CHAPTER-4

Dissipation of Tebuconazole in soil

4.1 Introduction

Pesticides reach the soil surface mainly when sprayed on crops or direct application on the soil. The dissipation of pesticides from the soil surface may reduce, or persistence may increase when it is used repeatedly at recommended field rate or maximum field rate of applications.

Amongst the different group of fungicide products used in India, "Folicur 25EC", formulation product of Tebuconazole is comparably more used triazole group of fungicide product. Tebuconazole is stable to elevated temperature in environment, photolysis by direct sunlight, and hydrolysis in pure water under sterile condition at 22 °C (Tomlin CDS., 2009). It is hydrolytically and photolytic stable due to the lack of functional groups and it does not contain chromophores so it is absorb at wavelength > 290 nm (Tebuconazole, PubChem CID 86102). The pKa value of tebuconazole in water at 25 °C is 5 ± 0.1(Cadkova E., et al. 2013). It is considered as a neutral or very weak base.

Dissipation of pesticides from soil is mainly by degradation. Apart from sorption (retention), dissipation is the second most important process used to predict the fate of pesticides in soils (Boesten, J.J.T.I. et al., 1991). The understanding of fate of pesticides is essential for rational decision-taking regarding their authorization (Hasan S. et al., 2014). Therefore, Standard laboratory dissipation study has been designed to investigate the dissipation behavior and soil factors that influence the dissipation of tebuconazole (often expressed as first-order half-life or DT50, which is the time required for 50% of the initial concentration to degrade) in three types of Indian soil under biotic and abiotic conditions. The dissipation of pesticides in soil is influenced by physical-chemical properties of soils (such as pH and organic carbon content), biological properties of soils (activity and distribution of microorganisms),

pesticide concentrations and environmental conditions that control soil temperature and moisture contents. The rates of dissipation also depend upon the properties of the chemical. The variation in dissipation rate is expected, and numerous studies have provided evidence for soil to soil variation in dissipation rate of pesticides (Walker, A. et al., 2001). A greater understanding of the factors that influence dissipation rate is required to ensure the safe use of new and existing pesticide. Some studies have demonstrated a possible influence of pH on degradation. The degradation of many neutral compounds has been shown to be faster at high pH (Kah, M. et al., 2006). But the common rules are not applicable to all pesticides.

The published study on degradation rates of tebuconazole in Tifton loamy sand soil during laboratory incubation suggested that the tebuconazole is not persistent, with soil half life, 49 days (Strickland T. C. et *al*., 2004). However, the unpublished degradation data summarized in registration documents and a risk assessment prepared by the working group of joint FAO/WHO meeting on pesticide residues (JMPR, 1994) have suggested that the compound is persistent, with aerobic soil metabolism half-lives ranged from 289 to 610 days (Tomlin, CDS., 2009; Walker, A.. et al., 2001 and Kah, M. et al., 2006). Further, it is not known how these data relate to environmental conditions in one of the product's major uses regions of Gujarat state. To assess adequately the human and ecological risks of tebuconazole use and to evaluate the potential for residue persistence in soils in the regions, soil dissipation studies are needed which reflect local soil and environment conditions.

The objective was thus to investigate dissipation kinetics of tebuconazole fungicide collected from alluvial soil, deep black clayey soil as well as black soils from tropical agro-climatic zone of Gujarat state as per OECD test guideline (OECD No. 307, 2002) The principle focus was to derive the dissipation time (DT_{50}) of

tebuconazole in different cropland soils under field stipulated laboratory condition, and compare them to values that were reported in the literature in order to gain a better understanding about the dynamics of the tebuconazole degradation in soils. It was decided to investigate dissipation in sterile soils to evaluate the microbial contribution in dissipation.

4.2 Materials and Methods

4.2.1 Chemicals and Reagents

- Ethyl acetate and anhydrous sodium sulphate (Analytical Reagent Grade) were purchased from Merck India.
- Tebuconazole standard (99.24% purity) was obtained by United Phosphorus Ltd (UPL) Mumbai, India and stock solution of tebuconazole (500 µg/ml) was prepared in ethyl acetate.
- Folicur 25EC formulation product was purchased from Bayer Crop Science, India and Folicur 25EC was diluted to concentration 43.17 µg/ml (w/v, A.I.).

Tebuconazole sample solutions used for abiotic study were sterilized by passing through 0.2 µm membrane filter, using sterilized glass wares under aseptic condition in a laminar flow chamber.

4.2.2 Instruments and Operating Conditions

- Capillary gas chromatograph (GC) equipped with nitrogen-phosphorous selective detector (NPD) and split mode injector (Perkin Elmer, USA, and Model, Clarus 500) was used for analysis of tebuconazole residue. The operating condition is given below,

Column : Dura Bond mid-polar fused-silica capillary with low bleed (DB-35 column: packing with 35% Phenyl-methyl polysiloxane,

Column dimension : 30 m × 0.25 mm I.D., 0.25 micron film thickness)

Instrument software : Total Chrom

Instrument operating temperature parameters;

Column Oven : 200 to 275 °C @ 25 °C/min (hold for 8.0 min)

Injector port : 260 °C

NPD Detector : 290 °C

Carrier gas and its flow rate: Helium and 1.0 ml/min

Hydrogen gas flow rate: 1.5 ml/min

Air flow rate : 95 ml/min

Injected volume : 2µL

Split ratio : vent: column, 2:1

- Ultrasonic water bath (Fixed Frequency 35 kHz, Spectra lab, India) was used for sonication/extraction.
- Round bottom type tube of 150 ml capacity with interchangeable polypropylene cap was used as sample container.
- Refrigerated centrifuge (Rota 4RV/FM, Plasto Crafts, India) was used for centrifugation of samples.
- The Autoclave and biological oxygen demand (BOD) with digital temperature controller (Somax, India) was used for soil and water samples sterilization and incubation.

4.2.3 Soils

Three types of top soil (alluvial, black and deep-black clayey of paddy crop, mango orchid and peanut crop fields) from a depth of 0-15 cm were obtained from Paria, Vansada and Bardoli regions of South Gujarat using a hand auger. The zone is a tropical agro-climatic zone of heavy and medium rainfall. The pesticides are not

used since last six months in in collected soils. Collected soil samples were passed through 2.0 mm sieve and characterized for different physico-chemical properties. The data of soils characterization is given below,

Table 4.1: Physicochemical properties of three types of Indian soil used in experiment

Location	Organic Carbon (%)	Total Nitrogen (%)	pH	Total Sand Content (%)	Clay Content (%)	Soil Types
Paria	2.5	0.1086	5.91	35	21	Silt Loam (Alluvial soil)
Vansada	0.7	0.0456	7.30	40	43	Clay Loam (Black soil)
Bardoli	1.3	0.0921	4.88	22	68	Clay (Deep Black Clayey)

The details of soil characterization methods are described in Chapter 2.

4.2.4 Extraction and Clean up of samples

About 50 g soil samples were placed in Erlenmeyer flasks and it was spiked with 0.1, 0.2 and 1.0 ml from tebuconazole stock solution (concentration, 43.17 µg/ml). The concentration of fortified soil samples were 0.0864 µg/g (at recommended field rate, FR), 0.173 µg/g (2-times field rate, 2FR) and 0.863 µg/g (10-times field rate application, 10FR). The contents were then thoroughly mixed. The moisture content in each flask was maintained to 60% of its field capacity by adding distilled water once every three days during the experiment. The entire experiment was conducted in closed BOD incubators at temperature 30 ± 2 °C, after wrapping each flask with aluminum foil to avoid photodegradation.

At each time interval, the whole 50 g soil samples were transferred into screw-capped round bottom type tubes to extract the fungicide by sonication followed by centrifugation. The triplicate samples (50 g) at each treatment were extracted by adding distilled water (20 ml) and ethyl acetate (50 ml) and then sonicated for 15 min

(at frequency of 35 kHz) at temperature 30 ± 2 °C. The tubes were centrifuged at 4000 rpm for 10 min. The organic layer was separated; moisture from the extracts was removed by passing through sufficient amount of sodium sulphate and then cleaned up by passing through 0.2 µm PTFE syringe filter. An aliquot of filtrate was evaporated to dryness under a gentle stream of nitrogen, reconstituted in ethyl acetate (1 ml), and analyzed using capillary GC-NPD.

4.2.5 Analysis and Recovery Study

The tebuconazole residue was quantified in fortified soil samples by comparing the peak area of sample with that of standard and the amount of the residue was calculated. The amount of tebuconazole residue in sample µg/g (in ppm) was calculated using the following formula.

$$Residue\ in\ \mu g/g\ \ (ppm) = \frac{A_s W_1 V_1}{A_{std} W_2 V_2}$$

Where,

A_s = Peak area of tebuconazole in the sample

A_{std} = Peak area of the tebuconazole standard

W_1 = Weight of the tebuconazole standard compound injected (µg)

V_1 = Total volume of sample injected (subtract, in µl)

W_2 = Weight (g) or volume (ml) of the substrate taken

V_2 = Volume of sample (subtract) injected (µl)

R_f = Recovery Factor

The results of recoveries were ranged from 98.7 - 99.1%, 98.3 - 98.5% and 97.4 - 98.0% in Paria, Vansada and Bardoli location soils (alluvial, black and deep black clayey soil) respectively at spiked concentration level 0.0864 and 0.863 µg/g.

4.2.6 Dissipation Experiments for Non-Sterile and Sterile Soils

For all analyses, the well characterized field soil samples (50 g) of Paria, Vansada and Bardoli locations were performed to investigate the dissipation pattern and effect of each factor (SOC, soil pH, soil clay, concentration and role of microorganisms) on tebuconazole dissipation.

About 50g soil samples from the three locations under study were placed in Erlenmeyer flasks and spiking was done with Folicur 25EC stock solution (concentration, 43.17 µg/ml) at recommended field rate, FR (0.0864 µg/g), 2-times field rate, 2FR (0.173 µg/g) and ten times field rate application, 10FR(0.863 µg/g). The conversion of the field application to µg of pesticide per g of soil was made by assuming an even distribution of the pesticide compound in 0-15 cm layer with soil density 1.5g/cm^3.

Sampling of soils for analysis was done at time intervals, 0, 3, 7, 15, 30, 45, 60, 75 and 90 days. A control was run for each soil at each time interval. The experimental protocol is summarized below:

No. of soil location = 3 (Bardoli, Vansda and Paria)

Application rates = 3 (FR, 2FR and 10FR)

3 replicate experiments for each soil at each application rate.

Sampling time interval = 9 (0, 3, 7, 15, 30,45,60,75 and 90 days)

Calculation of Field Rate Application:

The application of Folicur 25EC (i.e. 25.9% Tebuconazole Active ingredient (A.I) contents) product was recommended 750 ml/hectare for crops. It means, 194.25 g (= 194.25 x 10^6 µg) tebuconazole active ingredient was applied in one hectare field (equivalent to 2.24 x 10^9 g soil).

Resulting in fortification of 0.086 µg tebuconazole in 1 g of soil. After fortification of soils, the contents were then thoroughly mixed before incubating in the dark at 30 ± 2 ºC. The moisture content in each flask was maintained to 60% of its field capacity by adding distilled water once every three days during the experiment. The entire experiment was conducted in closed BOD incubators at temperature 30 ± 2 °C after wrapping each individual flask with aluminum foil in order to avoid photodegradation.

To establish the role of microorganisms in the degradation of the fungicide, the same experiment was also conducted on all three soil types under sterile conditions. The samples were sterilized on three consecutive days by autoclaving at 121 ºC for 20 min (Sukul P., et al., 2001). Spiking procedure in sterile soil was similar to what was used in non-sterile treatments, except that sterile distilled water was used to maintain the moisture content at 60% of its field capacity for sterile condition experiment. All equipment used during sterile treatment was swabbed with methanol, and autoclaved. All operations related to sterile treatments were performed inside a laminar flow cabinet. The same experimental protocol as one under non sterile conditions was followed for sterile soils.

4.2.7 Data and Statistical Analysis

The dissipation of tebuconazole in soils under varied treatments was modeled using simple first-order kinetics. Theoretically, the pesticide residues should fall natural logarithmically since the amount lost naturally per unit time is proportional to the total amount present at particular time. It was established that when residues were potted against time lapsed, a straight line was obtained that obeyed the general equation of straight line (Handa S. K. *et al.,* 1999) i.e. $y = mx + c$. The experimental data (Ln pesticide concentration versus time elapsed) were then subjected to sample

regression analysis. Equation 1, below represents the the concentration time relationship, where, Ct is the concentration of tebuconazole (µg/g) remaining in soil after time t (days), C_0 is the concentration of tebuconazole (µg/g) initially in soil at time zero (day), k is the slope of the linear plot as shown by regression equation. Which is actually the rate of degradation (day^{-1}) of pesticide with time t and t is time elapsed in days. Thus,

$$LnCt = LnC_0 - kt \quad (1)$$

Half-life is the time required to reduce the pesticide concentration to half of its initial concentration. Thus,

$$Ln\ (1/2C_0) = LnC_0 - kt$$

Therefore,

$$DT_{50} = \frac{Ln(2)}{k} \quad (2)$$

In addition, Pearson's correlation coefficient (r) was calculated to evaluate the influence of soil parameters on the rate of dissipation. All significant differences were accepted at levels $p < 0.05$.

4.3 Results and Discussion

The data of dissipation experiment along with half lives of tebuconazole in abiotic and biotic conditions of Paria, Vansda and Bardoli location soils are given in Table 4.2. Tebuconazole dissipation took place in sterilized soil, indicating the role of chemical and abiotic factors rather than photodegradation as the soils were kept in the dark during the experimental period. Higher dissipation in non-sterilized soil than in sterilized soil indicated the role of microbes in tebuconazole dissipation. However, there could also be the possibility of tebuconazole binding with humic substances or entrapment due to sequestration reaction. Generally, reversible or irreversible sorption and/ or sequestration mechanisms in combination with the microbially driven turnover

of soil organic matter strongly influence the pesticide mechanism although the key processes involved are still not understood precisely (Burauel P. et al., 2001).

Table 4.2: Regression equation, degradation rate constant (k/day), coefficient of determination (r^2) and half-life values of tebuconazole in Paria, Vansda and Bardoli soils under non-sterile and sterile soil conditions.

Treatment	Regression Equation	k/day	r^2	Half-life (days)
Non-sterile soil Conditions				
Paria Soil				
Tebuconazole FR	y = 4.3899 - 0.0201x	0.0201	0.985	34.48
Tebuconazole 2 FR	y = 5.1535 - 0.0198x	0.0198	0.985	35
Tebuconazole 10 FR	y = 6.7246 - 0.0192x	0.0192	0.996	36.09
Vansda Soil				
Tebuconazole FR	y = 4.4406 - 0.0150x	0.015	0.997	46.2
Tebuconazole 2 FR	y =5.1170 - 0.0145x	0.0145	0.998	47.79
Tebuconazole 10 FR	y =6.689 - 0.0144x	0.0144	0.993	48.13
Bardoli Soil				
Tebuconazole FR	y = 4.4075 - 0.0153x	0.0158	0.991	43.86
Tebuconazole 2 FR	y = 5.1086 - 0.0152x	0.0152	0.998	45.59
Tebuconazole 10 FR	y = 6.691 - 0.0149x	0.0149	0.994	46.51
Sterile soil Conditions				
Paria Soil				
Tebuconazole FR	y = 4.4126 - 0.0127x	0.0127	0.996	54.57
Tebuconazole 2FR	y = 5.1050 - 0.0123x	0.0123	0.997	56.34
Tebuconazole 10FR	y = 6.7543 - 0.0122x	0.0122	0.999	56.8
Vansda Soil				
Tebuconazole FR	y = 4.3905 - 0.0107x	0.0107	0.991	64.77
Tebuconazole 2FR	y = 5.0503 - 0.0105x	0.0105	0.971	66
Tebuconazole 10FR	y = 6.7092 - 0.0103x	0.0103	0.985	67.28
Bardoli Soil				
Tebuconazole FR	y = 4.3842 - 0.0116x	0.0116	0.974	59.74
Tebuconazole 2FR	y = 5.0850 - 0.0113x	0.0113	0.984	61.33
Tebuconazole 10FR	y = 6.7316 - 0.0110x	0.0110	0.998	63

The concentration of tebuconazole at zero day (4 hours after spiking) in non-sterilized soils ranged from 0.086 - 0.851µg/g (Paria soil), 0.085 – 0.848µg/g (Vansda soil) and 0.084 – 0.845 µg/g (Bardoli soil), while the values for sterilized soils ranged

from 0.086 - 0.856µg/g (Paria soil), 0.084 – 0.849µg/g (Vansda soil) and 0.085 – 0.851 µg/g (Bardoli soil) at the three different application rates. The tebuconazole residue in non-sterilized and sterilized Paria, Vansda and Bardoli soils persisted upto 90 days. The dissipation data of tebuconazole in Paria, Vansda and Bardoli soils under non-sterile and sterile conditions are plotted in Figures 4.1 to 4.3.

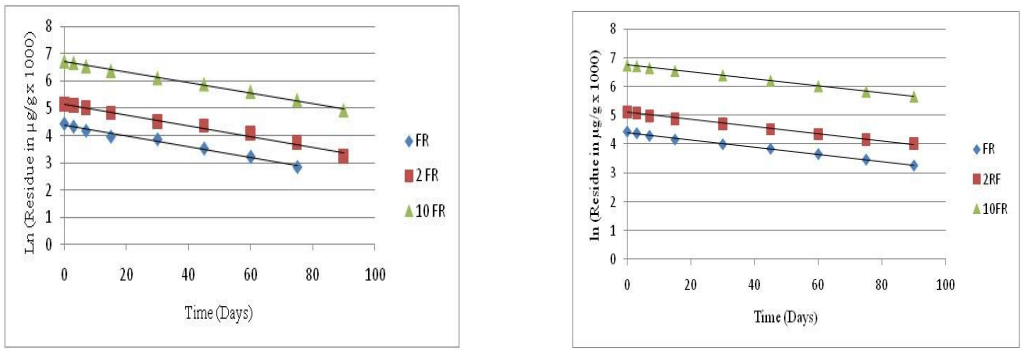

Figure 4.1: Plots of natural logarithm of tebuconazole residue concentration in Paria soil (alluvial soil) versus time at 0.0864 µg/g (field rate, FR), 0.1728 µg/g (2-times field rate, 2FR) and 0.863 µg/g (10-times field rate, 10FR) applications under non-sterile and sterile conditions respectively.

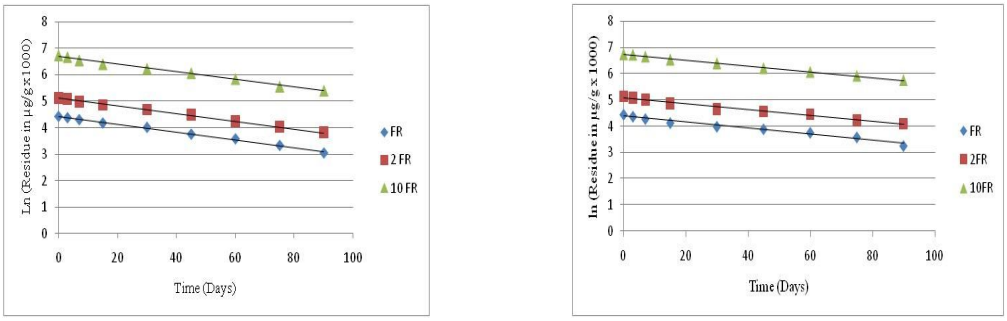

Figure 4.2: Plots of natural logarithm of tebuconazole residue concentration in Bardoli Soil (clay soil) versus time at 0.0864 µg/g (field rate, FR), 0.1728 µg/g (2-times field rate, 2FR) and 0.863 µg/g (10-times field rate, 10FR) applications under non-sterile and sterile conditions respectively.

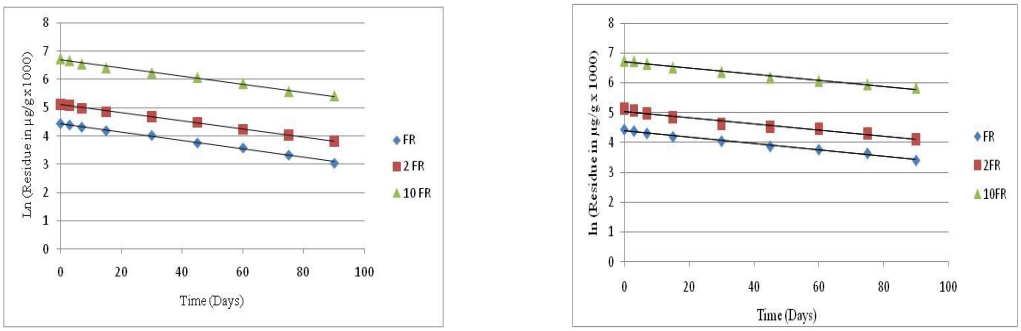

Figure 4.3: Plots of natural logarithm of tebuconazole residue concentration in Vansda soil (clay loam soil) versus time at 0.0864 µg/g (field rate, FR), 0.1728 µg/g (2-times field rate, 2FR) and 0.863 µg/g (10-times field rate, 10FR) applicationsm under non-sterile and sterile conditions respectively.

The dissipation rate constant of tebuconazole in non-sterilized Paria soil at three different application rates varied between 0.0192 to 0.0201/day, while that for the Vansda soil between 0.0144 to 0.0150/day and Bardoli soil were between 0.0149 to 0.0158/day. However in sterilized soils, the dissipation rate constant varied between 0.0122 to 0.0127/day, 0.0103 to 0.0107/day and 0.011 to 0.0116/day in Paria, Vansda and Bardoli soils respectively (Table 4.2). Higher the application rate of tebuconazole lower was the dissipation rate constants in non-sterile and sterile soils as seen in Figure 4.4. The reduced dissipation rates at higher initial concentrations in the present study could be attributed to limitation in the number of reaction sites in soil and toxic effects on microorganisms or enzyme inhibition (Prakash N. B. *et al.,* 2000).

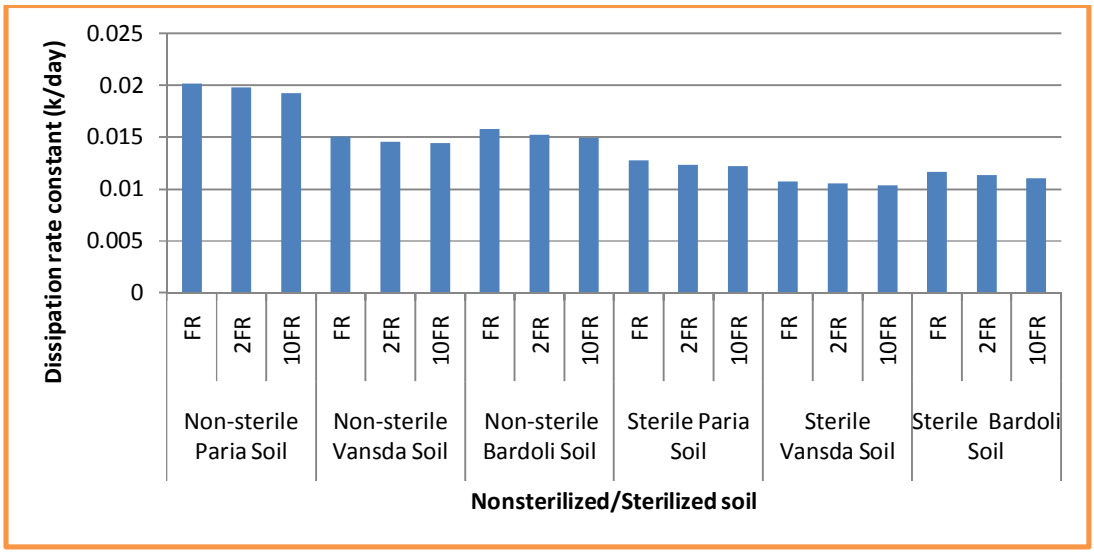

Figure 4.4: Dissipation Rate Constant (k/day) of tebuconazole in paria, vansda and bardoli soils under non-sterile and sterile conditions.

The half-life values calculated from the best-fit lines of the natural logarithm of tebuconazole residual concentrations versus incubation period, suggested first order reaction kinetics for tebuconazole dissipation in both sterilized and non-sterilized conditions for Paria, Vansda and Bardoli location soils.

The contribution of abiotic factors was also calculated by comparing percentage dissipation of tebuconazole in biotic and abiotic conditions in all the soils under study. The maximum contribution was observed in Paria soil wherein high dissipation could be attributed to the influence of microbial activity. The comparative roles of biotic and abiotic factors in dissipation of tebuconazole in Paria, Vansda and Bordoli soils are represented in figure 4.5.

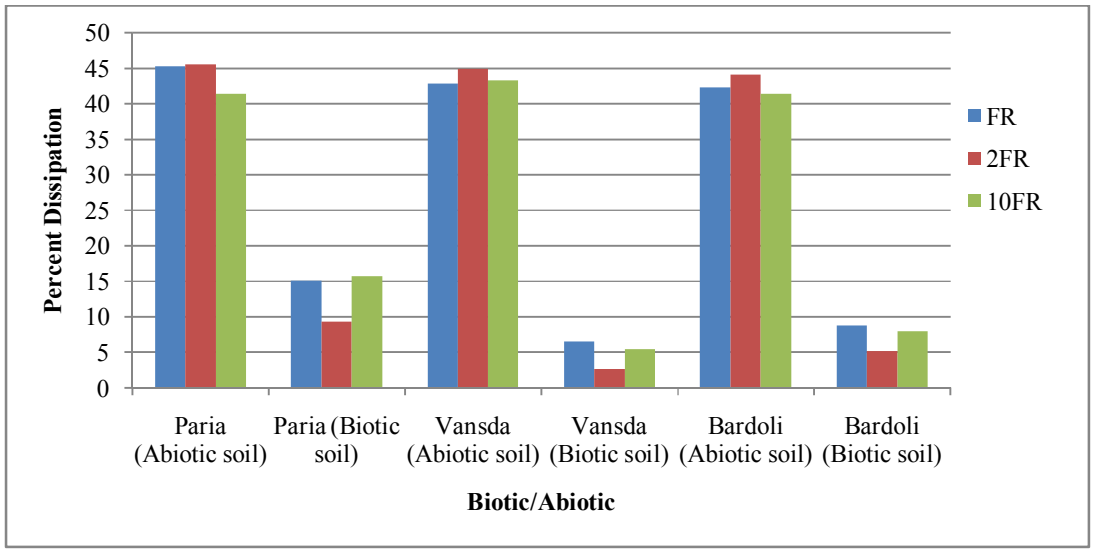

Figure 4.5: Comparative role of biotic and abiotic factors on dissipation of tebuconazole in Paris, Vansda and Bardoli soils at 45 day under laboratory condition.

Present study demonstrated that the soils biotic factors strongly influenced the dissipation of tebuconazole. A simple Pearson's correlation coefficient was calculated between soil variables and Dissipation Rate Constant (DRC) values (k) of tebuconazole for biotic and abiotic conditions. The *p*-test was carried out at 0.05 levels for the Pearson's correlation coefficient (r). A positive correlation between SOC content and degradation rates was observed for the tebuconazole (Table 4.3). An increase in the soil organic carbon (SOC) content resulted in faster tebuconazole dissipation in soils as reflection of enhanced bioactivity.

Table 4.3: Correlation coefficients between soil properties and dissipation rate constant of tebuconazole under non-sterile soil and sterile soil conditions.

[#]Mean dissipation rate constant (k) in non-sterile soil ± SD	[#]Mean dissipation rate constant (k) in sterile soil ± SD	Soil Properties			Location (Soil Types)
		Organic carbon contents (%)	pH	[¥]Clay content (%)	
0.0146 ± 0.0003	0.0105 ± 0.0002	0.7	7.3	43	Vansda (Black Soil)
0.0153 ± 0.0004	0.0113 ± 0.0002	1.3	4.88	68	Bardoli (Deep Black Clayey Soil)
0.0197 ± 0.0004	0.0124 ± 0.0002	2.5	5.91	21	Paria (Alluvial soil)
Correlation coefficient (r) between soil properties vs. k	–	0.978	0.21	0.773	–
–	Correlation coefficient (r) between soil properties vs. k	[*]0.995	0.495	0.546	–

***** indicate the significance (one trailed probability values) of a Pearson correlation coefficient at $p < 0.05$; [¥] indicate correlation with soil organic carbon ($r = 0.626$); [#] indicate the mean dissipation rate constant (k/day) of tebuconazole at FR, 2FR and 10FR application in each soil type; SD = Standard deviation.

The SOC content is either decrease the microbial mediated pesticide degradation by stimulating pesticide sorption processes or enhances the bioactivity of soil (Pesce, S. et al., 2004) by cometabolism (Wardle, D.A. et al., 1990a and Kah, M. et al., 2007). The stronger sorption properties of soil decreased the microbial mediated pesticide degradation by reducing the bioavailability of pesticides for degradation. Therefore, the faster dissipation of tebuconazole in Paria soil compared to other soils could be probably due to higher SOC content, resulting in enhanced bioactivity. However, no correlation was observed, with soils clay content with DRC of tebuconazole, probably this is due to weak correlation between SOC and clay contents ($r = 0.626$) (Table 4.3). Dissipation of the tebuconazole was slowest in Vansda soils compared to Paria and Bardoli soil which could be attributed to a multitude of factors

like soil organic carbon content (SOC), pH and clay content of which low SOC content may be attributed to slow degradation of tebuconazole.

The DT_{50} for all soils were obtained within the length of sampling time (90 days). The DT_{50} values for tebuconazole in non-sterile and sterile Paria soil decreased compared with the Bardoli and Vansda soils (Figure 4.6), which is consistent with the earlier assumption that higher soil organic carbon contents affected dissipation. Thus for non-sterile and sterile soils, the rate of dissipation of tebuconazole was found in the order to Paria > Bardoli > Vansda soil (Figure 4.4). The fastest dissipation of tebuconazole in non-sterile and sterile Paria soil could be attributed to higher soil organic carbon content. In sterile soils, the rate of dissipation was significantly influenced by soil organic carbon contents (Table 4.3). Therefore, the Vansda soil with high clay (43%) and lowest organic carbon content (0.7 %) exhibited the higher persistence in compare to the other two soils. The rate of degradation in both soil conditions was not significantly influenced by soil pH (Table 4.3). This relationship was also observed for triadimefon (Singh, N., 2005), propiconazole (Thorstensen C. W. et al., 2001) and tetraconazole (Alam, S. et al., 2013). The degradation behavior of other triazole fungicides, reported by other researchers also showed that most of the triazole fungicide are persistent in nature, dissipation followed first-order kinetics with half-lives of about 200 days for propiconazole and greater than 2 years for flutriafol, epoxiconazole, and triadimenol (Bromilow R. H. et al. 1999). The tebuconazole DT_{50} values obtained in present study were close to literature reported values as given below in Table 4.4,

Table 4.4: The DT_{50} values tebuconazole obtained from literature

Matrix	Field/Laboratory Conditions	DT50	Reference
Soil	Laboratory	49 days	Strickland T. C. et *al*., 2004
Soil	Field	43 to 5 days	Potter T. L. et al., 2005
Soil	Laboratory	45 days	White PM, et al., 2010
Grape leaves	Field	2.68 to 3.96 days	Jyot G, et al., 2010
Soils	Sterile soils under Laboratory	34.48 – 48.13 days	Present study
	Non-sterile soils under Laboratory	54.57 – 67.28 days	

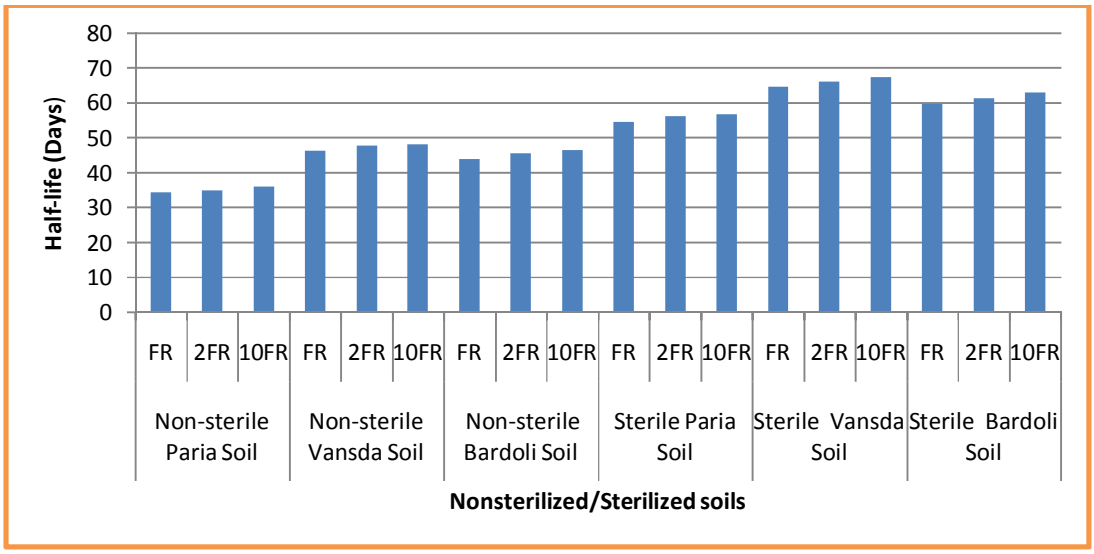

Figure 4.6: Half-life ($t_{1/2}$) values of tebuconazole in paria, vansda and bardoli soils under non-sterile and sterile conditions.

4.4 References

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