Original Research Article

Effect of Long Term Fertilization Practices on Tembotrione Movement in Soil: Assessment by Plant Bioassay

ABSTRACT

The herbicide behavior in soils is executed nowadays via the chemical extraction and instrumental analysis. However, the biological availability and the dynamics of herbicide in soil could be studied effectively through bioassay in the absence of sophisticated techniques and also *insitu* conditions. Tembotrione is one of the recently registered triketone herbicide and its movement behavior was not studied much in India. Hence the present study was executed to understand the influence of long term fertilization practices on its transport and movement using the bioassay technique involving Randomized Block Design (RBD). The plot wise soil samples were collected from the 50 years old long term fertilization experiment, processed and used to conduct the leaching column (made of PVC) experiment under controlled condition for 30 days. Soil samples were dissected depth wise and residue of tembotrione in each depth was obtained by conducting bioassay using green gram and sunflower as test species. For calibration curve preparation, a separate bioassay was conducted by fortifying tembotrione in control soil at different concentrations. The recorded plant parameters viz., germination percent and biomass after 15th day of both the test species predicted the tembotrione transport efficiently and the results of both the test species were comparable. The tembotrione has shown movement upto 30 cm soil depth and the concentration of residue decreased with increase in soil depth. The long term fertilization practices with the balanced nutrients supply viz., 100% NPK with hand weeding, Zn, FYM showed significant effect on the transport of tembotrione in soil at various depths.

The study concludes that the plant bioassay technique using green gram and sunflower could be very well used to investigate the transport behavior of Tembotrione in soil. Based on data, tembotrione was identified as moderately mobile compound in sandy clay loam and its movement has been altered by the long term fertilization practices.

Keywords: [Tembotrione, bioassay, transport, fertilization, greengram, sunflower]

1. INTRODUCTION

The persistence and movement of herbicides in the soils depend on the sorption and physical, chemical, and biological degradation of them contributes to the biogeochemical transformation of any molecule [1-3]. All these variables are affected by soil chemical, biological, and physical characteristics, and the chemical nature of the herbicides [3, 4]. The wide use of long persistence herbicides has caused residue accumulation in the environment through volatilization of toxic compounds, surface runoff, and leaching besides altering the soil biota [5-7].

Herbicide residues in soils can be determined by plant bioassays or by chemical methods. Chemical methods are specific, sensitive and quantify the total amount of herbicide residue in soil ([8-10]. However, they may be costly, requiring extraction solvents and sophisticated analytical equipment, and can be time consuming as well. Plant bioassays are simple,

inexpensive, and measure a phytotoxic portion of soil residual herbicide which typically varies with soil type and plant species.
A bioassay is a measure of a plant's response to the total herbicide residue in soil at a site-specific location [11]. A bioassay can determine whether or not a susceptible crop grown in a rotation will be damaged, even when current recommended recropping intervals are followed [12]. For example, mustard was found to be a good indicator of 2,4-D [13], and ryegrass (*Lolium multiflorum* Lam.) was chosen as the best indicator among several crops for linuron [14].
Tembotrione, a synthetic triketone was considered as 'eco-friendly' when they were marketed in the 2000s, as they result from hemi-synthesis of natural phytotoxin. Nevertheless, natural products can impose toxicity in the environment [15]. Tembotrione can be applied at pre and post-emergence stage due to which larger part of it goes to soil after application. Therefore, it is highly prone to contaminate environment due to its retention in soil and movement to ground water through aqueous phases present in soil [16-18].

This molecule is registered recently (during 2016) in India and frequently applied by the farmers particularly for maize crop to keep weed free. It has a systemic action on plants and inhibits the biosynthesis of 4-hydroxyphenylpyruvate dioxygenase (HPPD), an enzyme essential for carotenoid synthesis. If sensitive plants treated with this herbicide, a reduction of carotenoid concentration is observed in the leaves. Under this condition, chlorophyll and photosynthetic membranes are degraded by photo-oxidation, which results in the appearance of intoxication symptoms characterized by an intense whitish colouring of leaves, evolving to necrosis and plant death [19].

In this juncture, it is crucial to know the retention and transport processes of tembotrione in the soils of tropical semi arid region which could helps to modify the recommendation for efficient agronomic weed management and to predict the environmental impact of its residue in soil. Mostly the instrumental techniques are used to assay the herbicides behavior which involves skilled personnel and high cost. On other side, using indicator plants to detect its residue would helps to judge the influence of soil environment on their dynamic behavior beside rivet less cost and semi-skilled personnel. Identifying suitable indicator plant and judging parameters are also warranted otherwise the detection close to actual occurrence could not be achieved. Thus, the present study was planned to investigate the movement and transport behavior of tembotrione influenced by the long term fertilization practices in *typic Ustropept* soil.

2. MATERIAL AND METHODS

2.1. Soil collection

Soil samples were collected from each treatment plot (nine long term fertilization practices and control) at a depth of 0-30 in the field no. 36 (Long-term fertilizer experiment) of Tamil Nadu agricultural university, Coimbatore after the harvest of 114th Ragi crop. After drying the samples in shade, the soil particles were ground and processed through a 2 mm sieve.

2.2. Chemicals, reagents and standards

The formulation product of tembotrione (Laudis 34.4% w/w OD) of Bayer Crop Science India Limited, Mumbai was purchased from 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.

2.3. Bioassay for standard curve preparation and residue detection

The green gram (*Vigna radiata*) and sunflower (*Helianthus annuus*) were chosen as test species to conduct the bioassay. The bioassay using control soil was conducted by fortifying the tembotrione working standards of various concentrations ranged from 0 to 5.0 mg/kg. Control soil was filled in disposable cups at known weight and the tembotrione working concentrations were fortified on the top of cups and then sown with test species. This standard bioassay was replicated thrice. For assessing the tembotrione movement and transport, the above said plant species were sown @ 10 seeds per cup / depth in each depth of each column from column experiment. Growth parameters viz., percent germination and total plant biomass (g) on 4^{th} and 10^{th} day respectively were recorded to assess the tembotrione residue both in calibration and column experiment.

2.4. Column experiment and bioassay study

Columns (60 cm length and 60 cm internal diameter) were constructed from polyvinyl chloride (PVC) pipe columns were cut vertically into two and joined together using an adhesive tape. The lower end of the tube was covered with a muslin cloth. The soil of each fertilization plot was added to the columns in small increments (@ 50 g) and after each addition the soil was compacted with equal force to obtain a column of uniform compactness. The columns were packed to bulk densities of 1.33 Mg/m^3. Columns were pre treated with 100 ml distilled water one day before the herbicide application. Water was allowed to drain naturally. The tembotrione was applied at 20 g ai/ha on the surface of each column and sufficient water was added whenever the hairline cracks seen on the top of soil column. Each column was leached with 100 mm (70 mL) of distilled water at a time which was equivalent to average rainfall of 130 mm normally received during the experiment period in the study region. One column packed with soil was kept as control to which no herbicide was added. Each column was replicated twice. At the end of experimental period (30 days) adjective tape was cut and the column was split vertically by-passing metal wire along the joint. Then the soil in the column was sectioned based on depth viz., 0-15, 15-30 and 30-45 cm depth and were used to carry out plant bioassay.

2.5. Statistical analysis

The dose-response standard calibration curve (exponential regression) was constructed using the tembotrione concentration (X axis) versus growth parameters data (Y axis) obtained from the tembotrione fortified experiment individually for both the plant species with the help of MS excel software 7.0 version. The standard calibration curve regression equation was used to predict the tembotrione residue concentration at different depth by fitting the growth parameters viz., germination percent and biomass from column experiment bioassay. Residue calculated with the help of plant growth parameters of both the plant species were compared to assess the efficiency of residue quantity calculation to assess the transportation behavior of tembotrione in soil as modified by the various long term fertilization practices.

3. RESULTS AND DISCUSSION

3.1. Screening of test plants for bioassay

Two distinct plant species were evaluated for bioassay (green gram and sunflower) to assess the transport behavior of tembotrione in soil by fortifying various concentration of

tembotrione ranged from 0.10 to 5.00 mg/kg control soil. Irrespective of the tembotrione concentration and test species, there was not much difference in the growth parameters (Fig. 1) among the control and treated soil bioassay. Both the test species showed similar performance with respect to reduction of percent germination (29.9 and 35.0 % for sunflower and green gram respectively) in treated soil compared to control. However, the reduction in biomass for sunflower and green gram was found to be 38.8 and 50.5 percent, respectively and which showed that the green gram was more sensitive to the tembotrione than sunflower as bioassay species

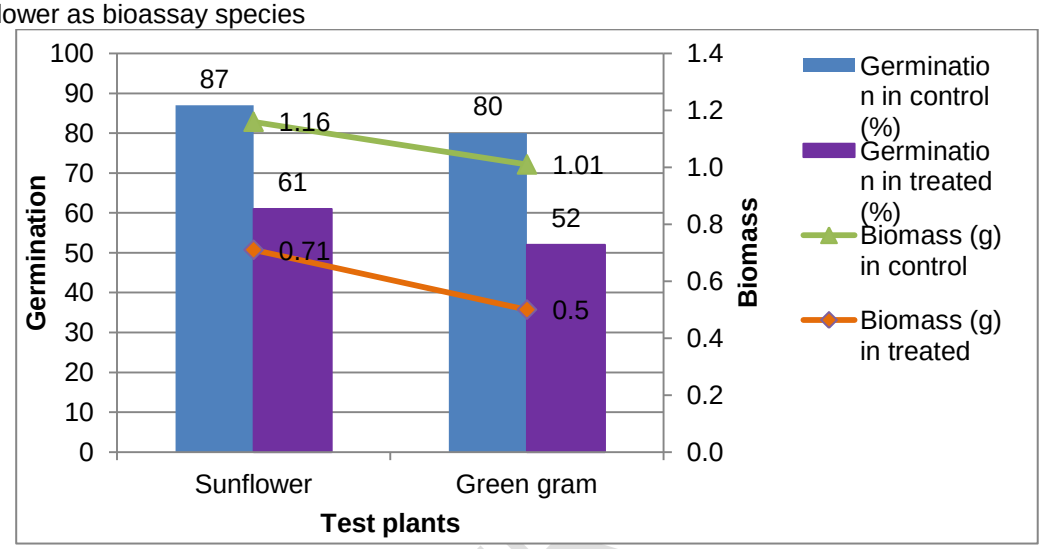


Fig 1. Effect of tembotrione on growth parameters of bioassay plants compared to control

3.2. Calibration standard curve and linearity assessment

Linearity is a technique for measuring the range in which the findings are proportionate to the analyte concentration in the samples, either directly or through an appropriate mathematical transformation. The control (no herbicide) soil was taken in polyvinyl chloride (PVC) pipe column with different tembotrione concentrations from 0 to 5.0 mg/kg, and green gram and sunflower (five plants in each cup) were performed for bioassay upto 15 days and growth parameters were recorded. The calibration curves were prepared by plotting the tembotirone concentrations in 'X' axis and the change in growth parameters in 'Y' axis obtained from the fortified bioassay experiment. The percentage reduction in germination and biomass of sunflower and green gram seeds with different tembotrione concentrations was linear across the range of 0 to 5.0 mg/kg. The exponential expression of the dose-response relationship for both the parameters from both the test species is presented in Fig 2. The best-fit estimated regression equation was y=80.142e-0.161x (R2=0.9519**) and y=1.030e-0.22x (R^2 = 0.954**) respectively for sunflower germination and biomass and y= 71.264e-0.189x (R2=0.8995*) and y=0.846e-0.35x (R^2 = 0.960**) for green gram germination and biomass. Similar results were obtained for tembotrione bioassay using indicator test species [20-21]. Faria et al. [20] screened tweleve plant species for the detection of temobtrione retention in soils and selected the two most efficient species viz., *Beta vulgaris* and (Y = -3.7763+104.2767*exp (0.441x); R2: 0.85) *Brassica oleracea* var. *capitata* (Y=-8.3042+109.8817*exp(0.3277x); R2=0.97) as indicator plants for detecting tembotrione in the soil. Using beet as Indicator plant, they determines sorption of this

herbicide and found that the shoot dry matter was the most adequate parameter for tembotrione detection (R2=>0.91*).

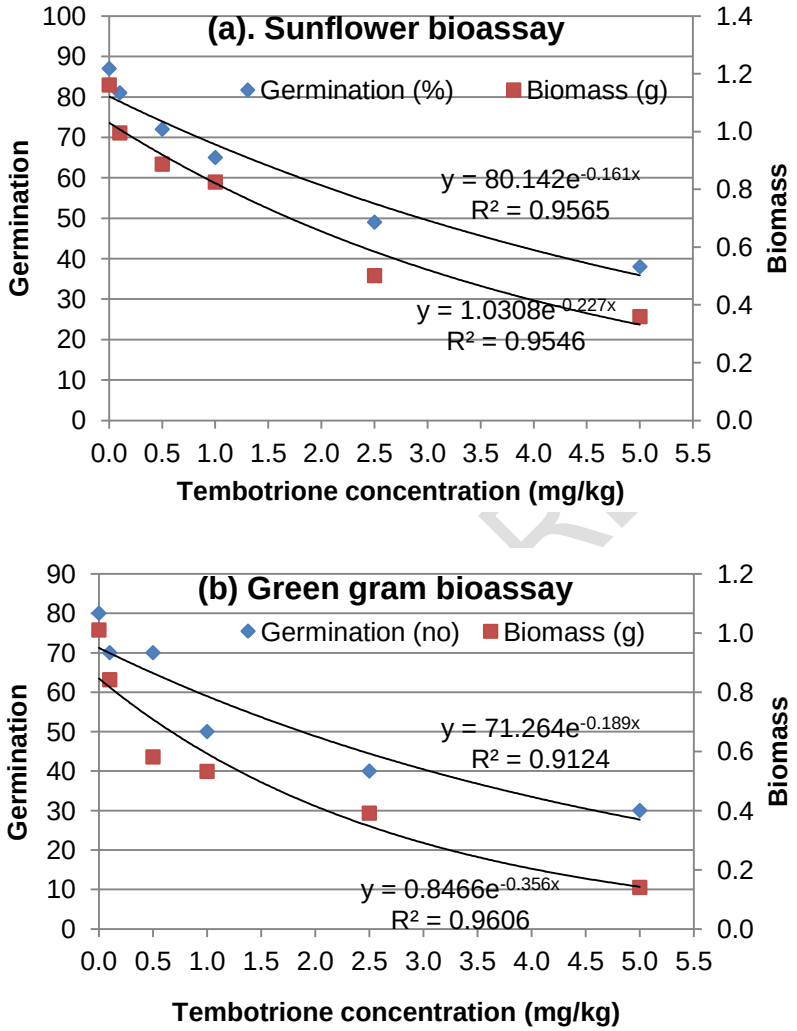


Fig 2. Calibration graph showing exponential plot of tembotrione concentration Vs plant parameters

3.3. Movement and transport of tembotrione in soil

The indicator plants bioassay using green gram and sunflower as test species was conducted to investigate the movement and transport of tembotrione in soils from long term fertilization practices plots with leaching column experiment last for 30 days. After 15 days, the growth parameters namely germination percentage and total biomass were recorded at different depths viz., 0-15, 15-30 and 30-45 cm depths of the leaching column. With the help of the calibration curve's regression equation, the tembotrione residue present in the leaching column at different depths was calculated and the efficiency of the growth parameters and test species were compared in assessing its movement and transport behavior in soil. Both the growth parameters of both the test species showed higher

tembotrione residue in top soil (0-15 cm) and then decreased with increased soil depth (Figures 3 and Table 1-2) irrespective of the long term fertilization practices. Results showed that the tembotrione is moderately mobile in the present experimental sandy clay loam soil since maximum residue was detected at 0-15 cm depth as assessed by all the bioassay parameters. Generally the pesticides are classified into different classes of leaching based on the Koc or Kd values and those with low values are identified to be highly mobile pesticides. Though USEPA [22] classify the tembotrione as highly mobile on based on its Koc of 14, it has been found to be moderately mobile molecule as major residue was detected in 0-15 cm by all the parameters of both the test species. This is against the findings of Rani et al. [23] who mentioned that the dissociated anionic products of tembotrione at alkaline pH are more resistant to hydrolysis and photolysis and may have longer persistence and greater offsite movement in the environment.

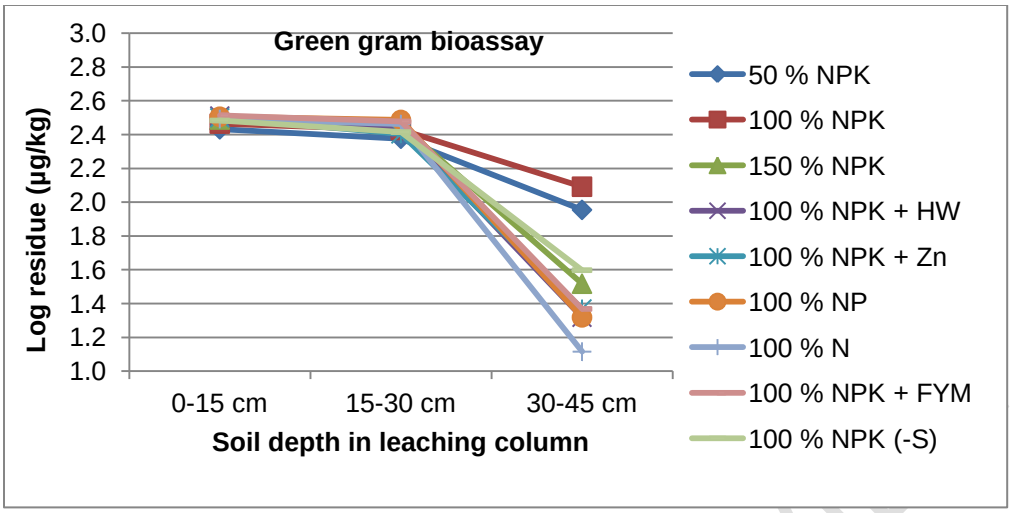
Table 1. Depth (cm) wise distribution of tembotrione residue (%) detected through bioassay technique

Test species	Growth parameters	Distribution of tembotrione residue (%)		
		0-15 cm	15-30 cm	30-45 cm
Green gram	Germination	49	45	6
	Biomass	50	43	5
Sunflower	Germination	54	33	13
	Biomass	50	43	7

3.4. Influence of long term fertilization practices on tembotrione transport in soil

The influence of long term fertilization practices on modifying the transport and movement behavior of tembotrione was assessed using the bioassay technique and the log residues at different depths under various long-term fertilization practices are presented in Figure 3. Irrespective of species and growth parameters, the tembotrione showed moderate leaching and was modified by the fertilization practices. The tembotrione residue detected at 0-15, 15-30 and 30-45 cm soil depth assessed by green gram was ranged from 0.23-0.31, 0.17-0.28 and 0.13-0.17 mg/kg respectively using germination and 0.31-0.36, 0.24-0.33 and 0.04-0.07 mg/kg respectively using biomass. Similarly the with the sunflower bioassay, the residue calculated was ranged from 0.24-0.27, 0.11-0.19 and 0.08-0.11 mg/kg respectively using germination and 0.32-0.38, 0.29-0.30 and 0.06-0.11 mg/kg respectively using whole plant biomass. This showed that both the species exposed that the tembotrione has moderate mobility in the present *Typic Ustropept* soil and is highly confined to 0-30 cm soil depth. Similar results were reported for sulcotrione and tembotrione where the movement was seen upto 25 cm soil depth depending on soil texture [22].

All the fertilization practices recorded similar quantity of tembotrione residue at surface soils of 0-15 and 15-30 cm except 100% NPK+FYM, 150% NPK and 100% NPK+ hand weeding (HW) practices. The variations were sharply observed among the fertilization practices only at 30-45 cm depth. While the 100% NPK had more residue at sub soil (30-45 cm), the 100% NPK+FYM and 100% N, 100%NPK+Zn and 100% NPK(-S) registered less residue. Hence the detailed interaction between the tembotrione movement and nutrients must be studied to understand their influence. Higher residue at surface depth in 100% NPK+FYM could be attributed to the higher organic carbon content in this plot. Simlar results were reported by Rouchaud et al. [24] who measured the mobility of mesotrione in the 0-20 cm and observed that the compound was not detected in the 15-20 cm and they conclude that the adsorption of mesotrione was greatest on the soils recently treated with organic fertilizers, and having a loam or sandy loam texture.



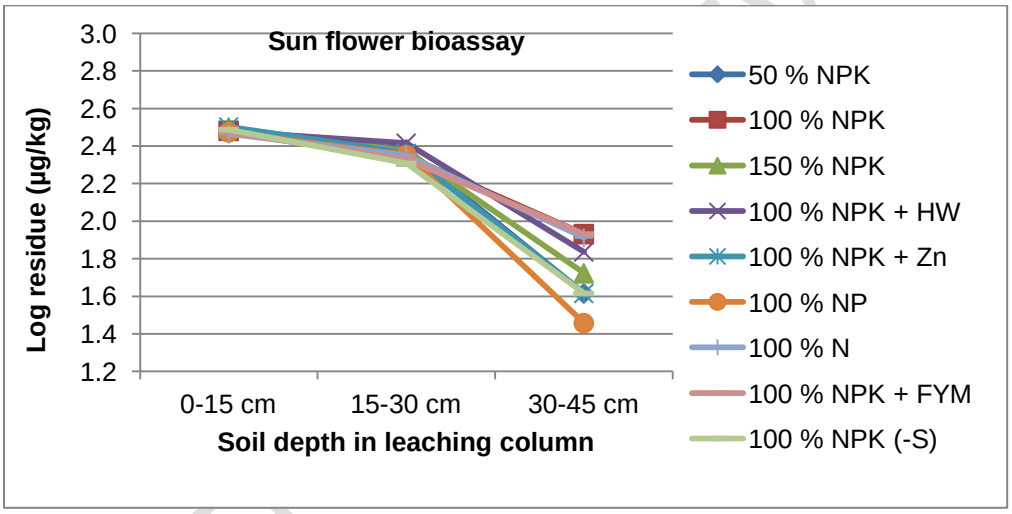


Fig 3. Effect of long term fertilization practices on the depth-wise tembotrione residue assessed using bioassay technique

Among the long term fertilization practices, 100% NPK, 100%NPK+HW, 100% NPK+FYM and 100% NPK (-S) practices showed higher tembotrione retention in all the depth irrespective of growth parameters and test species. This could be attributed to the variations in organic carbon status and nutrients availability of the soil (Data not given). Krutz et al. and Shaner et al. [25, 26]. also identified that the total organic carbon and pH are the two important factors control the sorption and degradation of herbicides in soil. Rouchad et al. [23] reported about faster dissipation of triketone herbicides in soil with higher OC and Rani et al. [22] observed slightly higher dissipation in clay loam soil with 0.48% OC than sandy loam soil 0.38% OC. They reported that the presence of higher OC content offered greater interaction between cation-anion species through ligand bridging and electrostatic interaction with metal oxides present in soil and hence tembotrione leaching has been hindered in the clay loam soil.

Table 2. Influence of long term fertilization practices on the depth (cm) wise distribution of tembotrione residue (%) detected through bioassay techniques

Treatments	Germination (%)			Biomass (g)		
	0-15 cm	0-15 cm	15-30 cm	0-15 cm	0-15 cm	15-30 cm
Green gram						
50 % NPK	43	32	25	47	46	7
100 % NPK	37	37	26	48	41	11
150 % NPK	52	48	0	47	44	10
100 % NPK + HW	53	47	0	52	42	6
100 % NPK + Zn	52	48	0	55	38	7
100 % NP	51	49	0	48	46	6
100 % N	48	47	5	53	47	0
100 % NPK + FYM	51	49	0	50	44	6
100 % NPK (-S)	53	47	0	49	40	0
Sunflower						
50 % NPK	47	37	16	55	45	0
100 % NPK	54	30	16	47	41	13
150 % NPK	59	41	0	48	38	14
100 % NPK + HW	51	39	11	46	43	11
100 % NPK + Zn	51	32	17	56	44	0
100 % NP	56	32	12	53	47	0
100 % N	53	33	14	46	41	13
100 % NPK + FYM	53	25	21	47	43	9
100 % NPK (-S)	58	24	19	54	46	0

4. CONCLUSION

The present study showed that the plant bioassay using green gram and sunflower as test species were sensitive enough to detect the movement and transport of tembotrione in soil. Tembotrione transport was restricted mostly to 30 cm and hence the molecule is can be identified as moderately mobile in sandy clay loam soil. Its movement and transport was influenced by the long term fertilization practices involving the balanced nutrients supply practices viz., 100% NPK with hand weeding, Zn and FYM. This indicates the existence of interaction between the soil-physico chemical properties and its movement in soil. Hence detailed investigation needs to be carried out to understand the transport behavior of tembotrione in soil as influenced by the different fertilization practices and to assess the risk of environmental contamination by the tembotrione.

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