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Dissipation Kinetics and Distribution of Carbosulfan and its Toxic Metabolites in Banana, cv. Nendran (AAB)

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A B S T R A C T

The pattern of dissipation and persistence of carbosulfan and its metabolites in banana, cv. Nendran (AAB), with treatments as absolute control (No carbosulfan); recommended dose of per plant application of 400 mg a.i. of carbosulfan, applied in the soil on 0, 60 and 150 days of planting was conducted in red loam soils.The matrix matched samples of different plant parts (leaves, fingers bunches and flower bud, central core of pseudostem and corm) were spiked, and satisfactorily validated for residues extraction QuEChERS method. The plants parts analyzed for residue at predetermined time intervals had residue of carbosulfan in the 1st to 4th leaves till day 20th and subsequent dissipation pattern prediction for BDL in 23.7 day indicated that, at recommended dose of application it is not safe to use the leaves within 23 days of application for serving or food packing (as commonly practiced in every households of Kerala).The residue of carbosulfan and their metabolites were in below detectable limit in blossom bud, flower bract alone, bunch on 15th day of emergence, bunch on 30th day of emergence, peel, bunch (fruit) on harvest, pseudo stem and corm, in recommended dose other additional doses. Metabolites of Carbosulfan residue existed in the 1st, 2nd and 3rdleaves between 5th and 20th day and it dissipated to below detectable limit on 25th day of application.

Introduction

Banana is one of the major fruit crops of India. It is consumed as fresh fruit and after cooking. Inner core of the pseudostem, blossom bud and even the rhizome are edible. Carbosulfan is among the important pesticide used by farmers, especially for the control of aphids

and weevil pests. Carbosulfan, (2,3-dihydro-2,2-dimethyl benzo- furan-7-yl [(dibutylaminothio) sulfanyl] N-methyl carbamate, a broad-spectrum systemic insecticide is recommended as a substitute for banned insecticides viz., phorate and carbofuran for the control of banana aphid, (the vector of bunchy top disease of banana) (KAU, 2011). The literature pertaining to the dissipation and movement of these chemicals in banana are scanty especially in Kerala. The study on the absorption, distribution, partitioning, and degradation pattern of carbosulfan in Nendran variety of banana in red loam acidic soils of Kerala, in the absence of data in acidic red loam soils also address safety concerns with respect to public health.

The metabolism of the insecticide carbosulfan to its carbofuran metabolite in oranges is rapid with dissipation of both pesticides in 3 days (Trevisan *et al.,* 2004). Their residues concentrate in the bagasse (peel + flavedo + albedo) are not penetrating into fruit interior, thus are not contaminating the juice. There is lack of adequate data to fix the MRL of carbosulfan in banana. Vijayan (2000) studied the pattern of the absorption, and degradation of carbofuran in nendran variety of banana. A similar work on banana has not been taken out following introduction of 2 granular insecticides replacing the banned insecticide (KAU, 2011; 2015). Persistence and dissipation of granular pre-mix broad spectrum systemic fungicides trifloxystrobin and tebuconazole on banana and soil was studied by Beevi *et al., (*2013).

Carbosulfan when applied under invitro conditions in pakchoi (*Brassica campestris*) was transformed to higher toxic metabolites including carbofuran (CAN), 3-hydroxycarbofuran (3-OH) and 3-ketocarbofuran (3-KETO) where as in cucumber (*Cucumis sativus* L.), were metabolised only to CAN and 3-OH (Chai *et al.,* 2015). In their study the degradation the time marking the disappearance of 50% of the pesticide less within 2.5 days and they suggested for the monitoring of metabolites as prior objective for carbosulfan, and different metabolites while assessing the risk of carbosulfan. None of the random samples analysed for presence pesticide residues in soils of banana growing tracts of different districts of Kerala, tested affirmative for carbosulfan (Paul *et al.,* 2015).

Carbosulfan residue under laboratory conditions in black, red and alluvial soils following application @ 5 and 10 mg kg^{-1} progressively declined to below detectable level (<0.01 mg Kg^{-1}) within 75 days in red and alluvial soil and 90 days in black soil. However, more than 95% of carbosulfan degraded within 60 days after incubation irrespective of the soil type and concentration (Rajeswaran *et al.,* 2005).

Materials and Methods

Result of the soil analysis of a randomised block design (RBD) field experiment for investigating dissipation and distribution of carbosulfan and their metabolites in banana var. Nendran (AAB) in the red soils, kaolinitic isohyperthermic, typic kandiustults (GOK, 2007)at Instructional Farm of College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India showed a sandy loam textured with silt of 8.7and clay 19.5 percent respectively. Plot was moderately acidic with a pH of 5.7, electrical conductivity of 0.4 dSm^{-1}, having medium organic carbon content of 1.5 percent, with high available P and K (196.1, 358.4 kg ha^{-1} respectively).

Essential micronutrients levels in the soils were in sufficiency range for Fe, Zn, Mn, and Cu extracted using dilute HCl). However, the soil was deficient in secondary nutrients viz., Ca, Mg and micronutrient B for which the

crop were cultivated and managed as per pop-KAU, 2011 but for the study treatments as T_1- Absolute control (No application carbosulfan), T_2- Recommended practices (RP_c) of 400 mg a.i. of carbosulfan per plant, applied thrice viz., on 0, 60 and 150 days of planting and T_3– Double dose of RP_c (i.e., RP_c x 2), applied as per above schedule of T_2. For this purpose, Agricultural insecticide grade commercial carbosulfan granules (i.e., Sheriff 6% G w/w manufactured and marketed by FMC India limited) was procured and used.

Chemicals and reagents

The analytical standards used were certified reference standards procured from Sigma Aldrich, Switzerland for Carbofuran (99.9 % w/w), Carbofuran-3-Keto (99.5 % w/w), Carbofuran-3-hydroxy (98.0 % w/w) and Carbosulfan (98.5 % w/w). HPLC grade solvents viz., acetone, ammonia solution, dichloromethane (HPLC grade); LC-MSgrade methanol and acetonitrile; AR grade magnesium sulphate, primary secondary amine, sodium chloride, sodium sulphate (anhydrous), calcium chloride anhydrous were used for residue analysis. Sodium sulphate, sodium chloride and magnesium sulphate were activated prior to use.

The celite and florsil-used were of chromatography grade, either of HPLC / LC-MSor AR grades. Calibrated equipment and instruments were used to optimise the meet performance criteria. Commercially available granular form of carbosulfan (Sheriff 6%G) formulation was used for soil application in the experimental plot.

Instrumentation

The Triple Quadrupole Mass Spectrometer (API 3200, AB Sciex, USA) attached to Ultra Performance Liquid Chromatograph (ACQUITY, Waters, USA) was used to analyse the clean extracts. The samples as well as standards were injected into the equipment for spectral matching and quantification of residues.

LC-MS/MS System

The chromatographic separation with Atlantis dC18 column (Waters, Ireland) with dimension 2.1 x 100 mm, 5 micron particle size, maintained at 40°C in the ACQUITY UPLC system of LC MS/MS. Elution was done using two eluents (solvent mixtures), *viz.*, from reservoirs Aand B as described below

10 per cent methanol in water + 0.1 per cent formic acid + 5 mM ammonium acetate

10 per cent water in methanol + 0.1 per cent formic acid + 5 mM ammonium acetate

The optimized gradient elution for flow rate of the mobile phase solvent system with an initial flow rate of 0.80 mL/ min was obtained with 80 percent flow from reservoir A and 20 percentage from B. The gradient elution of the compound mixture was monitored for 12 minutes.

The differential flow rates maintained for reservoir A being 80, 10, 5, 0, 80 and 80 percent at 4, 5, 9,10 and 12 minutes after injection with matching complementary flow rates from reservoir B.

The effluent from LC then enters into triple quadrupole API 3200 MS/MS system. System contains ion source gas 1 (at 50 psi), ion source gas 2 (at 40 psi) and curtain gas (at 30 psi) with ion source temperature of 550°C and ion spray voltage source of 5500 V. The residues were quantified in MS/MS system. For each analyte, two selective reaction monitoring (SRM) transitions were taken in positive mode.

Pesticide residues in the sample was calculated in (mg kg^{-1})

$$\frac{\text{Peak area of sample} \times \text{Concentration of standard injected} \times \text{Dilution factor}}{\text{Peak area of standard}}$$

Laboratory experiments to determine the accuracy, relative standard deviation (RSD value), linearity and limit of quantitation (LOQ) of the methods followed for estimation viz., QuEChERS (Anastassiades, 2007) were carried out to ascertain the method to be followed for extraction of residues from the field samples.

The finely chopped samples pieces of 250 g per replicate was macerated in a blender. In 50 ml centrifuge tube, 10 g of the ground sample were taken in to which 20 ml of HPLC grade acetonitrile was added and kept at ¯20°C for 20 minutes. The sample was then homogenised (Heidolph Silent Crusher-M) at 14000 rpm for 3-4 min. To this 4.5 g of activated sodium chloride was added and vortexed for 2 min on a rotospin and then centrifuged for 5 min at 2,500 rpm. In to a 50 ml centrifuge tube containing 5 g of pre-activated sodium sulphate, an aliquot of 12 ml clear upper layer of the supernatant of the centrifuged sample was transferred and vortexed for 2 min for removing traces of moisture, if any. Dispersive solid phase extraction (DSPE) was for cleaned up. From this, 8 ml of the upper layer was transferred in to a 15 ml centrifuge tube containing 0.125 g PSA, 0.8 g anhydrous magnesium sulphate, 0.05g end capped C18 and 0.025g GCB. The mixture was again vortexed for 2 min and centrifuged for 5 min at 2,500 rpm. The 5 ml of the centrifuged supernatant was transferred to turbovap tube maintained at 40 °C and 7.5 psi nitrogen flow using turbovap and evaporated to dryness. The residue was then reconstituted in 2 ml of methanol and filtered through a 0.2-micron PVDF syringe filter (13mm) was used for UPLC-MS/MS analysis.

Results and Discussion

The percentage recovery of pesticide residue extracted using through QuEChERS method from banana leaves, pseudostem, bunch finger, flower, and corm collected (from the plants specially maintained in control plots) for carbofuran, 3-keto carbofuran, 3-hydroxy carbofuran and carbosulfan ranged from 80.0 to 119.9 percent, while the corresponding. According to Beevi *et al.,* (2014), recovery values of pesticides in LC MS/MS ranged between 70-120 percent for 26 compounds tested, were considered to be satisfactory. The Linearity and Limit of Quantitation (LOQ) for recovery of residue from different parts of banana were was 0.01 to 1 ppm and 0.01ppm respectively. The findings in the present study are in agreement with results of Bruzzoniti *et al.,* (2014), Beevi *et al.,* (2014), Lehotay *et al.,* (2005) and Anastassiades *et al.,* (2007) and these value for all different matrices of banana is as per SANTE (2015) norms. The MRM set up of quantitative ions for carbofuran, 3-keto carbofuran, 3-hydroxy carbofuran and carbosulfan were found to be 123, 151.1, 181.1 and 160.1 respectively. For qualitative ions the set-up, the respective parameters yielded 165.2, 179.1, 163.1 and 118.1 respectively. The retention time for carbosulfan and its metabolites were 3-hydroxy carbofuran (1.06 min), 3-keto carbofuran (1.52 min), carbofuran (1.94 min) and carbosulfan (5,36 min)

Field studies on dissipation (absorption, translocation, distribution, persistence) of carbosulfan in banana leaves

Carbosulfan and its metabolites were not detected in treatment T_1, control during the period of observation in all the four leaves tested (Table-2 and Fig-1).

Table.1 Instrumental parameter setting and selection of SRM for quantitative and qualitative ions for carbosulfan and its metabolites in analyte matrix.

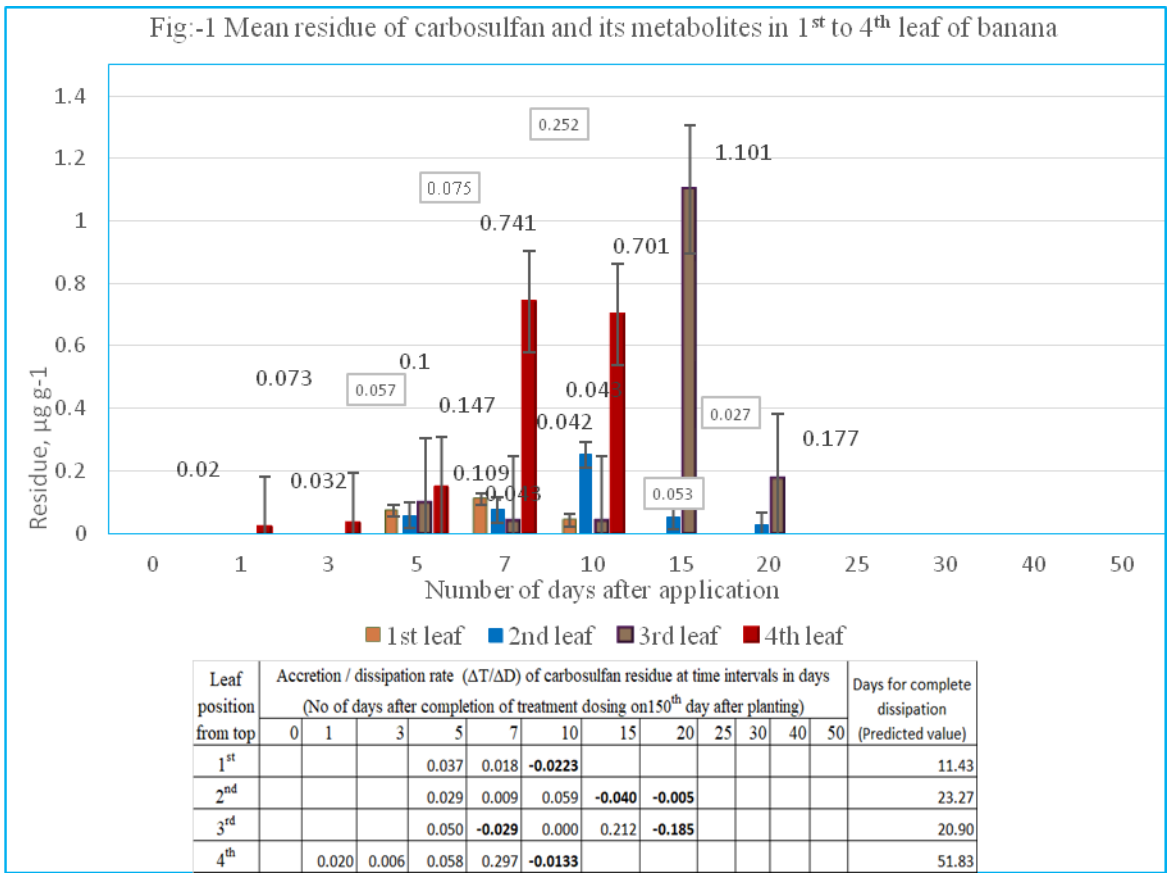
Instrument parameter	Molecule			
	Carbofuran	3-Keto carbofuran	3-hydroxy carbofuran	Carbosulfan
Retention time in minutes	1.94	1.52	1.06	5.36
Q1 Mass precursor ion	222.1	236.1	238.1	381.2
Q3 Daughter ion (quantitative)	123	151.1	181.1	160.1
Q3 Daughter ion (qualitative)	165.2	179.1	163.1	118.1
Declustering potential (Volt)	30	33	28	42
Collision energy(Volt) CE (quantitative)	29	23	16	22
Collision energy(Volt) CE (qualitative)	17	18	21	33
Collision cell exit potential (Volt) CXP	2	1	1	1
Entrance potential (Volt) EP	10	10	10	10
Collision cell entrance potential (Volt) CEP	26.3	22	24	31

Table.2 Mean residue of total carbosulfan and its metabolites in 1st to 4th leaf of banana, µg g^{-1}

Carbosulfan$^{\beta}$ in leaf (position from top)	**Time interval in days** (No of days after completion of treatment dosing on150th day after planting)												
	Before 0th **	***0th	1st	3rd	5th	7th	10th	15th	20th	25th	30th	40th	50th
Control*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
1st	BDL	BDL	BDL	BDL	**0.073**	**0.109**	**0.042**	BDL	BDL	BDL	BDL	BDL	BDL
2nd	BDL	BDL	BDL	BDL	**0.057**	**0.075**	**0.252**	**0.053**	**0.027**	BDL	BDL	BDL	BDL
3rd	BDL	BDL	BDL	BDL	**0.100**	**0.043**	**0.043**	**1.101**	**0.177**	BDL	BDL	BDL	BDL
4th	BDL	BDL	**0.020**	**0.032**	**0.147**	**0.741**	**0.701**	BDL	BDL	BDL	BDL	BDL	BDL

Foot note: *mean of all metabolites for carbosulfan; **-150th day before treatment imposition and *** - 2 hours after 3rd application and BDL-below detectable limit; $^{\beta}$carbosulfan and its metabolites; pop: - Package of practices Recommendations: Crops, KAU

Fig.1



Leaf position from top	Accretion / dissipation rate (ΔT/ΔD) of carbosulfan residue at time intervals in days (No of days after completion of treatment dosing on150th day after planting)												Days for complete dissipation (Predicted value)
	0	1	3	5	7	10	15	20	25	30	40	50	
1st				0.037	0.018	**-0.0223**							11.43
2nd				0.029	0.009	0.059	**-0.040**	**-0.005**					23.27
3rd				0.050	**-0.029**	0.000	0.212	**-0.185**					20.90
4th		0.020	0.006	0.058	0.297	**-0.0133**							51.83

Absorption and persistence of mean of metabolites of carbosulfan displayed a different pattern in different leaves, though, residue of "carbosulfan molecule" was not detected in any of the tested samples during the sampling period in the 1st, 2nd, 3rd and 4th leaf of banana at both the levels of application(Table-2 and Fig-1).

Table -2 indicate that mean of residues of mean carbosulfan was present in all the leaf only between i.e.,"total carbosulfan" was observed only between 5th and 10th day of application. In the fourth leaf it was present between 1st and 10th day where as in 2nd and 3rd leaf it was between 5th and 20th day dissipating to BDL on day 25th. In first leaf residue of carbosulfan was detected only between 5th and 10th day and on day 15th it was BDL. This clearly indicate early absorption translocation and accretion of metabolites to 4th leaf. Vijayan (2000) reported in a residue study of carbofuran that average total carbofuran in 3rd and 4th leaf of banana was 0.027µg g^{-1} on day 6th and on all other days of observation, it was BDL. In the present study the spread of detection period of residue was same for 2, 3rd and 4th leaf, however the translocation to 2nd and 3rd leaf was delayed further by 4 more days as compared to 4th leaf.

The net transformation or dissipation rate per day as indicated by ΔT/ΔD worked out by dividing the difference in residue levels of the sample between two adjacent observations divided by the number of days in between the observation, was positive for 5thand 7thday indicating an accretion i.e., absorption rate being higher than rate of and transformation and dissipation of the molecule. On 10th day it

was negative indication a dissipation rate exceeding the absorption rate. Assuming a continued dissipation rate of day 10, it could have dissipated completely by 11.9, 25.2, and 25.6 days in 1st, 2nd, 3rd leaf (Fig-1).

However, in 4th leaf the predicted day of complete disappearance was 51.83 days and was actually on BDL by 15th itself, indicating much higher rate of dissipation in the system. A thresh hold value of accumulation of the residue molecule in the cell may be the factor that decide the rate of dissipation. The trash hold value of residue in the cells, in the absence of fresh absorption from the soil, may decide rate of dissipation.

Field studies on dissipation (absorption, translocation, distribution, persistence) of carbosulfan in bunches, fruits and other plant parts of banana

Residue of carbosulfan and its metabolites were also not detected in the blossom bud, flower bract alone, bunch (on 15th day of emergence), bunch (on 30th day of emergence), peel, bunch on harvest, pseudo stem and corm of banana.

Vijayan (2000) reported in a residue study of carbofuran that average total carbofuran in 3rd and 4th leaf of banana was 0.027µg g^{-1} on day 6th and on all other days of observation, it was BDL. Rouchaud (1990) reported that carbosulfan, furathiocarb and carbofuran were absorbed from soil by the plant with similar intensities, and the plant metabolized carbosulfan mainly into carbofuran and 3-hydroxycarbofuran. According to Trevisan (2004) the carbosulfan metabolism to its carbofuran metabolite was rapid in 3 days, both analytes concentrated in the bagasse of oranges (peel + flavedo + albedo). Rajeswaran *et al.,* (2005), too did not observe any residue of carbosulfan in cotton leaves at harvest, when applied on 25th, 40th and 55th day after sowing at recommended dose as foliar spay.

In the 1st leaf when applied at recommended dose mean residue of carbofuran (sum of all the metabolites) was highest on day 7th (0.741µg g^{-1}) and was BDL on day 15th. The presence of carbosulfan in the 1st to 4th leaves till day 20th and subsequent dissipation pattern prediction for BDL in 23.3 days indicated that, leaves of banana applied with carbosulfan is not safe for use within 24 days of application for serving or food packing (as commonly practiced in every house holds of Kerala). Application of carbosulfan as per package of practice recommendation is will not result in a development of residue in the harvested food products such as fruit, (both raw and ripe), flower bud, inner core of the pseudo stem and even in the corm.

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