

LOW DOSES OF ATRAZINE CAUSE HORMESIS IN *TRIBULUS TERRESTRIS*

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Abstract

Growth stimulation due to ultra-low doses of different herbicides in several crop species has been observed both in controlled as well as in the field conditions; however available data of hermetic effects on weeds growth is very limited. This study investigates the promotive effect of low doses of atrazine on growth of Tribulus terrestris. Pot experiments were conducted at Agronomic Research Area, collage of Agriculture, University of Sargodha, during 2019. Six different concentrations of atrazine [0, 5, 10, 20, 40 and 80 g a.i. ha⁻¹] were applied as post emergence herbicides at 3- 4 leaves stage of the weeds under two different water regimes (60 % and 100 % water concentrations). Results revealed that increase in plant chlorophyll content, fresh weight, dry weight, root length and shoot length were observed after spraying of atrazine @ 5, 10 and 20 g a.i. ha⁻¹ at 100 % water concentration. Doses of atrazine above 20 g a.i. ha⁻¹ negatively affect the growth of Tribulus terrestris.

Keyword: Atrazine, dry weight, fresh weight, root length and shoot length

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INTRODUCTION

The stimulatory effect of sub-toxic doses of herbicides on crops has been studied as a possible means of increasing crop production. The general phenomenon of low doses of toxicants causing stimulatory responses is termed hormesis (Calabrese 2005). Hormetic responses of different toxicants have been reported in all groups of organisms, including higher plants (Calabrese 2005). To confirm the hormetic effects of herbicides, a study including 687 dose-response curves was conducted using 10 different herbicides and three plant species, and revealed that herbicides at low doses produced hormetic responses in 25–76% of the dose-response curves depending upon the plant species (Cedergreen et al. 2007). Herbicide hormesis can stimulate plant growth by up to 100%, but, on average, 20–30% growth stimulation occurred at herbicide doses below 10% of the recommended field rate (Cedergreen et al. 2005). Consistent hormetic responses of different herbicides in wheat, rye, pea, soybean, barnyard grass, chickpea, maize, and barley have been reported by different researchers (Wagner et al. 2003; Duke et al. 2006; Velini et al. 2008; Cedergreen 2008a; Abbas et al. 2015).

Like other triazine herbicides, atrazine functions by binding to the plastoquinone-binding protein in photosystem II, which animal's lack. Plant death results from starvation and oxidative damage caused by breakdown in the electron transport process. Oxidative damage is accelerated at high light intensity. Research addressing to the effects of herbicide hormesis on weed growth and weed crop competition are currently absent. Most of the published research is about the hormetic responses of crops against herbicides (Davies et al., 2003; Duke et al., 2006; Cedergreen et al., 2007; Velini et al., 2010; Belz et al., 2011). However, for practical implementation of herbicide hormesis to improve crop growth and yield, the effects of these lower doses on weed growth should also be considered. Research on responses of weeds to

hormetic doses of herbicides may also help us to understand the evolution of the mechanism of resistance, because for resistant biotypes of weeds the recommended field doses of some herbicides may also act as hormetic doses and can produce hormetic responses (Petersen et al., 2008). Therefore, this study was conducted to evaluate the promotive effect of different lower doses of atrazine on the growth of *Tribulus terrestris* L.

MATERIALS AND METHODS

Site and Soil

The research work was conducted in pots having 7 Kg soil to estimate the impact of ultra-low doses of Atrazine on *Tribulus terrestris* L under two water regimes at agronomy research area. College of Agriculture, University of Sargodha, Pakistan during summer season, 2019.

Soil Analysis

Before conducting of experiment and harvesting of crop soil analysis was done. By using soil auger soil sample was collected at the depth of 15 and 20 cm and additional information concerning to experimental area soil are displayed in table # 1.

Experimental design

An experiment was carried out in pots to check the effect of ultra-low dose of atrazine on *Tribulus terrestris* L under two irrigation regimes during summer season 2019. The experiment was laid out in pots under completely randomized design (CRD) with two factors; each factor replicated four times. Each pot having 7 kg of soil. The experiment was comprised of following treatments.

Treatments

Factor A: water regimes

Normal irrigation (100 % F. C)

Drought (60 % F.C)

Factor B: Weedicide doses

T₁ = 0.0 dose a.e. ha

$T_2 = 5.0 \text{ g a.e. ha}^{-1}$

$T_3 = 10.0 \text{ g a.e. ha}^{-1}$

$T_4 = 20.0 \text{ g a.e. ha}^{-1}$

$T_5 = 40.0 \text{ g a.e. ha}^{-1}$

$T_6 = 80.0 \text{ g a.e. ha}^{-1}$

Table # 1 Physio-chemical analysis of soil from experimental area

Characteristics	Soil sample
Soil Ph.	7.8
E.C _e (mS cm ⁻¹)	2.18
Organic matter (%)	0.97
N (%)	0.41
Available P (ppm)	8.4
Available K (ppm)	180
Saturation (%)	40
Texture	Loam

Procedure for maintaining water limited condition

Water limiting condition was maintained by calculating field capacity. Calculations were made by filling 7 kg air dry soil in each uniform size pot. Hole below the pot was blocked by placing the paper towel at the base prior to filling with soil. Sample of air dry soil was kept in oven at 105 °C for oven drying till constant weight. Soil in pot should be fully saturated with water and kept overnight till drainage of excess water from pot. Sample of soil will be weighed and kept in oven to get dry weight of soil. Weight of water in pot was determined by subtracting oven dry weight of soil from total weight of wet soil after drainage of excess water. Water which was present at the time of filling of soil in pot was added in it.

Quantity of water needed to maintain 100% F.C was determined by using following formula:

Water required to maintain 100 % F.C = (weight of water/ weight) × 100

Quantity of water need to maintain 60 % F.C was determined as follows

Water required to maintain 60 % F.C = (60/100) × water required to maintained 100 % F.C.

Target weight of each pot for each field capacity level will be maintained throughout the conduct of experiment.

Crop husbandry:

Tribulus terrestris L was sown on 06-09-2019. Before sowing the soil was ploughed manually by hand tools to loose the soil and prepared a good fine seed bed. Ten seeds/pot were planted manually to obtain maximum germination. Thinning was done ten days after germination of seeds leaving four plants/pot. Spray the herbicides on crop at 3-4 leaf stage after germination of crop. The plants were harvested from each pot 21st days after spray.

Ultra-low dose of weedicides

Ultra-low dose of all herbicides were applied with a backpack atomizer equipped with a flat- fan (tee jet 8002 Nozzle, spraying system company, wheaton, 1L) at four weeks after the emergence of *Tribulus terrestris* L. Calibration was done to deliver 250 L per

hectare at walking speed of 3.3 km per hectare.

Procedure for recording data

The data were recorded by using following standard procedure from each treatment.

Fresh weight (g)

Plant fresh weight of plants was examined at 21st day after spray from each treatment in each replication. An electric balance was used to measure fresh weight of plant.

Dry weight (g)

For dry weight four plants that were already uprooted for fresh weight placed at 60 °C for period of 48 hours in an oven. The dry weight of these plants was measured with the help of an electric balance and then take average.

Chlorophyll contents (mg)

Chlorophyll content of four plant from each pot was measured 21st days after spray by using photo meter then take average.

Shoot length (cm)

Shoot length of plant was observed 21 days after spray from ground level to shoot tip with the help of meter rod and then take average.

Root length (g)

Plants root length was recorded 21 days after spray with meter from start of root to root tip then take average.

Statistical analysis

The data recorded was analyzed statistically by using the MSTAT C for analysis of variance and significance of treatment mean was tested using Tukey's honestly significance difference (HSD) test at 5% probability level (steel *et al.*, 1997).

Results

Table 2.1: Effect of ultra-low dose of atrazine on chlorophyll content% (g) of *Tribulus terrestris* L under two irrigation regimes

Herbicide dose (g a.e. ha ⁻¹)	Plant chlorophyll content (%)		
	Normal irrigation	Drought	Mean
0.0 (Control)	33.26	32.73	32.99ab
5.0	32.15	32.74	32.45ab
10	34.58	33.82	34.20a
20	34.13	34.22	34.17a
40	29.63	28.88	29.25b
80	16.16	14.11	15.14c
Mean	29.98	29.42	

LSD: Herbicide= 4, Irrigation regimes =6.93, Herbicide× Irrigation regimes = 2.83

Mean sharing the same letter did not different with each other at 5% probability level

Table 2.2: Effect of ultra-low dose of atrazine on fresh weight (g) of *Tribulus terrestris* L under two irrigation regimes

Herbicide dose (g a.e. ha ⁻¹)	Plant fresh weight (g)		
	Normal irrigation	Drought	Mean
0.0 (Control)	8.59a	6.69c	7.64a
5.0	8.68a	6.87c	7.77a
10	8.84a	7.08c	7.96a
20	8.57a	6.93c	7.75a
40	7.78b	5.36e	6.57b
80	5.93d	5.48de	5.70c
Mean	8.06a	6.40b	

LSD: Herbicide= 0.72 , Irrigation regimes = 1.26 , Herbicide× Irrigation regimes =0.51
 Mean sharing the same letter did not different with each other at 5% probability level

Table 2.3: Effect of ultra-low dose of atrazine on dry weight (g) of *Tribulus terrestris* L under two irrigation regimes

Herbicide dose (g a.e. ha ⁻¹)	Plant dry weight (g)		
	Normal irrigation	Drought	Mean
0.0 (Control)	1.40	1.10	1.25a
5.0	1.45	1.14	1.29a
10	1.47	1.18	1.33a
20	1.43	1.14	1.28a
40	1.30	0.89	1.09b
80	1.09	0.83	0.96c
Mean	1.36a	1.05b	

LSD: Herbicide=0.12 , Irrigation regimes =0.22 , Herbicide× Irrigation regimes =0.09

Mean sharing the same letter did not different with each other at 5% probability level

Table 2.4: Effect of ultra-low dose of atrazine on root length (cm) of *Tribulus terrestris* L under two irrigation regimes

Herbicide dose (g a.e. ha ⁻¹)	Root length (cm)		
	Normal irrigation	Drought	Mean
0.0 (Control)	9.85	9.60	9.73a
5.0	9.80	9.44	9.62a
10	10.00	9.99	9.99a
20	9.91	9.55	9.73a
40	9.24	8.73	8.98a
80	6.88	7.80	7.34b

Mean	9.28	9.18
LSD: Herbicide= 1.40, Irrigation regimes =2.43, Herbicide× Irrigation regimes = 0.99		
Mean sharing the same letter did not different with each other at 5% probability level		

Table 2.5: Effect of ultra-low dose of atrazine on shoot length (cm) of *Tribulus terrestris* L under two irrigation regimes

Herbicide dose (g a.e. ha ⁻¹)	Plant fresh weight (g)		
	Normal irrigation	Drought	Mean
0.0 (Control)	14.31	13.63	13.97a
5.0	14.46	13.74	14.10a
10	14.73	14.16	14.44a
20	14.29	13.86	14.08a
40	12.96	10.73	11.84b
80	8.28	7.60	7.94c
Mean	13.17	12.29	

LSD: Herbicide= 1.36 , Irrigation regimes = 2.36 , Herbicide× Irrigation regimes =0.96

Mean sharing the same letter did not different with each other at 5% probability level

Results:

Chlorophyll content (%):

Chlorophyll is a natural green pigment found in photosynthetic organisms such as plants and algae. Chlorophyll is vital for photosynthesis, allowing plants to absorb energy from light. Quantity of chlorophyll per unit area is an indicator of photosynthetic capacity of a plant. Higher photosynthetic capacity means higher production rate. Data pertaining to the effect of ultra-low dose of atrazine on chlorophyll content (%) of leaves under two irrigation regimes recorded at 21 days after atrazine application is presented in table 2.1 (a). Data showed that ultra-low dose of atrazine at the rate of 10 g a.e ha⁻¹ produced significantly higher chlorophyll content than the treatments where the atrazine was applied at higher rates i.e., 40 and 80 g a.e. ha⁻¹. However, it is statistically at par with the treatments where the atrazine was applied at the rate of 0, 5 and 20 g a.e ha⁻¹. The lowest chlorophyll content was observed where

the atrazine was applied at the rate of 80 g a.e ha⁻¹.

In case of water regimes, normal irrigation treatment showed more chlorophyll content than drought treatment but these are non-significantly different with each other. The results showed that interaction between herbicidal treatment and irrigation regimes were non-significant.

Fresh weight:

Fresh weight of a crop is vegetative growth and yield determining traits of crops. Studies showed that plant biomass was due to more vegetative growth, number of branches, and number of flowers per plant which leads to higher crop yield. Data presented by table (2.2) showed that fresh weight was significantly affected by different levels of atrazine. Atrazine at 10 g a.e. gave highest fresh weight followed by 5, 20 and 0 g a.e. respectively. The minimum fresh weight was obtained in the treatment where the atrazine was applied at 80 g a.e.

The results revealed that normal irrigation gave significantly higher fresh weight (8.06 g) than drought condition (6.40 g). The interaction between different levels of treatments and irrigation regimes were found to be significant. However, the highest fresh weight was obtained where the atrazine was applied at 10 g a.e. under normal irrigation condition.

Dry weight (g):

Table (2.3) demonstrates that all treatments of atrazine and irrigation regimes had a vital effect on the dry weight (g). Results showed that ultra-low dose of atrazine (10 g a.e.) showed maximum dry weight followed by 5 and 20. The control treatment was also statistically at par with the treatments of atrazine at 5, 10 and 20 g a.e.

Again the normal irrigation showed statistically higher dry weight than drought. However the results remain non-significant for interactions of different atrazine levels and irrigation regimes.

Root length (cm):

Root length was also significantly affected by different levels of atrazine. However, the atrazine at the rate of 10 g a.e. showed maximum root length (9.99) as compared to other levels of atrazine. The minimum root length (7.34 cm) was observed where the atrazine was applied at its highest level i.e., 80 g a.e. Table (2.4) showed that normal irrigation showed higher root length than the drought conditions but the difference was non-significant. The interaction between herbicidal treatments and irrigation regimes was also non-significant.

Shoot length (cm):

Table (2.5) showed that atrazine at its lower levels i.e., 0,5,10 and 20 g a.e. produced significantly long shoots than

higher levels of atrazine (40 and 80 g a.e.). Normal irrigation also produced lengthy shoots (13.17 cm) than drought conditions (12.29 cm) but the difference was non-significant. Data showed that interaction between different levels of atrazine and irrigation regimes was remain non-significant.

Discussion:

Atrazine at the rate of 10 g a.e. produced statistically higher chlorophyll content, fresh weight, dry weight, root length and shoot length than atrazine applied at 40 and 80 g a.e. Treatments 0, 5 and 20 g a.e. were statistically at par with 10 g a.e. applied treatment. Normal irrigation produced statistically more chlorophyll content, fresh weight, dry weight, root length and shoot length than drought conditions.

There are very few researches available in the literature with results of hormesis caused by atrazine or some other herbicide with a hormetic effect on *Tribulus terrestris* L. Tavares et al. (2017) applied sub-doses of 2,4-D on pequi seedlings and showed hormetic effects based on the leaf area, specific leaf area, and leaf area ratio with doses of 3.3 g ha⁻¹, 1.65 g ha⁻¹, and 3.35 g ha⁻¹, respectively. In a study carried out by Silva (2014), the hormetic effect was shown on soybean crops, but with the application of sub-doses of glyphosate. In this case, the application of 3.6 g ha⁻¹ of glyphosate followed by a fresh application of 3.2 g ha⁻¹ after 14 days induced the maximum growth of the plant shoot, probably in response to the increase in the production of amino acids. Also, Silva (2014) verified that, for total dry mass, a dose of 7.2 g ha⁻¹ of glyphosate, applied 14 days after an application of an initial dose of 18.2 g ha⁻¹, provided a more evident hormetic effect.

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