

Uptake and Translocation of Fenitrothion and Thiobencarb in Rice Plant under Laboratory and Filed Conditions

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Abstract: This study investigate the uptake and translocation of fenitrothion insecticide and thiobencarb herbicide in rice plants. This study is carried out under laboratory conditions, two concentrations (1 and 10 µg/g) and lysimeter conditions at a concentration of 25 µg/g. Significant differences in pesticide uptake by rice were observed between the two laboratory treatments (1 and 10 µg/g) across all time intervals, as well as in the cumulative amounts. After reaching maximum concentrations, a decrease in uptake was noted, likely due to biodegradation and plant growth dilution. The average bio-concentration factor (BCF) of thiobencarb was higher than that of fenitrothion, indicating a greater ability of rice plants to absorb and accumulate thiobencarb. The BCFs for both fenitrothion and thiobencarb were higher at lower concentrations (4.85 and 5.67) compared to higher concentrations (0.55 and 0.89), suggesting that the uptake capacity was saturated at low concentrations, limiting the rice plants' ability to accumulate the pesticides at varying initial concentrations under laboratory conditions. The recovery rates of fenitrothion and thiobencarb from soil and rice plant samples, as determined by HPLC, exceeded 80%. After 10-week period, fenitrothion and thiobencarb were not detectable in soil or rice under field conditions. Additionally, two metabolites of fenitrothion were tentatively detected in rice plants, while two metabolites of thiobencarb were detected in soil, with concentrations of 1.5 and 1.0 µg/g for metabolites I and II, respectively. One thiobencarb metabolite (metabolite I) was detected in rice plants at a concentration of 5 µg/g.

Keywords: Pesticides, Rice plant, Translocation, Uptake

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Introduction

Pesticide uptake, translocation and persistence in plants can be a hazard not only to human health but also to ecosystems, thus there is considerable research interest in the prediction of these pesticide residues [1-3]. Uptake and translocation of pesticides in plants and degradation in soil are considered relatively important bioprocesses due to the possible production of toxic substances that can be hazardous to humans and the environment [4-7]. Therefore, in the recent there is a con-

siderable research interest to monitor and understand these bioprocesses [4,8]. Plants could have a high uptake capacity of many contaminants of emerging concern (CEC) in soil such as polycyclic aromatic hydrocarbons (PAH), pesticides, pharmaceutical, perfluorinated compounds, and engineered nano-materials. CEC bioaccumulation in the root is higher than in aerial parts [9]. Furthermore, various factors (species of plant, type of pollutant and microbial interactions) influence the overall uptake process. Also, environmental factors (temperature and soil erosion) can affect the CEC plant bioavailability [9].

The organic compounds transfer into plants occurred via two major pathways; (1) desorption from the soil followed by root uptake and (2) transfer from air via dry and wet particles deposition on surface of plant, followed by desorption into the inner plant parts [1,10-12]. Food crops in general are susceptible to contamination by different organic pesticides and wastes through root or foliar uptake [2,13-14]. Plants can be exposed to pollutants in different ways. Organic pollutants may enter plant roots by passive and/or active processes, then move in transpiration stream to other plant components [15,16]. Passive transport proceeds in the direction of decreasing compound potential and consists of a series of partitions between water of plant and organic matter of plant within different plant components [15,17]. Most nonionic pollutants enter plants largely through passive process [18]. Active transport, may proceed against the electrochemical potential gradient, occurs for various nutrients and inorganic/organic ions. The uptake magnitude and efficiency depend on source concentration and properties of contaminants, plant species and composition, exposure time to contaminants [14,17,19,20]. Lipid content of plants may have a significant influence on organic chemical storage [16]. The roots uptake of nonionic organic compounds is mechanistically similar to the chemical partition into non-living organic matter [15]. It was reported that sparingly soluble organic chemicals were poorly translocated within below-ground plant parts [17,21,22].

Systemic pesticides are defined as the pesticides able to enter plants and able to transport in the vascular system. Systemic distribution in plants can be achieved through uptake by roots or following foliar pesticide application. Studies of root-to-shoot translocation through xylem is poor because of the technical difficulties associated with plant root experiments [23,24]. Root uptake plays a significant role for both soil- and foliar-applied pesticides. Some pesticides that are usually foliar-applied can become systemic when root-applied [25]. Systemic compounds can be absorbed from soil by roots via the liquid or the vapor phase, the quantity taken up by each route depending essentially on the physico-chemical properties of the pesticide, as well-explained by Henry's law [26]. Usually, roots take up water and pesticides rapidly in the region (10~100 mm) behind the root tips [24]. One of the major factors in compound transport is thus the rate of passage of the compounds across the barrier membranes. Less-lipophilic compounds take the apoplastic pathway before reaching the endodermis, while more-lipophilic compounds tend to cross membranes and be partitioned into lipophilic tissue through the pathway [27]. Pesticide uptake into plant foliage varies with plant and pesticide types. Pesticide penetration into plant leaves is related to the physico-chemical properties of the compound, especially lipophilicity and molecular size. For a specific compound, uptake varies greatly with species of plant and there is no simple technique to quickly determine the leaf surface permeability of a plant. A good understanding of the foliar uptake process should help to rationally use pesticides and minimize their environmental negative impact [28].

Rice (*Oryza sativa* L.) is the most widely consumed staple food for about half of the world's population thus it is considered as the most important agricultural commodity in the world [29-31]. The massive use of pesticides in paddy fields to enhance rice production could cause risks to beneficial insects and deterioration of the natural environment as well as human health [32,33]. For proper use of pesticides in rice production, intensive studies are necessary not only to determine their efficiency as pesticides, but also to clarify their behavior in rice plants as well as in the environment where they might accumulate [34]. Thus, the uptake and translocation of pesticides by rice have been studied worldwide [17,35-38]. Uptake and translocation of three pesticides thiamethoxam, imidacloprid and difenoconazole in rice plants were studied at field rate (1FR) and (10FR) under laboratory conditions. The thiamethoxam and imidacloprid concentrations detected in rice leaves were much higher than those in roots. Concentrations of difenoconazole in roots were much higher than those in leaves. The bioconcentration factor (BCF), representing the capability of rice plants to accumulate contaminants from soil into plant tissues, ranged (1.9 to 224.3) for imidacloprid, (2.0 to 72.3) for thiamethoxam, and (0.4 to 3.2) for difenoconazole at different concentrations. Much higher BCFs were

found for thiamethoxam and imidacloprid at 10FR-treatment than those at 1FR-treatment. The translocation factors, evaluating the capability of rice plants to translocate contaminants from the rice roots to the above ground parts, ranged (0.02 to 0.2) for stems and (0.02 to 9.0) for leaves. The tested pesticides were poorly translocated from rice roots to stems, with a translocation factor (< 1). However, thiamethoxam and imidacloprid were well translocated from rice roots to leaves [35].

Materials and Methods

Tested pesticides

Fenitrothion (O, O-dimethyl O-4-nitro-m-tolyl phosphorothioate) is usage for controlling chewing and sucking insects on rice, orchard fruits, vegetables, cereals, cotton, and forest [39]. Thiobencarb (S-4-chlorobenzyl diethyl thiocarbamate) is a pre-emergence and early post-emergence herbicide for weed control in rice paddy fields [40,41].

Tested plant

Rice plant (*Oryza sativa*, variety Giza 101) was cultivated in the field lysimeter (built above the ground with bricks, 1.1 m length $\times$ 1.1 m width $\times$ 2.5 m depth. A coarse aggregate layer of 50 cm thickness was placed, then the lysimeter was filled with calcareous soil and a network of 4 cm diameter PVC pipes were connected with it to drain the leachates water at different depths every 30 cm) was cultivated a rate of 500 g seeds per lysimeter to study the plant uptake under field conditions and also cultivated in the field, then the seedlings (30 days age) were transplanted in containers (10 seedlings/replicate) to study the plant uptake under laboratory conditions.

Instrumentation

The used HPLC 1260 Infinity Series (Agilent Technologies) was equipped with pump (G1311C - 1260 Quat VL), detector (DAD; Diode Array Detector), and column (Zorbax Eclipse Plus C18, 3.5 μ m, 4.6 $\times$ 100 mm). The Thermo UV-Vis Spectrophotometer was used (Nicolet Evolution 100, Germany). The used sonicator was Model LBS 2-4 (5 L). Rotary evaporator (Bibby Scientific Limited, Stone, Staffordshire, ST15 0SA, UK) and orbital shaker (Bibby Sterilin Ltd., UK) were used. Centrifuge (Model 90-1, UK) and a digital balance (ADAM, PW-214, 0.0001-200 g, UK) were used.

Determination of Pesticide and recovery by HPLC

HPLC standard solution

Working standard solutions of fenitrothion and thiobencarb (100 μ g/g) were freshly prepared by appropriately diluting multiple stock solutions with acetonitrile-HPLC. The standard solution was determined immediately before the samples.

Determination by HPLC

Fenitrothion and thiobencarb residues in soil and rice plant samples of uptake experiments were quantitatively analyzed after extraction and clean-up using an Agilent 1260 HPLC Infinity system equipped with diode array detector (DAD). The samples were injected into HPLC with constant injected volumes of 20 μ l. The mobile phase was acetonitrile water (80:20). Residue amounts were calculated by comparing peak area of sample to peak area of standard solution.

Recovery assay by HPLC

Untreated soil and plant samples were homogenized and spiked with standard solutions of fenitrothion and thiobencarb

(10 µg/g). The samples were processed according to the treated samples. Results of the pesticides of uptake experiments were corrected according to the recovery rate. Blank analyses were performed to check interference from the matrix [42,43].

Rice plant uptake of tested pesticides in laboratory

Rice seedlings, at the age 30 days, 10 cm height were collected from the Abis farm of the Faculty of Agriculture, Alexandria University, Egypt [44,45], and acclimatization took place in the laboratory for a week. Two concentrations of 1 and 10 µg/g for fenitrothion and thiobencarb were prepared in a final volume of 2 L and placed in plastic containers (4 L). Two replicates were used for each concentration in addition to the control. Ten rice seedlings (1 g/seedling) were cultivated in each pesticide treatment. The concentration of pesticide residues in the aqueous media were determined at different time intervals (0, 4, 8, 12, 16, 20, 24 and 28 days) by UV-Vis Spectrophotometer. Daily transpiration and evaporation rates were calculated using a separate untreated experiment for 28 days. Independent experiments were performed to assess the breakdown of the tested pesticides in water. According to the pesticide concentration in the water with the time, the reduction of water due to evaporation and transpiration, and the degradation rate of pesticide in water, the quantity of the pesticide absorbed by plants was calculated [9,35,46].

Rice plant uptake pesticides in field

The field lysimeter was used to study the uptake of fenitrothion and thiobencarb by rice plants under field conditions by application rate 25 µg/g. Soil and rice plant samples were taken at 10th week of rice plant cultivation which, is the same time of pesticide application. Soil samples (250 g) were dried at room temperature. Ten grams of soil samples were taken, then 5 g of anhydrous sodium sulfate was added and grind for 5 minutes in a tray. Soil was extracted by sonication with 30 ml of dichloromethane-HPLC for 15 min. The liquid phase was separated from the soil by passing through 1.2-µm glass fiber filters. Soil residue was washed again twice with 20 ml dichloromethane-HPLC for 15 min by sonication. The samples were evaporated using rotary evaporator to dryness. The pesticide residue was dissolved in 1 ml acetonitrile-HPLC. The soil extracts were analyzed by HPLC [47,48]. Rice plant samples were washed with distilled water and air dried. It was taken 10 g of rice plants, cut and milled with 10 g of sodium sulfate anhydrous. The samples were extracted by sonication with 50 ml dichloromethane-HPLC and methanol-HPLC (1:1) for 15 min. The samples were filtrated through 1.2-µm glass fiber filters. The plant samples were extracted with another 50 ml dichloromethane-HPLC and methanol-HPLC (1:1) for 15 min by sonication. The filtrate was collected and passed through filter paper containing 1 g charcoal and then passed onto a silica gel column 30 cm in length and 1 cm diameter. The column was moistened with 30 mL acetonitrile. The samples were applied on the column and eluted by 50 mL dichloromethane-HPLC and methanol-HPLC (1:1). The solvents were evaporated, then the residues were redissolved in 1 ml acetonitrile-HPLC and determined by HPLC [49-51].

Results and Discussion

Rice plant uptake of tested pesticides in laboratory experiment

The availability of pesticides in plant micro-environment is of major importance in indicating plant uptake potential to the pesticides. Therefore, the uptake of rice plants was monitored throughout about 1 month under laboratory conditions. The rice seedlings were transplanted at age 30 days and grown in water medium without soil to avoid interacting of the adsorption process. Degradation of tested pesticides in water was determined during the experiment interval and it was considered in calculations. Also, daily transpiration and evaporation rates were calculated using a separate untreated experiment for 28 days and the equivalent water amount was added to the plant pots. According to the pesticide concentration in the water with the

time, the reduction of water due to evaporation and transpiration, and the degradation rate of pesticide in water, the quantity of fenitrothion and thiobencarb absorbed by rice plants (*Oryza sativa* L.) was investigated with a water-treated experiment at two application rates: 1 and 10 µg/g, equivalent to 1 fold (1F) and 10 fold (10F) recommended rates under laboratory conditions [9,35,46].

Table 1 and Fig. 1 show the uptake dynamic of fenitrothion by rice plants during the experimental period. None of the target pesticides were detected in the control. The amount of fenitrothion absorbed by plants increases with time. In the case of 1 µg/g-treatment, the amount of the pesticide increased to reach a maximum (5.18 µg/g plant) after 16 days then it reached a plateau until the end of experiment. About the 10 µg/g-treatment, the amount of fenitrothion absorbed increased to reach a maximum within the 8th to 16th day (about 6.4 µg/g plant), then decreased to a plateau corresponding to 5.1 µg/g plant (Fig. 1A). Significant differences were recorded between the two treatments for fenitrothion at all time intervals, however, the difference between the two treatments was low. Significant differences were found between cumulative amount of fenitrothion into plant tissues for the two treatments 1 and 10 µg/g (Table 1). The pesticides were detected in the rice plants grown in pesticides treated water soils [35]. The cumulative amount of fenitrothion was slightly higher in 10 µg/g-treatment compared to the low concentration-treatment. It was observed that the cumulative amount in the two treatments was increased with constant rate to reach a maximum amount absorbed 34.0 and 38.8 µg/g plant for 1 µg/g-treatment and 10 µg/g-treatment, respectively (Fig. 1B).

The uptake and accumulative amount of thiobencarb in rice plants were shown in Table 2 and Fig. 2. In general, a rapid rise was detected for thiobencarb in the first week and same time took to reach the maximum of the peaks in the two treatments (Fig. 2A). The uptake of thiobencarb reached a maximum within the 8th to 12th day period corresponding to about 8.7 µg/g plant in 1F-treatment and 13.5 µg/g plant in 10F-treatment. Moreover, a decrease in the uptake in the two treatments was observed after the concentrations reached the maximum, on the 24th day the uptake reached to 2.6 and 5.6 µg/g plant for low concentration and high concentration treatments, respectively. It was reported that a decrease of the pesticides was detected after the concentrations reached the highest point, which could probably be due to plant growth dilution and the biodegradation [52]. The accumulative amount of thiobencarb in low concentration-treatment in rice plants was two thirds that in high concentration-treatment, 39.7 and 62.5 µg/g plant (Fig. 2B).

On the other hand, the bioconcentration factor (BCF) was determined as the ratio of the chemical concentration in the rice plants to that in the water medium, $BCF = C_{\text{plant}} / C_{\text{soil}}$ [46,53]. In this study, BCF was calculated using $C_{\text{water medium}}$ instead C_{soil} . BCFs of the two pesticides are listed in Tables 1 and 2. Value of $BCF > 1$ indicates accumulation of the pesticides from medium to plants. The average BCFs of thiobencarb was greater than those of fenitrothion at both treatments, suggesting a higher capability of plants to accumulate thiobencarb than fenitrothion from medium. For fenitrothion and thiobencarb, the BCFs were 0.55 and 0.89 at 10F-treatment, whereas the BCFs were 4.85 and 5.67 at 1F-treatment, which could possibly be due

Table 1. Uptake and bioconcentration factor (±SE) of fenitrothion by rice plants

Time (day)	Uptake±SE (µg/g plant)		Accumulative±SE (µg/g plant)		BCF ±SE	
	1 µg/g	10 µg/g	1 µg/g	10 µg/g	1 µg/g	10 µg/g
4	4.13 ±0.01	4.89 ±0.06	4.13 ±0.01	4.89 ±0.06	4.13 ±0.01	0.49 ±0.01
8	5.05 ±0.44	6.43 ±0.78	9.17 ±0.45	11.32 ±0.71	5.05 ±0.63	0.64 ±0.11
12	5.12 ±0.41	6.34 ±0.28	14.29 ±0.04	17.66 ±1.00	5.12 ±0.58	0.63 ±0.04
16	5.18 ±0.62	5.64 ±0.09	19.47 ±0.58	23.30 ±0.91	5.18 ±0.88	0.56 ±0.01
20	4.95 ±0.09	5.35 ±0.11	24.41 ±0.49	28.65 ±0.81	4.95 ±0.13	0.54 ±0.02
24	4.78 ±0.12	4.98 ±0.08	29.19 ±0.36	33.63 ±0.88	4.78 ±0.17	0.50 ±0.01
28	4.77 ±0.55	5.15 ±0.07	33.97 ±0.19	38.78 ±0.82	4.77 ±0.78	0.52 ±0.01
BCF average					4.85	0.55

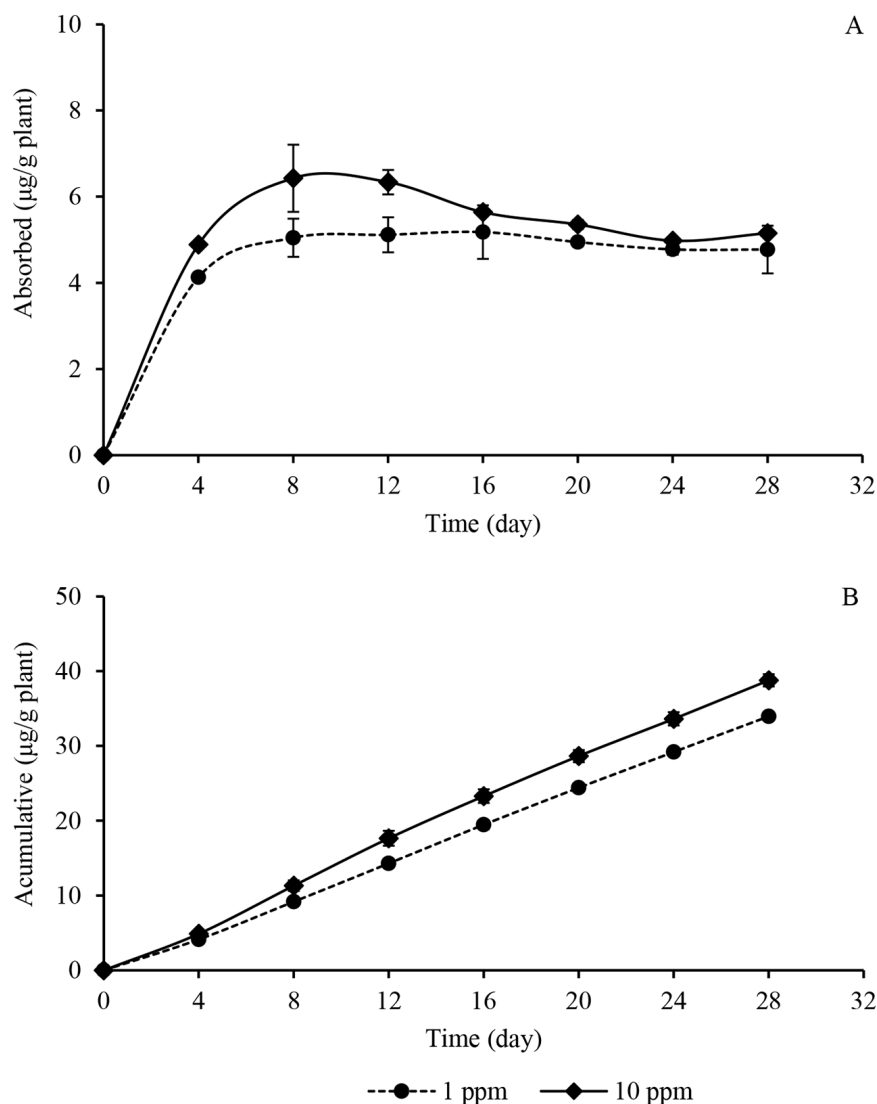
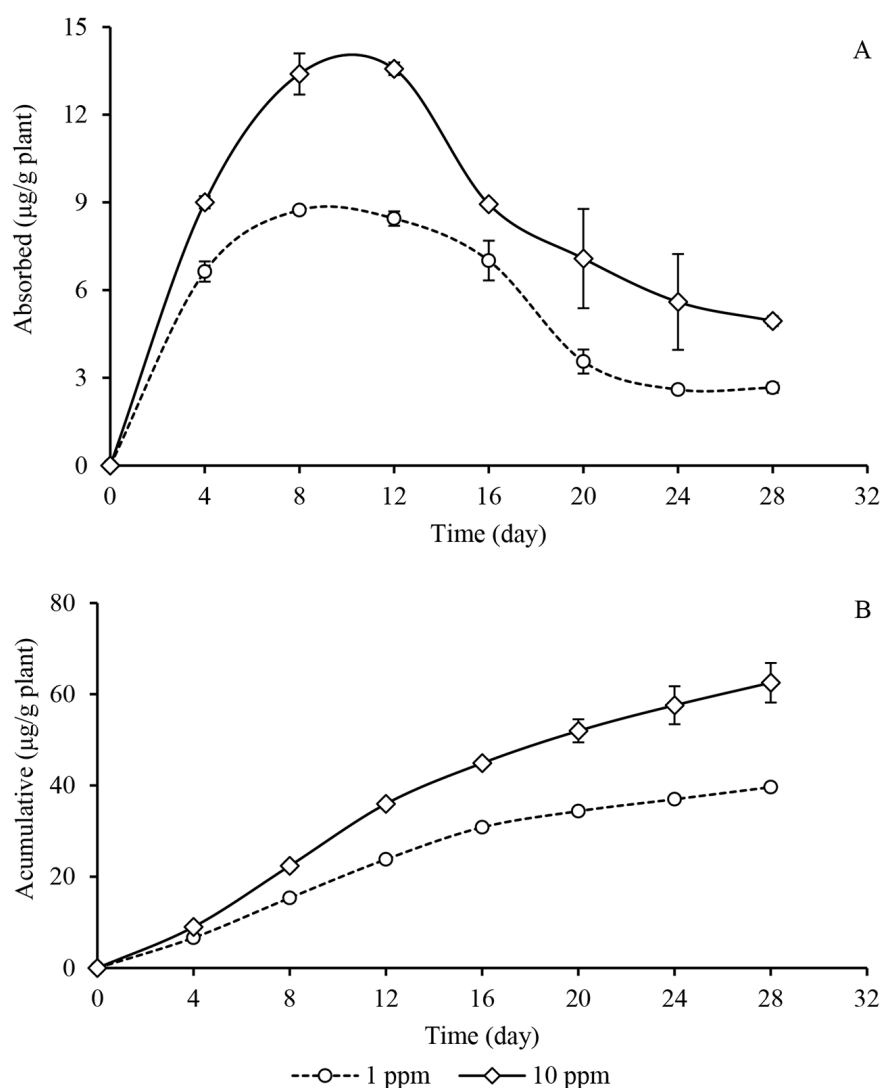


Fig. 1. Uptake (A) and accumulative (B) ($\pm$ SE) of fenitrothion by rice plants.

to the major uptake capacity saturated by the low concentration. The studies of uptake of pesticides from soil indicated that the BCFs were greater in low concentration, this observation differs from the results obtained by Broznic et al. [54], which reported less bioavailable pesticides at a lower concentration [54]. Moreover, the adsorption of pesticides on soil could also influence uptake and accumulation in plants [55-57]. Greater difference in BCFs while slight difference in amount absorbed were found between the two treatments of fenitrothion and thiobencarb, indicating limited capability for rice plants to accumulate fenitrothion and thiobencarb from medium to plants at different initial concentrations. It was indicated that rice plants were capable of absorption of imidacloprid, thiamethoxam and difenoconazole from soil. The BCFs of imidacloprid and thiamethoxam in plants were much higher than those of difenoconazole [35]. The results indicate that on the 28th day the cumulative amount of thiobencarb (39.7 and 62.5 $\mu\text{g/g}$ plant) was higher than that of fenitrothion (34.0 and 38.8 $\mu\text{g/g}$ plant) in low-treatment and high-treatment, respectively. Thiobencarb is easier to be taken up and accumulated in rice plants than fenitrothion, which could be attributed to the physicochemical properties. However, the values of partition coefficient octanol/water ($\log P$ or K_{ow}) are 3.42 and 3.32, both compounds have not coefficient of dissociation (pK_a), the water solubility 30 and 38 mg/L and the molecular weight 258 and 277 for thiobencarb and fenitrothion, respectively. It is relatively hard for certain pesticides to penetrate through bio-membranes and be absorption by the rice plants due to their high $\log K_{ow}$ and low pK_a [58].

Table 2. Uptake and bioconcentration factor ($\pm$ SE) of thiobencarb by rice plants

Time (day)	Uptake $\pm$ SE (μ g/g plant)		Accumulative $\pm$ SE (μ g/g plant)		BCF $\pm$ SE	
	1 μ g/g	10 μ g/g	1 μ g/g	10 μ g/g	1 μ g/g	10 μ g/g
4	6.64 $\pm$ 0.35	9.01 $\pm$ 0.21	6.64 $\pm$ 0.35	9.01 $\pm$ 0.21	6.64 $\pm$ 0.49	0.90 $\pm$ 0.03
8	8.74 $\pm$ 0.07	13.39 $\pm$ 0.70	15.37 $\pm$ 0.28	22.40 $\pm$ 0.91	8.74 $\pm$ 0.10	1.34 $\pm$ 0.10
12	8.45 $\pm$ 0.25	13.57 $\pm$ 0.22	23.82 $\pm$ 0.52	35.97 $\pm$ 0.70	8.45 $\pm$ 0.35	1.36 $\pm$ 0.03
16	7.01 $\pm$ 0.68	8.94 $\pm$ 0.13	30.84 $\pm$ 0.16	44.92 $\pm$ 0.83	7.01 $\pm$ 0.96	0.89 $\pm$ 0.02
20	3.56 $\pm$ 0.41	7.08 $\pm$ 1.70	34.39 $\pm$ 0.25	52.00 $\pm$ 2.53	3.56 $\pm$ 0.58	0.71 $\pm$ 0.24
24	2.60 $\pm$ 0.06	5.60 $\pm$ 1.64	37.00 $\pm$ 0.19	57.59 $\pm$ 4.16	2.60 $\pm$ 0.09	0.56 $\pm$ 0.23
28	2.66 $\pm$ 0.17	4.94 $\pm$ 0.17	39.66 $\pm$ 0.03	62.54 $\pm$ 4.33	2.66 $\pm$ 0.24	0.49 $\pm$ 0.02
BCF average					5.67	0.89

**Fig. 2. Uptake (A) and accumulative (B) ($\pm$ SE) of thiobencarb by rice plants.**

Rice plant uptake of tested pesticides in field

Residue peaks were tentatively identified based on retention times. Residue amounts were calculated by comparing peak

area of sample to standard peak of fenitrothion and thiobencarb [59]. The result of recovery percentage of fenitrothion and thiobencarb from soil and rice plant samples pretreated with pesticide concentrations indicated that the recovery % > 80%. In general, the recovery percentages were used to correct the results of tested pesticides. These results confirmed recovery data using HPLC obtained by Roy et al. [43], whereby recovery of fenitrothion was 98% alluvial soil [43], and Anyusheva et al. [60], who found recovery of fenitrothion 92±8% in paddy soil [60].

The results indicated that the concentration of fenitrothion residue in lysimeter soil at 10 weeks period was non-detectable (Table 3). Moreover, no metabolites were detected in soil samples. Fenitrothion is not persistent because of its rapid degradation by chemical, physical or biological means [61]. Also, the concentration of fenitrothion was determined in rice samples grown in an experimental field lysimeter and harvested over 10 weeks period following treatment with this insecticide. At the end of the experiment, the residue of the parent compound was undetectable while, two fenitrothion metabolites were detected with the retention times of 0.768 and 0.990 min., having concentrations of 0.96 and 3.72 µg/g plant, respectively. Moreover, BCF of both metabolites was 0.19 (Table 3). This result in general is in agreement with that of Fenoll et al. [62] and Roy et al. [43], they found that after 4 weeks following treatment with fenitrothion, the evolution of the two main fenitrothion metabolites (3-methyl-4-nitrophenol and fenitrothion-oxon) was detected [43,62]. The retention times were (18.07 min) for fenitrothion, (9.92 min) for 3-methyl-4-nitrophenol, and (16.19 min) for fenitrothion-oxon. The presence of these metabolites should be taken into account due to their reported toxicological properties [63-65]. It was reported that the oxidative pesticide de-sulphuration at the first step followed by hydrolytic cleavage of P-O-aryl linkage at the second step and demethylation at the third step [43].

Table 3. Data of HPLC chromatograms and residues of fenitrothion in rice plant and soil

Rt (min)	Detected residue	Soil	Rice plant	
		Residue (µg/g)	Residue (µg/g)	BCF
0.768	Metabolite I	-	0.96	0.04
0.99	Metabolite II	-	3.72	0.15
3.15	Parent compound	ND	ND	-

Rt: Retention time, ND: Non-detectable, -: No result

Table 4. Data of HPLC chromatograms and residues of thiobencarb in rice and soil

Rt (min)	Detected residue	Soil	Rice plant	
		Residue (µg/g)	Residue (µg/g)	BCF
0.708	Metabolite I	1.5	5.0	0.2
1.312	Metabolite II	1.0	-	-
5.325	Parent compound	ND	ND	-

Rt: Retention time, ND: Non-detectable, -: No result

The concentration of thiobencarb in soil and rice plant after 10 weeks period following treatment was non-detectable with concomitant formation of two metabolites in soil sample and one metabolite in the plant sample. Six and 4% of applied thiobencarb was detected in soil as metabolites I and II, respectively. Also, metabolite I of thiobencarb was detected in rice plants in concentration of 5 µg/g plant and its BCF was 0.2 (Table 4). Thiobencarb is rapidly metabolized after absorption by plants. It is metabolized during translocation in plants [66]. The potential degradation products were 4-chlorobenzylmethyl sulfone and thiobencarb sulfoxide [67,68]. Though 4-chlorobenzaldehyde was not identified in previous metabolic studies in rice [69], it has been identified in soil degradation studies [70,71].

Data Availability: All data are available in the main text or in the Supplementary Information.

Author Contributions: M.R.F. led the uptake and translocation experiments, performed the statistical analysis, and wrote the first manuscript; A.F.E. collected the data, conceived and designed the research; M.I.A. provide critical feedback, revised the manuscript.

Notes: The authors declare no conflict of interest.

Additional Information:

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