

*BIOLOGY FORUM*

# **Interaction of Continuous Fertilization Practices on Tembotrione Degradation in Tropical Soil of India**

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#### *ABSTRACT*

*Laboratory incubation experiment was conducted using the soil collected from Long Term Fertilization (LTF) Experiment plots cultivated with Finger millet-maize cropping sequence. The study was aimed to investigate the influence of different long-term fertilization practices and rates of TMB application on its persistence and degradation behavior in Typic Ustropept soil. The results are showed concentration of TMB residue on day 1, ranged from 0.459 to 0.622, 0.504-1.061, and 0.629-1.348 mg kg-1 across different LTF practices at 60, 120, and 240 g/ha application rates. Higher TMB residue was recorded by 100 % NPK (-S) practice at 60 and 240 g/ha rates and 150 % NPK at 120 g/ha rate and the 100% NPK+ FYM basis recorded lower TMB residue on 1st day of incubation. Tembotrione degradation follows 1st order kinetics of dissipation irrespective of dose and LTF practices. Application of 100%NPK+FYM and omission of K fertilizer practices followed the best fit with 2nd-order kinetics. More than 50 % of the initial tembotrione deposit dissipated on the 15th and 30th at 60 g/ha and at 120,240 g/ha doses respectively irrespective of LTF practices. Tembotrione dissipated with the degradation half-lives of 10.19 to 14.71 days. Halflife of tembotrione ranged from 9.39-22.27 days across the LTF practices and rates of its application. Tembotrione degradation half-life increased with the increased rate of application and the mean HL was calculated to be 9.39-13.72,14.26-20.51 and 18.94-22.27 days respectively at 60, 120 and 240 g/ha. Lowest and highest HL of 14.30 and 19.86 days was observed with 100%NPK(-S) and 100%NPK+FYM respectively and the tembotrione residue was detected in soil up to 60 days irrespective of its application rate. This indicates the existence of interaction between the nutrients management practices and its persistence in soil.* 

**Keywords:** *Dissipation, Tembotrione, Fertilization, Soil nutrients, FTIR, FYM*

# **INTRODUCTION**

The behavior of herbicides in the agriculture ecosystem is influenced directly or indirectly by intrinsic soil variables such as soil fertility and chemistry. In many instances, soil-applied nutrients and its quantity compete for herbicide adsorption sites in the soil which might alter the weed control efficiency and input cost.Since nutrients and herbicides play a vital role in cropweedinteractions, it is highly desirable to study the effect of various fertilization practices through an inorganic or integrated approach on herbicide dynamics in soil.

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An understanding of the dynamics of herbicides in the soil as affected by fertilization practices is important for maintaining soil health and sustainability which is a researchable issue constantly. Agreat decrease inthe atrazine mineralization due to the supply of mineral N at outsized concentration with or without organic amendments was also observed  $[1,2]$ . Fertilization of soil with urea-decreased pendimethalin volatilization but increased herbicide levels in soil solution and persistence, with a half-life approximately 70% higher than that found in unfertilized soil  $[3]$ . The nutrients like C, N, and P stimulate microbes to produce the essential enzymes which breakdown the contaminants; hence, the interaction of nutrient supplementation on chemical degradation becomes imperative for various researchers<sup>[4]</sup>. Carbon and nitrogen application in soil both as organic and inorganic causes atrazine to remain as a bound residue for 326 days<sup>[5]</sup>.

The interactive effect of fertilization particularly nitrogen fertilization on the persistence behavior of many herbicide molecules like atrazine, pendimethalin, 2,4-D, atrazine, chloracetanilines viz., butachlor etc has been investigated and published<sup>[6]</sup>. Similarly, the effect of nitrogen, phosphorus and micronutrient fertilization on the dissipation of glyphosate in soil was wellstudied<sup>[7]</sup>. However, the interaction of fertilizer nutrients on the dynamics of newer lowdose herbicides particularly under long-term fertilization practices was not studied.

Among the low-dose herbicides, the newer molecule registered in India during 2015 is tembotrione which is very widely used in maize and other minor millets like finger millet to regulate the grassy and broadleaf weeds as well as several dicotyledonous plants. It belongs to the triketone group and its application results in rapid bleaching and quick elimination of susceptible weeds. It disrupts carotenoid production by inhibiting the enzyme 4-hydroxy-phenylpyruvate dioxygenase (HPPD) which cause the bleaching of chlorophyll in plant $[8,9]$ .

Tembotrionehas apKa of 3.2, a Koc of 66 mL  $g<sup>-1</sup>$ , or mobility of 28 g L<sup>-1</sup>. Being a weak acidic compound and solubility 71  $g/L$  at 20 $^{\circ}$ C in water, it can exist in different tautomeric forms which largely depend upon the environmental conditions and soil  $pH^{[10]}$ . It is stable to hydrolysis at an environmental pH range of 5–9 but prone to photolysis in soil and water $[8]$ . Its degradation in soil strongly depends on pH and the highest degradation rate was observed in more alkaline soil $s^{[11,12]}$ . It has high mobility and varying soil persistence with ahalf-life of 6-14 days and 10- 22 days in the presence and absence of light respectively.Tembotrione may also have an impact on the microbial populations in the soil when used at high doses and repeatedly across an extended length of time $[13]$ , besides, showing an impact on the reproduction rate of earthworms $[14]$ . Priyaa Goalla et al. $[15]$  reported that the higher rate of tembotrione with atrazine hindered the enzymes dehydrogenase and alkaline phosphatase and the arbuscular mycorrhizal fungi population and root colonization in maize soil.

The dynamics of tembotrione in soil environment was investigated mostly under controlled conditions and also the interaction between the fertilization practices and tembotrione behavior was little studied. Most of the studies were undertaken with the old herbicide molecules which are applied at higher rates for efficient weed management. However, the effect of longterm fertilization practices on the persistence and dissipation behavior of new-generation and lowdose herbicides belongingto triketones is to be investigated in the Indian tropical climate. Hence the present study was formulated to find out the influence of long-term fertilization practices on the persistence and degradation behavior of tembotrione in soil.

## **MATERIALS AND METHODS**

## *Field experiment details and Soil Collection*

It is geographically positioned at 11° North latitude, 77° East longitude, with a height of 426.7 meters above mean sea level and belongs to the North Western agro-climatic zone of Tamil Nadu. The experimental field soil is a calcareous mixed black, sandy clay loam soil belongingto the Periyanaickenpalayam series and classified under Inceptisol (*VerticUstropept*) order.

Every year /season, the recommended dose of N,  $P_{2}O_{5}$ , and  $K_{2}O$  was applied at 250, 75, and 75 kg ha $^{-}$ <sup>1</sup>for maize and 250, 75, and 75 kg ha<sup>-1</sup> respectively as per CPG 2020 of TNAU. Urea, Single Super Phosphate (SSP), and Muriate of Potash are used as N, P, and K sources for all treatments except  $T_{0}$ (S free - DAP as P source) and hand weeding is done for  $T<sub>4</sub>$  (100% NPK + hand weeding). For INM  $(100\%$  NPK + FYM) plots, 10 t FYM ha<sup>-1</sup>is applied for every crop. In treatment  $T<sub>5</sub>$  the Zinc Sulphate  $@ 25 kg ha^{-1}$  was being used along with 100% RDF. Soil samples were collected from each treatment plot (nine long-term fertilization practices and control) at a depth of 0-30 from the Long-term fertilizer experiment located at the Eastern Block farm, back of the centenary main building of TNAU after the harvest of  $11<sup>th</sup>$  Ragi crop. About five kg soil was collected from each treatment plots (of three replications), shade dried, powdered and sieved through a 2 mm mesh sieve and stored to conduct laboratory experiments and leaching column experiments using tembotrione herbicide at different rates.

#### **Climate and weather**

Coimbatore is situated in semi-arid tropics and the mean annual rainfall is 674.2 mm and the mean maximum and minimum temperatures are 31.5°C and 21.3°C, respectively. Weather conditions that prevailed during the experimental period is furnished in Annexure I. The mean minimum and maximum temperature was 24.21 and 33.89 respectively with 52.9 mm total rainfall (Fig 1).



**Fig 3.** Weekly weather parameters observed during the experimental period

## *Chemicals, reagents and standards*

Tembotrione, 42% SC w/v  $(34.4\%$  SC w/w), a liquid product emulsion with safener isoxadifenethyl (LAUDIS) of Bayer Crop Science India Limited, Mumbai was purchased from the market and used for the study. Stock standard solutions (1000 mg/L) of tembotrione and the working solutions prepared using distilled water were used to conduct the bioassay calibration and column studies. It is 2-Chloro-4-(methylsulfonyl)-3- [(2,2,2- trifluoroethoxy) methyl]benzoyl)-1,3 cyclohexanedione and this herbicides belong to the triketone group.

#### *Incubation experiment*

The incubation experiment was conducted under laboratory conditions using plastic cups with a capacity of 250 g to study the persistence and dissipation behavior. Soil collected from each

treatment of LTFE trails was filled in plastic cups and wetted to field capacity. Tembotrione was applied at 3 rates viz., 60, 120 and 240 g/ha along with control. Each treatment was replicated twice. After herbicide application, the cups were wetted to field capacity at the weekly interval and the experiment was continued for 45 days. Soil samples were collected at different intervals viz., 1, 3, 5, 7,15, 30 and 45 days after herbicide application (DAHA) and stored to analyze the tembotrione residue.

## *Functional Groups in soil samples*

FT-IR 6800 JASCO, Japan, was used to examine the infrared spectra. Because it uses the ATR approach, this innovative apparatus does not require any sample pre-extraction or pretreatment. The functional groups of the soil samples were determined using FT-IR spectral bands in the  $400-4000$  cm<sup>-1</sup> spectral range.

## *Tembotrione extraction and HPLC detection*

The tembotrione from soil and suspension was extracted using acetonitrile: acetic acid 100:1 v/v and partitioned with dichloromethane by adapting the methodology described by Rani et al.[12]. The extracted tembotrione residues in soil and water was analyzed using the Shimadzu UFLC having quaternary pump (LC20AD), Degasser (DGU-20A), Thermostated column (CTO-10AS), Autosampler (Shimadzu SIL-20AC-HT ) and PDA detector (SPD-M20A) with Lab solutions software. The separation of tembotrione was achieved using  $C18$  column  $(3.0 \times 50 \text{ mm}, 2.7 \mu \text{m})$  by eluting with Acetonitrile: 0.05% orthophosphoric acid buffer (70/30 v/v) at 1.0 mL min<sup>-1</sup> flow rate. The compound was detected by Photo Diode Array Detector at 285 nm wavelength with a retention time of 6.59 min.

## *Method validation*

The validity of the analytical approach used for the extraction and detection of tembotrione in soil / leachate was confirmed through the recovery studies. A known weight of soil samples were fortified with known concentrations of tembotrione standards ranging from 0.001 to 5.0 mg  $L^{-1}$ . After 1 hour of fortification, a soil was subjected to extraction and clean up and then determined using HPLC as above.

Quantification of herbicide residue concentration was accomplished by comparing the peak height response for samples with peak height of the standard. The precision standard deviation of replicate analysis of standard spiked at different concentrations was used to calculate the limit of detection (LOD) and limit of quantification (LOQ).

#### *Statistical analysis of data*

The data collected on various characters for the present study during the course of the investigation was statistically analyzed as suggested by Gomez and Gomez<sup>[16]</sup>. Whenever the treatment differences were found significant by 'F' test, critical differences were worked out at 5 percent probability level and the values are furnished. Treatment differences that were non-significant are denoted as 'NS'. The Pearson correlation analysis was carried out between variables and constant parameters at a significance level of P=5.



**Fig 2.** Influence of rate of tembotrione application on its persistence in soil



**Fig 3.** Influence of LTF practices on tembotrione persistence in soil



**Fig 4.** FTIR spectra of soil from LTF practices viz., 150% NPK and 100%NPK+FYM measured using ATR window





**Fig 5.** FTIR spectra of soil from LTF practices viz., 100% NPK+Zn, 100%NPK (-S) and control



**Fig 6.** Influence of LTF practices on tembotrione dissipation rate in soil



**Fig 7. Influence of rate of tembotrione application on its dissipation rate in soil**

#### **Results and Discussion**

#### **Persistence and degradation of tembotrione in soil**

The tembotrione residue was analyzed on 1, 3, 7, 15, 30, 45 and 60 days after its application (Table 1 to 3). The initial deposit of TMB residues on day

1, ranged from 0.459 to 0.622, 0.504-1.061 and 0.629-1.348 mg kg<sup>-1</sup> across different treatments at 60, 120 and 240 g/ha rates to TMB application. The increased rate of TMB application increased its residue irrespective of LTF practices. Among all the long-term fertilization practices, higher TMB residue was recorded by 100 % NPK (-S) at 60 and 240 g/ha rate and 150 % NPK at 120 g/ ha rate and the 100% NPK+ FYM basis recorded lower TMB residue on  $1<sup>st</sup>$  day of incubation (Fig 2). The 150 % NPK continued to register high residue in soil at later days (up to  $15<sup>th</sup>$  day) of incubation  $@$  120 and 240 g/ha rates, the 100%NPK+Zn registered higher residue at later stages @ 60 g/ ha rates. Lower TMB residue concentration on 1 day was observed  $100\%$ NPK+FYM  $(10 \t{ h}a^{-1})$  (Fig 3). This could be due to the increased sorption of TMB by the higher organic carbon/matter in the soil correlated with the higher content of alkyl and carboxyl groups of the organic matter in the soil of this plot as reported by Dumas et al.<sup>[10]</sup> who stated that both mineral fractions and organic constituents (Fulvic substances) are involved in the adsorption of sulcotrione, a triketone herbicide in Vertisol. The composition of organic matter isa secondary factor responsible for the higher TMB residue in soil as sorption is favored by the high percentage of carboxylic substitution in aliphatic chains of soil  $OM<sup>[17]</sup>$ . This was proved by the presence of aromatic C-H bands, Aliphatic C-H (methyl and methylene) groups, C=O stretch, the anhydride of a carboxyl group, S=O stretching of sulfate compound and sulfonyl chloride and C = C stitching bond, N-O stretching nitro compound in its spectra obtained through FTIR (Fig 4). Further, the decreased soil pH locally due to the application of FYM might have increased the TMB sorption by decreasing the TMB ionization in soil. Also the higher sorption of TMB with higher organic matter occurs due to the hydrophobic interactions, as well as hydrogen bonds involving aromatic and aliphatic groups with the organic colloids of the soil $[18,19]$ .

The LTF practice, 100% NPK (-S) registered the higher TMB residue and could be due to the  $increased Ca<sup>2+</sup>$  in soil solution by the non application of S continuously for 50 years which plays a major role in the chemisorption of the ionic compounds. This has been also supported by the absence of sulfur functional group stretching peak  $2112 \text{ cm}^{-1}$ in this practice (Table 2  $\&$  Fig 5) when compared to other LTF practices viz., 100%NPK+FYM,

100%NPK+Zn. Similar results was reported in the literature<sup>[10]</sup> for the sorption of mesotrione it's anionic form was found toincrease logarithmically with Ca2+concentration. The favorable influence of liming effect on TMB degradation in soil was also observed by Madalao et al.<sup>[20]</sup>.

Apart from the 100%NPK+FYM practice, the other LTF practices viz., the omission of PK or K alone and 100%NPK+HW and 100%NPK+Zn also showed less residues than the balanced nutrients supplied practices. This showed the existence of definite interaction between the nutrients and TMB sorption and degradation in soil. As the present experimental soil is mixed calcareous montmorillonite clay type, the  $Ca^{2+}$  have keep the soil alkaline (above 8.0 pH) and decreased the TMB sorption $[10,17,21]$  The residue of TMB decreased with the time and it persists in soil up to 30 days at 60 g/ha rate and 45 days at 120 and 240 g/ha rate after its application irrespective of LTF practices and then becomes below the quantification limit. The detection of TMB residue in soil up to 90 days<sup>[20]</sup> and indicated the possibility of TMB carryover risks to successive crops.

The TMB dissipation rate was calculated at different application rates as influenced by the LTF practices using the initial deposition data on day 1 (Fig 6 & 7). The TMB dissipation rate on 3rd day was ranged from 7.91-30.93, 9.14-27.66 and 6.46-25.15 percent at 60, 120 and 240 g/ha application rates across different LTF practices. Irrespective of rate, the TMB dissipation was increased with the advancement in incubation time. The dissipation was high in 100% NPK(-S) on  $3<sup>rd</sup>$ ,  $7<sup>th</sup>$ ,  $30<sup>th</sup>$  and  $45<sup>th</sup>$  days after TMB application whereas the NPK+FYM practice showed higher dissipation on  $5<sup>th</sup>$  and  $15<sup>th</sup>$  day. This could also be attributed to the enhanced sorption as well as biological degradation facilitated by the increased supply of carbon and nitrogen for the microorganisms especially bacteria to grow fast and utilize TMB as source of energy. The faster dissipation of triketone herbicides in soil with higher OC content was reported by Rouchaud et al.  $[22]$  and Richard et al. $[23]$ . Rani et al. $[12]$  reported that the electrostatic interaction between anionic TMB with positive charge present with minerals and organic matter in soil at alkaline pH could be responsible for the faster dissipation of TMB in clay loam soil with the OC of 0.48%. The omission of K in the LTF practices showed moderate to

high dissipation rate and could be attributed to the increased Ca2+ in solution due to the reduced  $K^+$  ions and hence the TMB might have dissipated at a higher rate through the reduced sorption and enhanced chemical degradation. The reduced sorption of TMB could be attributed to the solubility of organic matter which is always related to the presence of polyvalent ions viz.,  $Al^{3+}$ , Fe<sup>3+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> or monovalent ions like Na<sup>+</sup> and  $K^{+[29]}$ . At high concentrations of ions in solution, these process increase and the solubility of organic matter is reduced by flocculation<sup>[24]</sup>. Barriuso et al.[25] reported that the nature of dissolved organic matter influences the adsorption and desorption of dimefuron, atrazine and carbetmine in soil. The low to moderate dissipation in the balanced fertilization practices viz., 100% NPK, 150 % NPK and 100% NPK+Zn showed that the TMB degradation could be modified by the balanced LTF practices and the source of nutrients. This showed that the supply or availability of nitrogen plays major role on TMB degradation in the soil and the promotion of atrazine and other heterocyclic compounds degradation through inorganic nitrogen starvation has also been reported [26,27]

More than 40 percent dissipation of the initial TMB residue was recorded on  $30<sup>th</sup>$  day irrespective of LTF practices and TMB applicationrates. The TMB dissipation rate increased with time and more than 70 percent dissipation occurred on 30th day after TMB application.When comparing the TMB dissipation at different application rate, the increased rate of TMB application, (Fig 6) as the degradation is always related negatively with dose of herbicide application<sup>[30]</sup>. The dissipation was found to be rapid at low rate of 60 g/ha and has been decreased with the increased rate of TMB application. The dissipation rate was found to 11.12, 13.57 and 16.24 percent on 3rd day and was 74.10, 77.32 and 95.42 percent on  $45<sup>th</sup>$  day respectively at 60, 120 and 240 g/ha. Similar results of slightly longer persistence of TMB residues in double dose (0.12 mg/kg) than single dose (0.06 mg/kg) during the later stages of degradation in clay loam soil $[12]$ .

#### **Dissipation kinetics of tembotrione in soil**

In the present investigation, irrespective rates of TMB rate and different LTF practices, the TMB dissipation profile fitted well to the first-order



**Fig 8.** Log plot of tembotrione residue in the soil as influenced by long term fertilization practices



**Fig 9.** Mean half life of tembotrione in soil as influenced by its rate of application



**Fig 10.** Mean half life of tembotrione in soil as influenced by long term fertilization practices

reaction kinetics (Fig 8). The intercept (a), slope (b) and half-life  $(T\frac{1}{2})$  and their lower and upper limits as obtained from first-order dissipation kinetics are presented in Tables 19-21. The lower and upper limit of TMB mean half-life (HL) ranged from 9.39-13.72, 15.19-20.51 and 18.94- 22.27 days respectively at 60, 120 and 240 g/ha TMB rates across different long term fertilization treatments. The computed values of the coefficient of determination  $(R^2)$  between log residues in soil and time varied from 0.771 to 0.989 (significant at  $P = 0.01$ ), indicating that the dissipation of TMB could be accounted by first-order kinetics. The highly significant fit of the 2<sup>nd</sup> order functions were observed for the treatments which 100%N, 100%NPK+FYM and 100%NPK(-S) at the lower rate of TMB application (60 g/ha) only. Whereas at recommended and double the recommended TMB rates of 120 and 240 g/ha, the TMB dissipation data fit well with the first-order kinetics except for 100%NPK+FYM which showed highly significant fit with 2nd order kinetics. A similar result of first order degradation kinetics of TMB in sandy loam and clay loam soils was observed by Rani et al.<sup>[12]</sup>. The degradation half-life (HL) of TMB was found to range from 9.39-22.29 days across the LTF practices and rates of TMB application. Increased rate of TMB application increased its degradation half-life (Fig  $9 \& 10$ ) and the mean HL was calculated to be 9.39-13.72,14.26-20.51 and 18.94-22.27 days respectively at 60, 120 and 240 g/ha application rate. The varied HL of 7.2 to 13.4 days<sup>[12]</sup>, 32-90 days<sup>[20]</sup> was reported for TMB in soil and stated that the variation is mainly attributed to the soil pH beside modified by the mineral and

**Table 1.** Effect of long term fertilization practices on tembotrione persistence (mg/kg) in soil @ 60 g/ ha application rate



\* BDL- Below Detectable Level

**Table 2.** Effect of long term fertilization practices on tembotrione persistence (mg/kg) in soil @ 120 g/ ha application rate



\* BDL- Below Detectable Level

**Table 3.** Effect of long term fertilization practices on tembotrione persistence (mg/kg) in soil @ 240 g ai/ha application rate



organic carbon of the soil. The mean HL across the LTF practices was found to be between 14.30-18.56 days irrespective of application rate (Fig 10) with the lowest and highest HL was in 100%NPK(-S) and 100%NPK+FYM respectively. This showed the positive interaction of organic carbon and the negative interaction of S free fertilization practices on decreasing and increasing respectively the TMB half life in soil. The influence of OC on enhanced TMB degradation with the HL of 13.4 days $[12]$  who stated that the soils having high OC content had boosted microbial degradation and hastened the dissipation of TMB by serving as a carbon source at the early stage and mentioned that the shifting of microbes on other sources of available carbon alleviated the dissipation rate. However, the later findings regarding the S free fertilization and increased TMB half-life needs to be explored in detail to understand the interaction and to assess the TMB efficiency on weed control in soil. The higher half life in the present investigation irrespective of rates and LTF practices could be attributed to the higher soil pH at which the TMB might present as anion and subjected to slow metabolism<sup>[12]</sup>. Trigo et al.<sup>[28]</sup> also mentioned that the reduction of TMB half-life with an increasing soil pH by the reduction of TMB sorption.

# **CONCLUSION**

Results of the conducted laboratory studies conclude that the tembotrione dissipated with the mean half-life of 14.30 and 18.55 days from the sandy clay loam soil of finger millet-maize cropping system. The continuous application of various fertilization practices and rate of tembotrione application has a significant influence on its dissipation in soil particularly the 100%NPK with FYM  $\omega$  10 t/ha and the 100%NPK  $(-S)$ , 100%+Zn and K omitted practices.This needs to be investigated in detail to understand the field persistence of tembotrione in soil exposed to various agronomic fertilization practices and to assess the risk of environmental contamination.

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