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GLYPHOSATE HERBICIDE CAUSES HORMESIS IN WHEAT

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ABSTRACT

The hormetic response of the most frequently used herbicide, glyphosate can be used to enhance crop growth and yield. A pot study was conducted twice to assess the effect of glyphosate on wheat at different doses (0, 1.8, 3.6, 7.2, 18, 36, 72, 180, 360 and 720 g a.e. ha^{-1}) sprayed at its 3-4 leaf stage. Observations recorded three weeks after spray and maturity to determine whether growth enhancement was sustained over time. Results revealed that glyphosate application enhanced wheat growth at low doses (up to 36 g a.e. ha^{-1}) which was sustained over time; conversely, higher doses caused phytotoxicity. Three weeks after spray, the highest plant height and shoot dry weight of wheat was observed at glyphosate dose of 18 g a.e. ha^{-1} when compared with the control. While, maximum number of tillers was observed at 36 and 180 g a.e. ha^{-1}. At maturity, a similar trend was observed on plant height, shoot dry weight and number of tillers. Glyphosate at the rate of 3.6 g a.e. ha^{-1} produced higher number of grains; while highest spike length, 1000-grain weight and grain weight plant^{-1} was observed with 18 g a.e. ha^{-1}. It was concluded that application of ultralow doses of glyphosate up to 36 g a.e. ha^{-1} to wheat at 3-4 leaf stage can stimulate growth.

Key words: Growth, herbicide hormesis, phytotoxicity**,** *Triticum aestivum*, yield.

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INTRODUCTION

The world population is increasing day by day which is projected to increase from 6 billion at present to approximately 9 billion in 2050 (Martindale and Trewavas, 2008; Murchie *et al.,* 2009). This increase in population is creating the gap between required food for enhanced population and availability of the cultivated land, thus demanding the research to facilitate new technologies for enhancing crop production per capita (Cedergreen *et al.,* 2009). The growth promoting potential of herbicides is known as herbicide hormesis may change the use of herbicides from crop protection to crop enhancement. Many scientists claim that herbicide hormesis is rule, it is not excepted behavior, and thus, it can provide evolutionary based response for crop enhancement (Stebbing, 1998; Calabrese and Baldwin, 2001; Abbas *et al.,* 2017). Literature showed that many herbicides can promote plant growth and yield when applied at lower doses (Schabenberger *et al.,* 1999; Davies *et al.,* 2003; Duke *et al.,* 2006; Cedergreen *et al.,* 2007; Velini *et al.,* 2008; Abbas *et al.,* 2015; Abbas *et al.,* 2016). Cedergreen *et al.* (2007) reported that different herbicides cause growth stimulation, but the magnitude of stimulation in growth is dependent on the dose of herbicide. Previous studies revealed that herbicide hormesis cause growth stimulation up to 30% in a controlled condition and up to 25% under field conditions (Cedergreen *et al.,* 2005).

Stimulatory responses of plants at a low dose of herbicides is very complex in nature, depending on several variables including type of crop, light, nutrients, temperature, CO_2, time of application and herbicide dose (Belz *et al.,* 2008; Cedergreen, 2008a; Belz and Cedergreen, 2010; Cedergreen and Olesen 2010). Therefore, growth promotion due to herbicides under non-controlled conditions is often unpredictable (Belz *et al.,* 2011; Belz and Leberle, 2012).

Glyphosate is one of the most used herbicides in the world which can consistently induce hormesis in different crops (Wagner *et al.,* 2003; Duke *et al.,* 2006; Abbas *et al.,* 2015). It acts by blocking the production of the aromatic amino acids and other secondary metabolites derivative to these aromatic amino acids, by stopping the synthesis of enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Duke, 1988). All non-transgenic crops are sensitive to glyphosate due to inhibition of EPSPS which causes accumulation of shikimate in plants tissues. Hormesis is commonly observed with glyphosate at low doses (Cedergreen, 2008a and b; Velini *et al.,* 2008, Abbas *et al.,* 2015, Nadeem *et al.,* 2016).

Several studies about herbicide hormesis have reported the hormetic effect of herbicides on early seedling growth of target plants. But, a few studies are available that show the hormetic effects of glyphosate sprayed at terminal growth stages of crop and grain yield (Cedergreen *et al.,* 2009). However, the literature reports about the effect of glyphosate application at early growth stages of the wheat on wheat growth over time and grain yield is not available. Therefore, this study was conducted to detect whether its stimulatory effect is sustained over time until wheat maturity.

MATERIALS AND METHODS

An experiment was conducted twice in a greenhouse at the Agronomic Research Area, University of Agriculture, Faisalabad, Pakistan, during 2014-2015. The experimental site was located about 31.25^o N latitude, 73.09^o E longitude, and altitude of 184 m. The mean minimum and maximum temperatures in the greenhouse during the experiment were 24^{o}C and 30^{o}C, respectively. The relative humidity ranged from 23-60%. The soil was collected from research fields near the Department of Agronomy, University of Agriculture, Faisalabad. Each pot was filled with 15 kg of soil. The soil was sandy loam with 1.10% organic matter content and pH of 8.0.

Glyphosate at ten different doses (0, 1.8, 3.6, 7.2, 18, 36, 72, 180, 360 and 720 g a.e. ha^{-1}) was sprayed three weeks after wheat emergence. Roundup ® (a product of Monsanto Pakistan AgriTech Pvt. Ltd.) was used as a source of glyphosate. The glyphosate was sprayed with a Knapsack hand sprayer using T-Jet nozzle at 30 psi pressure. The sprayer was calibrated, and the amount of water was calculated for one square matter. Pots receiving same treatment were placed in one square meter and spray was carried out. Completely randomized design having four replications was used and reshuffled each week to achieve uniform growth conditions for all plants. Ten seeds of wheat variety Lasani-2008 were sown in separate plastic pots (25 cm diameter and 45 cm depth). Three weeks after spraying five plants from each pot were removed to collect data for growth parameters. Remaining plants (five plants in each pot) were kept growing till the maturity. At start 1.2 liters of water was applied to each pot and then throughout the experimental period the pots were kept moistened. Data were recorded at three weeks after spraying and at grain harvest. Spike length, number of grains per spike, 1000-grain weight and grain weight was recorded at maturity.

Repeated experiments gave statistically similar results, so data were pooled. Pooled data were transformed before statistical analysis using square root transformation to normalize the data. Fisher's analysis of variance technique was used to analyze the transformed data, and comparison of treatments means was prepared by least significant difference test at 5% probability level (Steel *et al.,* 1997).

RESULTS AND DISCUSSION

Response of wheat to glyphosate

Results of growth traits three weeks after glyphosate application revealed that low doses of glyphosate imposed growth promotive effect on wheat (Table-1). The positive effect was dose dependent. Positive growth response of wheat plants was observed by increasing the dose of glyphosate up to 36 g a.e. ha^{-1}. However, further increase in dose suppressed the wheat growth (Fig. 1). The highest plant height of 31 cm was observed when glyphosate was applied at the rate of 18 g a.e. ha^{-1}. At a dose of 180 g a.e. ha^{-1}, glyphosate caused a significant reduction in plant height as compared to control (Fig. 1a). Tiller producing ability of wheat was also influenced by glyphosate application, but this trend was different, with maximum tillers $plant^{-1}$ (4) produced at 36 as well as 180 g a.e. ha^{-1} (Fig. 1b). Lower doses promoted shoot dry weight up to 72 g a.e. ha^{-1}, and further increase in dose caused a reduction in shoot dry biomass. Plants sprayed with glyphosate doses of 1.8 and 18 g a.e. ha^{-1} caused more increase in shoot dry weight (Fig. 1c). The increase in plant growth of wheat was observed three weeks after glyphosate application at 1.8 to 72 g a.e. ha^{-1} (Fig. 1). Increase in growth was similar to those of previous reports in which the stimulating effect of glyphosate against different crops was observed at low doses (Duke *et al.,* 2006; Velini *et al.,* 2008). Glyphosate interferes with auxin production and activity, which may alter growth behavior of wheat plants (Crozier *et al.,* 2000). Further studies are needed to understand the mechanism of the growth promoting effect of glyphosate. Few studies indirectly revealed that glyphosate hormetic responses are related to target site of herbicide action as the hormetic effect was just observed in glyphosate sensitive plants, but transgenic plants are resistant to glyphosate where active site of plants was blocked for glyphosate thus no hormetic responses were observed (Velini *et al.,* 2008). Cedergreen *et al.* (2010) reported that glyphosate application at low concentration promoted the growth of target plants by increasing the rate of photosynthesis. The increase in photosynthetic activity causes promoting effect on plant height, number of tillers and plant biomass. Similar to our results, previous studies have shown that glyphosate at low doses promotes crop growth but growth stimulation was inconsistent within different growth attributes (Cedergreen, 2008a). In most of the growth parameters, maximum stimulation was observed at glyphosate dose ranging from 18 to 36 g a.e. per ha. But in number of tillers per plant, the trend was slightly different. More number of tillers was detected at comparatively higher doses of glyphosate (Fig. 1b). Change in trend about number of tiller may be possibly due to inhibition of auxin which caused the increased production of tillers (Crozier *et al.,* 2000).

Response of wheat to glyphosate at maturity

Results regarding growth at crop maturity revealed that growth promotive effect of glyphosate was sustained over time. Different growth traits such as plant height, number of tillers and dry biomass were significantly affected at crop maturity (Table-2). Plant height was increased gradually by increasing glyphosate doses up to 72 g a.e. ha^{-1}. The tallest plants (63 cm) were detected at glyphosate dose of 18 g a.e. per ha. Further increase in glyphosate dose reduced plant height and minimum plant height (53 cm) was recorded at 180 g a.e. ha^{-1} as compared to control (Fig. 2a). Number of tillers observed at crop maturity was improved with increasing dose of glyphosate. The more number of tillers was produced at 180 g a.e. ha^{-1} (Fig. 2b). Results about dry biomass revealed that glyphosate at 1.8 to 72 g a.e. ha^{-1} increased biomass production of wheat. Further increase of glyphosate dose reduced the biomass production significantly over control. Highest biomass was observed at 7.2 as well as 18 g a.e. ha^{-1} (Fig. 2c). The pattern of growth caused by glyphosate in wheat at maturity was similar to growth effects at an earlier growth stage, showing that the hormetic effects were sustained over time (Fig. 2). This increase in growth may be due to increase in growth at the early vegetative stage which was observed by glyphosate application. Glyphosate hormesis boost early growth which kept treated plants taller than untreated till the maturity. In our study, early growth stimulation was sustained over time. It may also possible that early exposure of wheat plant to glyphosate induced defense mechanism in these plants that made the sprayed plants more tolerant to other stresses. As Calabrese *et al.* (2007) reported that initial exposure of plant to one stress makes it tolerant to other stresses.

Effect of glyphosate on wheat yield and yield attributes

Yield and different yield contributing parameters were significantly influenced by different doses of glyphosate (Table 3). Spike length of wheat was increased at low doses, and maximum spike length (11 cm) was detected at 18 g a.e. ha^{-1} followed by 36 g a.e. ha^{-1} (Fig. 3a). Number of grains per spike was also influenced by glyphosate spray. Plants sprayed with glyphosate dose of 3.6 g a.e. per ha produced maximum number of grains per spike (39) followed by 18 g a.e. ha^{-1} (Fig. 3b). Highest 1000-grain weight (39 g) of wheat was observed by glyphosate application at the rate of 18 g a.e. ha^{-1} (Fig. 3c). Grain yield of wheat was significantly increased with glyphosate application at 18 g a.e. ha^{-1}. Maximum grain weight (30% more than control) was achieved at 18 g a.e. ha^{-1}. Conversely, glyphosate doses above 72 g a.e. ha^{-1} significantly reduced grain yield

(Fig. 3d). Wheat yield and yield contributing parameters were boosted by low doses of glyphosate applied at early vegetative stage of wheat (Fig. 3). Up to 30% increase in grain weight of wheat was noticed with glyphosate dose of 18 g a.e. ha^{-1}. The increase in yield might be because of enhanced growth and development of wheat plants treated with low doses of glyphosate. As previous studies showed that glyphosate hormesis increases the photosynthetic rate of plants (Cedergreen *et al.,* 2010) which produce more photosynthates ultimately resulting in more yield. Abbas *et al.* (2015) reported that plants sprayed with low doses of glyphosate produced more grain yield as compared to control. Our results regarding number of grains per spike and 1000 grain weight in low dose glyphosate-treated plants were different from Cedergreen *et al.* (2008) who reported that glyphosate hormesis had no influence on number of grains and individual grain weight in barley. It might be due to the differential response of wheat than barley to glyphosate as literature showed that even glyphosate is non-selective herbicide but sensitivity to glyphosate among different crop species is different (Cerdeira and Duke, 2006; Gove *et al.,* 2007). Cedergreen *et al.* (2009) reported that glyphosate at low doses ranging from 2.5 to 20 g a.e. ha^{-1} increased barley grain yield up to 15%. Earlier studies have revealed that glyphosate induced hormesis in different crop species and increase in their growth and yield (Duke *et al.,* 2006; Cedergreen *et al.,* 2008). However, the unpredictability of stimulatory responses is a question for its practical implementation in the field to improve growth and yield. Cedergreen (2008a) found no increase in yield of barley (Hordeum vulgare). Belz *et al.* (2011) indicated that herbicides are not used for yield enhancement because of the unpredictability of results in the field. But according to Belz and Duke (2014) hormesis related practical constraints can be managed even under field conditions if we wisely deal with them and herbicide hormesis can be used efficiently. However, studies about the influence of glyphosate hormesis on the growth of associated weeds of wheat are needed. For example, Nadeem *et al.* (2016) reported that glyphosate hormesis might cause a significant increase in growth of broad-leaved weeds including *Coronopus didymus* L.*, Chenopodium album* L.*, Rumex dentatus* L. and *Lathyrus aphaca* L. of wheat. Foliar-applied glyphosate in the range 4 –32 g a.e. per ha increased growth and seed production ability of all tested weeds. The stimulatory effect of low doses of fenoxaprop-P-ethyl on growth and reproductive potential of narrow leaved weeds of wheat including littleseed canarygrass and wild oat have been observed (Abbas *et al.,* 2016). Therefore for particle use of glyphosate to enhance wheat production, effect of glyphosate on associated weeds of wheat should also be considered. Nadeem *et al.* (2016)

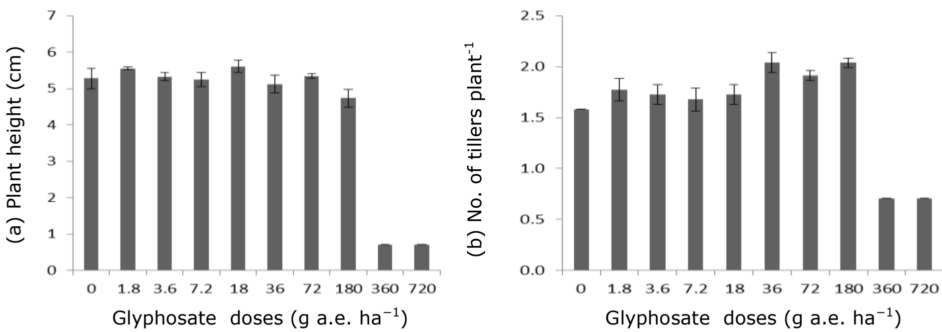
reported that glyphosate at low doses might increase the growth and reproductive potential of various broad-leaved weeds of wheat.

CONCLUSION

Hormetic effects of glyphosate, sprayed on 3-week-old wheat plants in the greenhouse were maintained until maturity, including enhancement of grain yield. The most effective glyphosate rate was 18 g a.e. per ha. Further studies are needed to determine the practicality of glyphosate as a wheat yield enhancer in the field.

Table-1. Mean square values from analysis of variance for the response of wheat growth to glyphosate three weeks after spray.

Source of variation	Plant height	No. of tillers	Shoot dry weight
Treatment	11.2910*	0.71572*	0.52171*
Error	0.0632	0.01293	0.00287



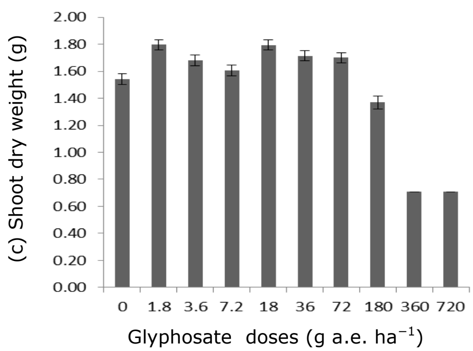
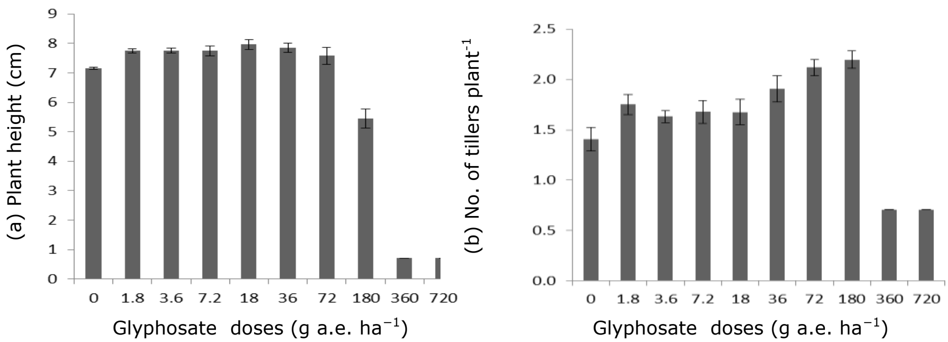


Figure 1(a-c). Hormetic effect of glyphosate on wheat growth three weeks after spraying. Square root transformation was used to make data normal. Bars indicate standard error of mean. Means are significantly different from the zero glyphosate treatment plants at the 95% confidence level.

Table-2. Mean square values from analysis of variance for the growth response of wheat to glyphosate at maturity

Source of variation	Plant height	No. of tillers	Shoot dry weight
Treatment	25.4932*	0.79122*	0.70415*
Error	0.0645	0.01964	0.01061



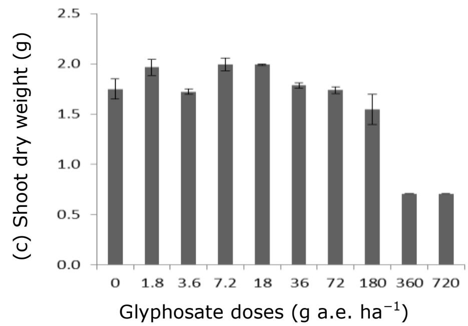


Figure 2 (a-c). Hormetic effect of glyphosate on wheat growth at maturity. Square root transformation is used to make data normal. Bars indicate standard error of mean. Means are significantly different from the zero glyphosate treatment plants at the 95% confidence level.

Table-3. Mean square values from analysis of variance for the hormetic effect of glyphosate on yield and yield attributes of wheat

Source of variation	Spike length	1000 grain weight	No. of grains/spike	Grain yield
Treatment	2.98039*	13.9948*	14.4820*	0.23331*
Error	0.01177	0.1103	0.4071	0.01048

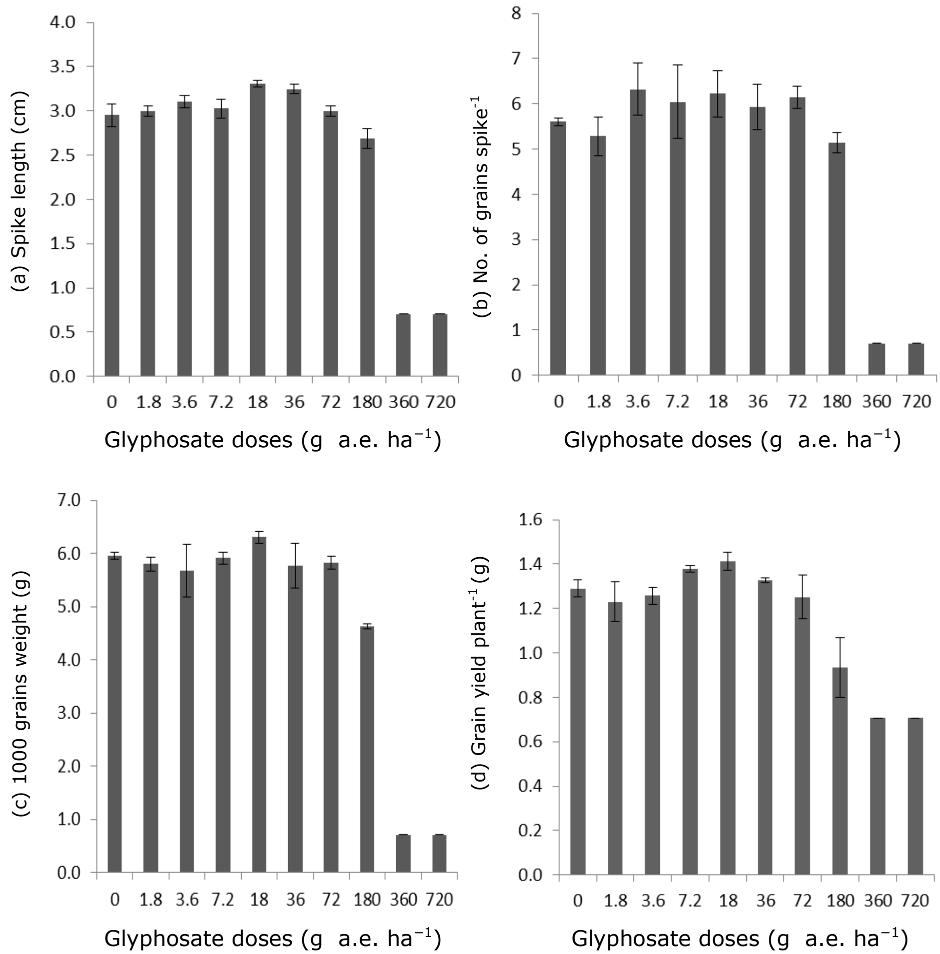


Figure 3. Hormetic effect of glyphosate on yield and yield attributes of wheat. Square root transformation is used to make data normal. Bars indicate standard error of mean. Means are significantly different from the zero glyphosate treatment plants at the 95% confidence level

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