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Hormetic Responses of niger (*Guizotia abyssinica* Cass.) to Imazethapyr: Toward Lower Herbicide Inputs and Sustainable Environmental Management

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Abstract

This field experiment, carried out in 2023, evaluated the effects of imazethapyr dosages)0, 0.375, 0.75, 1.5, 3, 6, 12, 25, 50 and 100 g a.i. ha^{-1}) on the activity of antioxidant enzymes catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX), growth and grain yield of niger plants. The results showed that there was no visible phytotoxicity at dosages of 0.375 to 0.75 g a.i. ha^{-1}. However, plant scorch increased significantly at 1.5 g and above. Some recovery was observed at dosages of 1.5 to 12 g while chlorosis at dosages of 25 to 100 became progressively more severe over time. Overall, imazethapyr application resulted in a reduction of photosynthetic pigments, growth parameters, and antioxidant enzyme activities. While high herbicide concentrations led to decreased grain yield and increased levels of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and proline, the opposite effects were observed at lower concentrations. Chlorophyll was more sensitive than carotenoids. Also, among antioxidant enzymes GPX showed a greater response to dosages of imazethapyr application. The results of this experiment confirmed Hormetic effects of imazethapyr on niger plants, as reactive oxygen species (ROS) production and consequently membrane lipid peroxidation were decreased while grain yield was increased at low herbicide concentrations. Moreover, the activity of CAT, compared to those of APX and GPX showed a stronger correlation with the Hormetic effects of imazethapyr. These findings highlight the potential for reducing imazethapyr concentrations in crop management, thereby lowering environmental contamination risks and contributing to agricultural sustainability.

Introduction

Imazethapyr, a member of the imidazolinone family, is a systemic herbicide that effectively controls a wide range of broadleaf weeds in legume crops [1, 2]. It reduces the synthesis of essential amino acids such as valine, leucine, and isoleucine by inhibiting the enzyme acetolactate synthase, thereby disrupting key metabolic processes and ultimately causing plant death [2, 3]. Beyond its impact on amino acid biosynthesis, imazethapyr can interfere with photosynthesis, induce oxidative stress, and impair reproductive development [4].

Low concentrations of herbicides have been reported to stimulate plant growth, improve yield, and enhance physiological and enzymatic functions, a phenomenon known as hormesis [5, 6]. Hormesis occurs when small amounts of stressors exert stimulatory rather than toxic effects [7, 8]. Such responses may positively affect cellular and molecular mechanisms, including photosynthesis, stomatal conductance, chlorophyll content, signaling pathways, and antioxidant enzyme activities, ultimately influencing crop productivity [9].

Meeting the dietary demands of the world's expanding population can be supported by rediscovering and utilizing underused crops such as niger (*Guizotia abyssinica* Cass.), which offers promising alternative food resources [10]. Niger is an oilseed crop with high adaptability to diverse environments and favorable oil quality, making it a suitable candidate for cultivation. It is predominantly grown in Ethiopia and India, where it contributes substantially to national oilseed production, accounting for

approximately 50% of total oilseed output in Ethiopia and about 3% in India [11]. Its seeds contain around 40% extractable edible oil, with a lipid profile rich in polyunsaturated fatty acids, particularly linoleic acid (C18:2), which comprises 75–80% of total fatty acids [12, 13]. Despite its nutritional potential, information on the physiological and biochemical characteristics of niger remains limited [14].

Although the phytotoxic effects of imazethapyr are well documented, there is still a significant knowledge gap regarding its potential hormetic effects at sublethal concentrations, particularly in under-studied crops such as niger. Imazethapyr is highly water-soluble, weakly adsorbed to soil particles, and exhibits low mammalian toxicity. The soil matrix acts as the primary environmental sink, and the extent of adsorption strongly influences its bioactivity, mobility, and persistence. Its Groundwater Ubiquity Score (GUS) ranges from 2.29 to 2.80, classifying it as a transition pesticide with moderate-to-high leaching potential [15]. This persistence raises ecological and rotational crop concerns and justifies further investigation of its physiological effects on non-target plants such as *Guizotia abyssinica*.

Physiological indicators such as photosynthetic pigments, antioxidant enzyme activity, MDA, H_2O_2, and proline content provide reliable insights into plant responses to stress. Antioxidant enzymes reflect defense capacity, MDA and H_2O_2 indicate oxidative damage, and proline accumulation is a common stress response linked to osmotic adjustment. Accordingly, this research investigated how lowering imazethapyr application rates influenced growth traits, photosynthetic pigment levels, antioxidant enzyme responses, MDA and H_2O_2 accumulation, proline concentration, and seed yield of niger plants.

Materials and Methods

Field experiment

Between July and December 2023, a field study was conducted at the research farm of Isfahan University of Technology, Iran (32.38° N, 51.39° E; 1620 m a.s.l.). The soil was a loam with organic matter content below 0.7%. Seeds of *Guizotia abyssinica* (local Isfahan genotype) were obtained from Isfahan Pakan Seed Company. Sowing took place on 22 July 2023, with 20 cm row spacing and 15 cm intra-row spacing, resulting in a plant density of 33 plants m^{-2} (Fig. 1). Each plot measured 1 m^2 and consisted of five rows. Irrigation was applied every four days until seedling establishment and subsequently based on 40% soil moisture depletion using drip tape irrigation, with a total of approximately 420 mm water applied during the growing season.

A dose–response experiment was arranged in a randomized complete block design with three replications. Ten rates of imazethapyr were tested (0, 0.375, 0.75, 1.5, 3, 6, 12, 25, 50, and 100 g a.i. ha^{-1}). The recommended field dose for legume crops in Iran is 50–70 g a.i. ha^{-1}, which was included as a reference. For 1 m^2 plots, these rates corresponded to 0.000375, 0.000750, 0.001500, 0.003000, 0.006000, 0.012000, 0.025000, 0.050000, and 0.100000 cc m^{-2}. Treatments were applied as foliar sprays three weeks after emergence at the 4–6 leaf stage. Imazethapyr (10% SL) was supplied by Aria Chemical Company and applied with a calibrated manual sprayer delivering 450 L ha^{-1} (45 mL per plot). Untreated

buffer rows and plastic shielding were used to minimize spray drift (Fig. 2). Samples were collected from the central three rows of each plot to avoid border effects.

Herbicide phytotoxicity assessment

Leaf chlorosis and other visible phytotoxicity symptoms were evaluated 10, 20, and 30 days after treatment (DAT) using a 0–100 rating scale (0 = no injury, 100 = plant death), following European Weed Research Association guidelines [16].

Determination of photosynthetic pigments

Leaf samples were collected 7 DAT to determine chlorophyll and carotenoid contents. Fresh fully expanded leaves (0.1 g) were homogenized in 10 mL of 80% (v/v) acetone. After filtration, absorbance was measured at 470, 646.8, and 663.2 nm using a UV–Vis spectrophotometer (U-1800, Hitachi, Japan). Pigment contents were calculated according to Lichtenthaler and Buschmann [17]:

$$\text{Chlorophyll a} = 12.25A_{663.2} - 2.79A_{646.8}$$

$$\text{Chlorophyll b} = 21.50A_{646.8} - 5.10A_{663.2}$$

$$\text{Carotenoids} = \frac{1000A_{470} - 1.82C_a - 85.02C_b}{198}$$

Proline content

Proline concentration was determined 7 DAT following Bates et al. [18]. Leaf tissue (0.5 g) was homogenized in 5 mL of 3% sulfosalicylic acid. Two milliliters of the extract were mixed with 2 mL glacial acetic acid and 2 mL acid ninhydrin and incubated at 100°C for 1 h. The chromophore was extracted with toluene, and absorbance was read at 520 nm. Proline content was calculated from a standard curve and expressed as mg g^{-1} fresh weight (FW).

Antioxidant enzyme activity

Enzyme assays were conducted 10 DAT using 0.1 g fresh leaves homogenized in 1 mL extraction buffer (50 mM potassium phosphate buffer, pH 7.0, containing 1% polyvinylpyrrolidone and 0.5% Triton X-100). The homogenate was centrifuged at 15,000 rpm for 20 min at 4°C, and the supernatant was used for enzyme assays.

Catalase (CAT, EC 1.11.1.6) activity was measured by monitoring the decrease in absorbance at 240 nm due to H_2O_2 decomposition [19]. Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined according to Nakano and Asada [20] by following the decline in absorbance at 290 nm. Guaiacol

peroxidase (GPX, EC 1.11.1.9) activity was assayed following Herzog and Fahimi [21] at 470 nm. Protein concentration was determined using the Bradford method [22] with bovine serum albumin as a standard.

Hydrogen peroxide content

H_2O_2 content was quantified 7 DAT according to Velikova et al. [23]. Leaf tissue (0.2 g) was homogenized in 2 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at 10,000 g for 10 min. A 0.5 mL aliquot of the supernatant was mixed with 0.5 mL of 10 mM phosphate buffer (pH 7.0) and 1 mL of 1 M KI. Absorbance was measured at 390 nm, and H_2O_2 concentration was calculated from a standard curve.

Lipid peroxidation (MDA content)

MDA content was measured 7 DAT as an indicator of lipid peroxidation following Heath and Packer [24]. Leaf tissue (0.2 g) was homogenized in 2 mL of 0.1% TCA and centrifuged at 10,000 g for 15 min. A 0.5 mL aliquot of the supernatant was mixed with 1 mL of 0.5% TBA in 20% TCA and incubated at 95°C for 30 min. After cooling and centrifugation, absorbance was read at 532 and 600 nm, and MDA content was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Growth parameters

At full flowering, plant height was measured from the base to the apex. Aerial biomass was harvested at early flowering (approximately 5 weeks after treatment), oven-dried at 75°C for 48 h, and weighed.

Yield and yield components

At physiological maturity, five randomly selected plants per plot were used to determine the number of sub-branches, inflorescences per plant, seeds per inflorescence, seed weight per inflorescence, and 1000-seed weight. Total seed yield per plot was then calculated and converted to kg ha^{-1}.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using SAS software (version 9.4). Treatment means were compared using the least significant difference (LSD) test at the 5% significance level. Graphs were prepared in Microsoft Excel 2016.

Results

Phytotoxicity and growth inhibition

Imazethapyr dosage had highly significant effects ($p \leq 0.01$) on chlorosis, plant height, dry biomass, and photosynthetic pigments, as well as grain yield and its components and antioxidant-related traits (Tables 1 and 2). Significant phytotoxicity was observed 10 DAT at 1.5 g a.i. ha^{-1} and higher (Table 3). No

phytotoxicity was evident at 0.375 and 0.75 g a.i. ha^{-1} (Fig. 3). Chlorosis at 1.5, 3, 6, and 12 g a.i. ha^{-1} 10 DAT was 7, 12, 23, and 42%, respectively, but symptoms declined over time (Fig. 4). At 1.5 g a.i. ha^{-1}, nearly complete recovery was observed by 20–30 DAT, whereas chlorosis at 25, 50, and 100 g a.i. ha^{-1} (48, 63, and 75% at 10 DAT) became more severe over time (Fig. 5).

Table 1
Analysis of variance for growth, pigment, and oxidative stress parameters of niger under different imazethapyr doses.

Source of Variation	**df**	**Chlorosis%**			**Height**	**Dry weight (g)**
		10D	**20D**	**30D**		
Treatment	9	2439**	3449**	3742**	7493**	417**
block	2	25.8ns	0.3ns	10.83ns	8.03ns	2.23ns
Error	18	11.01	22.67	9.90	24.2	5.50
		chlorophyll a	chlorophyll b	Total chlorophyll	a/b	carotenoid
Treatment	9	0.04**	0.006**	0.079**	0.248**	0.001**
block	2	0.0003ns	0.00008ns	0.0001ns	0.064ns	0.0002ns
Error	18	0.001	0.0004	0.002	0.075	0.0003

** Treatment effect significant at P ≤ 0.01.

* Treatment effect significant at P ≤ 0.05;

ns, not significant.

Table 2
Analysis of variance on grain yield and some physiological traits of niger plants

Source of Variation	df	Yield	NB	NI	NS	SW	w1000
Treatment	9	959884^{**}	92.8^{**}	2308^{**}	54069^{**}	0.71^{**}	7.07^{**}
block	2	2311^{ns}	5.7^{*}	27.7^{ns}	1185^{ns}	0.001^{ns}	0.41^{ns}
Error	18	2026	0.99	11.77	581	0.01	0.37
		MDA	H_2O_2	CAT	PAX	GPX	proline
Treatment	9	463^{**}	3736^{**}	0.093^{**}	5.75^{**}	2.76^{**}	0.217^{**}
block	2	79.26^{**}	113^{ns}	0.0046^{ns}	0.125^{ns}	0.149^{ns}	0.119^{**}
Error	18	10.9	45.73	0.005	0.24	0.300	0.016

** Treatment effect significant at P ≤ 0.01.

* Treatment effect significant at P ≤ 0.05;

ns, not significant.

Table 3
Effect of imazethapyr dose on chlorosis and phytotoxicity scores.

Imazethapyr dosages	Chlorosis%			Weight of 1000 grains	Number of branches	Number seed of inflorescence
g a.i. ha^{-1}	10D	20D	30D			
Control	0 ± 0.0^{g}	0 ± 0.0^{f}	0 ± 0.0^{g}	3.79 ± 0.79^{a}	8 ± 1.56^{c}	38.6 ± 7.35^{b}
0.375	0 ± 0.0^{g}	0 ± 0.0^{f}	0 ± 0.0^{g}	3.49 ± 0.20^{a}	14.6 ± 0.33^{a}	69.6 ± 0.88^{a}
0.75	0 ± 0.0^{g}	0 ± 0.0^{f}	0 ± 0.0^{g}	3.18 ± 0.03^{a}	16.0 ± 0.58^{a}	76.3 ± 0.87^{a}
1.5	6.6 ± 1.67^{f}	0 ± 0.0^{f}	0 ± 0.0^{g}	3.90 ± 0.49^{a}	11.0 ± 1.15^{b}	46.0 ± 3.45^{b}
3	11.6 ± 1.67^{f}	6.6 ± 1.67^{f}	5 ± 1.67^{f}	3.96 ± 0.35^{a}	7.60 ± 0.88^{cd}	30 ± 2.78^{c}
6	23.3 ± 1.67^{e}	17.6 ± 1.67^{e}	15 ± 1.67^{e}	4.10 ± 0.31^{a}	7.30 ± 0.67^{cd}	21.6 ± 1.45^{d}
12	41.6 ± 1.67^{d}	18.3 ± 1.67^{d}	21.6 ± 1.67^{d}	3.38 ± 0.30^{a}	5.60 ± 0.88^{de}	13 ± 0.58^{e}
25	48.3 ± 4.41^{c}	53.3 ± 4.41^{c}	56.6 ± 4.41^{c}	2.82 ± 0.82^{a}	4.60 ± 0.88^{e}	15 ± 1.53^{de}
50	63.3 ± 1.67^{b}	76.6 ± 3.33^{b}	83.3 ± 3.33^{b}	0 ± 0.0^{b}	0 ± 0.0^{f}	0 ± 0.0^{f}
100	75 ± 2.89^{a}	90 ± 5.77^{a}	90 ± 0.1^{a}	0 ± 0.0^{b}	0 ± 0.0^{f}	0 ± 0.0^{f}

The means within a column sharing the same letters are not significantly different according to the LSD test at $P \leq 0.05$ (mean ± standard error of the mean).

Photosynthetic pigments

Total chlorophyll content generally decreased with imazethapyr application (Table 4). The chlorophyll a/b ratio was higher in herbicide treatments up to 3 g and at 25–50 g compared with the control, but significantly higher only at 0.375 g. Reductions in carotenoids (6–42%) were smaller than those in chlorophyll (13–77%).

Table 4
Means of Photosynthetic pigments Chlorophyll a, b, Carotenoid, Total chlorophyll and Chlorophyll a/b of niger plant under different imazethapyr dosages.

Imazethapyr dosages g a.i. ha^{-1}	Chlorophyll a	Chlorophyll b	Carotenoid	Total chlorophyll	Chlorophyll a/b
Control	0.52 ± 0.019^{a}	0.25 ± 0.010^{a}	0.112 ± 0.016^{ab}	0.77 ± 0.028^{a}	2.08 ± 0.025^{bcd}
0.375	0.44 ± 0.034^{b}	0.17 ± 0.011^{c}	0.119 ± 0.010^{a}	0.61 ± 0.045^{b}	2.59 ± 0.035^{a}
0.75	0.47 ± 0.026^{ab}	0.2 ± 0.022^{b}	0.105 ± 0.016^{abc}	0.67 ± 0.019^{b}	2.35 ± 0.018^{ab}
1.5	0.33 ± 0.013^{c}	0.14 ± 0.006^{cd}	0.087 ± 0.012^{bcd}	0.47 ± 0.009^{c}	2.36 ± 0.339^{ab}
3	0.28 ± 0.016^{cd}	0.12 ± 0.004^{de}	0.087 ± 0.001^{cde}	0.40 ± 0.019^{cd}	2.33 ± 0.066^{abc}
6	$0.28 \pm .0.013^{cd}$	0.14 ± 0.005^{cd}	0.078 ± 0.001^{cde}	0.42 ± 0.016^{cd}	2.00 ± 0.072^{bcd}
12	0.23 ± 0.006^{d}	0.13 ± 0.016^{d}	0.054 ± 0.011^{e}	0.36 ± 0.016^{d}	1.77 ± 0.212^{cd}
25	0.28 ± 0.032^{cd}	0.13 ± 0.013^{d}	$0.087 \pm 0.006c^{de}$	0.41 ± 0.044^{cd}	2.15 ± 0.080^{bcd}
50	0.24 ± 0.001^{d}	0.11 ± 0.006^{de}	$0.077 \pm 0.002c^{de}$	0.35 ± 0.007^{d}	2.18 ± 0.094^{bcd}
100	0.16 ± 0.030^{e}	0.1 ± 0.006^{e}	0.065 ± 0.006^{de}	0.26 ± 0.034^{e}	1.60 ± 0.245^{d}

The means within a column sharing the same letters are not significantly different according to the LSD test at $P \leq 0.05$ (mean ± standard error of the mean).

Growth parameters

Plant height decreased with increasing imazethapyr dose, but the reduction was not significant at 0.75 and 1.5 g (Fig. 6). All herbicide treatments significantly reduced plant dry weight. Dry weight decreased by 35% at 0.375 and 0.75 g, by 49% at 1.5 g, and by more than 80% at 3 g and higher.

Proline content

Imazethapyr at 0.375, 0.75, and 1.5 g did not significantly affect proline content (Fig. 7). However, doses of 3 g and above caused a marked increase, exceeding 100% relative to the control. At 100 g, proline

content was up to 12 times higher than the control.

Antioxidant enzyme activities

All antioxidant enzymes showed increased activity in response to imazethapyr (Table 5). CAT and GPX activities at 0.375 g did not differ significantly from the control. Maximum CAT activity occurred at 3 g, whereas APX peaked at 100 g and GPX in the 6–100 g range. CAT activity was higher at lower imazethapyr concentrations, while APX and GPX were more responsive at higher doses. Overall, GPX exhibited the greatest relative increase.

MDA content and H_2O_2 concentration

Imazethapyr increased MDA and H_2O_2 contents, except at 0.375–0.75 g for MDA and 0.375–1.5 g for H_2O_2 (Table 5). MDA increased significantly from 1.5 g (25% increase) up to 195% at 100 g. H_2O_2 increased significantly from 3 g (44% increase) up to 179% at 50 g.

Table 5
Effect of imazethapyr dose on antioxidant enzyme activities, MDA, and H_2O_2.

Imazethapyr dosages	CAT	AXP	GPX	MDA	H_2O_2
g a.i. ha^{-1}	U mg^{-1} protein			µmol g^{-1}(FW)	
Control	0.16 ± 0.017^{d}	0.59 ± 0.04^{e}	0.04 ± 0.002^{d}	19.0 ± 1.49d^{e}	41.6 ± 2.50^{de}
0.375	0.17 ± 0.044^{d}	2.74 ± 0.38^{c}	0.05 ± 0.001^{d}	15.6 ± 1.21^{e}	31.8 ± 0.61^{ef}
0.75	0.47 ± 0.024^{bc}	2.18 ± 0.17^{cd}	0.95 ± 0.040^{b}	15.4 ± 0.95^{e}	22.4 ± 1.25^{f}
1.5	0.40 ± 0.013^{bc}	2.09 ± 0.09^{cd}	0.71 ± 0.022^{c}	23.8 ± 3.52^{d}	40.7 ± 1.78^{e}
3	0.70 ± 0.078^{a}	1.53 ± 0.29^{d}	0.81 ± 0.020^{bc}	32.7 ± 2.52^{c}	59.8 ± 1.40^{bc}
6	0.42 ± 0.051^{bc}	2.23 ± 0.21^{cd}	1.63 ± 0.104^{a}	33.2 ± 1.80^{c}	53.1 ± 1.97^{cd}
12	0.36 ± 0.046^{c}	1.69 ± 0.16^{d}	1.66 ± 0.90^{a}	41.4 ± 0.97^{b}	67.4 ± 6.68^{b}
25	0.35 ± 0.026^{c}	3.66 ± 0.22^{b}	1.53 ± 0.036^{a}	42.1 ± 3.29^{b}	69.5 ± 1.53^{b}
50	0.38 ± 0.051^{bc}	4.06 ± 0.16^{b}	1.56 ± 0.057^{a}	43.7 ± 1.79^{b}	116 ± 1.79^{a}
100	0.49 ± 0.036^{b}	5.32 ± 0.60^{a}	1.55 ± 0.067^{a}	56.1 ± 2.82^{a}	108 ± 10.31^{a}

The means within a column sharing the same letters are not significantly different according to the LSD test at $P \leq 0.05$ (mean ± standard error of the mean). Catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase activities (GPX), malondialdehyde (MDA), hydrogen peroxide (H_2O_2).

Yield and yield components

Imazethapyr at 0.375 and 0.75 g significantly increased grain yield by 12 and 35%, respectively (Fig. 8). These yield gains were associated with 82 and 100% increases in the number of branches and 80 and 98% increases in seeds per inflorescence (Table 3). At 3 g and higher, grain yield and these components were significantly reduced. No grain was produced at 50 and 100 g.

Discussion

Impact of low imazethapyr dosages on growth and physiological parameters

In this study, significant phototoxicity was observed at imazethapyr dosages of 1.5 g and higher. The phototoxicity imposed by imazethapyr in the current study was accompanied by a reduction in the content of photosynthetic pigments. This was also evident in the study of Shivani Grewal et al. [25], in which post-emergent application of imazethapyr decreased chlorophyll and carotenoids in lentil plants due to chloroplast damage. Moreover, Su et al. [26] reported reduced chlorophyll and net photosynthetic rate in maize seedlings by imazethapyr. The decrease in chlorophyll was attributed to the herbicide interfering with the production of key amino acids, which subsequently impairs chloroplast activity and the plant's overall metabolic processes [16]. However, the symptoms of chlorosis was to some extent diminished over time at dosages of 12 g a.i. ha^{-1} and lower. Similar results were obtained by Monjezi et al. [27], as they reported that the herbicide Bentazon damage was reduced over time. It is assumed that in this study, an increase in the activity of antioxidant enzymes could effectively eliminate ROS, thus overcoming oxidative stress induced by imazethapyr. However, higher concentrations of imazethapyr may still reach its site of action in the plant, resulting in visual injury and eventually death [28].

Impact of low imazethapyr dosages on biochemical parameters

The application of high imazethapyr dosages resulted in significantly increased H_2O_2 production in treated plants, indicating herbicide-induced oxidative stress through overproduction of reactive oxygen species (ROS), which can lead to cell membrane destruction. This was accompanied by elevated malondialdehyde levels, a recognized biomarker for oxidative stress. Conversely, low imazethapyr dosages decreased H_2O_2 levels, suggesting a positive Hormetic effect of the herbicide in controlling free radical production. This reduction was associated with a notable activation of antioxidant enzymes: CAT, APX and GPX. Other studies have similarly shown that certain herbicides can activate specific pathways that enhance antioxidant enzyme function, consequently reducing the ROS production [29]. The increased activity of CAT, APX and GPX with higher herbicide rates implies that antioxidant genes can be highly regulated by herbicides at the molecular level, enabling crops to activate antioxidant mechanisms to mitigate herbicide-induced stress [30]. However, at high herbicide concentrations, excessive ROS generation may overwhelm the detoxification capacity of antioxidant enzymes, leading to oxidative damage and decreased enzymatic efficiency [6]. In the current study, the highest CAT activity was observed at lower herbicide concentrations, while peak APX and GPX activities occurred at higher concentrations. This suggests a dose-dependent activation of the plant's antioxidant defense system in response to oxidative stress. Furthermore, it may indicate that CAT activation was more closely correlated with the hormetic effects of imazethapyr at lower concentrations compared to the other two enzymes studied. CAT and APX are acknowledged as primary enzymes responsible for eliminating H_2O_2, though their specific contributions may differ based on stress level, plant genotype, and the

responsiveness of particular tissues [16]. On the other hand, a dramatic increase in proline content was observed by higher imazethapyr dosages. Similarly, Kumar et al. [31], reported that increasing imazethapyr concentrations raised proline levels in lentil plants, indicating a direct relationship between the herbicide dosage and proline synthesis. Given that free proline acts as an osmoprotectant, a protein stabilizer, a scavenger of lipid peroxidation, hydroxyl radicals and (OH^-) and superoxide radicals (O_2) [32], its increased production have been related to plants' attempts to mitigate herbicide-induced stress [33]. However, the absence of a notable change in proline content at lower herbicide concentrations (at 0.275, 0.75 and 1.5 g a.i. ha^{-1}), supported the hypothesis that Hormetic effect of low imazethapyr dosages alleviates oxidative stress. The Hormetic response observed in our study aligned with previous studies, indicating that mild oxidative stress can enhance plant resilience to subsequent stress by triggering systemic acquired resistance and improving metabolic adaptability [34]. Thus, the ability of low imazethapyr dosages to reduce oxidative damage while maintaining crop productivity highlights a sustainable approach to herbicide use, minimizing environmental pollution risks through lower chemical inputs.

Impact of low imazethapyr dosages on yield and yield components

Low dosages of imazethapyr, ranging from 0.375 to 0.75 g, exhibited Hormetic effect that increased grain yield. This yield enhancement coincided with a noticeable rise in the number of sub-branches and the number of seeds per inflorescence at these low application rates. A significant body of recent research continues to validate the potential of herbicide hormesis to stimulate crop growth and yield. While the phenomenon has historically been challenging to leverage due to erratic results and difficulty in determining effective doses in the field [35, 36], a more nuanced understanding of application is emerging. Studies have shown that while low herbicide doses can consistently boost early-season vegetative metrics like plant biomass, leaf area, and nodulation [36, 37], this does not always translate to an increase in final grain yield [36]. However, Several studies have reported the potential for persistent hormetic effects induced by herbicides, resulting in enhanced plant growth and increased grain yield [35, 38]. According to Cesco et al. [39], exposure to low doses of glyphosate enhanced the reproductive performance of *Conyza sumatrensis* genotypes, leading to a 37% rise in the number of panicles and a 41% increase in grains produced per plant. In the current study, the increased grain yield observed at low imazethapyr concentrations correlated with an improved plant antioxidant defense system, resulting in decreased production of H_2O_2 and malondialdehyde, indicating alleviated oxidative stress. Although low dosages of imazethapyr (0.375–0.75 g) enhanced grain yield, they were also associated with a reduction in total biomass production. This may be linked to the inherently low harvest index of the plant species, indicating that a greater allocation of resources toward grain formation came at the expense of vegetative growth. These findings highlight a trade-off between optimizing yield efficiency and maintaining overall plant biomass, as only a limited portion of total biomass was ultimately allocated to grain production. On the other hand, despite a significant decrease in plant biomass due to herbicide application, the plants still maintained sufficiently improved photosynthetic capacity, attributed to

Hormetic effects, to produce higher grain yield. Crucially, the ability to achieve yield gains at such low herbicide concentrations also implies a reduction in chemical inputs, thereby lowering the risk of environmental contamination and supporting more sustainable crop management practices.

Effectiveness and resistance risk of low-dose herbicide applications

The results of the present study demonstrate that low doses of Imazethapyr (below 12 g a.i. ha^{-1}) caused only partial phytotoxic responses in *Guizotia abyssinica* and are therefore unlikely to provide effective control of sensitive weed species under field conditions. Although reduced rates may temporarily suppress susceptible weeds, they generally fail to eliminate all individuals, particularly those with natural tolerance to ALS-inhibiting herbicides. Previous research has shown that repeated sublethal exposures can select for individuals carrying resistance-conferring mutations in the acetolactate synthase (ALS) gene [40]. Consequently, the routine use of low-dose Imazethapyr could accelerate the evolution of herbicide-resistant weed populations. Therefore, while low-dose strategies may be economically attractive or environmentally motivated, they must be carefully managed and integrated with nonchemical weed control methods to prevent the development of resistance and to maintain long-term efficacy of Imazethapyr.

Conclusion

This study demonstrated that low concentrations of imazethapyr (0.375 and 0.75 g a.i. ha^{-1}) increased niger grain yield under field conditions by promoting sub-branch and capitulum development. These hormetic effects were associated with enhanced antioxidant enzyme activity and reduced oxidative stress, as indicated by lower H_2O_2 and MDA levels. In contrast, higher imazethapyr concentrations (≥ 3 g a.i. ha^{-1}) caused severe phytotoxicity, reduced photosynthetic pigments, stunted growth, and yield loss. Antioxidant enzymes showed dose-dependent and differential responses, with CAT more closely associated with hormetic effects at low doses. These findings suggest that carefully optimized low-dose imazethapyr applications can improve crop performance while reducing herbicide inputs and environmental contamination. Further research is needed to elucidate the molecular mechanisms underlying these responses and to refine application strategies for sustainable weed management.

Declarations

CRediT authorship contribution statement

Abbas Ghafori: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Morteza Zahedi**: Supervision, Conceptualization, Writing – review & editing. **Hassan Karimmojeni**: Conceptualization, Writing–review & editing. **Mette Sønderskov**: Conceptualization, Writing–review & editing. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contribution

Abbas Ghafori: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. Morteza Zahedi: Supervision, Conceptualization, Writing – review & editing. Hassan Karimmojeni: Conceptualization, Writing–review & editing. Mette Sønderskov: Conceptualization, Writing–review & editing. All authors read and approved the final manuscript.

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Data Availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

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Figures



Figure 1

Row spacing and intra-row spacing, and a planting density of plants m².



Figure 2

Untreated buffer rows and plastic shielding



Figure 3

A. phototoxicity observed at imazethapyr dosages of 1.5 g a.i. ha $^{-1}$. B & C No phytotoxicity evident at 0.375 and 0.75 g a.i.ha^{-1}.

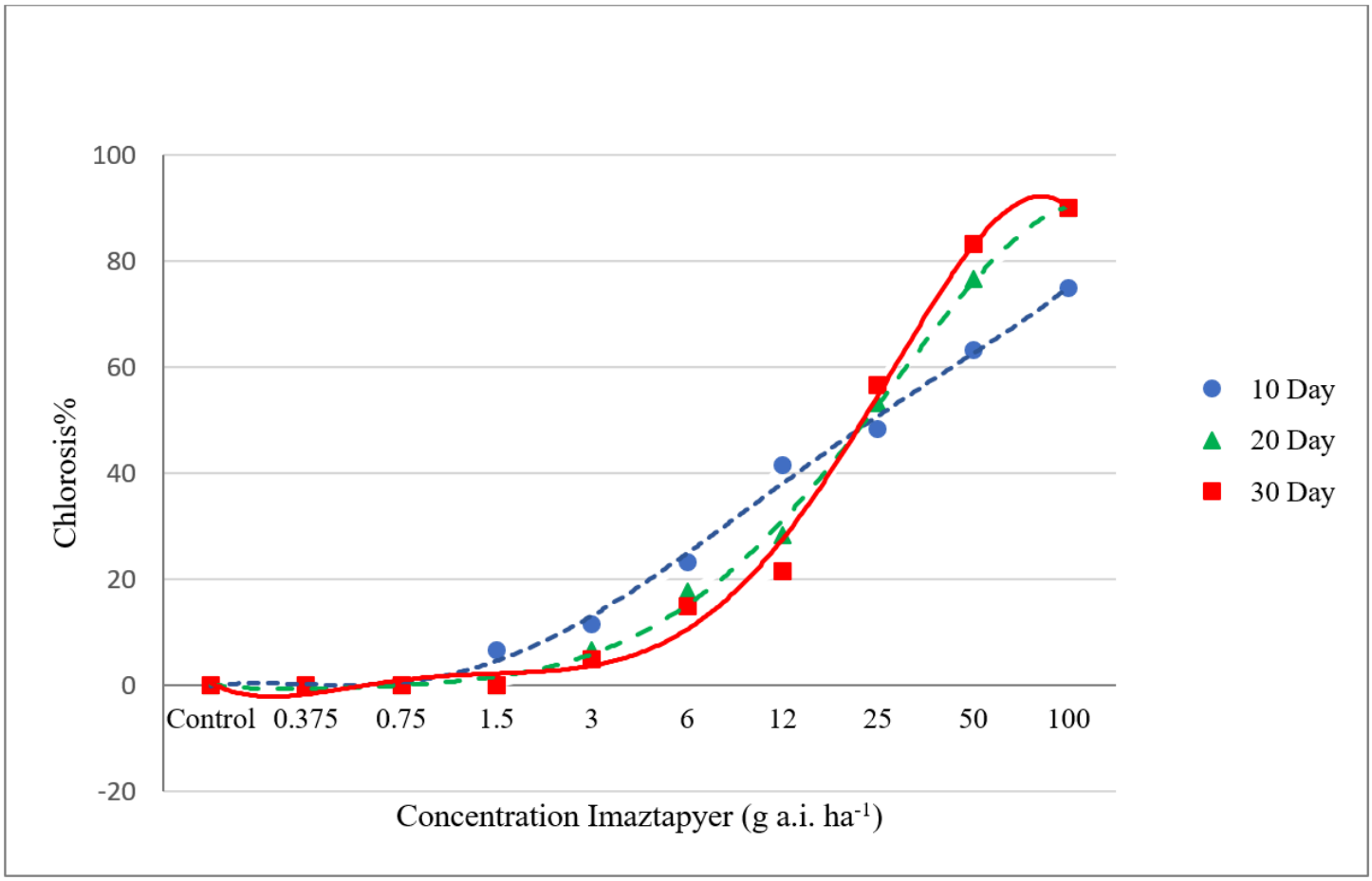


Figure 4

Effect of concentration of imazethapyr on Chlorosis Percentage.



Figure 5

Phototoxicity observed at imazethapyr dosages of A:12 g B: 25 g and C: 50 g a.i. ha $^{-1}$.

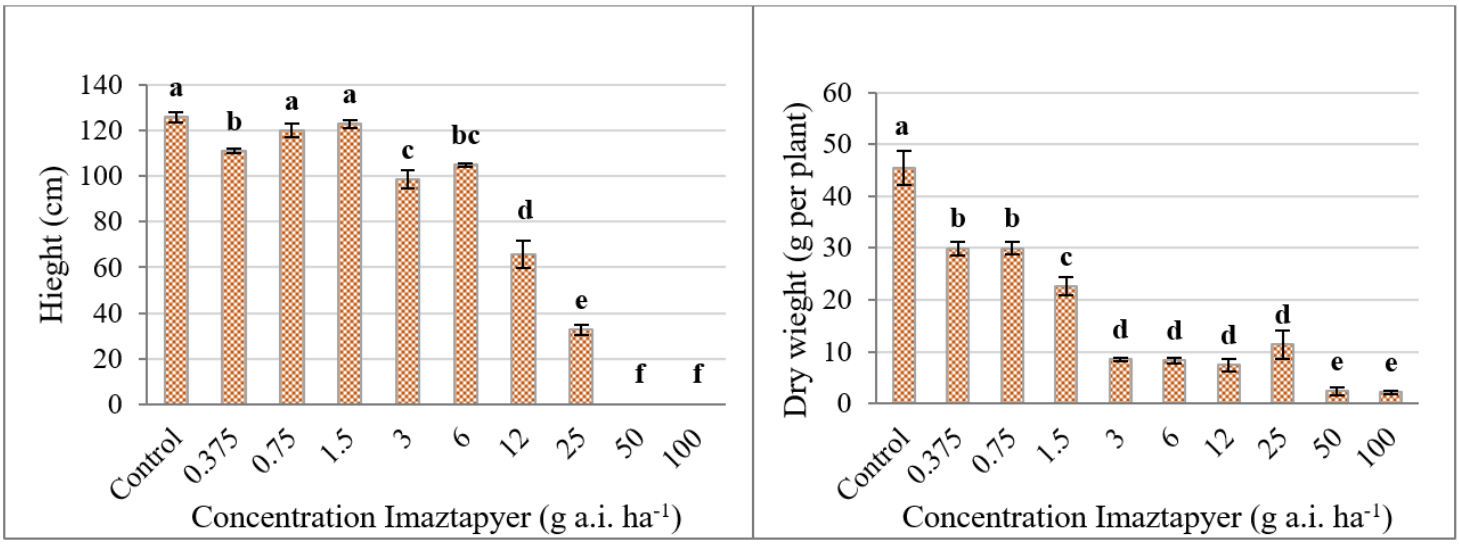


Figure 6

Effect of concentration of imazethapyr on height and dry weight. Different letters within columns in each parameter indicate significant differences at P < 0.05. Bars on the columns are means ± standard error.

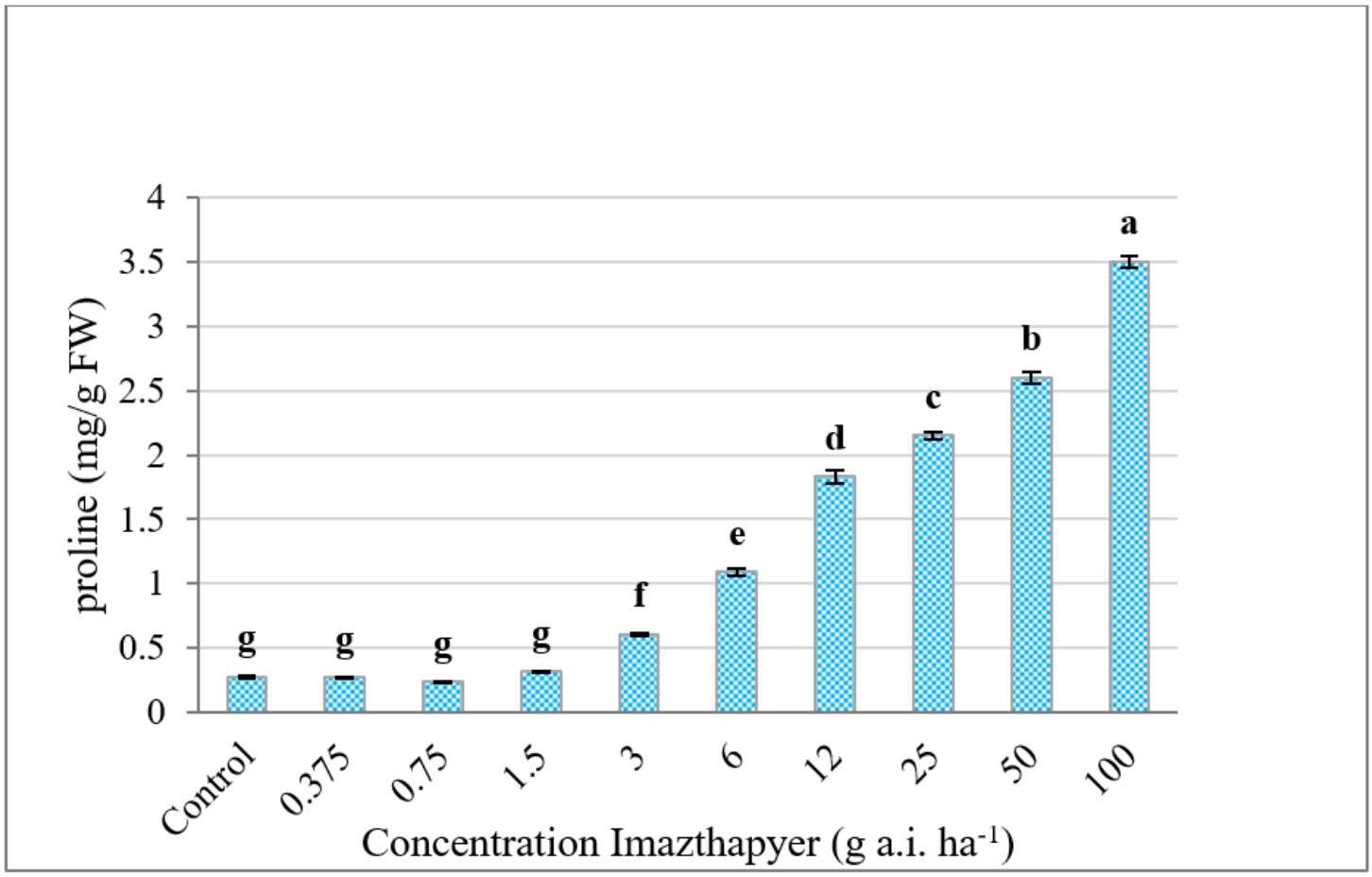


Figure 7

Effect of concentration of imazethapyr on Proline content (mg /g Fw). Different letters within columns in each parameter indicate significant differences at $P < 0.05$. Bars on the columns are means ± standard error.

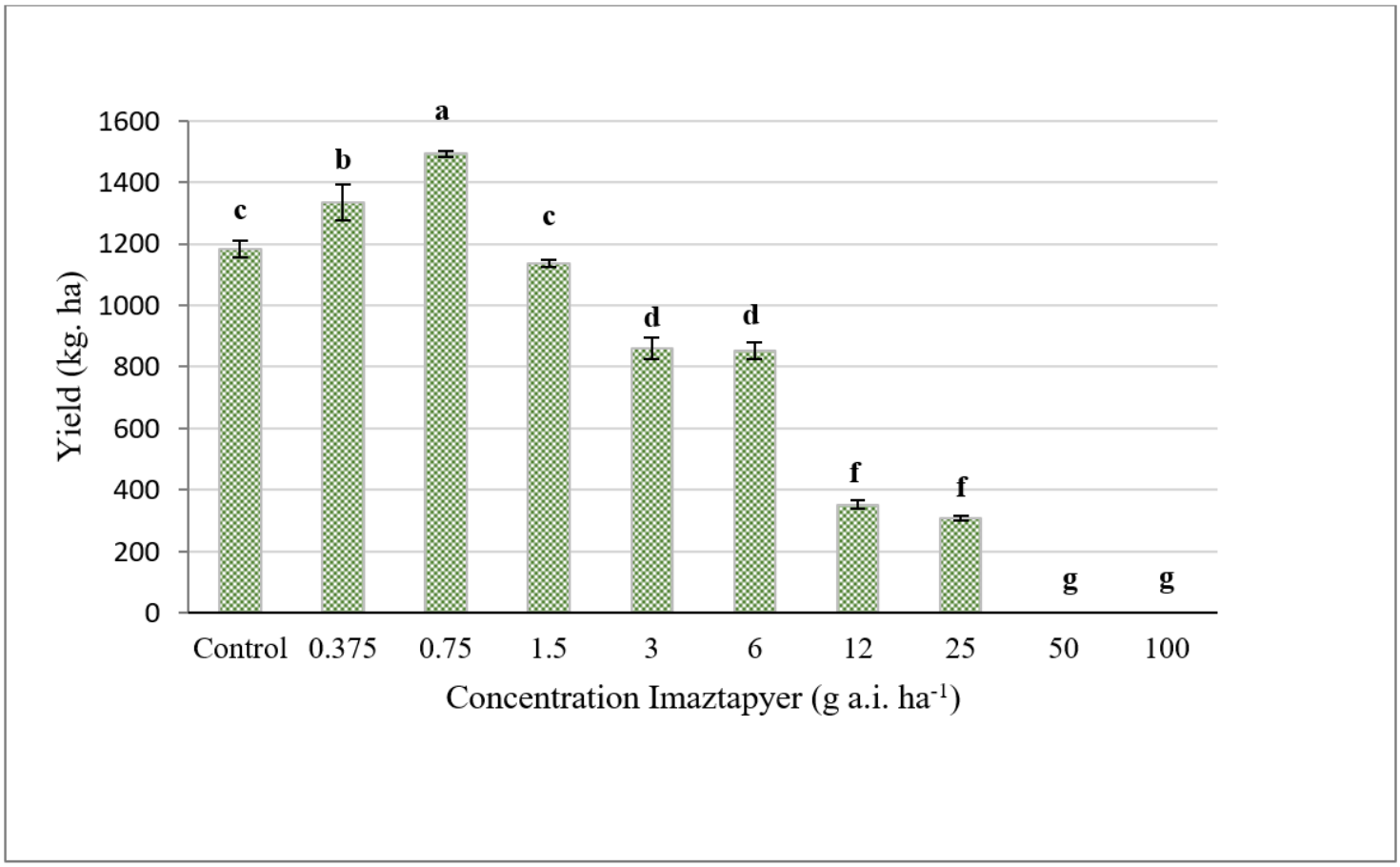


Figure 8

Effect of concentration imazethapyr on grain yield (kg ha^{-1}). Different letters within columns in each parameter indicate significant differences at $P < 0.05$. Bars on the columns are means ± standard error.