

1           **Kinetic field dissipation and fate of endosulfan after**  
2           **application on *Theobroma cacao* farm in tropical**  
3           **Southwestern Nigeria**

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20 **Abstract**

21 Endosulfan, 6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano,2,4,3-  
22 benzodioxathiepin-3-oxide is still a pesticide of choice for most cocoa farmers in Southwestern  
23 Nigeria, in spite of its persistence, bio-accumulative, toxicological properties and restriction.  
24 A single-treatment of 1.4 kg ai/ha (0.5% ai) of technical grade endosulfan (Thiodan, 35EC)  
25 was applied to 0.0227 hectare of cultivated *Theobroma cacao* L. (Cocoa) farm at the Cocoa  
26 Research Institute of Nigeria (CRIN). Levels of parent endosulfan ( $\alpha$ -,  $\beta$ -endosulfan) and  
27 major metabolite (endosulfan sulfate) were determined in vegetation and surrounding matrices  
28 at days 0, 7, 14, 21, 28, 42 and 60 using GC-MS. Their kinetic variables were determined.  
29 Order of  $\Sigma$ endosulfan distribution at day 0 was: dry foliage>fresh foliage>bark>Pods>soil (0-  
30 15cm). No residual endosulfan was found in cocoa seeds and sub-surface soil (15-30 cm). Low  
31 residual levels in pods on day 0 may be due to endogenous enzymatic breakdown, with  $\alpha$ -  
32 isomer more susceptible and  $\alpha/\beta$ -endosulfan ratio being 0.90. Fell dry foliage as mulch was  
33 predominantly the receiving matrix for non-target endosulfan sprayed. Volatilization was key  
34 in endosulfan dissipation between days 0 and 7 from foliage surfaces (> 60% loss), while  
35 dissipation trend were bi-phasic and tri-phasic for vegetation and soil respectively.  
36  $\Sigma$ endosulfan loss at terminal day ranged between 40.60% (topsoil) and 99.47% (fresh foliage).  
37 Iteratively computed half-lives ( $DT'_{50}$ ) ranged from 6.48 – 30.13d for  $\Sigma$ endosulfan in  
38 vegetation. Endosulfan was moderately persistent in pods – a potential source for cross  
39 contamination of seeds during harvest. Iteratively determined  $DT'_{50}$  and initial-final day  $DT_{50}$   
40 are highly correlated (R=0.9525; n= 28) and no significant difference (P=0.05) for both  
41 methods.

42 Key words: Endosulfan, *Theobroma cacao*; persistence; kinetics; half-life

43

## 44 **Introduction**

45 Endosulfan (6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-  
46 benzo(e)dioxathiepin-3-oxide) is an organochlorine pesticide (OCPs) of the cyclodiene  
47 subgroup, still been used by cocoa farmers in West African countries such as Ghana, Nigeria  
48 and Cote d'Ivoire in controlling insects, fungi and virus that causes defoliation and diseases in  
49 *Theobroma cocoa* (Padi and Owusu, 1998; Bateman, 2003; Bateman, 2008) despite its  
50 prohibition.

51 Commercially available endosulfan pesticide is a diastereomeric mixture of 70%  $\alpha$ -isomer and  
52 30%  $\beta$ -isomer (USEPA, 2002b). Both isomers are reported to exhibit similar insecticidal  
53 properties, however with different physicochemical properties (Schmidt *et al.*, 2001). It is one  
54 of the most frequently detected pesticides in the environment because of its propensity to  
55 undergo long-range transport (Halsall, 2004). It is persistent and has high potential for  
56 bioaccumulation in biota (USEPA, 2002b). Its ubiquitous existence in the environment and  
57 physical–chemical properties has caused endosulfan to be classified as a persistent organic  
58 pollutant (POP) under the Stockholm Convention (UNEP, 2011).

59 Dissipation and terrestrial fate of pesticides after application on plants are of great importance  
60 from environmental point of view. Environmental processes such as volatilization, transport,  
61 degradation, adsorption, bioaccumulation and bio-magnification (Weber *et al.*, 2010) are  
62 common phenomenon after the treatment of farm crops with pesticides. These processes also  
63 influence their level of persistence and contamination in the environment. Persistence of  
64 pesticide is measured by its half-life and best determined kinetically (OECD, 2014). This is  
65 used to assess its risk and adverse impact to human health. To achieve a more reliable result,  
66 field studies are preferable - where milieus of environmental factors amongst others are

67 involved in the interplay within a specified natural environment after the application of the  
68 pesticide.

69 The dissipation and residual levels of applied pesticides in food crops and different plant parts  
70 are dependent on phase partitioning, intermedia and intra-medium transport and degradation.  
71 Different assessment models for the determination of half-lives of pesticides in plants have  
72 been reported (Juraske *et al.*, 2008; Trapp and Legind, 2011; Fantke *et al.*, 2013). However,  
73 there are paucity of experimental data on pesticide-plant assessment and where available large  
74 variations in half-lives are reported per pesticide (Fantke *et al.*, 2014). Dissipation half-lives  
75 are either estimated from individual experimental data per pesticide or derived from other  
76 parameters, such as soil half-lives. The use of soil half-lives only for the estimation of plant  
77 half-lives is inappropriate, since dissipation in plants is a function of the pesticide properties  
78 (NAFTA/EPA, 2006; Jacobsen *et al.*, 2015), plant characteristics (Katagi, 2004, Fantke and  
79 Juraske, 2013), environmental factors and plant morphology (Yu *et al.*, 2006).

80 Many field dissipation studies on endosulfan pesticide residues have reported in the literature  
81 (Antonious *et al.*, 1998; UNEP 1999; GFEA, 2007; Ntow *et al.*, 2007; Rosenhadl *et al.*, 2009).  
82 However, most of these studies were carried out in the temperate region. Although, the  
83 dissipation studies of endosulfan in field grown tomato (*Lycopersicon esculentum L*) at  
84 Akumadan, Ghana (Ntow *et al.*, 2007) and in eggplant (*Solanum macrocarpon L*) cultivated in  
85 Southern Benin Republic (Rosenhadl *et al.*, 2009) have been reported, there is still paucity of  
86 information on residual endosulfan and its' metabolites (endosulfan sulfate, diol, lactone, etc.)  
87 in cultivated plants, crops and soils within the tropical region.

88 Moreover, no kinetic field dissipation and persistence studies on the magnitude of residual  
89 endosulfan and its' metabolites in parts of *T. cacao* plant (leaves, stem bark, pods and seeds)  
90 and its surrounding matrix (fell dry leaves and cropped soils) have been reported. This study

91 therefore is aimed to investigate the dissipation and environmental fate of endosulfan in  
92 cultivated *T. cacao* farm after a single double-dose treatment with 1.4 kg active ingredient  
93 (ai)/ha (i.e., 0.5% ai).

94 Emphasis was laid on the relationship between chemo-kinetic variables obtained and the  
95 persistence of total endosulfan, parent isomers ( $\alpha$ - and  $\beta$ -endosulfan) and major metabolite -  
96 endosulfan sulfate (Figure 1) in the various environmental matrices monitored.

## 97 **Materials and methods**

### 98 Description of Study area

99 The experimental site was located within the Cocoa Research Institute of Nigeria (CRIN) (7°  
100 14'N; 3° 52'E) Oyo State, Nigeria. This area exhibits the typical tropical climate with average  
101 high temperatures (33 °C) and high relative humidity (72 – 76%)(Babalola, 2013), with two  
102 major seasons – rainy (March – October) and dry (November – February). Temperatures are  
103 highest at the end of the dry season (January or February). The average annual rainfall in the  
104 study area is 1500 mm, while vegetation is rain forest, with composition of mainly large tall  
105 crowned trees, mixed with thick under growths (Aikpokpodion *et al.*, 2010).

### 106 Field experiment and design

107 The field experiment was conducted between mid-October and mid-December 2012. The mean  
108 daily minimum temperature during this period was  $20.7 \pm 0.2^{\circ}\text{C}$ , while the daily maximum  
109 temperature was  $28.2 \pm 0.2^{\circ}\text{C}$ . No heavy rainfall was recorded during the study period.  
110 However, slight showers and dew were common occurrence during this period.

111 There is no known history of use of OCPs in the area chosen for study. The loss of applied  
112 endosulfan pesticide from plant surfaces and impacted soils were studied on the same plots.  
113 Plant surface samples comprising of fresh foliage, stem bark, pods and cocoa bean seeds (from  
114 mucilage of sampled cocoa pods) and surrounding fell dry cocoa leaves and soils (0-15 and 15-

115 30 cm depths) were monitored. Composite samples were analyzed per plot. Minimum  
116 distortion of the cropped surrounding was ensured during sample collection of dry cocoa leaves  
117 (that were previously part of the plant) and soils. In order to allow for a true and proper  
118 agricultural practice, fell dry leaves on farmland were left to serve as mulch to the soil - this is  
119 very useful especially in the tropics during the dry season. Samples were collected on the first  
120 day (day 0) 45 minutes after spraying, while subsequent samples were collected at 7, 14, 21,  
121 28, 42 and 60 d.

122 Commercial endosulfan, Thiodan EC 35 (usually comprising of a mix of  $\alpha$ - and  $\beta$ -isomers with  
123 a formulation of 7 + 3) was used in this study (Weir *et al.*, 2006). The pesticide was applied as  
124 water emulsion (mixture of water and commercial endosulfan) using a calibrated PB-10  
125 knapsack hand-operated dispenser.

#### 126 Experimental Procedure

127 Five sub-plots (0.00455 ha/plot) [i.e., approximately (3 x 3) m<sup>2</sup> x 5 trees per plot] each  
128 containing five (5) cocoa trees with matured pods were marked for easy identification.  
129 Replicate plots were well spaced apart (80 - 100 meters) to minimize the effect of drifting after  
130 spraying of pesticide. Marked plots were sprayed with 1.4 kg ai/ha (0.5% ai) of endosulfan  
131 (commercial grade). *Theobroma cacao* tree trunks were sprayed from the bottom (around the  
132 sides) to the canopy to achieve equal and adequate spread of pesticide (Spraying time was 3-5  
133 minutes per tree). The *Theobroma cacao* trees were all matured (over 15 years old), with a  
134 height range of 2 – 3 meters and canopy coverage of 2.55 m<sup>2</sup>. Prior to spraying, baseline  
135 concentrations of parent endosulfan and endosulfan sulfate in plant parts and surrounding  
136 matrices were determined (as control samples).

#### 137 Sample collection

138 Representative samples from plant surfaces, fell dry cocoa leaves and soils were collected  
139 randomly after pesticide treatment from each of the designated plots on sampling days.  
140 Samples were wrapped first with aluminum foil, then with cellophane and kept in an ice chest  
141 cooler and transported to the laboratory, stored in the refrigerator at -4°C before endosulfan  
142 extraction. A total of 735 samples was collected – this comprising of fresh leaves, stem bark,  
143 cocoa pods, seeds, fell dry leaves and soils (0-15 cm and 15-30cm) in triplicate per plot for the  
144 seven sampling time amplitudes aforementioned. Each sample type was composited per plot  
145 and assayed in triplicates.

#### 146 Reagents and materials

147 Extraction solvents - dichloromethane (DCM), n-hexane, acetone, petroleum spirit and  
148 acetonitrile (all of analytical grades) and pure  $\alpha$ -endosulfan (99.6%),  $\beta$ - endosulfan (99.9%) and  
149 endosulfan sulphate (99.9%) standards were purchased from Sigma-Aldrich (St Louis, USA),  
150 Sodium sulfate (anhydrous) and silica gel 60 extra-pure (60 – 120 mesh) for column  
151 chromatography were from BDH limited (Poole, England). Thiodan EC 35 (commercial-grade  
152 endosulfan, manufactured by Bessen Chemical Co., Ltd., Nanajing, Jiangsu, CHINA) was  
153 purchased at Dugbe market, Ibadan, Southwestern Nigeria from an Agro-chemical vendor.

#### 154 Preparation of standard solutions

155 Stock solutions for  $\alpha$ -endosulfan,  $\beta$ -endosulfan and endosulfan sulfate reference standards were  
156 prepared by weighing 50 mg of each into separate 50 mL volumetric flask, 5 mL  
157 hexane;isopropyl alcohol (1:1) mixture was added and shaken gently to homogenize. These  
158 were made to volume with same solvent mixture to obtain reference standard stock  
159 concentration of 1000  $\mu\text{g mL}^{-1}$  for each pesticide. All working concentrations for each standard  
160 were prepared from these stocks.

#### 161 Samples pre-treatment

162 Fresh leaves, fell dry leaves, pods, cocoa seeds and stem bark were separately blended and  
163 homogenized using a kitchen blender, before extraction. The seeds and bark were chopped into  
164 small bits before being blended, while the pods were peeled before blending. After each  
165 blending, the cup of the blender was thoroughly cleaned and rinsed with acetone to prevent  
166 cross contamination.

#### 167 Sample Extraction and Clean Up

168 Modified methods of USEPA 3570 (2002a) and Yeboah *et al.*, (2003) were employed for the  
169 extraction of endosulfan from cocoa plant tissues and cropped soils. A mixture of ethyl  
170 acetate:petroleum spirit (3:2) and that of acetone: petroleum spirit (1:1) were used for  
171 extraction of endosulfan in plant tissues and soil samples respectively. To 2 g of blended plant  
172 tissues or soil samples, 1 g of anhydrous sodium sulfate was added in an amber extraction  
173 flask. This was shaken vigorously with 10 mL of extraction solvent mixtures for 45 minutes  
174 using a Thermo Scientific reciprocating/orbital shaker; model MaQ at 80 - 100 r/minute.  
175 Aliquot, 5 mL of extract (equivalent to 1 g of sample) was transferred into 10 mL beaker and  
176 evaporate with gentle stream of nitrogen gas to 1 mL residue. To residue was added 5 mL of  
177 hexane, homogenized and reduce to 1 mL using nitrogen gas. The residue extract was then  
178 cleaned by passing through a glass column (dimension, 120mm (l) × 12 mm (i.d)) packed with  
179 activated silica gel (60 -120 mesh) previously mixed with 10 % (w/w) of distilled water and a  
180 bed of 0.5 g of anhydrous sodium sulfate on top.. Elution was first with 10 mL of  
181 hexane:dichloromethane (1:4) and then with 5 mL of dichloromethane: hexane: acetonitrile  
182 (50:49.65:0.35) at a flow rate of 1 mL/min. The combined eluate was reduced to 2 mL after  
183 addition of 1 mL of iso-octane using a rotary evaporator. All traces of CH<sub>2</sub>Cl<sub>2</sub> were ensured  
184 replaced with hexane before Gas Chromatography-Mass Spectroscopy (GC-MS) analysis.

#### 185 Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis



186 Hexane reconstituted cleaned extract was analysed with Thermo-Finnigan Trace GC Ultra  
187 (Waltham, MA, USA) equipped with a AS 2000 Tray Auto-sampler (Thermoquest), splitless  
188 injector, coupled to an ion trap mass spectrometer (MS) (Polaris Q). Xcalibur was the data  
189 software processor. Chromatographic separation was achieved with a HP-5MS capillary  
190 column of 30m length  $\times$  0.25mm i.d.  $\times$  0.25 $\mu$ m film thickness (Agilent J&W Scientific Co.,  
191 Folsom, CA, USA). The oven temperature was programmed, which was initially held at 80°C  
192 for 5 minutes, and was increased to 200°C at a rate of 20°C/min, held for 5 minutes and then  
193 raised to 280°C at a rate of 10°C/min and held for 2 min. The flow rate of the carrier gas  
194 (helium, 99.99% purity) was kept constant at 1.18 mL/min. Splitless injection mode at an  
195 injection temperature of 250°C was carried out at a pressure of 79.5 kPa. The linear velocity  
196 and total flow were 10.0 cm/sec and 32.7 mL/min respectively. The interface line and ion  
197 source temperatures were 260°C and 250°C respectively.

198 Calibration graph of  $\alpha$ -,  $\beta$ -endosulfan and endosulfan sulfate for kinetic studies

199 A six point calibration curve was carried out for  $\alpha$ -,  $\beta$ -endosulfan and endosulfan sulfate in  
200 hexane and iso-propyl alcohol mixture (1:1). The working concentrations for standards used for  
201 calibration ranged from 20 - 1200  $\mu$ g/L. Calibration graph showed good regression coefficient  
202 ( $r^2$ ), values were 0.9989, 0.9976 and 0.9965 for  $\alpha$ -,  $\beta$ -endosulfan and endosulfan sulfate  
203 respectively, while their retention times (RTs) were 18.61, 20.26 and 21.18 minutes  
204 respectively.

205 Data analysis

206 The rate of dissipation/degradation ( $k$ ) and half-life ( $t_{1/2}$  or  $DT_{50}$  for field studies) for residual  
207 concentrations of  $\Sigma$ endosulfan (sum of  $\alpha$ -endosulfan,  $\beta$ -endosulfan and endosulfan sulfate),  $\alpha$ -  
208 endosulfan and  $\beta$ -endosulfan were independently determined applying equation (i), assuming

209 first order kinetics, while equation (ii) for endosulfan sulfate determination. Calculations were  
210 done iteratively and between days 0 (initial) and 60 (final);

$$211 \quad C_{(t)} = C_{(0)}e^{-kt} \quad (i)$$

$$212 \quad C_{(t)} = C_{(max)}(1 - e^{-kt}) \quad (ii)$$

213 Where  $C_{(t)}$  is the residual concentration of pesticides on vegetation at time  $t$ ;  $C_{(0)}$  is the  
214 concentration of pesticides at time zero i.e.,  $t = 0$ ;  $C_{(max)}$  is maximum concentration attained by  
215 metabolite;  $t =$  time (days);  $k$  is the dissipation rate constant and  $DT_{50}$  (or  $t_{1/2}$ ) is the field  
216 dissipation half-life. [Note:  $DT'_{50}$  and  $k'$  represented calculations obtained iteratively ( $d_{0 \rightarrow 60}$ ),  
217 while  $DT_{50}$  and  $k$  obtained from initial-final day calculation ( $C_{0\&60}$ )]

218 The fitting of model curves and kinetic variables to the dissipation-degradation data were  
219 performed using nonlinear regression (OriginPro8-Data Analysis and Graphing Workspace,  
220 Version 8E, Software, China.) and linear regression (Microsoft Excel 2010).

#### 221 Quality assurance

222 To validate methodology, portions of cocoa vegetation and soil samples for baseline  
223 determination were used for recovery experiments. Blank samples were spiked at 125 and 500  
224  $\mu\text{gkg}^{-1}$  concentrations. Each spiked sample type and levels were replicated thrice. The  
225 sensitivity of method was expressed by the limit of detection (LOD) and the limit of  
226 quantification (LOQ). The LOD and LOQ were determined by evaluating the lowest  
227 concentrations of the analyte that can be detected and measured respectively. They were  
228 calculated using the equations,  $\text{LOD} = 3.3S_a/b$ ;  $\text{LOQ} = 10S_a/b$  (where  $S_a$  is the standard  
229 deviation of the intercept of regression line, and  $b$  is the slope of the regression line) (Bohm et.  
230 al., 2010; Shrivastava and Gupta, 2011).

231 Also, identified peaks for  $\alpha$ -endosulfan,  $\beta$ -endosulfan and endosulfan sulfate obtained for each  
232 matrix was confirmed by selected molecular ion peaks at  $m/z$  values using the National

233 Institute of Standards and Technology (NIST) search library (Stein 1995, Vaikosen et al.,  
234 2018).

235 Determination of physico-chemical properties

236 The following physico-chemical properties of the farm soil – soil moisture, pH, total organic  
237 carbon (TOC), cations exchangeable capacity (CEC) and particle size distribution were  
238 determined. The soil moisture content was determined by gravimetric method (Reynolds 1970),  
239 while particle size was by sieve analysis (ISO 2001). Soil pH was measured with glass  
240 electrodes in 1:10, soil:water suspensions (i.e., 10% w/v). The exchangeable cations ( $\text{Ca}^{2+}$ ,  
241  $\text{Mg}^{2+}$ ,  $\text{K}^+$  and  $\text{Na}^+$ ) were assayed by adaption of the Thomas method (Thomas, 1982). The TOC  
242 was determined using modified Walkey-Black titration method (Walkey and Black, 1934;  
243 Gelman et al., 2011).

### 244 **3. Results and discussion**

245 Physico-chemical properties of cocoa farm soil

246 The farm soil was basic and pH values were 8.04 and 7.95 for top (0 -15 cm) and sub-surface  
247 (15 - 30 cm) soils respectively. Cocoa trees have been reported to grow well in soils with pH  
248 ranging from 5 to 8.0 (Wood and Lass, 2011). The total organic carbon (TOC) was 2.03% and  
249 1.70 % for top and sub-surface soils respectively, while corresponding C:N ratios were 15:1  
250 and 16:1. The cation exchange capacities (CEC) were 25.268 and 22.923 meq/100g for top and  
251 sub-surface soils respectively. Soil texture was loamy. Particle size distribution for topsoil was  
252 clay – 13.65%, silt - 16.84% and sand - 69.51% respectively, while sub-surface was as follows:  
253 13.61%, 17.59% and 68.80%. The moisture content ranged from 30 °C to 38 °C.

254 Validation of analytical method

255 The calibration curves for standards were found to be linear over the concentration range  
256 applied. The linearity was good as indicated by the regression coefficient obtained which

257 ranged between 0.9968 and 0.9989. Also, Table 1, showed that the values obtained in the  
258 recovery studies for parent endosulfan and the sulfate metabolite, ranged between  $87.9 \pm 1.7$  and  
259  $119.2 \pm 3.4\%$  for all matrices, while coefficient of variation (CV) as %RSD were  $\leq 4.9\%$  – these  
260 values indicated that method of analysis was highly reliable and reproducible. The LOQ and  
261 LOD values were  $0.001 \mu\text{gg}^{-1}$  and  $0.0003 \mu\text{gg}^{-1}$  respectively; this also showed good sensitivity  
262 of the method applied for the analysis of the pesticides.

263 *Theobroma cacao* vegetation

264 Distribution of residual concentration of endosulfan

265 Figure 2 (a, b, c, d and e) shows the residual concentrations of total ( $\Sigma$ ) endosulfan on cocoa  
266 vegetation (fresh leaves, stem bark, pods and seeds) on day 0, values ranged from  $<0.001$  to  
267  $97.01 \mu\text{gg}^{-1}$ .  $\Sigma$ endosulfan was due to parent isomers as no metabolite was found in leaves, bark,  
268 pods and seeds. The highest residual level of  $\Sigma$ endosulfan was recorded on fresh leaves, while  
269 initial concentrations of  $\alpha$ -endosulfan and  $\beta$ -endosulfan were at  $66.51 \pm 17.48 \mu\text{gg}^{-1}$  and  
270  $30.50 \pm 8.24 \mu\text{gg}^{-1}$  respectively. Endosulfan was not detected in the cocoa seeds ( $<0.001 \mu\text{gg}^{-1}$ ).  
271 The order of  $\Sigma$ endosulfan residual concentrations was fresh leaves  $>$ bark  $>$  pods  $>$  seeds. This  
272 trend may be due to the exposed surface areas, shapes and position of each of these plant parts  
273 on the *T. cacao* tree; High residual levels on the leaves were due to its large and flat surface  
274 area, which are horizontal positioned. In addition, the epicuticular waxy nature of plant leaves  
275 may have enhanced the initial distribution of endosulfan on the fresh leaves. Plant leaves are  
276 reported to contain predominately long-chain polyester that accumulates lipophilic substances  
277 such as OCPs (Reischl *et al.*, 1989; Calamari *et al.*, 1991).

278 Residual concentrations of  $\alpha$ - and  $\beta$ -endosulfan isomers in fresh leaves decreased rapidly  
279 between day 0 and 7, while its metabolite - endosulfan sulfate was formed on day 7 (Figure  
280 2a). The percentage dissipation was 71.64% for  $\Sigma$ endosulfan, while parent isomers  $\alpha$ - and  $\beta$ -  
281 isomers were 70.58% and 80.24%, respectively. These values agreed with the dissipation of

282 endosulfan from foliar part of cotton (Kennedy *et al.*, 2001) and tomato plants (Ntow *et al.*,  
283 2007) after 7 days of treatment. The National Research Council, Canada (NRC) reported that in  
284 most fruits and vegetables, 50% of the parent residue is lost within 3 to 7 days after application  
285 (NRC, 1975). This rapid loss may be attributed to volatilization, although some level of  
286 degradation occurred, since endosulfan sulfate was found at day 7. Higher percentage  
287 disappearance was observed for  $\beta$ -endosulfan compared to the  $\alpha$ -isomer in fresh leaves. This  
288 may be due to the conversion of the  $\beta$ -isomer to  $\alpha$ -isomer (Rice *et al.*, 1997; Hapeman *et al.*,  
289 1997; Schmidt *et al.*, 2001).

290 For plant parts like stem bark, percentage dissipation was 20.26%, 50.55% and 24.01% for  
291  $\Sigma$ endosulfan,  $\alpha$ - and  $\beta$ -endosulfan respectively, while pods values were 12.36%, 16.27% and  
292 15.92% respectively. The order of loss was fresh leaves >bark > pods.

293 The relatively higher dissipation from fresh leaves compared to stem bark and pods may be due  
294 to the morphology of the cocoa plant, where the foliage and branches form a canopy; thereby  
295 screening the stem and pods from direct sunlight and air movement and reducing the  
296 dissipation of endosulfan. In addition, the horizontal positioning of the foliar lamina would  
297 enhance endosulfan volatilization from and photo-degradation in the leaves (Raha *et al.*, 1993,  
298 Antonious *et al.*, 1998).

299 Terminal concentrations of endosulfan and ratios

300 Figure 3 shows the residual concentrations and percentage dissipation for  $\Sigma$ endosulfan,  $\alpha$ -  
301 endosulfan and  $\beta$ -endosulfan in *T. cacao* vegetation at day 60. Residual concentrations in  
302 cocoa foliage were  $0.11 \mu\text{gg}^{-1}$  (99.84%),  $0.12 \mu\text{gg}^{-1}$  (99.62%), and  $0.51 \mu\text{gg}^{-1}$  (99.47%) for  $\alpha$ -  
303 endosulfan,  $\beta$ -endosulfan and  $\Sigma$ endosulfan respectively (with percentage dissipation in  
304 parenthesis), while in stem bark residual amounts were  $0.17 \mu\text{gg}^{-1}$  (99.57%),  $0.07 \mu\text{gg}^{-1}$   
305 (99.58%) and  $2.51 \mu\text{gg}^{-1}$  (95.67%); pods were  $0.16 \mu\text{gg}^{-1}$  (80.78%),  $0.20 \mu\text{gg}^{-1}$  (82.87%) and  
306  $0.76 \mu\text{gg}^{-1}$  (61.13%) for  $\alpha$ -endosulfan,  $\beta$ -endosulfan and  $\Sigma$ endosulfan respectively. This

307 implies that the pods had higher residual levels of endosulfan than the leaves. High carotenoid  
308 levels have been reported to be responsible for retention of chlorinated hydrocarbons in the  
309 body and peel of vegetables (Miglioranza *et al.*, 1999). The residual concentrations of both  
310 isomers at day 60 showed no distinct significant difference in the foliage and pods; however, a  
311 significant differential was observed in the stem bark. This was evident in the  $\alpha$ -isomer/ $\beta$ -  
312 isomer concentration ratios at day 60 (Figure 4a, 4b & 4c). The  $\alpha/\beta$  ratio of parent endosulfan  
313 and endosulfan sulfate/ $\Sigma$ endosulfan ratio are used as indicators of weathering and aging of  
314 technical grade endosulfan in the environment (Kennedy *et al.*, 2001; Malik *et al.*, 2009). The  
315  $\alpha/\beta$ -endosulfan ratio at this period for stem bark was 2.37 compared to 0.90 and 0.80 on fresh  
316 foliar and pods respectively at day 60. However, it is pertinent to mention that the initial  
317 concentration of  $\alpha$ - and  $\beta$ -isomers in pods at day 0, were  $0.93 \pm 0.30 \mu\text{gg}^{-1}$  and  $1.03 \pm 0.31 \mu\text{gg}^{-1}$   
318 respectively – this gave a ratio of 0.90 compared to 2.3 expected for technical grade endosulfan  
319 applied at the start of the experiment (Figures 2c and 4c). The  $\alpha/\beta$ -endossulfan ratio in fresh  
320 foliage and stem bark on day 0 were 2.18 and 2.34 respectively (Figure 4a and 4b), these are  
321 comparable to the expected ratio for commercial grade endosulfan (7:3 ratio). This drastic  
322 deviation observed in the pods may have been due to an initial rapid enzymatic action on the  
323 pesticide (Weir *et al.*, 2006; Ortiz-Hernandez *et al.*, 2013), with the  $\alpha$ -isomer being more  
324 susceptible to enzymatic breakdown. However, the level of the metabolite endosulfan sulfate  
325 was below detection limit ( $< 0.001 \mu\text{gg}^{-1}$ ) on day 0. The non-detection of the sulfate metabolite  
326 may be due to fast degradation of the parent compound to other metabolites like endosulfan  
327 diol, endosulfan hydroxyl carboxylic acid, endosulfan ether, and endosulfan lactone (UNEP,  
328 2009). In addition, in aqueous environment, endosulfan diol is the predominant metabolite and  
329 where the sulfate is formed it is further metabolized to endosulfan diol (USEPA, 2002b). The  
330 moisture content of cocoa husk is about 14% (w/w) (Daud *et al.*, 2013), this may have  
331 facilitated the hydrolysis of endosulfan in pod tissues to form the diol metabolite. Besides, the

332 presence of some endogenous biological enzymes such as lignin peroxidases and pectin methyl  
333 esterase (Falade et al., 2016, Kameshwar and Qin 2018) may have enhanced the rapid  
334 degradation of endosulfan observed on day 0 in pods (Wolejko et al., 2017). These other  
335 metabolites were not determined; endosulfan sulfate is reported to be the major metabolite of  
336 endosulfan - which also is an intermediary metabolite to the formation of other metabolites in  
337 plants and animals via the endosulfan diol route.

338 The relatively lower residual concentration of the  $\alpha$ -isomer in the fresh leaves (with  $\alpha/\beta$  ratio <  
339 1.0) at day 60 and the rapid decline in residual concentration from day 7, may be due to the  
340 physiochemical properties of both isomers. The  $\alpha$ -isomer is more volatile than the  $\beta$ -isomer,  
341 with vapour pressures at 20°C are 0.006 mmHg and 0.003 mmHg respectively. This may  
342 account for the relative persistence of  $\beta$ -endosulfan in this environment. The rapid decrease in  
343  $\alpha/\beta$ -isomer ratio from day 7 (>3.0) to day 60, after an initial increase from 2.3 (day 0), depicted  
344 a faster rate of disappearance of  $\alpha$ -isomer relative to the  $\beta$ -isomer. In addition, this initial  
345 increase in the ratio between days 0 and 7, may be due to an early conversion of the  $\beta$ -isomer  
346 to the  $\alpha$ -isomer (Tiwari and Guha 2013). It has been reported that residues of parent isomers  
347 are generally negligible after 2-3 weeks of application of 1.0 -100 mg/kg parent endosulfan,  
348 with  $\alpha$ -isomer being less persistent than the  $\beta$ -isomer (NRC, 1975).

349 Formation and disappearance of metabolite - endosulfan sulfate

350 Endosulfan sulfate was not detected in all the components assayed on day 0; however, various  
351 levels were recorded on day 7 except in cocoa bean. The concentrations of endosulfan sulfate  
352 were  $1.92 \pm 0.65 \mu\text{gg}^{-1}$ ,  $12.96 \pm 3.70 \mu\text{gg}^{-1}$  and  $0.07 \pm 0.05 \mu\text{gg}^{-1}$  on fresh leaves, bark and pods  
353 respectively. Levels of endosulfan sulfate was observed to have increased in almost all the  
354 parts - due to build-up as time progressed, with significant decline in the concentrations of the  
355 parent compounds. Highest concentrations were observed on days 14 and 42 for fresh leaves  
356 and pods respectively, while on *T. cacao* bark it was observed at day 7 and these persisted with

357 decline through day 60. High levels of the metabolite on the bark may be due to the  
358 morphological nature of the cocoa bark which has crevices or small grooves that may have trap  
359 pesticides and restricted oxidative action and effect of air movement on residual endosulfan.  
360 On day 7, 1.98%, 22.31% and 3.73% of the initial  $\Sigma$ endosulfan sprayed was oxidized to  
361 endosulfan sulfate in the foliar, stem bark and pods respectively; this constituted 6.98%  
362 (foliar), 28.02% (bark) and 4.25% (pods) of  $\Sigma$ endosulfan at this period (Figures 2a, 2b and 2c).  
363 Its contribution to  $\Sigma$ endosulfan increased steadily to 65.13%, 90.20% and 55.09% in cocoa  
364 leaves, stem bark and pods respectively at the terminal period (with ratios of endosulfan sulfate  
365 to  $\Sigma$ endosulfan for vegetation matrices  $\geq 0.55$ ). Ratios of endosulfan sulfate/ $\Sigma$ endosulfan and  
366 endosulfan sulfate/ $(\alpha+\beta)$  endosulfan could be used as markers for weathering and degradation  
367 of applied technical grade endosulfan. The endosulfan sulfate/ $(\alpha+\beta)$  endosulfan ratio also rose  
368 steadily from day 7 through day 60 as more metabolites were being formed.

369 On plant surfaces endosulfan is oxidized to endosulfan sulfate (Antonious *et al.*, 1998). In most  
370 plant studies on endosulfan and its' metabolites, endosulfan sulfate residue tend to increase  
371 relative to the parent isomers and other metabolites, thereby exhibiting more persistence.  
372 Endosulfan sulfate has been reported more persistent than parent endosulfan (Camacho-Morales  
373 & Sanchez 2016). The disappearance of > 98% of the pesticide from fresh leaves, pod and bark  
374 at day 60, indicated that residual endosulfan was due to topical treatment and not through  
375 translocation via root uptake from soil to aerial parts.

376 Distribution of residual endosulfan on dry foliage and soils

377 Figures 2d and 2e show the mean concentration of  $\Sigma$ endosulfan on fell dry foliage and topsoils  
378 (0-15cm) at day 0:  $59.25 \pm 37.89 \mu\text{gg}^{-1}$  and  $1.88 \pm 1.05 \mu\text{gg}^{-1}$  respectively, while levels in sub-  
379 surface soils (15-30 cm) was  $<0.001\mu\text{gg}^{-1}$ . The initial levels of  $\alpha$ -and  $\beta$ -endosulfan on dry fell



380 foliage were  $108.77 \pm 25.72 \mu\text{gg}^{-1}$  and  $50.48 \pm 12.16 \mu\text{gg}^{-1}$  respectively, while topsoil (0-15cm)  
381 values were  $1.24 \pm 0.65 \mu\text{gg}^{-1}$  and  $0.64 \pm 0.40 \mu\text{gg}^{-1}$  for  $\alpha$ - and  $\beta$ -endosulfan respectively.

382 Comparatively, more than eighty-fold magnitude of residual  $\Sigma$ endosulfan was found on fell dry  
383 foliage relative to surrounding topsoil on day 0. The higher concentration of  $\Sigma$ endosulfan was  
384 as a result of fell dry leaves covering the soil in cocoa farms. As a normal practice, they are left  
385 on topsoil to serve as mulch especially in the tropics. The dry leaves are the initial receiving  
386 surface for non-target endosulfan sprayed, around the cocoa tree, thereby restricting large  
387 amount of the pesticide reaching the top soil after its application. This was evident in the very  
388 high concentration of endosulfan on the dry leaves at day 0, while metabolite - endosulfan  
389 sulfate was  $< 0.001 \mu\text{gg}^{-1}$  for both matrices.

390 Between day 0 and day 7, residual concentrations of  $\alpha$ -endosulfan,  $\beta$ -endosulfan and  
391  $\Sigma$ endosulfan, on dry foliage decreased rapidly, with 64.51%, 46.71% and 55.50% losses  
392 respectively. These losses were mainly due to volatilization as amount of  $\Sigma$ endosulfan  
393 accounted for as residue was 44.50%, with endosulfan sulfate constituting only 7.57% of the  
394 residual concentration on day 7 and 3.37% with respect to day 0 (Figure 2d). The parent  
395 compound was more predominant. The vapour pressure of 0.83 mPa at 20°C for technical  
396 grade endosulfan indicates that it has an intermediate to high volatility under field conditions  
397 (Tomlin 2000). The Henry's law constants of  $4.54 \times 10^{-5} \text{ atm.m}^3/\text{mole}$  and  $4.39 \times 10^{-5}$   
398  $\text{atm.m}^3/\text{mol}$  and the corresponding  $1/H$  values of 540 and 560 for  $\alpha$ - and  $\beta$ -isomers  
399 respectively, indicated that both isomers have the potential to volatilize from water or moist  
400 soil surfaces (Mackay *et al.*, 1997). These physico-chemical properties must have accounted  
401 for the high dissipation of endosulfan from dry leaves.

402 The percentage dissipation from the soil at depth 0 - 15cm was 36.50% ( $0.79 \pm 0.27 \mu\text{gg}^{-1}$ ),  
403 9.11% ( $0.58 \pm 0.16 \mu\text{gg}^{-1}$ ) and 15.98% ( $1.58 \pm 0.55 \mu\text{gg}^{-1}$ ) for  $\alpha$ -,  $\beta$ -endosulfan and

404  $\Sigma$ endosulfan respectively on day 7, with residual concentrations in parentheses. About 84.02%  
405 of  $\Sigma$ endosulfan at the start of the experiment was accounted for on day 7 by residual  
406 concentrations of  $\alpha$ -,  $\beta$ -isomers and endosulfan sulfate. This suggests that a small proportion of  
407 initial concentration at the topsoil was lost by volatilization and degradation. The low  
408 dissipation from topsoil may have been due to the mulching of topsoil from direct air  
409 movement and heat energy from sunlight. In addition, some quantities of sprayed endosulfan  
410 from fell dry leaves and *Theobroma cacao* tree canopy may have drained on the topsoil after  
411 collection of day 0 samples, thus replenishing levels of the pesticide. The dissipation of  
412  $\Sigma$ endosulfan from the soil exhibited a three-phase process (i.e., tri-phasic phenomenon) (Figure  
413 2e). A gradual decrease from day 0 to day 14 ( $1.88 \rightarrow 1.49 \mu\text{gg}^{-1}$ ) was observed, followed by a  
414 rapid increase between days 14 and 21 ( $1.49 \rightarrow 2.38 \mu\text{gg}^{-1}$ ) and a gradual decline through day  
415 60. This implied that only 20.78% of  $\Sigma$ endosulfan disappeared after 14 days, with over 59.5%  
416 and 26.0% increase on day 21 with respect to days 14 and day 0 respectively. This abnormal  
417 trend may have resulted from dew and slight shower that fell during the week; thereby washing  
418 residues from the canopy (leaves, pods and stem) and fell dry foliage to the soil (Wauchope *et*  
419 *al.*, 2004; Ciglasch *et al.*, 2006). The order of individual contribution to  $\Sigma$ endosulfan was  
420 endosulfan sulfate (52.06%) >  $\beta$ -isomer (26.81%) >  $\alpha$ -isomer (21.23%). A rapid decrease was  
421 observed between days 21 and 28 for  $\alpha$ - and  $\beta$ -endosulfans. This sharp decline in  $\alpha$ - and  $\beta$ -  
422 isomers contents may have resulted from increased microbial activity and hydrolytic action  
423 resulting from the slight rain and dew that was observed during week 3 (Tiwari and Guha  
424 2013). Percentage disappearance between days 21 and 28 was 43.43% and 32.31% for  $\alpha$ - and  
425  $\beta$ -isomers respectively, while endosulfan sulfate recorded 2.43% - this suggests greater  
426 persistence when compared to parent compounds. These declines were mainly due to  
427 biodegradation, with minimum volatilization caused by air current on loose topsoils as a result  
428 of mulching. The order of persistence was  $\alpha$ -isomer <  $\beta$ -isomer < endosulfan sulfate. A slow

429 reduction in residual concentrations was observed for  $\alpha$ -,  $\beta$ -isomers and endosulfan sulfate  
430 from day 28 to day 60. Final residual contents in cropped soil were  $0.11 \mu\text{gg}^{-1}$ ,  $0.16 \mu\text{gg}^{-1}$ ,  $0.85$   
431  $\mu\text{gg}^{-1}$  and  $1.12 \mu\text{gg}^{-1}$  for  $\alpha$ -,  $\beta$ -isomers, endosulfan sulfate and  $\Sigma$ endosulfan respectively, with  
432 the metabolite contributing 75.96% to  $\Sigma$ endosulfan residue at this terminal period. The level of  
433 metabolite formed (by oxidative, photolytic, hydrolytic and microbial actions) from parent  
434 endosulfan in topsoil was  $0.31 \mu\text{gg}^{-1}$  on day 7 - reaching a peak concentration of  $1.24 \mu\text{gg}^{-1}$  on  
435 day 21 and  $0.85 \mu\text{gg}^{-1}$  at terminal (day 60). A moderate percentage degradation/disappearance  
436 of 31.53% was observed for endosulfan sulfate between peak concentration (day 21) and final  
437 concentration (day 60) over a period of 40 days - this also depicted persistence when compared  
438 to parent compounds. The ratios of endosulfan isomers and its metabolite are key in assessing  
439 the fate of technical grade endosulfan in the environment, which also is dependent on their  
440 individual physicochemical properties in soil. The  $\alpha/\beta$ -endosulfan ratio on day 0 was  $\sim 2.0$ .  
441 This dropped rapidly to  $< 1.0$  on day 14, followed by a gradual decline to  $< 0.70$  on day 60  
442 (Figure 3e). Again this portrays the  $\beta$ -isomer being more persistent in the soil. The  $\alpha$ -isomer is  
443 reported to be more susceptible to microbial and hydrolytic degradation (Ghadiri and Rose,  
444 2001), while  $\beta$ -isomer has more adsorptive and less volatile properties (Rice *et al.*, 2002;  
445 USEPA, 2002b). The endosulfan sulfate/ $(\alpha+\beta)$ -endosulfan residual ratios increased rapidly  
446 from day 0 to day 60 – giving almost a linear-increasing trend, with a value  $> 3.0$  at terminal  
447 day. This implies that metabolite was more predominant and persistent than the parent  
448 compound. The endosulfan sulfate/ $\Sigma$ endosulfan ratio ranged between 0.13 (day 7) and 0.76  
449 (day 60), which intermittently revealed the amount of metabolite being contributed to  
450  $\Sigma$ endosulfan as dissipation progressed, while at terminal period, endosulfan sulfate was the  
451 dominant compound – contributing 76% to  $\Sigma$ endosulfan in soil.

452 The order of percentage dissipation of  $\Sigma$ endosulfan from all field matrices at day 60 was  
453 99.47% ( $0.51\mu\text{gg}^{-1}$ ), 98.92% ( $1.71 \mu\text{gg}^{-1}$ ), 95.67% ( $2.51\mu\text{gg}^{-1}$ ), 61.13% ( $0.76\mu\text{gg}^{-1}$ ), 40.60%

454 (1.12  $\mu\text{g g}^{-1}$ ) for fresh foliage, dry foliage, stem bark, pods and soil respectively, with residual  
455 concentrations order being stem bark > dry foliage > soil (0 – 15 cm) > pods > fresh foliage.

456 Chemo-kinetic parameters–dissipation rate constant and terrestrial field half-life

457 The kinetics of pesticide dissipation under terrestrial field application is mostly described as  
458 first-order reactions (Tiwari and Guha 2013; OECD, 2014). The rate of dissipation (or  
459 degradation) ( $k$ ) and field half-life ( $DT_{50}$ ) for  $\Sigma$ endosulfan,  $\alpha$ -endosulfan,  $\beta$ -endosulfan and  
460 endosulfan sulfate in vegetation (fresh leaves, bark and pods) and surrounding matrices (dry  
461 foliage and soil) were determined iteratively - taking successive residual concentrations from  
462 day 0 (initial) through each sampling day (7, 14, 21, 28, 42 and 60) ( $k'$  and  $DT'_{50}$ ) into  
463 consideration; and between days 0 (initial concentration) and 60 (final residual concentration)  
464 ( $k$  and  $DT_{50}$ ).

465 Kinetic variables for vegetation

466 The dissipation rate constant ( $k'$ ) of  $\Sigma$ endosulfan in fresh foliage, bark and pods were 0.107 d<sup>-1</sup>  
467 <sup>1</sup>, 0.073 d<sup>-1</sup> and 0.023 d<sup>-1</sup> respectively, with corresponding  $DT'_{50}$  (field half-life) values as  
468 follows 6.48 d, 9.49 d and 30.13 d (Table 2). The order of  $DT'_{50}$  values in cocoa vegetation was  
469 fresh foliage < stem bark < pods. The order is likely due to greater surface area and exposure of  
470 leaves to direct air movement (or wind), heat from sunlight, surface wash-off by rain or dew  
471 and oxidation due to availability of oxygen, compared to the pods and bark, which are often  
472 shaded by the trees' canopy. In addition to the aforementioned, cocoa stem barks are rough,  
473 often with shallow crevices and grooves (hence further shielding), this may have accounted for  
474 the relatively higher  $DT'_{50}$  value recorded for the stem bark. Total endosulfan seemed to persist  
475 most in the cocoa pods (highest  $DT_{50}$ ). Ghadiri *et al.*, (1995), reported that the half-life of  
476 endosulfan in most fruits and vegetables is to be three to seven days. The dissipation rate  
477 constant  $k'$  for all vegetation parts (fresh leaves, bark and pods, except cocoa seeds) ranged

478 from  $0.036 - 0.167 \text{ d}^{-1}$ ,  $0.036 - 0.160 \text{ d}^{-1}$  and  $0.032 - 0.049 \text{ d}^{-1}$  for  $\alpha$ -endosulfan,  $\beta$ -endosulfan  
479 and endosulfan sulfate respectively, with corresponding field half-lives range of 4.15–19.25d,  
480 4.33 – 19.22 d and 13.36 –21.67 d. The field half-lives,  $DT'_{50}$  obtained for  $\alpha$ -endosulfan on  
481 fresh foliage, bark and pods were 4.15, 5.02 and 19.25 d respectively, with corresponding  
482 values for  $\beta$ -endosulfan being 4.33, 6.34 and 19.22 d (Table 2). The difference in half-life  
483 between both isomers was almost insignificant; however the  $\beta$ -isomer showed more persistence  
484 in fresh foliage and stem bark. This difference may have been due to the slight difference in  
485 their vapour pressure and action of volatilization. The  $\alpha$ -isomer is reported to be more volatile  
486 and dissipative (Siddique *et al.*, 2003), while the  $\beta$ -isomer exhibits relatively more persistent  
487 character.

488 There is a divide from literature on the preferential degradation of both isomers (Tiwari and  
489 Guha 2013). Kwon *et al.*, (2002) and Sethunathan *et al.*, (2004) reported faster degradation for  
490 the  $\alpha$ -isomer, while  $\beta$ -isomer was reported to exhibit faster rate by Walse *et al.*, 2003. The half-  
491 life values observed in stem bark and fresh foliage may be due to fast dissipation of the  $\alpha$ -  
492 isomer, followed by a very slow conversion of  $\beta$ -isomer to  $\alpha$ -isomer (Rice *et al.*, 1997;  
493 Schmidt *et al.*, 2001); while there may have been an inter-conversion between  $\alpha$ - and  $\beta$ -isomers  
494 in the pods. The conversion of  $\alpha$ -endosulfan to  $\beta$ -endosulfan under field conditions have been  
495 reported (Mukherjee and Gopal, 1994). However, it was obvious that the  $\beta$ -isomer was  
496 favoured in the inter-conversion between both isomers in pods at day 60 (Figure 3c). The  
497 higher field half-lives recorded in pods compared to other plant tissues may be due to diffusion  
498 of the applied pesticide into the soft tissues of the pods, thus leading to accumulation and  
499 persistence. Parent endosulfan and metabolites - endosulfan diol, ether and sulfate have been  
500 found to penetrate plant tissues and translocated from leaves to the roots of bean and sugar beet  
501 plants (Beard and Ware, 1969). Also, endosulfan is reported to have a log  $K_{ow}$  value of 3.55

502 (Mackay *et al.* 1997), thereby having a high potential to be bioaccumulated in biota (CCME,  
503 2010).

504 The half-life values obtained in this study for *Theobroma cacao* vegetation (fresh foliar, stem  
505 bark and cocoa pods) were significantly higher than values reported in field grown tomato  
506 (*Lycopersicon esculentum* L.) at Akumadan, Ghana (Ntow *et al.*, 2007) and eggplant (*Solanum*  
507 *macrocarpon* L.) grown in Southern Benin, West Africa (Rosendahl *et al.*, 2009). The wide  
508 difference may be due mainly to their morphological differences, for example, wider surface  
509 leaf lamina, canopy and greater waxy leaf cuticle in *Theobroma cacao*, cultivation practice  
510 and as well as higher dose concentration for crop treatment – all of these are likely to favour  
511 higher foliar half-life in *Theobroma cacao*.

512 Kinetic variables of surrounding matrixes

513 In soil (0-15cm), half-lives were 12.16 d ( $\alpha$ -endosulfan), 16.75 d ( $\beta$ -endosulfan ), 26.30 d  
514 (endosulfan sulfate) and 36.47 d ( $\Sigma$ endosulfan), with the  $\beta$ -isomer being more persistent than  
515  $\alpha$ -isomer. The order of persistence amongst the parent compound and metabolite was  $\alpha$ -  
516 endosulfan <  $\beta$ -endosulfan < endosulfan SO<sub>4</sub> in this study. Endosulfan sulfate is reported more  
517 persistent and toxic than parent compound, with half-life two or more times longer than its  
518 parent isomers, while estimated half-lives for the combined residues – total endosulfan, ranged  
519 from 9 months to 6 years (US EPA, 2002b). The  $DT'_{50}$  calculated for endosulfan sulfate was  
520 >1.5 times longer than values obtained for  $\alpha$ - and  $\beta$ -endosulfan respectively (Table 2). Previous  
521 studies have shown that the two isomers have different degradation times in soil. Half-lives of  
522 35 d and 150 d have been reported for  $\alpha$ - and  $\beta$ -endosulfan respectively, under neutral  
523 conditions, while under acidic environments they tend to persist longer (CCME, 2010).  
524 Degradation rate in soil is pH dependent; alkaline conditions favour degradation, whereas  
525 acidic conditions slow down the process (Ghadiri *et al.*, 1995). The soil pH at 0-15 cm depth

526 was alkaline (8.04), this may have accounted for lower half-life values obtained in this study in  
527 addition to the tropical environment. The half-life values reported by CCME (2010) were for  
528 studies carried out in temperate region. Degradation of pesticides is also influenced by  
529 temperature, thus lower half-lives are expected in the tropics. Lower half-lives for  $\alpha$ -,  $\beta$ -  
530 endosulfan and  $\Sigma$ endosulfan were reported in tropical West African farms soils at Akumadan,  
531 Ghana (Ntow *et al.*, 2007). Also, values obtained from another field study in southern Republic  
532 of Benin, West African were comparable with values observed in this study (Rosendahl *et al.*,  
533 2009). There is no previous terrestrial field dissipation (TFD) study reported for endosulfan on  
534 cropped soil in the Nigeria environment. However, there are half-life values reported for other  
535 OCPs such as DDT -8.7 w or 60.9 d, Aldrin -3.5 w or 24.5 d and lindane - 7.1 w or 49.7 d  
536 (Osibanjo, 2003).

537 Finally, no half-life was computed for bottom soil (15-30cm), since no residual parent  
538 compound and metabolite were detected ( $< 0.001 \mu\text{g g}^{-1}$ ). This observation is in consonance  
539 with Kathpal *et al.*, (1997), who reported that parent endosulfan and metabolites were confined  
540 to 0-10 cm depth in a terrestrial field study on bare cotton soil under sub-tropical conditions in  
541 Northern India.

#### 542 Comparative field half-life

543 The half-life  $DT'_{50}$ , (computed iteratively) values for  $\Sigma$ endosulfan were 6.48 d , 9.49 d and  
544 30.13 d, in fresh foliage, bark and pods respectively, while corresponding  $DT_{50}$  (computed with  
545  $t_{0d}$  and  $t_{60d}$ ) values were 7.97 d, 13.33 d and 43.31 d. The  $DT'_{50}$  and  $DT_{50}$  values for  $\alpha$ -  
546 endosulfan in fresh foliage, bark and pods were 4.15 d and 6.42 d; 5.02 d and 7.61 d; 19.25d  
547 and 23.90 d respectively, while corresponding  $\beta$ -endosulfan half-lives were 4.33 d and 7.45 d;  
548 6.34 d and 7.61 d; 19.22 d and 24.75 d for  $DT'_{50}$  and  $DT_{50}$  respectively. The  $DT'_{50}$  values of  
549 both isomers for cocoa vegetation were all significantly lower than  $DT_{50}$ . The same trend was  
550 observed for the surrounding matrices (top soil and fell dry foliage).

551 It is pertinent that due consideration be given to residual concentrations of parent compound,  
552 the formation and subsequent degradation of endosulfan sulfate as the process progresses from  
553 day 0 to 60 and the interaction between the pesticide and its' environmental components. The  
554 consideration of the initial and final concentrations (i.e,  $t_{0d}$  and  $t_{60d}$ ) only, would not adequately  
555 account for the phenomenal changes that would have occurred at intervals between successive  
556 sampling amplitudes from day 0 through the terminal day; hence kinetic variables should be  
557 determined iteratively for field kinetic studies. However, statistically, there was strong  
558 correlation between  $DT'_{50}$  and  $DT_{50}$  values ( $R= 0.9525$ ;  $n= 28$ ), while there was no significant  
559 difference between both methods of calculation of half-lives for parent isomers and metabolite  
560 ( $P = 0.05$ ) using the paired t-test.

#### 561 Assessment of Persistence

562 Persistence of pesticides is assessed based on half-lives and classified as non-persistent ( $< 30$   
563 d), moderately persistent (30-100 d) and persistent ( $> 100$  d) (EXTOXNET, 1993; Kerle *et al.*  
564 2007). In this study, endosulfan was non-persistent in fresh leaves, dry foliage and stem bark,  
565 while moderately persistent in cocoa pod and soil (0-15cm). The implication of the latter is  
566 two-fold. Firstly, persistence in soil may lead to residual build-up of endosulfan concentration  
567 over time and subsequently uptake by the roots, followed by translocation to various parts of  
568 the cocoa plant, where it is bio-accumulated. Secondly, there is high probability of residual  
569 endosulfan in the cocoa pod being transferred to the seed during harvest (cross contamination).  
570 This may be the major source of OCPs contamination of cocoa beans which affected the  
571 quality and rating of Nigerian cocoa in the world market in the 1980s and 1990s.

#### 572 ***Risk assessment of Theobroma cacao seeds and vegetation***

573 In this study, the level of total endosulfan found in *Theobroma cacao* seeds was  $< 0.001\mu\text{gg}^{-1}$   
574 (or  $\text{mgkg}^{-1}$ ) at day 60. The maximum residue limit (MRL) for endosulfan as stipulated by the



575 FAO/WHO is  $0.10 \text{ mgkg}^{-1}$  (EC, 2011, FAO/WHO, 1989). This implied that the quality of  
576 *Theobroma cacao* beans harvested during this study was satisfactory. This finding was  
577 comparable to previous terrestrial field trials by FAO/WHO in Brazil and Ghana where  
578 residual concentration was reported as ND or  $<0.01 \text{ mgkg}^{-1}$  (FAO, 2006). However, Oyekunle  
579 et al., (2017) and Aikpokpodion et al., (2012), have reported residual values which exceeded  
580 the stipulated FAO/WHO MRL for total endosulfan in the assessment of cocoa beans harvested  
581 from farms in Southwestern, Nigeria.

582 In considering the use of fresh leaves, cocoa pods (or husks) and fell dry leaves as green forage  
583 and fodder for livestock; the residual values in *Theobroma cacao* tissues at day 60 - fresh  
584 foliage -  $0.51 \mu\text{gg}^{-1}$ ; fell dry foliage (as mulch) -  $1.71 \mu\text{gg}^{-1}$  and pods -  $0.76 \mu\text{gg}^{-1}$  were all  
585 above the maximum residue limits of  $0.1 \text{ mgkg}^{-1}$  and  $0.300 \text{ ppm}$  ( $0.3 \text{ mgkg}^{-1}$ ) set by the  
586 Australian Government (AQIS, 2003) for pulse and primary animal feeds respectively.  
587 Therefore, the aforementioned parts do not meet the international standards as forage and  
588 fodder.

## 589 **Conclusion**

590 This study has shown that the fell dry foliage left as mulch on *Theobroma cacao* farm soils was  
591 predominantly the receiving surface for most non-target endosulfan sprayed. This phenomenon  
592 restricted large portion of the pesticide from reaching the topsoil on day 0 and subsequently  
593 restricted volatilization of the pesticide from topsoil. The epicuticular waxy nature and  
594 horizontal position of the lamina of the fresh foliar favoured high distribution of endosulfan at  
595 day 0. Endosulfan was not found in cocoa seed and sub-surface soil (15 -30 cm). Volatilization  
596 was key in terrestrial field dissipation of endosulfan especially at first period (between days 0  
597 and 7) in *Theobroma cacao* foliage surfaces; only marginal percentage ( $<3.5\%$ ) of initial  
598 concentration was accounted for as metabolite endosulfan sulfate. Dissipation trend over 60

599 days was distinctly biphasic and bi-continuum for fresh leaves, fell dry foliage and bark, while  
600 tri-phasic and tri-continuum in pods and soil (0-15cm) matrices.  
601 Residual concentrations in *Theobroma cacao* vegetation were due to topical application and  
602 not uptake from the soil. Rate of dissipation from vegetation surfaces occurred much faster  
603 than from soil. Amongst the vegetative parts, the dissipation rate constant ( $k$ ) was least in pod.  
604 Half-lives determined iteratively ( $DT'_{50}$ ) were higher than values calculated by initial-final day.  
605 The  $\beta$ -isomer was more persistent than  $\alpha$ -isomer, while the endosulfan sulfate was the most  
606 persistent. A rapid endogenous enzymatic breakdown of endosulfan was observed in pods, with  
607 the  $\alpha$ -isomer more susceptible; as  $\alpha/\beta$ -endosulfan ratio was 0.90 on day 0. Total endosulfan  
608 was moderately persistent in pods – a potential source for cross contamination of seeds during  
609 harvest. Iteratively determined  $DT'_{50}$  and initial-final day  $DT_{50}$  are highly correlated ( $R =$   
610  $0.9525$ ;  $n = 28$ ) and no significant difference ( $P=0.05$ ) for both methods.  
611 The use of fell leaves as mulch in cocoa farms restricted extensively the contamination of the  
612 topsoil.

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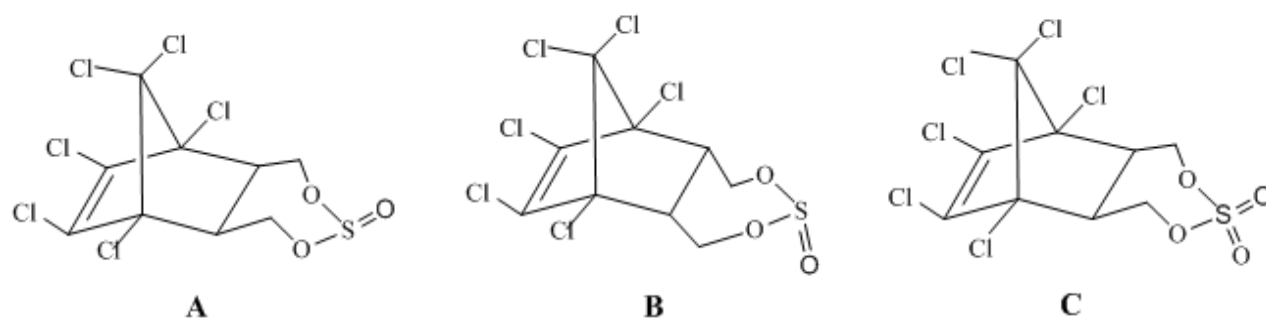
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Figure 1. Molecular structures of  $\alpha$ -endosulfan (A),  $\beta$ -endosulfan (B) & endosulfan sulfate (C)

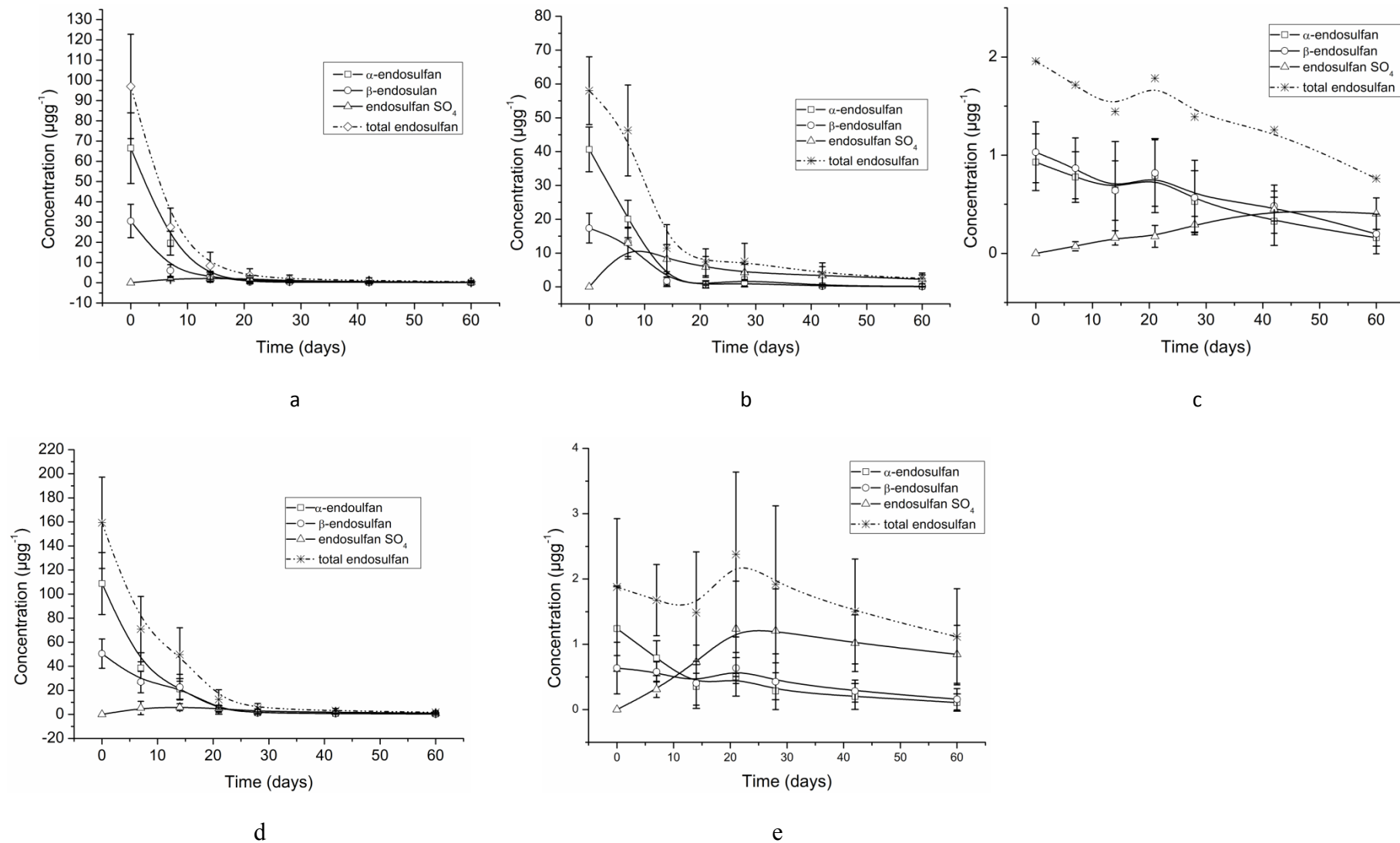


Figure 2: Residual concentration of  $\alpha$ -,  $\beta$ -endosulfan, endosulfan  $\text{SO}_4$  and total endosulfan in (a) fresh leaves (b) stem bark (c) pods (d) dry leaves (e) soil (0-15cm) over 60 days

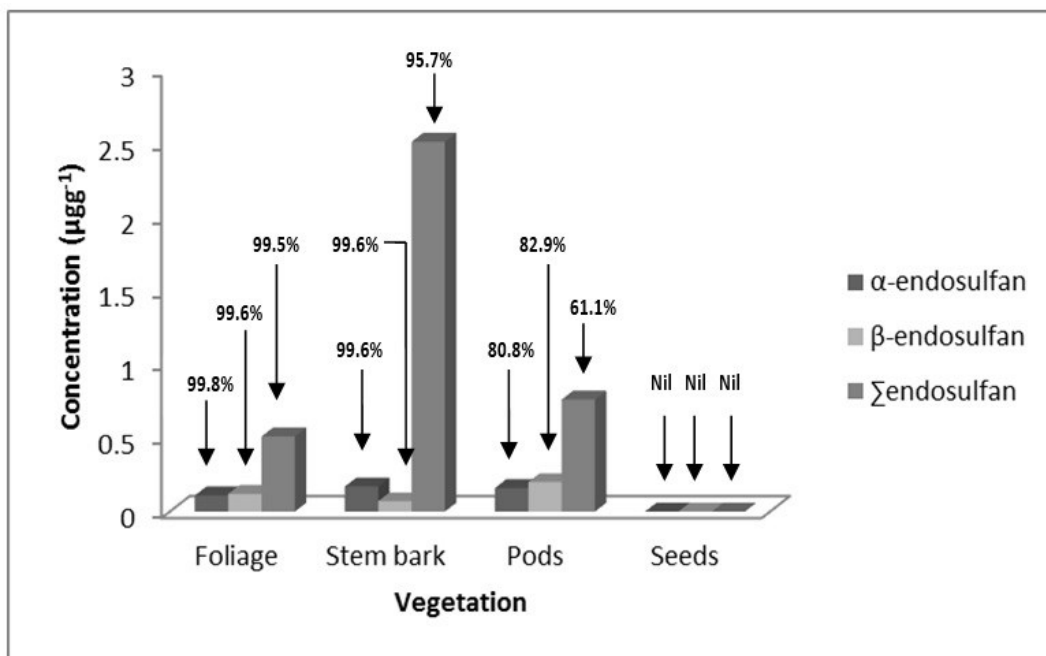
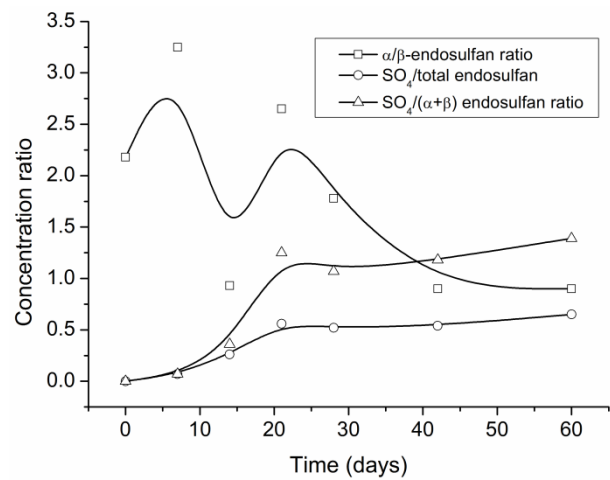
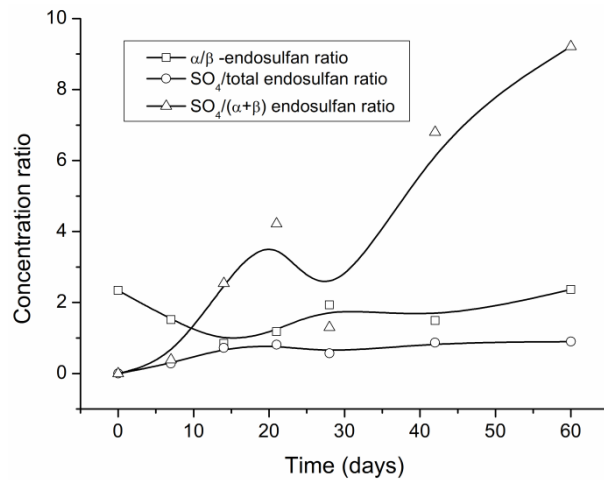


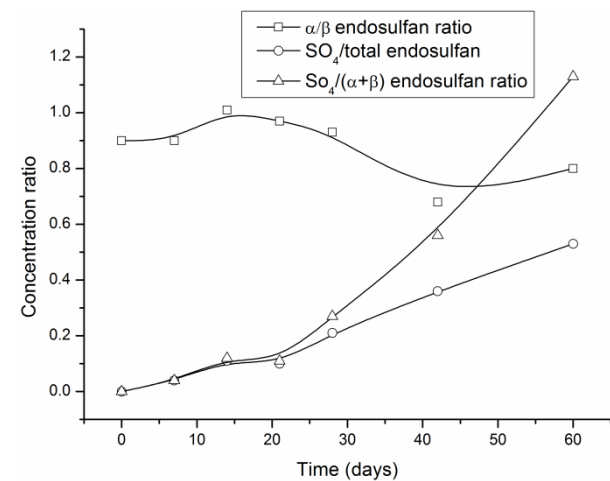
Figure 3. Residual concentrations and percentage dissipation of parent isomers and  $\Sigma$ endosulfan at day 60



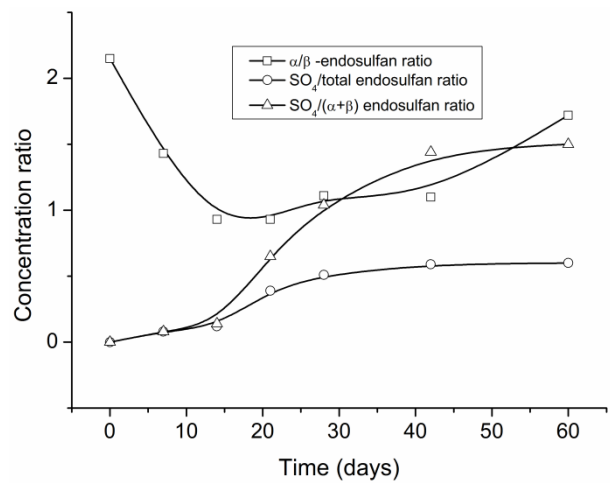
a



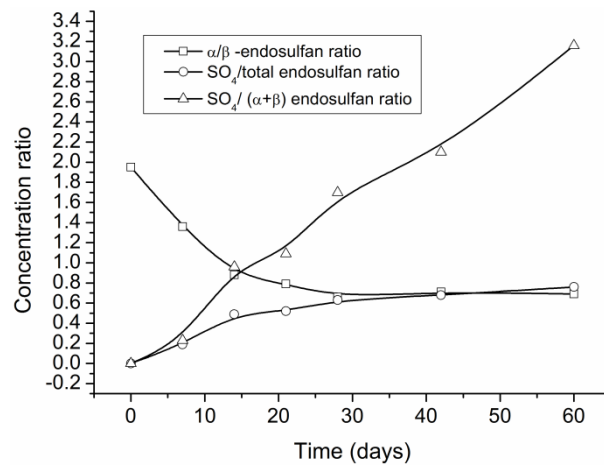
b



c



d



e

Figure 4: Ratios of  $\alpha/\beta$ -endosulfan, endo  $SO_4$ /total endo and endo  $SO_4/(\alpha+\beta)$  endosulfan in a) fresh leaves, b) stem bark, c) cocoa pods, d) dry leaves and, e) soil 0-15cm

**Table 1: Recovery study for  $\alpha$ -endosulfan,  $\beta$ -endosulfan and endosulfan sulfate in *Theobroma cacao* plant and surrounding matrix**

		THEOBROMA CACAO VEGETATION TYPE										SURROUNDING MATRIX			
		FRESH LEAVES		STEM BARK		PODS		SEEDS		DRY LEAVES		SOIL			
PESTICIDE	Amount of pure pesticide spiked ( $\mu\text{gkg}^{-1}$ )	Total quantity of pesticide found ( $\mu\text{gkg}^{-1}$ )	Percent recovery of pesticide spiked (%)	Total quantity of pesticide found ( $\mu\text{gkg}^{-1}$ )	Percent recovery of pesticide spiked (%)	Total quantity of pesticide found ( $\mu\text{gkg}^{-1}$ )	Percent recovery of pesticide spiked (%)	Total quantity of pesticide found ( $\mu\text{gkg}^{-1}$ )	Percent recovery of pesticide spiked (%)	Total quantity of pesticide found ( $\mu\text{gkg}^{-1}$ )	Percent recovery of pesticide spiked (%)	0-15 cm		15-30 cm	
		Total quantity of pesticide found ( $\mu\text{gkg}^{-1}$ )	Percent recovery of pesticide spiked (%)	Total quantity of pesticide found ( $\mu\text{gkg}^{-1}$ )	Percent recovery of pesticide spiked (%)	Total quantity of pesticide found ( $\mu\text{gkg}^{-1}$ )	Percent recovery of pesticide spiked (%)	Total quantity of pesticide found ( $\mu\text{gkg}^{-1}$ )	Percent recovery of pesticide spiked (%)	Total quantity of pesticide found ( $\mu\text{gkg}^{-1}$ )	Percent recovery of pesticide spiked (%)	Total quantity of pesticide found ( $\mu\text{gkg}^{-1}$ )	Percent recovery of pesticide spiked (%)	Total quantity of pesticide found ( $\mu\text{gkg}^{-1}$ )	Percent recovery of pesticide spiked (%)
$\alpha$ -endosulfan	250	244.2 $\pm$ 9.9 %RSD=4.0	97.7 $\pm$ 3.9	238.2 $\pm$ 9.6 %RSD=4.0	95.3 $\pm$ 3.9	234.3 $\pm$ 2.9 %RSD=1.2	93.7 $\pm$ 1.2	248.5 $\pm$ 2.6 %RSD=1.0	99.4 $\pm$ 1.0	249.9 $\pm$ 5.2 %RSD=2.1	99.9 $\pm$ 2.1	245.6 $\pm$ 7.4 %RSD=3.0	98.2 $\pm$ 3.0	252.6 $\pm$ 9.9 %RSD=3.9	101.1 $\pm$ 4.0
	500	502.9 $\pm$ 9.2 %RSD=1.8	100.6 $\pm$ 1.8	506.5 $\pm$ 6.8 %RSD=1.4	101.3 $\pm$ 1.4	458.1 $\pm$ 9.6 %RSD=2.1	91.6 $\pm$ 1.9	513.3 $\pm$ 12.6 %RSD=2.5	102.7 $\pm$ 2.5	502.9 $\pm$ 6.6 %RSD=1.3	100.6 $\pm$ 1.3	505.6 $\pm$ 6.4 %RSD=1.3	101.1 $\pm$ 1.3	515.9 $\pm$ 8.7 %RSD=1.7	103.2 $\pm$ 1.7
$\beta$ -endosulfan	250	242.1 $\pm$ 7.0 %RSD=2.9	96.8 $\pm$ 2.8	242.0 $\pm$ 10.6 %RSD=4.4	96.8 $\pm$ 4.2	219.8 $\pm$ 9.6 %RSD=4.4	87.9 $\pm$ 3.8	238.8 $\pm$ 2.0 %RSD=0.9	95.5 $\pm$ 0.8	267.4 $\pm$ 6.0 %RSD=2.3	106.9 $\pm$ 2.4	239.9 $\pm$ 3.5 %RSD=1.5	96.0 $\pm$ 1.4	249.0 $\pm$ 7.8 %RSD=3.1	99.6 $\pm$ 3.1
	500	487.3 $\pm$ 11.1 %RSD=2.3	97.46 $\pm$ 2.2	488.1 $\pm$ 10.5 %RSD=2.2	97.6 $\pm$ 2.1	451.4 $\pm$ 13.0 %RSD=2.9	90.3 $\pm$ 2.6	483.9 $\pm$ 6.2 %RSD=1.3	96.8 $\pm$ 1.2	501.0 $\pm$ 3.4 %RSD=0.7	100.2 $\pm$ 0.7	500.3 $\pm$ 18.9 %RSD=3.8	100.1 $\pm$ 3.8	486.7 $\pm$ 8.3 %RSD=1.7	97.3 $\pm$ 1.7
Endosulfan SO <sub>4</sub>	250	253.7 $\pm$ 4.7 %RSD=1.9	101.5 $\pm$ 1.9	242.2 $\pm$ 10.4 %RSD=4.3	96.9 $\pm$ 4.2	235.7 $\pm$ 3.0 %RSD=1.3	94.3 $\pm$ 1.2	254.7 $\pm$ 4.5 %RSD=1.8	101.9 $\pm$ 1.8	255.0 $\pm$ 5.0 %RSD=2.0	102.0 $\pm$ 2.0	248.0 $\pm$ 12.1 %RSD=4.9	99.2 $\pm$ 4.9	298.1 $\pm$ 8.5 %RSD=2.3	119.2 $\pm$ 3.4
	500	487.5 $\pm$ 10.3 %RSD=2.1	97.5 $\pm$ 2.1	508.5 $\pm$ 16.6 %RSD=3.3	101.7 $\pm$ 3.3	438.6 $\pm$ 8.6 %RSD=2.0	87.9 $\pm$ 1.7	492.5 $\pm$ 11.4 %RSD=2.3	98.5 $\pm$ 2.3	491.1 $\pm$ 7.2 %RSD=1.5	98.2 $\pm$ 1.4	482.5 $\pm$ 15.3 %RSD=3.2	96.5 $\pm$ 3.1	506.8 $\pm$ 6.8 %RSD=1.3	104.1 $\pm$ 1.4

Note: Each spiked sample types and levels were replicated thrice

**Table 2: Chemo-kinetic parameters for endosulfan in cocoa farm**

Plant Part	Σendosulfan			α-endosulfan			β-endosulfan			Endosulfan sulfate		
	<i>chemo-kinetic variables calculated iteratively from <math>d_0 \rightarrow d_{60}</math></i>											
	$k'$ (d <sup>-1</sup> )	$DT'_{50}$ (d)	R <sup>2</sup>	$k'$ (d <sup>-1</sup> )	$DT'_{50}$ (d)	R <sup>2</sup>	$k'$ (d <sup>-1</sup> )	$DT'_{50}$ (d)	R <sup>2</sup>	$k'$ (d <sup>-1</sup> )	$DT'_{50}$ (d)	R <sup>2</sup>
Fresh foliage	0.107	6.48	0.884	0.167	4.15	0.883	0.160	4.33	0.813	0.052	13.36	0.939
Bark	0.073	9.49	0.855	0.138	5.02	0.793	0.109	6.34	0.904	0.049	14.09	0.930
Pods	0.023	30.13	0.858	0.036	19.25	0.924	0.036	19.22	0.892	0.032	21.67	0.780
Seed	-	-	-	-	-	-	-	-	-	-	-	-
Dry leaves	0.094	7.37	0.905	0.133	5.21	0.879	0.100	6.95	0.922	0.031	19.25	0.937
Soil (0-15cm)	0.019	36.47	0.341	0.057	12.16	0.915	0.041	16.75	0.840	0.026	26.30	0.215
Soil (15-30cm)	-	-	-	-	-	-	-	-	-	-	-	-
	<i>chemo-kinetic variables calculated from <math>d_0</math> &amp; <math>d_{60}</math></i>											
	$k$ (d <sup>-1</sup> )	$DT_{50}$ (d)	R <sup>2</sup>	$k$ (d <sup>-1</sup> )	$DT_{50}$ (d)	R <sup>2</sup>	$k$ (d <sup>-1</sup> )	$DT_{50}$ (d)	R <sup>2</sup>	$k$ (d <sup>-1</sup> )	$DT_{50}$ (d)	R <sup>2</sup>
Fresh foliage	0.087	7.97	-	0.108	6.42	-	0.093	7.45	-	0.036	19.25	-
Bark	0.052	13.33	-	0.091	7.61	-	0.091	7.61	-	0.033	21.00	-
Pods	0.016	43.31	-	0.029	23.90	-	0.028	24.75	-	0.032	21.66	-
Seed	-	-	-	-	-	-	-	-	-	-	-	-
Dry leaves	0.076	9.12	-	0.092	7.53	-	0.088	7.87	-	0.032	21.66	-
Soil (0-15)	0.009	77.00	-	0.041	16.90	-	0.023	30.13	-	0.019	36.47	-
Soil (15-30cm)	-	-	-	-	-	-	-	-	-	-	-	-

**Legend:**  $k'$  – dissipation rate constant calculated iteratively on sampling days ( $d_0 \rightarrow d_i \rightarrow d_{60}$ );  $k$  – dissipation rate constant calculated using initial and final concentrations ( $d_0$  &  $d_{60}$ );  $DT'_{50}$  – field half-life (iteratively);  $DT_{50}$  – field half-life (initial-final);  $R^2$  – regression coefficient or correlation coefficient