, Zobiole et al. (2009) found that glyphosate reduced the total amount of macro and micronutrients in GR soybeans tissues. In the same view as Coutinho and Mazo (2005), Zablotowicz and Reddy (2007) documented that the extent of injury is correlated with levels of AMPA formed within the plant. This primary phytotoxic metabolite is also toxic to plants (Reddy et al., 2004). In the current study, this could explain the reduction in chlorophyll content as the doses of glyphosate increase. However, glyphosate-immobilized Mg could also be a mechanism, since chlorophyll is dependent on Mg for its formation (Beale, 1978).

Conclusion

Knowledge on crop damages caused by soil pollution from glyphosate is of great importance. Results showed a link between high doses of soil glyphosate and deleterious effects on plants. From the presented results, it can be concluded that low glyphosate rate had mitigated effect on both plants. Application at the recommended concentration (0.4 g) and beyond had negatively influenced almost all the studied parameters. Some detrimental effects were more pronounced from one plant species to another. However, damages caused by glyphosate in the field that are attributed mainly to excess application may be due also to other complex processes involving the soil-plant systems. Further studies should be undertaken comparing different formulations of the product, test plants and soils.

Conflict of interest statement

Authors declare that they have no conflict of interests.

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