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Residual effects of butisanstar and clopyralid herbicides on *Gossypium hirsutum* L. growth: insights from a pot experiment

SOLTANI TOULAROUD Ali Ashraf¹, GOLCHIN Ahmad² and ROUHI-KELARLOU Tohid^{2*}

Abstract

Background The intensive use of herbicides in agriculture raises concerns about their residual impacts on non-target crops such as cotton (*Gossypium hirsutum* L.), which is often rotated with cereals, sugar beet, and canola. Butisanstar (BUT) and clopyralid (CLO) are widely used for broadleaf weed control in these rotations. However, how residual herbicide activity influences cotton growth and development is not well understood. This study evaluated these residual effects by measuring multiple growth parameters in a greenhouse. Cotton was grown for 40 days in soil incubated for 90 days with herbicide treatments arranged in a factorial design (type: BUT, CLO, and their combination; dose: 0, ½, 1, 2, and 5 × recommended field dose [RFD]).

Results Herbicide residues reduced cotton growth in a dose-dependent manner, with greater inhibition at higher doses. The combined BUT + CLO treatment produced the strongest negative effects, followed by CLO and then BUT alone. Compared with controls, seedling emergence declined by 12%–83%, root length by 12%–87%, plant height by 10%–84%, and chlorophyll index by 12%–80% across treatments from ½ × RFD BUT to 5 × RFD BUT + CLO. Root and shoot biomass also decreased significantly. Under the 5 × RFD combined treatment, shoot N, P, and K concentrations dropped by 48%, 78%, and 70%, respectively, relative to the control.

Conclusions Even low levels of residual BUT and CLO impair cotton growth. To mitigate these effects, it should avoid planting cotton on recently treated soils, leave sufficient intervals between herbicide application and cotton planting, and apply soil amendments to boost microbial degradation. These measures are essential for sustaining soil health and cotton productivity.

Keywords Cotton, Herbicide residues, Growth traits, Nutrient concentrations, Seedling emergence

Introduction

Clopyralid (CLO) 30% SL, chemically known as 3,6-dichloropyridine-2-carboxylic acid ($C_6H_3Cl_2NO_2$), is formulated as a water-soluble solution (Kucharski et al. 2015). It is a

selective, systemic herbicide absorbed through both leaves and roots, and translocated via the vascular system to affect the entire plant (Roberts et al. 1998). CLO induces symptoms such as leaf twisting, tissue swelling, and root cracking by disrupting hormonal balance and cell wall flexibility, ultimately inhibiting plant growth (Tomco et al. 2016; Rose et al. 2016). Typically applied post-emergence at 600–800 mL·hm⁻², it effectively controls broadleaf weeds in sugar beet, canola, and cereal crops (Tomlin 1994; Gillespie et al. 2011). Notably, CLO is highly persistent in soil and resistant to microbial degradation, with an average half-life of approximately 40 days, although this varies

*Correspondence:

Rouhi-Kelarlou Tohid
tohid.rouhik@gmail.com

¹ Department of Soil Science, Faculty of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran

² Department of Soil Science, Faculty of Agriculture, University of Zanjan, Zanjan, Iran



depending on environmental and soil conditions. This persistence increases the risk of phytotoxicity to subsequent rotational crops (Gillespie et al. 2011).

Butisanstar (BUT) 41% SC is a pre-emergence herbicide composed of two active ingredients: metazachlor ($C_{14}H_{16}ClN_3O$, 29.5% w/w), a chloroacetanilide, and quinmerac ($C_{11}H_8ClNO_2$, 7.4% w/w), a synthetic auxin of the quinoline carboxylic acid group. It is formulated as an off-white suspension. Registered in 2008 for use in Iranian rapeseed fields, it is applied at 2 500 mL·hm⁻² post-planting, following initial irrigation, to control both broadleaf and grass weeds. The herbicidal activity of BUT results from the combined action of its two components. Metazachlor inhibits the biosynthesis of very-long-chain fatty acids (> 18 C) outside the chloroplast (Böger 2003). Although its precise mode of action remains unclear, metazachlor is believed to interfere with cell division and elongation by inhibiting protein, fatty acid, and lignin synthesis (Roberts et al. 1998). Quinmerac, absorbed primarily through roots and also through stems and leaves, inhibits root elongation and exhibits auxin-like effects (Roberts et al. 1998). The half-lives of these compounds vary with soil and environmental conditions. For instance, in loamy soil, metazachlor has a half-life of 18 days under sunlight but 78 days in darkness (Mantzios et al. 2017), while quinmerac's half-life ranges between 22 and 35 days in clay loam soil at 15 °C and 10 °C, respectively (Roberts et al. 1998).

Herbicides are cornerstones of integrated weed management, accounting for nearly 50% of global pesticide use (Vats 2015; FAO 2022). However, their intensive and prolonged use, coupled with their chemical persistence, often leads to contamination of soil and non-target organisms. It is estimated that less than 5% of applied herbicides reach their intended targets, while the remaining 95% may affect surrounding ecosystems (Sebiomo et al. 2011; Meena et al. 2020). Therefore, evaluating plant and soil microbial responses to herbicide exposure is essential for understanding the environmental behavior and long-term effects of these residues. Although herbicides are effective in controlling weeds, their residues can significantly alter soil microbial parameters, including population dynamics, biomass carbon, respiration rates, and enzymatic activities. These factors in turn influence soil fertility and plant performance (Hristeva et al. 2015; Medo et al. 2021; Meena et al. 2021). A pot experiment conducted by Rouhi-Kelarlou et al. (2023a, 2024b) assessed the effects of CLO, BUT, and their combination on soil microbial and enzymatic properties at multiple time points (10, 30, 60, and 90 days after application). The results revealed a dose-dependent decline in microbial activity, with bacterial populations being more sensitive than fungal ones. Microbial biomass carbon, respiration,

and enzymatic activities (e.g., dehydrogenase and catalase) were all negatively affected, especially under the combined CLO + and BUT treatment.

While herbicide residues can improve long-term weed control efficiency, their persistence in soil poses a threat to sensitive rotational crops, complicating sustainable farming systems. Plant bioassays are widely used to assess herbicide impact on non-target crops. Compared with analytical techniques such as high performance liquid chromatography (HPLC), bioassays offer a simpler, cost-effective, and biologically meaningful method for detecting herbicide activity (O'Sullivan 2005). These bioassays are often combined with microbial assessments to better understand the ecological implications. For instance, Tomkiel et al. (2019) reported that flufenacet and isoxaflutole significantly reduced microbial abundance, enzymatic activity, and maize biomass, with stronger effects observed at higher concentrations and shorter incubation periods (30 days) than at lower doses and longer intervals (60 days). Similarly, O'Sullivan et al. (1999) found that when applying flumetsulam and CLO at post-emergence in maize for two consecutive years, it caused yield loss in cabbage but had no adverse effects on potatoes, tomatoes, lettuce, peppers, beans, or cucumbers.

Cotton, a broadleaf dicot, is a vital cash crop that supplies natural fiber for the textile industry and seed oil for both industrial and edible uses (Pala et al. 2021). In Iran, it is cultivated across nearly 20 000 hectares of the Moghan Plain in Ardabil Province, usually in rotation with wheat, corn, rapeseed, and sugar beet. Herbicides like BUT and CLO are frequently used in such planting systems. However, growing concerns exist about their residual toxicity on cotton as a non-target crop. The present study aimed to assess the potential risks of residual herbicide exposure on cotton, focusing on morphological traits during early growth stages. A plant bioassay approach was employed, using soil pre-treated with herbicides and incubated for 90 days, without direct chemical residue analysis.

Materials and methods

Study area and soil properties

The soil used in this study was collected from the surface layer (0–10 cm depth) in the Moghan Plain (47°54' E, 39°35' N; 79 m above sea level), a flat region characterized by alluvial soils formed by sedimentation from the Aras River. The selected soil had no prior history of herbicide application with either BUT or CLO. Key physicochemical properties (Table 1) were determined using standard procedures (Carter et al. 2007). The soil was nonsaline, loamy in texture, slightly alkaline (pH > 7), and relatively low in organic matter.

Table 1 Physicochemical properties of the experimental soil

P _{avail} /(mg·kg ⁻¹)	K _{avail} /(mg·kg ⁻¹)	Nt/%	SP/%	FC/%	CCE/%	OC/%	Sand/%	Silt/%	Clay/%	EC/(dS·m ⁻¹)	pH (1:25)
8.83	519	0.05	42.2	32	20.6	0.46	41	33.6	25.4	3.99	7.52

EC: Electrical conductivity, OC: Organic carbon, CCE: Calcium carbonate equivalent, FC: Field capacity (pot), SP: Saturation percentage, Nt: Total nitrogen, K_{avail}: Available potassium, P_{avail}: Available phosphorus.

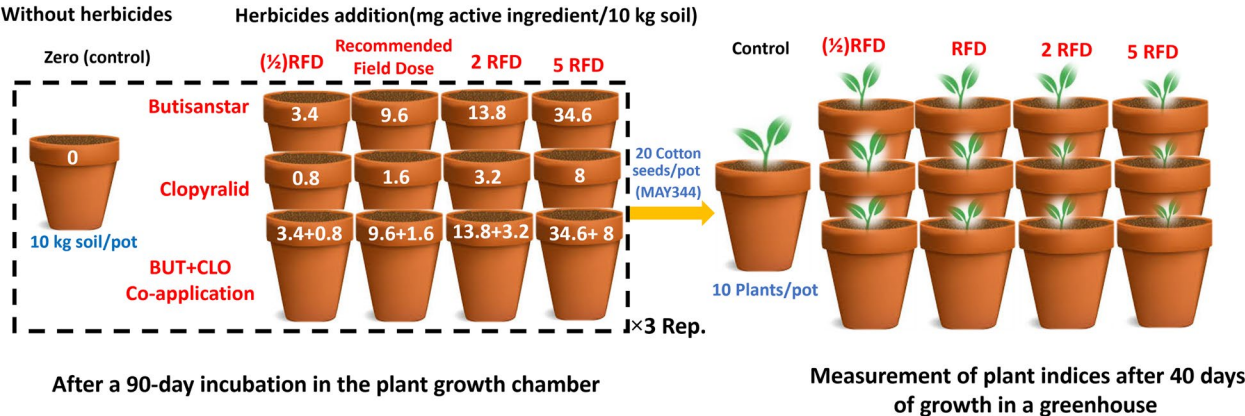


Fig. 1 Schematic representation of the experimental treatment arrangement

Experimental design, treatments, and soil preparation

This study was conducted as a factorial experiment within a completely randomized design (CRD), comprising 13 treatments, each with three replications, which had a total of 39 experimental pots (Fig. 1). Stock solutions of each herbicide were prepared at a concentration of 1 mg·mL⁻¹. To explore a range of potential responses, the selected doses for BUT were 3.4, 6.8, 13.6, and 34.2 mg (active ingredient) per 10 kg of soil, while CLO was applied at 0.8, 1.6, 3.2, and 8 mg per 10 kg soil. These represented ½ recommended field dose (RFD), RFD, 2 RFD, and 5 RFD, respectively. Combination treatments of CLO + BUT were also included at equivalent doses. These application levels were chosen to simulate both standard agricultural practices and possible deviations observed under field conditions. While the RFD reflects manufacturer-recommended rates, higher doses were included to mimic scenarios where farmers may apply excessive or repeated herbicide treatments, particularly in the context of herbicide-resistant weed populations.

The soil was sieved through a 4.75 mm mesh and weighed to obtain 10 kg of dry soil per treatment. It was then spread as a thin layer on a plastic sheet. Herbicide solutions were prepared by diluting the required amounts in distilled water to a final volume of 500 mL. These solutions were uniformly applied using a manual sprayer and thoroughly mixed to ensure even distribution. The treated soil was transferred to plastic pots (22 cm × 26 cm each). Control pots received 500 mL of distilled water

without herbicide. All pots were placed in the growth chamber of the Soil Science and Engineering Department at Mohaghegh Ardabili University and incubated for 90 days under controlled conditions: temperature (25 ± 2) °C, relative humidity 50%–60%, and soil moisture maintained at approximately 60% of field capacity. Temperature and humidity were continuously monitored using automated sensors, and soil moisture was adjusted daily by weighing the pots.

Measurement of plant traits

Following the 90-day incubation period, 20 seeds of the cotton cultivar MAY344 were sown at a depth of 3–4 cm per pot to evaluate the residual herbicide effects under a rotational cropping scenario. Cotton, being a warm-season crop, requires around 30 °C for optimal seed germination and seedling development (Barpete et al. 2015). The pots were randomly arranged in a greenhouse and maintained under controlled conditions for 40 days at (30 ± 2) °C, 50%–60% RH, 16h light/8h dark photoperiod, and 60% field capacity moisture. Seedling emergence (SE) was calculated for each treatment using Eq. 1. Seedlings were counted daily from the appearance of the first seedling until three consecutive days of stable counts were recorded (Schroeder et al. 2023). One week after emergence (2–3 leaf stage), seedlings were thinned to 10 plants per pot (Fig. 1).

Table 2 Analysis of variance (mean squares) for the effects of herbicide type and dose on cotton growth traits and nutrient concentrations

Sources of variation	df	Seedling emergence	Chlorophyll index	Plant height	Root length	Shoot biomass		Root biomass		Nitrogen	Phosphorus	Potassium
						Fresh	Dry	Dry	Fresh			
Herbicide type (H)	2	1 072**	466**	114**	200.1**	167**	37.4**	0.24 ^{ns}	10.1**	0.87**	0.04**	2.01**
Herbicide dose (HD)	4	2 355**	1 749**	884**	149.7**	1 265**	170.5**	2.46**	72.2**	3.62**	0.12**	6.37**
H × HD	8	144.36**	44.2**	38.2**	13.42**	53 ^{ns}	3.02 ^{ns}	0.02 ^{ns}	1.27 ^{ns}	0.07**	0.00**	0.22**
Error	30	6.3	4.3	14.6	3.2	27.9	1.6	0.08	1.3	0.00	0.00	0.01
Coefficient of variation		0.33	0.36	0.34	0.47	0.40	0.62	0.68	0.54	0.20	0.40	0.30

df: degree of freedom. Significant was set at the 5% probability level, *: $P \leq 0.05$; **: $P \leq 0.01$, ns: non-significance.

$$\text{Seedling emergence(SE)} = \frac{\text{Number of emerged seeds}}{\text{Total number of seeds sown}} \times 100 \quad (1)$$

Prior to harvest, plant traits were measured, including plant height using a ruler and chlorophyll content using a handheld chlorophyll meter (CL-01 Hansatech-SPAD), with the results expressed in SPAD units. After 40 days, plants were harvested at the soil surface, and the root were carefully washed. Root length and fresh biomass (shoot and root) were measured using a digital scale with 0.001 kg precision. For dry weight, samples were oven-dried at 70 °C for 48 h, then ground and sieved for further nutrient analysis. Wet digestion with a strong acid mixture (Jones et al. 1990) was used to prepare plant extracts. N was quantified using the Kjeldahl method, P was determined by the Vanado-Molybdate Yellow Color Method with a spectrophotometer ($\lambda = 470$ nm), and K was measured using a flame photometer.

Statistical analysis

All statistical analyses were conducted using the General Linear Model (GLM) in SPSS version 26. One-way analysis of variance (ANOVA) was used to test for treatment effects. Duncan's multiple range test was used for mean separation at a 5% significance level ($P < 0.05$). Pearson's correlation coefficients were calculated to evaluate the relationships between herbicide treatments and plant parameters. Figures and charts were created using Microsoft Excel 2016.

Results

Herbicide residual effects on cotton growth traits

The data presented in Table 2 indicate significant main effects of both types of herbicide and herbicide dose, as well as their interaction ($H \times HD$), on several cotton growth traits. The analysis of variance (ANOVA) revealed that the interaction between herbicide type and dose did not significantly affect shoot and root biomass at the 5% probability level, suggesting that these two factors independently influenced biomass. In contrast, other growth traits showed highly significant differences ($P < 0.01$). The wide range of herbicide doses and their varying impacts on growth responses led to greater data variability, as reflected in the higher coefficients of variation.

The results of the present study revealed significant effects of herbicide type, herbicide dose, and their interaction on SE, chlorophyll index, root length, and plant height, all at the 1% significance level ($P \leq 0.01$) (Table 2). Overall, the combined herbicide treatments exerted a progressively stronger inhibitory effect on plant indices compared with individual treatments. Regardless of herbicide type, there was a clear negative correlation

between increasing herbicide dose and the reduction in plant growth parameters (Figs. 2 and 3).

Regarding the early effects of herbicide residues, a notable reduction in SE percentage was observed comparing to the control soil, even at $\frac{1}{2}$ RFD following 90 days of incubation. SE percentages ranged from 75% in the control, 47%–66% in the treatment of BUT, 34%–55.8% in the treatment of CLO, and 13%–51% in the combined BUT + CLO treatments. The decline in SE percentage ranged from 12% at $\frac{1}{2}$ RFD of BUT to 83% at 5 RFD of the BUT + CLO combination. These findings underscore the strong phytotoxicity and inhibitory effects of herbicide residues, with higher SE percentages in soils treated with BUT alone compared with CLO and the combination treatment (Fig. 2).

Similarly, the chlorophyll index was significantly reduced under all herbicide treatments compared with the control. The decline was most pronounced in the BUT + CLO treatment. The chlorophyll index ranged from 58.7 SPAD in the control pot, with the lowest and the highest values recorded at 5 RFD and $\frac{1}{2}$ RFD, respectively. Index values ranged from 29.2–51.4 SPAD in BUT treatments, 22.5–42.1 SPAD in CLO treatments, and 12–38.2 SPAD in the combined treatments (Fig. 3). The reduction in chlorophyll index ranged from 12% at $\frac{1}{2}$ RFD of BUT treatment to 80% at 5 RFD of BUT + CLO treatment. The negative correlation was observed between herbicide treatment and SE as well as the chlorophyll index ($r = -0.33^*$ and $r = -0.41^*$). And the correlation of herbicide dose with SE and chlorophyll index was stronger ($r = -0.90^{**}$ and $r = -0.84^{**}$) (Table 3).

The analysis of mean squares (Table 2) indicated that, in contrast to other measured traits, root length was primarily influenced by herbicide type rather than dose. No statistically significant differences were observed among the $\frac{1}{2}$ RFD, RFD, and 2 RFD doses for each herbicide. However, slight numerical differences in root length were still noted across these treatments (Fig. 3). Among all the plant traits assessed, root length showed the strongest negative correlation with herbicide type ($r = -0.52^{**}$), highlighting the direct impact of residual herbicides on root development. This suggested that root length may serve as a sensitive biological indicator of herbicide contamination, reflecting the plant's vulnerability to chemical exposure and residual toxicity (Table 3). In the control treatment, the average root length was 17.57 cm, while the lowest and highest values were recorded under the 5 RFD and $\frac{1}{2}$ RFD treatments, respectively. The root lengths ranged from 9.75–15.4 cm in BUT treatments, 8.07–14.33 cm in CLO treatments, and 2.24–6.1 cm in the BUT + CLO treatments (Fig. 3). The overall reduction in root length ranged from 12% at $\frac{1}{2}$ RFD of BUT treatment to 87% at 5 RFD of the BUT + CLO treatments.

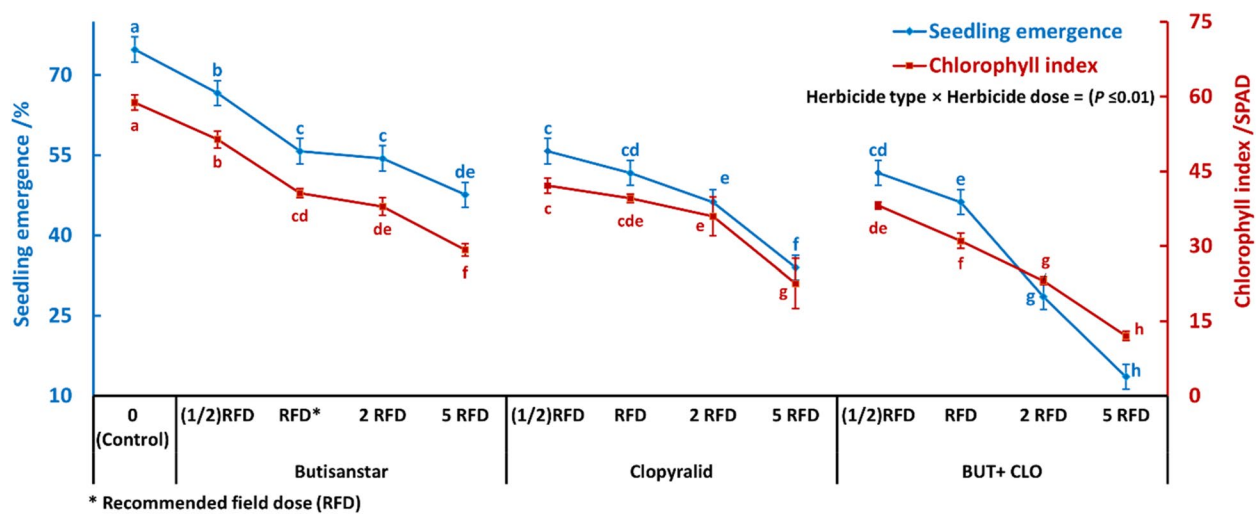


Fig. 2 Impact of various doses (zero, recommended field dose (RFD), 1/2RFD, 2RFD, and 5RFD) of butisanstar (BUT), clopyralid (CLO) or two herbicides in combination on the seedling emergence percentage and chlorophyll index. The line bars represent the means of three replicates $\pm$ standard deviations, with different letters indicating significant differences (Duncan's test; $P \leq 0.05$)

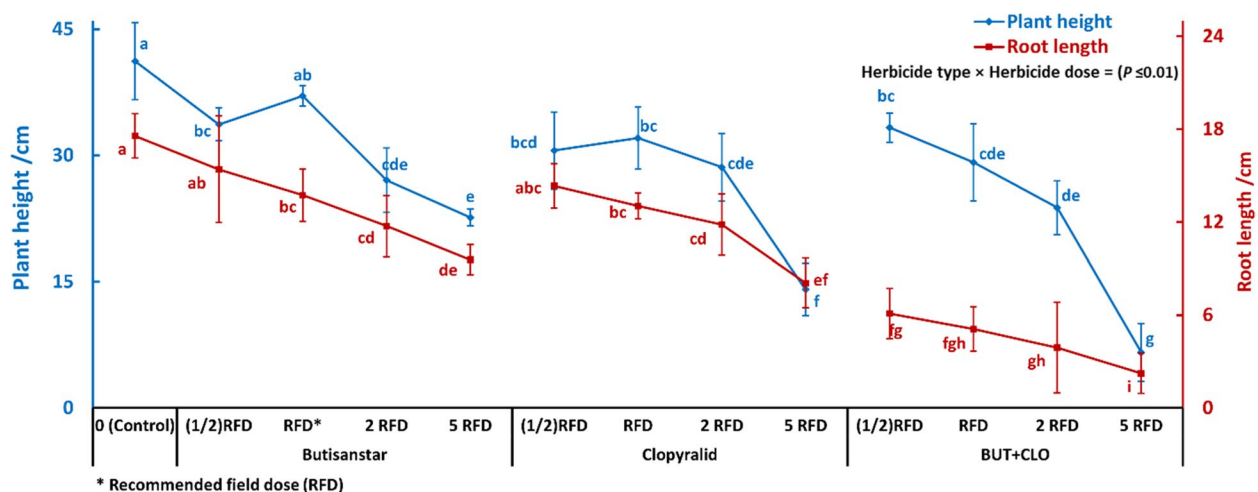


Fig. 3 Impact of various doses (zero, recommended field dose (RFD), 1/2RFD, 2RFD, and 5RFD) of butisanstar (BUT), clopyralid (CLO) or two herbicides in combination on root length and plant height. The line bars represent the means of three replicates $\pm$ standard deviations, with different letters indicating significant differences (Duncan's test; $P \leq 0.05$)

Similarly, plant height exhibited a decreasing trend with increasing herbicide dose. Plants in the control group reached an average height of 41.21 cm, while values ranged from 22.62–37.05 cm in BUT treatments, 14.08–32.06 cm for CLO treatments, and 6.61–33.31 cm in the combination treatment (Fig. 3). The reduction in plant height spanned from 10% at 1/2 RFD of BUT treatment to 84% at 5 RFD of the BUT +CLO treatment. Overall, the significant reductions in both root length and plant height compared with the control strongly underline the phytotoxic effects of herbicide residues

in soil, with the most severe impacts observed at higher application doses.

Herbicide residual effects on cotton shoot and root biomass

The analysis of mean squares (Table 2) revealed that, unlike most other measured plant indices, the interaction between herbicide type and dose did not have a statistically significant effect on shoot and root biomass (fresh or dry) at the 5% probability level. Notably, root dry weight was also unaffected by herbicide type alone. However, the analysis clearly indicated that

Table 3 Pearson's correlation coefficients between plant growth parameters

	Herbicide	Dose	Chlorophyll index	Shoot biomass		Root biomass		Plant height	Root length	Seedling emergence	Nitrogen	Phosphorus	Potassium
				Fresh	Dry	Fresh	Dry						
Herbicide	1												
Dose	0	1											
Chlorophyll index	-0.33*	-0.90**	1										
Fresh shoot	-0.20	-0.86**	0.87**	1									
Dry shoot	-0.29	-0.88**	0.93**		1								
Dry root	-0.19	-0.84**	0.85**	0.84**		0.89**	1						
Fresh root	-0.24	-0.88**	0.92**	0.86**		0.87**							
Plant height	-0.22	-0.84**	0.88**	0.87**		0.91**		1					
Root length	-0.52**	-0.68**	0.87**	0.83**		0.84**	0.71**	0.82**	1				
Seedling emergence	-0.41*	-0.84**	0.97**	0.73**		0.80**	0.69**	0.78**		1			
Nitrogen	-0.32**	-0.93**	0.97**	0.88**		0.92**	0.85**	0.89**	0.86**		1		
Phosphorus	-0.39**	-0.88**	0.97**	0.88**		0.92**	0.85**	0.91**	0.86**	0.95**		1	
Potassium	-0.36**	-0.90**	0.95**	0.85**		0.95**	0.86**	0.91**	0.86**	0.96**	0.98**		1
				0.88**		0.93**	0.85**	0.90**	0.84**	0.96**	0.98**	0.97**	

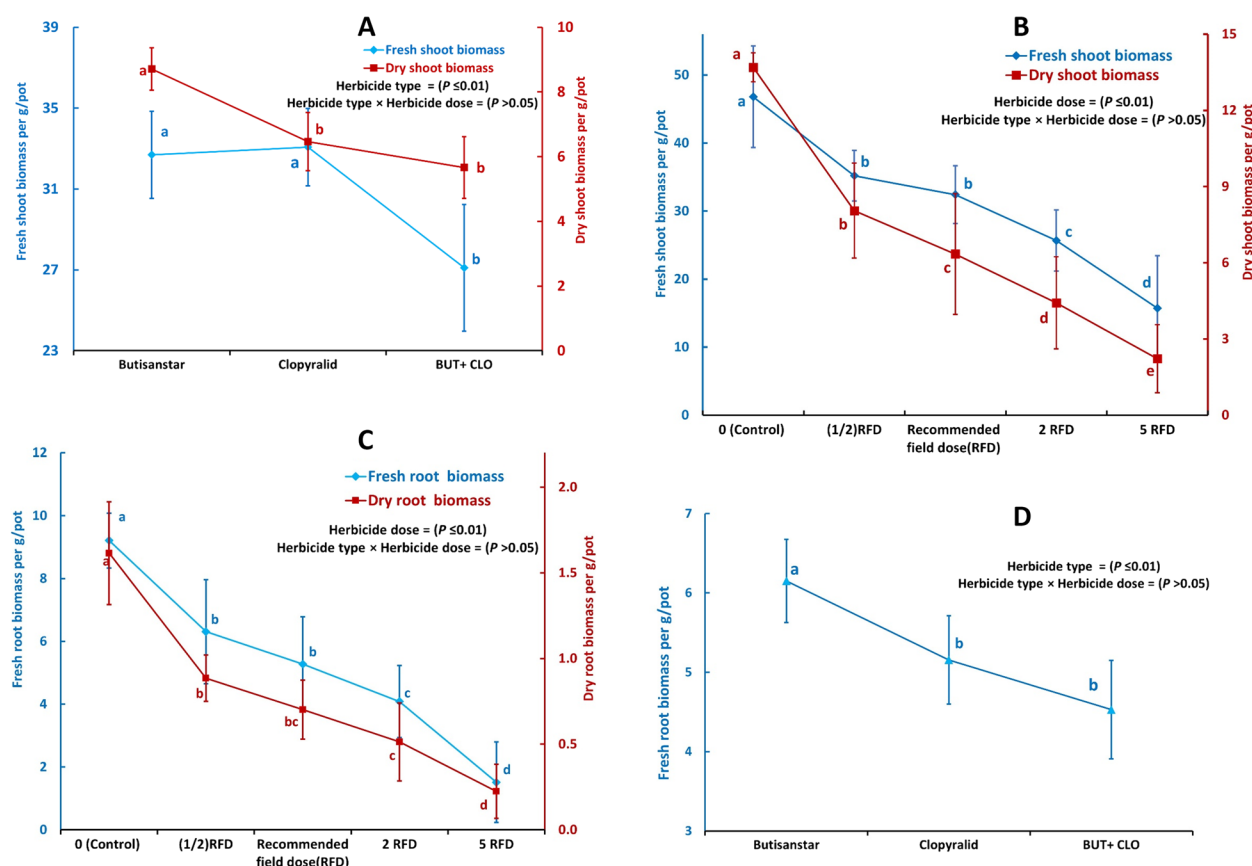


Fig. 4 Impact of various doses (zero, recommended field dose (RFD), ½RFD, 2RFD, and 5RFD) of butisanstar (BUT) (A), clopyralid (CLO) (B), and in combination (C) on the fresh and dry biomass of cotton shoots and roots. The line bars represent the means of three replicates $\pm$ standard deviations, with different letters indicating significant differences (Duncan's test; $P \leq 0.05$)

herbicide dose had a stronger influence than herbicide type on both shoot and root biomass. In line with this, a strong negative correlation was observed between herbicide dose and shoot biomass (fresh $r = -0.86^{**}$; dry $r = -0.88^{**}$) as well as root biomass (fresh $r = -0.84^{**}$; dry $r = -0.88^{**}$), suggesting a direct inverse relationship between herbicide dose and biomass accumulation. In contrast, a weak negative correlation was found between biomass and herbicide type, with correlation coefficients of $r = -0.20^{**}$ (fresh) and $r = -0.29^{**}$ (dry) for shoot biomass, and $r = -0.19^{**}$ (fresh) and $r = -0.24^{**}$ (dry) for root biomass (Table 3).

Even at half the recommended field dose (½ RFD), herbicide treatments caused a noticeable decline in both fresh and dry biomass compared with the untreated control. As shown in Fig. 4A and D, the highest shoot and root biomass values were recorded under the BUT treatment (shoot: fresh 32.7 g.pot⁻¹, dry 8.7 g.pot⁻¹; root: fresh 6.1 g.pot⁻¹). In contrast, the lowest values were found under the combined herbicide treatment (shoot: fresh 27.1 g.pot⁻¹, dry 5.7 g.pot⁻¹; root: fresh 4.5 g.pot⁻¹).

When examining dose levels, the maximum biomass was recorded in soils treated with ½ RFD (shoot: fresh 35.2 g.pot⁻¹, dry 8.1 g.pot⁻¹; root: fresh 6.3 g.pot⁻¹, dry 0.89 g.pot⁻¹), while the minimum biomass was observed at 5RFD (shoot: fresh 14.7 g.pot⁻¹, dry 2.2 g.pot⁻¹; root: fresh 1.52 g.pot⁻¹, dry 0.23 g.pot⁻¹) (Fig. 4B and C).

Herbicide residual effects on cotton shoot nutrient concentrations

Although some studies have reported increased nutrient concentrations in response to herbicide application (Hala et al. 2011; Jaysawal et al. 2023), the present study demonstrated that herbicide residues significantly reduced the concentrations of N, P, and K in cotton shoots (Fig. 5). These reductions were statistically significant ($P < 0.01$) even at the ½ RFD level, with further declines observed at 5 RFD. N level reductions ranged from 5% of ½ RFD treatment to 37% at 5 RFD of BUT treatment, 11% to 40% in CLO treatments, and 19% to 48% for the CLO + BUT treatments (Fig. 5A).

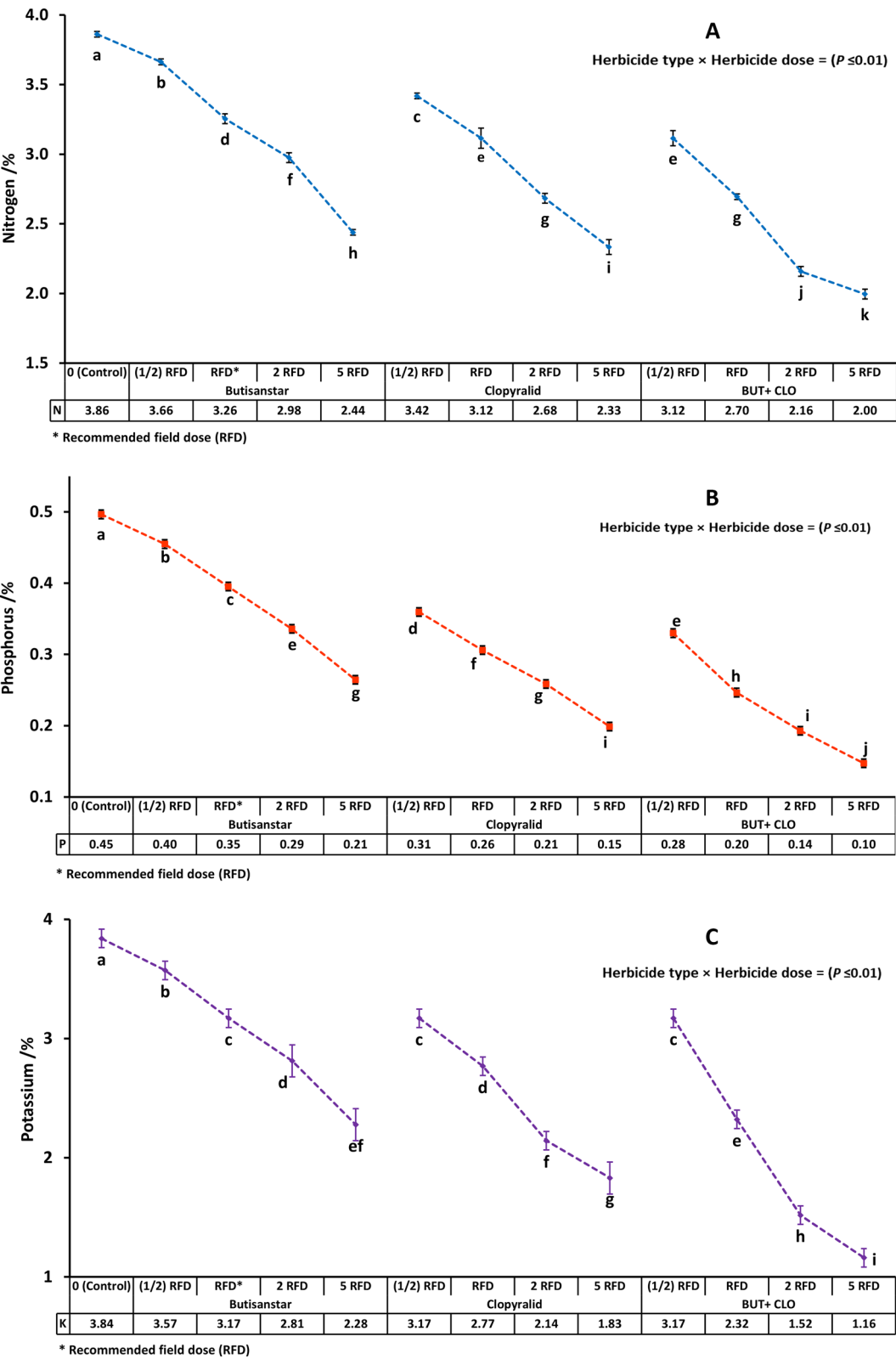


Fig. 5 Impact of various doses (zero, recommended field dose (RFD), ½RFD, 2RFD, and 5RFD) of butisanstar (A) and clopyralid (B), and in combination (C) on nitrogen, phosphorus, and potassium concentrations in shoots. The line bars represent the means of three replicates ± standard deviations, with different letters indicating significant differences (Duncan's test; $P \leq 0.05$)

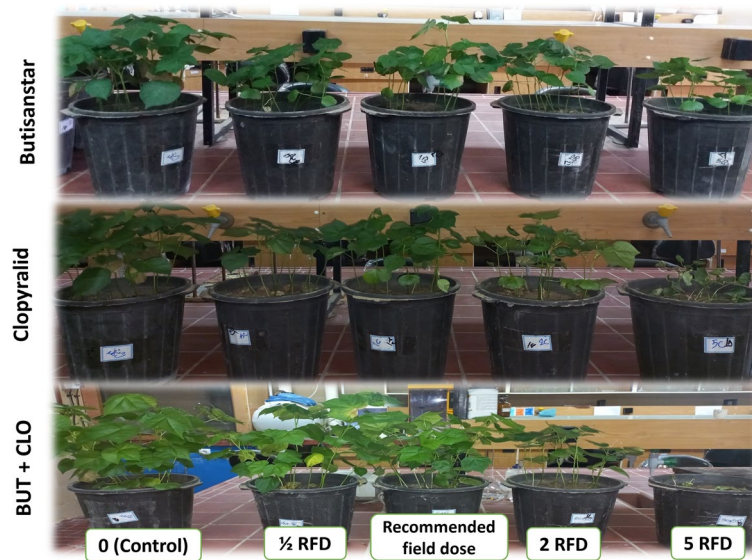


Fig. 6 Cotton growth in soil treated with various doses of butisanstar and clopyralid herbicides at the experiment pot

Similarly, P levels decreased by 11% to 53% of BUT treatments, 31% to 67% of CLO treatments, and 38% to 78% under the combined treatments (Fig. 5B). K concentrations declined by 7% to 41% with BUT treatments, 17% to 52% with CLO treatments, and 17% to 70% under the CLO + BUT treatments (Fig. 5C). These findings clearly indicated that the combined residual effects of CLO and BUT resulted in the greater nutrient depletion than either herbicide alone. A strong negative correlation was observed between herbicide dose and shoot nutrient concentrations, including N ($r = -0.93^{**}$), P ($r = -0.88^{**}$), and K ($r = -0.90^{**}$), indicating an inverse relationship between herbicide dose and nutrient uptake (Table 3). In contrast, herbicide type exhibited a weaker negative correlation with nutrient concentrations: N ($r = -0.32^{**}$), P ($r = -0.39^{**}$), and K ($r = -0.36^{**}$). These results suggested that herbicide dose exerts a stronger influence on nutrient depletion in cotton shoots than herbicide type.

Figure 6 illustrated the growth status of cotton plants at 40 days after sowing in the experimental pots. Visual assessments clearly showed that increasing herbicide doses, regardless of the herbicide type, led to smaller leaves, incomplete morphological development, and weaker stem structure. Among all treatments, the 5RFD of BUT + CLO treatment produced the most severe inhibitory effect, resulting in the poorest overall plant growth. These findings highlight the substantial phytotoxicity of herbicide residues, particularly the adverse effect of CLO on cotton development. The persistence of these herbicides in soil allowed their toxic effects to remain active throughout the 90 days incubation period,

ultimately leading to significant growth suppression in cotton.

Pearson's correlation coefficients

The data presented in Table 3 demonstrated a statistically significant negative correlation ($P < 0.01$) among plant indices and herbicide doses, whereas the correlation with the herbicide type was weaker. This indicated an inverse relationship between herbicide doses and plant indices. Additionally, the higher correlation values associated with the herbicide doses suggested that plant indices are more strongly influenced by the dose than by the type of herbicide. In line with this, the greater mean square values for the "herbicide dose" factor, as shown in Table 2, highlight its more substantial impact on plant indices compared with the "herbicide type" factor.

Discussion

Evaluating the residual effects of herbicides on crop cultivation is essential for preventing unintended damage to subsequent crops and maintaining environmental sustainability. Herbicides differ in their soil persistence, with some exhibiting extended longevity that enables them to remain active throughout the growing season to be absorbed via plant roots (Janaki et al. 2015; Mohammadnezhad et al. 2017). However, this prolonged persistence can become a risk, particularly in rotational systems, by causing phytotoxicity to sensitive follow-up crops (Moyer et al. 2001). The current study demonstrated that BUT and CLO exhibit considerable residual activity, leading to significant reductions

in plant growth indices compared with the untreated control. These effects were dose-dependent, with more pronounced phytotoxicity observed at higher herbicide concentrations.

Among the two herbicides tested, CLO had a stronger inhibitory effect than BUT, a difference likely attributable to their distinct chemical stability and soil half-lives. Although both herbicides are designed to control broadleaf weeds, their non-selectivity toward other broadleaf species such as cotton makes crops in the same category particularly vulnerable. This vulnerability is especially pronounced during early growth stages, where direct root contact with contaminated soil can impair seed emergence and root elongation. The root system was particularly affected, supporting the idea that roots are sensitive and reliable indicators of herbicide-induced phytotoxicity (Mehdizadeh et al. 2019). Studies such as Mehdizadeh et al. (2016) have shown similar findings, reporting that sulfosulfuron and tribenuron-methyl residues reduced both root and shoot biomass in canola and sugar beet. A compromised root system impairs water and nutrient absorption, initiating a cascade of physiological and biochemical disturbances. These observations align with Felix et al. (2005), who found that CLO and flumetsulam significantly damaged rotational potato crops in corn systems. Similarly, Carriquiry et al. (2024) reported that herbicide mixtures such as glyphosate + dicamba + clethodim and flumioxazin + metolachlor reduced biomass accumulation, nutrient cycling, and root nodulation in soybean. Notably, elevated doses hindered P uptake and reduced P concentrations, even when applied at recommended field rates. Additionally, some herbicide residues can disrupt soil biological properties, including microbial abundance and enzymatic functions, ultimately affecting soil fertility and crop productivity (Nguyen et al. 2018; Sun et al. 2022; Wang et al. 2024). The negative impact on microbial populations may further amplify the direct phytotoxic effects on plant performance. The residual presence of herbicides also contributes to abiotic stress conditions, which compromise plant metabolism, nutrient uptake, and photosynthetic efficiency. These factors were evident in the reductions in chlorophyll index, biomass, and plant height observed in this study. Herbicides may affect nutrient acquisition by disrupting root-microbe interactions (e.g., rhizobial symbiosis), nutrient cycling processes (e.g., nitrogen, phosphorus, sulfur), organic matter decomposition, and root system development. As a result, nutrient deficiencies further decrease crop productivity (Jaybhaye et al. 2020; Brochado et al. 2023; Carriquiry et al. 2024). The combination of direct phytotoxic effects and microbial disruption intensifies the detrimental consequences on cotton growth. Supporting this, field studies on CLO and

dicamba have shown reduced tuber yield and biomass in potatoes due to their long half-lives in soil (Seefeldt et al. 2014). Similarly, herbicides such as chlorsulfuron, triasulfuron, CLO, and pyroxasulfone, when used in wheat fields, significantly reduced nodulation, nitrogen fixation, shoot weight, root length, and total biomass in legumes grown under dryland conditions, even 8–10 months post-application (Yates et al. 2024).

Conclusion

The persistence of herbicides in soil is a critical factor affecting agricultural productivity, particularly in rotational cropping systems. The results of this study confirm the phytotoxic effects of BUT and CLO, with CLO showing a more pronounced residual impact due to its longer persistence. The observed dose-dependent decline in cotton growth traits underscores the potential risks of residual herbicides in soil. To mitigate these adverse effects, it is essential to consider the herbicide application history before planting cotton. If CLO or BUT was previously applied to crops such as cereals, canola, or sugar beet, cotton as the following rotation crop is not recommended. An appropriate waiting interval should be implemented to allow for degradation of residual herbicides. In addition, the application of organic soil amendments can enhance microbial activity, which may in turn accelerate herbicide breakdown. These amendments also promote healthier plant development and greater stress tolerance, thereby helping to reduce the phytotoxic impacts on cotton. For future research, it is advisable to explore the potential of engineered nanoparticles in enhancing the degradation of herbicide residues and minimizing their adverse effects on sensitive crops like cotton, given the recent advancements in nanotechnology-based soil remediation.

Abbreviations

BUT	Butisanstar herbicide
CLO	Clopyralid herbicide
BUT + CLO	Butisanstar + Clopyralid
RFD	Recommended field dose
SE	Seedling emergence

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Authors' contributions

All authors contributed to the study conception and design. Investigation, laboratory analysis, data collection, and statistical analysis were performed by Soltani Toularoud AA and Rouhi-Kelarlou T. Shapes and graphics were drawn, the original draft was translated, and the manuscript was edited by Rouhi-Kelarlou T, Golchin A. All authors read and approved the final manuscript.

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Data availability

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

Declarations**Ethics approval and consent to participate**

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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