https://doi.org/10.17221/223/2023-PSE

# Herbicides and nutrients interaction on earthworm activity in tomato cultivated soil and toxicity appraisal

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**Citation**: Yerra P., Janaki P., Ramalakshmi A., Jagadeeswaran R., Sankari A., Arthanari P.M. (2023): Herbicides and nutrients interaction on earthworm activity in tomato cultivated soil and toxicity appraisal. Plant Soil Environ., 69: 429–436.

**Abstract:** While nutrients are administered through various sources and combinations, herbicides are used for pre-plant and pre-emergence weed control in the tomato fields. Therefore, a study was conducted to understand the effects of nutrient fertilisation through inorganic and organic sources along with farmers practice and the application of three herbicides, namely glyphosate, pendimethalin, and metribuzin, individually or in combinations, on earthworm activity in tomato crops. The herbicides caused a significant reduction in earthworm biomass and escapement to the lower 15 cm depth. Application of pendimethalin and metribuzin to soils inorganically fertilised with major and micronutrients resulted in low survival rates and a high ecological risk quotient. The effect was attenuated when farmyard manure was applied. The study suggests that when 2 to 3 herbicides are applied in succession and combined with inorganic fertilisers as nutrient sources, stringent measures like adding organic nutrient sources, applying the correct herbicide combination, etc., must be followed to reduce their toxicity to earthworms. This helps to protect and sustain earthworm activity and biodiversity in the soil.

**Keywords:** *Solanum lycopersicum* L.; vegetable; pesticide; bioindicator; organic nutrients; tank mixing herbicides

The tomato (*Solanum lycopersicum* L.) is the second most important vegetable grown in the world. In its cultivation, herbicides such as glyphosate, pendimethalin, and metribuzin are frequently used to control various weeds. On the other hand, fertilisers are widely used to promote the growth and production of tomatoes. Therefore, the simultaneous application of two major agro-inputs, namely pesticides and fertilisers, may have either antagonistic or synergistic interactions between them and accordingly affect the non-target organisms such as earthworms in the soil. Several reports have been published on the effects of herbicides on earthworm activity (Stepic et al. 2013) caused by single or sequential applications to field crops such as cereals, legumes, and oilseeds. However, the influence of different herbicides with different nutrient fertiliser application practices on earthworm activity in vegetable-cultivated soils is lacking or very little published.

Earthworms are the most important organisms disturbed by agrochemicals, and these bioindicators help detect the toxic effects of various chemi-

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cals under different conditions in the soil (Lydy and Linck 2003, Schmidt et al. 2023). Because they are able to ingest toxic substances through their skin or by consuming large amounts of soil, a significant decline in earthworm population occurs due to the soil-applied xenobiotic compounds, i.e., pesticides and fertilisers (Miglani and Bisht 2019). Herbicides also affect the feeding behaviour of earthworms, depending on the exposure time, chemical nature, and soil organic matter content (Zarea and Karimi 2012). Triazine herbicides such as atrazine showed higher toxicity to *Eisenea fetida* than glyphosate, which had no or low toxicity depending on the additives in the formulations (Lydy and Linck 2003, Zarea and Karimi 2012, Schmidt et al. 2023). Increased earthworm mortality by metribuzin at higher doses due to increased mucus secretion (lethal concentration ($LC_{50}$) of 8.48 mg a.i. (active ingredient)/kg) has also been documented (Samadi Kalkhoran et al. 2022). Herbicides also affect earthworm activity indirectly by distressing the organic matter availability. An increase in earthworm weight and density in the presence of corn residues despite the atrazine applied dose and by the leguminous residues like faba bean than barley residues were reported by Farenhorst (2003) and Treder et al. (2020).

The herbicides commonly used in vegetable crops are glyphosate, pendimethalin, and metribuzin. While glyphosate (N-(phosphonomethyl)glycine) has recently brought under restricted use, metribuzin (4-amino-6-tert-butyl-3-(methylthio)-1,2,4-triazin-5(4H)-one) and pendimethalin (N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine), a triazinone and dinitroaniline herbicides respectively used pre-emergently for weed control. While pendimethalin is sprayed to manage annual grasses, metribuzin is recommended to manage broad-leaved weeds in cropped fields. However, tomato growers are applying two to three herbicides as a tank mix to have broader weed control (Robinson et al. 2006, Anshu et al. 2020), which might have a negative impact on earthworm density and growth. Therefore, the present study investigated the interactive effect of herbicides and nutrient supply through inorganic fertilisers and organic sources applied in tomatoes on earthworms' activity and toxicity in soil.

## MATERIAL AND METHODS

**Details on soil type and pot experiment.** An organised pot experiment was conducted using the processed, 2 mm sieved soil collected from the herbicide residue-free field. On the other side, the sub-sample from the processed soil was analysed for physico-chemical characteristics. The soil is classified texturally as mixed sandy loam in nature and belongs to the Typical Rhodustalf group. It has an alkaline pH of 7.55 and a low electrical conductivity (EC) of 0.215 dS/m, a medium content of available nitrogen (N) (131 mg/kg) and a high content of available phosphorus (P) (15 mg/kg) and potassium (K) (210 mg/kg) and has organic carbon of 5.1 g/kg and cation exchange capacity (CEC) of 14.9 $cmol_+$/kg. The tomato cultivar Darsh was grown during the winter season (October–February 2022–2023) in a controlled, transparent glasshouse at the Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore, India. Initially, 7 kg pots were filled with the calculated amount of homogenised soil, maintaining an optimum bulk density of 1.35 $t/m^3$ and moistening to field capacity. Tomato seeds were sown in the growing bed, and seedlings were transplanted after 30 days with 2 seedlings per pot. Pots were irrigated to field capacity moisture every 4–6 days, depending on the prevailing rainfall, relative humidity and temperature during the growing season. The pest and disease were managed per the crop production guide (CPG 2020) recommendation for tomato cultivation in Tamil Nadu, India.

**Imposing fertiliser nutrients and herbicide treatments.** The experiment was laid out in factorial completely randomised block design (FCRD) with four fertiliser nutrients application (NFA) practices as factor 1 and seven herbicide treatments as factor 2 (Table 1). Treatments were replicated three times, and fertiliser nutrient practices were imposed based on soil test (ST) crop response techniques targeting 90 t/ha fruit yield. The fertilisers used to supply the required quantity of NPK were urea, superphosphate, potassium chloride and farm yard manure (FYM) as per the treatments. The glyphosate (GLYPH PRO 41% SL) was applied 10 days before transplanting tomato seedlings. The pendimethalin (STOMP 50% EC) and metribuzin (TATA metric 70% Wettable Powder (WP)) were applied pre-emergently on $3^{rd}$ day after transplanting tomato seedlings. Herbicides were sprayed on the soil surface using a knapsack sprayer with a flat fan nozzle that adopted the recommended spray volume of 500 L/ha (CPG 2020).

**Earthworm activity assessment and toxicity appraisal.** Earthworm *Euginea fetida* Pers. was in-

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Table 1. Details of factors imposed in the experiment

| Factor | Acronym used | Detail |
|---|---|---|
| Factor 1: Fertiliser nutrients application (FNA) practices | ST-NPK | soil test based N, P, K alone @ 700, 382 and 723 mg/pot, respectively |
| | ST-NPK + MN | in addition to ST-NPK, micronutrients fertilisers were applied *viz.*, $ZnSO_4$ 175 mg, $FeSO_4$ 175 mg, borax (sodium tetraborate salt) 35 mg and $CuSO_4$ 13 mg per pot |
| | ST-NPK + FYM | soil test-based NPK was supplied through farm yard manure (25 t/ha) and inorganic fertiliser sources |
| | farmers practice | general recommendation available for tomato cultivar cultivation in Tamil Nadu, India, was adopted by farmers for major nutrients along with micronutrients (700 mg N, 382 mg P, 723 mg K, 175 mg $ZnSO_4$ and 35 mg borax per pot) |
| Factor 2: Herbicide treatments as single or in combinations and sequence | PP Glyphosate | pre-plant application of glyphosate @ 7.5 L/ha |
| | PE Pendimethalin | pre-emergence application of pendimethalin @ 1 000 g a.i./ha |
| | PE Metribuzin | pre-emergence application of metribuzin @ 500 g a.i./ha |
| | PP Glyphosate fb PE pendimethalin | pre-plant glyphosate followed by (fb) pre-emergence pendimethalin |
| | PP Glyphosate fb PE Metribuzin | PP glyphosate followed by pre-emergence metribuzin. |
| | PE Glyphosate fb PP pendimethalin + metribuzin | PP glyphosate followed by pre-emergence pendimethalin and metribuzin as a tank mix |
| | control | no herbicide |

ST – soil test; NPK – urea, superphosphate, potassium chloride; MN – micronutrients; FYM – farm yard manure; PP – pre-plant; PE – pre emergence; fb – followed by

troduced to all the pots @ 5 Nos/pot and allowed 5 days for acclimatisation before tomato transplanting. Earthworms' weight and numbers in each pot were noted at transplanting tomato (day 1). Then earthworm activity, *viz*., survival rate, depth of habitat, worm weight, and escape from the pot, was recorded 7, 15 and 30 days after tomato transplanting. The safety of earthworms was assessed, as reported by Bhandari et al. (2021). The risk factor is considered high if risk quotient (RQs) > 1, moderate for RQs 0.1–1 and low for RQs < 0.1. Effects of the herbicides on earthworms were classified as harmless (< 50% mortality), slightly harmful (50–79% mortality), moderately harmful (80–89% mortality) and harmful (> 90% mortality) based on literature (Nasreen et al. 2000).

**Herbicide residue extraction from soil.** Both pendimethalin and metribuzin were simultaneously extracted using methanol from the soil and tomato fruits by shaking and centrifuging in a rotary orbital shaker. Afterwards, it was filtered and concentrated to 10 mL by evaporation at 40 °C, and the content was partitioned using hexane and aqueous NaCl thrice. Organic portions were pooled and dehydrated *via* anhydrous sodium sulfate. Then, it dried to a moist level, re-dissolved in acetonitrile and analysed in high-performance liquid chromatography (HPLC). Glyphosate was extracted and analysed, as detailed by Brindhavani et al. (2020), using HPLC. The extraction and detection method was validated by performing recovery studies before analysing unknown samples, as described by Janaki et al. (2016) for bensulfuron methyl.

**Statistical analysis.** Data collected were analysed by two-way analysis of variance (ANOVA) using R statistical software (version 4.2.2. package doe bioresearch, https://posit.co/download/rstudio-desktop/). Graphical visualisation was performed by MS Excel (https://www.microsoft.com/en-in/microsoft-365/excel). Heat map and principle component (PC) analyses were carried out using the online clusVis tool (https://biit.cs.ut.ee/clustvis/). Correlations among the variables were assessed by performing Pearson correlation coefficients and probability analysis.

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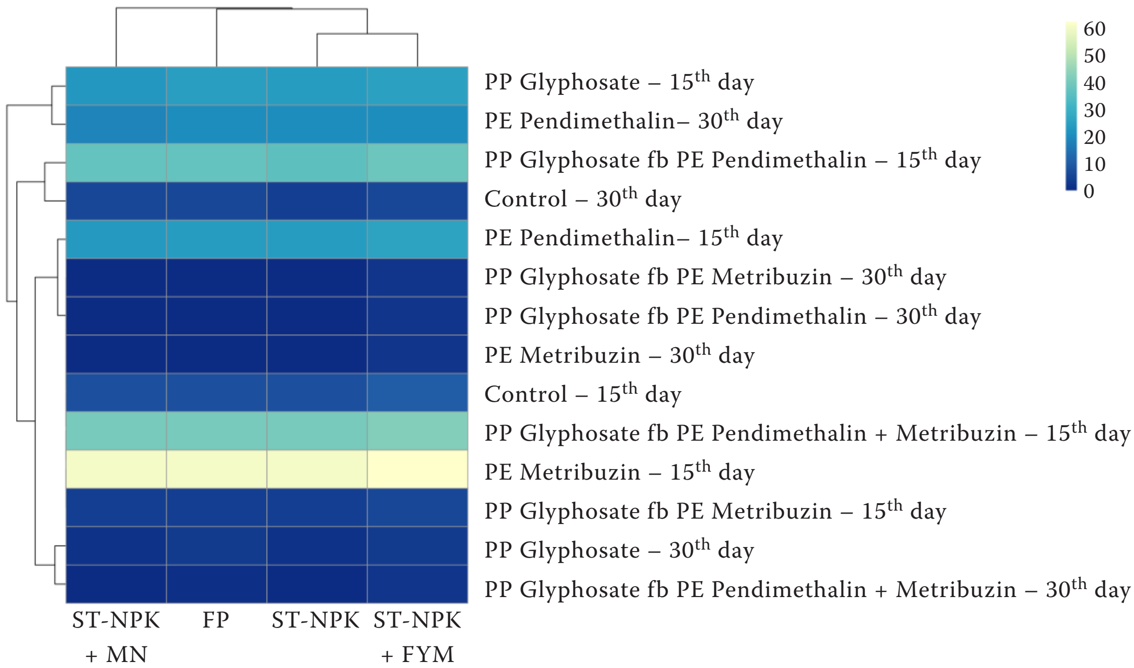


Figure 1. Heat map analysis shows the change in earthworm numbers as influenced by the NFA practices and herbicides at different time intervals (unit variance scaling is applied to rows). Both rows and columns are clustered using correlation distance and average linkage; light colour indicates more effect on earthworm numbers, and increased colour gradation indicates reduced toxicity. ST – soil test; NPK – urea, superphosphate, potassium chloride; MN – micronutrient; FP – farmers practice; FYM – farm yard manure; PP – pre-plant; PE – pre emergence; fb – followed by

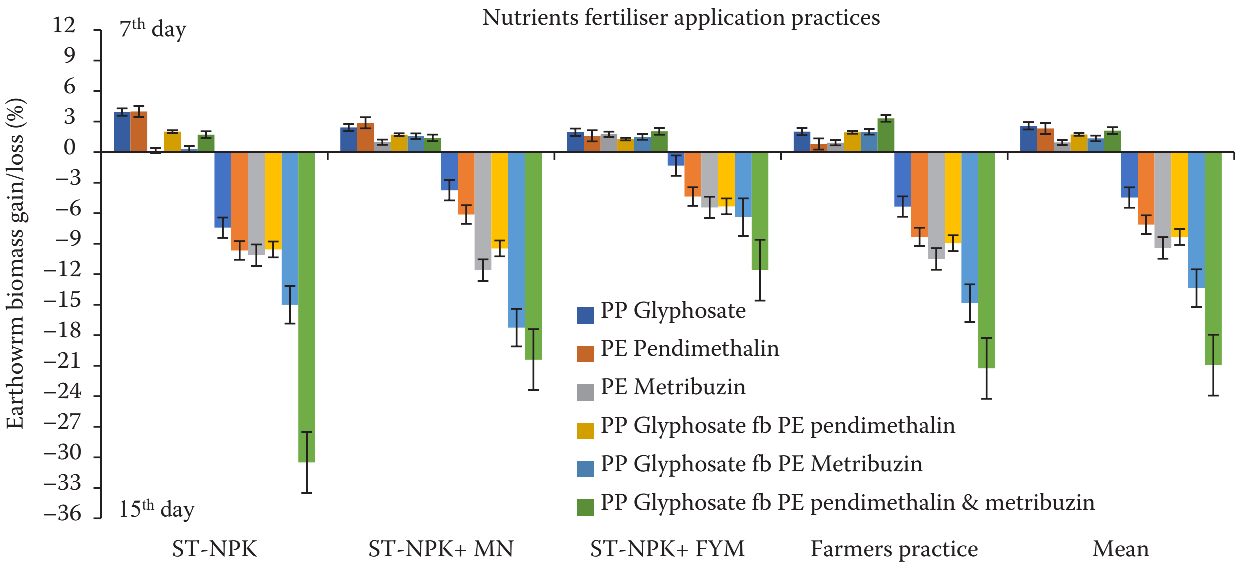


Figure 2. Effect of fertiliser nutrients application practices and herbicides on earthworms biomass in tomato planted soil (error bars indicate standard error variation among the same herbicide treatment across nutrients management practice; positive bars represent the earthworms biomass gain on the 7th day after herbicide application; negative bars represent earthworm biomass loss occurs on 15th day after herbicides application; in X axis various fertiliser nutrients management practices followed for the study and mean data of all practices are represented)y. ST – soil test; NPK – urea, superphosphate, potassium chloride; MN – micronutrient; FYM – farm yard manure; PP – pre-plant; PE – pre emergence; fb – followed by

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## RESULTS AND DISCUSSION

Exposure to different herbicides or their combinations significantly reduced earthworm biomass and numbers, which was also altered by NFA practices (Table 1, Figures 1 and 2). All introduced worms survived on day 7 and then decreased on day 15 (40–80%) and day 30 (20–40%) in herbicide-treated soil, regardless of NFA practices (Table 1). On days 7 and 15, all worms migrated to the subsurface and settled at 15 cm in the herbicide-treated soil, regardless of the NFA practices. This could be due to the avoidance behaviour of earthworms to overcome the harmful effect of herbicide residues, which could be present in a toxic concentration in the surface soil. Adelasoye et al. (2017) observed similar escape behaviour of *E. fetida* in maize-based cropping systems to deeper soil layers and even attachment to the cover due to pendimethalin spray. On day 30, only a few surviving worms were seen at 5 cm depth under glyphosate alone and in the control soil across different NFA practices. In contrast, it was observed at 10–15 cm in metribuzin-treated soil and 15 cm in pendimethalin alone or their combination sprayed soils. On the 15$^{th}$ and 30$^{th}$ day, the number of earthworms surviving (Table 1) was higher in the soil of ST-NPK + FYM with glyphosate and with PP glyphosate followed by PE pendimethalin + metribuzin as a tank mix. A single or sequential spray of pendimethalin or metribuzin after PP glyphosate decreased the earthworm survival rate by 40–60% on the 15$^{th}$ and 0–20% on the 30$^{th}$ day. This confirmed the higher toxicity of pendimethalin and metribuzin to earthworms compared to glyphosate. High earthworm lethality by pendimethalin than glyphosate was documented by Santos et al. (2012) and Anshu et al. (2020). Heatmap (Figure 1) and PC analyses showed that earthworm numbers were higher under ST-NPK + FYM practices and contributed to 76.9% variation regardless of time interval. In comparison, the other three practices contributed 13.8% variation only. Despite NFA practices, PE metribuzin reduced earthworm numbers the most on day 15 compared to PP glyphosate fb PE pendimethalin + metribuzin as a tank mix. The PP glyphosate fb PE pendimethalin, PE pendimethalin, and PP glyphosate also reduced the earthworm population on day 15. This confirmed the more toxic effect of metribuzin on earthworms than pendimethalin and metribuzin or their combined application as a tank mix after glyphosate.

Avoidance behaviour was assessed (Schmidt et al. 2023) by determining the number of worms at 0–10 cm depth across all treatments and excluding worms that lived in the lower 15 cm. While 100% avoidance was observed on day 7, it was 80–100% and 20–60% on days 15 and 30, respectively. The low avoidance on day 30 compared to day 15 could be due to high mortality and low survival (Table 1) caused by chronic toxicity of herbicide residues (Sanyal et al. 2016). At day 30, at ST-NPK and ST-NPK + MN, soils with all herbicides except glyphosate alone were avoided by about 40% and 60%, respectively. At ST-NPK + FYM and FP, 20–40% avoidance was noticed regardless of herbicides, with a higher percentage for metribuzin or its combinations, followed by pendimethalin and glyphosate. A similar higher toxicity of metribuzin than halosulfuron and/or flumioxazin to earthworms was reported by Samadi Kalkhoran et al. (2022).

The change in earthworm biomass on days 7 and 15 under different NFA practices was recorded compared to day 1 (Figure 2). Earthworms showed an increase in biomass on day 7 compared to day 1, and all herbicide-treated soils showed a smaller increase than the control. Among the NFA practices, ST-NPK recorded the highest biomass increase of 1.72–3.94% and the lowest increase of 1.96–2.04% by ST-NPK + FYM soil (Figure 2). Among the herbicides or their combinations, the highest and lowest biomass change was observed for pendimethalin and metribuzin, respectively, when applied singly or followed by glyphosate in sequence. Earthworm biomass loss was observed on the 15$^{th}$ day (Figure 2) and was highest in three herbicide-treated soils, followed by soils treated with metribuzin with or without glyphosate. Biomass loss ranged from 1.336% to 30.66%, regardless of NFA practices and herbicides. However, biomass gain was found in the control plot and was 2.45, 2.10, 4.88, and 3.27% in ST-NPK, ST-NPK + MN, ST-NPK + FYM, and FP practices imposed soils, respectively. The higher increase in ST-NPK + FYM showed the positive effect of the addition of organic matter (FYM) compared to inorganic fertilisers alone practices on earthworm development. Yasmin and D'Souza (2007) documented the negative effects of the pesticides carbendazim, dimethoate, and glyphosate and their mixtures on *E. fetida* growth and reproduction.

On day 30, a drastic decrease in biomass (98–100%) was observed (data not shown) as many worms escaped from the soil and were also poisoned by herbicide residues (Table 2) by, affecting the worm metabolism. This was confirmed by the positive cor-

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Table 2. Mean herbicide residue concentration in soil on the 15th day after transplanting tomato

| Herbicides treatment | Residue (mg/kg) | | | |
|---|---|---|---|---|
| | total | glyphosate | pendimethalin | metribuzin |
| PP Glyphosate | 0.433 | 0.494 | – | – |
| PE Pendimethalin | 0.487 | – | 0.488 | – |
| PE Metribuzin | 0.347 | – | – | 0.348 |
| PP Glyphosate fb PE pendimethalin | 0.800 | 0.441 | 0.359 | – |
| PP Glyphosate fb PE Metribuzin | 0.771 | 0.433 | – | 0.338 |
| PP Glyphosate fb PE pendimethalin + metribuzin | 0.924 | 0.403 | 0.231 | 0.390 |
| Control | – | – | – | – |

PP – pre-plant; PE – pre emergence; fb – followed by

relation between the number of surviving earthworms and their biomass in the soil on day 30 ($y = 0.311x + 0.043$; $R^2 = 0.783^*$). Our findings are consistent with the results of previous studies with herbicides such as metolachlor (Xu et al. 2010) and metribuzin (Samadi Kalkhoran et al. 2022), in which prolonged exposure of earthworms affected worm metabolism (e.g., mucus secretion) and decreased body weight. The lethal effect of herbicides on earthworms was reduced at ST-NPK + FYM practice, where organic matter supply may have helped worms grow. The positive and significant correlation between organic matter content and earthworm biomass change confirmed the results with $R^2$ values of 0.967** and 0.686* on the 7th and 15th day, respectively. Low avoidance behaviour has been reported for metribuzin and glyphosate in soils with high organic content (Zarea and Karimi 2012, Schmidt et al. 2023). Reduced feeding by earthworms due to decreased intestinal α-amylase activity by residual toxicity of pendimethalin was documented by Sanyal et al. (2016). On day 30, cocoon formation and young earthworms were observed in the soil of all pots, which were absent in all herbicide pots, while 4–6 cocoons were found in the control soil. A similar, destructive effect of glyphosate and atrazine on the viability of cocoons and earthworm biodiversity was documented by Bon et al. (2006) and Kumar and Kumawat (2018).

The toxicity of the studied herbicides to earthworms, based on different parameters, is in decreasing order of metribuzin > pendimethalin > glyphosate. This could be due to the lower lethal concentration of metribuzin (of 0.0172 g a.i./$cm^2$) in earthworms compared to glyphosate (0.066–0.30 g a.i./$cm^2$), as reported by Samadi Kalkhoran et al. (2022) and Piola et al. (2013). The $LC_{50}$ of 1.77 to 1.93 L/ha or 0.022 mg/kg soil was reported

Table 3. Survival rate (%) of earthworms in soil imposed with different fertiliser nutrients application (NFA) practices and herbicides on the 15th and 30th day of tomato planting

| NFA practices/herbicide treatment | ST-NPK | | ST-NPK + MN | | ST-NPK + FYM | | FP | |
|---|---|---|---|---|---|---|---|---|
| | 15 D | 30 D | 15 D | 30 D | 15 D | 30 D | 15 D | 30 D |
| PP Glyphosate | 80 | 20 | 80 | 20 | 80 | 40 | 80 | 40 |
| PE Pendimethalin | 60 | 20 | 60 | 20 | 60 | 20 | 60 | 20 |
| PE Metribuzin | 60 | 0 | 60 | 0 | 60 | 0 | 60 | 0 |
| PP Glyphosate fb PE pendimethalin | 60 | 0 | 60 | 0 | 60 | 20 | 60 | 0 |
| PP Glyphosate fb PE Metribuzin | 40 | 0 | 40 | 0 | 60 | 20 | 40 | 0 |
| PP Glyphosate fb PE pendimethalin + metribuzin | 40 | 0 | 40 | 0 | 40 | 40 | 40 | 20 |
| Control | 80 | 40 | 80 | 60 | 100 | 60 | 80 | 60 |
| **Statistical analysis** | SED | | CD ($P$ = 0.05) | | SED | | CD ($P$ = 0.05) | |
| NFA strategies (N) | 1.0 | | 2.0 | | 2.1 | | 4.3 | |
| Herbicides (H) | 1.6 | | 3.8 | | 3.9 | | 7.9 | |
| Interaction (N × H) | 2.6 | | 5.3 | | 5.6 | | 11.3 | |

PP – pre-plant; PE – pre emergence; fb – followed by; D – day; SED – standard error difference; CD – critical difference

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for pendimethalin by Adelasoye et al. (2017) and Sanyal et al. (2016). The negative effect of pendimethalin and metribuzin on biomass change, survival rate and avoidance behaviour of earthworms was attributed to the chronic toxicity caused by the herbicide residues in the soil (Table 3). This was confirmed by the significant and negative correlation between total herbicide residue concentration on day 15 and earthworm biomass ($r$ = –0.713**) and number ($r$ = –0.531*) on day 30. Among the individual molecules, pendimethalin showed a significant relationship (($r$ = –0.0.454 and –0.569**), followed by metribuzin and glyphosate.

Based on the mean percent earthworm mortality in the soils of the different NFA practices, glyphosate was ranked as slightly harmful (70% mortality). In comparison, pendimethalin (80% mortality) and metribuzin (95–100% mortality) were ranked as moderately harmful when applied individually. The herbicide safety ranking given to earthworms based on the present study is glyphosate > pendimethalin > metribuzin. The soil ecological risk quotient for the applied herbicides was determined according to the reports of Bhandari et al. (2021) and were 0.50, 0.58, and 0.03 for pendimethalin, metribuzin, and glyphosate, respectively. According to the RQs safety classification, glyphosate was considered safe as the RQs value was < 0.03, while the other two herbicides were considered moderate in risk as the values ranged from 0.1 to 1.0, as reported by Bhandari et al. (2021). Therefore, the risk to earthworms associated with pendimethalin and metribuzin spray in tomato-grown soil should not be disregarded and must be applied according to the recommendations without indiscriminate use.

The results of the present study show that the herbicides pendimethalin and metribuzin are harmless to earthworm biodiversity when applied to the tomato field at the recommended rate along with nutrients supply through FYM 25 t/ha and inorganic fertilisers adopting soil test-based NPK practice. Based on ecological risk assessment and mortality, glyphosate was found to be safer than pendimethalin and metribuzin. Caution should also be used when applying two herbicides pre-emergently by a tank mix in succession after glyphosate. Continued monitoring is needed to assess the risk of chronic toxicity of pendimethalin and metribuzin residues to earthworm activity and soil biodiversity.

**Acknowledgement.** The authors acknowledge the Professor and Head, Nammazhvar Organic Farming Research Centre, for permitting the use of LC-MS-MS analytical facilities and the Vice-Chancellor, Dean (SPGS), Professor and Head (SS&AC), TNAU, Coimbatore, for motivating the Ph.D. scholar (author 1) to publish the research work.

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Received: June 2, 2023
Accepted: September 9, 2023
Published online: September 25, 2023