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

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Abstract

Endosulfan, 6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano,2,4,3-benzodioxathiepin-3-oxide is still a pesticide of choice for most cocoa farmers in Southwestern Nigeria, in spite of its persistence, bio-accumulative, toxicological properties and restriction.

A single-treatment of 1.4 kg ai/ha (0.5% ai) of technical grade endosulfan (Thiodan, 35EC) was applied to 0.0227 hectare of cultivated *Theobroma cacao* L. (Cocoa) farm at the Cocoa Research Institute of Nigeria (CRIN). Levels of parent endosulfan (α-, β-endosulfan) and major metabolite (endosulfan sulfate) were determined in vegetation and surrounding matrices at days 0, 7, 14, 21, 28, 42 and 60 using GC-MS. Their kinetic variables were determined. Order of ∑endosulfan distribution at day 0 was: dry foliage>fresh foliage>bark>pods>soil (0-15cm). No residual endosulfan was found in cocoa seeds and sub-surface soil (15-30 cm). Low residual levels in pods on day 0 may be due to endogenous enzymatic breakdown, with α-isomer more susceptible and α/β-endosulfan ratio being 0.90. Fell dry foliage as mulch was predominantly the receiving matrix for non-target endosulfan sprayed. Volatilization was key in endosulfan dissipation between days 0 and 7 from foliage surfaces (> 60% loss), while dissipation trend were bi-phasic and tri-phasic for vegetation and soil respectively. ∑endosulfan loss at terminal day ranged between 40.60% (topsoil) and 99.47% (fresh foliage). Iteratively computed half-lives (DT'_{50}) ranged from 6.48 – 30.13d for ∑endosulfan in vegetation. Endosulfan was moderately persistent in pods – a potential source for cross contamination of seeds during harvest. Iteratively determined DT'_{50} and initial-final day DT_{50} are highly correlated (R=0.9525; n= 28) and no significant difference (P=0.05) for both methods.

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

Introduction

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

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

Dissipation and terrestrial fate of pesticides after application on plants are of great importance from environmental point of view. Environmental processes such as volatilization, transport, degradation, adsorption, bioaccumulation and bio-magnification (Weber *et al.,* 2010) are common phenomenon after the treatment of farm crops with pesticides. These processes also influence their level of persistence and contamination in the environment. Persistence of pesticide is measured by its half-life and best determined kinetically (OECD, 2014). This is used to assess its risk and adverse impact to human health. To achieve a more reliable result, 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 (α- and β-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 ± 0.2^{o}C, while the daily maximum
109 temperature was 28.2 ± 0.2^{o}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-

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

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

Experimental Procedure

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

Sample collection

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

Reagents and materials

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

Preparation of standard solutions

Stock solutions for α-endosulfan, β-endosulfan and endosulfan sulfate reference standards were prepared by weighing 50 mg of each into separate 50 mL volumetric flask, 5 mL hexane;isopropyl alcohol (1:1) mixture was added and shaken gently to homogenize. These were made to volume with same solvent mixture to obtain reference standard stock concentration of 1000 μgmL^{-1} for each pesticide. All working concentrations for each standard were prepared from these stocks.

Samples pre-treatment

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

Sample Extraction and Clean Up

Modified methods of USEPA 3570 (2002a) and Yeboah *et al.,* (2003) were employed for the extraction of endosulfan from cocoa plant tissues and cropped soils. A mixture of ethyl acetate:petroleum spirit (3:2) and that of acetone: petroleum spirit (1:1) were used for extraction of endosulfan in plant tissues and soil samples respectively. To 2 g of blended plant tissues or soil samples, 1 g of anhydrous sodium sulfate was added in an amber extraction flask. This was shaken vigorously with 10 mL of extraction solvent mixtures for 45 minutes using a Thermo Scientific reciprocating/orbital shaker; model MaQ at 80 - 100 r/minute. Aliquot, 5 mL of extract (equivalent to 1 g of sample) was transferred into 10 mL beaker and evaporate with gentle stream of nitrogen gas to 1 mL residue. To residue was added 5 mL of hexane, homogenized and reduce to 1 mL using nitrogen gas. The residue extract was then cleaned by passing through a glass column (dimension, 120mm (l) × 12 mm (i.d)) packed with activated silica gel (60 -120 mesh) previously mixed with 10 % (w/w) of distilled water and a bed of 0.5 g of anhydrous sodium sulfate on top.. Elution was first with 10 mL of hexane:dichloromethane (1:4) and then with 5 mL of dichloromethane: hexane: acetonitrile (50:49.65:0.35) at a flow rate of 1 mL/min. The combined eluate was reduced to 2 mL after addition of 1 mL of iso-octane using a rotary evaporator. All traces of CH_2Cl_2 were ensured replaced with hexane before Gas Chromatography-Mass Spectroscopy (GC-MS) analysis.

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

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

Calibration graph of α-, β-endosulfan and endosulfan sulfate for kinetic studies

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

Data analysis

The rate of dissipation/degradation (k) and half-life ($t_{1/2}$ or DT_{50} for field studies) for residual concentrations of ∑endosulfan (sum of α-endosulfan, β-endosulfan and endosulfan sulfate), α-endosulfan and β-endosulfan were independently determined applying equation (i), assuming

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

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

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

Where $C_{(t)}$ is the residual concentration of pesticides on vegetation at time t; $C_{(0)}$ is the concentration of pesticides at time zero i.e., t = 0; $C_{(max)}$ is maximum concentration attained by metabolite; t = time (days); k is the dissipation rate constant and DT_{50} (or $t_{1/2}$) is the field dissipation half-life. [Note: DT'_{50} and k' represented calculations obtained iteratively ($d_{0\rightarrow60}$), while DT_{50} and k obtained from initial-final day calculation ($C_{0\&60}$)]

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

Quality assurance

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

Also, identified peaks for α-endosulfan, β-endosulfan and endosulfan sulfate obtained for each matrix was confirmed by selected molecular ion peaks at m/z values using the National

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

Determination of physico-chemical properties

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

3. Results and discussion

Physico-chemical properties of cocoa farm soil

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

Validation of analytical method

The calibration curves for standards were found to be linear over the concentration range 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±1.7 and
259 119.2±3.4% for all matrices, while coefficient of variation (CV) as %RSD were ≤ 4.9% – these
260 values indicated that method of analysis was highly reliable and reproducible. The LOQ and
261 LOD values were 0.001 μgg^{-1} and 0.0003 μgg^{-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 (∑) endosulfan on cocoa
266 vegetation (fresh leaves, stem bark, pods and seeds) on day 0, values ranged from <0.001 to
267 97.01 $\mu gg^{-1.}$ ∑endosulfan was due to parent isomers as no metabolite was found in leaves, bark,
268 pods and seeds. The highest residual level of ∑endosulfan was recorded on fresh leaves, while
269 initial concentrations of α-endosulfan and β-endosulfan were at 66.51±17.48 μgg^{-1} and
270 30.50±8.24 μgg^{-1} respectively. Endosulfan was not detected in the cocoa seeds (<0.001 μgg^{-1}).
271 The order of ∑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 α- and β-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 ∑endosulfan, while parent isomers α- and β-
281 isomers were 70.58% and 80.24%, respectively. These values agreed with the dissipation of

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

For plant parts like stem bark, percentage dissipation was 20.26%, 50.55% and 24.01% for ∑endosulfan, α- and β-endosulfan respectively, while pods values were 12.36%, 16.27% and 15.92% respectively. The order of loss was fresh leaves >bark >pods.

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

Terminal concentrations of endosulfan and ratios

Figure 3 shows the residual concentrations and percentage dissipation for ∑endosulfan, α-endosulfan and β-endosulfan in *T. cacao* vegetation at day 60. Residual concentrations in cocoa foliage were 0.11 μgg^{-1} (99.84%), 0.12 μgg^{-1} (99.62%), and 0.51 μgg^{-1} (99.47%) for α-endosulfan, β-endosulfan and ∑endosulfan respectively (with percentage dissipation in parenthesis), while in stem bark residual amounts were 0.17μgg^{-1} (99.57%), 0.07 μgg^{-1} (99.58%) and 2.51 μgg^{-1} (95.67%); pods were 0.16 μgg^{-1} (80.78%), 0.20 μgg^{-1} (82.87%) and 0.76 μgg^{-1} (61.13%) for α-endosulfan, β-endosulfan and ∑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 α-isomer/β-
312 isomer concentration ratios at day 60 (Figure 4a, 4b &4c). The α/β ratio of parent endosulfan
313 and endosulfan sulfate/∑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 α-/β-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 α- and β-isomers in pods at day 0, were 0.93±0.30 μgg^{-1} and 1.03±0.31 μgg^{-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 α/β-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 α-isomer being more
324 susceptible to enzymatic breakdown. However, the level of the metabolite endosulfan sulfate
325 was below detection limit (< 0.001 μgg^{-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

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

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

Formation and disappearance of metabolite - endosulfan sulfate

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

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

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

Distribution of residual endosulfan on dry foliage and soils

Figures 2d and 2e show the mean concentration of ∑endosulfan on fell dry foliage and topsoils (0-15cm) at day 0: 59.25 ± 37.89 $\mu g g^{-1}$ and 1.88 ± 1.05 $\mu g g^{-1}$ respectively, while levels in sub-surface soils (15-30 cm) was <0.001$\mu g g^{-1}$. The initial levels of α-and β-endosulfan on dry fell

foliage were 108.77 ± 25.72 μgg^{-1} and 50.48 ± 12.16 μgg^{-1} respectively, while topsoil (0-15cm) values were 1.24 ± 0.65 μgg^{-1} and 0.64 ± 0.40 μgg^{-1} for α- and β-endosulfan respectively.

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

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

The percentage dissipation from the soil at depth 0 - 15cm was 36.50% (0.79 ± 0.27 μgg^{-1}), 9.11% (0.58 ± 0.16 μgg^{-1}) and 15.98% (1.58 ± 0.55 μgg^{-1}) for α-, β-endosulfan and

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

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

The order of percentage dissipation of ∑endosulfan from all field matrices at day 60 was 99.47% (0.51μgg^{-1}), 98.92% (1.71 μgg^{-1}), 95.67% (2.51μgg^{-1}), 61.13% (0.76ugg^{-1}), 40.60%

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

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

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

Kinetic variables for vegetation

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

478 from 0.036 – 0.167 d^{-1}, 0.036 – 0.160 d^{-1} and 0.032– 0.049 d^{-1} for α-endosulfan, β-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 α-endosulfan on
481 fresh foliage, bark and pods were 4.15, 5.02 and 19.25 d respectively, with corresponding
482 values for β-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 β-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 α-isomer is reported to be more volatile
486 and dissipative (Siddique *et al*., 2003), while the β-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 α-isomer, while β-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 α-
492 isomer, followed by a very slow conversion of β-isomer to α-isomer (Rice *et al.,* 1997;
493 Schmidt *et al*., 2001); while there may have been an inter-conversion between α- and β-isomers
494 in the pods. The conversion of α-endosulfan to β-endosulfan under field conditions have been
495 reported (Mukherjee and Gopal, 1994). However, it was obvious that the β-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

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

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

Kinetic variables of surrounding matrixes

In soil (0-15cm), half-lives were 12.16 d (α-endosulfan), 16.75 d (β-endosulfan), 26.30 d (endosulfan sulfate) and 36.47 d (∑endosulfan), with the β-isomer being more persistent than α-isomer. The order of persistence amongst the parent compound and metabolite was α-endosulfan <β-endosulfan <endosulfan SO_4 in this study. Endosulfan sulfate is reported more persistent and toxic than parent compound, with half-life two or more times longer than its parent isomers, while estimated half-lives for the combined residues – total endosulfan, ranged from 9 months to 6 years (US EPA, 2002b). The DT'_{50} calculated for endosulfan sulfate was >1.5 times longer than values obtained for α- and β-endosulfan respectively (Table 2). Previous studies have shown that the two isomers have different degradation times in soil. Half-lives of 35 d and 150 d have been reported for α- and β-endosulfan respectively, under neutral conditions, while under acidic environments they tend to persist longer (CCME, 2010). Degradation rate in soil is pH dependent; alkaline conditions favour degradation, whereas 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 α-, β-
530 endosulfan and ∑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 $\mu g g^{-1}$). 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 ∑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 α-
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 β-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 < 0.001μgg^{-1}
574 (or $mgkg^{-1}$) at day 60. The maximum residue limit (MRL) for endosulfan as stipulated by the

575 FAO/WHO is 0.10 $mgkg^{-1}$ (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$mgkg^{-1}$ (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 μgg^{-1}; fell dry foliage (as mulch) - 1.71 μgg^{-1} and pods - 0.76 ugg^{-1} were all
585 above the maximum residue limits of 0.1 $mgkg^{-1}$ and 0.300ppm (0.3 $mgkg^{-1}$) 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 β-isomer was more persistent than α-isomer, while the endosulfan sulfate was the most
606 persistent. A rapid endogenous enzymatic breakdown of endosulfan was observed in pods, with
607 the α-isomer more susceptible; as α/β-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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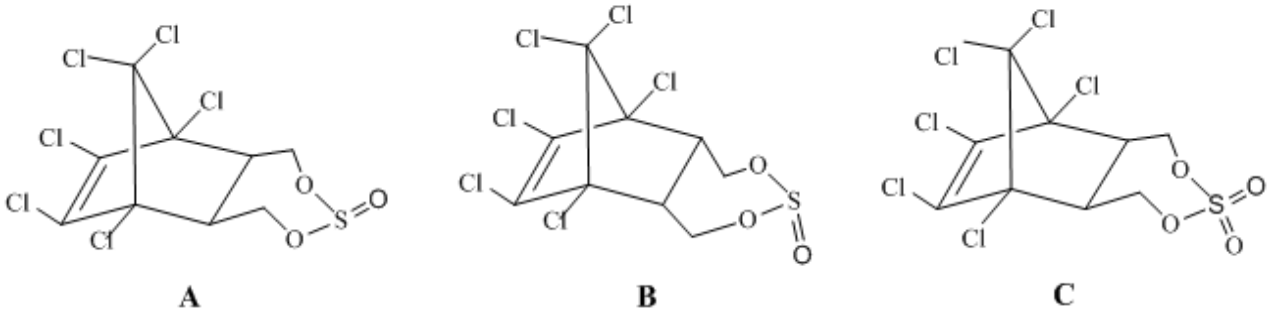

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890 Figure 1. Molecular structures of α-endosulfan (A), β-endosulfan (B) & endosulfan sulfate (C)

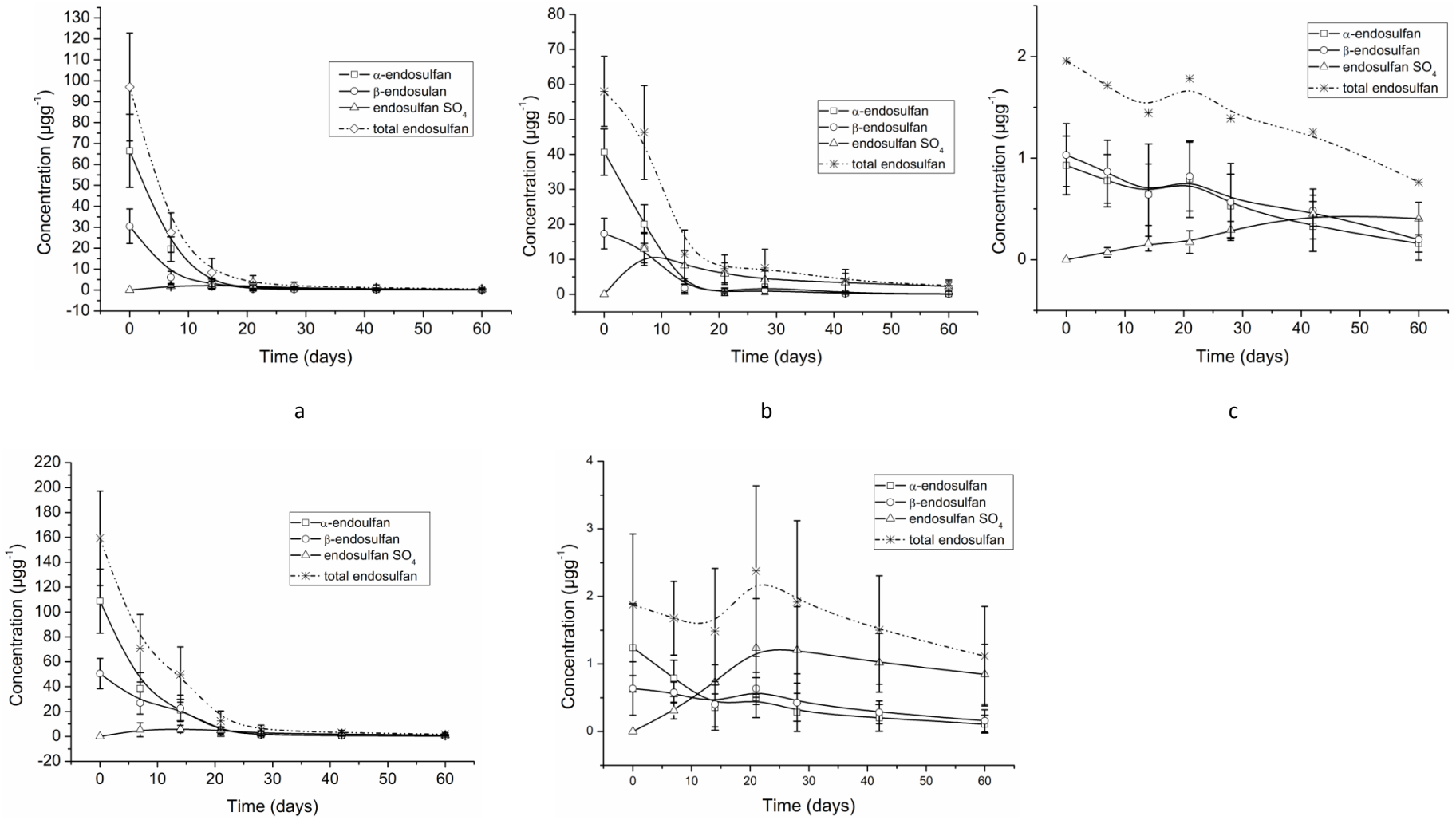

Figure 2: Residual concentration of α-, β-endosulfan, endosulfan SO_4 and total endosulfan in (a) fresh leaves (b) stem bark (c) pods (d) dry leaves (e) soil (0-15cm) over 60 days

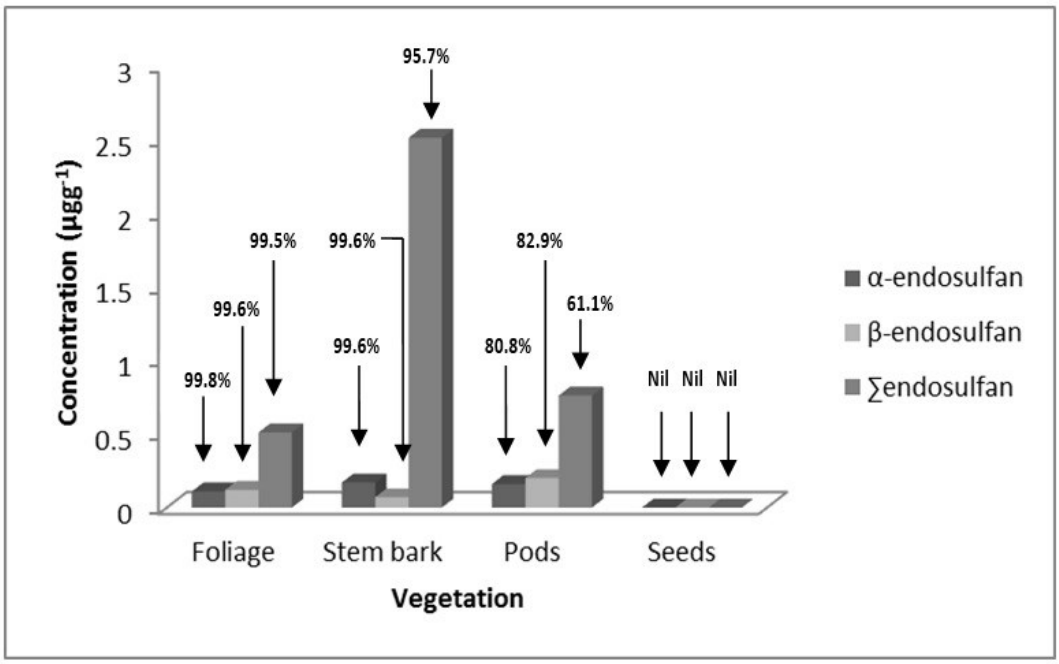

Figure 3. Residual concentrations and percentage dissipation of parent isomers and Σendosulfan at day 60

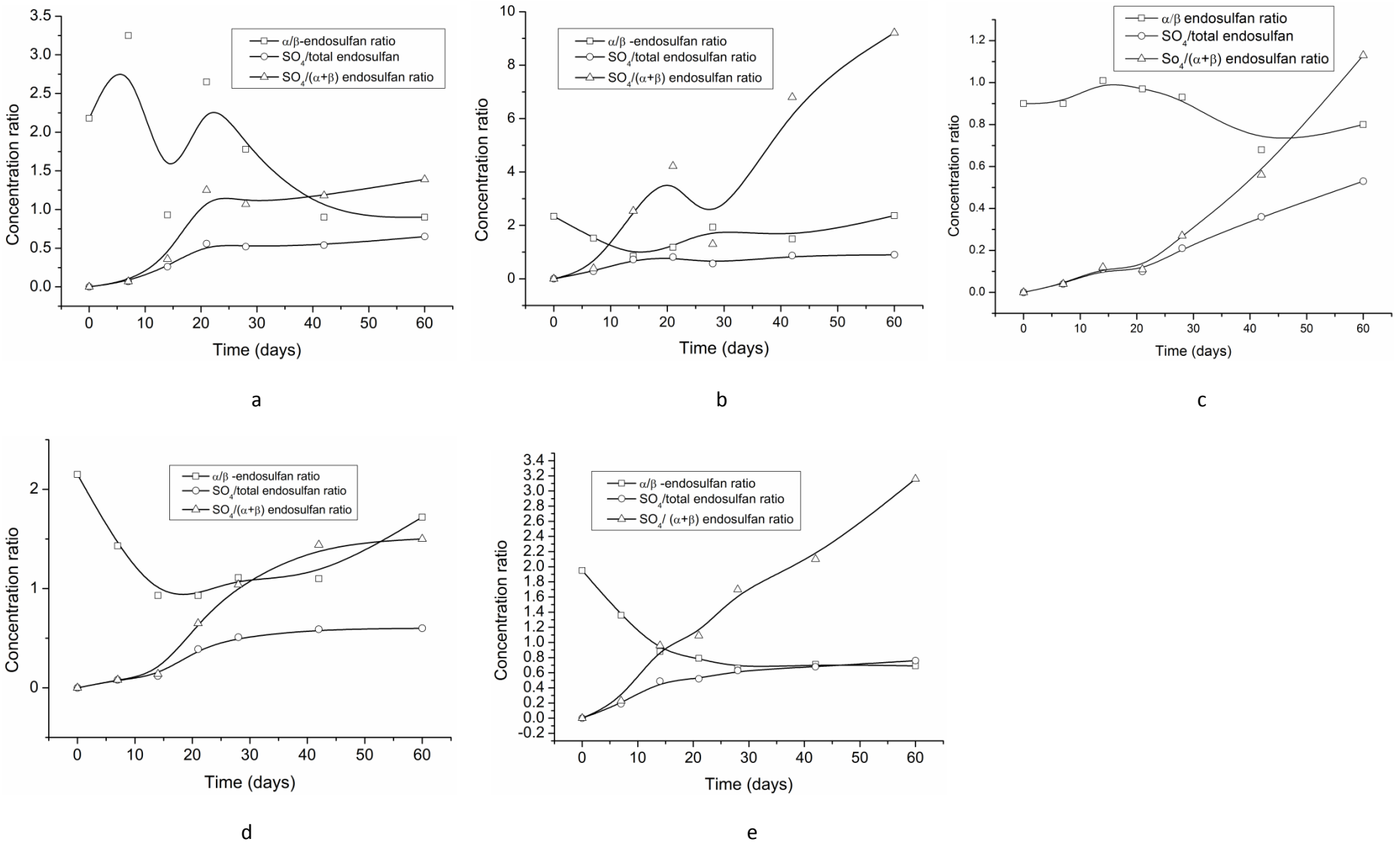

Figure 4: Ratios of α/β-endosulfan, endo SO_4/total endo and endo SO_4/ (α+β) endosulfan in a) fresh leaves, b) stem bark, c) cocoa pods, d) dry leaves and, e) soil 0-15cm

Table 1: Recovery study for α-endosulfan, β-endosulfan and endosulfan sulfate in *Theobroma cacao* plant and surrounding matrix

PESTICIDE	Amount of pure pesticide spiked (μgkg^{-})	*THEOBROMA CACAO* VEGETATION TYPE										SURROUNDING MATRIX			
		FRESH LEAVES		STEM BARK		PODS		SEEDS		DRY LEAVES		SOIL			
												0-15 cm		15-30 cm	
		Total quantity of pesticide found (μgkg^{-1})	Percent recovery of pesticide spiked (%)	Total quantity of pesticide found (μgkg^{-})	Percent recovery of pesticide spiked (%)	Total quantity of pesticide found (μgkg^{-})	Percent recovery of pesticide spiked (%)	Total quantity of pesticide found (μgkg^{-})	Percent recovery of pesticide spiked (%)	Total quantity of pesticide found (μgkg^{-})	Percent recovery of pesticide spiked (%)	Total quantity of pesticide found (μgkg^{-})	Percent recovery of pesticide spiked (%)	Total quantity of pesticide found (μgkg^{-})	Percent recovery of pesticide spiked (%)
α-endosulfan	250	244.2±9.9 %RSD=4.0	97.7±3.9	238.2±9.6 %RSD=4.0	95.3±3.9	234.3±2.9 %RSD=1.2	93.7±1.2	248.5±2.6 %RSD=1.0	99.4±1.0	249.9±5.2 %RSD=2.1	99.9±2.1	245.6±7.4 %RSD=3.0	98.2±3.0	252.6±9.9 %RSD=3.9	101.1±4.0
	500	502.9±9.2 %RSD=1.8	100.6±1.8	506.5±6.8 %RSD=1.4	101.3±1.4	458.1±9.6 %RSD=2.1	91.6±1.9	513.3±12.6 %RSD=2.5	102.7±2.5	502.9±6.6 %RSD=1.3	100.6±1.3	505.6±6.4 %RSD=1.3	101.1±1.3	515.9±8.7 %RSD=1.7	103.2±1.7
β-endosulfan	250	242.1±7.0 %RSD=2.9	96.8±2.8	242.0±10.6 %RSD=4.4	96.8±4.2	219.8±9.6 %RSD=4.4	87.9±3.8	238.8±2.0 %RSD=0.9	95.5±0.8	267.4±6.0 %RSD=2.3	106.9±2.4	239.9±3.5 %RSD=1.5	96.0±1.4	249.0±7.8 %RSD=3.1	99.6±3.1
	500	487.3±11.1 %RSD=2.3	97.46±2.2	488.1±10.5 %RSD=2.2	97.6±2.1	451.4±13.0 %RSD=2.9	90.3±2.6	483.9±6.2 %RSD=1.3	96.8±1.2	501.0±3.4 %RSD=0.7	100.2±0.7	500.3±18.9 %RSD=3.8	100.1±3.8	486.7±8.3 %RSD=1.7	97.3±1.7
Endosulfan SO_4	250	253.7±4.7 %RSD=1.9	101.5±1.9	242.2±10.4 %RSD=4.3	96.9±4.2	235.7±3.0 %RSD=1.3	94.3±1.2	254.7±4.5 %RSD=1.8	101.9±1.8	255.0±5.0 %RSD=2.0	102.0±2.0	248.0±12.1 %RSD=4.9	99.2±4.9	298.1±8.5 %RSD=2.3	119.2±3.4
	500	487.5±10.3 %RSD=2.1	97.5±2.1	508.5±16.6 %RSD=3.3	101.7±3.3	438.6±8.6 %RSD=2.0	87.9±1.7	492.5±11.4 %RSD=2.3	98.5±2.3	491.1±7.2 %RSD=1.5	98.2±1.4	482.5±15.3 %RSD=3.2	96.5±3.1	506.8±6.8 %RSD=1.3	104.1.±1.4

Note: Each spiked sample types and levels were replicated thrice

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

Plant Part	∑endosulfan			α-endosulfan			β-endosulfan			Endosulfan sulfate		
	chemo-kinetic variables calculated iteratively from $d_0 \rightarrow d_{60}$											
	k' (d^{-1})	DT'_{50} (d)	R^2	k' (d^{-1})	DT'_{50} (d)	R^2	k' (d^{-1})	DT'_{50} (d)	R^2	k' (d^{-1})	DT'_{50} (d)	R^2
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)	-	-	-	-	-	-	-	-	-	-	-	-
	chemo-kinetic variables calculated from d_0 & d_{60}											
	k (d^{-1})	DT_{50} (d)	R^2	k (d^{-1})	DT_{50} (d)	R^2	k (d^{-1})	DT_{50} (d)	R^2	k (d^{-1})	DT_{50} (d)	R^2
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