



RESEARCH ARTICLE

Synergistic effects of herbicide safener on tembotrione behavior in irrigated Inceptisols, weed obstruction and maize productivity

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ABSTRACT

Lab and field experiments were conducted to investigate the synergistic effects of safener isoxadifen-ethyl on tembotrione (TBT) persistence, sorption and leaching behavior in maize cultivated Inceptisol. Batch sorption indicated that the most stable time of its sorption in Inceptisol is 12 h irrespective of safener addition. Tembotrione showed moderate affinity to soil solids with K_{foc} values of 17.08 - 26.29. Tembotrione dissipation followed first-order kinetics with a mean half-life of 15 days and was influenced by application rate, safener, soil pH and organic carbon content. Terminal residue in maize grain was below minimum residue limit set by European Food Safety Standard (0.02 mg/kg), though detected (0.012 mg/kg) above detection limit in soil applied with double recommended rate of TBT. The weed control efficiency was 62.37 and 61.35 percent respectively in recommended and double recommended rate and produced higher grain yield too. Study authenticated that the TBT 120 g/ha with safener, isoxadifen-ethyl 1000 mL/ha is safe to soil environment and provide effective weed control in maize under tropical Inceptisols.

Keywords: Dissipation, HPLC Isoxadifen-ethyl, Maize, Safener, Sorption, Synergistic effects, Tembotrione

INTRODUCTION

Tembotrione [2-{2-chloro-4-mesyl-3-[(2,2,2-trifluoroethoxy) methyl] benzoyl} cyclohexane -1,3-dione] is a new generation low dose herbicide belongs to synthetic triketone family. It is a selective post-emergence herbicide applied to control grasses and broad-leaved weeds in maize (Santel 2009). This contact herbicide acts by disrupting carotenoid biosynthesis through inhibition of 4-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme (Schulte and Kocher 2009). Its application to maize field is recommended along with safener isoxadifen-ethyl as it enhances tolerance of cereal crops to herbicides by effectively inducing cellular xenobiotic detoxification mechanisms besides improving the selectivity (Sun *et al.* 2017, Gonsioriewicz Rigo *et al.* 2021). Though the safeners enhance the crop safety, they might also show phytotoxicity to crops when its concentration is high. Combined application of herbicide foramsulfuron with isoxadifen has increased its translocation to corn parts and its adsorption in soil (Bunting *et al.* 2004). Hence it is essential to know the synergistic influence of safener, isoxadifen-ethyl on the persistence and sorption of TBT in tropical Inceptisols of semi-arid region.

Tembotrione is stable to hydrolysis at environmental pH range of 5–9 but prone to photolysis in soil and water (USEPA 2007). Its degradation in soil strongly depends on pH and highest degradation rate was observed in more alkaline soils (AGES 2012, Rani *et al.* 2020). The loss of cyclohexane dione moiety from the benzene ring and to non-toxic phenol compounds under feasible environment is degradation pathway of tembotrione (TBT) in soil environment (Dumas *et al.* 2017 and Rani *et al.* 2020). In aerobic soils, it degrades biologically with a half-life ranged from 4 to 56 days (Macbean 2008, USEPA 2007). In acid soil, it undergoes photo degradation following biphasic pattern with rapid initial dissipation to 60% by 3 days and subsequent distinct slow dissipation with photolysis half-life of 0.7 days (AGES, 2012). Tembotrione and its metabolite also showed first order dissipation kinetics in clay loam and sandy loam soils with half-life from 7.2 to 13.4 days under controlled conditions (Rani *et al.* 2020). Being a weak acidic compound with a pKa value 3.17–2.98 and solubility 71 g/L at 20°C in water, it can exist in different tautomeric forms which largely depend upon the environmental conditions and soil pH (Dumas *et al.* 2017). Since it is not adsorbed to the suspended solids and soil sediments and released into water due to the estimated K_{foc} of 14, it has high mobility in soil and the potential to leach into ground

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water (USEPA 2007). Maximum TBT retention in 15–25 cm soil depth with limited leaching was also observed in clay loam soil than the sandy loam soil (Rani *et al.* 2020) though its metabolite showed appreciable leaching potential in sandy loam soil. It is generally established that sorption checks the pesticide degradation by reducing their partition into the soil liquid phase (Farmer and Aochi 1987).

Herbicide safeners, are chemical agents applied along with herbicides to increase the tolerance of crops to herbicides without affecting the weed control effectiveness mainly through providing selectivity and counteraction of the residual action of soil applied herbicides (Jablonkai 2013). Mostly the safeners were developed to increase tolerance of monocotyledon cereal plants and applied along with pre-emergence herbicides. Trends toward post-emergence herbicide treatments and the use of high-activity herbicide molecules have led to the development of safeners with post-emergence application in winter cereals which were subsequently evaluated and recommended for application in wheat, rice and maize (Rosinger *et al.* 2012 and Jablonkai 2013).

In India, tembotrione (TBT) has been registered for use in maize as pre- and early post-emergence herbicide. Mostly the published literatures revealed only the bio efficacy of tembotrione in tropical condition and its dissipation behavior in acidic soils. No published or only limited information on its dynamic behavior in high pH soil of semi-arid tropical condition. Therefore, field and laboratory experiments were undertaken to investigate the behavior of TBT in irrigated Inceptisol and its terminal residues in maize, weed obstruction and its productivity when applied with safener, isoxadifen-ethyl.

MATERIALS AND METHODS

Weather and climate

The normal weather of the experimental location was documented every day. Maximum temperature in the cropping period ranged from 29.9°C to 36.4°C with a mean of 32.5°C. The minimum temperature ranged from 19.6°C to 24.4°C with a mean of 21.71°C. A total rainfall of 283.10 mm was received during the cropping period. The mean sunshine hours during the cropping period were 6.1 h and the mean morning and evening relative humidity were 85.5 and 48.7 percent, respectively.

Field experiment details

Field experiment was conducted at TNAU Eastern block farm, Coimbatore which has a semi -

arid and tropical climate with dry summer and cool winter. The experimental farm is positioned at 11°0'27" N Latitude, 76°56'29" E Longitude at an altitude of 426.7 m above mean sea level in the Western Zone of Tamil Nadu. The experiment was laid out in randomized block design with five treatments replicated four times. The size of each plot was 4 × 5 m. Maize (*Zea Mays* L.) hybrid 'CO 6' of TNAU was sown during winter (*Rabi*) 2016-17. The test chemical Laudis 42% SC (34.4% W/W), manufactured by the M/S. Bayer's Crop Science Ltd., was applied as early post-emergence (2-3 leaves stages of weeds) at two rates, *viz.* 120 and 240 g/ha with and without herbicide safener isoxadifen-ethyl (0.25 % V/V) at 1000 and 2000 mL/ha as an early post-emergence spray in maize field on 15th day after sowing (DAS) with the help of a knapsack sprayer. Safener isoxadifen-ethyl was applied as per the treatment along with the TBT herbicide. Control treatment without herbicide was maintained as weed free for comparison. The soil samples were collected at 0 (2 h), 1, 3, 5, 10, 30, 45 and 60 day time intervals after its application and at maize harvest (120 days after herbicide application) from all the treated and untreated (control) plots. The soil of the experimental field was mixed black calcareous, moderately deep and well-drained, sandy clay loam in texture and classified as Inceptisol (*Typic Ustropept*). It has alkaline pH 8.45 and EC 0.32 dS/m and low in available N and high in available P and K status and 0.54% organic carbon.

Soil samples were drawn randomly from 0-15 cm depth using a tube auger from five selected spots in each plot and pooled to have approximately 500 g. Soil sample of each plot was homogenized by blending conscientiously and spread on a clean plastic sheet for size reduction. It was portioned into four equal quarters and opposite two quarters was discarded by retaining the other two quarters. Quartering and removing process was repeated to reach 100 g representative sample for final analysis. Collected soil samples were air-dried, powdered and passed through a 2 mm sieve before analysis. Maize cobs were harvested from net plot, processed and yield was expressed in kg/ha 14% moisture content. Three whole plant samples constituting about 500 g was randomly collected from each plot during harvest, cut into small pieces, homogenized for representative sub sampling. Similarly, the maize cobs were collected from each plot, thrashed and processed for analysis. About 50 g grain and stover from each treatment was sub sampled and powdered for residue analysis.

Laboratory experiment details

The batch adsorption experiment was conducted to determine the equilibrium time for assessing the TBT sorption (mg/L) with and without safener (2 ml/L) in soil at an interval of 6, 12 and 18 h. Both the herbicide formulation and safener were mixed well and used for the study. Bulk soil samples collected from the experimental field before the start of experiment was processed and used for the lab study. For adsorption studies, five grams of soil samples were placed in required number of centrifuge tubes and to which different concentrations of herbicides active ingredients (0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0 mg/L) prepared in 0.01M CaCl₂ were added with 1:5 soil: solution ratio and the experiment was duplicated for each time interval. The soil solution suspension in centrifuge tubes was shaken in an end over end shaker at ambient temperature to attain equilibrium. At each interval, two sets of each treatment were removed and centrifuged at 5000 rpm for 10 minutes maintained at 20°C and the supernatant was filtered through Paul nylon membrane filters of < 2 μm. Filtrate was extracted for tembotrione residue and determined in HPLC-DAD. The amount adsorbed (mg/kg) was calculated using the formulae

$$C_s = (C_i - C_e) * v / m$$

where C_s = the amount of solute adsorbed from the solution. v = Volume of the adsorbate, C_i = the concentration added (mg/L), C_e = the concentration in equilibrium solution (mg/L) and m = the weight in gram of the adsorbent.

Tembotrione extraction and clean up

A valid homogenized representative laboratory sub sample of soil was taken in centrifuge tube and vortexed with known volume of acetonitrile: 0.1 % acetic acid (50/50 v/v) as reported by Rani *et al* (2020). The mixture was shaken in orbital shaker for 1 hr at 60 rpm and then centrifuged at 2500 rpm for 5 minutes. Supernatant was filtered, transferred to separating funnel and partitioned using known volume of petroleum ether twice. Petroleum-ethyl layer was collected and pooled for clean-up. Powdered and homogenized maize grain and straw were extracted using distilled water by shaking an hour and centrifuging at 5000 rpm for 5 minutes. Supernatant was filtered and partitioned using hexane and ethyl acetate (2:3 v/v ratio) and 5% NaCl. Organic layer was collected and re-partitioned with di-chloromethane twice. Pooled dichloromethane layer was subjected to clean up. Extracted TBT residue was cleaned up using Na₂SO₄ and dried in rotary vacuum evaporator to moist condition.

Residue was re-dissolved in 2 ml acetonitrile for HPLC analysis. Tembotrione residue from adsorption studies was extracted following the method as described for soil samples.

HPLC conditions for tembotrione determination

The tembotrione residue was determined by Agilent HPLC (1200 series) equipped with Diode Array Detector (DAD), binary pump and auto sampler with rheodyne injection system. The separation of compound was performed using Agilent Eclipse XDB-C 18, 5 μm, 4.6 × 150 mm column kept in thermo stated oven maintained at 30°C. The instrument connected to a computer records the response in terms of peak area using the Ezchrome software. The mobile phase consists of 80:20% (v/v) acetonitrile and 0.02% ortho phosphoric acid at flow rate of 0.5 mL min⁻¹ was used in binary mode to separate the TBT. The absorption was measured at 285 nm by injecting 10 μl sample.

Method validation

Stock solution of TBT (100 mg/L) was prepared in acetonitrile using certified reference material. The working standards of TBT were prepared by serial dilution from the stock solution. Then, 0.5 μL of each working standards were injected into HPLC and the peak area was measured for linearity check study. Validation of method was executed in terms of recovery studies before analyzing unknown samples as reported by Janaki *et al.* (2013).

Data analysis

For linear sorption isotherms, the TBT sorption coefficient, K_d [L/kg] was calculated by $K_d = C_s / C_e$ (Janaki *et al.* 2013) where C_s is the amount of herbicide sorbed by the soil [mg/kg] and C_e is the herbicide concentration of the soil solution at equilibrium [mg/L]. The value of K_d was determined from the slope of the linear plots of sorbed Vs aqueous TBT concentrations. The amount of TBT sorption per unit soil organic carbon (SOC), K_{oc}, or per unit of clay, K_c, was determined using the formula K_{oc} or $K_c = (K_d / \text{SOC or clay } \%) \times 100$.

Tembotrione degradation was described using the first order kinetics equation as described by Janaki *et al.* (2013). The half-life of the herbicide molecule was determined using the equation $T_{1/2} = 0.6931/k$. Where 'k' is the degradation constant.

RESULTS AND DISCUSSION

Under the optimized HPLC conditions, the tembotrione was detected at 8.42 ± 0.2 minutes as

sharp peak. The limit of detection (LOD) and limit of quantification (LOQ) by HPLC was, 0.01 and 0.05 mg/kg, respectively. Similar LOQ for maize straw was reported by Su *et al.* (2020). Analytical calibration curve was linear ($r^2=0.9970^{**}$) in the range of 0.01 to 1.00 mg/L. Tembotrione recovery from spiked soil and maize parts with three concentrations from 0.01 to 0.5 mg/kg was more than 80 per cent confirmed the suitability of the extraction protocol for TBT from different matrices.

Tembotrione behavior in soil under laboratory conditions

Results divulged the influence of safener on time required to reach TBT sorption equilibrium. Soil solution reached equilibrium by 6 and 12 hr (**Figure 1**), respectively in TBT alone and TBT with safener added treatments. In case of TBT alone treatment, much change was not observed on 12 and 18 hr compared to 6 hr. Similarly, the TBT with safener applied treatment did not show much change on 18 h when compared to 12 hr. This time-dependent adsorption can be a result of physical and chemical nonequilibrium and has been attributed to several factors, such as diffusive mass transfer resistances, rate-limited adsorption reactions etc. (Jensen and Escudey 2019). In the present study, the variation in

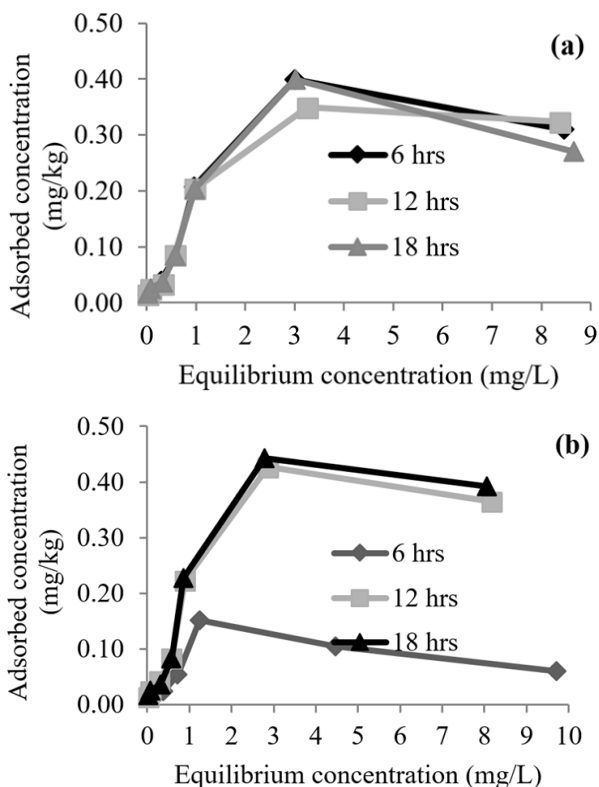


Figure 1. Tembotrione adsorption in maize soil revealed by batch equilibrium experiment (a. tembotrione alone; b. tembotrione +isoxadifenethy)

Table 1. Tembotrione adsorption isotherms parameters by simple linear model

Treatment	Isotherm parameters			
	K _d (L/g)	R ²	K _{oc}	K _c
6 h				
Tembotrione	0.177	0.714	16.22	0.263
Tembotrione + isoxadifen-ethyl	0.025	0.253	5.110	0.080
12 h				
Tembotrione	0.170	0.796	15.33	0.249
Tembotrione + isoxadifen-ethyl	0.091	0.754	18.48	0.300
18 h				
Tembotrione	0.170	0.648	15.32	0.249
Tembotrione + isoxadifen-ethyl	0.096	0.770	19.49	0.320

time and adsorption rate could be ascribed to the slow release of TBT to soil solution freely due to the modification of TBT sorption behavior by the safener isoxadifen-ethyl. It was confirmed by the significant correlation between TBT sorbed and equilibrium concentrations (**Table 1**).

Sorption data at 6 hr and 12 hr fitted to the linear function showed good correlation between equilibrium and sorbed concentration in TBT alone applied soil whereas 12 and 18 hr interval showed significant correlation in tembotrione with safener applied soil. This again confirmed that the inclusion of safener isoxadifen-ethyl increased the time required to reach the equilibrium solution concentration (**Table 3**). The results were also confirmed by the distribution coefficient (K_d), r² and K_{oc} (15.32 – 16.22) values which was high in the TBT alone received soil. Similar K_{oc} value of 12 was reported by USEPA (2007). Estimated low K_{oc} value suggests that TBT is expected to have very high mobility in soil due to the low adsorption capacity. Its low interaction with soil properties like organic carbon, carbonates, metal oxides, clay and its type etc., could be attributed to its chemical nature particularly negative charge (Gabriela Zemeka 2015) of its tautomeric form in the experimental soil pH of 8.54.

The Freundlich empirical constant (K_f) representing the soil sorption capacity (**Figure 2**) for a given range of herbicide concentration was higher at 6 hr (0.914) and 12 hr (1.288) for TBT alone and TBT with isoxadifen-ethyl treatments, respectively (**Table 2**). The Freundlich parameter (n) values, was inferior to 1 in both the treatments and showed ‘L’ type sorption. This signified a moderate affinity between soil solids and TBT and a decreased availability of adsorption sites with increased liquid

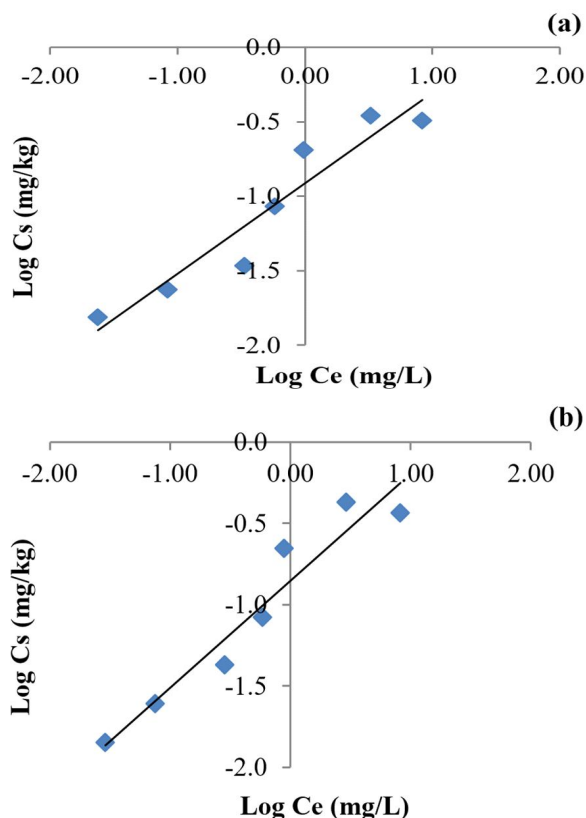


Figure 2. Linear plots of Freundlich isotherm for tembotrione adsorption in maize soil after 12 h of equilibrium (a. tembotrione alone; b. tembotrione + isoxadifenethy)

Treatment	Isotherm parameters			
	K_f (L/g)	n	R^2	K_{foc}
6 h				
Tembotrione	0.892	0.611	0.909	18.20
Tembotrione + isoxadifen-ethyl	1.288	0.369	0.648	26.29
12 h				
Tembotrione	0.914	0.608	0.908	18.65
Tembotrione + isoxadifen-ethyl	0.852	0.656	0.925	17.39
18 h				
Tembotrione	0.809	0.037	0.003	16.51
Tembotrione + isoxadifen-ethyl	0.837	0.598	0.883	17.08

concentration (Faria *et al.* 2019). The R^2 of TBT sorption in both the treatments were >0.95 at 12 hr and proved that the most stable time of TBT sorption in Inceptisol is 12 h irrespective of safener addition. Gabriela Zemeka (2015) reported 6 hr as stable time for TBT adsorption in Vertisol. The calculated K_{foc} values were ranged from 17.08 to 26.29 with higher values in TBT with isoxadifen-ethyl treatment.

Being weak acid, only the part in the molecular form could be adsorbed and hence easily repelled by the negative groups on the surface of soil (Faria *et al.*

2019). The presence of organic matter and metal ions in high pH soils might also affect the herbicides sorption as the precipitation of metal ions reduces its interaction with herbicides. If not precipitates, the metal cations raise the herbicides sorption with residual negative charges as a result of electrostatic attraction (Rani *et al.* 2020). Thus, the present sorption study reveals that the TBT has moderate sorption behavior in Inceptisol and is modified by the soil pH besides the clay and organic matter content.

Tembotrione dissipation in soil and terminal residue in maize

Initial TBT deposit detected on day 0 (2 hr after TBT application) ranged from 0.0404, 0.1245, 0.0521 and 0.1745 mg/kg soil at 120, 240 g/ha without and with safener, respectively. Tembotrione 240 g/ha + 2000 ml safener recorded higher residue compared to 120 g/ha with or without safener. Residue detected in soil increased from 0 to 5 days and then decreased and ranged from 0.0801-0.2641 mg/kg on 5th day. It persisted in soil up to 45 and 60 days after application corresponds to lower and higher rates irrespective of safener addition (Figure 3) and then the residues became below the quantification limit. Similar results were reported by Dyson *et al.* (2002) for the mesotrione which persist in soil up to 32 days and were depending on the environmental conditions and type of soil. Stability of TBT to hydrolysis at environmental pH range of 5–9 (USEPA, 2007), might be responsible for its persistence in soil up to 60 days. Results also showed that the application of TBT with safener enhanced its persistence in soil and could be ascribed to the increased addition of TBT residue to soil through decomposing weeds which might have absorbed and retained more residue due to selectivity of safener. Wichert *et al.* (1999) also reported that the foliar uptake of triketone by weed species is rapid and 40 to 70% of the mesotrione chemical being absorbed within an hour of application.

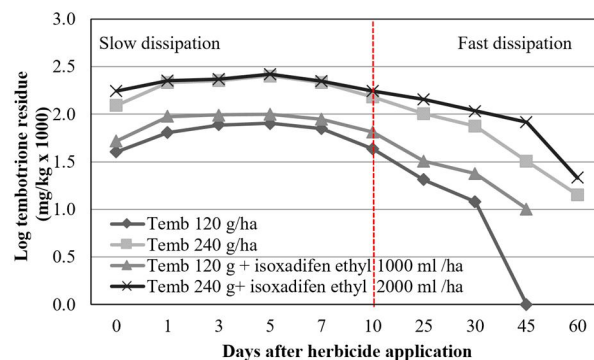


Figure 3. Degradation of tembotrione in maize cultivated Inceptisol (Temb – Tembotrione)

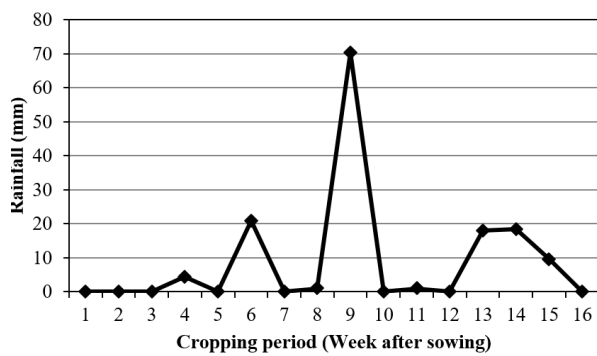


Figure 4. Rainfall received during the maize cultivation period

Dissipation of TBT was arrived using the highest residue detected on 5th day (Table 3). More than 50 per cent of the residue has dissipated from soil on 25th day in all the plots except at higher rate with safener and 50% dissipation occurs on 30th day in the plot that received 240 g/ha and safener 2000 mL/ha. This could be ascribed to the enhanced degradation and leaching of TBT by the high rainfall occurred during 6th and 9th weeks after its spray corresponds to 21 and 70.5 mm, respectively (Figure 4). Residue was not detected on day 90 of application. Tembotrione dissipated slowly from soil when it was applied with safener, and 36 and 34% was noted respectively in 120 and 240 g/ha plots on day 10 after application. At the above two rates, 85 and 71% dissipation were observed without safener

plots and 76 and 59% with safener plots on day 30. Kucharski and Sadowski (2009) found addition of adjuvants reduced the degradation rate of phenmedipham in soil. More than 90% of the applied TBT dissipated from soil on day 60 in double the rate applied plot and on 45th day from the recommended rate plot irrespective of safener addition. Similar longer persistence of TBT residues in double dose was observed by Rani *et al.* (2020) during later stages of its degradation in clay loam and sandy loam soils.

Tembotrione dissipation profile best followed the first-order and 1.5th order reaction kinetics (Table 3 and 4). The coefficient of determination (R^2) between log residues and time varied from 0.9773** - 0.9920** (significant at $P = 0.01$), 0.9685** - 0.8891* (significant at $P = 0.05$) and 0.9328** - 0.7905* (significant at $P = 0.05$), for 1st, 1.5th and 2nd orders, respectively. This indicated that the TBT dissipation could be well accounted by first-order kinetics followed by 1.5th order. Similar first order kinetics of TBT was also observed in clay loam and sandy loam soils by Rani *et al.* (2020). Mean half-life of TBT ranged from 9.1 to 20.5 days and increased with increased rate and safener addition in the present experiment. Half-life of 6 to 14 days and 10 to 22 days respectively in soil exposed to sunlight and or dark was reported in literature (EFSA, 2013 and Barchanska *et al.* 2016) for TBT degradation. Higher half-life in safener applied plot could be ascribed to the reduced leaching of TBT through soil profile.

Table 3. Persistence of tembotrione in maize cultivated Inceptisol

Time (DAA)	Residue concentration (mg/kg) ±SD ^a			
	Tembotrione 120 g/ha	Tembotrione 240 g/ha	Tembotrione 120 g/ha + 1000 ml isoxadifen-ethyl	Tembotrione 240 g/ha+ 2000 ml isoxadifen-ethyl
0	0.0404± 0.009	0.1245± 0.008	0.0521± 0.011	0.1745± 0.009
1	0.0642± 0.008	0.2148± 0.007	0.0945± 0.010	0.2248± 0.010
3	0.0768± 0.011	0.2245± 0.009	0.0987± 0.009	0.2343± 0.009
5	0.0801± 0.008	0.2541± 0.011	0.1002± 0.008	0.2641± 0.011
7	0.0708 ± 0.007 (11.6)	0.2145± 0.010 (15.6)	0.0886± 0.007 (11.6)	0.2219± 0.009 (16.0)
10	0.0432 ± 0.009 (46.1)	0.1513± 0.008 (40.5)	0.0646± 0.008 (35.5)	0.1745± 0.008 (33.9)
25	0.0204± 0.011 (74.5)	0.1004± 0.009 (60.5)	0.0318± 0.007 (68.2)	0.1425± 0.009 (46.4)
30	0.012± 0.006 (85.0)	0.0745± 0.007 (70.6)	0.0237± 0.006 (76.4)	0.1084± 0.007 (59.0)
45	BDL	0.032± 0.007 (87.4)	0.0101± 0.006 (90.0)	0.0825± 0.008 (68.8)
60	BDL	0.0142± 0.011 (94.4)	BDL	0.02141± 0.011 (91.9)
90	BDL	BDL	BDL	BDL

^aAverage of three replicates; SD: standard deviation; figures in parentheses designate % dissipation; BDL- below detectable level, DAA: Days after application

Table 4. Half-life, correlation coefficient and regression equation tembotrione degradation in maize cultivated Inceptisol

Tembotrione treatment	Half-life (days)	Regression equation	Correlation coefficient r^2
Tembotrione 120 g/ha	9.75	Y=2.2071+0.0763	0.9748
Tembotrione 240 g/ha	14.44	Y=1.2132+0.048	0.9773
Tembotrione 120 g/ha + 1000 ml isoxadifen-ethyl	12.12	Y=2.0273+0.0572	0.9920
Tembotrione 240 g/ha + 2000 ml isoxadifen-ethyl	20.51	Y=1.2225+0.0338	0.9485

Kucharski and Sadowski (2009) found that the DT_{50} value for phenmedipham + oil adjuvant mixture was about 11 days higher in comparison with phenmedipham alone. In the present experimental Inceptisol, the TBT degraded moderately with a mean half-life of 14 days which could have been strongly influenced by the higher soil pH (AGES 2012, Rani *et al.* 2020). Alkaline pH of soil could have accelerated the chemical degradation of TBT by detaching the cyclohexane dione moiety from benzene ring and further to non-toxic phenol compounds (Dumas *et al.* 2017 and Rani *et al.* 2020). Medium organic carbon (OC) content (5.4 g/kg soil) of the present experimental alkaline Inceptisol might have also hastened the TBT degradation. This could be attributed to the electrostatic interaction between anionic TBT with positive charge present with minerals and organic matter in soil at alkaline pH particularly the divalent calcium ions dominating in the present experimental soil. Rani *et al.* (2020) stated that TBT degradation rate is highly influenced by organic matter content and pH of the soil and observed a slightly higher dissipation in clay loam soil with 0.48% OC than sandy loam soil with 0.38% OC.

Terminal residues of TBT in soil and maize plant parts at harvest (120 DAS) was determined since the herbicides applied in soil are also taken in by the plants and metabolized or accumulated as such in different plant parts. In the present study, TBT terminal residues were seen below the detectable limit (0.01 mg/kg) in soil irrespective of rates and safener addition. However, the residue was detected above BDL (0.012 mg/kg) in the soil of plot that received TBT 240 g/ha and safener 2000 mL/ha. This showed that the increased rate of TBT and safener might have increased its persistence time by reducing its solubility and leaching from soil. Kucharski and Sadowski (2009) found that the addition of adjuvants caused an increase of the phenmedipham residues in soil and roots of sugar beet and reduced its degradation rate in soil. It is generally established that sorption checks the pesticide degradation by reducing their partition into the soil liquid phase (Farmer and Aochi 1987). Detectable residue (>0.01 ppm) was not found on day 120 of application in maize grain and stover at any of the rate which is well below the existing MRL (0.02 mg/kg) proposed by European Union for tembotrione residue in maize grain (EFSA 2018). This could be attributed to the capacity of maize plant to metabolize TBT into dihydroxy tembotrione and subsequently benzoic acid which is the dominant metabolite found in maize at maturity (EFSA, 2018).

Weed obstruction, phytotoxicity to maize and yield

Significant variation in total weed density and dry weight was observed among the TBT applied plots at 40 days after sowing (Table 5 and Figure 5). Tembotrione applied with safener controls the weeds appreciably than without safener plots. This showed that the safener isoxadifen-ethyl helps in the selectivity of weeds and crop to TBT and hence provided efficient weed control. Though increased dose of TBT decreased the weed density (5.17 no./m²) and dry weight (4.91 g/m²), TBT applied at 120 g/ha with isoxadifen 1000 mL/ha also gave effective weed control efficiency of 62.37 per cent without phytotoxicity to maize plant. Singh *et al.* (2012) noticed that tank mix of TBT 120 g/ha as PoE along with safener recorded significantly lower weed density and higher weed control efficiency. Weed control efficiency was found to range from 30.43 to 50.05% in the TBT applied plots but without safener isoxadifen-ethyl.

Maize tolerance to applied TBT with and without safener isoxadifen-ethyl was observed on 1, 3, 7 and 15 days and evaluated using phytotoxicity scorings. Tembotrione applied at higher rate of 240 g/ha with or

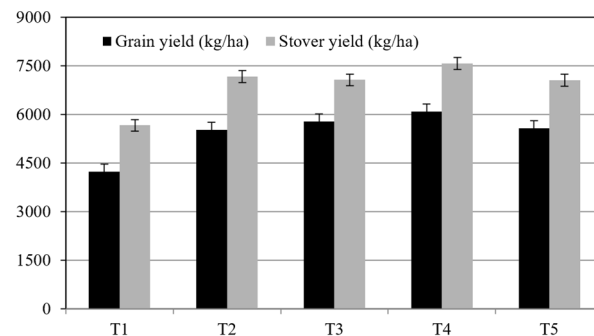


Figure 5. Synergistic effect of safener isoxadifen-ethyl and tembotrione on maize yield in Inceptisol (T1-Control; T2-Tembotrione 120 g/ha; T3-Tembotrione 240 g/ha; T4-Tembotrione 120 g/ha + 1000 ml safener; T5-Tembotrione 240 g/ha + 2000 ml safener; Error bar indicates standard error deviation)

Treatment	Total weed* density (no/m ²)	Total weed dry* weight (g/m ²)	Weed control efficiency (%)
Control	8.23 (65.80)	8.88 (76.92)	-
Tembotrione 120 g/ha	6.90 (45.78)	6.53 (40.76)	30.43
Tembotrione 240 g/ha	5.90 (32.87)	6.22 (36.70)	50.05
Tembotrione 120 g/ha + 1000 ml isoxadifen-ethyl	5.23 (25.42)	5.34 (26.54)	62.37
Tembotrione 240 g/ha + 2000 ml isoxadifen-ethyl	5.17 (24.76)	4.91 (22.10)	61.35
SED	0.24	0.27	-
LSD (p=0.05)	0.48	0.55	-

*Data subjected to SQRT ($\sqrt{x+2}$) transformation – Figures in parentheses are original values

without safener showed chlorosis and necrosis to maize on 3rd day. Symptom was visualized even after 7 days with a rating one in TBT applied plot (240 g/ha) without safener. Affected plants recovered completely after 10 days in all plots and the phytotoxicity was not evident thereafter. However, the maize is tolerant to TBT when applied at recommended rate of 120 g/ha. Present results are in line with the findings of Ankush Kumar *et al.* (2017) who have not observed TBT phytotoxicity on maize when applied at recommended rate. On 7th day, symptoms were not seen in the plot applied with TBT 240 g/ha with safener. This could be ascribed to the enhanced metabolism of TBT in maize induced by the isoxadifen-ethyl as it is a safener (Santel 2009, Schulte and Kocher 2009).

Maize grain yield was increased by the TBT irrespective of rate and isoxadifen-ethyl addition. Grain and stover yield increased from 31-44 and 25-34 % respectively due to TBT and safener addition compared to weed control (hand weeded) plot. Tembotrione applied with isoxadifen-ethyl at recommended rate 120 g/ha produced significantly higher maize grain and stover yield when compared to 240 g/ha with or without safener (**Figure 5**). This could be attributed to the increased weed control through the improved weed and crop selectivity by TBT persuade by the co-application of safener isoxadifen-ethyl. Significantly higher grain yield in maize by the TBT application 120 g/ha as PoE along with safener was also reported by Singh *et al.* (2012). Higher maize grain yield of 3.77 t/ha in the TBT 120 g/ha + safener 1000 mL/ha plot compared to without safener (3.61 t/ha) was observed by Ankush Kumar *et al.* (2017). Decreased of grain and stover yield (9 and 8 percent respectively) in the plot received higher TBT (240 g/ha) with isoxadifen-ethyl (2000 mL/ha) could be ascribed to the increased phytotoxicity. This was confirmed by the negative and significant correlation of crop phytotoxicity rating on 3rd day with maize stover biomass ($r^2 = -0.732^{**}$). Similarly, Ankush Kumar *et al.* (2017) reported higher maize grain yield of 3.77 t/ha in the TBT 120 g/ha + safener 1000 mL/ha plot compared to no safener (3.61 t/ha).

The study revealed that the TBT sorption in sandy clay loam Inceptisol with alkaline pH followed 'L' type sorption with stable sorption time of 12 hr and dissipated following first-order kinetics with a mean half-life 14.79 days. Its dissipation was found to be rate dependent and safener inclusion and was highly influenced by the soil pH and organic carbon content. Tembotrione terminal residue was detected

above BDL (0.012 mg/kg) in soil received 240 g/ha with safener 2000 mL/ha. The recommended rate of TBT applied with safener controls the weed appreciably and produced higher grain yield without phyto-toxicity. The study authenticated that the TBT at 240 g/ha with safener isoxadifen-ethyl 1000 mL/ha is relatively safe to soil environment and producing effective weed control in Indian tropical Inceptisols.

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