RESEARCH ARTICLE

EFFECT OF GLYPHOSATE HERBICIDE ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF *VIGNA MUNGO* L.

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Abstract: A field experiment was conducted to evaluate the effect of Glyphosate on different physiological and biochemical parameters of *Vigna mungo* L. The results obtained from this study revealed that the low amount of application of glyphosate (50 ppm and 100 ppm) of glyphosate have stimulatory effect on plant growth but adversely affect the growth parameters at higher concentration (>100 ppm). At higher concentration glyphosate decrease the protein, chlorophyll and leghaemoglobin contents of plants and interrupt the *Rhizobium*-legume symbiosis. Hence, the present study can conclude that glyphosate in the limited amount (50ppm and 100ppm) can enhance the productivity of plant *Vigna mungo* L.

Keywords: Glyphosate, *Vigna mungo* L., *Rhizobium*, Herbicides, Weed control methods

INTRODUCTION

Legumes belong to an important family Fabaceae (legume family) of flowering plants (angiosperms) with more than 650 genera and 18000 species. It is a large and economically important family. The name Fabaceae comes from the genus *Faba* now included into *Vicia*. Leguminosae is an older name still considered valid (Burkitt *et al.*, 1985), and refers to the typical fruit of these plants, called legumes.

Legumes are second only to cereals as a source of nutrition for humans and animals (Erdman and Fordyce, 1989).Legumes as food has high protein contents and used as food worldwide. On a worldwide scale, legumes provide 22% protein, 32% fat and oil, and 7% carbohydrates in terms of human nutrition. In terms of livestock nutrition, they provide 38% protein, 16% lipids and 5% carbohydrates. The beneficial effect of legumes in agriculture has been recognized even before the principles of crop rotation were established (Herridge, 1982)

India is the largest producer and consumer of *Vigna mungo* L. It occupies a unique position in Indian agriculture. Among the pulses, it stands fourth in production and acreage (Deepalakshmi, *et al.*, 2004). India produces about 1.5 million tons of Urd annually from about 2.5 million hectares of area with an average productivity of 400 kg per hectare. It contains the perfect combination of all nutrients, which include proteins (25-26%), carbohydrates (60%), fat (1.5%), minerals, amino acids and vitamins (Karamany, 2006).

Weeds are one of the major biological constraints in crop production and therefore, their control is important. Pynenburg *et al.* (2011) found that seed yield in common bean (*Phaseolus vulgaris*) gets reduced up to 85% as a result of season-long weed competition. Weeds reduce the yield of legumes crops by competing with legume plants for nutrition, water and space. To overcome such type of problems we use different mechanical, physical and chemical strategies to check or inhibit the growth of weeds. Weed control by chemicals is still the predominant component of weed management.

Herbicides are unique in that they are designed to kill plants. Sufficient high doses kill both crop and weed, while small doses have no effect upon crop and weed. The action of herbicides is usually determined by its chemical and physical properties, its effect on plant metabolism, the types of plant and the environment. Herbicides target key enzyme in the plant metabolic pathway, which disrupts plant food production and eventually kills it. The time and methods of application of herbicide are determined by its mode of action (Tu *et al.*, 2001).

Glyphosate [N-(phosphonomethyl) glycine] is commercially-available products as a white crystalline powder, which is commonly used as a broadleaf herbicide (weed killer), It is often available in liquid formulations (U.S. Environmental Protection Agency, 2005).

Glyphosate is a post-emergent, systemic and non-selective (or broad-spectrum) herbicide used in both agricultural and non-agricultural areas. Recommended application rates do not exceed 5.8 kg active ingredient per hectare. It is used to kill all plant types including grass perennials, and woody plants. It is mainly absorbed into the plant through the leaves and then transported throughout the plant where it acts on the plant's enzyme system. It acts as a potent inhibitor of the shikimic acid pathway for biosynthesis of aromatic amino acids. It is a competitive inhibitor of 5-enolpyruvilshikimate-3-phosphate (EPSP) synthase with respect to phosphoenolpyruvate (PEP) and noncompetitive with respect to shkimate-3-phosphate (S3P) (Coruzzi and Last, 2002).

Glyphosate is moderately persistent in soil, with an estimated average half-life of 47 days (Wauchope *et al.*, 1992; WSSA, 1994). It is strongly absorbed to most soils, even those with lower organic and clay contents, and it is highly soluble in water. Field and

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laboratory studies show that it does not leach appreciably, and has the low potential for runoff (except as adsorbed to colloidal matter) (Wauchope *et al.*, 1992).

MATERIAL AND METHOD

The present investigation carried out on the effect of Glyphosate herbicide on *Vigna mungo* (L.) plants which was undertaken with a view to have data from field experiments on the beneficial/adverse effects of Glyphosate herbicide with special emphasis on changes in physio and biochemical properties of *Vigna mungo* (L.). The details of the investigation are described as follow.

Geographical Situation

Meerut district is situated between 290 01N latitudes and 770 45E longitudes at an altitude of 237 meters above sea level. The C.C.S. University is situated at the distance of about 10km from Meerut city railway station and near about 12km on Delhi-Dehradun highway. The total geographical area of Meerut district is 2564 km2. The district falls under the western plain zone of Uttar Pradesh, sub-region of upper Gangetic plain.

Experimental site

Field experiments were conducted during the Kharif season in the month of March to June in 2016 to evaluate the response of Glyphosate herbicide on the Physio- chemical properties and yield of *Vigna mungo* L. The seeds of *Vigna mungo* L. were grown in the field of Botany Department, C.C.S. University, Meerut. The experiments were designed in 10 plots of equal size: (1 control and nine treated with glyphosate herbicide).

Material used

1. IARI certified seeds of PU-31 variety of *Vigna mungo* (L.).
2. Glyphosate herbicide (Round up) brought from IARI New Delhi.

Other Details (Experimental Details):

1. Total no. of blocks- 10
2. Control block- 1
3. Total no. of treated plots- 9
4. Plot size (area of plot) – 500 × 500 cm.

Fifty healthy seeds of *Vigna mungo* L. were sown in every plot. All plots were irrigated with tap water. The seed germination percentages were calculated after counting the difference between germinated (coming out of soil) and non-germinated seeds (remaining inside soil, non emergent).

Figure-A

Figure-B

Figure-C

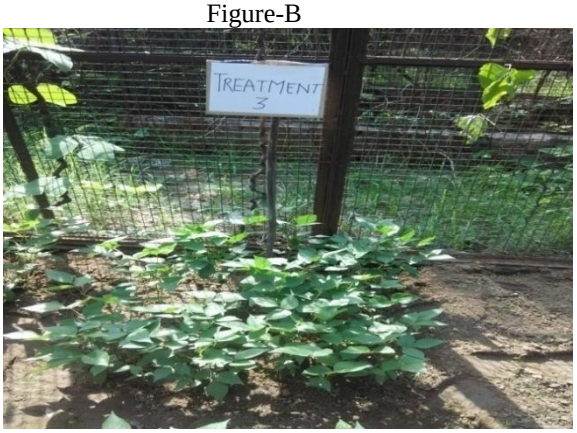

Figure-D

Figure-E

Figure-F

Germination %

The seed germination percentage was calculated by the given formula-

$$\text{Germination percentage} = \frac{\text{Seeds germinated} \times 100}{\text{Total seeds}}$$

Nodulation: Plants from nine plots for each treatment and control were removed 30 days after seeding (DAS) and were observed for the extent of nodulation for observation.

Determination of protein

The protein was estimated by the method adopted by Bradford (1976).The following formula was used for the measurement of protein content-

$$\text{Protien}\left(\frac{\text{Mg}}{\text{g}}\right) = \frac{\text{O.D.} \times \text{Factor} \times \text{Dilution (if any)} \times 1000}{100 \times \text{Total volume/volume of replicate}}$$

Leghaemoglobin content

Leghaemoglobin quantities of the nodules were measured spectrophotometrically as haemochromogen according to the method of **Bergersen (1980).** Leghaemoglobin content was calculated by using the following formula-

$$\text{LB: Protein} = \frac{\text{LB/g fresh weight of nodule X 100}}{\text{Protein/g/ fresh weight of nodules}}$$

Estimationof chlorophyll content

Chlorophyll content was estimated by using **Arnon's method.**For calculation the following formula was used -

$$\text{Chl. a (mg/g f } wt.) = \frac{12.7\ (A663) - 2.69\ (A645) \times V}{1000 \times W}$$

$$\text{Chl. b (mg/g f } wt.) = \frac{22.9\ (A645) - 4.89\ (A663) \times V}{1000 \times W}$$

$$\text{Total Chl. (mg/g f } wt.) = \frac{20.2\ (A645) - 8.02\ (A663) \times V}{1000 \times W}$$

$$\text{Carotenoid} = \frac{7.6\ (A445) - 8.02\ (A663) \times V}{1000 \times W}$$

Where,

V = final volume of chlorophyll extract

A = absorbance at specific wavelength

W = fresh weight of tissue extract

Estimation of total nitrogen (snell and snell, 1954)

Total nitrogen was estimated by the method as suggested by Snell and Snell, (1967).

RESULT AND DISCUSSION

Germination Percentage

It was noticed that application of glyphosate herbicide increases the germination percentage of seeds in 50 and 100 ppm treatments as compared to control and 150ppm (Fig. 1). According to Cavusoglu *et al.* (2011) 100, 250 and 500 mg l-1 doses of glyphosate caused 24%, 40% and 60%, reduction in seed germination and the root length of *Allium cepa*. Similar results were also reported by Zaidi *et al.* (2005) that Glyphosate and metribuzin herbicide (2 g a.i. kg–1) applied pre-emergence completely reduced the germination percentage. Failure to germinate at a higher concentration of metribuzin herbicide could be attributed to the rupturing of seed testa and damage to the cell membranes, leading to the efflux of nutrients and other cellular contents. Similar evidence of phytotoxic activity of the pre-emergent application of herbicides on germination and plant growth of soybean has previously been reported (Rennie and Dubetz, 1984).

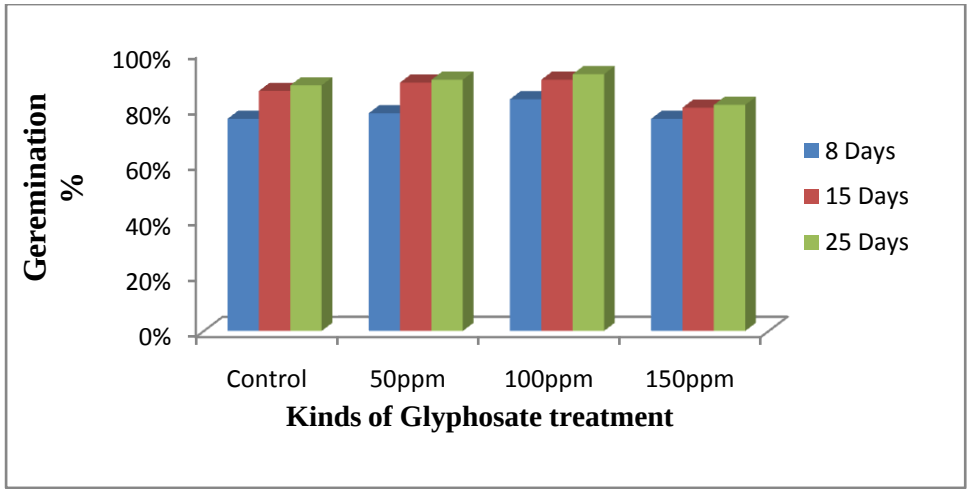

Figure-1. Effect of glyphosate on the germination percentage of *Vigna mungo* (L.) after 30 days of sowing

Nodulation: Maximum no. of nodules were found in the case of 100ppm solution treatment as compare to control and other treatments. Besides number, weight (dry and fresh) of nodules was also got affected by the treatments. Maximum weight of nodules (dry and fresh) was found in 100ppm solution treatment. Application of 50ppm and 100ppm solution caused a significant increase in nodule number and weight of nodules(Fig 2&3). Eberbach and Douglas, (1983) in their study found the lack of inhibitory effect on nodulation observed with glyphosate could be due to its rapid inactivation in soils or its rapid translocation, along with photosynthate, to a distant metabolic sink.

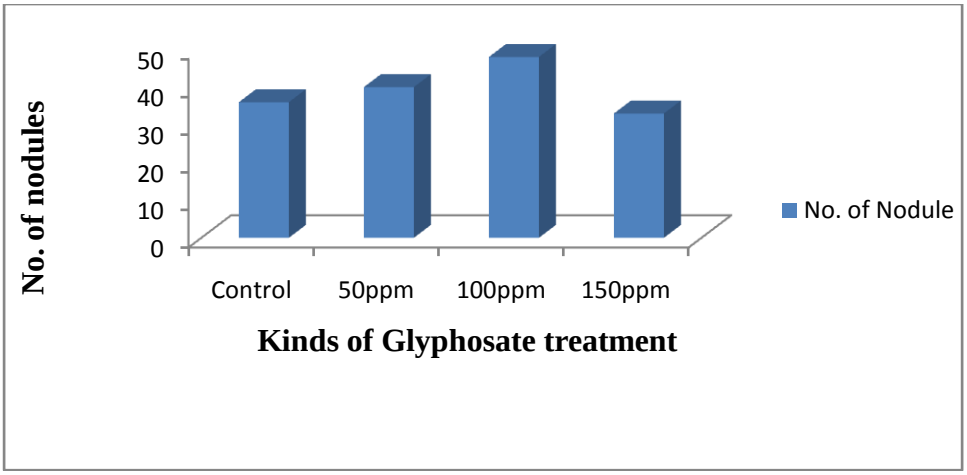

Figure-2. Effect of glyphosate on the Nodule number of *Vigna mungo* (L.) after 30 days of sowing

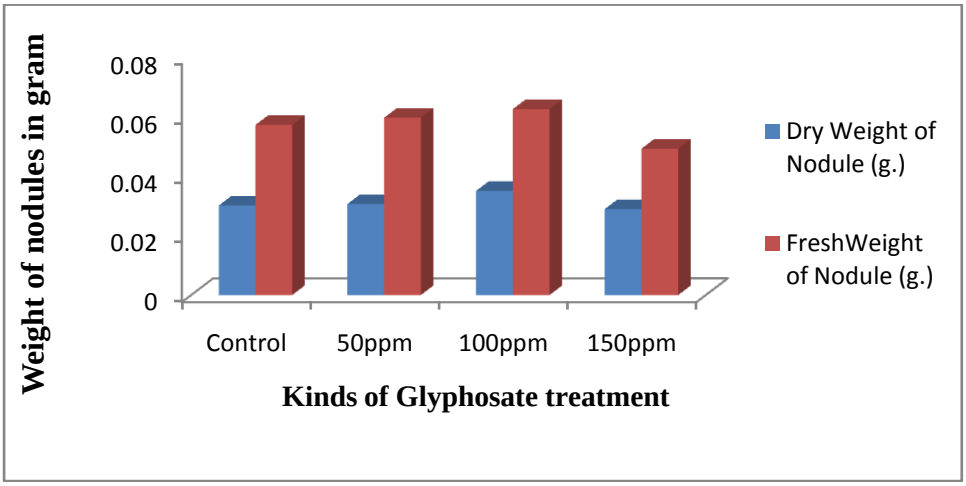

Figure-3. Effect of glyphosate on the Nodule fresh and dry weight of of *Vigna mungo* (L.) after 30 days of sowing

Plant Growth

The plant growth is measured by the length of its root and shoots. Maximum root and shoot length were measured at 100 ppm concentration as compare all other treatments (50 and 150ppm) and control.Application of glyphosate above the 100 ppm level caused a decrease in the plant length (Fig-4). Similarly, Shaban *et al.*, (1987) reported glyphosate was decreasing the plant height of faba bean. They suggested that glyphosate may increase the level of ethylene. Others (Stenley *et al.*, 1973) reported that ethylene inhibits cell division of meristematic tissues and noticed that plants exposed to ethylene-induced inhibition of stem height, so as a result, plant height got decreased when treated with glyphosate.

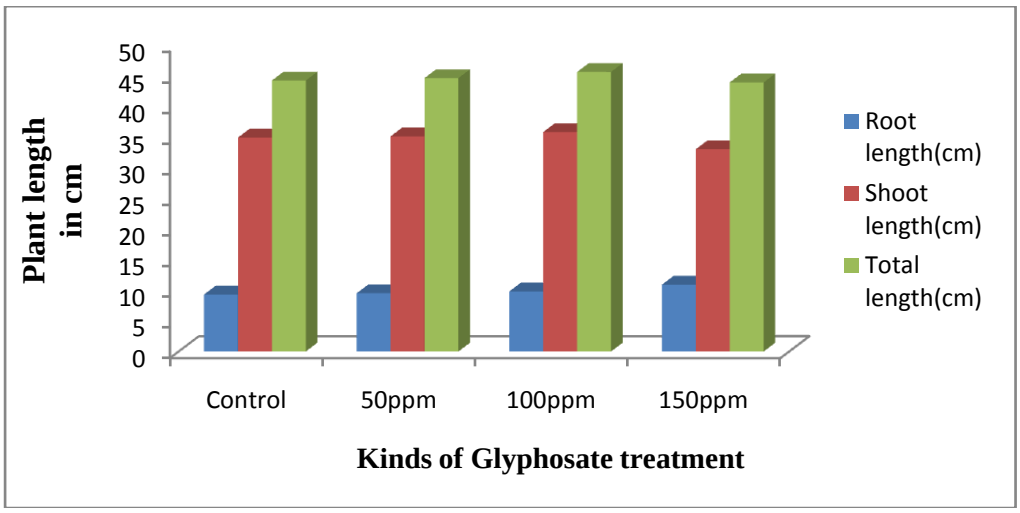

Figure-4. Effect of glyphosate on the growth of *Vigna mungo* (L.) after 30 days of sowing

The fresh and dry weight of root and shoot

Maximum fresh and dry weight of shoot and root of plant. were observed in 50ppm and 100ppm treatment as compared to control and 150ppm solution treatments. Wyszkowska (2002) also referred Treflan and Glyphosate as decreasing the fresh weight of bean plants compared with control and wheat plants compared with control respectively. These results are in agreement with reports which determined that herbicide application to the soil adversely affect physiological characteristics in crop plants. Treflan and Glyphosate caused a significant reduction in shoot dry weight in both bean and wheat plants. Treflan and Glyphosate application to wheat plants caused marked decrease in root fresh weight While, Ridomil increased root fresh weight (Vaughn, and Lehnen. 1991). Fresh and dry weights adversely affected as the duration and concentration of Glyphosate increased.

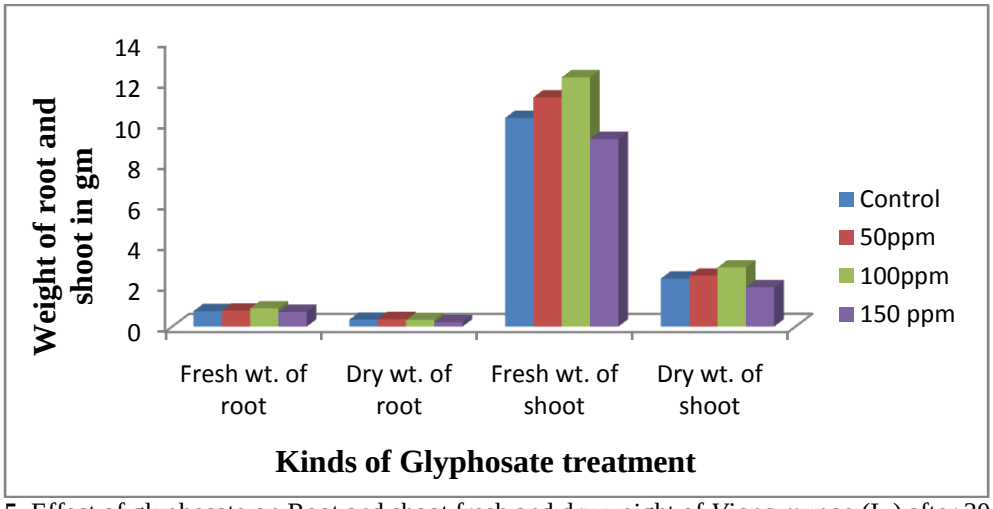

Figure-5. Effect of glyphosate on Root and shoot fresh and dry weight of *Vigna mungo* (L.) after 30 days of sowing

Chlorophyll

Impact of glyphosate was found to increase the level of chlorophyll in first and second treatment and decrease in third treatment as compared to control(Fig-6). Huang *et al.* (2012) also observed the similar results when they applied the different

concentration of glyphosate. Their result showed that Chl a & chl b content were remarkably decreased by increasing the concentration and treatment time of glyphosate. The glyphosate metabolite AMPA (Aminomethylphosphonic acid) can temporarily reduce chlorophyll content (causing yellowing or chlorosis) and photosynthesis in GR(Growth resistant) soybeans. Glyphosate may prevent chlorophyll synthesis indirectly by decreasing the Mg content in leaves, as shown by Cakmak *et al.* (2009), which leads to a decreased chlorophyll content and photosynthetic rate (Zobiole *et al.*, 2012). Indeed, the incorporation of Mg by Mg-chelatase in the porphyrin structure is a necessary step leading to the synthesis of chlorophyll molecules.

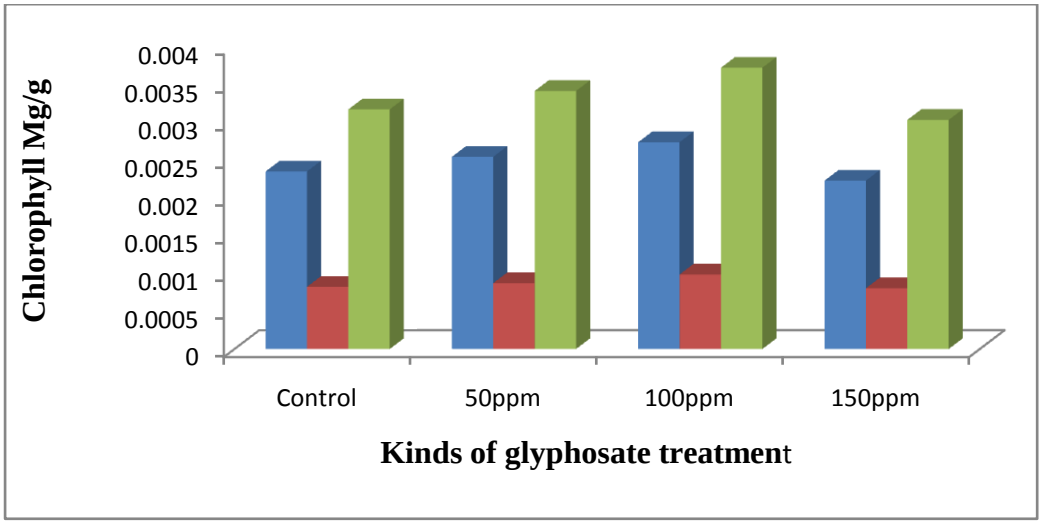

Figure-6. Effect of glyphosate on Chlorophyll content of *Vigna mungo* (L.) after 30 days of Sowing

Nitrogen content

It was noticed that application of glyphosate in limited amount increases the nitrogen content but when concentration increased nitrogen content decreased gradually (Fig-7). Glyphosate has also been observed to induce a similar reduction at higher concentration in Nitrogenase activity & nodulation of soybeans while not affecting legume growth or total plant nitrogen content with low amount of glyphosate applied(Eberbach,1998).Increase the concentration of Glyphosate may induce nutritional disturbances by immobilizing certain nutrients in plants or interfering with their uptake and translocation (Cakmak *et al.* 2009). Glyphosate can also influence N metabolism through direct effects on the rhizobial symbiont or indirectly by affecting the physiology of the host plant. Zaidi *et al.*(2005) observed that nitrogen contents declined with increasing dose rates for Glyphosate and other herbicides individually at both 35 and 60 DAS.

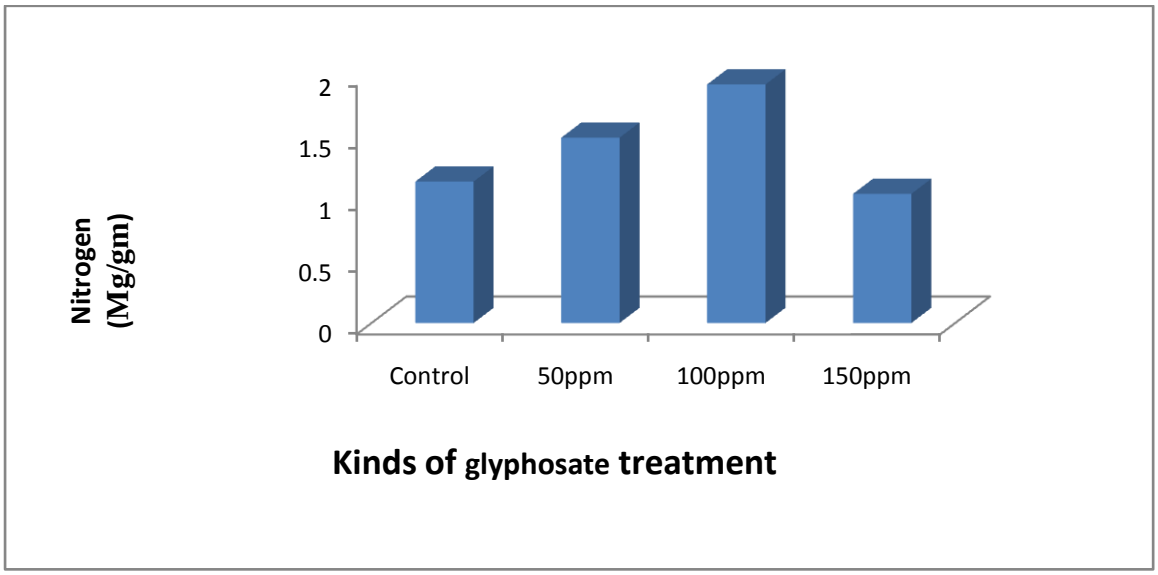

Figure-7. Effect of glyphosate on Nitrogen content of *Vigna mungo* (L.) after 30 days of sowing

Protein content

In this experiment, it was observed that addition of the low amount of glyphosate increases the protein content as compared to control and treatment III but as the concentration of glyphosate increased the protein content of plants significantly reduced (Fig-8). Eberbach(1998) observed that results obtained with plants that resist the phytotoxic effect of herbicides suggest that the resistance observed in the plants may be due to the ability of cells to rapidly detoxify the herbicide at a certain level. After a critical level, the detoxifying capacity of microorganism lost and reduction in growth occur. Reduction in protein content may be due to the higher amount of glyphosate and its direct involvement in inhibition of amino acid synthesis pathway. Glyphosate inhibits an enzyme pathway, the shikimic acid pathway, preventing plants from synthesizing three aromatic amino acids (Phenylalanine, Tryptophan, and Tyrosine). These amino acids are essential for growth and survival of most plants. EPSP (5-enolpyruvylshikimate3phosphate) is the key enzyme inhibited by glyphosate synthase (Cox, 2008). In addition, glyphosate has been proposed to interfere with ALA biosynthesis by controlling the conversion of alpha-ketoglutarate to ALA (ð-aminolevulinic acid) or the condensation of glycine with succinyl-CoA to form ALA and CO2 (Kitchen, 1980). Zaidi et al (2005) also observed the similar result that grain protein is significantly reduced with increased in the concentration of glyphosate.

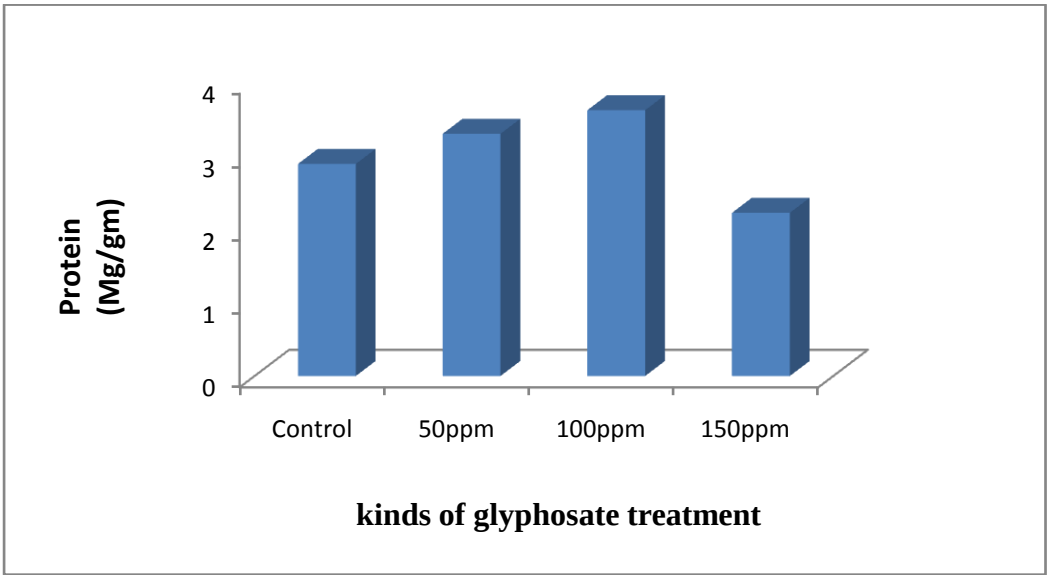

Figure-8. Effect of glyphosate on Protein content of *Vigna mungo* (L.) after 30 days of sowing

Leghaemoglobin content

Impact of glyphosate was found to increases the level of leghaemoglobin in first and second treatment and decreases in third treatment as compare to control (Fig-9).Glyphosate application rapidly stimulates soil microbial activity as measured by C&N mineralization by Haney et al(2000).Glyphosate that reaches the soil surface should be quickly degraded by soil microorganisms without adversely affecting them but this activity is restricted to a limited concentration of this herbicide and high concentration adversely affected the microorganism activity.

Aside from plants, microorganisms also possess EPSPS enzymes and are therefore susceptible to glyphosate (Fischer *et al.*, 1986) at higher concentration. For example, the soybean N-fixing symbiont *Bradyrhizobium japonicum* possesses a GS EPSPS (Glyphosate sensitive 5-enolpyruvylshikimate3phosphate synthase) and accumulates shikimate and hydroxybenzoic acids, such as protocatechuic and gallic acids, upon exposure to glyphosate. This leads to growth inhibition and induces death at high glyphosate concentrations (de María *et al.*, 2006).So glyphosate might impair with nitrogen fixation efficiency of symbiotic bacteria and their symbiosis with host plant which can disturb the formation of leghaemoglobin but further studies are required to explore the impact of Glyphosate on biological nitrogen fixation and leghaemoglobin content of plants.

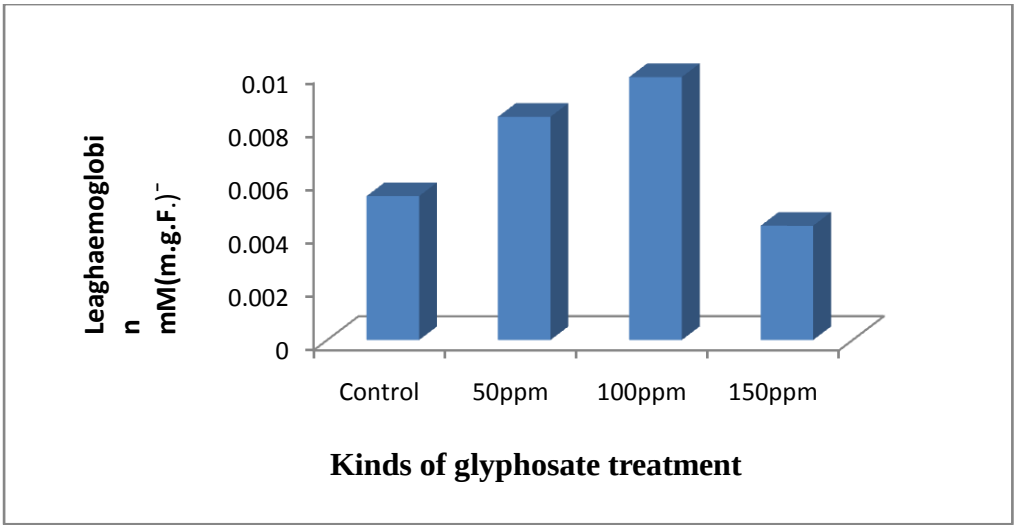

Figure-9. Effect of glyphosate on Leghaemoglobin content of *Vigna mungo* (L.) after 30 days of sowing

CONCLUSION

It can be concluded from the present study that application of glyphosate to certain limit increases the availability of many chemicals/nutrients in soil by killing certain weeds but when the concentration of Glyphosate is increased it adversely affects *Rhizobium* population and interrupt the legume-*rhizobium* symbiosis and biological nitrogen fixation efficiency of legumes. However, Glyphosate can be used to remove the weeds at low concentration but further study is required to explore the actual effect of glyphosate on plant metabolism and growth. Glyphosate can be replaced by using eco-friendly bioherbicides to overcome its adverse effect on soil microflora and plants growth.

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