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Sequential and combined spray of herbicides to tomato field on weed reduction, fruit parameters and carryover residues

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Abstract: Tomatoes are in great demand worldwide and consumed due to their nutritional and sensory qualities. Weed infestation poses a great challenge in tomato production, prompting growers to employ two to three herbicides in combinations and sequences for comprehensive control. Consequently, this study was undertaken to investigate the effects of glyphosate, pendimethalin, and metribuzin when applied individually or in sequential combinations in tomato fields. The herbicides significantly reduced the weed density and dry biomass and enhanced the weed control efficiency (WCE) compared to control. A tank mix spray of pendimethalin and metribuzin following glyphosate gave significantly higher WCE (80–91%) and fruit yield (88.47 t/ha). The tomato quality parameters were unaffected by the herbicides. The terminal residues in fruits were found below the safe limit of 0.1 mg/kg for glyphosate and 0.01 mg/kg for pendimethalin and metribuzin. Moreover, there was no evidence of residual carryover toxicity from the applied herbicides, as confirmed by the plant bioassay and instrumental techniques. However, continuous spraying of herbicides repeatedly in succession and in combination necessitates long-term monitoring to assess the potential development of herbicide-resistant weeds, the bio-magnification of residues in soil, their transfer to tomato fruits and the impact on the food chain.

Keywords: fruit quality; herbicides persistence; phytotoxicity; tank mix; weed control

Tomatoes are grown in an area of 85.20 million ha with a production of 21 t and productivity of 2.47 kg/ha (FAOSTAT 2021) in India. It is a significant staple daily diet across the world for being a rich source of health-promoting bioactive constituents such as lycopene, ascorbic acid, and β-carotene, besides culinary purposes. It is valued for having potent antioxidants (Yin et al. 2019) and anticancer prop-

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erties (Sgherri et al. 2008). The tomato crop is very sensitive to weed competition, especially during the early stages after transplanting, with severe effects on its growth, development, and flowering, resulting in a significant loss of yield and quality (Olayinka et al. 2017; Mennan et al. 2020). For broad-spectrum weed control, multiple herbicides are used sequentially and in combination as they cause 30–40% crop yield loss (Rao, Chauhan 2015). Effective control of weed flora without yield loss by sequential application of pre- and post-emergence herbicides has also been reported in rice (Singh et al. 2016; Ramesh et al. 2017; Janaki et al. 2019).

In India, herbicides such as glyphosate, pendimethalin, and metribuzin are commonly used to control different weed groups from field preparation to crop establishment, sequentially or in combination, due to their selectivity and different modes of action. Glyphosate, a broad-spectrum systemic herbicide and desiccant, is widely used on both cultivated and uncultivated land. It is degraded in most soils with an estimated half-life of 7 to 60 days, mainly by microbial mediation. The rate and extent of degradation are influenced by sorption behaviour, which depends on soil properties such as organic matter, clay content, microbial activity, mineralisation, etc. (Kanissery et al. 2019). Metribuzin controls weeds by inhibiting photosynthesis and is sprayed as pre- and post-emergence in crops. It is moderately adsorbed to soil particles, and adsorption decreases as soil pH increases (Peek, Appleby 1989), with moderate persistence. Unlike glyphosate, metribuzin exhibits high mobility in soil and is subject to photolysis depending on soil type and climatic conditions. It is mostly biodegraded with a half-life of 30 to 120 days in different soils (Wauchope et al. 1992). Also, a half-life of 9.11 to 21.15 days in tomato-cultivated neutral to moderately alkaline Indian soils was documented (Saritha et al. 2017). Pendimethalin is sprayed to curtail annual grasses and certain broadleaf plants. It is immobile in soil and degrades in the environment by binding to the soil, microbial-mediated metabolism, and volatilisation (USEPA 1997; Kaur, Bhullar 2015). It has moderate to high persistence and has restricted lateral and downward movement with a field half-life of 30 days (Lee et al. 2000). Additionally, its half-life in soil depends mainly on the time interval between application and the first rain event and ranges from 10.5 to 31.5 days (Alister et al. 2009).

In the published literature, the effects of individual herbicides on weed reduction, the persistence of residues in the soil, and product quality have been documented primarily for field crops and only a few for vegetables such as potatoes, peas, onions, garlic, etc. Little information has been compiled and published from the tomato-grown field. Hence, the current study was conducted to understand the effects of sequential and combined application of three herbicides on weed control efficiency (WCE), residue persistence, and yield and quality of tomatoes under semi-arid Indian conditions.

MATERIAL AND METHODS

Field and herbicides details. The field trial was conducted during the winter season (October 2022 – January 2023) at Devarayapuram, Coimbatore (11°00'13.08" North longitude, 76°48'5.64" East latitude). Tomato F1 hybrid ‘Darsh Gold’ (*Solanum lycopersicum*) purchased from a local agrochemical shop was cultivated as an experimental species. The selected field was divided into 21 plots (each 3 × 3 m^2 in size), and treatments were imposed in triplicate in a randomised block design. The treatments were glyphosate alone (GLY), pendimethalin alone (PEND), metribuzin alone (METRI), glyphosate followed by pendimethalin (GLY + PEND), glyphosate followed by metribuzin (GLY + METRI), glyphosate followed by pendimethalin and metribuzin as a tank mix (GLY + PEND + METRI), and control (no herbicide). Ten days prior to transplanting (weeds were in the active growth stage with 5–6 leaves) of tomato seedlings, GLY 7.5 L/ha (GLYPH PRO 41% SL) was sprayed as a pre-plant herbicide to facilitate field preparation and reduce weed threat in the tomato field according to the treatments. Tomato seedlings were planted in ridges 60 × 30 cm apart after irrigation. On the third day after transplanting, PENDI 1000 mL/ha (STOMP 50% EC) and METRI 500 g/ha (TATA metri 70% WP) were sprayed as pre-emergence herbicides according to the treatments. The herbicides were sprayed on with a flat fan nozzle fitted in a backpack sprayer, and the amount of water was calculated based on the recommended spray rate of 500 L/ha in India. Soil samples were collected from the experimental field before commanding treatments and processed with a 2-mm sieve to remove debris and heterogeneity. The processed homogeneous soils were subsampled and analysed for

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their physicochemical properties. The soil is classified as Alfisol and has a sandy loam texture, a medium content of available nitrogen (263 kg/ha) and a high content of available phosphorus (30 kg/ha) and potassium (420 kg/ha). The soil is non-saline [EC (electrical conductivity) 0.22 dS/m], alkaline reactive (pH 7.55), and has a CEC (cation exchange capacity) of 12.8 C.mol (P^+)/kg soil.

Metrological conditions. Weather parameters that prevailed during tomato cultivation from planting to first fruit harvest were documented weekly (Figure 1). The maximum and minimum temperatures recorded were 27.92–30.86 °C and 20.25–22.66 °C, respectively. A total of 301.50 mm of precipitation fell on 32 days, ranging from 0.50–69.002 mm. The weekly average duration of sunshine ranged from 1.18 to 7.40 hours.

Weed parameter data. Weeds were recorded species-by-species in a 1 m × 1 m quadrant from four randomly selected plants in each plot. Weeds that fell within the quadrant frame were counted, and mean values were expressed in numbers per m^2 (Nos./m^2). The density of grasses, sedges, broadleaf weeds and total weeds was recorded 40 days after transplanting (DAT) and expressed in Nos./m^2 after square root transformation (Dey, Pandit 2020). Samples were dried in the shade and later in a hot air oven at 80 °C for 72 h and then weighed. The dry weight was expressed in g/m^2. WCE was calculated according to the procedure described by Mani et al. (1973).

$$WCE\% = (WDc - WDt)/WDc \times 100\% \quad (1)$$

where: *WCE* – weed control efficiency (%), *WDc* – weed biomass (g/m^2) in the control plot, *WDt* – weed biomass (g/m^2) in the treated plot.

Tomato growth and yield. At the end of the growing season (60 days after tomato planting), the plant height in each treatment was measured in cm from the ground to the plant tip. The number of days to 50% flowering and the total number of fruit per cluster were counted in each treatment, and the average was calculated. Half-ripened tomato fruits were picked and weighed from each plot during each harvest. A total of six harvests were conducted, and the combined fruit weight from each treatment during each harvest was calculated and expressed in t/ha.

Tomato quality. The tomato fruits picked during the first harvest (75 DAT tomato plants) were used to determine several tomato fruit quality parameters. These include total soluble solids (TSS) content and pH, ascorbic acid content (mg/100 g), titratable acidity (%), lycopene content (mg/kg), β-carotene content (mg/100 g), and fruit shelf life, which were determined adopting published methods (Ranganna 1986; Siueia et al. 2020).

Carryover toxicity assessment. Replicated treatment-wise soil from the tomato experimental field was collected after the fifth harvest, covering the surface to 15 cm depth. It was used to conduct the plant bioassay in the pot using indicator crops, such as sun-

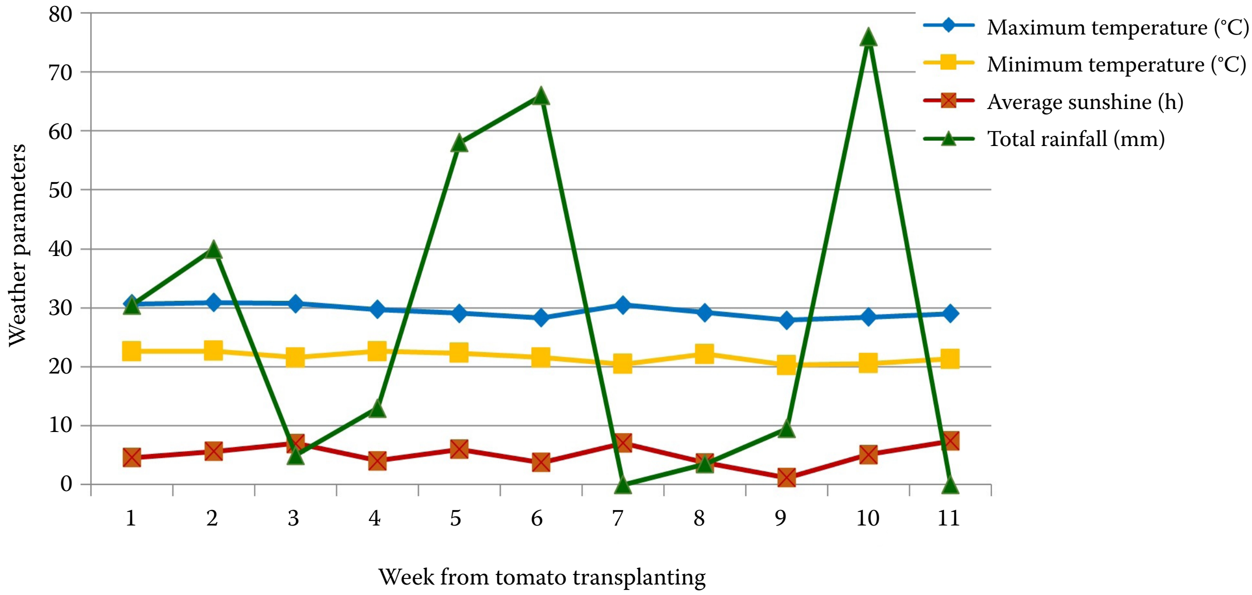

Figure 1. Weather parameters existed during the tomato-planted period

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flower and green gram. About 250 g of soil was filled in a 1/2 kg pot in 3 replications for each species and compacted to achieve the field bulk density. Then, the indicator species (10 per pot) were sown after watering to field capacity. The carryover toxicity to the succeeding crops was tested by growing them for up to 20 days and monitoring for variation in germination, plant height, and occurrence of herbicide toxicity symptoms, viz., yellowing, curling, cupping, twisting, etc., using visual scoring (Bhargavi et al. 2024). On the 20^{th} day, the plants were removed from each pot, and the total fresh biomass per pot was weighed.

Herbicides residue appraisal. Soil samples were randomly collected at the time of the first harvest of tomato fruits from a depth of 0–15 mm at 5 locations in each plot, pooled and processed for analysis (Janaki et al. 2016). Tomato fruits were harvested from five selected plants covering the entire plot, leaving out the outer edge, and stored at –4 °C after homogenisation and volume reduction. The fruits were crushed with pestle and mortar, sampled and then extracted for herbicide residues. Residues of PENDI and METRI were extracted from soil and tomato fruits and analysed according to the methodology outlined by Yerra et al. (2023). GLY was extracted and analysed using liquid chromatography (LC 20 model, Shimadzu Corporation, Japan), as detailed by Brindhavani et al. (2020). The accuracy, repeatability, and linearity of the extraction and detection techniques were validated by performing recovery studies using blank soil and tomato fruit of the control plot as described by Janaki et al. (2016) for bensulfuron methyl.

Residues were analysed using Shimadzu ultra-fast liquid chromatography (UFLC) equipped with a quaternary pump, thermo-stated column, autosampler and PDA/MS detector. PENDI and METRI were separated using an Agilent C18 column (4.6 × 150 mm, 5.0 μm) and acetonitrile: water as the mobile phase (70 : 30 v/v @ 0.8 mL/min flow rate for PENDI; 80 : 20 v/v @ 0.7 mL/min flow rate for METRI) by injection of 10 μL of sample, keeping the thermostat at 40 °C. PENDI and METRI were detected at 239 and 280 nm, respectively, in the PDA detector (Figure 2), with the identified retention time of 7.35 + 0.2 min and 5.05 + 0.2 min, respectively. GLY was separated using an Agilent HILIC column (4.6 × 100 mm, 3.5 μm) and determined in a UFLC with MS detector (Figure 3). Acetonitrile: 0.1% formic acid (95/5%) was used

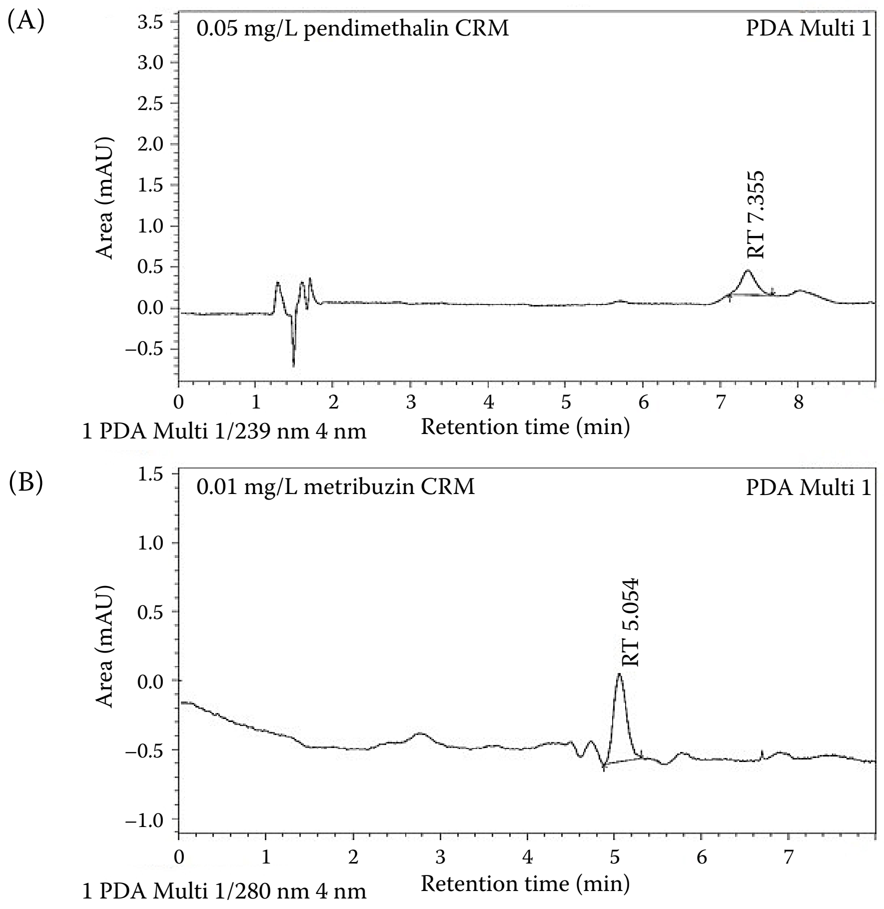

Figure 2. Standard chromatograms of (A) pendimethalin and (B) metribuzin detected by UFLC-PDA

UFLC-PDA – ultra-fast liquid chromatography-photo diode array detector; CRM – certified reference material; RT – retention time

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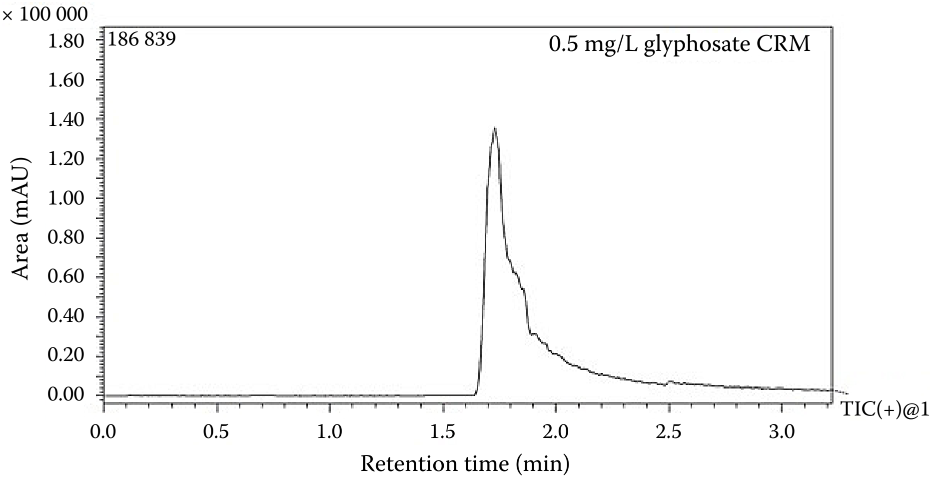

Figure 3. Standard chromatogram of glyphosate detected by LC-MS-MS
LC-MS-MS – liquid chromatography-double mass spectrometer; CRM – certified reference material; TIC – total ion chromatogram

as the mobile phase at a flow rate of 0.4 mL/min and an injection volume of 10 μL.

Statistical analysis. Data collected during the study were analysed using the R statistical software version 4.2.2. package doebioresearch using two-way analysis of variance (ANOVA). The Duncan multiple-range test (DMRT) was used to compare the means, with statistical significance determined at $P < 0.05$. Graphical plotting was also performed with R statistical software. Correlations between variables were assessed using Pearson correlation coefficients and probability analyses.

RESULTS AND DISCUSSION

Weed flora, weed density and biomass. Weed flora viz., *Trianthema portulacastrum*, *Parthenium hysterophorus*, *Cyperus rotundus*, *Alternanthera bettzickiana* (Regal) Voss, a spreading perennial, and *Cynodon dactylon* belongs to broadleaf sedges, weeds, and grasses were observed in the experimental field. Weed density and biomass were examined on 30 and 45 DAT tomato, and found that different herbicide sprays significantly changed weed parameters (Tables 1 and 2).

Spraying METRI or PENDI alone or in sequence following GLY led to significant reduction in both broadleaf and sedges density (15.70–18.40 No./m^2) and dry weight (9.90–13.08 g/m^2) at 30 DAT compared to GLY (21.05 No./m^2; 15.91 g/m^2) or no herbicide (22.40 No./m^2; 22.77 g/m^2). Similar trends were observed on 45 DAT. Effective reduction in weed density and dry biomass was also observed up to 45 DAT when METRI and PENDI were applied sequentially after GLY (Figures 4 and 5). On average, a reduction of 14, 39, 43, 46, and 47% in weed density and 25, 38, 41, 49, and 44% in weed dry weight was observed, respectively, in the GLY, PENDI, METRI,

Table 1. Effect of sequential and combined spray of herbicides on weed density (No./m^2) recorded in tomato field

Treatments	30 DAT*				45 DAT*			
	BLW	sedges	grasses	total	BLW	sedges	grasses	total
Glyphosate (GLY)	11.70	7.35	2.00	19.05	12.29	8.83	2.24	21.12
Pendimethalin (PENDI)	6.86	7.14	1.73	14.00	8.60	8.06	1.73	16.66
Metribuzin (METRI)	5.66	7.42	1.73	13.07	7.07	8.12	1.73	15.20
GLY + PENDI	5.66	6.86	0.00	12.51	6.71	7.86	1.73	14.57
GLY + METRI	5.39	6.71	0.00	12.09	6.63	7.35	1.73	13.98
GLY + PENDI + METRI	4.58	6.56	0.00	11.14	6.40	7.35	1.73	13.75
Control (no herbicide)	14.21	8.19	0.00	22.40	17.38	10.10	2.45	27.48
Mean	7.72	7.17	1.03	14.89	9.30	8.83	1.90	17.53
CD (P = 0.05)	0.094	0.493	0.013	0.103	0.266	0.074	0.039	0.210

CD – critical difference; BLW – broadleaf weeds; DAT – days after transplanting
*square root transformed values (original density +2)

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Table 2. Effect of sequential and combined spray of herbicides on weed dry weight (g/m2) recorded in tomato field

Treatments	30 DAT*				45 DAT*			
	BLW	sedges	grasses	total	BLW	sedges	grasses	total
Glyphosate (GLY)	8.19	5.83	1.89	14.02	9.66	4.98	1.91	14.63
Pendimethalin (PENDI)	5.20	6.08	1.81	11.28	7.28	4.76	1.82	12.04
Metribuzin (METRI)	5.10	5.48	1.77	10.58	6.48	4.75	1.77	11.23
GLY + PENDI	5.00	4.90	0.00	9.90	6.00	4.64	1.41	10.64
GLY + METRI	4.58	5.92	1.69	10.50	5.73	4.62	1.71	10.35
GLY + PENDI + METRI	2.45	4.47	1.62	6.92	5.31	4.36	1.69	9.67
Control (no herbicide)	14.21	6.56	2.00	20.77	13.96	6.08	2.27	20.05
Mean	6.39	5.60	1.54	11.99	7.77	4.88	1.79	12.65
CD (*P* = 0.05)	0.197	0.061	0.089	0.317	0.178	0.172	0.022	0.411

CD – critical difference; BLW – broadleaf weeds; DAT – days after transplanting
*square root transformed values (original density +2)

GLY + PENDI, and GLY + METRI treatments compared to the control.

Tomato growth and fruit yield. Tomato growth parameters, i.e. seedling establishment and phytotoxicity symptoms such as necrosis, scaling or curling of leaves, distortion, and stunting, were not observed by the herbicide spray. As they were applied at the recommended dose and sequentially, the plant did not exhibit any symptoms of phytotoxicity. However, variations were observed in plant height and

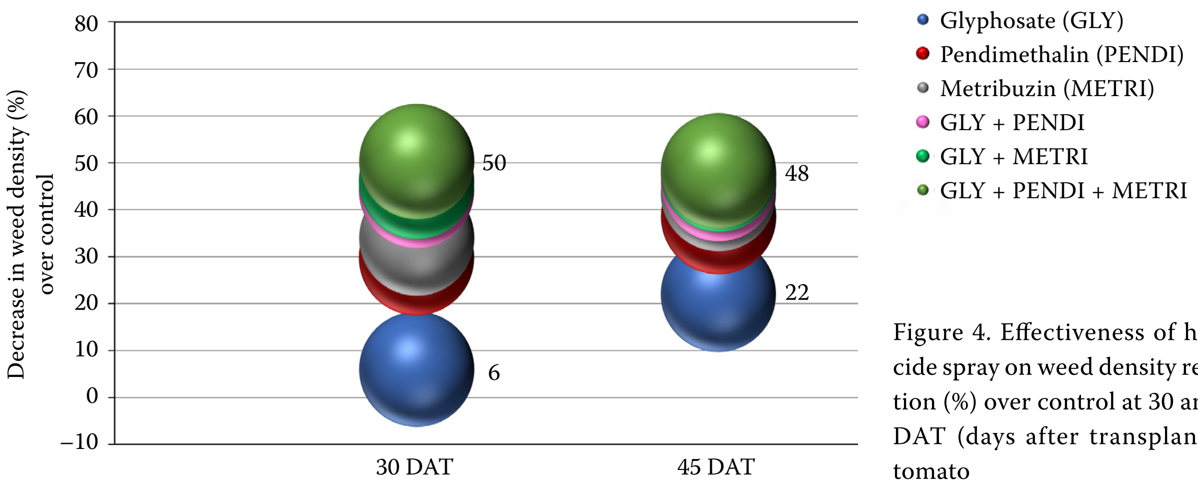

Figure 4. Effectiveness of herbicide spray on weed density reduction (%) over control at 30 and 45 DAT (days after transplanting) tomato

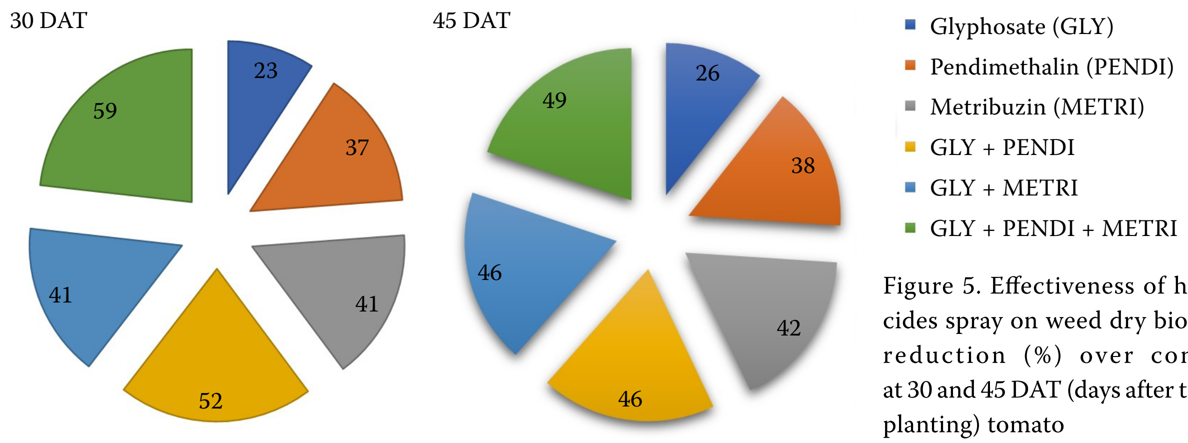

Figure 5. Effectiveness of herbicides spray on weed dry biomass reduction (%) over control at 30 and 45 DAT (days after transplanting) tomato

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number of clusters. Among the three herbicides, a pre-emergence spray of METRI resulted in better growth and higher fruit yield and was on par with PENDI alone. Similarly, when PENDI or METRI was sprayed following GLY, there was an increase in plant height and number of clusters per plant (Figure 6) at 45 DAT compared to a single spray of each herbicide. The number of fruits per plant and fruit yield was significantly increased by herbicide spray, and the plants treated with PENDI or METRI following GLY application produced a higher fruit yield of 82.67 and 83.07 t/ha, respectively, compared to their single application (80.20 and 80.47 t/ha, respectively). The effectiveness of combined and sequential spray of PENDI, METRI after GLY was also confirmed by the positive and significant correlation of fruit yield with weed dry weight and tomato growth parameters (Table 3), specially plant height and number of clusters.

Fruit quality and herbicide residue accumulation. The effects of herbicides on tomato fruit quality parameters, such as titratable acidity, β-carotenoids, lycopene, ascorbic acid, TSS, and shelf life, were analysed (data not given). Titratable acidity (%), total soluble salts (%), ascorbic acid (mg/100 g), and lycopene (mg/kg) ranged from 0.51 to 0.56, 4.39 and 4.94, 4.91 and 4.96, 43.87 and 44.98, and 4.43 and 4.71, respectively, and were found to be not significant for herbicides treatment. Fruit harvested at the first harvest was also subjected to herbicide residue analysis and was found below the detectable level (0.05 mg/kg).

Terminal residues in soil and carryover assessment. The soil of the tomato field was tested for herbicide residues at the time of the first harvest and examined for possible residue carryover trends by chromatography technique. Residues of all three herbicides were found to be below the detection limit of 0.05 mg/kg for PENDI and METRI and 0.1 mg/kg soil for GLY.

Plant bioassay results showed (Table 4) that germination percentage of green gram and sunflower did not vary significantly among the herbicide treatments (90–95 %) and control soil, which recorded 100%. The highest germination percentage was recorded by the control and all the herbicides treated soil were on par with the control. Similar

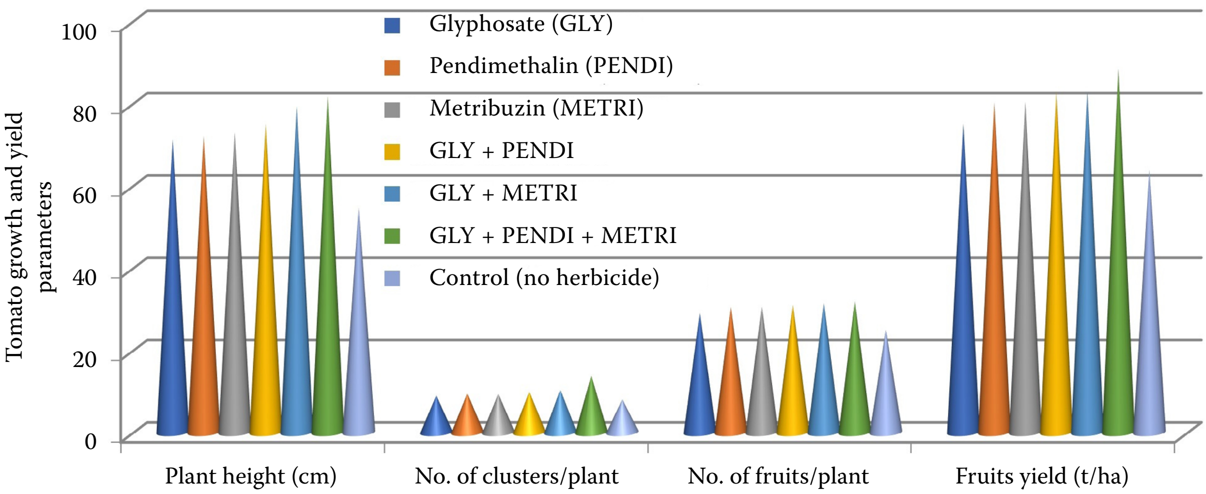

Figure 6. Effect of sequence and combined spray of herbicides on growth and yield parameters of tomato

Table 3. Regression analysis showing the relationship between tomato fruit yield (t/ha) and weed parameters and tomato growth parameters

Growth parameters	Weed biomass (g/m^2) 30 DAT	Weed biomass (g/m^2) 45 DAT	Tomato plant height (cm)	No. of clusters/plant	No. of fruits/plant
Regression equation	$y = -0.536x + 54.88$	$y = -0.491x + 53.48$	$y = 1.086x - 13.53$	$y = 0.294x + 6.59$	$y = 0.186x - 4.844$
R^2	0.875*	0.958**	0.947**	0.964**	0.641

DAT – days after transplanting

*, **significant at 5% level, 1% level, respectively

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Table 4. Residual carryover toxicity of the applied herbicides in tomato fields to the sensitive plant species

Treatments	Green gram			Sunflower		
	germination (%)	plant height (cm)	total biomass (g/pot)	germination (%)	plant height (cm)	total biomass (g/pot)
Glyphosate (GLY)	95	7.7	3.84	90	9.8	6.92
Pendimethalin (PENDI)	95	7.7	3.88	95	10.1	6.72
Metribuzin (METRI)	90	7.5	3.76	90	9.7	6.84
GLY + PENDI	95	8.1	3.85	95	10.2	6.56
GLY + METRI	90	8.0	3.81	90	9.9	7.04
GLY + PENDI + METRI	95	8.1	3.95	95	9.9	6.65
Control (no herbicide)	100	8.2	4.01	100	11.1	7.14
Mean	94	5.6	3.90	94	10.1	6.80
CD (P = 0.05)	ns	ns	ns	ns	ns	ns

CD – critical difference; ns – non-significant

results were noticed with plant height (7.5–8.2 cm and 9.7–11.1 cm for green gram and sunflower) and total biomass (3.76–4.01 g and 9.7–11.1 g for green gram and sunflower) of both the test species in all the herbicides treated soil. However, the control (no herbicide) treated soil exhibited higher values.

The tomato holds a significant position in global agriculture due to its widespread demand and consumption, which are attributed to its nutritional and sensory attributes. Yet, tomato production encounters significant hurdles in weed management, leading growers to employ herbicides either sequentially or in combinations to attain comprehensive weed control. Despite their extensive use, the potential impacts of these herbicides on weed reduction, tomato growth, fruit quality, and residue carryover have remained largely unknown. Our study sought to shed light on these critical aspects by investigating the effects of GLY, PENDI, and METRI when applied sequentially and in combination in tomato fields.

The weed density and biomass on 30 and 45 DAT tomato were significantly controlled by the herbicides applied sequentially. In particular, the density and dry biomass of grasses were very low and absent. Although GLY was applied as a pre-plant herbicide to aid in field preparation and weed load reduction, the subsequent pre-emergence spray of PENDI or METRI after GLY effectively suppressed the weed growth during the critical tomato growing period (up to 45 days). This showed the efficiency of herbicide combinations in eliminating weed species while creating unfavourable situations for the development of weed resistance-specific herbicides. The studied combination of GLY following PENDI and METRI is an excellent choice for weed control programs in tomatoes as it reduces selection pressure on weeds. Tiwari et al. (2017) also reported improved weed control, higher rice yield, and lower weed biomass by the combined spray of GLY, PENDI and METRI at the recommended doses compared to their individual applications. Maximum WCE of 91 and 80% was achieved on 30 and 45 DAT, respectively, by spraying GLY as pre-plant followed by a tank mix of PENDI and METRI as pre-emergence. Among three herbicides, GLY alone achieved a lower WCE of 49–61% on 30 and 45 DAT and PENDI and METRI as a single spray achieved 67–76% and 72–77%, respectively (Figure 7). Although the individual PENDI and METRI spray after GLY achieved 2–8% more WCE, the GLY followed by a tank mix spray of PENDI and METRI increased WCE by 30–32%.

The growth parameters of tomatoes provided significant insights into the effects of herbicide application. Importantly, the cautious application of herbicides at recommended doses ensured the absence of adverse effects, such as phytotoxic symptoms, on the tomato plants. This suggests that the herbicide treatments were compatible with the healthy growth of the tomato crop. However, noticeable variations in plant height and the number of clusters at different growth stages were observed. WCE by PENDI and METRI and higher growth and production in soybean and okra were also documented in previous studies (Rajasree et al. 2017; Alcántara-de la Cruz et al. 2019). Moreover, the number of fruits per plant and fruit yield were significantly

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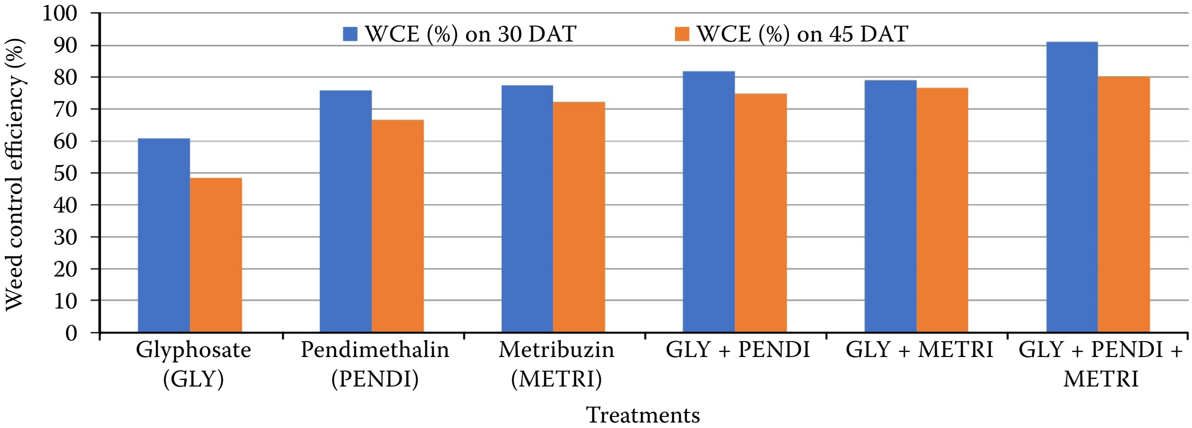

Figure 7. Effectiveness of herbicide spray on weed control efficiency (WCE, %) over control at 30 and 45 DAT (days after transplanting) tomato

increased by the herbicides and the plants treated with PENDI or METRI after GLY application produced higher fruit yield of 82.67 and 83.07 t/ha, respectively, compared to their individual spray (80.20 and 80.47 t/ha, respectively). The higher WCE, reducing competition for resources such as nutrients and water, likely contributed to the increased tomato growth and yield attributes. WCE and higher tomato yield with the sequential quizalofop-ethyl application after pre-emergence PENDI spray was also reported by Reddy et al. (2018).

The tomato fruit quality was unaffected by the herbicides, and such a non-significant effect of herbicides on tomato fruit quality (TSS, pH, etc.) was also previously reported by Mohamed and Ali (1984). However, the β-carotene content (mg/100 g) was low in GLY (1.47) and control (1.45) and ranged from 1.51 to 1.59 in herbicide treatments. These variations could be due to the differences in the duration of fruit formation and ripening, which occurred earlier in herbicide-treated plants due to the increased availability of resources such as nutrients and water and their utilisation, resulting from reduced weed competition. The fruit's shelf life ranged from 14.8 to 14.9 days among all treatments. The non-deleterious effect of herbicide (METRI) on sugar beet quality traits such as sugar content and root production was reported by Abbas et al. (2016).

The analysis of herbicide residue in tomato fruits confirmed that all herbicides remained well below the established maximum residue limit (MRL) of 0.05 mg/kg, as set for various products (cereals, onions, and tomatoes) by FSSAI (2020). Additionally, the PENDI and METRI residues were below the MRL of 0.05–0.2 mg/kg, established by the CODEX (FAO 2021) for various vegetables and 0.1 mg/kg in tomato paste as established by USEPA. GLY residue was also not detected in fruit and was below the MRL of 0.1 mg/kg, set by CODEX (FAO 2021). These findings suggest that the pre-emergence spraying of PENDI or METRI, either individually or sequentially following GLY at recommended doses, is safe for tomatoes, as their residues were well below the established MRL by CODEX and FSSAI. Given that all herbicides were applied at recommended doses, their residues did not persist in tomato fruit at toxic concentrations. Moreover, the plant's ability to metabolise METRI through deamination and subsequent conjugation could have contributed to its residue below MRL (Saritha et al. 2017). Similarly, PENDI, may not have been translocated to the plants, as it rapidly degraded in the soil through the oxidation of 4-methyl group (benzene ring) and *N*-1-ethylpropyl group in the amine moiety (USEPA 1997).

The soil from each treated plot underwent herbicide residue at the time of the first harvest and was examined for potential carryover toxicity. Residues of all three herbicides were found to be below the detection limit (BDL) of 0.05 mg/kg for PENDI and METRI, and 0.1 mg/kg for GLY. Given that GLY is foliar active and was applied 10 days prior to transplanting as a pre-emergence, its residue was BDL. This could be ascribed to its strong sorption to soil complex, resulting in limited availability to organisms due to poor distribution between the aqueous and solid phases. The rapid initial dissipation rate of GLY due to its higher adsorption, associated with clay content and CEC, was documented by Okada et al. (2019). The presence of METRI and PENDI residues at BDL could be attributed to their

https://doi.org/10.17221/41/2024-HORTSCI

degradation into metabolites and nontoxic products by chemical or biological means or loss from soil by volatilisation and leaching, favoured by soil properties and climatic conditions such as precipitation and temperature. Chemical degradation of PENDI by oxidation of benzene ring and *N*-l-ethylpropyl group (USEPA 1997; Tandon 2016) and deamination of METRI to deaminometribuzin (DA) in sandy soils and diketometribuzin by oxidative desulphurisation in clay soils was documented by Khoury et al. (2003). The low organic carbon content (0.50%) of the present field soil and high rainfall (Figure 1) during the second week of tomato planting (40 mm distributed over 5 days), corresponding to 4 days after METRI application, may have increased its loss through leaching. Ready leaching of METRI due to its lower affinity has also been reported by Majumdar and Singh (2007). The absence of detectable PENDI residues in the soil at harvest could be attributed to increased biodegradation or chemical degradation into its metabolites (Kočárek et al. 2016).

While the tested herbicides were found to be BDL (0.1–0.05 mg/kg) in soil, there is a concern regarding the potential carryover toxicity of herbicide residues to the subsequent crops due to desorption from the soil complex. Therefore, a plant bioassay was carried out using soil samples collected from each plot 60 days after tomato planting with green gram and sunflower as test species. The results (Table 4) indicated that the germination percentage of green gram and sunflower 20 days after sowing did not vary significantly among the herbicide treatments (ranging from 90% to 95%) and control soil, which recorded 100% germination. Similar outcomes were observed for plant height and total biomass of both the test species in all the herbicide-treated soil. This suggests the absence of residual phytotoxicity from the applied herbicides. These findings align with the research of Janaki et al. (2015) and Janaki et al. (2019), who reported that PENDI and METRI did not exhibit any carryover toxicity to succeeding crops when applied at recommended doses of 1 000 and 750 g/ha, respectively.

CONCLUSION

In the current study, herbicide applications, particularly those of METRI and PENDI, whether used alone or sequentially following GLY, notably decreased weed density and biomass compared to control and GLY alone, providing effective control for up to 45 days. The herbicide applications positively influenced the tomato yield parameters and fruit yield. Moreover, herbicides showed no significant effect on fruit quality parameters, indicating their safety for tomato cultivation when applied individually and or in combination with sequential spray. Residue levels in the fruit remained below MRL and complied with the residue limits established by various regulatory agencies such as USEPA, FSSAI, and CODEX.

No residue carryover toxicity was observed, as confirmed by both plant bioassay and instrumental techniques, regardless of the application method. Nevertheless, continuous spraying of herbicides as a mixture in repeated sequences on tomato fields warrants long-term monitoring to assess the development of herbicide-resistant weeds, the potential biomagnification of herbicide residues in soil, and their implications for the ecosystem and food chain.

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