Dissipation and distribution of fipronil, carbosulfan and their metabolites in banana var. Nendran (AAB) and soil

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by

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THESIS

Submitted in partial fulfillment of the requirements for the degree of

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COLLEGE OF AGRICULTURE, VELLAYANI.

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DECLARATION

I, hereby declare that this thesis entitled **"DISSIPATION AND DISTRIBUTION OF FIPRONIL, CARBOSULFAN AND THEIR METABOLITES IN BANANA VAR. NENDRAN (AAB) AND SOIL"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title, of any other University or Society.

VISVESWARAN S.
(2015-21-002)

Vellayani
Date: 28-07-2021

CERTIFICATE

Certified that this thesis entitled **"DISSIPATION AND DISTRIBUTION OF FIPRONIL, CARBOSULFAN AND THEIR METABOLITES IN BANANA VAR. NENDRAN (AAB) AND SOIL"** is a bonafide record of research work done by Mr. Visveswaran S., (2015-21-002) under my guidance and supervision and that it has not previously formed the basis for award of any degree, diploma, fellowship or associateship to him.

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We, the undersigned members of the advisory committee of Mr. VISVESWARAN S., (2015-21-002), a candidate for the degree of Doctor of Philosophy in Agriculture with major in Soil Science and Agricultural Chemistry, agree that the thesis entitled **"DISSIPATION AND DISTRIBUTION OF FIPRONIL, CARBOSULFAN AND THEIR METABOLITES IN BANANA VAR. NENDRAN (AAB) AND SOIL"** may be submitted by Mr. VISVESWARAN S. (2015-21-002), in partial fulfilment of the requirement for the degree.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
@	At the rate of
a.i.	Active ingredient
Av.	Available
BD	Bulk density
BDL	Below detectable limit
B	Boron
Ca	Calcium
CD	Critical difference
cfu	Colony forming unit
CEC	Cation exchange Capacity
cm	Centi meter
Cu	Copper
DCM	Dichloromethane
dS	Deci Siemens
DSPE	Dispersive solid phase extraction
EC	Electrical conductivity
et al.	And others
EPA	Environmental protection agency
FAO	Food and Agricultural Organisation
FSSAI	Food safety standards authority of India
Fe	Iron
g	Gram
GOI	Government of India
GOK	Government of Kerala
h	Hour
ha	Hectare
K	Potassium
KAU	Kerala Agricultural University
kg	Kilogram
kg ha^{-1}	Kilogram per hectare
LC-MS/MS	Liquid chromatogram-Mass spectrometer /Mass spectrometer
LOQ	Limit of quantitation
Fig.	Figure
Mg	Magnesium (as element)
mg	Milligram
Mg	Mega gram (as unit of mass)
mg kg^{-1}	Milligram per kilogram
Min	Minute
ml	Milli litre
Mn	Manganese

MRL	Maximum residue limit
N	Nitrogen
NS	Not significant
^{o}C	Degree Celsius
P	Phosphorus
PD	Particle density
PVDF	Poly vinylidene fluoride
pop	Package of Practices Recommendations Crops, Kerala
rpm	Revolutions per minute
S	Sulphur
TPD	Net transformation or dissipation rate per day
UPLC	Ultra-performance Liquid Chromatogram
US	United States
viz.	Namely
WHC	Water holding capacity
WHO	World Health Organisation
Zn	Zinc
µg	Microgram

1. INTRODCUTION

India is the largest producer of banana (*Musa* sp.) in the world with a record production of 30.8 million tons in the year 2018 (Indiastat, 2018). Banana is one among the most preferred food crops globally after rice, wheat and maize and believed to be the oldest fruit crop of India (Kuchi, 2017). The present-day edible banana and plantains are believed to have originated in South-East Asian and western Pacific regions since their inedible, seed-bearing, diploid ancestors are still found in the natural forest vegetation (Robinson and Sauco, 2010). About 350 varieties of banana occur naturally, among which Nendran (Musa sp. AAB) is the one of the most preferred varieties for consumption in the state of Kerala. The high economic returns fetched per rupee invested, consumer preference and market demand make Nendran banana cultivation popular among the farming community of the state.

Nendran is relished as a fresh fruit and also consumed after cooking. Almost all parts of Nendran are edible that includes rhizomes, blossom bud and rind. Nendran, being a rich source of carbohydrates, proteins, minerals, vitamins and antioxidants like anthocyanin, polyphenols, flavonoids and tannins, forms a part of daily diet of Keralites in one form or another (Siji and Nandini, 2017). More than 20 different types of bananas are grown in Kerala. The crop is highly infested with a wide variety of pests, necessitating chemical interventions for their timely control.

In general, plantains are susceptible to the attack of different type of weevils and bunchy top disease spread by aphids that cause heavy crop loss. The restriction imposed on the use of pesticides bearing red label (extremely toxic category) and selected highly toxic chemicals in Kerala necessitates the use of newer molecules that are environmentally safe and effective in controlling the pest. Among the different alternatives suggested for the control of insect pests in banana, carbosulfan and fipronil are the most popular chemicals used by farmers.

Control of pests by the use of pesticide has been one of the most important contributions of modern science. The use of pesticide has become almost inevitable wherever intensive commercial agriculture is adopted using high yielding varieties of crops. Carbosulfan, a broad-spectrum systemic insecticide is recommended as a substitute for phorate and carbofuran for the control of banana aphid, (the vector of bunchy top disease of banana (KAU, 2011). It is ideally used for control of sucking insects (Rai *et al.,* 2014) in many crops and also, has significantly reduced nematode cyst population in wheat especially when used in seed treatments. Its application was found to increase significantly the shoot weight and grain weight of wheat. Geng *et al.* (2018) proposed a minimum pre-harvest interval of 12 days for carbosulfan to guarantee safe consumption of cucumber.

Fipronil, another systemic insecticide belonging to phenyl pyrazole group is also a substitute for above two banned insecticides for the control of banana rhizome weevil (KAU, 2015). These are available in granular form, enabling easy application in soil for banana. It is a broad-spectrum insecticide that disrupts the insect central nervous system by blocking the passage of chloride ions through the GABA receptor and glutamate-gated chloride (GluCl) channels, components of the central nervous system. There are reports of lesser contamination of ground water by use of Fipronil since fine textured soils quickly sorb fipronil and migration of fipronil through soil profile would be low (Singh *et., al* 2015). Fipronil and their degradation products were found to persist in sandy loam and clay loam soils for a few days to several days (USEPA, 1996, Zhu *et al.*, 2004). Bonmatin *et al.* (2015) suggested that other non-target organisms, particularly those inhabiting soils, aquatic habitats, or herbivorous insects feeding on non-crop plants will also inevitably receive exposure, although data are generally lacking for fipronil and neonicotinoid groups of pesticides.

With the introduction of new recommended molecules, it is necessary to find out the safety, absorption, translocation and dissipation of these substances both in soils as well as in plants. Nendran banana cultivation is widely practiced in red loam soils. Since almost

all parts of the crop are consumed, the studies on half-life and disappearance pattern of these chemicals need to be evaluated. Alkaline hydrolysis is one of the important factors that contribute in metabolism and disintegration of pesticides. Even though in vitro studies on dissipation and disintegration of carbosulfan and its residues on fruit juices are available in other fruit crops, only few such studies are conducted on banana. The present study gains importance as literature available on the residue of carbosulfan and fipronil in different parts of banana, are scanty and Food Safety and Standards Authority of India (FSSAI) has not recommended / has not fixed maximum residue limit (MRL) value for these compounds in banana. In addition, farmers are in dire need for a suitable chemical alternative for combating the insect pest problem in banana and hence are in search for a safe chemical alternative. Hence a study on the absorption, translocation and persistence of fipronil and carbosulfan in different parts of banana plant when applied in the rhizosphere of banana grown in the red soil was under taken, with the following objectives

1. To standardize methodology for estimation of fipronil and carbosulfan in red loam soil and banana.
2. To study the dissipation, metabolism and persistence of fipronil and carbosulfan in red loam soil of Vellayani.
3. To study the impact of carbosulfan and fipronil on soil organisms in red loam soils of Vellayani.
4. To study the absorption, distribution, partitioning, and degradation pattern of fipronil and carbosulfan in Nendran variety of banana.

2. REVIEW OF LITERATURE

India produces 30.46 million tonnes of bananas annually contributing 20% share of world production, the majority being used for internal consumption with export share remaining only at 0.6 % (FAO, 2021). In Kerala, it is cultivated in an area of 62108 ha (GOK, 2017). With the intensification of cultivation practices by modern man using advanced inputs like fertilizers and plant protection chemicals, the production, as well as the productivity increased appreciably with a corresponding increase in the pest and disease incidence. This has resulted in the use of more pesticide for controlling various pests. In general, total pesticide consumption in India and Kerala during the year 2018-19 are 59650 and 995 metric tons, respectively (GOI_1, 2020). During this period, the total fipronil and carbosulfan use in the country were 548 and 12 metric tonnes, respectively (GOI_2, 2020).

Good spread of rainfall for over 7 months, comparatively low cost of production and good adaptability to homestead cultivation makes banana, a popular crop in the state. There are about 350 natural varieties of banana among which nendran is one of the most preferred both in terms of cultivation as well as consumption in the state of Kerala.

Banana plant has many uses, apart from its preference as fruit crop. Unripe banana finds its place in many common dishes of Kerala (Pushpangadan *et al*., 1989; Mohapatra *et al*., 2010). In Kerala and in many other states in India, the food is served in banana leaves, especially during ceremonial occasions. Fibre obtained from the pseudostem is used in the cottage industry for making handicraft products, while the inner core of pseudostem, flower bud and corm are used as a vegetable as well as medicine in Ayurveda and naturopathy. The dried and powdered green fruit is used as baby food as it is classified under protective foods with very high nutritive value. Sweet and salty delicious chips are prepared from nature green fruits of nendran variety are monopoly to Kerala. Processed foods like jam, candy and even wine are prepared from ripe banana (Akubor *et al*., 2003).

Prophylactic use of systemic insecticides like carbosulfan and fipronil are recommended as ad hoc practices for their control (KAU, 2015).

Fipronil, is (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]4[(trifluoromethyl) sulfinyl]-1H-pyrazole-3-carbo nitrile (Tingle et al., 2003).), a phenyl pyrazole insecticide, is recommended for the control of banana rhizome weevil when applied as soil application of fipronil 0.3 G formulation at the rate of 10 g per plant (equivalent to 30 mg ai per plant) each

time with the first application at the time of planting, followed by application at 3 and 5 months after planting (KAU, 2015).

Carbosulfan, is (2,3-dihydro-2,2-dimethylbenzo-furan-7-yl[(dibutylaminothio) sulfanyl]N-methylcarbamate, a broad-spectrum systemic carbamate insecticide recommended for the control of banana aphid, the vector of bunchy top disease of banana by soil application of 400 mg ai of carbosulfan 6 G (i.e., at the rate 400 mg per plant), applied thrice, on 0, 60 and 150 days of planting as (KAU, 2011).

The intensive pesticides usages may result in development of resistance in the pest (Pimentel *et al.*, 1992; Widawsky *et al*., 1998), human health problems (Kamel and Hoppin, 2004) and also lead to environmental damages (Hao and Yang, 2013).

2.1 Pesticide residue analysis

There are several techniques being used for pesticide detection (Sharma, 2013). It may be from old quantitative titration analysis through conventional or traditional methods to advanced detection techniques. Lykken (1963) pointed out that sampling is an important consideration and problem of collecting and preparing samples of a broad spectrum of crops treated with a pesticide chemical and grown in experimental plots seems to be the same regardless of the end-use, except for those aspects that are altered by the design of the experiment.

Omeroglu *et al*. (2012) stated that pesticide residue analysis includes two main facets as sampling performed outside the laboratory and laboratory operations comprising of sample preparation, sample size reduction, sample processing, extraction, clean-up, and chromatographic determination.

Sharma, (2013) described the six important steps in pesticide residue analysis as sampling, extraction and clean-up, identification and quantification, confirmatory analysis and fortification and recovery trails.

According to Lykken (1963), the important considerations in sampling are the source of the sample (which may range from sparsely-covered rangeland to a banana plantation), the size of the raw commodity itself (which may vary from a mustard seed to a watermelon), the nature and stability of the raw commodity (which may range from milk to dried beans), and the method of applying the pesticide (which may vary from hand-

application in soil and plant to aerial sprays), the intended use of the crop, on crop behaviour of the applied chemical and size of the gross sample needed or obtainable.

Samsidar *et al*. (2018) pointed out that conventional analytical methods are gas chromatography (GC), high-performance liquid chromatography (HPLC) and detection using various types of detectors including a combination of analytical techniques using mass spectrometers in tandem, enzyme-linked immune assay (ELISA) and capillary electrophoresis which has high sensitivity and selectivity at low detection limits. However, these methods are limited with shortcomings such as complicated, laborious, costly instruments and highly skilled manpower. More advanced rapid detection methods involve the use of specific sensors. Such sensors include an electrochemical, optical, piezoelectric and molecular imprinted polymer.

Songa and Okonkwo (2016) also suggested that other methods to determine residue of pesticides via unconventional methods have several advantages including rapid, simple and low-cost operation, high sensitivity and selectivity, and onsite detection.

It was noted by Zheng *et al*. (2018) and Zheng *et al*. (2015) that, the detection limits of sensors, particularly enzyme-based biosensors so far cannot reach the same detection level as compared to conventional methods. Hence these advanced methods cannot be precisely used for quantification but are very fast and easy to detect onsite in a semi-quantitative mode.

Bruzzoniti et *al*. (2014) recommended QuEChERS method, a single stage extraction and clean-up technique originally developed for recovering pesticide residues from fruits and vegetables. The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method is an extraction and clean-up technique originally developed by Anastassiades *et al*. (2003) and later revalidated by Lehotay *et al*. (2005) and Anastassiades *et al*. (2007) for the retrieval of more than 200 pesticide residues from fruits and vegetables.

Some of the usually employed solvents for extraction are hexane (Cabras *et al.,* 2001; Sharma, 2013), acetone, dichloromethane (Juan *et al.,* 2006), and acetonitrile (Li and Yuan, 2008), and ethyl acetate or cyclohexane (Sannino *et al.,* 2004) either individually or in a mixture of different proportions. In the case of soil samples, extraction with dilute mineral acids is also commonly recommended.

Extraction and clean-up methods of pesticides from samples may involve one or more extraction steps (Sharma, 2013) such as blending and sonication, microwave-assisted extraction (MAE), Pressurised liquid extraction, supercritical fluid extraction (SPE) and also invariably involving one or more clean up steps such as liquid-liquid extraction (LLE), solid-phase extraction (SPE), matrix solid-phase extraction and quick-easy-cheap-effective-rugged-safe (QuEChERS) extraction (Anastassiades *et al*., 2003), solid-phase micro-extraction (SMPE), and certain other micro-extraction methods (Sharma, 2013; Samsidar *et al*., 2018). The micro-extraction comprises dispersive liquid-liquid microextraction (DLLME) (Farajzadeh and Mogaddam, 2016; Wang *et al*., 2016[b]), single-drop microextraction (SDME) (Amde *et al*., 2015), continuous flow microextraction (CFME) (Wu *et al*., 2016), hollow fiber-liquid phase microextraction (HF-LPME) (Curbelo *et al*., 2013), a combination of SPE and DLLME (Ahmadi *et al*., 2015; Shamsipur *et al*., 2016) and solid-liquid extraction (SLE) (Costa *et al*., 2015).

The term "clean up" refers to the steps or procedures adopted to isolate the pesticides from other impurities or co-extractives present in the sample extract (Sharma, 2013). This step is very essential because co-extractives will render identification difficult and qualification of pesticide residue in the next stage. When unclear extracts are injected into GLC or HLPC the co-extractives can cause extra peaks, or poor peak resolution, deterioration of columns, loss of detector sensitivity. Similar interferences may creep in when spectrophotometric; thin layer chromatographic techniques are used for identification and quantification. Also, the degree or level of clean-up should be very high so that highly sensitive, expensive instruments used in subsequent steps are not adversely affected (Rejczak and Tuzimski, 2017). This may be achieved by solvent partition, for example, partitioning between hexane and dimethylformamide or acetonitrile to remove fatty co extractives.

An additional clean-up step may be based on adsorption chromatography, like example, thin layer chromatography or column chromatography. Adsorbents like silica gel, alumina, activated charcoal, florisil, and magnesia, and celite etc., may be used either individually or in mixtures or with dispersive solid phase extraction clean-up using primary secondary amine along with zirconia-coated silica particles for extract purification (Rejczak and Tuzimski 2017).

Zheng *et al*. (2018), described a modified QuEChERS method making use of C18 sorbent coupled with PSA during purification for the target analysis of cymiazole, fipronil, coumaphos, fluvalinate, amitraz, and its metabolite, to extract them effectively from acacia honey, chestnut honey, manuka honey, and royal jelly.

Li *et al.* (2020) reviewed and found that GC-ECD is quite good fluorine-rich analytes, GC-MS method has better selectivity and sensitivity, but they take a long time for separation. They also pointed out that, LC-based methods have the advantage in fast separation, but the LC-UV method is low in sensitivity and referred to LC-MS as the most preferred technique though it requires expensive instrumentation.

Methods like enzyme inhibition and bio-assay principles are also utilized for residue determination. Watanabe *et al*. (2006) found ELISA showing reasonable sensitivity (I_{50} 0.6 ng/g; limit of detection 0.053 ng/g) and high selectivity for acetamiprid as against other neonicotinoid analogs (thiacloprid amide).

Soler *et al*. (2006) reported that the LC–MS/MS method developed by them is simple, rapid, and suitable for the quantification and confirmation of carbosulfan and main metabolites in orange were at levels lower than 10 μg kg^{-1}. Beevi *et al*. (2014) reported a recovery of 71.13, 107.38 and 86.25 % at LOQ level for fipronil, carbofuran and carbosulfan from soil along Cardomom Hills in Kerala.

2.1.1 General properties of Fipronil and Carbosulfan

2.1.1.1 Fipronil

Fipronil is a non-systemic, chiral, phenylpyrazole chemical with broad-spectrum insecticidal action against several insects. Technical grade fipronil is a white powder with a mouldy odour (Tomlin, 2009 and USEPA, 1996) with a molecular weight of 437.2 g/mol, water solubility 0.0019 g/l (1.9 mg/l) (pH 5); at 20 °C.

2.1.1.2 Carbosulfan

Carbosulfan is a viscous brown liquid with a solubility of 7.88 e-07 M (Pubchem[1], 2005). It is often used as an insecticide and as a nematicide. Carbosulfan is synthesised from the reaction of carbofuran with dibutyl amine and sulphur dichloride (Unger, 1996) wherein the carbofuran is obtained synthesised from the thermodynamic reactions between catechol, 2 methyl allyl chloride and methyl isocyanate. Carbofuran was first synthesized by W. G.

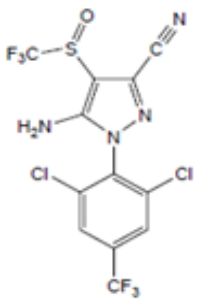
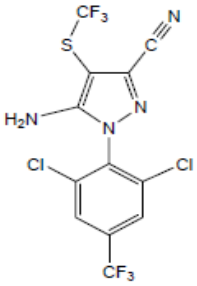
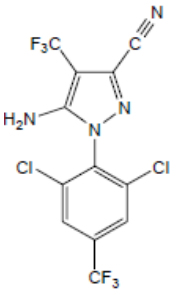
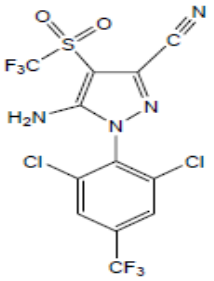
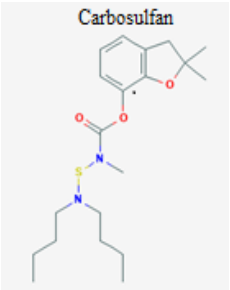
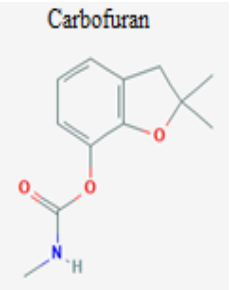
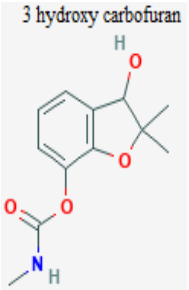
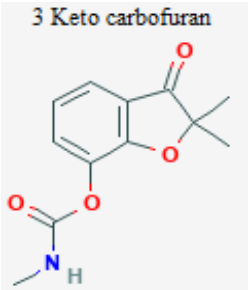
Scharpf in the laboratory of FMC corporation United States and registered for agricultural use in COD in the year 1968 (Unger, 1996).

General structure, molecular weight of fipronil, carbosulfan and their metabolites are given in the table.1 (Pubchem[1-8], 2005; 2006; 2007).

2.2 Fipronil and its toxic metabolites in plant and soil

Fipronil was discovered by Rhône-Poulenc Ag Company (now Bayer Crop Science) in the year 1987, launched in the year 1993 (Tomlin, 2009), and registered in the U.S. in 1996 (Ware, 2000) and is out of patent regime however, facing the demand of banning it from use in agriculture due to environmental concerns. It is available in numerous dry and wet forms, as impregnated materials and ready-to use-concentrates.

Table.1. Chemical structures of fipronil, carbosulfan and their major toxic metabolites

Molecular structure	Fipronil 	Fipronil-sulfide 	Fipronil-desulfinyl 	Fipronil-sulfone 
Formula	$C_{12}H_4Cl_2F_6N_4OS$	$C_{12}H_4Cl2F_6N_4S$	$C_{12}H_4Cl_2F_6N_4$	$C_{12}H_4Cl_2F_6N_4O_2S$
Molecular weight	437.1 g/mol	421.1 g/mol	389.08 g/mol	453.1 g/mol
Molecular structure	Carbosulfan 	Carbofuran 	3 hydroxy carbofuran 	3 Keto carbofuran 
Formula	$C_{20}H_{32}N_2O_3S$	$C_{12}H_{15}NO_3$	$C_{12}H_{15}NO_4$	$C_{12}H_{13}NO_4$
Molecular weight	380.5 g/mol	221.25 g/mol	237.25 g/mol	235.24 g/mol

Based on Unger (1996) it can be presumed that fipronil is formed by the multistage reaction involving 2,6 dichloro 4 trifluro methyl analine; ethyl 2,3, dicyanopropionate;

sodium nitrite; sulfuric acid; trifluromethyl sulphonyl chloride and meta chloro per benzoic acid.

Numerous products containing fipronil are currently available for use in agricultural, non-agricultural, and residential premises (Fent, 2004). Non-agricultural uses of fipronil include control of coleopteran larvae in soils on golf courses and other commercial turf grasses, and control of insects in food-handling establishments. Fipronil is used residentially to control fleas, ticks, and mites on domestic animals and as a mound treatment to control ants.

2.2.1 Physico-chemical properties of Fipronil

Fipronil is a white solid substance with a solubility of 1.9 mg l^{-1} in distilled water, all at 20 °C. A word for attention i.e., signals words for products containing fipronil may range from "Caution" to "Danger" (Tomlin, 2009). Fipronil is toxic to insects by contact or ingestion. Fipronil blocks and disrupt GABA-gated chloride channels, which lead to prevention of uptake of chloride ion, resulting in the excess of neuronal stimulation and death of the targeted insect resulting in disruption of the GABA receptor in the neuron (Cole *et al.,*1993; Ratra and Casida, 2001[a]).

Fipronil exhibits differential binding affinity for GABA receptor subunits, with a higher binding affinity for insect receptor complexes compared to mammalian complexes. The lower binding affinity for mammalian receptors enhances selectivity for insects and increases the margin of safety for people and animals. (Cole *et al.,* 1993; Ratra *et al.* 2001[b]; Hainzl *et al.,* 1998). Being a broad-spectrum insecticide, fipronil upon contact or ingestion disrupts the insect central nervous system by blocking the passage of chloride ions through the GABA receptor and glutamate-gated chloride (GluCl) channels, components of the central nervous system (Bonmatin *et al.*, 2015).

Fipronil degrades photochemically under environmental conditions to the desulfinyl derivative as the major photoproduct and the detrifluoromethylsulfinyl, sulfone, and sulfide compounds as minor products. Metabolism of fipronil in mice yields the sulfone but not the other derivatives.

Gunasekara *et al*. (2007) reported that the fipronil has undergone dissipation in the environment through the process mediated by microbes through reduction reactions and photolysis from foliage surface to form fipronil sulfide. It forms fipronil disulfonyl through

photolysis from soil and foliage. Dissipation products of fipronil also include the formation of fipronil sulfone through the soil biotic transformation, soil oxidation process and photolysis from foliage surface and fipronil amide.

Fipronil-sulfone, the primary biological metabolite of fipronil, is reported to be 20 times more active at mammalian chloride channels than at insect chloride channels. (Zhao *et al.* 2001). Fipronil-sulfone is reportedly 6 times more potent in blocking vertebrate GABA-gated chloride channels than fipronil, but demonstrates similar toxicity to the parent compound in mammals (Hainzl *et al.,* 1998).

Fipronil-desulfinyl, the primary environmental metabolite (photoproduct) of fipronil, is 9-10 times more active at the mammalian chloride channel than the parent compound, reducing the selectivity between insects and humans when exposed to this metabolite. (Hainzl and Casida, 1996; Hainzl *et al.* 1998)

2.2.2 Application of Fipronil in Agriculture:

In agriculture, fipronil is used on both agricultural and horticultural crops to protect against lepidopteron and orthopteran pests. It is registered for outdoor use, seed treatment, soil treatment, in-furrow treatment, and bait treatment. Fipronil is used to control ants, beetles, cockroaches, fleas, ticks, termites, mole crickets, thrips, rootworms, weevils, and many other insects. (Fent, 2014; Tomlin, 2006; USEPA 1996). Uses for individual fipronil products vary widely. Always read and follow the label when applying pesticide products. Fipronil is used in granular turf products, seed treatments, topical pet care products, gel baits, liquid termiticides, and in agriculture.

Martinez *et al*. (2014) observed that mortality of rhinoceros beetle in oil palms of Brazil would be better managed with fipronil application over lambda cyhalothrin, imidacloprid and thiamethoxam. Adrasha *et al.* (2015) observed that though fipronil was most effective in controlling the root white grub of arecanut.

In wheat grown areas infested with termite, study conducted by Singh[3] et al. (2015) reported a significantly highest per centage of wheat seed germination was recorded in application with fipronil 5 SC (95%) closely followed by thiamethoxam 35 FS (92.66%) and carbosulfan 25 DS (92.33 %), Bifenthrin 10 EC (90.33 %), Imidacloprid 70 WS (90.00 %) and Chlorpyriphos 20 EC (89.66 %), whereas, grain yield (g/m row and q/ha) was significantly higher in treated plot with fipronil compared to other treatments

Babar *et al.* (2016) concluded that lambda cyhalothrin, profenphos and quinalphos spray or soil application of DDVP, phorate were much more effective than fipronil in controlling cotton flower boll midge, *Dasineura gossipii*. These indicate that fipronil is more a contact insecticide.

2.2.3 Acute Toxicity of Fipronil

Technical grade fipronil is considered moderately toxic by ingestion with an oral LD_{50} of 97 mg/kg in rats and an LD_{50} of 91 mg/ kg in mice (Tingle *et al*., 2003). The lowest dose that produced adverse effects (LOAEL) was 7.5 mg/kg when fed to rats with a single dose of fipronil by gavage (introducing the chemical into the stomach through a tube) at a dose of 0, 2.5, 7.5, or 25.0 mg/kg. At that dose, male rats displayed decreased hind limb splay (spread wide apart) at 7 hours following administration. Researches also observed decreased body weight gain, decreased food consumption and food efficiency, and decreased grooming among female rats at 7 days after the single 7.5 mg/kg dose. All treatment-related effects resolved by 14 days following the single dose, except decreased grooming among female rats. The acute NOAEL (no observable adverse effect) for fipronil was 2.5 mg/kg. The acute oral LD_{50} of fipronil-desulfinyl (primary photo-degradate) in rats is 15 and 18 mg/kg for females and males, respectively (USEPA, 2009).

The primary metabolite of fipronil in armyworms, mice, and presumably other insects and vertebrates is the fipronil sulfone derivative (Hainzl and Casida, 1996; Hainzl *et al.* 1998). Mice injected with fipronil was found contain the sulfone derivative in the brain, liver, kidney, fat, and faeces (Hainzl and Casida, 1996), indicating its ability to cross blood brain barrier.

Fipronil is the first phenylpyrazole partly selective insecticide acting on sensitive nerve introduced for pest control (Moffat, 1993; Tomlin, 1994; Burris *et al.*,1994) suggested that Fipronil at low dosage provides long-term protection against major orthopterous and lepidopterous pests on crops and coleopterous larvae in soil. The pests it may control in a given agricultural and urban environments to include pests such as locust, crickets, termites, beetles, cockroaches, fleas, etc. (Tingle *et al*., 2003), suggests that it is acting more as broad-spectrum insecticide. AS per Codex standards, 2003 MRL of fipronil and its metabolite for banana, cabbage and rice were 0.02 mg/kg, 0.005 mg/kg, 0.01 mg/kg.

Mortensen *et al.* (2015), reported that fipronil is not systemic, however later, discussed in field studies employing seeds treated with fipronil or soil-incorporated fipronil

products and suggested that fipronil is taken up by the root and translocated into the plant. According to their review "it may be true during early growth stages of some plants that, fipronil tends not to reach the upper parts of plants with near the frequency of certain neonicotinoids, which would be predicted to be systemic based on physicochemical properties alone. Fipronil and neonicotinoid insecticides have different insecticidal modes of action and differ greatly in their physico-chemical properties. Hence, it is erroneous to include fipronil and neonicotinoids in the same category and call them collectively systemic insecticides".

2.2.4 Adsorption, mobility, persistence and dissipation of Fipronil in soil

According to Tingle *et al*. (2003), fipronil degrades slowly on vegetation and relatively slowly in soil and in water, with a half-life ranging between 36 hr and 7.3 months depending on substrate and conditions. It is relatively immobile in soil and has low potential to leach into groundwater. One of its main degradation products, fipronil desulfinyl, is generally more toxic than the parent compound and is very persistent. There is evidence that fipronil and some of its degradants may bioaccumulate, particularly in fish.

Ying and kookana, (2001) noted that average soil sorption coefficient, (K_{oc}) value for fipronil for eight soil types was 825 ± 214, and the K_{oc} values for fipronil-sulfide and fipronil-desulfinyl were 3946 ± 2165 and 2010 ± 1370, respectively. They are found to have a half-life of fipronil is 122-128 days in aerobic soils. Under aerobic conditions, naturally occurring soil organisms break down fipronil to form fipronil-sulfone. Fipronil can also be hydrolysed to form fipronil-amide (USEPA, 1996). The K_{oc} values for fipronil range from 427-1248 in sandy loam, but will vary depending on clay and organic carbon content of the soil.

Saini *et al*., (2015) reported that application of fipronil at 50 and 100 g a.i. ha^{-1} in sandy loam soils had a dissipation half-life of 10.81 and 9.9 days, with the dissipation following a biphasic 1^{st} order kinetics offering an LOQ value of 0.001 mg kg^{-1} and with the LOD of 0.0003 mg kg^{-1}.

Photo degradation under intense UV radiation (i.e., sunlight) of fipronil on soil surfaces lead to formation of fipronil-desulfinyl, and has a measured half-life of 34 days in loamy soil. However, soil particles beneath the surface under field conditions have higher residence time (USEPA, 1996; Bobe *et al*. 1998). Similarly, the reported $T_{1/2}$ of fipronil in

sandy loam was 122 days with fipronil- amide and fipronil-sulfone accounting for 27-38% and 14-24% of the total by-products, respectively (USEPA, 1996).

It is seen that fipronil was degraded by microorganisms in the non-sterile clay loam soil, which lead to the formation of the metabolite, MB45950 (Zhu *et al*., 2004). The half-lives in non-sterile clay loam soil were 9.72 and 8.78 d at 25 and 35 °C, respectively compared to 33.51 and 32.07 d at 25 and 35 °C, respectively in the sterile soil. Fipronil did not adversely affect the microbes once soil microbes adapted to the presence of fipronil in the clay loam soil.

According to Shuai *et al*. (2011), the concentration of fipronil in the leachate from the three soils are negatively correlated with soil organic carbon content. The half-life of fipronil in the soils ranged from 28 to 34 days when soil moisture content was 75% of field capacities and that 10.7–23.5% of the degraded fipronil was transformed into the two metabolites (fipronil sulfide and fipronil sulfone). They concluded Fipronil showed large losses through leaching but small losses via runoff owing to low volumes of runoff water generated and/or negligible particle-facilitated transport of fipronil. The half-life values of fipronil in all three soils were similar.

Ramı´rez, (2012) pointed out that as on that day, there are no reports of ecotoxicological damage. They observed degradation products in the agricultural soils as, desulfinyl and sulfone fipronil.

Verma e*t al.* (2014) observed that under in vitro conditions and sandy loam soils applied with fipronil and then subjected to natural light and UV light have fipronil dissipated in biphasic first-order kinetics. The dissipation from each medium increased with increasing of PH 5,7 and 9 and with half-life period of 14.2, 9.3 and 6.7 days respectively with corresponding persistence of 0.87 0.48 0.12 respectively on 40^{th} day before attaining BDL on 60^{th} day.

In studies to determine the fate of fipronil in soil, researchers found "no evidence of volatility" of fipronil or fipronil metabolites. (USEPA,1996).

In latosolic red and riverside and clay soils with high organic matter content in the range of 0.44 to 20.9 %, accumulation and persistence of fipronil and its metabolite where correlated with increasing organic matter content. (Yu *et al*., 2013).

Low mobility of fipronil in the soil diminishes its chance to reach the ground water. The mobility of fipronil mainly confine only up to upper six inches of soil, and hence significant lateral movement is also not expected (Tomlin, 2000; USEPA, 1996; Ying 2006). Inao *et al*. (2018) reported that limit of quantitation in water was 0.01 to 0.05 μg L^{-1} for fipronil and fipronil sulfone in soils it was 0.025 to 2.125 μg kg^{-1} and 0.2 to 1 μg kg^{-1} respectively.

Defang (1989) found that in simulated laboratory conditions, microorganisms in rice soil were effective in degrading the pesticide to some extent, but the degradation of carbofuran in soil could not be accelerated when applied repeatedly. The number of bacteria, actinomycetes and fungi in the treated soil increased 2.3 to 4.4 times as compared with untreated soil.

In a study Zhu *et al*. (2004) observed that sterile clay loam soil lacked the microbes responsible for the metabolism of fipronil as evidenced by smaller values of the rate constant and greater values of half-life time of fipronil. The degradation of fipronil in the clay loam soil lasted for a few days to several days with the appearance of metabolite, fipronil-sulfide with no further degradation of metabolite

Bobe[1] *et al*. (1998) reported that the adsorption of fipronil, a phenylpyrazole, on two Sahelian soils (Saguia and Banizoumbou in Niger) and a Mediterranean soil (Montpellier) is dependent on the level of organic matter and its adsorption isotherm followed the S type curve is characteristic of soils with low organic matter. Bobe[1] *et al.* (1998) noted that fipronil and its metabolites in sub-Saharan soil when applied for locust control did not move beyond 10 cm depth, except for the amide, which is not considered a toxicologically significant metabolite.

Singh[1] *et al*. (2015) observed that fine textured soils quickly sorb fipronil and hence the migration of fipronil through soil profile would be low with little danger to contaminate ground waters.

From these observations it may be noted that alkaline hydrolysis is one of the most contributing factors in metabolism and disintegration of pesticides. Such an information on knowledge gap with respect to fipronil in acidic red loam soils are not comprehended fully. Hence the present study may also be able to address aspects of safety concerns with respect to public health.

2.2.5 Absorption, translocation, metabolism, persistence and dissipation of fipronil in plant.

Fipronil is not well absorbed by plants after soil treatment (about 5%) and partially degraded in plants to the sulfone and amide derivatives. Fipronil applied to foliage partially photodegrades to form fipronil-desulfinyl. However, Yu *et al*. (2013) suggested duckweed as bioindicator for fipronil biodegradation.

Hainzl and Casida (1996) found that the photodegradation product of fipronil after 12 h and 93 h of exposure to was Desulfinyl fipronil 45% and 95% of the total residues on pea and pear leaves to August sunlight and 67% on corn leaves after 93 h of exposure to November sunlight, in the latter case with 13% detrifluoromethyl sulfinyl fipronil. Thus, desulfinyl fipronil is likely to be a major persisting residue on foliage-treated crops.

Aajoud *et al*. (2006) has observed transpiration depended fipronil uptake in sunflower under aqua culture. Under the said soil conditions (20% organic carbon), the partition coefficient between soil and water (K_d) was found to be equal to 386 ± 30. The average rate of fipronil transfer from soil water to seedlings was from 2 to 2.6 times lower than water transfer. During the 3-week experiment, 55% of recovered labelled compounds was in the parent form and 35% had been converted to lipophilic metabolites, with either a 4-CF_3-SO_2 or 4-CF_3-S substituent, which are also very potent lipophilic insecticides. This indicate about the possible uptake of fipronil by sunflower seedlings under agronomic conditions is mainly controlled by the physicochemical characteristics of the seed-coating mixture.

Xavier *et al.* (2014) found that the half-life of fipronil at single and double dose in fresh chilli pepper was 4.22 and 4.32 days and the waiting period was 25.9 and 30.6 days, respectively. Among the metabolites of fipronil, fipronil desulfinyl and fipronil sulfone had maximum residues in fresh and dried chilli, respectively, followed by fipronil sulfide.

Inao *et al*. (2018) reported that limit of quantitation in plant it was 2.1 to 1 μg kg^{-1}. The residual levels in rice plants were similar to those in root zone soils rather than inter plant soil (area between plant to plant).

Kumar and Singh (2013) reported that the residues in paddy samples at harvest which consisted of paddy straw, rice grains, bran and husk which were applied with fipronil at the

rate 45 and 180 mg a.i. ha^{-1} has been BDL, even in samples with the four times higher levels of application.

Hakme *et al*. (2018) reported that market samples of pear fruits showed the carbofuran residue of 0.062 and 0.032 μg kg^{-1} (where MRL is 0.001 μg kg^{-1}). However, only 2 samples out of 69 samples of various types of fruits showed the presence of fipronil sulfone.

The roots treated with 1, 2.5, 5 and 10 ppm of fipronil insecticide within 6, 12 and 24 hours had diminished the mitotic index clearly in each treatment group in comparison with control and enhanced the abnormality cell percentage in almost all of the concentrations (Karaismailoglu, 2017).

Kumar and Singh (2013) found that concentration of fipronil in paddy when applied with Regent 0.3G @ 45 a.i. ha^{-1} was 6.6 mg kg^{-1} on seventh day, 2.86 mg kg^{-1} on 45^{th} day and was below detectable limit on 60^{th} day where LOQ was 0.01 mg kg^{-1}. They have identified the metabolites sulfone, sulphide, amide and de sulphonyl in the samples of paddy plants. Total residue of fipronil and its metabolites in paddy after 7 days of application at the rate 180 g a.i. ha^{-1} was 19.85. They suggested the degradation process occurs through oxidation reduction hydrolysis photolysis, respectively. The presence of sulfone sulfide in significantly higher amounts as compared to other metabolite in the paddy plants clearly demonstrate that the oxidation reduction process plays major role in the metabolism of fipronil.

Mohapatra, (2010) observed that the residue of fipronil and its metabolites in the leaves of grapes where below detectable limit on 0.01 mg kg^{-1}.

2.2.6 Residues of Fipronil in water

Fipronil degrades rapidly in water when exposed to UV light to form fipronil-desulfinyl. Under these conditions, fipronil has a half-life of 4 to 12 hours (Bobe[1] *et al.*, 1998; Ngim and Crosby, 2000). Fipronil is stable to hydrolysis at pH 5 and pH 7. However, its degradation in alkaline conditions is directly proportional to increasing pH values. Fipronil-amide is the primary residue formed from hydrolysis. (USEPA 1996.; Jackson *et al*., 2009; Bobe[2] *et al.*, 1998; Ngim and Crosby, 2000)

Thuyet *et al*. (2013) observed that half-life estimates of fipronil at 7 and 10th day were 87.9 and 13.2 days in water in Tokyo where fipronil sulfone was the major degradation metabolite detected in HPLC analysis.

Araujo *et al*. (2013) observed the absence of matrix effect on various water samples for the extraction of fipronil with LOD 1.39 ηg l^{-1}. This indicates possibility of injection of ultra-filtered water into HPLC for analysis of residue of dissolved pesticide content.

Fipronil content in surface water ranged from 0.829 to 5.290 µg/L in southwestern Louisiana during March through April, which corresponds to the timing of releases of rice field tailwater. Fipronil degradation products accumulated in riverbed sediment while the parent compound was absent. (USGS, 2003).

Fipronil-desulfinyl photodegrades in aerated and static water and recorded half-life values of 120 (± 18) hours and 149 (± 39) hours, respectively (Ngim and Crosby, 2000). Fipronil and fipronil-desulfinyl are less volatile than water and can concentrate under field conditions. (Jackson *et al*., 2009; Tomlin 2006; USEPA,1996).

2.2.7 Aerial contamination with Fipronil

The vapor pressure for fipronil is 3.7 x 10^{-4} MPa at 25 °C. Photodegradation studies in soil found no evidence of volatility of fipronil or its metabolites. (USEPA,1996). No indoor fate data were found (USEPA, 1996.).

2.2.8. Residue of Fipronil in food and agricultural commodities

Agricultural commodities which contain a residue above their respective maximum residue level result in health hazard to the consumers (Chaudhary and Singh, 2016). Brinjal tomato and potato samples obtained from 7 places did not contain detectable amount of fipronil and organo chlorines gas chromatographic analysis.

Mohapatra *et al*. (2010) observed that the residue of fipronil and its metabolites in the berries of grapes where below detectable limit on 0.01 mg kg^{-1}, on 7 days after application, when applied at recommended doses.

Xavier *et al*. (2014), observed that half-life of fipronil at single and double dose in fresh chili pepper was 4.22 and 4.3 two days and waiting period of 25.9 and 30.6 days and observed 3 metabolites of applied fipronil viz., that is fipronil desulfinyl, fipronil sulfide and fipronil sulfone. They found that the fipronil desulfinyl content increased from 0th to 3rd day

due to conversion of applied fipronil to fipronil disulfonyl. Other metabolites too increased during the period.

2.2.9 Effect of Fipronil in Nontarget organism

On the basis of increases in thyroid follicular cell tumours in both sexes of the rat, US, EPA classified fipronil as "Group C- possible human carcinogen" (USEPA 2009). However no human data were found on carcinogenic, teratogenic and reproductive effect of fipronil. The whole-blood half-life of fipronil in rats ranged from about 6.2-8.3 days after a single 4 mg/kg oral dose and decreased significantly to 2.1-2.3 days after a single 150 mg/kg oral dose. (USEPA, 1996).

For fipronil MRL is fixed as 0.005 mg/kg and 0.01mg/kg of body weight and ADI as 0-0.0002 mg/kg of body weight for fipronil and fipronil-desulfinyl (FAO, 1997; FAO-CODEX, 2020[b]).

Fipronil is highly toxic to bobwhite quail and pheasants, with an acute oral LD_{50} of 11.3 mg/kg and 31.0 mg/kg, respectively. Fipronil also has high sub-acute toxicity with a 5-day dietary LC_{50} of 49 mg/kg in bobwhite quail (Jackson *et al.*, 2009; Tomlin 2006). Fipronil is practically non-toxic to mallard ducks with no documented acute, sub-acute, or chronic effects. (Jackson *et al.*, 2009; Tomlin 2006; USEPA,1996). The fipronil-sulfone metabolite is highly toxic to upland game birds and moderately toxic to Waterfowl by ingestion. Fipronil is highly to very highly toxic to marine and freshwater fish. The 96-hour LC_{50} is 0.246 mg/L for rainbow trout, 0.083 mg/L for bluegill sunfish, and 0.130 mg/L for sheepshead minnows. Fipronil-sulfone is 6.3 and 3.3 times more toxic to rainbow trout and bluegill sunfish, respectively, than the parent compound (Jackson *et al.*, 2009; USEPA,1996). Fipronil-desulfinyl, the primary photodegradate of fipronil, has been measured in the fat, brain, liver, kidney, skin, and faeces of mice, rats and lactating goats after oral exposure or injection (WHO, 1997; Hainzl *et al*. 1998).

According to Adrasha e*t al.* (2015), though fipronil was most effective in controlling the route white grub of arecanut, it had an adverse effect on non-target arthropods and earthworms in surface soils. Yu *et al*. (2013) suggested earthworm as a bioindicator. Earthworm population was proportional to organic matter, clay and depth. Population of earth worms at different soil depths were found to be in the order 15 to 20 > 10 to 15 > 5-10 >0- 5 cm.

Bonmatin *et al*. (2015) suggested that other non-target organisms, particularly those inhabiting soils, aquatic habitats or herbivorous insects feeding on non-crop plants in farmland, will also inevitably receive exposure, although data are generally lacking for fipronil and neonicotinoid groups of pesticides.

A significant reduction in cases of spore germination, vegetative growth of *Metarrhizium anisopliae* treated by hexaflumuron whereas pyriproxyfen and fipronil showed relatively little fungal inhibition at the concentrations of 50 and 100 ppm, similarly to the control. However, fipronil induced the lowest level of inhibition on the germination, vegetative growth and sporulation of M. anisopliae in vitro (Rashid *et al*., 2010).

Pascual and Schneider (2019) contested the claims of Holder *et al*. (2018). Holder et al speculate that fipronil (a phenylpyrazole insecticide), rather than imidaclopride (a neonicotinoid), caused mass mortalities of honeybees (*Apis mellifera*) in France, where soil application of these insecticides is the only recommended practice. However according to Pascual and Schneider (2019), fixing environmentally realistic residue concentrations of 5 ppb for 2 neonicotinoids and fipronil by Holder *et al.* (2018) do not have any reference or realistic data, use of simplistic extrapolation of lab data to environment sampling data and no supportive data to consider fipronil and neonicotinoids as "these systemic insecticides by providing presence of these compounds in plants after a soil application," and hence due to of such inadequacies, the main claim and title of the paper of Holder *et al.* (2018) are highly speculative and not adequately supported by reliable data.

2.3. Carbosulfan and its toxic metabolites in plant and soil

The chemical structure of carbosulfan and its metabolites are given in the table.1. Carbofuran is an odourless white crystalline solid (PubChem[5]. 2005). 3-Hydroxy-carbofuran is a metabolite of Carbofuran in plants, insects and mammals. It is a known environmental transformation product of Carbofuran and Carbosulfan. Its IUPAC name is (3-hydroxy-2,2-dimethyl-3H-1-benzofuran-7-yl) N-methylcarbamate (PubChem[7], 2005). 3-Ketocarbofuran or 3-Oxo-carbofuran ((2,2-dimethyl-3-oxo-1-benzofuran-7-yl) N-methylcarbamate) is a metabolite of Carbofuran in plants, insects, and mammals and is a known environmental transformation product of Carbofuran and Carbosulfan (PubChem[8], 2005). It is having an LD_{50} value of 295 mg/kg.

Carbosulfan has known environmental transformation products that include 3-OH-carbofuran, 3-ketocarbofuran, carbofuran, carbofuranphenol, 7-phenol and Dibutyl-amine

(FAO, 1997). A number of bacteria, capable of degrading carbofuran (*Pseudomonas spp, Flavobacterium spp, Achromobacterium spp, Sphingomonas spp, Arthrobacter spp*) have been isolated and characterized in an effort to better understand the bacterial role to remove carbofuran from the environment (Porto et. al, 2011). Carbofuran was converted first to carbofuran phenol and this was transformed to 2-hydroxy-3-(3-methyl propan-2-ol) phenol by *Sphingomonas sp*. (Kim *et al*., 2004) and Arthrobacter sp. (Schrijver and Mot, 1999).

2.3.1 Acute Toxicity and safety levels of carbosulfan

Carbosulfan has an oral LD_{50} value of 51 mg/kg in rats (PubChem[5]. 2005). However, it may be noted that as per FAO-CODEX[a] (2020), MRL for carbosulfan in banana has not been finalised in the list, although MRL for carbosulfan in mandarins, oranges, citrus pulp spices, roots, rhizomes were finalised as 0.1 mg/kg. As per decision of year 1986, the ADI of carbosulfan is 0.01 mg/kg body weight.

As per FAO-CODEX[b], (2020), carbofuran residues (which is a combination of residue of Carbofuran and 3-hydroxy carbofuran and expressed as carbofuran) are not fat-soluble and its MRL has been decided as 0.01 mg/kg in bananas with ADI of 0.001ppm mg/kg body weight in the year of decision i.e., 2008.

Acute toxicity tests performed by Iesce *et al*. (2006) on the rotifer *Brachionus calyciflorus* and two crustaceans, *Daphnia magna* and *Thamnocephalus platyurus*, while chronic tests comprised a producer, the alga *Pseudokirchneriella subcapitata* and a consumer, the crustacean *Ceriodaphnia dubia* lead to the suggestion that the various species utilized were not in the same order of sensitivity to these residues and that all three pesticides and phenol-4 derivatives exhibited acute and higher chronic toxicity towards the aquatic organisms tested.

2.3.2 Agricultural use of Carbosulfan

Bhan and Kanwar (2012) noted that carbosulfan is ideally used for control of sucking insects in many crops. They found that in wheat crop, all the treatments reduced cyst population over control but this reduction was significant only in soil application of carbofuran 3G (1.5 kg a.i./ha) and seed treatment with carbosulfan 25 ST (2% a.i. w/w). Seed treatment with carbosulfan and soil application of carbofuran proved equally effective in reducing cyst population. *Azotobacter chroococcum* alone or in combination with carbosulfan (1 or 2%) was not effective in controlling nematode, *Heterodera avenae*. They

also observed that shoot weight and grain weight increased significantly over control only in carbofuran and Azotobacter + carbosulfan treatments while other growth characters were on par in all other treatments.

Approved use of different formulations of carbosulfan (PPQS-GOI$_3$, 2020) in agricultural crops include only three crops. They are rice i.e., paddy (for controlling Stem borer, Gall midge, Green leaf hopper, Leaf folder, White Back Plant Hopper and Brown plant hopper), chillies (for controlling Leaf folder and white aphid) and in cotton (for controlling Jassids, Aphids, Thrips).

However, its immediate metabolite carbofuran which is also available in different formulations ((PPQS-GOI$_3$, 2020) are used for the controlling different pests of about 25 different crops i.e., Barley (Aphid, Cyst nematode and Jassids), Bajra (Shoot fly), Sorghum (Shoot fly and Stem borer), Jute (Nematodes) Groundnut (Pod borer and White grub), French bean (White grub), Potato (Aphid and Jassids), Tomato (Whitefly fly), Apple (Woolly aphid), Citrus (Nematode and Leaf miner) Maize (Stem borer, Shoot fly, Thrips), Paddy (Brown plant hopper, Gall midge, Stem borer, Green leaf hopper, Hispa and Nematodes), Mustard (Mustard leaf miner and Whitefly) Soybean (Root knot nematode), Sugarcane (Top borer) Bhindi (Okra-Jassids), Chilli (Aphid and Thrips), Cabbage (Nematode), Wheat (Ear cockle nematode, Cereal cyst nematode), Brinjal (Root knot nematode, Reniform nematode), Banana (Rhizome weevil, Aphid, Nematode), Peach (Leaf curl aphid), Mandarins (Soft greens scale), French bean (White grubs, Grey & Stem weevil), Pea (Shoot fly & Aphid) and Tea (Cock chafer grub).

Carbofuran being banned in Kerala for use in agriculture has now been substituted this with carbosulfan granular formulation (KAU, 2015; 2016).

In green gram, decline of nematode population was maximum in the treatment with combined application of *Trichoderma harzianum* @ 1.25 kg/ha + *Glomus fasciculatum* @ 300 spores/m^2 + carbosulfan 25 ST @ 1.5 % w/w + vermicompost 1.25 t/ha (Singh and Mahanta, 2013). As per Sharma (2013), the soil application of carbofuran @ 30 kg/ha at pre-blooming stage followed by post bloom spray of cypermethrin @ 562.5 ml ha^{-1} is very effective and safe against *L. erysimi* infesting *Brassica campestris var. sarson* (Mustard).

In 2011, field efficacy of several insecticides applied using mix sand broadcasting was studied at two sites in Guangxi Province in China, at Longan Base Qin *et al*. (2013) noted that 20% Chlorantraniliprole SC (225 ml ha^{-1}, 30% Durivo SC (600 ml ha^{-1}), 15%

Chlorpyrifos GR (18.75 kg/ha), 5% Carbosulfan GR (60 kg/ha) and 5% Terbufos GR (60 kg/ha) controlled sugarcane borers by 83.61%, 97.34%, 72.72%, 61.68% and 97.90%, respectively. No significant differences were found between Chlorantraniliprole, Durivo and Terbufos. However, they noted that in Wuming Base, Chlorantraniliprole, Durivo, Carbosulfan and Terbufos did not differ significantly.

Nimisha and Nisha (2019) observed that the banana yield was higher in treatment of using un-pared sword sucker as planting material which was subjected to bio fumigation with crop residues of cabbage (9.97 kg plant^{-1}), paring + mulching with green leaves of *Gliricidia maculate* (9.63 kg plant^{-1}) and chemical, carbosulfan (10.30 kg plant^{-1}). Even then the population of free living and predatory nematodes were significantly higher in all treatments than untreated and chemical check i.e., pared sword sucker subjected to paring with subsequent carbosulfan treatment.

Nengming *et al.* (2019) observed that the carbosulfan residue in the celery crop, applied with 20% carbosulfan EC (180 g/ha) when sprayed once has a safety interval 5 day, the same crop sprayed more than two times, the safety interval of more than 14 days.

2.3.3 Adsorption, mobility, persistence and dissipation of carbosulfan in soil

Rouchaud *et al.* (1990), found that among the soil applied carbosulfan, furathiocarb, and carbofuran in brussels sprouts, cauliflower crops and sugar beet, carbosulfan and furathiocarb were transformed into carbofuran, which then was transformed mainly into 3-ketocarbofuran, carbofuran phenol, and 3-ketocarbofuran phenol. In the soil of the carbosulfan treated crop, the carbosulfan and carbofuran concentrations were similar. In the carbosulfan treated soil, the concentrations of the sum carbosulfan + carbofuran were higher than the carbofuran concentrations in the carbofuran treated soil.

Sahoo *et al*. (1998), studied the role of microorganisms in the degradation of carbosulfan and carbofuran by selective enrichment of microorganisms degrading either or both insecticides by repeated application of the insecticides, individually or in combination to flooded soil. They found an accelerated rate of hydrolysis in soil suspensions collected later to six applications of the insecticides. The order carbosulfan treated > carbofuran treated > carbosulfan + carbofuran treated. The rate of degradation of carbofuran in this experiment was found to be in the order carbofuran retreated > carbosulfan + carbofuran retreated > carbosulfan retreated soil. Further they found that rapid degradation of

carbosulfan was brought about by microorganism in non-sterilized enrichment culture than that in sterilized one.

Here according to Sahoo *et al*. (1998), carbosulfan was more resistant to degradation by the enrichment culture than carbofuran and attributed this to the presence of N-S-N linkages with carbosulfan molecule. However, this observation was relative and that both carbosulfan and carbofuran could readily be degraded by both carbosulfan- and carbofuran-retreated soil.

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).

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 and fipronil (Paul *et al.*, 2015). Dhanya *et al.* (2015), in a study on the persistence of carbosulfan in laterite and coastal alluvium soils using emulsifiable concentrate and granule formulations found that degradation of carbosulfan was also dependant on the content of soil organic matter and the kind of formulation. The coastal alluvium soil with higher organic matter (0.84%) showed less persistence with granule formulation. In laterite soil and coastal alluvial soils, the residues persisted up to 20-30 days, 20 days respectively at the higher treatment level. Higher Lower level of application resulted in faster dissipation as compared to higher levels. The maximum residue was obtained on the 7^{th} day (0.223- 1.2 ppm). The half-lives were 9.88, 10.5 and 11.5 days respectively at 1, 2.5 and 5 ppm levels of application. They observed that the maximum residues obtained for EC formulation was much higher than the granule formulation. The increase in the residue level up to 7 days from granules may be due to the controlled release of carbosulfan, owing to slow disintegration of granules. The half-lives obtained for granules were higher than EC formulation. It was also observed that higher the increase in the treatment level, higher the retention of carbosulfan and half-life in the soil.

Merlinkamala and Chandrasekaran (2015), noted that carbosulfan at 5 and 10 μg g^{-1} levels degraded to BDL at 75 days after application in red and alluvial soils and 90 days after application in black soil indicating the higher persistence of carbosulfan in black soil.

Longer persistence observed in black soils may also due to adsorption of carbosulfan to soil colloids. They noted that soil organic matter was the most important factor influencing the adsorption of pesticides in soil.

Garg *et al*. (2018) found that isolated soil bacteria CISH I-1 and CISH C-1 from mango (Mangifera indica L.) orchard with capability to degrade imidacloprid and carbosulfan. These strains of bacteria, were categorized as gram negative, rod-shaped and catalase positive bacteria belonging to *Pseudomonas sp*. These stains have the ability to use these insecticides as carbon source for their growth and survival. Increase in bacterial growth was recorded up to 8 and 12 days in imidacloprid and carbosulfan containing media, respectively. The HPLC analysis revealed that 97 and 91 per cent of imidacloprid and carbosulfan were degraded in soil containing CISH I-1 and CISH C-1, respectively and suggested more study for their final identification and large-scale use in mango orchard soil for post application enhancement of pesticide degradation.

2.3.4 Absorption, translocation, metabolism, persistence and dissipation of carbosulfan in plants

In an experiment, Rouchaud (1990) observed that carbosulfan, furathiocarb and carbofuran were taken up from soil by the plant with similar intensities and plant metabolized them mainly into carbofuran and 3-hydroxycarbofuran. In the plant foliage of carbosulfan or furathiocarb treated fields, the concentrations of carbosulfan or furathiocarb were similar to the ones of carbofuran simultaneously present. The insecticide compounds concentrations in the plant foliage were higher when they were higher in the soil. They found that at harvest, no residue of either carbosulfan, furathiocarb, carbofuran, or of its metabolites were observed in the “flower” of cauliflower, or in the brussels sprouts themselves.

The breakdown of the insecticide carbosulfan to its carbofuran metabolite in oranges is fast with dissipation of both pesticides in 3 days (Trevisan *et al*, 2004) and the transformation of carbofuran to 3-hydroxy-carbofuran was of low intensity or this metabolite has quickly dissipated. According to them, carbosulfan insecticide, used in citrus crops according to good agricultural practices, is acceptable for fruit consumption in compliance with the Brazilian pesticide residue legislation. 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 sufficient data to fix the MRL of carbosulfan in banana. Vijayan (2000) studied the pattern of the absorption, and degradation of carbofuran a metabolite of carbosulfan 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 namely furdan and phorate (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*. (2015).

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 this 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.

Christopher *et al.* (2001)., noted that carbofuran was found to be mineralization by pure cultures of bacteria *Arthrobacter sp*. and fungi. Microbial degradation of carbamates occurs readily in soil. Accelerated degradation of certain carbamate insecticides has led to ineffectiveness of these compounds, e.g., control of *Phyloxera* in vineyards by carbofuran. Bacterial isolates from several genera (*Arthrobacter, Achromobacter, Azospirillum, Bacillus, and Pseudomonas*) can hydrolyse various insecticidal carbamates. The persistence of carbofuran was increased when these esterase inhibitors were combined with the cytochrome P-450 inhibitor, piperonyl butoxide. This synergism is due to the inhibition of both major mechanisms of carbofuran degradation, i.e., hydrolysis and oxidation.

According to Rajeswaran *et al.* (2005)., the harvest time residues estimate in cotton seed, lint, oil and soil after three applications of carbosulfan at 15 days interval on cotton @ 250, 500 and 1000 g a.i.ha^{-1} using GLC equipped with ^{63}Ni ECD, with an interval of last spray and harvest of 60 days were found to be in below detectable limit, in all cotton samples.

In a study, Merlinkamala and Kennedy (2017) found that in brinjal fruits, the carbosulfan residues were 0.05, 0.12 and 0.27 µg g^{-1} at 15 DAT at the first harvest of the fruits after three rounds of spraying when applied at three different concentrations viz., 250, 500 and 1000 g a.i. ha^{-1} respectively. However, the residues were below detectable limit at all the levels of application at the third harvest of the produce. The residues were below the

maximum permissible residue limit (MRL) of 0.1 μg g^{-1} at the recommended dose of 250 g a.i. ha^{-1} even at the first harvest itself making it as a suitable insecticide for managing brinjal pests without any risk. They found that initial deposit of carbosulfan at doses@ 250 and 500 g a.i. ha^{-1} were 11.29, 19.75 μg g^{-1} respectively, one hour after spraying.

Geng *et al.* (2018) reported that carbosulfan was metabolized to carbofuran, dibutylamine, 3-hydroxycarbofuran and 3-ketocarbofuran and dimethoate was degraded to omethoate in cucumber fruits and leaves. The dissipation of carbosulfan, carbofuran, 3-hydroxycarbofuran and dimethoate fitted first-order kinetics well, with R^2 ranging from 0.912 to 0.992 and their half-lives were 2.6, 2.7, 2.4 and 5.2 days in cucumber fruits and 2.8, 3.0, 4.6 and 2.5 days in leaves, respectively. The estimated daily intakes of the active ingredients and their relevant metabolites were 0.1–4% of the corresponding acceptable daily intakes. Acute oral exposure to carbofuran (a metabolite of carbosulfan) represented 367% of the acute reference dose (ARfD) for 1–6-year-old Chinese children and 227% for the general Chinese population. A minimum pre-harvest interval of 12 days for carbosulfan is proposed to ensure safe consumption of cucumber. The slow dissipation rate of omethoate in cucumber reveals that a longer pre-harvest interval (≥ 27 days) is necessary to prevent dietary risk when dimethoate is applied to cucumber.

2.3.5 Residue of carbosulfan in water

Park *et al* (2009) reported presence of carbofuran at the rate 1.25 ppb (in June 2005), Fipronil - 0.78 ppb (in July) and 1.20 to 1.6 ppb (in August) in samples collected from stream water during the growing cultivation season at the Neungchon watershed (where these chemicals were in use for plant protection) in Korea. In regard to the detected pesticides, the concentration of individual pesticides measured in surface water of the study areas never exceeded guidelines for agriculture chemicals concerning water quality-effluent from paddy fields.

Kumar *et al.* (2015) worked out ground water ubiquity score (GUS) technique to predict the ability of a chemical to contaminate groundwater and was used for assessing leaching potential of pesticides in Indian soils. On the basis of estimated GUS values, atrazine, metalyxyl, imidcloprid are in the category of pesticides with the high to very high potential to contaminate groundwater. GUS is an easy tool to predict the leaching potential of pesticides in Indian soils and follow the results got from the field experiments.

Photolysis of carbosulfan is evident from the observations of Iesce *et al. (*2006), and that under sunlight, irradiated water treated with benfuracarb and carbosulfan gave off carbofuran and carbofuran-phenol, while only carbofuran was detected in the dark experiments. The latter was degraded to phenol by exposure to sunlight. Effects of pH, humic acid and KNO_3 were evaluated by kinetics on dilute solutions in the dark and by UV irradiation, which evidenced the lability of the pesticide at pH 9. Their study on the hydrolysis and photolysis of benfuracarb and carbosulfan under natural conditions gives leads that there is selective decay to carbofuran and/or phenol-4 derivative. Carbofuran is found to be more persistent and toxic. They suggested that the decay of benfuracarb and carbosulfan to carbofuran and the relative stability of carbofuran may account for the detection of carbofuran in water, fruits and vegetables.

Aravinna *et al*. (2006) reported the status of residue levels of commonly used pesticides in 228 samples collected from surface water reservoirs and shallow groundwater from 2003 to 2006 in Walawe area (WA) which is a major agricultural area in Sri Lanka. Among with monitored pesticides method detection limits (as μg / L) were 0.07, 0.004, 0.1, 0.1 and 0.1 for carbosulfan, fipronil, carbofuran, 7-hydroxycarbofuran, and 3-ketocarbofuran respectively. Pesticide residues were not detected in surface water samples which were fed by paddy cultivated catchments, sugar cane cultivated catchment and those fed by mainly non-agricultural catchment in these regions. They also reported that applied pesticides of did not reach to shallow boundary wells, located one to three metre distance from treated paddy fields. Residues of Chlorpyrifos, Dimethoate, Propanil, MCPA and 2.4D were detected in field samples but their residue levels were below detection limits at first dilution points within 100 m distance. These suggests rapid conversion of applied chemical due to environmental interaction.

Fipronil and its less mobile, but well-known transformation products viz., fipronil-sulfide (MB 45950), fipronil sulfone (MB 46136) and fipronil desulfinyl, (MB 46513) were not detected in the ground waters. Kiefer, (2019) attributed this to the high LOQs (10-50 ng l^{-1}), low mobility (log_D pH7: > 4) and to the low application amounts several years ago.

2.3.5 Aerial deposition of residues of carbosulfan

Pesticides are known to move from treated agricultural areas into the broader environment, then they can reach to the non-target organisms. Once pesticides enter the atmosphere, they may be transported long distances. Tiryaki and Temur (2010) in a review

noted that the escapes of pesticides chemicals into the atmosphere represents an economic loss to the user, inefficient control of pests and introduction of possible environmental contamination. Pesticides enter the atmosphere either by application drift, post-application vapour losses or wind erosion of pesticide treated soil. They and their photodegradation products may be transported long distances before the removal processes of atmospheric wet and dry deposition return them to the earth's surface (Cessna *et al*. 2005, Cessna *et al*. 2006).

Pesticides frequently detected in the atmosphere are (i) organochlorine insecticides: resistant to environmental degradation, (ii) organophosphate insecticides: not long-lived in environment, (iii) triazine herbicides: heavily-used herbicides, persistent in environment, (iv) acetanilide herbicides: used heavily, but not as persistent as triazine (Toth and Buhler, 2009).

Thus, the movement of pesticides from the site of application, environmental behaviour of pesticide and transformations of pesticide in the soil-plant system are a resultant interaction of soil plant atmosphere chrono sequence continuum. However, the literature speaks little about the presence of carbosulfan and its metabolites in the atmosphere. Yusa *et al.* (2019) reviewed that, carbofuran content in ambient air samples ranged from not detected to 8.1 ng m^{-3}.

2.3.7 Residues in food commodities

Bhattacherjee (2013) noted that, carbosulfan sprayed at 0.05 % to a mango hybrid (H-1000) orchard during fruit development stage, dissipated to 0.049 mg kg^{-1} in fruit peel at the time of harvest from an initial residue of 5.296 mg kg^{-1} resulting in 99 % loss after 45 days of spray. The rate of dissipation followed first-order kinetics in whole fruit (peel plus pulp) but not in fruit pulp alone. The dissipation was slower in whole fruit. Initial residue of carbosulfan in fruit pulp was 0.082 mg kg^{-1}, which increased to 0.898 mg kg^{-1} after 10 days of spraying and started decreasing slowly.

2.3.8 Non target organisms

Carbofuran on contact with skin may cause skin and eye burns (PubChem. 2005[6]). When exposed to heat or flames it may emit toxic oxides of nitrogen. It is toxic by inhalation, skin contact, and/ or ingestion. It is used as a pesticide. Carbofuran is a carbamate ester and a member of 1-benzofurans. It has a role as an EC 3.1.1.7 (acetylcholinesterase) inhibitor,

a carbamate insecticide, an EC 3.1.1.8 (cholinesterase) inhibitor, an avicide. It is a systemic agricultural insecticide, acaricide and nematicide.

In two unalike studies, Silva *et al*. (2009) and Santos and Forrer (2011) observed that the earthworms avoided the soil "Typic Hapludox" treated with different levels of carbofuran. The pesticide also affected the production of juvenile specimens in both soils. In Typic Hapludox soil with a lower organic matter content, carbofuran has more pronounced effect on different aspects of the earthworms' life, most likely due to the higher bioavailability of the pesticide in the soil solution. Even the very small amount of carbofuran residue do not assure lack of toxicity. Thus, the effect of carbofuran on evasion and reproduction was evidently higher in the soil with lower OM, as indicated. They observed larger bioaccumulation of ^{14}C-pesticides in earthworms in soils and substrates with lower organic matter content.

Thus, from the preceding discussions and the fruit and inner core of pseudostem are consumed raw or cooked and even as the leaf is used for serving the food, harmful residue of the applied insecticide should not be there in these produces. All aspects of safety also need to be assured before giving a recommendation for chemical control of pest and disease. This should also lead to production of commodities which as per the codex Alimentarius standards. Considering these aspects in cognizance present investigation was designed mainly to gather information about the dissipation and distribution of fipronil and carbosulfan and their toxic metabolites in different plant parts and soil.

3. MATERIALS AND METHODS

A randomised block design (RBD) field experiment for investigating dissipation and distribution of fipronil, carbosulfan and their metabolites in banana var. Nendran (AAB) and soil were undertaken to generate sufficient number of samples required by the laboratory for analysis as per the technical program. The study was conducted at Instructional Farm of College of Agriculture, Vellayani in the Agro ecological unit, AEU-8 of Kerala during 2016-17.

Analysis of physico-chemical properties of soil, were carried out with the facilities of Laboratory of Department of Soil Science and Agricultural Chemistry, College of agriculture, Vellayani, Thiruvananthapuram and College of Horticulture, Vellanikkara, Thrissur. Residue analysis of the samples were carried out at the All-India Network Project on Pesticide Residue Laboratory, College of Agriculture, Vellayani. Assessment of population of microorganisms *viz*., fungi, bacteria and actinomycetes were carried out in the Laboratory of Department of Agricultural Microbiology, College of Agriculture, Vellayani.

3.1 Soil analysis for physico-chemical properties and layout of field experiment

Soil samples from the selected location of field study, were collected by using a core sampler up to a depth of 15 cm from the surface randomly, representing each replication and pooled together to get a composite sample as per quartering reduction technique, retaining about 500 g of the sample after removing the stones, pebble etc. These samples were gently crushed, air dried and sieved using 2 mm sieve and used for the analysis of initial soil properties of the field. The methodology adopted for the analysis of physico-chemical characteristics of the soil are presented in the table 2.

3.2 Field studies on dissipation (absorption, translocation, distribution, persistence) of fipronil and carbosulfan in banana plant and soil

Field experiments were conducted during the period, August 2016 to April 2017. The layout of the experiment is given in fig.1. Land preparation was started on 13/8/2016 and planting was done on 27/8/2016. The crop was maintained as per package of practices recommendations, Kerala Agricultural University (POP) for variety Nendran banana planted at a spacing of 2 x 2m under irrigated condition except for the part of treatment

imposition followed as per technical program. Climatic / weather parameters data are presented in the table 7 (in chapter 4-Results). Weather during the growth period was predominantly dry. Hence irrigation was scheduled on alternate days.

Table 2. Methodology followed in the analysis of soil physico-chemical properties

Sl. No.	Soil Characteristics	Methodology	Reference
1	Particle size analysis	Hydrometer method	Bouyoucos (1951)
2	Soil reaction (pH)	1:2.5 soil water (w: v) suspension using pH meter	Jackson (1958)
3	Electrical conductivity (EC)	EC of above suspension using conductivity bridge	Jackson (1958)
4	Bulk density	Core sampler method	Gupta and Dakshinamoorthy (1980)
5	Particle density	Pycnometer	Gupta and Dakshinamoorthy (1980)
6	Water holding capacity	Core sampler method	Gupta and Dakshinamoorthy (1980)
7	Organic carbon	Walkley and Black	Jackson (1958)
8	Available P	Bray No1 extraction*	Jackson (1958)
9	Exchangeable K	Neutral normal ammonium acetate extraction*	Jackson (1958)
10	Exchangeable Ca and Mg	Neutral normal ammonium acetate extraction*	Jackson (1958)
11	Available S	0.01% $CaCl_2$ extraction followed by turbidimetric estimation method	Tabatabai (1982); Massoumi and Cornfield (1963)
12	Available Fe, Zn, Mn, and Cu	0.1 M HCl extraction*	Sims and Johnson (1991)
13	Available B	Hot water extraction*	Jackson (1958)

* Extraction followed by instrumental analysis using ICP-OES.

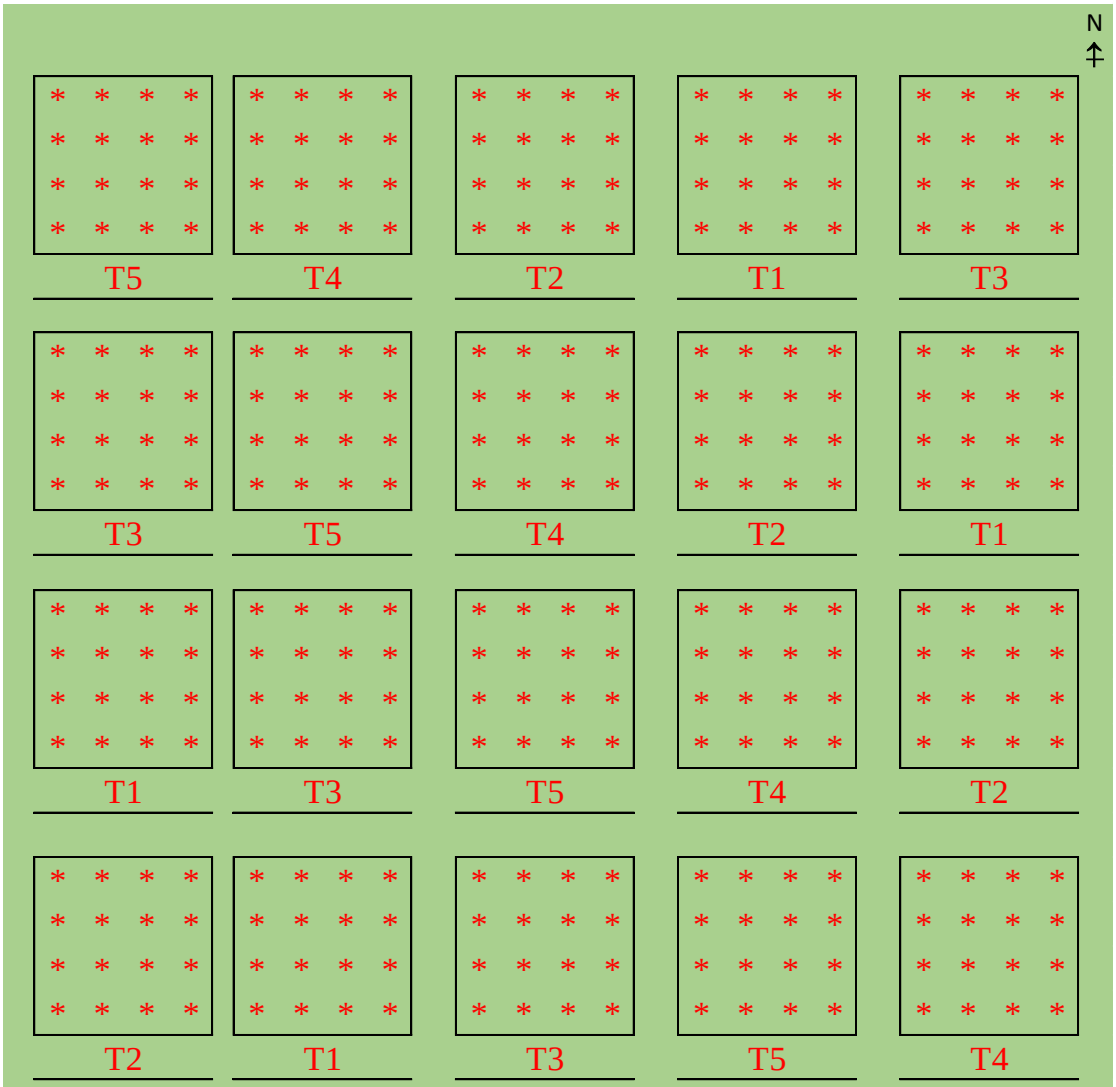


Fig.1. Layout of the experiment

Field particulars, design of experiments and treatment imposed are as given below.

Location : College of Agriculture, Vellayani,
Soil type : Red loam
Crop : Banana
Variety : Nendran
Design : RBD
Treatments : 5
Replication : 4
Plot size : 8m x 8m (for 16 plants)

Treatments:

T_1- Absolute control (No application of carbosulfan and fipronil)

T_2- Recommended practices (RP_f) of 30 mg *ai* of fipronil per plant, applied thrice viz., on 0, 60 and 150 days of planting.

T_3– Double dose of RP i.e., RP_f x 2, applied as per above schedule of T_2.

T_4- Recommended practice of 400 mg ai of carbosulfan per plant, applied thrice, on 0, 60 and 150 days of planting (RP_c) (KAU, 2011).

T_5- Double dose of RP i.e., RP_c x 2, applied as per above schedule of T_4

3.2.1 Method of soil application:

"Agricultural insecticide grade" commercial fipronil granules i.e., "Regent 0.3 GR (0.3% w/w)" manufactured and marketed by Bayer Crop Sciences India Ltd, were procured and applied at specific dose calculated as per the treatment schedule. Similarly, agricultural insecticide grade commercial carbosulfan granules i.e., Sheriff 6G (6 % w/w) manufactured and marketed by FMC India limited were procured and applied as per the schedule of treatment.

At the time of planting, granules were mixed with 100 g of air-dry soil and spread uniformly around the corm. The granules were mixed with 100 g of air-dry soil and applied around the basin at a radial distance of 45-50 cm around the plant on the 60^{th} and 150^{th} days of planting.

For T_2, a single dose weighing 10 g of Regent per plant was applied at the time of planting, 60 days after planting and 150 days after planting. For T_3 double dose weighing 20 g each of Regent 0.3 G was used.

For T_4, a single dose weighing 6.66 g of carbosulfan 6G formulation i.e., sheriff 6G was applied at the time of planting, 60 days after planting and 150 days after planting. For T5 double dose weighing 13.32 g each of sheriff 6 G was used.

Sufficient number of additional plants were maintained in each plot to undertake the experiments as per repeat application of treatment after bunching described under following para 3.3 and 3.4.

3.2.2 Repeat application of a dose after bunching

At the time of complete emergence of bunches, 4 plants from those additionally maintained for the purpose in each plot were applied with an additional dose of last (3^{rd}) application of T_2, T_3, T_4, and T_5 levels of carbosulfan and fipronil. Residues in blossom bud, flower bract alone, bunch (on 15^{th} day of emergence), bunch (on 30^{th} day of emergence), bunch on harvest, peel and edible portion of the pseudo-stem and corm were analysed. Residues in bunches were separately analysed in peel and pulp samples which were collected after 30 days of bunching and harvest.

3.2.3 Pseudostem injection after bunching

In addition, eight banana plants were also maintained in the field (as per para 3.2). Among these, 4 plants each were injected with five times the recommended dose (2000 mg a.i.) of carbosulfan and (150 mg a.i.) of fipronil. This dose was administered two weeks prior to bunch emergence at a height of 1m above the ground level. Measured quantity of the pesticide was injected very slowly into the central portion of the pseudostem ensuring no outward flow of injected liquid, using a multi aperture injection syringe, made for the purpose, procured from Central Tuber Crop Research Institute, (CTCRI, Sreekaryam, Thiruvananthapuram) (see Plate 1. in Appendix 1). Samples of bunches were collected starting with the complete emergence of bunches at 15 days intervals and for residues analysis. Peel and pulp of the banana fruits were separately analysed for residue.

3.3 Leaf sample collection and analysis of residues

The 1^{st}, 2^{nd}, 3^{rd} and 4^{th} leaves from the last fully opened leaf in the apex of crown were sampled and separately labelled and packed for estimation of residues. The sampling days were 0_i, 0_{2h}, 1, 3, 5, 7, 10, 15, 20, 25, 30, 40 and 50 days after last application. Day 0_i indicate the initial sample taken before application and 0_{2h} is the sample taken 2 hours after application. Thus, two samples were taken on day 0. The fingers from bunches were collected on 15^{th} and 30^{th} day after complete emergence of effective bunches and then on final harvest of bunches. The blossom bud (flower bud), central core of pseudo stem, bunches at harvest and rhizomes at harvest were collected from each treatment for the analysis of residues. The collected samples were packed in polythene sheets after wiping with pH neutral tissue papers and residues were extracted in suitable solvent system and

Plate 1. Leaf and soil sample collection

methodology as described under 3.5

3.4 Soil sample collection

Soil samples for residue analysis were collected by using a core sampler at 15 cm radial distance from the periphery of each plant, from every treatment and each replicate on $0_{i,}$ 0_{2h}, 1, 3, 5, 7, 10, 15, 20, 25, 30, 40, and 50 days of application on 150^{th} day of treatment imposition to banana and analysed for the respective residues. After removing pebbles, cobble if any, a 500 g soil sample was collected from which 250 g was used for estimation of residues. The extraction was accomplished using a standard procedure as described in para 3.6. The rest 250 g was used for estimation of enzymes like phosphatase, urease, and dehydrogenase. The population of earth worms, carabids and microorganisms *viz*., fungi, bacteria and actinomycetes were evaluated on 10^{th} day after the 2^{nd} application (60^{th} day of planting) of treatments.

3.5 Method validation and residue analysis

The methodology for residue estimation of carbosulfan and fipronil were standardized by using samples spiked with pure analytical standards of these chemicals followed by extraction and clean up. For this purpose, validation of experimental residue analytical methods was conducted by comparing a conventional extraction method adopted by Paul *et al*. (2015) for plant samples and Varghese *et al.* (2016) for soil samples against modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) method by Lehotay *et al.* (2005).

3.5.1 Laboratory glasswares, Chemicals and reagents

For performing residue analysis of fipronil, carbosulfan along with their metabolites in the laboratory, all the glasswares used were of Class A grade with certification, where-ever possible. All the standard flasks, pipette, burette and weighing balances were calibrated for ensuring required precision. For initial soil physical-chemical characterisation, AR grade chemical reagents were used.

All glasswares used for analysis were washed initially with tap water and were immersed overnight in 1 per cent laboline and then again washed with tap water. After that, these were dipped in boiling water for 2 hrs and rinsed with acetone and were kept in an oven at 50^{o}C for 3 hrs for drying. The plastic tubes were similarly washed and kept at room

temperature for drying. Syringes were thoroughly pre-rinsed with acetone and then methanol before use. The solvents and other reagents used for residue analysis were either of HPLC / LC-MS/MS or AR grade. Sodium sulphate, sodium chloride and magnesium sulphate were activated prior to use. All equipment and instruments were calibrated to meet performance criteria. List of, solvent, reagent, lab wares and equipment used in the laboratory are given in table 3.

Table 3. List of solvent, reagent, lab wares and equipment with their respective grade

Laboratory glasswares	**Chemical reagents (from E Merck)**	**Equipment**
Beakers 100, 250 and 500 ml	Acetone HPLC grade	Analytical balance with precision 0.00001mg
Centrifuge tubes 15 ml and 50 ml	Acetonitrile LCMS/MS grade	Laboratory centrifuge
Class A pipettes 0.5 ml, 1ml, 2ml, 10ml and 20 ml	Magnesium sulphate (anhydrous) AR grade	Mechanical shaker
Conical flasks and standard flasks Class A 250 ml, 500 ml and 1 l	Methanol LCMS/MS grade	Vortex shaker
Graduated test tubes 5 ml and 10 ml	Primary Secondary Amine	Turbo Vap Evaporator
Class A Micropipettes 100µl, 1 ml and 5 ml	Sodium Chloride (AR grade)	Rotary vacuum flash evaporator
Separating funnels 750 ml and 1 l	Ammonia solution HPLC grade	Ultra-performance Liquid Chromatograph-Mass spectrometer / Mass spectrometer
Hypodermic syringe 10 ml and Randisc PDVF filters (E-Merk)	Dichloromethane HPLC grade	
Hypodermic syringe 20 ml and with special 3 bore holes at equal-distance in the needle	Sodium Sulphate (anhydrous)	
	Calcium Chloride (anhydrous)	
	Celite and Florsil-Chromatography grade)	

3.5.1.1. Procurement of Certified Reference Material (CRM)

Certified reference materials of insecticides used for quantitation in the study were obtained from various sources as detailed below:

Table 4. List of certified reference materials

Sl. No.	Molecule and details	CAS no / Grade if any	Source of supply
1	Fipronil desulfinyl (98.5 % w/w)	111246-15-2	Bayer Crop Science, Germany
2	Fipronil (98.4 % w/w)	120067-37-3	Sigma Aldrich, Switzerland
3	Fipronil sulphide (98 % w/w/)	120067-83-6	Bayer Crop Science, Germany
4	Fipronil sulfone (99.7 % w/w)	120068-36-2	Bayer Crop Science, Germany
5	Carbofuran (99.9 % w/w)	1563-66-2 Pestanal®	Sigma Aldrich, Switzerland
6	Carbofuran-3-Keto (99.5 % w/w)	16709-30-1 Pestanal®	”
7	Carbofuran-3-hydroxy (98.0 % w/w)	16655-82-6 Pestanal®	”
8	Carbosulfan (98.5 % w/w)	55285-14-8 Pestanal®	”

A volume of 250 ml of analytical standards of 400 ppm concentration were prepared by accurately weighing the CRM considering their percentage purity.

3.5.2 Preparation of mixture of insecticide standards

The steps involved in the preparation of standard mixture of fipronil, its metabolites and carbosulfan and its metabolites were as follows.

3.5.2.1 Preparation of standard solution

Standard stock solutions of fipronil, its metabolites, carbosulfan and its metabolites (400 mg l^{-1}) were prepared separately in methanol and stored at -20^{o}C in deep freezer. The amount of CRM required for preparation of the standard solution was calculated using the formula:

Weight of CRM in mg

$$= \frac{\text{Required concentration of CRM in mg l}^{-1} \times 100 \times \text{Required volume in ml}}{\text{Percentage purity of CRM} \times 1000}$$

3.5.2.2 Intermediate stock solution and working standards

Intermediate standards of 100 mg kg^{-1} of fipronil and carbosulfan and their metabolites were prepared by diluting the required quantity of stock solution with methanol.

Aliquots of intermediate standards were taken in separate standard flasks in order to prepare working standards of 10 mg kg^{-1} of each compound. From this working standard (10 mg kg^{-1}), a mixture of all these compounds were prepared in methanol solvent and stored in refrigerator for further use. From this, a series of working standards were prepared to obtain 1, 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01 mg kg^{-1} concentrations making up the final volume with matrix match preparation for respective samples. The individual standards of different insecticides were injected in the Liquid Chromatography-Mass Spectrometer and a calibration curve was prepared by plotting concentration *vs.* peak area.

3.5.3 Recovery Experiment

Laboratory experiments were carried out to ascertain the accuracy, RSD value, linearity and limit of quantitation (LOQ) of the methods followed for estimation (viz., QuEChERS and conventional) and to ascertain the method to be followed for extraction or residues analysis from the field samples.

In general, the same procedure / steps were adopted for estimation of residues in all samples, both in field studies and in recovery studies. In both cases, matrix matched standards were prepared out of matrix extracted from respective samples (viz., soil, leaves, bunches, flower bud, inner core of pseudo-stem, and corm), collected from additional plants grown under control treatment protocol and adopting the procedures for extraction and clean-up of the sample matrix. For these matrix matched standards, no concentrating procedures were adopted. Thus, QuEChERS method and conventional method using dichloromethane/acetone extraction matrices were prepared. Before the sampling date, sufficient quantities of such matrices were prepared and stored at -20°C for next day's use. Detailed procedures adopted are described in the following paragraphs.

A recovery experiment was conducted to standardize the procedure for extraction and clean up processes. The experiment was conducted by adding a known quantity of insecticide mixture to the sample matrices and trying the extraction process using QuEChERS method and conventional method using dichloromethane /acetone extraction as described in para 3.5.3.1.1 to 3.5.3.4.2

3.5.3.1.1 QuEChERS method of extraction and clean-up from plant sample

Additional plants maintained in the control plots were used for collection of different sample matrices. Sufficiently before the first harvest, from each sample type, 500 g (on fresh weight basis) was collected randomly, so as to complete the recovery and matrix match study. This was used for spiking at various levels in the recovery study. The samples were extracted and cleaned up as per the QuEChERS method for estimating the residues of the insecticides (Lehotay *et al*., 2005). The samples were cut into small pieces of 250 g per replicate and it was macerated in a blender. Ten grams of the ground sample was then taken from each replication in a 50 ml centrifuge tube, 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 minutes. Activated sodium chloride (4.5 g) was added to the homogenised sample and vortexed for 2 minutes on a rotospin.

3.5.3.1.2 QuEChERS method of clean-up from different matrix after extraction

Subsequently, the above contents were centrifuged at 2,500 rpm for 5 minutes. An aliquot of 12 ml clear upper layer of the sample was transferred into a 50 ml centrifuge tube prefilled with 5 g pre-activated sodium sulphate and vortexed for 2 minutes for removing traces of moisture, if any. The extract was cleaned up by dispersive solid phase extraction (DSPE).

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, 0.05 g end capped C18 and 0.025 g GCB. The mixture was again vortexed for 2 minutes and centrifuged for 5 minutes at 2,500 rpm.

3.5.3.1.3 Concentration of the cleaned-up extract

From the supernatant liquid extract cleaned as per para 3.5.3.1.2, 5ml was transferred to turbovap tube and evaporated to dryness at 40 °C and 7.5 psi nitrogen flow under a gentle stream of nitrogen using turbovap setup. The residue was then reconstituted in 2 ml of methanol and filtered through a 0.2-micron PVDF syringe filter (13mm) for UPLC-MS/MS analysis.

3.5.3.2 Conventional method of extraction and clean-up from plant samples

Another set of control sample of all matrices were extracted following sequential extraction using acetone. A 25 g macerated sample of these matrices was sequentially extracted thrice using 50 ml of acetone each time. The extract was filtered through Whatman No-40 filter paper to a 250 ml stoppered jointed conical flask each time. The combined extracts were concentrated in a rotary vacuum flash evaporator and the aqueous residue was transferred to a 250 ml separatory funnel, and diluted with 100 ml distilled water after addition of 10 g NaCl.

The liquid phase extraction was carried out by three sequential extractions using 50, 25 and 25 ml each of dichloromethane (DCM). The lower DCM was collected by passing through anhydrous sodium sulphate in a 250 ml jointed conical flask.

The combined DCM extract was concentrated in a rotary vacuum flash evaporator at 50 °C till the entire DCM fraction was removed. It was then made up to 2ml using HPLC grade methanol for estimation of residues using LC-MS/MS.

3.5.3.3. Extraction of residues from soil by QuEChERS and conventional method

3.5.3.3.1 QuEChERS method

Soil samples (1 kg) collected from control plots were mixed and dried in shade and sieved through 2 mm sieve, from which 10 g is used for recovery study. The soil samples were extracted using acetonitrile by QuEChERS method as well as by acetone extraction method and the efficiency of extraction was assessed and compared for adoption in the study. Here also matrix matched working standards were prepared.

QuEChERS method employs acetonitrile extraction of spiked pesticides from soil. For this purpose, 10 g of air dried, sieved (2 mm) soil was weighed in 50 ml centrifuge tube and spiked with the standard insecticide mixture and evaporated to release the solvent vapours. The soil samples were spiked with 0.01, 0.02 and 0.05 and 0.10 ml each of 10 mg kg^{-1} solution to get 0.01, 0.02 0.05 and 0.10 mg kg^{-1} levels of each of the spiked compounds. To this, 4 g magnesium sulphate, followed by 1 g sodium chloride and 20 ml acetonitrile were added, shaken for 2 minutes in a vortex shaker and was centrifuged for 4 minutes at 3300 rpm. From this a 10 ml supernatant was transferred to a 15 ml centrifuge tube using a micropipette and 0.25 g primary secondary amine and 1.5 g magnesium sulphate were added and was shaken for 30 seconds in a vortex followed by centrifugation at 4400 rpm for 10 minutes. After the centrifugation, 4 ml of the cleaned supernatant extract was transferred to a turbo tube and evaporated to dryness at 40°C using turbovap with nitrogen flushing. The dry residue was re-dissolved in methanol and the volume made up to 1 ml, filtered through 0.22 µm PVDF syringe filter and passed to a vial which was wrapped with parafilm to avoid evaporation.

3.5.3.3.2 Extraction of residue by conventional method from soil

Another set of spiked samples were also extracted in a similar manner using acetone followed by partitioning of the concentrated extract with Dichloromethane and finally made up in methanol for estimation using UPLC-MS/MS.

Soil sample weighing 25 g was taken in 100 ml conical flask and spiked with a known concentration of pesticide standards. Residues were extracted sequentially thrice using 50 ml acetone, 50 ml acetone hexane mixture (1:1) and 50 ml n-hexane. The extracts were collected in a 250 ml conical flask and combined extracts were concentrated using RVFE at 50°C. The concentrated extract is transferred to a 250 ml separatory funnel by repeated washing with 100 ml distilled water, followed by an addition of 10 g NaCl. The aqueous layer was extracted sequentially thrice using 25 ml DCM every time. Each time the DCM layer was collected in a 250 ml joint conical flask after passing through a funnel packed with 10 g anhydrous sodium sulphate. The DCM fraction was concentrated to dryness in a nitrogen evaporator using a jet of N gas and was made up to 2 ml using HPLC grade methanol, from which residue estimation was performed using UPLC-MS/MS.

3.5.3.4 Recovery studies by fortification of plant samples with standard insecticide mixture

3.5.3.4.1 Recovery studies by fortification of plant samples with carbosulfan standard mixture

Fresh plant sample weighing 500 g (viz., leaves, bunches, flower bud, pseudo-stem and corm as the case may be) were collected from the additionally maintained plants in the control plot. The samples were macerated in a high-speed blender and from these, 28 samples of 10 g each were taken separately in labelled 50 ml centrifuge tube. The samples were spiked at 4 different levels in six replicates with one un spiked control for each level of spiking. Samples were spiked using 0.01, 0.02 and 0.05 and 0.10 ml each of 10 mg kg^{-1} of the standard mixture of carbosulfan to get 0.01, 0.02 0.05 and 0.1 mg kg^{-1} levels of each of the spiked compounds, respectively. Extraction, clean-up and concentration of these were done by using procedures as per para 3.5.3.1.1 to 3.5.3.1.3. The final residue was then reconstituted in 1.5 ml methanol for analysis using LC-MS/MS to find out the recovery of each of the spiked compounds by this method. Separate sets for different samples were prepared and analysed following the procedure for QuEChERS and conventional method.

Another set of samples were spiked similarly for assessing the efficiency of extraction of fipronil and its metabolites from various plant samples from banana, after spiking at four levels in six replicates.

3.5.3.4.2 Recovery studies by fortification of soil samples

Precisely weighed 10 g each of air dried, sieved (2 mm) soil samples, collected from the identified control plots of the experimental area, were taken for the recovery study of carbosulfan and fipronil from soil. A total of 28 samples, each of 10 g were collected in 50 ml centrifuge tubes. A set of six centrifuge tubes each from these were spiked separately with 0.01, 0.0.2 and .05 ml and 0.10 each of 10 mg kg^{-1} working standard mixture of carbosulfan to get 0.01, 0.02, 0.05 and 0.1 mg kg^{-1} levels of spiking, respectively. The spiked soil was thoroughly shaken for 10 minutes to ensure uniform homogenization. After one hour extraction, clean up and concentration, the spiking of these samples was carried out following the procedure described as in para 3.5.4.3.2.

Another set of soil sample were spiked similarly for assessing the efficiency of extraction of fipronil and its metabolites from soil samples, after spiking at four levels in six replicates.

3.5.4. Estimation

The cleaned extracts were analysed on an Ultra Performance Liquid Chromatograph equipped with Triple Quadrupole Mass Spectrometer (Sciex- API 3200). The samples as well as standards were injected into the equipment for spectral matching and quantification of residues.

3.5.4.1. *LC-MS System*

The ACQUITY UPLC system was used for chromatographic separation with a column size of 15 cm length maintained at 40°C. Elution was done using two eluents (solvent mixtures), *viz.*,

A: 10 per cent methanol in water + 0.1 per cent formic acid + 50 mM ammonium acetate

B: 10 per cent water in methanol + 0.1 per cent formic acid + 50 mM ammonium acetate

The flow rate remained constant at 0.8 ml min^{-1} and the injection volume was 10 µl.

The effluent from LC was then introduced 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 5000 V. The residues were quantified in MS/MS system. For each analyte, two Multiple Reaction Monitoring (MRM) transitions were taken.

3.5.4.2 *Residue Quantification and Recovery Experiment*

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

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

Dilution factor (DF) =

$$\frac{\text{Volume of solvent added} \times \text{Final volume of the extract}}{\text{Weight of sample} \times \text{Volume of extract taken for concentration}}$$

$$\text{Percentage Recovery (\%)} = \frac{\text{Concentration of pesticide residue recovered} \times 100}{\text{Concentration of pesticide residue added}}$$

$$\text{Relative Standard Deviation (RSD)} = \frac{\text{Standard deviation} \times 100}{\text{Mean Recovery}}$$

A method with recovery percentage in a range of 80-120 % and the Relative Standard Deviation (RSD) value < 20 % were selected for the study (Beevi *et al.*, 2014). Limit of quantitation (LOQ) and Limit of Detection (LOD) were also calculated for both the methods. The concentration of fipronil and carbosulfan and their metabolites in the sample were derived from the calibration curve and chromatogram of the standards of respective compounds.

3.6 Analysis of experimental field samples

Fresh samples of the plant parts, each weighing 250 g (viz., leaves, bunches, flower bud, pseudo-stem and corm as the case may be) were collected on 0_i, 0_{2h}, 1, 3, 5, 7, 10, 15, 20 25, 30, 40 and 50 days after 3rd application of treatments. From this 10 g sample in triplicate were taken for estimation of residues. After thorough mixing, the sample residues were extracted using 20 ml acetonitrile. The rest of the procedures are same as in para 3.5.3.1.1 and 3.5.3.1.2. The final residue was then dissolved, reconstituted and volume made up with 1.5 ml of methanol.

Soil samples weighing 500 g were collected from the rhizosphere of banana at 2 spots lying radially opposite, 50 cm from the banana plant. These were sieved and analysed for residues of either carbosulfan or fipronil on 0_i, 0_{2h}, 1, 3, 5, 7, 10, 15, 20 and 25, 30, 40 and 50 days of application following the procedures outlined in para 3.5.3.4.3. The residue data were used for assessing the persistence of the compound.

3.7 Effect of carbosulfan and fipronil residues on soil fungi, bacteria and actinomycetes

Growth of fungi, bacteria and actinomycetes under the influence of treatments were studied by sampling the soil on 10th day after application of second dose of treatments imposed on 60th day of planting. These samples were subjected to serial dilution technique (Johnson and Curl, 1972). The dilutions, four numbers each per replication were inoculated to respective media plate in petri plates under aseptic conditions in laminar air flow chamber. Plates were incubated at 28^{o} C till maximum formation of countable colonies without visible

distortions. Numbers of colonies formed were counted using a colony counter. Details of the media for respective micro-organism are given below.

Table 5. Details of media used in serial dilution technique

Sl. No.	Micro-organism	Dilution from which counts are made	Media used
1	Bacteria	10^{-4}, 10^{-5} and 10^{-6}	Nutrient agar medium (Timonin, 1940)
2	Fungi	10^{-2}, 10^{-3}, and 10^{-4}	Martins' Rose Bengal agar medium (Martin, 1950)
3	Actinomycetes	10^{-2}, 10^{-3}, and 10^{-4}	Ken knight agar medium (Timonin, 1940)

3.8 Effect of carbosulfan and fipronil residues on soil enzymes urease, phosphatase and dehydrogenase

The activity of enzymes viz., urease, phosphatase, dehydrogenase as influenced by the application of treatments were studied using enzyme assay method by sampling the soil on the 10^{th} day after application of second dose of treatments imposed on 60^{th} day of planting.

Urease enzyme activity was measured by estimating the amount of urea hydrolysed in μg g^{-1} of soil per hour by using the procedure described by Broadbent *et al*. (1958).

Acid phosphatase activity of the soil was determined by using the method adopted by Tabatabai and Bremner (1969) by measuring the p- nitrophenol released (in μg) per gram of soil per hour after the addition of p-nitrophenyl phosphate to the substrate containing soil.

Dehydrogenase activity of the soil was measured in terms of μg of triphenyl formazan formed per gram of soil after the addition of triphenyl tetrazolium chloride followed by and incubation for 24 hours as described by Casida et al. (1964).

3.9 Effect of carbosulfan and fipronil residues on soil *viz*., earthworms, carabids by enumeration of macro-organisms

The activity of earthworms as influenced by the application of treatments was studied by sampling the soil on the 10^{th} day after application of second dose of treatments imposed on 60^{th} day of planting. Following two methods were used for counting the number of earthworms per unit area

a) Direct observation in treatment plots in a volume of soil collected from 1 x 1 x

0.2 m (length x breadth x depth). The soil samples were replaced after counting the actual numbers of earth worms

b) Cow dung slurry-soaked jute bag method. Jute bag of size 0.6 m x 0.6 m dipped in cow dung slurry for 30 minutes was placed on the opposite side of above proposed method area (para 3.9.a) on previous evening of the 10th day and observation was taken for the soil collected from the jute bag spread area at a depth of 20 cm.

Effect of application of treatments on carabid population was studied for a period of 10 days by observing the daily counts of carabids fallen on a plastic cup trap placed at ground level around plants under different treatments imposed on the 60th day of planting.

3.10 Incidence of pest and diseases if any will be monitored for adopting control measures as per KAU, 2011

3.11 Biometric observations

3.11.1 Plant growth characters

Plant growth characters were observed and recorded from the experimental plot at fortnightly intervals up to the emergence of bunch and at the harvest stage.

3.11.1.1 Height of the plant

Height of the plant was measured in centimetres from the base of the stem of the soil level up to the axle of the youngest leaf.

3.11.1.2 Girth of the pseudo stem

Girth of pseudo stem was taken at 20 cm above the ground level by measuring the circumference.

3.11.1.3 Number of active leaves per plant

Number of fully open functional leaves per plant was counted and recorded.

3.11.1.4. Length and breadth of the 4th active leaf

Length and breadth of the fully opened 4th active leaf from the top of the plant are measured from which length to breadth ratio was worked out.

3.11.1.5 Number of days for appearance of the bunch

Number of days for appearance of the bunch at terminal axis of the plant from the date of plating was noted.

3.11.1.6 Weight of the blossom buds

Weight of the Blossom buds (flower buds) just after the completion of opening of economically viable finger hand bearing bracts / floret (visual assessment).

3.11.1.7 Weight of bunch, rhizome (corm) and pseudo stem

Weight of bunch, rhizome (corm) and pseudo stem were noted and expressed in kilogram.

3.12 Statistical analysis

Analysis of variance, critical difference and coefficient of variation, DMRT for randomized block design (RBD) as applicable to the tabulated data as per Gomaz and Gomaz (1984), were followed for

a) the biometric observations,

b) effect of carbosulfan and fipronil residues on soil enzymes urease, phosphatase, dehydrogenase, soil fungi, bacteria and actinomycetes.

4. Results

Results of the experiments on the dissipation, metabolism and persistence of fipronil and carbosulfan in banana, cv. Nendran (AAB), and in soil and its impact on soil organisms in red loamy soil conducted at College of Agriculture, Vellayani are presented in this chapter. Technical supervision and application of treatments were ensured as per approved technical program presented in the materials and methods (para 3.5)

Physico-chemical properties of soil, are presented in the table-6. Results of residue analysis of the respective samples are presented in various tables under section 4.1 to 4.10. Results of effect of treatment on population of macro and microorganisms *viz*., fungi, bacteria and actinomycetes, are presented under section 4.6.4.

4.1 Soil analysis for physico-chemical properties

The physico-chemical analysis of the soil revealed that the surface soil is sandy loam in texture. It can be classified as kaolinitic isohyperthermic, typic kandiustults (GOK, 2007). Soils of the experimental plots are moderately acidic with a pH of 5.9. The organic carbon content was found to be 1.5 per cent. Available P and K were high, and found to be 196.1, 358.4 kg ha^{-1} respectively. Extractable Ca and Mg were recorded as 39 and 90.3 mg kg^{-1} and available S was 11.70 mg kg^{-1}. Essential micronutrients viz., HCl extractable Fe, Zn, Mn, Cu and hot water extractable B content of the soil was 217.8, 3.8, 11.4, 1.5 and 0.4 mg kg^{-1} respectively**.**

The electrical conductivity of the site of experiment was 0.4 dS m^{-1}**.** The bulk and particle densities of the soil**s** were 1.24 and 2.39 Mg m^{-3} with a water holding capacity of 31.92 per cent. The coarse sand and fine sand content of the soil together constituted 71.8 per cent. The silt and clay contents were 8.7 and 19.5 per cent respectively. The soils of the experimental plot, thus grouped under sandy loam, found to be deficient in secondary nutrients viz., Ca, Mg and micronutrient B were managed as per package of practices recommendation crops- KAU, (2016).

Table 6. Soil properties of the experimental plots (Average values n=4)

Sl. No.	Property	Unit	Mean
1	pH		5.9
2	Electrical conductivity	dSm^{-1}	0.4
3	Bulk density	$Mg\ m^{-3}$	1.24
4	Particle density	$Mg\ m^{-3}$	2.39
5	Water holding capacity	%	31.92
6	Coarse sand	%	52.20
7	Fine sand	%	19.60
8	Silt	%	8.70
9	Clay	%	19.50
10	Textural class	Sandy loam	
11	Organic carbon	%	1.5
12	Available P	$(kg\ ha^{-1})$	196.1
13	Available K	$(kg\ ha^{-1})$	358.4
14	Exchangeable Ca	$(mg\ kg^{-1})$	393
15	Exchangeable Mg	$(mg\ kg^{-1})$	90.3
16	Available S	$(mg\ kg^{-1})$	11.70
17	Available Fe	$(mg\ kg^{-1})$	217.8
18	Available Mn	$(mg\ kg^{-1})$	3.8
19	Available Zn	$(mg\ kg^{-1})$	11.4
20	Available Cu	$(mg\ kg^{-1})$	1.5
21	Hot water extractable B	$(mg\ kg^{-1})$	0.4

Weather parameters viz., maximum, minimum, mean temperature in °C, rainfall in mm received and pan evaporation recorded at fortnightly intervals are presented in the table-7. The trends of weather parameters are presented in fig 2.

During the growth period, only 186.7 mm rainfall was received and cumulative evapotranspiration was as high as 869.9 mm. Hence irrigation was scheduled on alternate days at the rate 40 l/plant, except on rainy days during the period of the experiment.

Table 7. Weather parameters during the experimental period

Time interval in fortnightly interval and at harvest	Maximum temperature (°C)	Minimum temperature (°C)	Rainfall (mm)	Evaporation (mm)
2	31.7 (30.8-32.2)	24.5 (24.0-24.8)	2.2	55.7
4	31.8 (31.0-32.2)	24.6 (24.2-24.8)	2.6	59.9
6	31.7 (31.4-32.0)	24.5 (24.4-24.8)	0.0	59.5
8	31.8 (30.8-32.4)	24.3 (23.6-24.6)	12.0	58.8
10	31.7 (31.2-32.4)	24.0 (23.0-24.4)	30.0	50.4
12	31.8 (30.4-32.6)	24.1 (24.2-24.4)	2.0	50.5
14	32.1 (32.6-32.8)	23.8 (24.2-24.7)	34.2	42.7
16	31.5 (32.0-32.2)	23.5 (22.9-)24.7	9.8	50.6
18	33.0 (31.6-34.2)	23.8 (23.0-25.2)	18.8	54.3
20	32.7 (31.8-33.8)	22.7 (21.0-23.7)	0.0	51.0
22	32.5 (31.6-33.2)	22.6 (19.1-24.8)	6.0	51.3
24	31.9 (31.2-32.4)	21.4 (19.8-24.1)	10.0	57.8
26	33.0 (31.6-33.6)	21.7 (19.1-24.9)	0.0	66.4
28	33.1 (32.2-34.4)	24.3 (22.5-25.2)	59.1	62.2
30	33.3 (32.6-33.6)	24.3 (22.9-25.3)	0.0	62.6
Harvest 31st week	34.0 (33.8-34.2)	24.5 (23.9-25.6)	0.0	36.2
Total			**186.7**	**869.9**

Values in parenthesis represent range. * -Average

Plate 2. Mass spectrum of carbosulfan and its metabolites

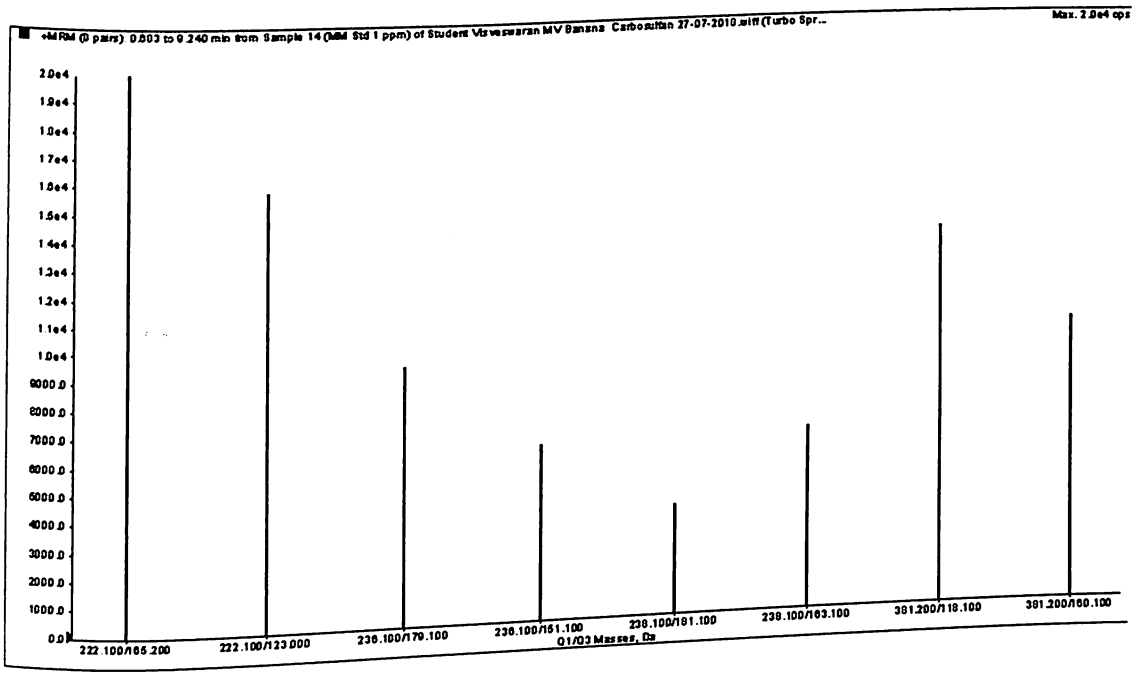


Plate 3. Mass spectrum of Fipronil and its metabolites

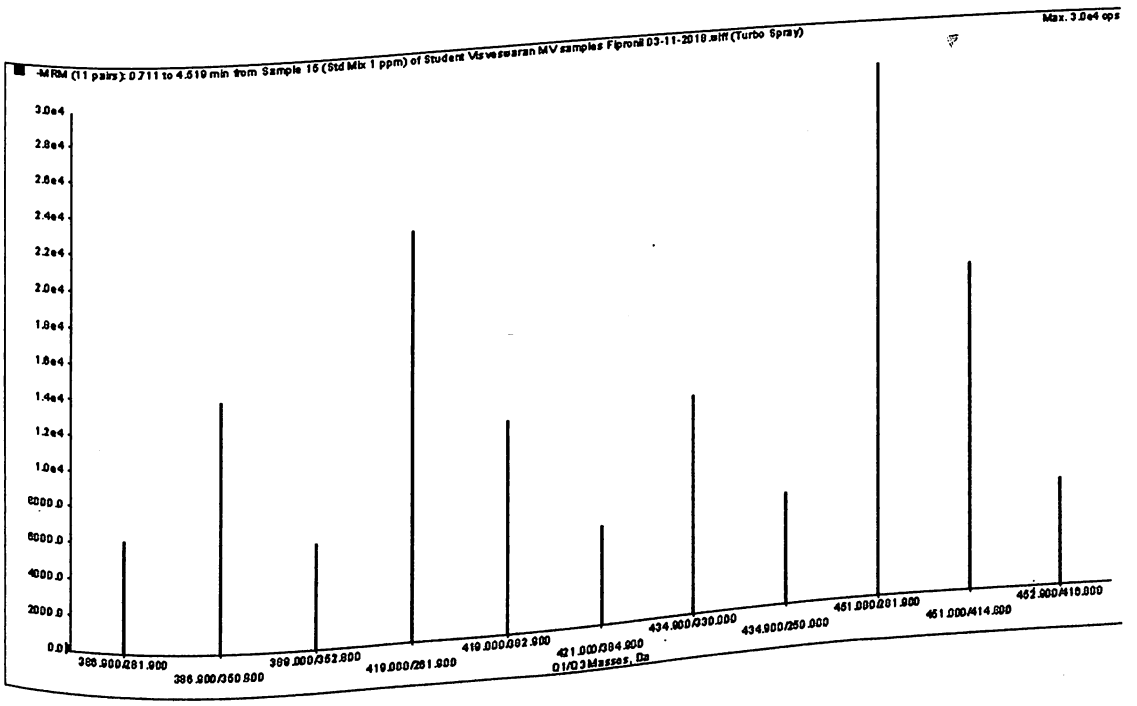
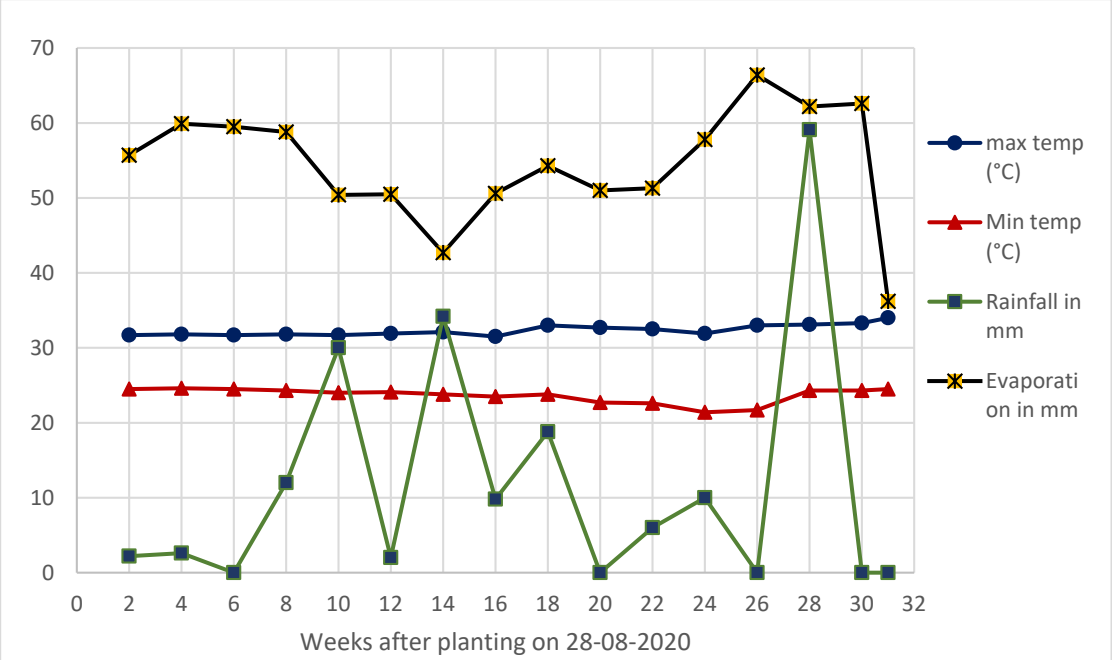


Fig. 2: Weather parameters during the experimental period



4.2 Method validation and optimization of LC-MS/MS parameters for estimation of fipronil residues from banana and soil

The methodology for residue estimation of fipronil and its metabolites from various samples types were standardized by using control samples spiked with pure analytical standards of fipronil desulfinyl, fipronil, fipronil sulphide and fipronil sulfone followed by extraction and clean up using suitable techniques.

The sample matrix extracted from different sample types viz., banana leaves, pseudo-stem, bunch finger, flower, corm and soil from control plots were utilised for the purpose before and at respective stages of the sampling from the control plots of field experiments. Percentage recovery, RSD value, linearity and repeatability and limit of quantitation (LOQ) for each of the compounds tried are presented in tables 11 to 15.

4.2.1 Optimization instrument parameters: UPLC gradient elution for flow rate for fipronil and its metabolites

Multi composition mobile phase constituents in the binary reservoirs of the UPLC denoted as A* and B** of instrument at variable proportions of pumping pressure in time sequence for fipronil are presented as table 8. The optimized gradient elution for flow

rate of the mobile phase solvent system with an initial flow rate of 0.75 ml / min was obtained with 80 per cent flow from reservoir A* and 20 per cent from B**. The gradient elution of the compound mixture was monitored for 8 minutes at differential flow rates as shown in table 9. Fitting injection volume at optimized performance was found to be 10 µl.

Table 8. UPLC gradient elution flow rate for fipronil and their metabolites maintained in the experiment.

Time in minutes	Flow rate M ml per min	A* %	B** %
Initial	0.75	80	20
1.0	0.75	50	50
2.0	0.75	30	70
4.0	0.75	10	90
6.0	0.75	00	100
8.0	0.75	80	20

*A: 10 % methanol in water + 5 mM ammonium acetate + 0.1 % formic acid
**B: 10 % in water in methanol+ 5 mM ammonium acetate + 0.1 % formic acid

4.2.2 Optimization of MS/MS parameters: Selection of multiple reaction monitoring (MRM) for quantitative ion for fipronil and its metabolites

The multiple reaction monitoring for qualitative and quantitative ions through setting up of MS parameters for compound-source for fipronil and its metabolites, through product ion scan and precursor ion scan were optimized with source parameters using electrospray ionization in negative ionization mode are presented in the table-9. The ion source gas (GS1) and GS2- were set at 50 psi with -4500 V and with curtain gas set at 15 psi, maintaining the source temperature at 550°C. At the retention time, quantitative daughter ions (Q3) for the metabolites were, 281.9, 330.0, 261.9 and 414.8 for fipronil desulfinyl, fipronil sulphide, fipronil and fipronil sulfone respectively. Similarly, the respective quantitative daughter ions were 350.9, 250.0, 382.9 and 281.9. The order of appearance of peak (i.e., retention time) for fipronil and its toxic metabolites were in the

respective order of fipronil desulfinyl (3.08 min), fipronil (3.17 min), fipronil sulphide (3.28 min) and fipronil sulfone (3.43 min).

Table 9. Optimized instrumental parameter setting and selection of multiple reaction monitoring (MRM) for injected quantitative and qualitative ions retained for 200ms for fipronil and its metabolites in analyte matrix

Instrument parameter	Molecule			
	Fipronil desulfinyl	Fipronil sulphide	Fipronil	Fipronil sulfone
Retention time in minutes	3.08	3.17	3.28	3.43
Q1 Mass precursor ion	386.90	434.90	419.00	451.00
Q3 Daughter ion(quantitative)	281.90	330.00	261.90	414.80
Q3 Daughter ion (qualitative)	350.90	250.00	382.90	281.90
DP Volt	-35.00	-36.00	-30.00	-29.00
EP Volt	-5.00	-6.00	-9.00	-5.00
CEP Volt	-26.00	-23.00	-24.00	-24.00
CE Volt (quantitative)	-43.00	-23.00	-38.00	-23.00
CE Volt (qualitative)	-26.00	-36.00	-17.00	-37.00
CXP Volt	-6.00	-6.00	-6.00	-7.00

DP-declustering potential, EP-entrance potential, CEP-collision cell entrance potential, CE-collision energy, CXP-collision cell exit potential

4.2.3 Percentage recovery, RSD value, linearity, repeatability and limit of quantitation (LOQ) for fipronil

4.2.3.1 Fingers of banana bunches

Mean of method validation parameters with matrix match samples of fingers of bunches collected from the specially maintained control plots at the respective stages of harvest for parameters viz., percentage recovery, RSD value, linearity and repeatability for the two tried methods of extraction of residues of pesticides (conventional and QuEChERS method) for fipronil and its metabolites in “LC-MS/MS (UPLC Waters Aquity+ AB Sciex API 3200) triple quadrupole mass analyser are presented in table-10. From the table it can be seen that extraction and clean up using QuEChERS has resulted in better accuracy (80.9 to 113.2 %) and precision of estimation of fipronil desulfinyl

(2.8-12.9), fipronil (4.1-12.5), fipronil sulphide (5.5-12.8) and fipronil sulfone (2.2-12.8). It can be seen that each one has a narrow and better adoptable ranges of percentage recovery and RSD values than the values obtained for the same molecules estimated using conventional method.

Table 10. Comparison of method validation parameters for recovery of fipronil and its metabolites from fingers of bunches from banana

Sl. No.	Molecule	Extraction method	Percentage recovery (Accuracy)	RSD value (Precision)	Linearity ppm	Limit of Quantitation (LOQ) ppm
1	Fipronil desulfinyl	QuEChERS	83.9-101.3	2.8-12.9	0.01 to 1	0.01
		Conventional	79-117.8	3.9-15.9	0.05 to 1	0.05
2	Fipronil sulphide	QuEChERS	93.6-105.8	5.5-12.8	0.01 to 1	0.01
		Conventional	68.5-116.5	5.6-14.1	0.05 to 1	0.05
3	Fipronil	QuEChERS	80.9-113.2	4.1-12.5	0.01 to 1	0.01
		Conventional	73.0-114.7	2.8-20.6	0.05 to 1	0.05
4	Fipronil sulfone	QuEChERS	92.9-101.9	2.2-12.8	0.01 to 1	0.01
		Conventional	71.4-112.8	2.6-20.0	0.05 to 1	0.05

Linearity estimation for the molecules of fipronil desulfinyl, fipronil, fipronil sulphide, and fipronil sulfone using matrix matched standards extracted through QuEChERS method was found to be in the range of 0.01 to 1.0 ppm which resulted in a limit of quantitation (LOQ) of 0.01 ppm. The same estimations using conventional methods yielded a linearity range of 0.05 to 1 ppm and LOQ of 0.05 ppm. Hence QuEChERS method was found to be better for the extraction of the molecules of fipronil and its metabolites from fingers of banana bunches.

4.2.3.2 Leaves of banana

The result of method validation performed for recovery of residues of fipronil and its metabolites from banana leaf revealed that the QuEChERS method yields better performance with respect to accuracy range of 80-119.9 per cent, RSD value of less than 14.7 per cent, linearity range of 0.01 to 1.00 ppm and LOQ of 0.01 ppm (table-11). However, in conventional method, the accuracy value ranged between 71.3 to 119 %,

Table 11. Comparison of method validation parameters for recovery of fipronil and its metabolites from leaves of banana

Sl. No.	Molecule	Extraction method	Percentage recovery (Accuracy)	RSD value (Precision)	Linearity ppm	Limit of Quantitation (LOQ) ppm
1	Fipronil desulfinyl	QuEChERS	80-97.1	3.9-12.8	0.01 to 1	0.01
		Conventional	71.3-110.8	3.9-19.9	0.05 to 1	0.05
2	Fipronil sulphide	QuEChERS	94.8-112.9	3-13.3	0.01 to 1	0.01
		Conventional	78.3-111.8	3.9-18.1	0.05 to 1	0.05
3	Fipronil	QuEChERS	99.6-119.9	2.3-14.7	0.01 to 1	0.01
		Conventional	76.2-118.2	6.4-14.2	0.05 to 1	0.05
4	Fipronil sulfone	QuEChERS	90-114.7	1.8-12.4	0.01 to 1	0.01
		Conventional	79.1-119.1	5.2-17.2	0.05 to 1	0.05

RSD value 3.9 to 19.9 per cent, linearity ranged from 0.05 to 1.0 ppm and LOQ was found to be 0.05 ppm. Hence QuEChERS method was found to be superior to conventional method for extraction and estimation of residues of fipronil and its metabolites from banana leaves and hence adopted for estimation of residues of these molecules in the leaf samples of the experimental plot.

4.2.3.3 Pseudostem of banana

Method validation parameters for recovery of fipronil and its metabolites from inner core of pseudostem of banana are presented in the table 12.

Table 12. Comparison of method validation parameters for recovery of fipronil and its metabolites from inner core of pseudo stem of banana

Sl. No.	Molecule	Extraction method	Percentage recovery (Accuracy)	RSD value (Precision)	Linearity ppm	Limit of Quantitation (LOQ) ppm
1	Fipronil desulfinyl	QuEChERS	83.3-105.9	1.1-16.2	0.01 to 1	0.01
		Conventional	74.3-113.2	3.3-17.9	0.05 to 1	0.05
2	Fipronil sulphide	QuEChERS	94.8-112.9	0.4-17.1	0.01 to 1	0.01
		Conventional	80.1-112.1	2.8-18.3	0.05 to 1	0.05
3	Fipronil	QuEChERS	80.6-114.7	2-13.9	0.01 to 1	0.01
		Conventional	79.4-109.9	2.8-28.7	0.05 to 1	0.05
4	Fipronil sulfone	QuEChERS	82.7-107.3	1.5-16.1	0.01 to 1	0.01
		Conventional	78.9-114.3	1.8-12.4	0.05 to 1	0.05

From table-12, it is clear that method validation performed for extraction and recovery of fipronil and its metabolites from pseudostem following QuEChERS resulted

in better performance with respect to accuracy range of 80.6 - 114.7 percent, RSD value less than 17.1percent, linearity range of 0.01 to 1.00 ppm and LOQ of 0.01 ppm. In conventional methods the accuracy value ranged between 74.3 and 114.3 per cent and LOQ was found to be 0.05 ppm. Hence QuEChERS method was found to be better than the conventional method for extraction and estimation of fipronil from pseudostem of banana.

4.2.3.4 Flower bud of banana

Method validation performed for extraction and recovery of fipronil and its metabolites from flower bud or inflorescence following QuEChERS provided better performance (table. 13) with respect to accuracy range of 80 to 115.3 per cent, RSD value (less than 13.4 per cent), linearity range of 0.01 to 1.00 ppm and LOQ of 0.01 ppm. In conventional methods the accuracy value ranged between 71.3 and 117.6 percent with an RSD value less than 22.7 and LOQ was found to be 0.05 ppm. Hence QuEChERS method was found to be better than the conventional method for extraction and estimation of fipronil from flower bud of banana over the conventional method.

Table 13. Comparison of method validation parameters for recovery of fipronil and its metabolites from flower bud of banana

Sl. No.	Molecule	Extraction method	Percentage recovery (Accuracy)	RSD value (Precision)	Linearity ppm	Limit of Quantitation (LOQ) ppm
1	Fipronil desulfinyl	QuEChERS	82.5-106.9	0.7-12.9	0.01 to 1	0.01
		Conventional	71.3-110.8	3.9-19.9	0.05 to 1	0.05
2	Fipronil sulphide	QuEChERS	88.7-115.3	0.5-13.4	0.01 to 1	0.01
		Conventional	75.6-116.3	3.4-17.3	0.05 to 1	0.05
3	Fipronil	QuEChERS	80-100.3	0.4-12.9	0.01 to 1	0.01
		Conventional	79-115.1	4.8-17.1	0.05 to 1	0.05
4	Fipronil sulfone	QuEChERS	93.4-115.2	0.5-13.1	0.01 to 1	0.01
		Conventional	78.4-117.6	2.4-22.7	0.05 to 1	0.05

4.2.4 Corm of banana

From the table-14, it is seen that, method validation performed for extraction and recovery of fipronil and its metabolites from corm of banana, following QuEChERS has shown better performance with respect to accuracy ranged of 80.2 to 117.1 per cent, RSD value less than 15.3 per cent, linearity range of 0.01 to 1.00 ppm and LOQ (0.01 ppm). In the conventional method, the accuracy value ranged between 70.8 and 117.9 per cent and LOQ was found to be 0.05 ppm. Hence QuEChERS method was preferred and selected over the conventional method for extraction and estimation of fipronil from corm of banana.

Table 14. Comparison of method validation parameters for recovery of fipronil and its metabolites from corm of banana

Sl. No.	Molecule	Extraction method	Percentage recovery (Accuracy)	RSD value (Precision)	Linearity ppm	Limit of Quantitation (LOQ) ppm
1	Fipronil desulfinyl	QuEChERS	89-108.4	5.1-12.6	0.01 to 1	0.01
		Conventional	79-118.8	3.9-19.8	0.05 to 1	0.05
2	Fipronil sulphide	QuEChERS	83.4-116.6	6.1-12.5	0.01 to 1	0.01
		Conventional	75.4-117.5	2.6-13.1	0.05 to 1	0.05
3	Fipronil	QuEChERS	81-117.1	2.5-14.8	0.01 to 1	0.01
		Conventional	72.6-117.9	2.8-17.6	0.05 to 1	0.05
4	Fipronil sulfone	QuEChERS	80.2-108.6	5.8-15.3	0.01 to 1	0.01
		Conventional	70.8-116.8	2.4-19.7	0.05 to 1	0.05

4.2.4.1 Soil

Method validation performed for extraction and recovery of fipronil and its metabolites from soil following QuEChERS method has resulted in better performance with respect to accuracy range of 80.1 to 97.1 percent, RSD value less than 13.2 percent, linearity range of 0.01 to 1.00 ppm and LOQ 0.01 ppm (table-15). As seen before the conventional method showed a lower accuracy value ranging between 55 to 85 per cent and LOQ of 0.05 ppm. Hence QuEChERS method was found to be better than the conventional method for extraction and estimation of fipronil and its metabolites from soil.

Table 15. Comparison of method validation parameters for recovery of fipronil and its metabolites from soil

Sl. No.	Molecule	Extraction method	Percentage recovery (Accuracy)	RSD value (Precision)	Linearity ppm	Limit of Quantitation (LOQ) ppm
1	Fipronil desulfinyl	QuEChERS	80.1-97.1	1-13.6	0.01-1	0.01
		Conventional	55-85	13-20	0.05 to 1	0.05
2	Fipronil sulphide	QuEChERS	80.1-94.6	2-13.4	0.01-1	0.01
		Conventional	61-83.4	14.2-17	0.05 to 1	0.05
3	Fipronil	QuEChERS	80.7-91.1	3.3-13.3	0.01-1	0.01
		Conventional	66-82.4	11-18.7	0.05 to 1	0.05
4	Fipronil sulfone	QuEChERS	82.3-94.9	0.6-13.2	0.01-1	0.01
		Conventional	67-84.2	9-16.5	0.05 to 1	0.05

4.3 Optimization of instrument parameters: UPLC gradient elution for flow rate for carbosulfan and its metabolites

Multi composition mobile phase constituents in the binary reservoirs of the UPLC denoted as A* and B** of instrument at variable proportions of pumping pressure in time sequence for fipronil are presented as table-16. The optimized gradient elution for flow rate of the mobile phase solvent system with an initial flow rate of 0.8 ml / min was obtained with 80 per cent flow from reservoir A* and 20 per cent from B**. The gradient elution of the compound mixture was monitored for 12 minutes at differential flow rates as shown in table 16. Fitting injection volume at optimized performance was found to be 10 µl.

Table 16. UPLC gradient elution flow rate for carbosulfan and its metabolites maintained in the experiment

Time in minutes	Flow rate	A* %	B** %
Initial	0.80	80	20
4.00	0.80	10	90
5.00	0.80	5	95
9.00	0.80	0	100
10.0	0.80	80	20
12.00	0.80	80	20

*A: 10 % methanol in water + 5 mM ammonium acetate + 0.1 % formic acid

**B: 10 % water in methanol + 5 mM ammonium acetate + 0.1 % formic acid

4.3.1 Optimization of instrument parameters: Selection of multiple reaction monitoring (MRM) for quantitative ion for carbosulfan and its metabolites

The appearance of peak for carbosulfan and its toxic metabolites was in the order of carbofuran (1.94), carbofuran-3-keto (1.52 min), carbofuran-3-hydroxy (1.06 min) and carbosulfan (5.36 min) as presented in the Table-17. UPLC with binary reservoirs holding multi composition mobile phase constituents are denoted as A* and B** of instrument with adjustable proportions of pumping pressure in time sequence for fipronil are presented as table-17. The optimized gradient elution for flow rate of the mobile phase solvent system with an initial flow rate of 0.8 ml / min was again obtained with 80 per cent flow from reservoir A* and 20 per cent from B**. As in the case of fipronil the gradient elution of the compound mixture was monitored for 12 minutes at differential flow rates as shown in table 17. Suitable injection volume at optimized performance was found to be 10 µl.

Table 17. Optimized instrumental parameter setting and selection of multiple reaction monitoring (MRM) for qualitative ions for 200 ms for carbosulfan and its metabolites in analyte matrix

Molecule	Molecule			
	Carbofuran	3-Keto carbofuran	3-hydroxy carbofuran	Carbosulfan
Retention time	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
DP Volt	30	33	28	42
CE Volt (quantitative)	29	23	16	22
CE Volt (qualitative)	17	18	21	33
CXP Volt	2	1	1	1
EP Volt	10	10	10	10
CEP Volt	26.3	22	24	31

DP-declustering potential, EP-entrance potential, CEP-collision cell entrance potential, CE-collision energy, CXP-collision cell exit potential

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 set-up, the respective parameters yielded 165.2, 179.1, 163.1 and 118.1 respectively. The emergence of peaks of carbosulfan and its metabolites after

injection in minutes were in the ascending order for 3-hydroxy carbofuran (1.06), 3-keto carbofuran (1.52), carbofuran (1.94) and carbosulfan (5.36).

4.3.2 Percentage recovery, RSD value, linearity, repeatability and LOQ of carbosulfan

The methodology for residue estimation of carbosulfan and its metabolites from various samples were standardized using control samples spiked with pure analytical standards of carbofuran, carbofuran-3-keto, carbofuran-3-hydroxy and carbosulfan followed by extraction and clean up using suitable techniques described in para 3.

The sample matrix from different sample types viz., banana leaves, pseudo-stem, bunch finger, flower, corm and soil from control plots were utilised for the purpose before and at respective stages of the sampling. Percentage recovery, standard deviation, RSD value, linearity and repeatability and LOQ for each of the compounds tried are presented in the tables 18 to 23.

4.3.2.1 Fingers of banana bunches

Method validation parameters (with matrix match samples using fingers of bunches collected from additional control samples) viz., percentage recovery, RSD value, Linearity and repeatability for the two methods of extraction of pesticides residues (conventional and QuEChERS method) for carbosulfan and its metabolites in LC-MS/MS (UPLC Waters Aquity + AB Sciex API 3200) triple quadrupole mass analyser are presented in the table 18.

The result of the method validation performed in the extraction matrix of fingers of banana revealed that the recovery of carbosulfan and its metabolites by following QuEChERS method has performed better with an accuracy range of 80.9 to 113.2 %, RSD value less than 12. 9 per cent, linearity range of 0.01 to 1.00 ppm and LOQ of 0.01 ppm. In conventional methods the accuracy value ranged between 68.5 to 117.8 per cent and LOQ was found to be 0.05 ppm. Hence QuEChERS method was found superior to the conventional method for extraction and estimation of fipronil from fingers of the bunches.

Table 18. Comparison of method validation parameters for recovery of carbosulfan and its metabolites from fingers of bunches of banana

Sl. No.	Molecule	Extraction method	Percentage recovery (Accuracy)	RSD value (Precision)	Linearity ppm	Limit of Quantitation (LOQ) ppm
1	Carbofuran	QuEChERS	83.9-101.3	2.8-12.9	0.01 to 1	0.01
		Conventional	79-117.8	3.9-15.9	0.05 to 1	0.05
2	Carbofuran-3-keto	QuEChERS	93.6-105.8	5.5-12.8	0.01 to 1	0.01
		Conventional	68.5-116.5	5.6-14.1	0.05 to 1	0.05
3	Carbofuran-3-hydroxy	QuEChERS	80.9-113.2	4.1-12.5	0.01 to 1	0.01
		Conventional	73.0-114.7	2.8-20.6	0.05 to 1	0.05
4	Carbosulfan	QuEChERS	92.9-101.9	2.2-12.8	0.01 to 1	0.01
		Conventional	71.4-112.8	2.6-20.0	0.05 to 1	0.05

4.3.2.2 Leaves of banana plants

Method validation parameters for recovery of carbosulfan and its metabolites from leaves of banana plant are presented in table 19.

Table 19: Comparison of method validation parameters for recovery of carbosulfan and its metabolites from leaves of banana

Sl. No.	Molecule	Extraction method	Percentage recovery (Accuracy)	RSD value (Precision)	Linearity ppm	Limit of Quantitation (LOQ) ppm
1	Carbofuran	QuEChERS	80-97.1	3.9-12.8	0.01 to 1	0.01
		Conventional	71.3-110.8	3.9-19.9	0.05 to 1	0.05
2	Carbofuran-3-keto	QuEChERS	94.8-112.9	3-13.3	0.01 to 1	0.01
		Conventional	78.3-111.8	3.9-18.1	0.05 to 1	0.05
3	Carbofuran-3-hydroxy	QuEChERS	99.6-119.9	2.3-14.7	0.01 to 1	0.01
		Conventional	76.2-118.2	6.4-14.2	0.05 to 1	0.05
4	Carbosulfan	QuEChERS	90-114.7	1.8-12.4	0.01 to 1	0.01
		Conventional	79.1-119.1	5.2-17.2	0.05 to 1	0.05

The result of the method validation experiment performed for recovery parameters of extraction and clean-up of carbosulfan and its metabolites in leaves of banana plant by following QuEChERS method has revealed an accuracy range of 80 to 119.9 per cent, RSD value less than 14.7 per cent, linearity range of 0.01 to 1.00 ppm and LOQ of 0.01 ppm (table-19) are better than the conventional method. In the conventional method, the accuracy value ranged between 71.3 to 119.1 % and LOQ was found to be 0.05 ppm. Hence QuEChERS method was found superior over the conventional method for extraction and estimation of fipronil from leaves of banana plant.

4.3.2.3 Inner core of pseudostem of banana plant

Method validation experiment for recovery parameters of carbosulfan and its metabolites by following QuEChERS method has resulted in better performance with respect to accuracy range of 80.1 - 114.7 per cent, RSD valueless than 18.3 per cent, linearity range of 0.01 to 1.00 ppm and LOQ of 0.01 ppm (table-20). In conventional methods the accuracy value ranged between 74.3 to 114.3% and LOQ was found to be 0.05 ppm. Hence, QuEChERS method was found superior over the conventional method for extraction and estimation of carbosulfan and its metabolites from pseudostem of banana.

Table 20. Comparison of method validation parameters for recovery of carbosulfan and its metabolites from inner core of pseudo stem of banana

Sl. No.	Molecule	Extraction method	Percentage recovery (Accuracy)	RSD value (Precision)	Linearity ppm	Limit of Quantitation (LOQ) ppm
1	Carbofuran	QuEChERS	83.3-105.9	1.1-16.2	0.01 to 1	0.01
		Conventional	74.3-113.2	3.3-17.9	0.05 to 1	0.05
2	Carbofuran-3-keto	QuEChERS	94.8-112.9	0.4-17.1	0.01 to 1	0.01
		Conventional	80.1-112.1	2.8-18.3	0.05 to 1	0.05
3	Carbofuran-3-hydroxy	QuEChERS	80.6-114.7	2-13.9	0.01 to 1	0.01
		Conventional	79.4-109.9	2.8-28.7	0.05 to 1	0.05
4	Carbosulfan	QuEChERS	82.7-107.3	1.5-16.1	0.01 to 1	0.01
		Conventional	78.9-114.3	1.8-12.4	0.05 to 1	0.05

4.3.2.4. Flower bud of banana

Method validation experiment conducted for recovery parameters of extraction and clean-up of carbosulfan and its metabolites in flower bud of banana plant by following QuEChERS method has shown that the accuracy range of 80 to 115.3, RSD value less than 13.4 per cent, linearity range of 0.01 to 1.00 ppm and LOQ of 0.01 ppm (table-21) are better than the values obtained for the conventional method (the accuracy value ranged between 71.3 to 119.9 % and LOQ was found to be 0.05 ppm). Hence QuEChERS method was found better than the conventional method for extraction and estimation of fipronil from flower bud of banana.

Table 21. Comparison of method validation parameters for recovery of carbosulfan and its metabolites from flower bud of banana

Sl. No.	Molecule	Extraction method	Percentage recovery (Accuracy)	RSD value (Precision)	Linearity ppm	Limit of Quantitation (LOQ) ppm
1	Carbofuran	QuEChERS	82.5-106.9	0.7-12.9	0.01 to 1	0.01
		Conventional	71.3-110.8	3.9-19.9	0.05 to 1	0.05
2	Carbofuran-3-keto	QuEChERS	88.7-115.3	0.5-13.4	0.01 to 1	0.01
		Conventional	75.6-116.3	3.4-17.3	0.05 to 1	0.05
3	Carbofuran-3-hydroxy	QuEChERS	80-100.3	0.4-12.9	0.01 to 1	0.01
		Conventional	79-115.1	4.8-17.1	0.05 to 1	0.05
4	Carbosulfan	QuEChERS	93.4-115.2	0.5-13.1	0.01 to 1	0.01
		Conventional	78.4-117.6	2.4-22.7	0.05 to 1	0.05

Accuracy value ranged from 71.3 to 117.6 percent and LOQ was found to be 0.05 ppm. Hence QuEChERS method was found to be better than the conventional method for extraction and estimation of carbosulfan and its metabolites from inflorescence.

4.3.2.5 Corm of banana

The result of the method validation experiment performed for recovery parameters of extraction and clean-up of carbosulfan and its metabolites from corm of banana plant by following QuEChERS method has found to have better range of accuracy range of 80.2 to 117.1 per cent, RSD value less than 15.3 percent, linearity range from 0.01 to 1.00 ppm and LOQ of 0.01 ppm (table-22) are better than the conventional method. In conventional methods, the accuracy value ranged between 70.8 and 118.8 per cent. LOQ was found to be 0.05 ppm. Hence QuEChERS method was found superior to conventional method for extraction and estimation of fipronil from corm of banana plant.

Table 22. Comparison of method validation parameters for recovery of carbosulfan and its metabolites from corm of banana

Sl. No.	Molecule	Extraction method	Percentage recovery (Accuracy)	RSD value (Precision)	Linearity ppm	Limit of Quantitation (LOQ) ppm
1	Carbofuran	QuEChERS	89-108.4	5.1-12.6	0.01 to 1	0.01
		Conventional	79-118.8	3.9-19.9	0.05 to 1	0.05
2	Carbofuran-3-keto	QuEChERS	83.4-116.6	2.6-13.1	0.01 to 1	0.01
		Conventional	75.4-117.5	6.1-12.5	0.05 to 1	0.05
3	Carbofuran-3-hydroxy	QuEChERS	81-117.1	2.5-14.8	0.01 to 1	0.01
		Conventional	72.6-117.9	2.8-17.6	0.05 to 1	0.05
4	Carbosulfan	QuEChERS	80.2-108.6	5.8-15.3	0.01 to 1	0.01
		Conventional	70.8-116.8	2.4-19.7	0.05 to 1	0.05

4.3.2.6 Soil

Method validation experiment conducted for recovery parameters of extraction and clean-up of carbosulfan and its metabolites soil by following QuEChERS method has shown that the accuracy range of 80.1 to 119.9, RSD value less than 14.7 per cent, linearity ranges from 0.01 to 1.00 ppm and LOQ of 0.01 ppm (table-23) are better than the conventional method.

Table 23. Comparison of method validation parameters for recovery of carbosulfan and its metabolites from soil

Sl. No.	Molecule	Extraction method	Percentage recovery (Accuracy)	RSD value, (Precision)	Linearity ppm	Limit of Quantitation (LOQ) ppm
1	Carbofuran	QuEChERS	80.1-97.1	3.9-12.8	0.01 to 1	0.01
		Conventional	69-120.8	3.9-20.9	0.05 to 1	0.05
2	Carbofuran-3-keto	QuEChERS	94.7-112.9	3-13.3	0.01 to 1	0.01
		Conventional	65.5-114.5	2.6-13.1	0.05 to 1	0.05
3	Carbofuran-3-hydroxy	QuEChERS	99.6-119.9	2.3-14.7	0.01 to 1	0.01
		Conventional	70-87.7	2.8-20.6	0.05 to 1	0.05
4	Carbosulfan	QuEChERS	90-114.7	1.8-12.4	0.01 to 1	0.01
		Conventional	71.4-117.6	2.4-22.7	0.05 to 1	0.05

In conventional methods the accuracy value ranged between 65.5 and 120.8 per cent and LOQ was found to be 0.05 ppm.

Thus, from the forgoing results presented in the para 4.2. – 4.3.2.6, it has been found that for extraction of carbosulfan and their metabolites from various plant parts of banana and soil, QuEChERS method is found to be give better percentage recovery (Accuracy), RSD value (Precision), Linearity and LOQ over conventional method.

4.4 Field studies on dissipation (absorption, translocation, distribution and persistence) of fipronil in banana leaves

In table 24, the residue status of fipronil and its toxic metabolites viz., fipronil - desulfinyl, fipronil sulphide and fipronil sulfone in 1st, 2nd and 3rd leaves of banana at various intervals of sampling for the different treatments, T_1 (control), T_2 recommended dose of application and T_3 double the recommended dose are presented.

It can be seen from the table 24 that residue of fipronil and its metabolites analysed were below the BDL of the equipment as per the procedure followed and for the 1st leaf

from 2nd hour after application till the end of observation period on the 50th day of application of the 3rd dose of the treatment. Similar observations were obtained for second and third leaf as well (table-24). The treatment T_1 and T_2 samples too did not show any residue of fipronil or its metabolites in all four leaves (also refer table-25), throughout the period of sampling and analysis.

Table 24. Residue of fipronil and its metabolites in 1st, 2nd and 3rd leaf of banana, µg g^{-1}

Treatment and Molecule	**Time interval in days** (No of days after completion of treatment dosing on150th day after planting)												
	Before 0_i **	*** 0_{2h}	1st	3rd	5th	7th	10th	15th	20th	25th	30th	40th	50th
T_1 control*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_2- (POP) Fipronil desulfinyl$^{\alpha}$-	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Fipronil sulphide$^{\alpha}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Fipronil$^{\alpha}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Fipronil sulfone$^{\alpha}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_2-Total Fipronil*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_3-(2x POP) Fipronil desulfinyl$^{\alpha}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Fipronil sulfide$^{\alpha}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Fipronil$^{\alpha}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Fipronil sulfone$^{\alpha}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_3-Total Fipronil*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

*Foot note: *mean residue of total fipronil were found to be BDL;*
***-150th day before treatment imposition; *** - 2 hours after 3rd application and BDL-below detectable limit; POP: - Package of practices Recommendations: Crops, KAU*

Residue of fipronil and its metabolites in the 4th leaf of banana are presented in table 25. In case of the 4th leaf, fipronil applied at double the recommended dose recorded a value of 0.034 ppm on the 40th day of application and was BDL on the 50th day. In the 4th leaf other toxic metabolites of fipronil viz., fipronil desulfinyl, fipronil sulfide, fipronil

sulfone and fipronil were BDL, showing the same trend as noticed in case of 1st, 2nd and 3rd leaf.

As in the case of first leaf, the mean of total fipronil (i.e., sum of fipronil desulfinyl, fipronil sulphide and fipronil sulfone and fipronil) on the 2nd and 3rd leaves also were below detectable limit (table 24 and table 30)

4.5 Field studies on dissipation (absorption, translocation, distribution, persistence) of carbosulfan

4.5.1 In banana leaves

The results of analysis of residues of Carbosulfan (cs-1) and its toxic metabolites viz., carbofuran-1(cf-1), carbofuran 3 keto and carbofuran 3 hydroxy in 1st, 2nd, 3rd and 4th leaves of banana at different time intervals in 1st, 2nd, 3rd and 4th leaves of banana are presented in table 26, 27, 28, and 29 and respective mean total fipronil and carbosulfan in tables 29, 30, 31 and 32.

Table 25. Residue of fipronil and its metabolites in 4th leaf of banana, μg g^{-1}

Treatment and molecule	**Time interval in days** (No of days after completion of treatment dosing on150th day after planting)												
	Before 0_i**	*** 0_{2h}	1st	3rd	5th	7th	10th	15th	20th	25th	30th	40th	50th
T_1 control*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_2- (POP) Fipronil desulfinyl$^{\alpha}$-	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Fipronil sulphide$^{\alpha}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Fipronil$^{\alpha}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Fipronil sulfone$^{\alpha}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_2-Total Fipronil*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_3-(2 x POP) Fipronil desulfinyl$^{\alpha}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Fipronil sulfide$^{\alpha}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Fipronil$^{\alpha}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.034	BDL
Fipronil sulfone$^{\alpha}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_3-Total Fipronil*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.034	BDL

Foot note: *mean of total fipronil were BDL; ** - 150th day before 3rd application;
*** 2 hours after 3rd application and BDL - below detectable limit; $^{\alpha}$ Fipronil and its metabolites;
POP: - Package of practices Recommendations: Crops, KAU

Carbosulfan and its metabolites too, were not detected in treatment T_1, control during the period of observation in all the four leaves tested. Unlike pattern of absorption and persistence of the fipronil, carbosulfan and its metabolites displayed a different pattern in different leaves.

Residue of carbofuran was present only on the 7th day at 0.0797 µg g^{-1} in the 1st leaf when applied at recommended dose (T_4) (Table-26). However, when applied at double the recommended dose (T_5), the residues were detected from 5th day onwards at a concentration of 0.388 ppm which subsequently got dissipated to 0.268 ppm on 7th day, 0.231on 10th day, 0.232 on 15th day, 0.167 20th day, 0.08 on 25th and persisted up to 30th day with a content of 0.023 µg g^{-1}, which got further dissipated to BDL on 40th day.

Table 26. Mean residue of carbosulfan and its metabolites in 1st leaf of banana, µg g^{-1}

Treatment and Molecule	**Time interval in days** (No of days after completion of treatment dosing on150th day after planting)												
	Before 0_i**	*** 0_{2h}	1st	3rd	5th	7th	10th	15th	20th	25th	30th	40th	50th
T_1 control*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_4-pop Carbofuran$^{\beta}$ **(Cf)**	BDL	BDL	BDL	BDL	BDL	0.0797	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Carbofuran-3 keto$^{\beta}$ **(Cf,3-keto)**	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Carbofuran-3 hydroxy$^{\beta}$ (**Cf ,3-hydroxy**)	BDL	BDL	BDL	BDL	0.073	0.03	0.042	BDL	BDL	BDL	BDL	BDL	BDL
Carbosulfan$^{\beta}$ (**Cs)**	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_4-Total Carbosulfan$^{\beta}$ (**Cs)***	BDL	BDL	BDL	BDL	0.073	0.109	0.042	BDL	BDL	BDL	BDL	BDL	BDL
$T_5$2x pop- Cf	BDL	BDL	BDL	BDL	0.388	0.268	0.231	0.232	0.167	0.08	0.023	BDL	BDL
Cf,3- keto$^{\beta}$	BDL	BDL	BDL	BDL	0.033	0.131	0.018	0.0123	BDL	BDL	BDL	BDL	BDL
Cf,3-hydroxy$^{\beta}$	BDL	BDL	BDL	BDL	0.550	0.683	0.275	0.117	0.123	0.11	0.088	0.017	BDL
Cs 1$^{\beta}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_5-Total Cs Carbosulfan$^{\beta}$*	BDL	BDL	BDL	BDL	0.971	1.082	0.524	0.349	0.29	0.19	0.123	0.017	BDL

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

The residues of carbofuran 3-keto could not be detected even on 50th day of application at the recommended rate (T_4). However, at double dose (T_5) though the residues were BDL up to 3rd day, it was 0.033 μg g^{-1} day on 5th day which further tend to increase to 0.131 μg g^{-1} on the 7th day. On the 15th day 0.0123 μg g^{-1} was detected which further dissipated to BDL on 20th day.

Carbofuran-3 hydroxy was detected only on 5th, 7th, and 10th day of application at T_4 level. The residues were 0.073, 0.03 and 0.04 respectively, on these days and no residue was detected in subsequent samples. At T_5 level, up to 3rd day the residue was BDL, while the samples collected from 5th to 40th day were detected with residues at 0.550, .0653, 0.275, 0.117, 0.123, 0.11,0.088 and 0.017 ppm on 5th, 7th, 10th, 15th, 20th, 25th, 30th and 40th day's samples. Here again the peak level of residue was noted on the 7th day (0.683 μg g^{-1}) and dissipated to lowest level of 0.017 μg g^{-1} on the 40th day and gradually dissipated to BDL by 50th day.

No residue of carbosulfan molecule was detected in T_4 and T_5 during the sampling period in the 1st, 2nd, 3rd and 4th leaf of banana at both the levels of application.

In the 2nd leaf, residue of carbofuran was found to be 0.054 and 0.048 μg g^{-1} on 7th and 10th day respectively when applied at recommended dose (T_4) (Table-27), whereas when applied at double the recommended dose (T_5) it was detected in leaves from 5th day (0.37) and persisted up to 40th day (0.019 μg g^{-1}) and the maximum detected being 0.827 μg g^{-1}, observed on 10th day, which got dissipated to BDL on 50th day.

In the 2nd leaf, residue of carbofuran 3-keto could not be detected up to 50th day of application at the recommended rate (T_4). But at T_5 rate of application though it recorded BDL on 3rd day, it was 0.013 μg g^{-1} on 5th day and increased to a highest value of 0.14 μg g^{-1} on 15th day, which further declined to 0.038 on 25th day and finally to BDL on 30th day.

Carbofuran-3 hydroxy were detected only on 5th, 7th, 10th,15th and 20th day of application at T_4 level in the 2nd leaf (Table 27). The highest value recorded was 0.057 μg g^{-1} on day 7 and it dissipated to BDL on 25th day. At T_5 level, up to 3rd day it was BDL and residues were detected from 5th to 40th day (0.168, 0.782, 0.573, 0.461, 0.303, 0.274, 0.131 and 0.089 μg g^{-1}), peak value being 0.782 μg g^{-1} observed on 7th day and dissipated to BDL by 50th day.

Table 27. Mean residue of carbosulfan and its metabolites in 2nd leaf of Banana, µg g^{-1}

Treatment and molecule	**Time interval in days** (No of days after completion of treatment dosing on150th day after planting)												
	Before 0_i**	*** 0_{2h}	1st	3rd	5th	7th	10th	15th	20th	25th	30th	40th	50th
T_1 control*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_4-pop Carbofuran$^{\beta}$ **(Cf)**	BDL	BDL	BDL	BDL	BDL	0.054	0.048	BDL	BDL	BDL	BDL	BDL	BDL
Carbofuran-3 keto$^{\beta}$ **(Cf, 3- keto)**	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Carbofuran-3 hydroxy$^{\beta}$ (**Cf ,3-hydroxy**)	BDL	BDL	BDL	BDL	0.057	0.020	0.203	0.053	0.027	BDL	BDL	BDL	BDL
Carbosulfan$^{\beta}$ (**Cs)**	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_4-Total Carbosulfan$^{\beta}$ (Cs)*	BDL	BDL	BDL	BDL	0.057	0.075	0.252	0.053	0.027	BDL	BDL	BDL	BDL
T_5- 2 x pop- Cf	BDL	BDL	BDL	BDL	0.370	0.497	0.827	0.233	0.111	0.022	0.105	0.019	BDL
Cf,3- keto$^{\beta}$	BDL	BDL	BDL	BDL	0.012	0.062	0.053	0.140	0.027	0.038	BDL	BDL	BDL
Cf ,3-hydroxy$^{\beta}$	BDL	BDL	BDL	BDL	0.168	0.782	0.573	0.461	0.303	0.274	0.131	0.089	BDL
Cs 1$^{\beta}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_5-Total Cs Carbosulfan$^{\beta}$*	BDL	BDL	BDL	BDL	0.551	1.340	1.452	0.835	0.441	0.334	0.236	0.108	BDL

Foot note: *mean of all metabolites for carbosulfan was worked to be zero;
-150th day before treatment imposition and * - 2 hours after 3rd application and BDL - below detectable limit; POP: - Package of Practices Recommendations: Crops, KAU

In the 3rd leaf with T_4 treatment, carbofuran residue was detected on 5th, 15th and 20th day (0.072, 0.362 and 0.022 µgg^{-1}, respectively) and was below detectable level on 25th day (Table-28). It was observed that the residue was below detectable limit on 7th and 10th day. With T_5, the residues persisted through 5th day to 40th day, registering peak on 7th and 10th (0.609 µgg^{-1}) day which gradually dissipated to BDL by 50th day.

Residues of carbofuran-3- keto when applied as per T_4 in 3rd leaf were found on 15th and 20th day (0.073 and 0.013 µg g^{-1}) and then dissipated to BDL. In T_5, the residue was detected from 5th to 40th day, with a peak residue value of 0.099 on 30th day which was observed to be BDL by 50th day.

In 3rd leaf, carbofuran-3 hydroxy were detected during the period 5th to 20th day of application at T_4 level of application, peak being observed on 15th day (0.667 µg g^{-1}). At T_5 levels of application, on day 5th it was 0.192 µgg^{-1} and on 40th day residue was (0.211 µg g^{-1}) and dissipating to BDL by 50th day. It can be seen that at both level of

carbosulfan application, maximum level of residues was recorded on the 15th day of application.

Table 28. Mean residue of carbosulfan and its metabolites in 3rd leaf of banana, μg g^{-1}

Treatment and molecule	**Time interval in days** (No of days after completion of treatment dosing on150th day after planting)												
	Before 0_i**	*** 0_{2h}	**1st**	**3rd**	**5th**	**7th**	**10th**	**15th**	**20th**	**25th**	**30th**	**40th**	**50th**
T_1 control*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_4-pop Carbofuran$^{\beta}$ **(Cf)**	BDL	BDL	BDL	BDL	0.072	BDL	BDL	0.362	0.022	BDL	BDL	BDL	BDL
Carbofuran-3 keto$^{\beta}$ **(Cf,3- keto)**	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.073	0.013	BDL	BDL	BDL	BDL
Carbofuran-3 hydroxy$^{\beta}$ (**Cf ,3-hydroxy**)	BDL	BDL	BDL	BDL	0.027	0.043	0.043	0.667	0.142	BDL	BDL	0.01	BDL
Carbosulfan$^{\beta}$ (**Cs)**	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_4-Total Carbosulfan$^{\beta}$ **(Cs)***	BDL	BDL	BDL	BDL	0.100	0.043	0.043	1.101	0.177	BDL	BDL	BDL	BDL
T_5 2 x pop- Cf	BDL	BDL	BDL	BDL	0.404	0.609	0.609	0.352	0.260	0.096	0.172	0.078	BDL
Cf,3- keto$^{\beta}$	BDL	BDL	BDL	BDL	0.014	0.090	0.090	0.078	0.033	0.042	0.099	0.046	BDL
Cf ,3-hydroxy$^{\beta}$	BDL	BDL	BDL	BDL	0.192	0.864	0.864	0.567	0.387	0.251	0.621	0.211	BDL
Cs 1$^{\beta}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_5-Total Cs Carbosulfan$^{\beta}$*	BDL	BDL	BDL	BDL	0.611	1.562	1.562	0.997	0.681	0.389	0.892	0.335	BDL

Foot note: *mean of all metabolites for both fipronil and carbosulfan were worked to be zero;
- 150th day before application and * - 2 hours after 3rd application and BDL-below detectable limit;
$^{\beta}$ carbosulfan and its metabolites; POP: - Package of practices Recommendations: Crops, KAU.

In the 4th leaf, carbofuran-1 residues persisted through 5th, 7th and 10th days at 0.108, 0.212 and 0.180 μg g^{-1} respectively (Table-29), then dropping to BDL on 15th day, at T_4 level. For T_5 level, the residues persisted from 1st day to 20th day of application, (0.012, 0.319, 0.378, 0.311, 0.452, 0.282 and 0.055 μg g^{-1} respectively) with the peak value observed on 10th day.

Carbofuran-3- keto when applied as per T_4, persisted in the 4th leaf, on 7th and 10th day (0.075 and 0.080 μg g^{-1}) and then dissipated to BDL on day 15. However, in T_5 remained as residue from 5th to 20th day, attaining a peak on 7th day (0.238 μg g^{-1}) and registering BDL on day 25th.

Carbofuran-3 hydroxy residues in 4th leaf at T_4 level of application were detected during the period of even from the 1st day. Peak value for residue was noted on 7th day of application (0.453 μg g^{-1}) and dissipated to 0.441μg g^{-1} on 10th day, before attaining BDL on 15th day itself. At T_5 levels, carbofuran-3 hydroxy residues were found during the period of 1st to 20th day, reaching BDL on 25th day. It can be seen that at both level of treatment, maximum of residues carbofuran-3 hydroxy were recorded on 7th day on application. In 4th leaf too, carbosulfan residue was absent throughout the period of observation.

Table 29. Mean residue of carbosulfan and their metabolites in 4th leaf of banana, μg g^{-1}

Treatment and molecule	**Time interval in days** (No of days after completion of treatment dosing on150th day after planting)												
	Before 0_i**	*** 0_{2h}	1st	3rd	5th	7th	10th	15th	20th	25th	30th	40th	50th
T_1 control*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_4-pop Carbofuran$^{\beta}$ **(Cf)**	BDL	BDL	BDL	BDL	0.108	0.212	0.180	BDL	BDL	BDL	BDL	BDL	BDL
Carbofuran-3 keto$^{\beta}$ **(Cf,3- keto)**	BDL	BDL	BDL	BDL	BDL	0.075	0.080	BDL	BDL	BDL	BDL	BDL	BDL
Carbofuran-3 hydroxy$^{\beta}$ (**Cf ,3-hydroxy**)	BDL	BDL	0.020	0.032	0.040	0.453	0.441	BDL	BDL	BDL	BDL	BDL	BDL
Carbosulfan$^{\beta}$ (**Cs)**	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_4-Total Carbosulfan$^{\beta}$ **(Cs)***	BDL	BDL	0.020	0.032	0.147	0.741	0.701	BDL	BDL	BDL	BDL	BDL	BDL
T_5 2 x pop- Cf	BDL	BDL	0.012	0.319	0.378	0.311	0.452	0.282	0.055	BDL	BDL	BDL	BDL
Cf,3- keto$^{\beta}$	BDL	BDL	BDL	BDL	0.018	0.238	0.067	0.087	0.043	BDL	BDL	BDL	BDL
Cf ,3-hydroxy$^{\beta}$	BDL	BDL	0.036	0.161	0.217	1.012	0.630	0.524	0.162	BDL	BDL	BDL	BDL
Cs 1$^{\beta}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_5-Total Cs Carbosulfan$^{\beta}$*	BDL	BDL	0.048	0.480	0.613	1.561	1.149	0.894	0.260	BDL	BDL	BDL	BDL

Foot note: *mean of all metabolites for both fipronil and carbosulfan were worked to be zero;
** -150th day before application; *** - 2 hours after 3rd application and BDL-below detectable limit; $^{\beta}$ carbosulfan or its metabolites; POP: - Package of practices Recommendations: Crops, KAU.

Comparison of residue of total fipronil and total carbosulfan in 1st leaf are given in the table-30. In contrast to total fipronil (sum of fipronil and its metabolites for a sample), total carbosulfan (sum carbosulfan and its metabolites for a sample) was present in the 1st leaf from 5th to 10th day in $T_4$with a peak residue of 0.109 μg g^{-1}on 7th day. In the T_5 total carbosulfan persisted through 5th day to 40th day with a peak value of 1.082 μg g^{-1}, being observed again on 7th day.

Table 30. Mean residue of total fipronil and total carbosulfan in 1st leaf of banana after pre-bunching application, µg g^{-1}

Time interval in days	Treatment and Molecule				
	T1 control*	T2- Fipronil -pop	T3- Fipronil- 2 x pop	T4- Carbosulfan- pop	T5- Ccarbosulfan- 2 x pop
Before 0_i**	BDL	BDL	BDL	BDL	BDL
*** 0_{2h}	BDL	BDL	BDL	BDL	BDL
1st	BDL	BDL	BDL	BDL	BDL
3rd	BDL	BDL	BDL	BDL	BDL
5th	BDL	BDL	BDL	0.042	0.97
7th	BDL	BDL	BDL	0.109	1.082
10th	BDL	BDL	BDL	0.073	0.524
15th	BDL	BDL	BDL	BDL	0.349
20th	BDL	BDL	BDL	BDL	0.29
25th	BDL	BDL	BDL	BDL	0.19
30th	BDL	BDL	BDL	BDL	0.123
40th	BDL	BDL	BDL	BDL	0.017
50th	BDL	BDL	BDL	BDL	BDL

*Mean of all metabolites for both fipronil and carbosulfan were BDL, ** - 150th day before 3rd application and *** - 2 hours after 3rd application and BDL-below detectable limit.

Table 31 shows the comparison of residue of fipronil and carbosulfan in 2nd leaf. Total carbosulfan residues were present in the 2nd leaf from 5th to 20th day in T_4 and from 5th to 40th day in T_5. No residue of fipronil was detected in any of the samples throughout the period of study. In both T_4 and T_5 maximum carbosulfan residue were noted on 10th day with a residue value of 0.252 and 1.452 µg g^{-1} respectively.

Table 31. Mean residue of fipronil and carbosulfan in 2nd leaf of banana, after pre-bunching application, µg g^{-1}

Time interval in days	Treatment and Molecule				
	control* (T_1)	Fipronil - pop (T_2)	Fipronil- 2 x pop (T_3)	Carbosulfan- pop (T_4)	Carbosulfan- 2 x pop (T_5)
Before 0_i**	BDL	BDL	BDL	BDL	BDL
*** 0_{2h}	BDL	BDL	BDL	BDL	BDL
1st	BDL	BDL	BDL	BDL	BDL
3rd	BDL	BDL	BDL	BDL	BDL
5th	BDL	BDL	BDL	0.057	0.551
7th	BDL	BDL	BDL	0.075	1.34
10th	BDL	BDL	BDL	0.252	1.452
15th	BDL	BDL	BDL	0.053	0.835
20th	BDL	BDL	BDL	0.027	1.452
25th	BDL	BDL	BDL	BDL	0.334
30th	BDL	BDL	BDL	BDL	0.236
40th	BDL	BDL	BDL	BDL	0.108
50th	BDL	BDL	BDL	BDL	BDL

Foot note: BDL: -below detectable limit; **pop**: - Package of Practices recommendation crops; ** - On 150th day before treatment imposition. *** - 2 hours after treatment imposition on 150th day of planting.

Mean residue of fipronil and carbosulfan in 3rd leaf are given in table-32. Total

Table 32. Mean residue of total fipronil and carbosulfan in 3rd leaf of banana, after pre-bunching application, µg g^{-1}

Time interval in days	Treatment and Molecule				
	control* (T1)	Fipronil - pop (T2)	Fipronil- 2 x pop (T3)	Carbosulfan- pop (T4)	Carbosulfan- 2 x pop (T5)
Before 0_i**	BDL	BDL	BDL	BDL	BDL
*** 0_{2h}	BDL	BDL	BDL	BDL	BDL
1st	BDL	BDL	BDL	BDL	BDL
3rd	BDL	BDL	BDL	BDL	BDL
5th	BDL	BDL	BDL	0.043	0.611
7th	BDL	BDL	BDL	0.1	0.703
10th	BDL	BDL	BDL	1.101	1.562
15th	BDL	BDL	BDL	0.177	0.997
20th	BDL	BDL	BDL	BDL	0.892
25th	BDL	BDL	BDL	BDL	0.389
30th	BDL	BDL	BDL	BDL	0.387
40th	BDL	BDL	BDL	BDL	BDL
50th	BDL	BDL	BDL	BDL	BDL

Foot note: BDL**:** -below detectable limit; **pop**: -Package of Practices recommendation crops. **- 150th day before treatment imposition. *** - 2 hours after treatment imposition on 150th day of planting.

Table 33. Mean residue of fipronil and carbosulfan in 4th leaf of banana, µg g^{-1}

Time interval in days	Treatment and Molecule				
	control* (T1)	Fipronil -pop (T2)	Fipronil- 2 x pop (T3)	Carbosulfan- pop (T4)	Carbosulfan- 2 x pop (T5)
Before 0_i**	BDL	BDL	BDL	BDL	BDL
*** 0_{2h}	BDL	BDL	BDL	BDL	BDL
1st	BDL	BDL	BDL	BDL	BDL
3rd	BDL	BDL	BDL	0.02	0.048
5th	BDL	BDL	BDL	0.032	0.48
7th	BDL	BDL	BDL	0.147	0.613
10th	BDL	BDL	BDL	0.741	1.561
15th	BDL	BDL	BDL	0.701	1.149
20th	BDL	BDL	BDL	0.02	0.894
25th	BDL	BDL	BDL	BDL	0.26
30th	BDL	BDL	BDL	BDL	BDL
40th	BDL	BDL	BDL	BDL	BDL
50th	BDL	BDL	BDL	BDL	BDL

Foot note: **BDL:** -below detectable limit; **pop**: -Package of Practices recommendation crops. **-150th day before treatment imposition. *** - 2 hours after before treatment imposition on 150th day of planting.

carbosulfan residues were present in the 3rd leaf from 5th to 15th day in T_4 level and from 5th day to 30th day in T_5 level. During the period of study, no residue of fipronil was detected in any of the samples. In both T_4 and T_5 maximum carbosulfan residue were note on 10th day with a residue value of 1.101 and 1.562 µg g^{-1}, respectively.

On comparing fipronil and carbosulfan residues in 4th leaf as given in the table-33, there was no fipronil residues detected. But total carbosulfan residues were seen in the 4thleaf from 3rd to 20th days in T_4 level and from 3rddday to 25th days in T_5 level. The maximum carbosulfan residue in both T_4 and T_5 were detected on 10th day with a residue value of 0.741 and 1.561 μg g^{-1}, respectively.

4.5.2 Field studies on dissipation of fipronil and carbosulfan in various plant parts other than leaves after bunching

Data on the persistence of total fipronil and carbosulfan in various plant parts other than leaves after bunching are presented in table-34. It was observed that fipronil and carbosulfan and their metabolites did not persist in any of the plant parts analysed viz., blossom bud, flower bract alone, bunch on 15th day of emergence, bunch on 30th day of emergence, banana fruit peel, bunch, pseudo stem and corm on harvest.

Table 34. Mean residue of total fipronil and carbosulfan in various plant parts other than leaves after bunching

Treatment and molecule	Residues (μg g^{-1})							
	Blossom bud	Flower bract alone	Bunch (on 15th day of emergence)	Bunch (on 30th day of emergence)	Peel	Bunch on Harvest	Pseudo stem	Corm
T_1 control	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_2- fipronil-pop	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_3- fipronil-2xpop	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_4-carbosulfan-pop	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_5-carbosulfan-2xpop	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Foot note: **BDL: -**below detectable limit; **pop**: -Package of Practices recommendation crops
2xpop: - double the dose of pop recommendation.

4.6 Residues in soils

4.6.1 Fipronil and its metabolites

The results of analysis of rhizosphere soils for the estimation of residues of fipronil, fipronil desulfinyl, fipronil sulfide and fipronil sulfone (as metabolites of fipronil) are presented in table 35 and their corresponding mean values in the table 37. Fipronil desulfinyl metabolite was not detected in rhizosphere soil for both T_2 and T_3.

Table 35. Mean residue of fipronil and its toxic metabolites in rhizosphere soil, μg g^{-1}

Treatment and molecule	**Time interval in days** (No of days after completion of treatment dosing on150th day after planting)												
	Before ** 0_i	*** 0_{2h}	1st	3rd	5th	7th	10th	15th	20th	25th	30th	40th	50th
T_1 control*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_2- Fipronil desulfinyl$^{\alpha}$- POP	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Fipronil sulphide$^{\alpha}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Fipronil$^{\alpha}$	BDL	0.121	0.059	0.051	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Fipronil sulfone$^{\alpha}$	BDL	0.025	0.036	BDL	0.032	0.031	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_2-Total Fipronil*	BDL	0.146	0.095	0.051	0.032	0.031	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_3--2x POP Fipronil desulfinyl$^{\alpha}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Fipronil sulfide$^{\alpha}$	BDL	0.328	BDL	0.089	BDL	0.027	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Fipronil$^{\alpha}$	BDL	0.866	0.605	0.217	0.385	0.185	0.279	0.117	0.066	0.059	0.095	0.084	BDL
Fipronil sulfone$^{\alpha}$	BDL	0.261	0.089	0.186	0.048	0.125	BDL	0.067	0.088	0.071	BDL	BDL	BDL
T_3-Total Fipronil*	BDL	1.456	0.694	0.492	0.433	0.336	0.279	0.184	0.154	0.130	0.095	0.084	BDL

Foot note: *mean of all metabolites for fipronil were worked to be zero; **-150**th** day before treatment imposition and *** - 2 hours after 3rd application and BDL-below detectable limit; $^{\alpha}$Fipronil and or its metabolite; POP: - Package of practices Recommendations: Crops, KAU

Fipronil sulfide was not detected in rhizosphere soil at normal rate of application in the soil (T_2). However, it was detected up to 3rd day in soil treated at double rate of application (T_3). The maximum concentration observed was on 0th day at 0.328 μg g^{-1} level.

In T_2, fipronil molecule remained as such in the rhizosphere soil from the time of application to 3rd day dissipating from 0.121 μg g^{-1} on 0th day to 0.051 μg g^{-1} and finally reaching BDL on 5th day. The pattern of dissipation of fipronil at double level of application in soil was different from that in T_2. In T_3 with double rate of application, has

resulted in a longer persistence of fipronil molecule till 40th day (0.084 μg g^{-1}), recording the highest residue on day zero (0.0866 μg g^{-1}) and dissipated to BDL by 50th day.

Fipronil sulphone metabolite, was detected right from 0th to 7th day samples except for 3rd day sample. The maximum residue value of 0.036 μg g^{-1} was recorded on the 1st day. Fipronil sulfone residues were highest on day zero (0.261 μg g^{-1}) which was absent on 30th day, but presenting a residue of 0.071 μg g^{-1}on 25th day. However in between, on day 10th fipronil sulfone was at below detectable limit again registering 0.067 μg g^{-1}on 15th day.

It was found that at T_2 level, total content of fipronil and its toxic metabolites persisted up to 10th day (0.019 μg g^{-1}; table-37), the highest residue level being found on day zero (0.146 μg g^{-1}). At T_3 level it persisted for a longer period up to 40th day (0.084 μg g$^{-1)}$ with initial highest level on day zero (1.456 μg g^{-1}).

4.6.2 Carbosulfan and its metabolites in soil

The results of analysis of rhizosphere soils for estimation of residues of carbosulfan, carbofuran(cf-1), carbofuran 3-keto and carbofuran 3hydroxy (as metabolites of carbosulfan) are presented in table 36 and the mean values for comparison with fipronil in the table 37.

In the case of carbosulfan treatment in soil, no residue of 3-keto carbofuran was detected in soil treated at normal (T_4) and double dose (T_5) of carbosulfan.

Carbofuran, a primary metabolite of carbosulfan was not detected in soil treated with normal doses of carbosulfan (T_4), but was detected on 0th day at double dose of application with no residues detected in the subsequent samples.

Residues of carbofuran-3 hydroxy (Cf, 3-hydroxy) dissipated to BDL on 7th day recording a peak on day one in T_4 (0.129 μg g^{-1}) and day zero in T_5 (1.949 μg g^{-1}). The level of residues detected in T_5 was higher when compared to T_4

Residue of carbosulfan (Cs) too dissipated to BDL on day 10th, both recording peak concentration on 0th day in both T_4 (0.22 μg g^{-1}) and T_5 (3.804 μg g^{-1}) treatments.

In soil, the residues of total carbosulfan and its metabolites were highest on the day zero and dissipated to below detectable limit on 10th day.

Table 36. Mean residue of carbosulfan and their toxic metabolites in soil, µg g^{-1}

Treatment and molecule	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
T1 control*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T4-POP- Cf Carbofuran$^{\beta}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Carbofuran-3 keto$^{\beta}$ (Cf,3- keto)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Cf ,3-hydroxy$^{\beta}$	BDL	0.095	0.129	0.032	0.020	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Carbosulfan$^{\beta}$ **(Cs)**	BDL	0.220	0.158	0.051	0.020	0.015	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T4-Total- Cs* Carbosulfan$^{\beta}$	BDL	0.315	0.287	0.083	0.04	0.015	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T5-2x POP- **Cf**	BDL	0.027	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Cf,3- keto$^{\beta}$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Cf ,3- hydroxy$^{\beta}$	BDL	1.949	0.118	0.267	0.042	0.057	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Cs 1$^{\beta}$	BDL	3.804	0.359	0.309	0.123	0.120	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T5-Total Cs Carbosulfan$^{\beta}$*	BDL	5.78	0.477	0.576	0.165	0.177	BDL	BDL	BDL	BDL	BDL	BDL	BDL

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

Table 37**.** Mean residue of fipronil and carbosulfan in rhizosphere soil, µg g^{-1}

Treatment and molecule	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
T_1 control	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_2- Fi-pop	BDL	0.146	0.095	0.051	0.032	0.031	0.019	BDL	BDL	BDL	BDL	BDL	BDL
T_3- Fi-2xpop	BDL	1.456	0.694	0.492	0.433	0.336	0.279	0.184	0.154	0.130	0.095	0.084	BDL
T_4-CS-pop	BDL	0.220	0.158	0.051	0.020	0.015	BDL	BDL	BDL	BDL	BDL	BDL	BDL
T_5-Cs-2xpop	BDL	5.78	0.477	0.576	0.165	0.177	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Foot note: BDL**:** -below detectable limit; pop: -Package of Practices recommendation crops **-150th day before treatment imposition. *** -2 hours after treatment imposition on 150th day of planting. Fip-pop: -Fipronil as per T_2 POP dose; Fip-pop: - fipronil as per T_3 POP-twice the dose Cs-pop carbosulfan as per T_4-POP dose; Cs-2x pop: - carbosulfan as per T_5 POP-twice the dose.

4.6.3 Effect of fipronil and carbosulfan on soil enzymes

Effect of fipronil and carbosulfan on soil enzyme activity in samples collected on 10th day of second soil application of treatments each at normal and double doses are presented in the table 38. Soil urease activity on 10th day was significantly influenced by the treatment at double doses as evidenced from a lower urease activity in these soils when

compared with the normal dose of application. The urease activity in T_3 was lower than T_2 and that in T_5 was lower than T_4 as well as in control. However, dehydrogenase activity and acid phosphatase activity were not significantly influenced by the treatments, as evident from the table.

Table 38. Effect of fipronil and carbosulfan on soil enzymes on 10th day after 2nd application.

Treatment and molecule	Soil enzyme		
	Urease (µg of urea hydrolysed g^{-1} of soil hr^{-1})	Dehydrogenase (µg of TPF hydrolysed g^{-1} of soil 24 hrs^{-1})	Acid phosphatase (µg of p-nitrophenol released g^{-1} of soil hr^{-1})
T_1 control	45.1[b]	63.7	40.6
T2- fipronil-pop	47.3[a]	65.3	41.5
T3- fipronil-2xpop	45.5b[b]	64.5	40.7
T4-carbosulfan-pop	47.0[a]	67.1	42.6
T5-carbosulfan- 2xpop	45.4[b]	67.3	42.9
CD (at 0.05)	0.978	ns	ns
SE(d)	0.449	na	na
CV	1.38	3.84	4.26

Foot note: pop: -Package of Practices recommendation crops
Fip-pop: -Fipronil as per T2 POP dose; Fip-pop: -twice the dose of fipronil;
Cs-pop carbosulfan as per T4-POP dose; Cs-2x pop: - twice the dose of carbosulfan.

4.6.4 Effect of fipronil and carbosulfan on soil microorganisms

Logarithmic transformed values of the counts representing effect of soil application of fipronil and carbosulfan on soil microorganism are presented table 39 (Plate No 4 to 6). After 10 days of application in soil, it was observed that population of actinomycetes, bacteria and fungi population were higher in T_1, followed by T_2 and T_4 compared to the higher doses of test chemicals. The application of fipronil or carbosulfan as per treatment schedule on 60th day of planting (i.e., 10 days after 2nd application) at both at single and double doses, did not affect the population of actinomycetes significantly. However, actinomycetes population were highest in T_1-control (1.23 x 1.0E+05 cfu g^{-1}) and least in T_4 (1.17 x 1.0E+05 cfu g^{-1}) (appendix table i). The data presented in table 36 highlights the significant influence of fipronil and carbosulfan compounds on the population of bacteria and fungi where in the control samples of soil recorded maximum population of these microorganisms.

The bacterial population in T_1 was significantly higher than all other treatments. Among the treatments, carbosulfan had more inhibitory influence on bacterial population and maximum inhibition was observed in T_4 followed by T_5, T_3 and T_2.

Maximum inhibition of fungal population was observed in treatment with fipronil wherein maximum inhibition was observed in T_2 followed by T_3, T_4 and T_5 and the population of fungi in these treatments were found to be on par.

Table 39. Effect of fipronil and carbosulfan on soil microorganism 10th day after 2nd application.

Effect of residues on soil microorganisms on 70th day of planting			
	Actinomycetes	Bacteria	Fungi
Treatment and molecule	Mean of logarithmic value of the counts		
T_1 Control	4.07	7.1[a]	4.73[a]
T_2- 30 mg a.i. fipronil / plant	4.06	6.8[b]	4.35[c]
T3- 60 mg a.i. fipronil/plant	4.06	6.3[c]	4.37[c]
T_4- 400 mg a.i. Carbosulfan/plant	4.05	6.1[d]	4.39b[c]
T_5- 800 mg a.i. Carbosulfan/plant	4.05	6.3[c]	4.44[b]
CD (0.05)	ns	0.145	0.064
SE(d)	na	0.039	0.050
CV (%)	2.063	0.84	0.17

4.6.5 Effect of fipronil and carbosulfan on soil organisms

Carabid beetles and earthworms were not trapped in the respective traps or methods set for the purpose (Plate-10)

4.7 Additional soil application of treatments after bunch emergence

Banana plants maintained to study the residue and persistence of fipronil and carbosulfan after application on 0th, 60th and 150th day were subjected to an additional dose of fipronil (in T_2 and T_3 treatment) and carbosulfan (in T_4 and T_5 treatment), respectively during bunch emergence. The residues present in various plant parts are presented in table 40. Even after additional application of treatments just after bunch emergence, no residue of carbosulfan and fipronil and their metabolites were present in various harvested parts of banana plant.

Plate 4. Effect of treatment on bacterial growth in the soil

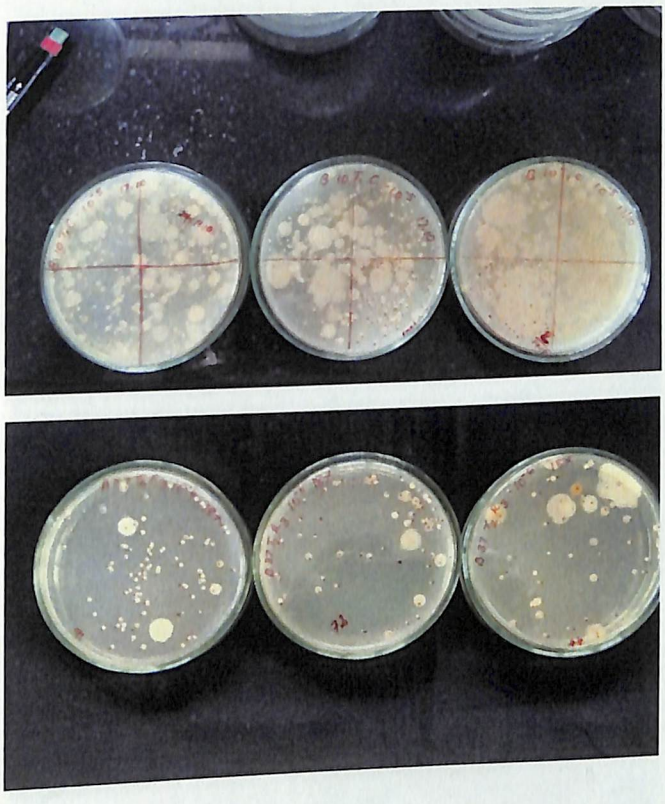


Plate 5. Effect of treatment on actinomycetes growth in the soil

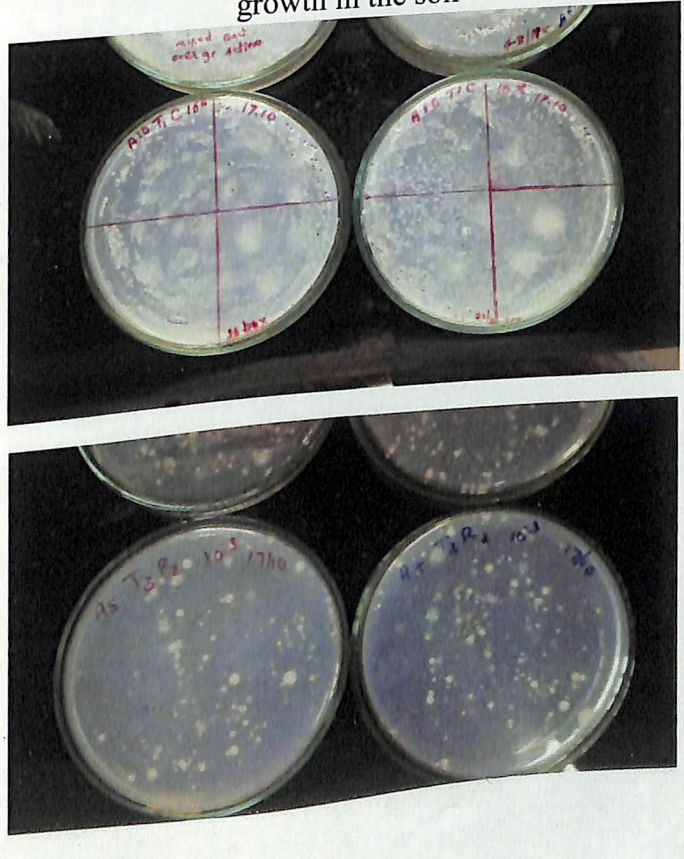


Plate 6. Effect of treatment on actinomycetes growth in the soil

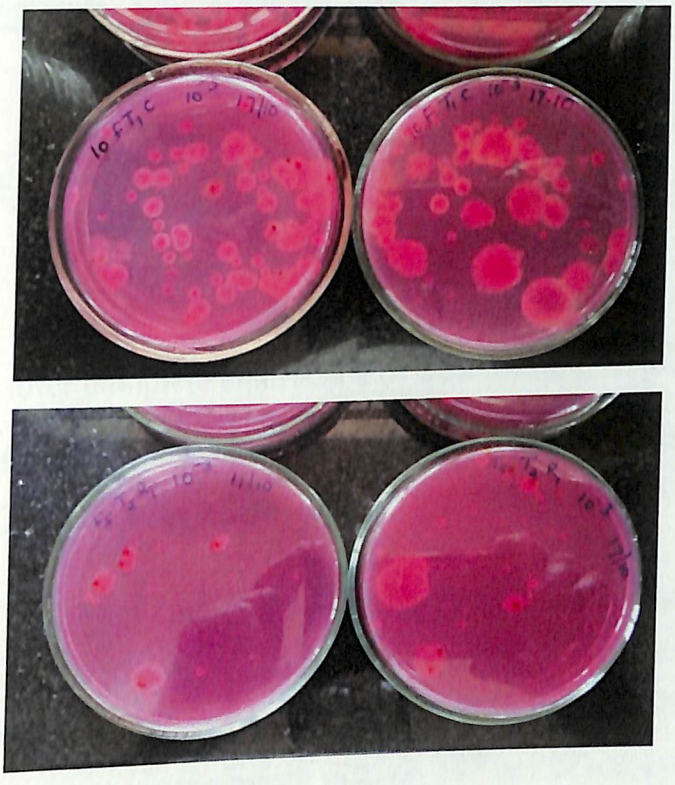


Table 40. Residue of carbosulfan and fipronil and their metabolites in banana at harvest due to additional application of treatment at bunching.

Treatment and molecule	Mean residue (μg g^{-1})				
	Flower bud*	Peel alone	Fingers of Bunch on Harvest	Pseudo stem	Corm
T_1 Control	BDL	BDL	BDL	BDL	BDL
T_2- 30 mg ai fipronil / plant	BDL	BDL	BDL	BDL	BDL
T_3- 60 mg ai fipronil/plant	BDL	BDL	BDL	BDL	BDL
T_4- 400 mg ai Carbosulfan/plant	BDL	BDL	BDL	BDL	BDL
T_5- 800 mg ai Carbosulfan/plant	BDL	BDL	BDL	BDL	BDL

***** Harvested 3 days after complete emergence of fruit forming fingers

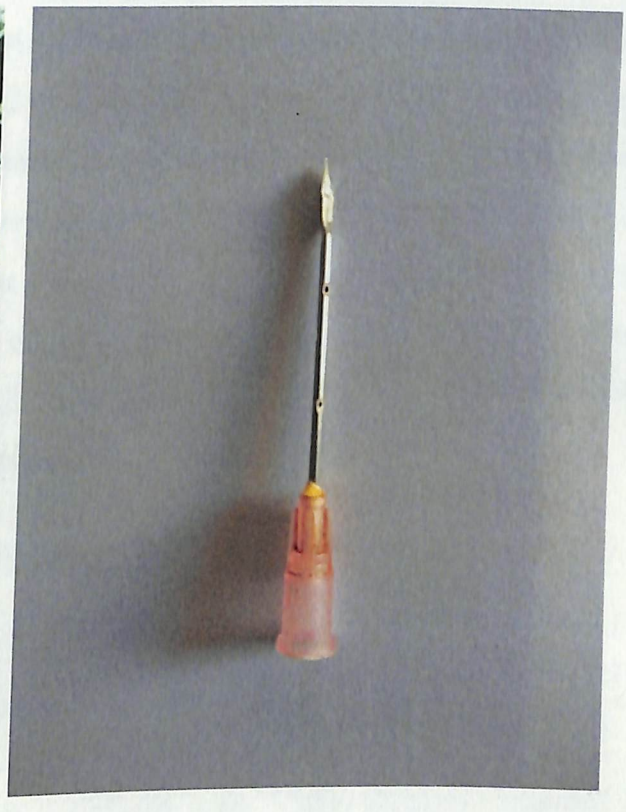
4.8 Effect of pseudostem injection of fipronil and carbosulfan at the rate five times the recommended dose at bunch emergence

The residue of fipronil and carbosulfan in bunches following application of 2000 mg a.i. of carbosulfan and 150 mg a.i. of fipronil injected into the pseudo stem at the time of bunch emergence (as per para 3.2.3.) are presented in the table-41. It is observed that carbosulfan residue persisted in the bunch pulp on 15th day of emergence after five times the recommended dose application (0.0128 μg g^{-1}) and dissipated to BDL in the sample harvested after 30 days emergence of bunch. Residues of fipronil and its metabolites were not detected in the samples. The residue of fipronil and carbosulfan were not detected in flower bud, flower bract alone, bunch pulp and in the peel.

Table 41. Effect of application of five times the recommended dose as pseudo stem injection at bunch emergence stage on residue levels in inflorescence and bunch

Residue of fipronil and carbosulfan, μg g^{-1}						
Treatment and molecule	flower bud	Flower bract alone	Bunch pulp alone (on 15th day of emergence)	Bunch (on 30th day of emergence)	Peel	Bunch pulp alone
T_1 control	BDL	BDL	BDL	BDL	BDL	BDL
T_2- fipronil	BDL	BDL	BDL	BDL	BDL	BDL
T_4-carbosulfan	BDL	BDL	0.0128	BDL	BDL	BDL

Plate 7. Application of 5-time dose as pseudo stem injection using special needle from (CTCRI, Sreekaryam, Trivandrum



4.9 Effect of treatments on biometric observations and yield parameters

4.9.1 Effect of treatments on plant height of banana

The different levels of treatments with fipronil and carbosulfan did not result in any significant differences in the plant height throughout the period of observations taken at weekly intervals and finally at harvest (table42). Maximus height was observed in treatment T_2 (279 cm) followed by T_4 (252 cm), T_3 (248 cm), T_1 (243.3 cm) and the least for T_5 (235.5 cm). Plants receiving T_2 treatment recorded the maximum height throughout the period of observation over the plants which received other treatments. How ever this was no significantly higher that the height attained by the plants in other treatment. It is seen that height observed at harvest was slightly lower than that observed at 22 weeks after planting in all the cases. Plants under treatment T_5 registered the lowest height, though not significant over the other treatment.

Table 42. Effect of treatment on plant height of banana at fortnightly intervals

Treatment	Plant height, cm											
	Time interval in fortnightly interval and at harvest											
	2	4	6	8	10	12	14	16	18	20	22	Harvest
T_1 control	21.3	43.0	64.3	85.5	106.8	128.5	149.8	171.0	192.3	225.0	249.0	243.3
T_2- fipronil-pop	25.5	51.0	76.5	102.0	127.5	153.0	178.5	204.0	229.3	267.8	285.8	279.0
T_3- fipronil-2xpop	22.3	44.8	67.0	89.8	112.3	134.5	156.8	179.3	201.5	235.8	251.0	248.0
T_4-carbosulfan-pop	23.0	46.3	69.3	92.0	115.3	138.3	161.3	184.5	207.5	242.5	257.5	252.0
T_5-carbosulfan 2xpop	22.3	44.0	66.3	88.5	110.5	132.3	154.8	176.5	198.5	232.3	245.8	235.5
CD (0.05)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	19.74	19.85	19.79	19.66	19.75	19.70	19.71	19.63	19.71	19.60	17.34	17.98

4.9.2 Effect of treatments on plant girth of banana

Imposed treatments do not have any significant effects on the girth of the trunk of banana (table 43), highest girth was observed to be 56 cm for T_2 and the least for T_1 (53 cm).

Table 43. Effect of treatment on girth of banana trunk at fortnightly intervals

	Girth, cm											
	Fortnightly time intervals and at harvest											
Treatment	2	4	6	8	10	12	14	16	18	20	22	Harvest
T_1 control	3.8	7.8	13.5	17.8	22.5	27.3	33.0	38.0	43.0	48.5	53.3	53
T_2- fipronil-pop	3.8	9.0	14.8	20.0	24.8	30.8	36.3	42.5	47.3	54.0	56.3	56
T_3- fipronil-2xpop	3.8	8.3	14.0	18.8	23.5	28.8	34.5	40.3	45.0	51.3	54.5	54.25
T_4-carbosulfan-pop	3.8	8.3	14.0	18.8	23.3	28.8	34.3	40.0	44.8	51.0	54	54
T_5-carbosulfan 2xpop	3.8	8.3	14.0	18.8	23.0	29.3	34.3	39.8	45.0	51.0	54	54
CD (0.05)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	14.6	11.4	9.8	9.3	8.9	9.2	8.1	8.4	8.3	8.6	6.5	6.5

4.9.3 Effect of treatments on number of active leaves of banana

The effect of treatment on the number of active leaves during the growth period are presented in the table 44. Treatment T_2 registered the highest value (7.3) and T_1 with lowest value of 6.5 numbers at harvest, however there was no significant differences between the treatments

Table 44. Effect of treatments on number of active leaves of banana

	Number of active leaves, numbers											
	Time interval in fortnightly interval and at harvest											
Treatment	2	4	6	8	10	12	14	16	18	20	22	Harvest
T_1 control	0.3	1.8	3.5	4.5	6.0	7.0	8.0	9.3	10.5	11.5	11.5	6.5
T_2- fipronil-pop	1.0	2.0	4.3	5.8	7.0	8.0	9.0	10.3	12.3	13.3	13.0	7.3
T_3- fipronil-2xpop	0.5	2.0	3.8	4.8	6.5	7.5	8.5	10.0	11.0	12.3	12.5	7.0
T_4-carbosulfan-pop	0.5	2.0	4.0	5.3	6.5	7.5	8.8	10.3	11.8	12.8	12.3	7.0
T_5-carbosulfan 2xpop	0.3	2.0	3.8	5.0	6.3	7.3	8.3	9.8	10.8	12.0	12.3	7.0
CD (0.05)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	9.01	11.5	12.3	11.3	8.2	7.1	9.3	6.4	8.4	7.9	5.4	5.2

4.9.4 Effect of treatments on length of index (4th) leaf of banana

Length of index leaf i.e., 4th leaf at the time of observations at fortnight intervals are presented in the table 45. Due to non-formation of index leaf, observation on 2nd week of planting for length and breadth of index leaves were not presented in the table. Treatments did not result in any significant difference. However, banana plants which received T_2 treatment has exhibited highest value for length throughout the growing period. At harvest also, these samples recorded the maximum values (134.8 cm) and the least was recorded by those plants which received treatment T_4.

Table 45: Effect of treatment on length of index leaf (4th) of banana

	Length of index leaf, cm										
	Time interval in fortnightly interval and at harvest										
Treatment	4	6	8	10	12	14	16	18	20	22	Harvest
T_1 control	31.3	41.3	55.0	68.5	82.0	95.5	108.5	118.5	132.0	132.0	126.3
T_2- fipronil-pop	33.3	44.0	58.3	72.8	87.5	101.8	116.0	126.8	141.0	141.0	134.8
T_3- fipronil-2xpop	31.5	41.3	54.8	68.0	82.0	95.5	108.5	118.5	132.0	132.0	126.0
T_4-carbosulfan-pop	30.0	39.3	52.0	65.3	78.0	91.0	103.8	113.0	125.8	125.8	120.0
T_5-carbosulfan 2xpop	33.0	43.3	57.0	71.5	85.8	99.8	113.5	124.3	138.0	138.0	131.5
CD (0.05)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	7.1	7.4	7.2	7.3	7.3	7.1	7.2	7.4	7.2	7.2	7.3

4.9.5 Effect of treatments on breadth of index (4th) leaf of banana

In table 46, effect of fipronil and carbosulfan on breadth of the index leaf of banana are given. It can be seen that from 4th week till harvest, there exist a significant difference in the breadth of the leaf, between the treatments T_1, T_2 and T_1, T_5. Treatment T_2 exhibited the highest value and T_1 exhibited the lowest value for leaf breadth throughout the period. T_2 (64.3 cm) was significantly higher than other treatments. T_1 was significantly lower than T_2 and T_5. However, T_1 (56.3 cm), T_3 (60.0 cm) and T4 (58.8 cm) were on par at the time of harvest. Also, there was no significant difference between T_2 and T_5 at the time of harvest. Similar trend was not noticed during different phases of growth other than the observation at harvest.

Table 46: Effect of treatment on breadth of index leaf (4th) of banana

Treatment	Breadth of index leaf, cm										
	Time interval in fortnightly interval and at harvest										
	4	6	8	10	12	14	16	18	20	22	Harvest
T1 control	23.0c	26.5c	30.5c	35.0c	39.0c	43.0c	46.8c	51.3c	57.0c	58.8c	56.3c
T2- fipronil-pop	26.5a	30.8a	35.0a	40.0a	44.5a	49.3a	54.0a	58.3a	65.5a	67.3a	64.3a
T3- fipronil-2xpop	24.0bc	27.8bc	31.8ac	36.5bc	40.5bc	45.0bc	48.8bc	52.8ab	59.5bc	63.0abc	60.0abc
T4-carbosulfan-pop	23.5bc	26.8c	30.8bc	35.0c	39.0C	43.0c	47.0c	51.3b	57.3c	61.5bc	58.8bc
T5-carbo-sulfan 2xpop	25.8ab	29.8ab	34.0ab	38.8ab	43.0ab	47.8ab	52.3ab	56.8ab	63.5ab	65.5ab	62.8ab
CD (0.05)	2.42	2.93	3.32	3.46	3.99	4.12	4.82	5.54	5.86	4.67	5.45
SE(d)	1.11	1.34	1.52	1.59	1.83	1.89	2.21	2.54	2.54	2.15	2.08
CV (%)	6.38	6.72	6.65	6.07	6.30	5.85	6.29	6.65	6.28	4.81	4.88

Table 47. Effect of treatment on length to breadth ratio of index leaf (4th) of banana

Treatment	Length breadth ratio										
	Time interval in fortnightly interval and at harvest										
	4	6	8	10	12	14	16	18	20	22	Harvest
T1. Control	1.37	1.57	1.81	1.97	2.11	2.23	2.33	2.32	2.33	2.26	2.25
T2- fipronil-pop	1.26	1.44	1.67	1.83	1.97	2.07	2.16	2.18	2.16	2.10	2.10
T3- fipronil-2xpop	1.32	1.50	1.74	1.87	2.04	2.14	2.24	2.26	2.23	2.10	2.11
T4-carbosulfan-pop	1.28	1.47	1.69	1.87	2.00	2.12	2.21	2.21	2.20	2.05	2.05
T5 carbosulfan 2xpop	1.29	1.46	1.68	1.85	2.00	2.09	2.18	2.19	2.18	2.11	2.10
CD (0.05)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
SE(d)	na	na	na	na	na	na	na	na	na	na	na
CV (%)	5.44	8.79	5.83	6.08	6.64	5.36	6.37	7.09	7.42	5.97	6.33

observation from 4th to 22nd week. Throughout the period of observation, the T2 samples recorded significantly higher breadth of index leaf. Similar trend of significant differences was not noticed during different phases of observation from 6th to 22nd week and at the time of harvest.

4.9.6 Effect of treatments on length to breadth ratio of index (4th) leaf of banana

The effect of treatment on ratio of length to breadth of index leaf is presented in the table 47. It was noted that over the period of growth, treatments did not show any significant difference on the ratio of length to breadth ratio. At the time of harvest length to breadth ratio varied between 2.05 and 2.25.

4.9.6 Effect of treatments on breadth of index (4th) leaf of banana blossom bud, bunch, pseudostem and corm weight of banana

Weight of blossom bud, bunch, pseudo-stem and corm under different treatments are given in table 48. Weight of blossom bud were significantly higher under T_2 (1.79 kg) and T_4 (2.02 kg) and that of flower buds from T_1, T_3, and T_5 were on par. Weight of blossom bud was highest in T_4. Bunch weight of T_2 (12.21 kg) and Pseudo stem weight (37.84 kg) and corm weight (6.4 kg) of T_2 were also significantly higher than others.

Table 48. Effect of treatment on blossom bud, bunch, pseudostem and corm weight of banana

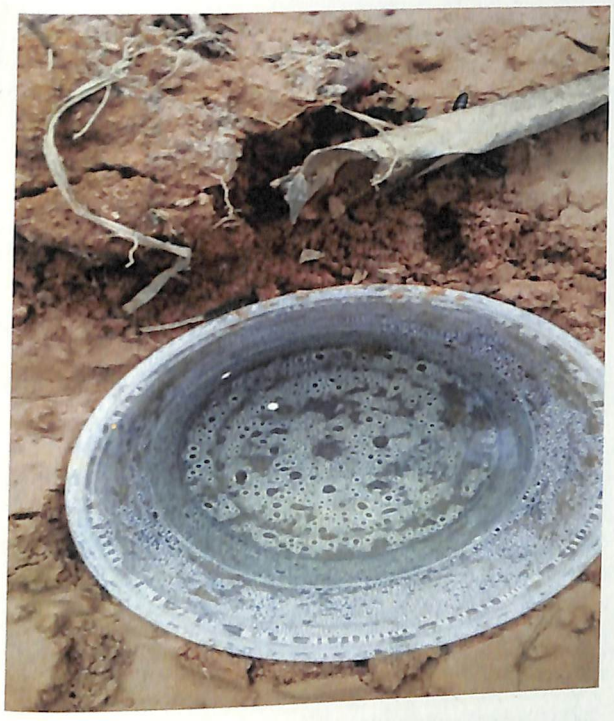
Treatment	Weight of the plant part (kg)			
	Blossom bud	Bunch	Pseudo-stem	Corm
T_1 control	1.52^{b}	11.11^{d}	34.44^{d}	5.8^{d}
T_2- fipronil-pop	1.79^{ab}	12.21^{a}	37.84^{a}	6.4^{a}
T_3- fipronil-2xpop	1.62^{b}	11.73^{b}	36.37^{b}	6.b^{b}
T_4-carbosulfan-pop	2.02^{a}	11.28^{c}	34.95^{c}	5.9^{c}
T_5-carbosulfan-2xpop	1.70^{b}	10.84^{e}	33.60^{e}	5.7^{e}
CD (0.05)	0.50	0.48	0.547	0.482
SE(d)	0.147	0.07	0.13	0.02
CV (%)	11.63	0.55	0.56	0.56

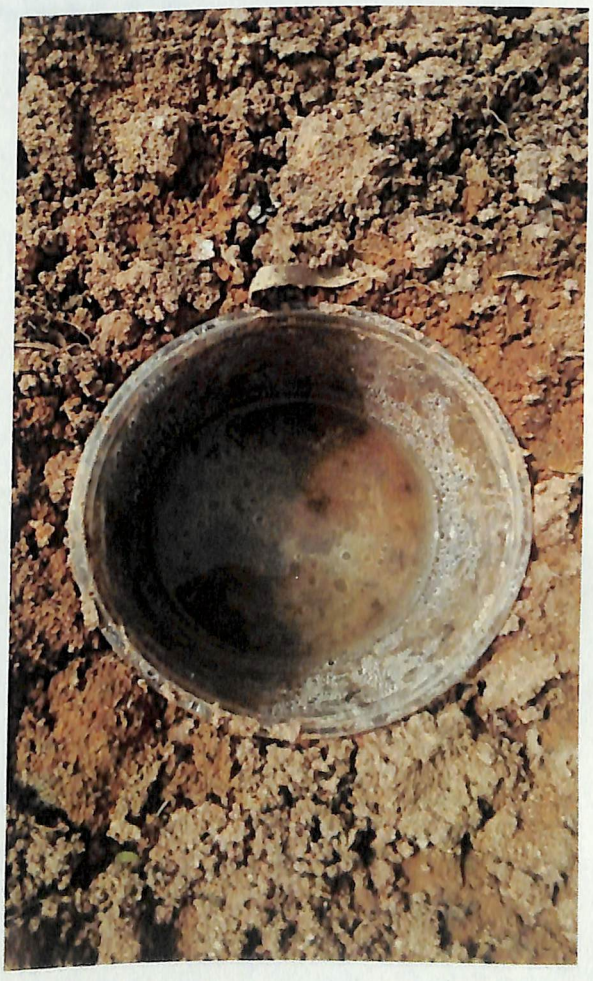
4.10. Other items of observations:

Emergence of bunch started on 6th day after 22nd week and was completed on 2nd February 2017 in all the plots under various treatments and replication.

Incidence of pest and disease: Leaf spot symptoms of sigatoka were observed in certain plants on the 16th week on planting and appropriate control measures were taken as per package of practice recommendation of KAU, 2016.

Plate 8. Pit fall traps for carabids (modified)





5. Discussion

Banana, cv. Nendran (AAB) (*Musa spp*) is the most preferred fruit in Kerala. It is used both for table (consumed) and culinary (consumed after cooking) purposes. Cultivation of the crop is faced with many challenges among which insect pests like pseudo-stem weevil and rhizome weevil pose severe threat and crop damage (Ostmark,1974). The insecticides recommended for control of weevil pests of banana were soil application of granular forms of phorate and carbofuran (KAU, 2011). Some of the insecticides recommended earlier for insect control have been banned from use and were not available for the use in banana. However, due to widespread infestation and consequent damage of the crop leading to economic loss to farmers KAU suggested various ad-hoc alternatives for the control of these pests in banana. Among the alternatives suggested carbosulfan and fipronil in granular form are the recommended insecticides.

Fipronil, 5-amino-1-(2,6-dichloro-α, α, α, -trifluoro p-tolyl)-4-trifluoro-methyl sulfinyl pyrazole-3-carbonitril, belonging to phenyl pyrazole group is also a substitute for carbofuran and phorate, the two banned insecticides for the control of banana rhizome weevil (KAU, 2015). 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 phorate and carbofuran for the control of banana aphid, (the vector of bunchy top disease of banana) (KAU, 2011).

Considering the toxicological relevance of the use of these compounds when used in banana, a set of experiments were undertaken to investigate the absorption, translocation, dissipation or metabolism of fipronil and carbosulfan when applied to soil as per the recommended dose and its double rates in soil from August 2016- to April, 2017. In these experiments an additional single application at bunch emergence as against the recommendation and application at five times, the one-time recommended dose into the pseudostem as an injection to assess the presence of residues in the edible commodity during harvest were also included. The main objective of this study was to

work out the dissipation and residue status of applied chemicals and their toxic metabolites in edible portions of banana, leaf and soil.

5.1. Soil Characteristics of the experimental plot

The surface soil of the experimental plot is sandy loam in texture and belongs to kaolinitic isohyperthermic, typic kandiustults (GOK, 2007). Soils of the experimental plots have a pH of 5.7 in the acidic range and were classified as moderately acidic. The organic carbon content was 1.5 per cent. It belongs to class 9 of high organic matter. Available P and available K were 196.1, 358.4 kg ha^{-1} respectively, requiring only 54 percent and 25 percent each of P_2O_5 and K_2O, respectively, of the recommended dose of N, P and K (KAU, 2011). Extractable Ca and Mg were 393 and 90.3 mg kg^{-1}, respectively whereas available S was 11.70 mg kg^{-1}. Essential micronutrients viz., HCl extractable Fe, Zn, Mn, Cu were in the sufficiency level, while hot water extractable B content of the soil was low (0.4 mg kg^{-1}). Except for Mg and boron, all extractable secondary and micronutrients were in sufficiency level and hence Mg and B were supplemented as $MgSO_4$ and borax as per KAU, (2016).

From table 2 (results) the weather parameters during the growth period were comparatively dry, receiving only 186.7 mm rainfall with cumulative pan evaporation was 869.9 mm, which warranted irrigation (on alternate days except days of precipitation) throughout the period of growth.

5.2 Method validation and optimization of LC-MS/MS parameters for estimation of fipronil, carbosulfan and residues of their metabolites from banana and soil.

The methodology for residue estimation of carbosulfan and fipronil were standardized by using samples spiked with pure analytical standards of these chemicals followed by extraction and clean up using suitable techniques. The sample matrix from different sample types viz., banana leaves, pseudo-stem, bunch finger, flower, corm and soil collected from the additional plants from control plots at respective stages were utilized for the purpose of method validation.

Mean of method validation parameters for fipronil and its metabolites with matrix match samples of banana leaves, pseudo-stem, bunch finger, flower, corm and soil, collected from the specially maintained control plots at the respective stages of

harvest for parameters viz., percentage recovery, relative standard deviation (RSD) value, linearity and repeatability are presented in tables 3 to 8. To assess the effectiveness of extraction and clean up processes, two methods were tried for the extraction of residues by both conventional and QuEChERS method for analysis of fipronil and its metabolites using LC-MS/MS (UPLC Waters Aquity + AB Sciex API 3200) triple quadrupole mass analyser. From the tables it is seen that the percentage recovery of estimation of fipronil desulfinyl, fipronil, fipronil sulphide, and fipronil sulfone obtained through QuEChERS method ranged from 80.0 to 119.9 percent while the corresponding values for precision ranged from 0.4 to 12.9 percent, which were in the acceptable range and thereby increasing the possibility of handling more samples per unit time. Dutta et al. (2008) obtained a recovery of fipronil and its metabolites from cabbage samples which ranged from 80.84 to 88.3 $\pm$6.8%. Seiber (2011) suggested that efforts are needed to advance and improve rapid method technique like QuEChERS that require minimal sample preparation and are suitable for in situ estimations. Beevi *et al.* (2014) contented that, the recovery of pesticides in LC MS/MS ranged between 70-120 percent may be treated as satisfactory on obtaining for value in that range for all the 26 compounds including fipronil (71.13 per cent), carbofuran (107.38 per cent) and carbosulfan (86.25 per cent) when tested at the respective LOQ.

From the above discussions, it is concluded that QuEChERS method yielded a value for accuracy in the acceptable range (70-120 per cent) better than values obtained for the same molecule estimated using the conventional method.

For carbosulfan, percentage recovery, RSD value, linearity and repeatability and limit of quantitation of each of the method tried are presented in tables 12-17. Carbosulfan and its metabolites also yielded similar results for these parameters and QuEChERS method was found more ideal owing to better recovery and RSD values associated with low solvent consumption and faster analysis and hence chosen for all analytical procedures in the study.

In the entire matrix matched recovery studies for the above 6 different types of matrices confirmed that linearity (ranged from 0.01 to 1.00 $\mu g\ g^{-1}$) and limit of

quantitation LOQ (0.01μg g^{-1}) obtained has a definite edge over the conventional method. In conventional method, the linearity ranged between 0.05 to 1.00 μg g^{-1} and LOQ was found to be 0.05μg g^{-1}. In this connection, Beevi *et al.* (2014) reported a LOQ of 0.05 μg g^{-1} in the estimation of residues following QuEChERS method of extraction for carbofuran, carbosulfan and fipronil in soil followed by detection and quantification using LC-MS/MS. 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). In the present study, for estimation of residues of fipronil and carbosulfan from all different matrices of banana is as per Sante (2016) norms.

Hence, QuEChERS method was adopted for extraction, clean up and estimation of residues in all the samples of leaves, fingers of bunches, pseudostem, flower (blossom bud), corm, and soil from the field experiment plot.

5.3 Field studies on dissipation (absorption, translocation, distribution, persistence) of fipronil in banana

Samples of 1st, 2nd, 3rd and 4th leaves of banana collected for residue analysis of fipronil and its metabolites at two different levels of application in soil viz., normally recommended dose and its double rate are depicted in the table 24.

Residue status of fipronil and its toxic metabolites viz., fipronil desulfinyl, fipronil sulphide and fipronil sulfone in 1st, 2nd 3rd and 4th leaves of banana, applied thrice with single or double dose of fipronil were found to be BDL even on day 50 after application.

Mortensen *et al.* (2015) too suggested that fipronil cannot be classified as a systemic insecticide, though there are reports which suggested that fipronil is taken up by the root and translocated into the plant (Bonmatin *et al.*, 2015). However, no toxic metabolite of fipronil was detected in any of the leaf samples, indicating a faster metabolism and dissipation of fipronil in banana, both at normal and double doses, thereby ensuring safety from its toxic residues in leaf samples. The results are in agreement with the findings of Dutta *et al.*, (2008), in cabbage where fipronil got

dissipated with a half-life of 7.5-7.6 days and suggested that fipronil applied cabbage is safe for consumption, only when it is soil incorporated.

Application of fipronil even at double dose did not leave any residues in first 3 banana leaves, indicating that there is no effective symplastic translocation of fipronil or its toxic metabolites to these leaves when applied to the rhizosphere soil. This may also be attributed to the nature of the crop having height 2 meters. At double the recommended dose of application fipronil was present in the fourth leaf only on 40^{th} day of application (0.034 ppm) (Table 21) and the same was BDL by 50^{th} day.

5.4 Field studies on dissipation (absorption, translocation, distribution, persistence) of carbosulfan and its metabolites in banana

Samples of 1^{st}, 2^{nd}, 3^{rd} and 4^{th} leaves of banana were collected for residue analysis of carbosulfan and its metabolites. Persistence of total carbosulfan molecule (carbosulfan and its metabolites - carbofuran, 3-hydroxy carbofuran and 3-keto carbofuran) in the 1st, 2^{nd}, 3^{rd} and 4^{th} leaves of banana at two different levels, viz., normally recommended dose, T_4 and its double rate, T_5 are depicted in the fig. 2 to 5. The ratio, residue observed on treatment level of double the application of POP to that of POP (as indicated by T_5/T_4 for carbosulfan and T_3/T_2 for fipronil) was worked out for each day of observation with a value for residue. The net transformation or dissipation rate per day (TPD) i.e., rate of dissipation, was worked out. TPD is obtained by dividing the difference in residue concentration between two consecutive observations (ΔT_4 and ΔT_5 representing treatment 4 and 5 respectively) with number of days between the interval of observation (ΔD). A positive value for TPD indicates a higher rate of absorption from the soil than the rate of dissipation of the residue within the plant system. A negative value of TPD indicate a lower rate of absorption from the soil than the rate of dissipation of the residue with in the plant system. These values are depicted as a footer table to the graph in the respective figures.

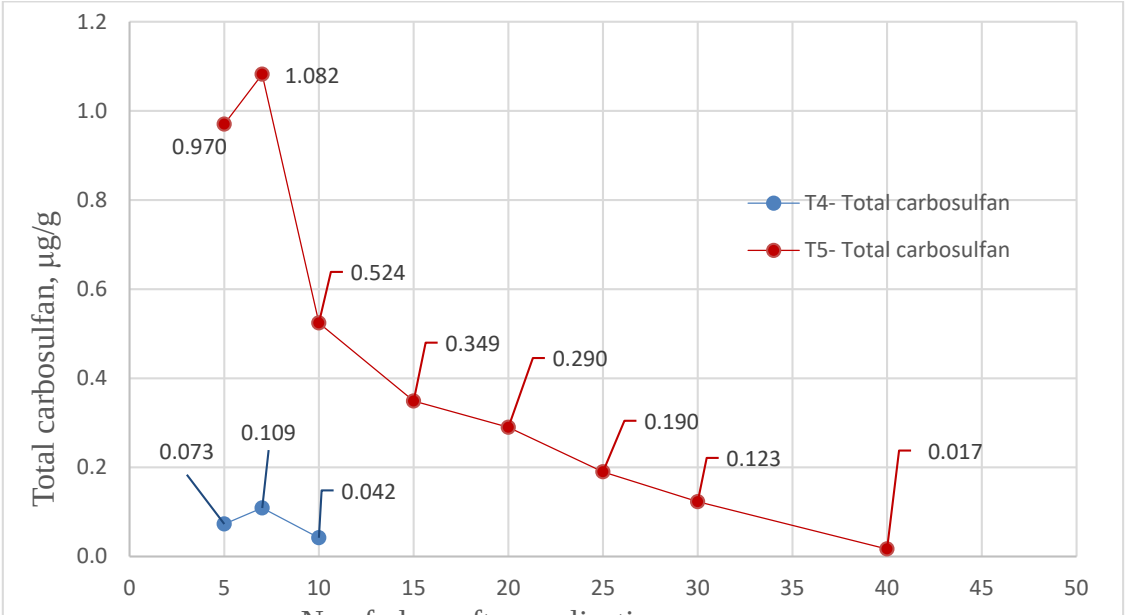
5.4.1 Total carbosulfan and its metabolites in the 1^{st} leaf of banana

It is seen that in the first leaf (fig. 2), on the 5^{th} day, the total metabolites of carbosulfan at double the rate of application was 13.3 times higher than the normal

dose. For 7th and 10th day residue was higher by 9.9 and 12.5 times, respectively. The net transformation (TPD), as indicated (in the fig. 2) by $\Delta T_4/\Delta D$ was positive for 5th day for T_4 and it was negative on 7h and 10th days before reaching BDL by day 15. Absorbed residue attained maximum level on 7th day in both T4 and T5 treatments. In the 1st leaf for T_5, it was positive only on 5th day, however continued to remain negative from day 7th to day 40th day before attaining BDL by day 50.

The TPD of 1st leaf on day 10 suggests that the residue would have dissipated completely on 15.25 days after application in T_4. In T_5, a similar assumption of dissipation rate of 0.002 between day 40 and 50 has led to conclude that complete dissipation in the first leaf might have occurred only by 48.5 days of application. Geng *et al*. (2018) reported that in green house cucumber the half-life of carbosulfan in leaves as 2.8 days.

Fig. 3. Mean residue of total carbosulfan and its metabolites in 1st leaf of banana



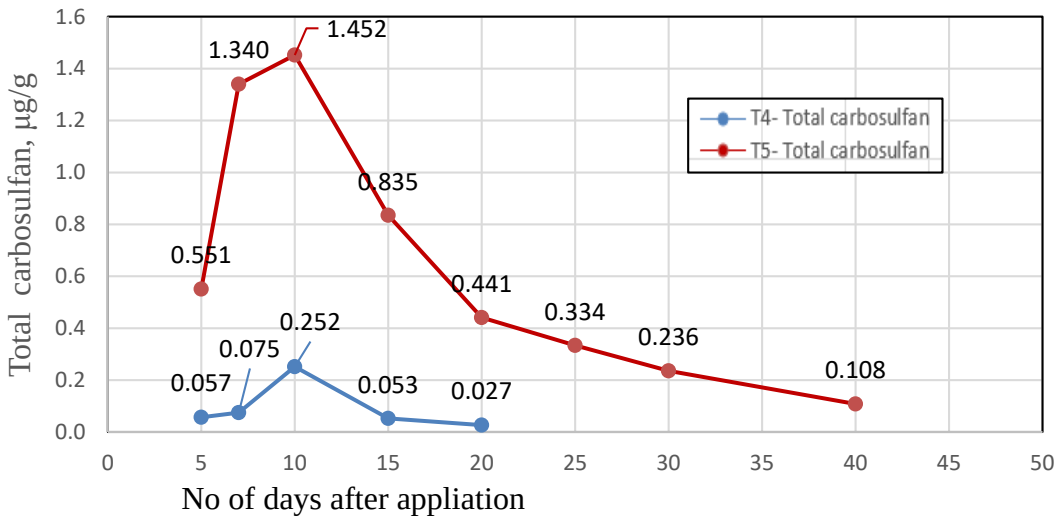
Parameter	Days after application									
	3	5	7	10	15	20	25	30	40	50
$T_5/T4$		13.3	9.9	12.5						
$\Delta T_4/\Delta D$	0.037	0.018	-0.022	-0.008						
$\Delta T_5/\Delta D$	0.485	0.056	-0.186	-0.035	-0.012	-0.020	-0.013	-0.011	-0.002	

5.4.2 Total Carbosulfan and its metabolites in the second leaf of banana

In the 2nd leaf, on 7th day the total of carbosulfan metabolites were 17.9 times higher in T_5 (fig. 3) where-in, the rate of dissipation was positive on 5th, 7th and 10th day and then transforming to negative dissipation rate from day 15 before reaching BDL on day 45. The dissipation rate in 2nd leaf for T_4 and T_5 remained positive till day 10.

Higher content of the metabolites in T_5 coincides with double the level of application in the soil suggests that, the rate of absorption and transformation of the applied molecule was at sub optimal level in T_4 due to lower concentration of residues for saturating the exchange in the sites of the soil matrix. Also, this lower concentration in T_4 would have led to a higher proportion of adsorption of the applied molecule, on the organic matter-clay complex (OMCC) surfaces together with higher tenacity to hold the applied or transformed molecules may be a reason for a higher residue in the leaf. This higher tenacity of the molecule to the OMCC would have prevented the absorption of molecule by the plant root. The tenacity might get reduced with further increase in the concentration of the applied molecule. This would have led to increased concentration in the leaves.

Fig. 4. Mean residue of total carbosulfan and its metabolites in 2nd leaf of banana



Parameter	Days after application									
	3	5	7	10	15	20	25	30	40	50
T_5 /T4		9.7	17.9	5.8						
$\Delta T_4 / \Delta D$	0.029	0.009	0.059	-0.040	-0.005	-0.005				
$\Delta T_5 / \Delta D$	0.276	0.395	0.037	0.014	-0.137	-0.100	-0.020	-0.013	-0.011	

In the second leaf too, TPD as indicated by $\Delta T_4/\Delta D$ was positive from 5th to 7th. In the 2nd leaf for T_5, $\Delta T_5/\Delta D$ was positive on day 5 to 10, however continued to remain negative through day 20 to day 40 before attaining BDL on day 50. Un like first leaf, in the 2nd leaf, the peak absorption of residues at both levels were observed on day 10th.

The rate of dissipation on day 10 for 2nd leaf indicates that, at that rate of dissipation the whole molecules would have dissipated completely on 20.67 days after application at T_4 level. In the case of T_5 level, based on dissipation rate of 40th day, the molecule might have assumed to be completely dissipated on 49.8 days after application.

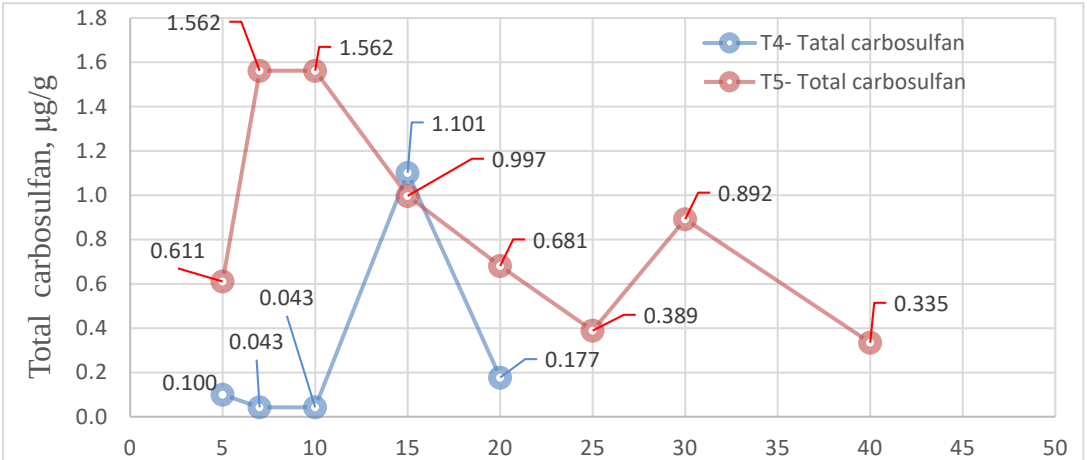
It may be noted that the calculated half-life for each interval of observation was highly varying with respect to interval of observation, may be due to crop physiology as affected by load of absorbed molecule, dissipation rate of molecule with in the plant and soil dependent factors affecting release of molecule. Hence an utmost care is required in estimation of half-life of absorbed molecules in banana and its application, which are being managed in tropical climatic conditions with heavy irrigation, intermittent with multiple periods of heavy rainfall affecting the already high growth dilution factor for this crop.

5.4.3. Total carbosulfan and its metabolites in the third leaf of banana

In 3rd leaf, the total carbosulfan metabolites were 36.3 times higher in T_5 (Fig. 4) and it was 6.1 and 16.3 times higher on 5th and 10th day respectively in comparison to T_4. In T_4, dissipation rate on day 5 was negative due to the decline in the level of residue on 7th day. The residue level remained same on 7th and 10th day on 3rd leaf showing a dissipation rate of zero, that is a state where rate of absorption from the soil and its translocation to the leaf might be same as the rate of the dissipation and disappearance of toxic metabolites from the leaf. There was an increased rate of absorption from the soil on day 7 onwards, reaching a peak on day10. It remained static for the next 3 days and gradually increased to 0.212 µg per day on 15th day. Thereafter, the residue level attained BDL by day 25.

Based on the dissipation rate on the penultimate day of observation of residue, it may be presumed that the residue in 3rd leaf for total carbosulfan would have fully dissipated in 20.83 days in T_4 application and in 39.89 days in T_5 application.

Fig. 5. Mean residue of total carbosulfan and its metabolites in 3rd leaf of banana



Parameter	Days after application									
	3	5	7	10	15	20	25	30	40	50
T_5 /T4		6.1	36.3	36.3						
$\Delta T_4 / \Delta D$	0.050	0.0	0.0	0.212	-0.185	0				
$\Delta T_5 / \Delta D$	0.306	0.5	0.0	-0.113	-0.063	-0.058	0.101	-0.056	-0.034	

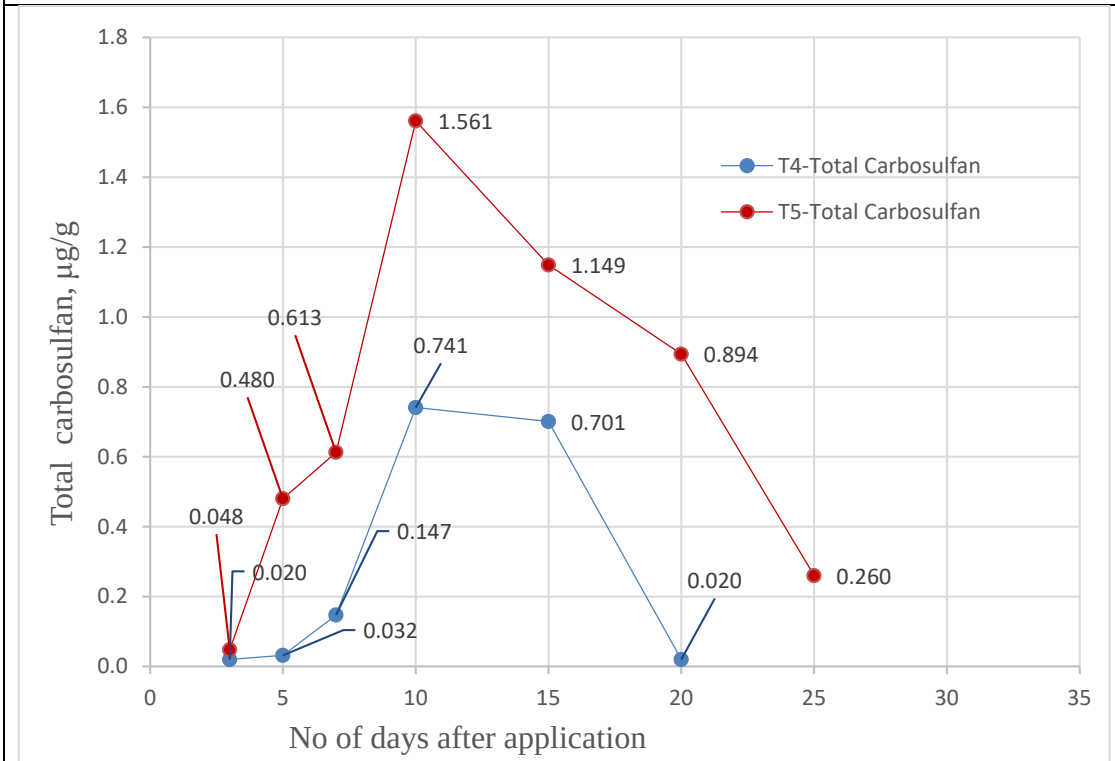
5.4.4 Total carbosulfan and its metabolites in the fourth leaf of banana

Mean residue of total carbosulfan and its metabolites in 4th leaf of banana, presented in the fig-5 depicts the pattern of dissipation of total carbosulfan lasting up to 20th and 25th day in T_4 and T_5 level of application respectively. The T_5 level of application had resulted in 15 times absorption of carbosulfan on the 5th day in comparison to T_4 level and $\Delta T_4/\Delta D$ was positive up to day 10 indicating a higher rate of absorption than the rate of dissipation within the plant. In 4th leaf, on day 15 it was negative and at that rate of dissipation, the carbosulfan would have dissipated fully by 22.5 days in T_4 level of application. In this leaf at T_5 level complete dissipation would have occurred by day 30, which is in concurrence with the recording made on day 30.

Vijayan (2000) reported in a residue study of carbofuran that the average total carbofuran in 3rd and 4th leaf of banana was 0.027 µg 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 *et at.* (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.

Fig. 6. Mean residue of total carbosulfan and its metabolites in 4th leaf of banana



Parameter	Days after application								
	1	3	5	7	10	15	20	25	30
T_5/T_4		2.4	15.0	4.2	2.1	1.6	44.7		
$\Delta T_4/\Delta D$	0.010	0.006	0.058	0.198	-0.008	-0.136	-0.004		
$\Delta T_5/\Delta D$	0.024	0.216	0.067	0.316	-0.082	-0.051	-0.127	-0.052	

It can be seen that translocation of carbosulfan (total) in different leaves were in the order $L_3 > L_4 > L_2 > L_1$ for both level of application. Carbosulfan persisted up to 10 days in the 1st leaf, at recommended dose of application. In 2nd, 3rd and 4th leaves it was present till 20th day, attaining BDL by 25th day, indicating that it is not safe to use the leaves within 25 days of application of recommended dose of carbosulfan for serving or packing food (which is very common practice in households of Kerala).

At T5 level of application, residue of carbosulfan (total) in 1st and 2nd leaves persisted till the 40th day. In case of 3rd and 4th leaves, at this level of application, carbosulfan (total) persisted till 30th and 25th day respectively.

5.4.5. Carbofuran residue in the first leaf

Mean residue of carbofuran molecule in the 1st four leaves of banana plant are depicted in Fig. 6 to 9. The residue of carbofuran in the 1st leaf at T_4 treatment was detected only on 7th day of application (fig-6) and on this day, it was 3.36 times higher in T_5 level of application. At this T_5 level, on day 3, it recorded the highest carbofuran residue of 0.388 μg g^{-1}. Though dissipation rate was positive on day 3 and 10, on 5th, 7th, 15th, 20th, 25th and 30th days it was negative. Based on the dissipation rates, at T_4 and T_5 level of application, carbofuran residue in the 1st leaf would have dissipated fully on day 10 and 32.09 respectively.

5.4.6. Carbofuran residue in the second leaf

Residues of carbofuran in 2nd leaf was present only on day 7 and 10 (Fig-7). On these days, translocation to the 2nd leaf at T_5 level was higher by 9.2 and 17.2 times, when compared to that in the T_4 level of application, indicating, a higher rate of translocation to leaf 2nd from the absorption pool of the molecule from the soil at higher level of application. In the 2nd leaf, at T_5 level of application, dissipation rate of carbofuran molecule was positive up to day 10 and was recording negative dissipation rate in between on 10th and 30th day except on day 25 where it was positive.

Considering the dissipation rate of carbosulfan in T_4 level in the 2nd leaf on the penultimate day of observation of BDL i.e., day 7th, it can be inferred that, in 2nd leaf, carbofuran metabolite would have dissipated fully only by 34th day. However, carbofuran was BDL on day 15th indicating that, the rate of dissipation was much higher after day 10th. However, in T_5 level, the carbofuran residue should have dissipated fully by 42.11 day.

5.4.7. Carbofuran residue in the third leaf

Carbofuran residue in the 3rd leaf was present only on day 5, 15 and 20, without any detectable residues on day 7 and 10 and again going below the detectable level on day 25 (Fig-8). The T_5/T_4 value was highest on day 20th, again indicating differential absorption and dissipation rate of residue molecule for the metabolites too. The

dissipation rate was positive on day 5 and 25 of T_5 level of application indicating a higher rate of absorption and rate of transformation or rate dissipation. It can be assumed from the dissipation rate of day 30th for the T_5 level that, the entire carbosulfan would have dissipated completely on the 48.67th day of application. In T_4 level, it should have happened on day 20.32 itself.

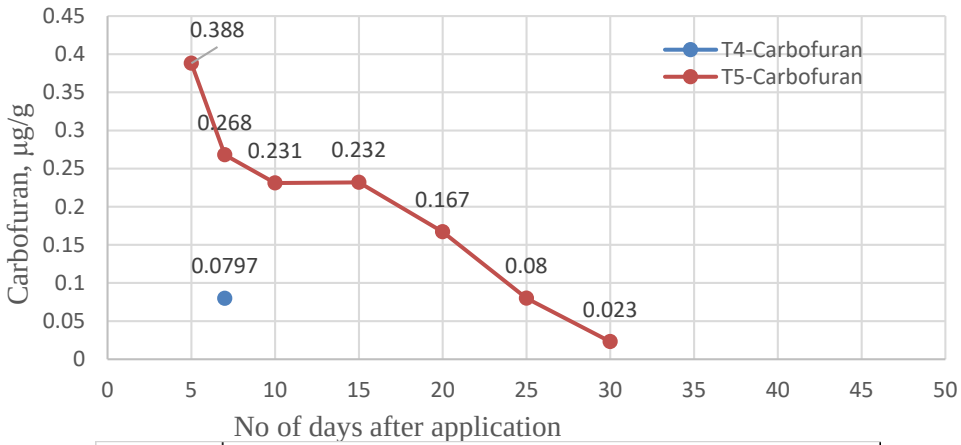
Vijayan (2000) reported that the average of carbofuran molecule in the pooled sample of 3rd and 4th leaf was BDL throughout the period of observation of 63 days, however a higher level of application at 7th month has resulted in persistence throughout the period.

5.4.8. Carbofuran residue in the fourth leaf

In 4th leaf, at T_4 level of application, carbofuran residues were present only on day 5 to day 10 and dissipated to BDL on 15th day. Considering the rate of dissipation on day 7, it is presumable that it would have dissipated completely on 11.63 days at T_4 level of application.

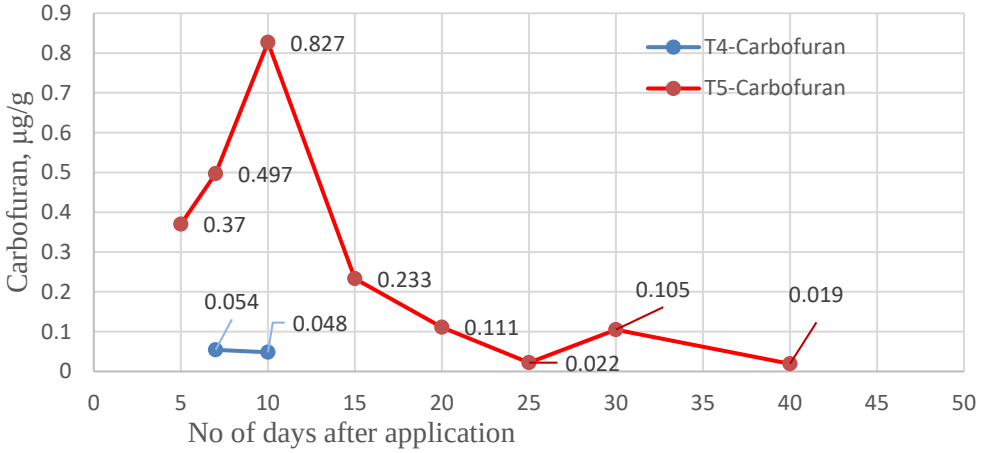
On day 5th, the carbosulfan level in the 4th leaf was 3.5 times higher in T_5 than T_4 samples (fig-9). At T_5 level of application, the residue dissipated fully on 21.1 day itself. At this level of application, the carbofuran started showing in the 4th leaf at day 1st after application, hinting immediate transformation of applied carbosulfan to carbofuran and other metabolites (as suggested by Rouchaud *et al.*, 1990 and Trevisan *et al.*, 2004 in different crops) either in soil or while being absorbed and translocated by in the plant.

Fig. 7. Mean residue of carbofuran in 1st leaf of banana



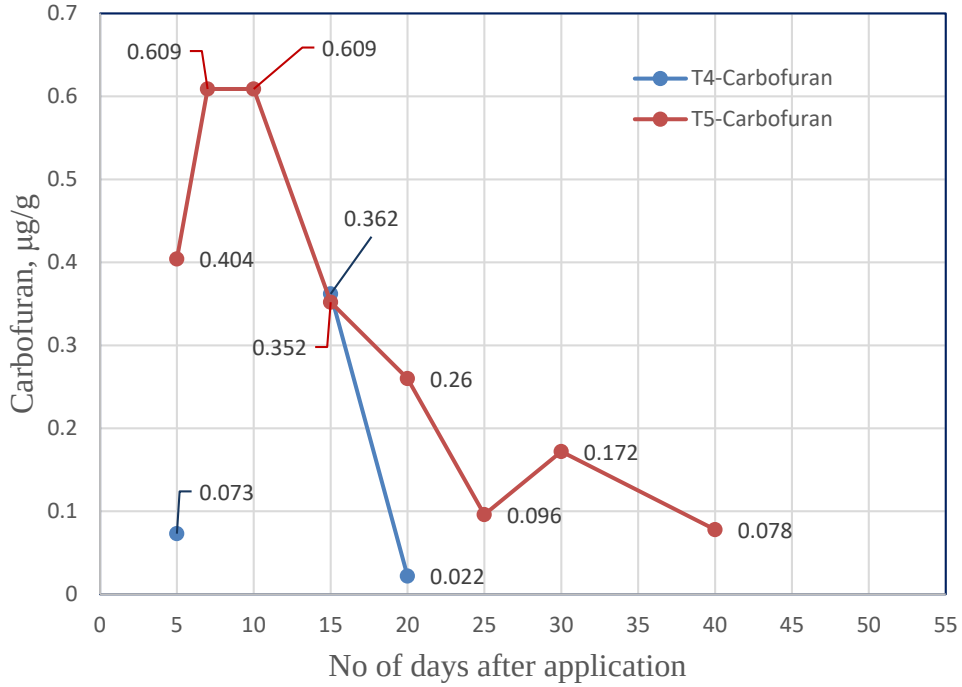
Parameter	Days after application								
	3	5	7	10	15	20	25	30	40
T_5 /T4			3.363						
ΔT_4 /ΔD		0.04	-0.027						
ΔT_5 /ΔD	0.194	-0.06	-0.012	0.0002	-0.01	-0.02	-0.011	-0.002	

Fig. 8. Mean residue of carbofuran in 2nd leaf of Banana



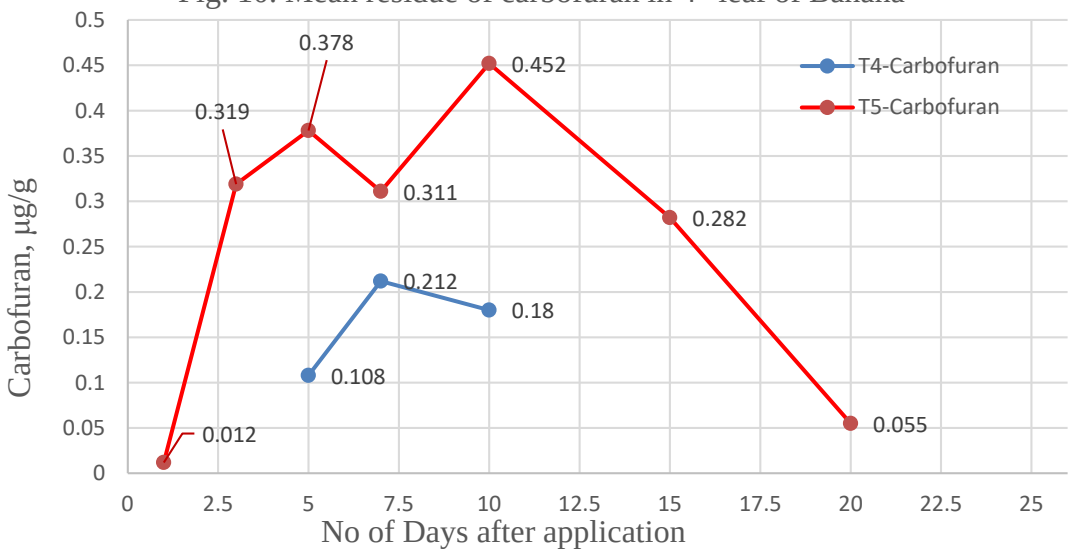
Parameter	Days after application									
	3	5	7	10	15	20	25	30	40	50
T_5/T_4			9.2	17.2						
$\Delta T_4/\Delta D$		0.027	-0.002	-0.0096						
$\Delta T_5/\Delta D$	0.185	0.064	0.110	-0.119	-0.024	-0.018	0.017	-0.009	-0.002	

Fig. 9. Mean Residue of carbofuran in 3rd leaf of Banana



Parameter	Days after application									
	3	5	7	10	15	20	25	30	40	50
T_5/T_4		5.534			0.972	11.818				
$\Delta T_4/\Delta D$	0.037	-0.037	0.0	0.072	-0.068					
$\Delta T_5/\Delta D$	0.202	0.103	0.0	-0.051	-0.018	-0.033	0.015	-0.009	-0.0078	

Fig. 10. Mean residue of carbofuran in 4th leaf of Banana



Parameter	Days after application								
	0	1	3	5	7	10	15	20	25
T_5/T_4				3.5	1.467	2.511			
$\Delta T_4/\Delta D$			0.054	0.052	-0.011	-0.04			
$\Delta T_5/\Delta D$	0.012	0.154	0.03	-0.03	0.047	-0.03	-0.05	-0.011	

5.4.9. 3-keto carbofuran residue in the leaves

Among the metabolites of carbosulfan, residues of 3-keto carbofuran at T_4 level of application were not at all observed in 1st and 2nd leaf (fig-10). It was present on day 7th and 10th in the 3rd leaf and 15th and 20th day in 4th leaf. However, at double the dose of application T_5 it was present in 1st to 4th leaves (Fig-10). At double the dose of application (T_5) in the 1st leaf it persisted through day 5th to 15th finally dissipating to BDL on day 20th. The dissipation rate of day15 (fig-11) suggests that 3-keto carbofuran residues might have completely dissipated on 21.15 days of application in the first leaf at T_5 level of application. Vijayan, (2000) reported residue of 3 keto carbofuran only on day 6th in pooled sample of 3rd and 4th leaves of banana after soil application of phorate at recommended practices. He also observed that, higher rate of phorate as soil application in 7th month also produced residue of 3-keto carbofuran only on 3rd day after application.

In 2nd leaf at T_5 level of application, 3-keto carbofuran persisted through day 5th to day 40th and was exhibiting fluctuating transformation per day (TPD) of positive (accretion) and negative (dissipation rate) value on every alternate day of observation. 3-keto carbofuran was absent in 2nd leaf at T_4 level of application.

Fig. 11. Residue of 3-keto carbofuran in 1st to 4th leaves of banana on single dose application (T-4)

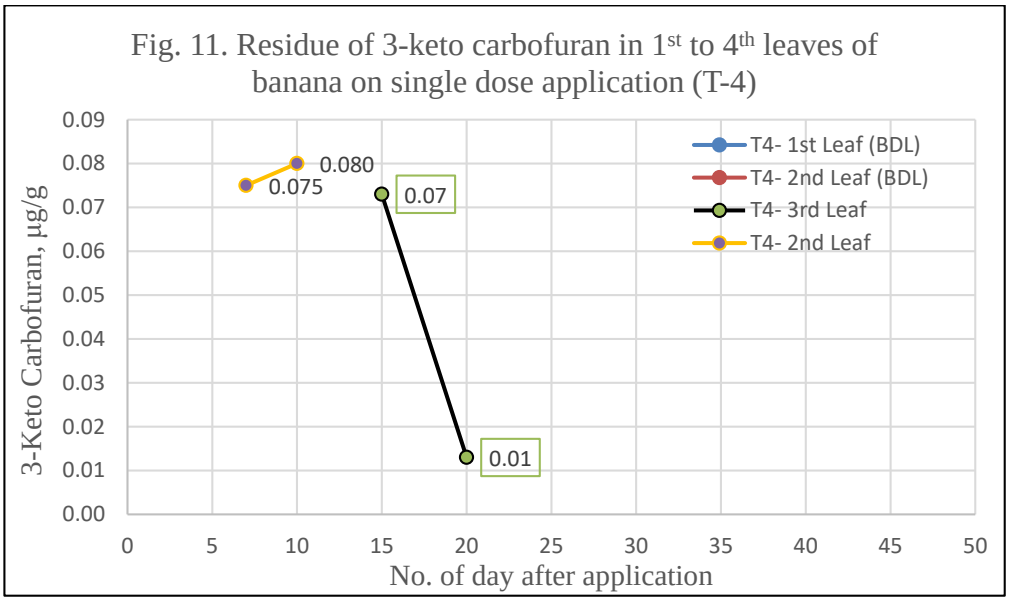
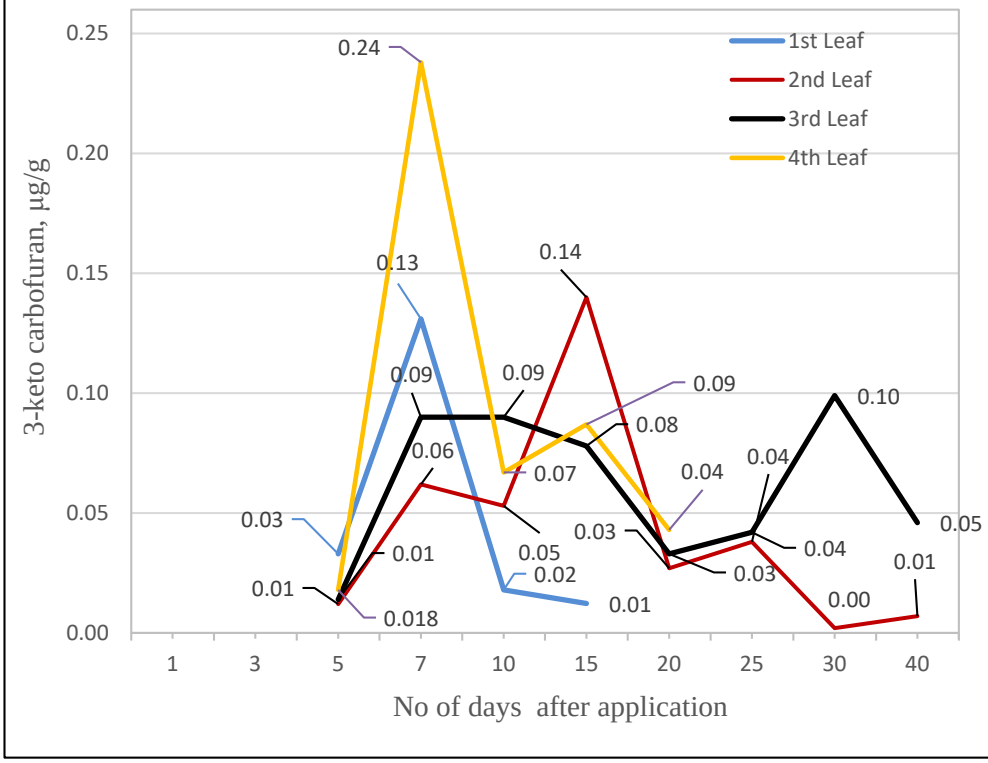
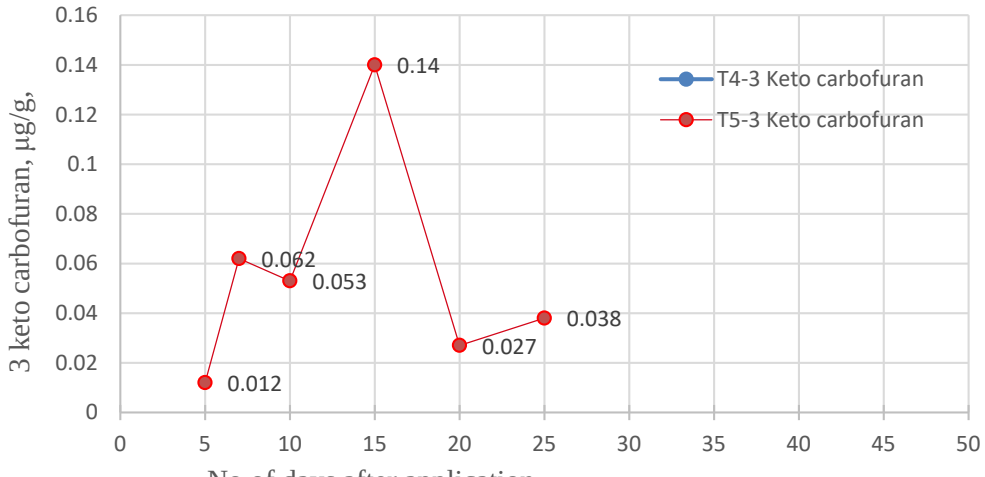


Fig.12. Residue of 3-keto carbofuran in 1st to 4th leaves of banana at double dose of application (T-5)



In the 3rd leaf, the residue of 3-keto carbofuran was almost equal on day 15th at T_4 and T_5 level of application. In this leaf, it was present on day 15th and 20 only at T_4 level of

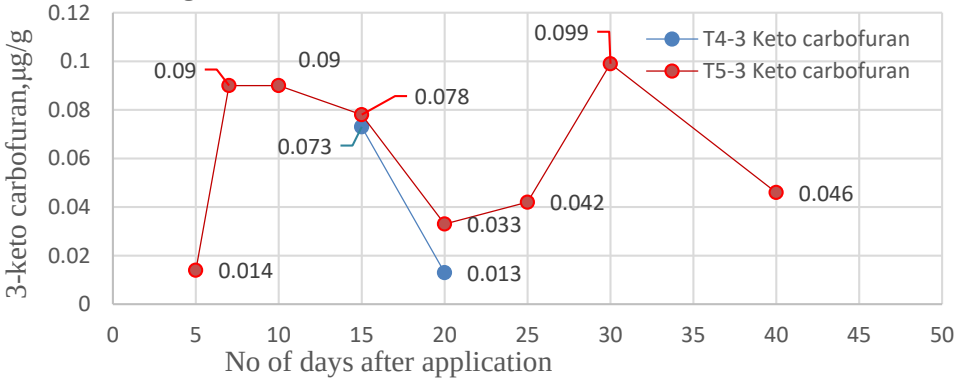
Fig.13 Mean residue of 3 keto carbofuran in 2nd leaf of Banana



Parameter	Days after application							
	3	5	7	10	15	20	25	30
$\Delta T_5/\Delta D$	0.006	0.025	-0.003	0.02	-0.02	0.002	-0.008	

application. Rate of dissipation on day 15th for T_4 level indicates that, this residue would have fully dissipated on 21.08 days of application. The same in T_5 level of application would have ensued on day 49.2 of application. In the 3rd leaf too, at T_5 level the residues of 3-keto carbofuran were exhibiting fluctuating transformation per day (TPD) of positive (accretion) and negative (dissipation rate) value on different days of observation up to 40 days.

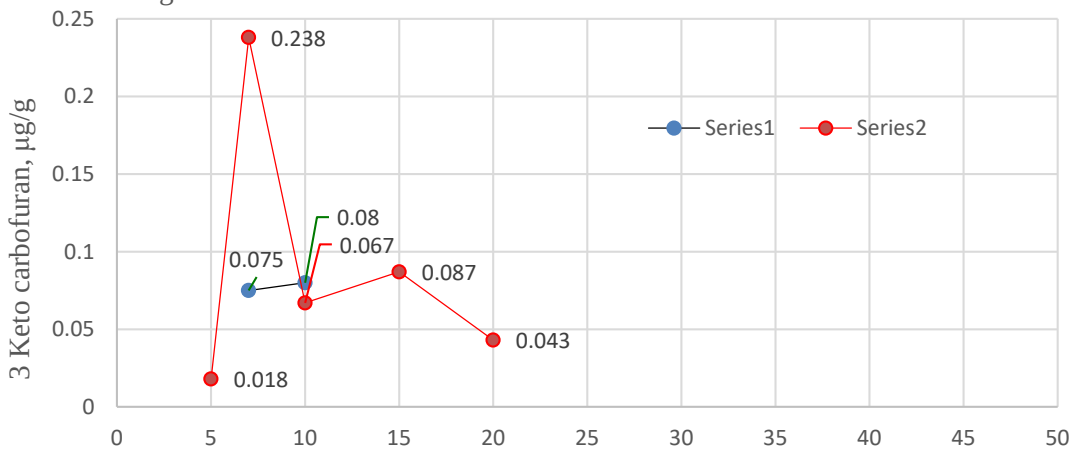
Fig.14. Mean residue of 3-keto carbofuran in 3rd leaf of Banana



Parameter	Days after application									
	3	5	7	10	15	20	25	30	40	50
T_5/T_4				0	1.0685	2.538				
$\Delta T_4/\Delta D$				0.015	-0.012	-0.003				
$\Delta T_5/\Delta D$	0.007	0.038	0.0	-0.002	-0.01	0.002	0.011	-0.005	-0.005	

In the 4th leaf at T_4 level of application, 3-keto carbofuran was present on 7th and 10thdays, exhibiting a slight positive TPD value on day 7th indicating accretion of the residue on day 10th (fig-14). At T_5 level 3-keto carbofuran residue in this leaf was extant between day 5 and 20 of application. TPD value of T_5 level application in this leaf was positive on day 5 and day 10 and having a negative TPD (dissipation) in between on day 7th day. On extrapolation of the dissipation rate of day 15, it can be presumed that, this level of application on leaf 4th, 3-keto carbofuran would have dissipated fully on 24.78 days.

Fig.15 Mean residue of 3-keto carbofuran in leaf 4th of Banana



Parameter	Days after application						
	3	5	7	10	15	20	25
T_5/T_4	0	0	3.173	0.8375	0	0	0
$\Delta T_4/\Delta D$	0	0.04	0.002	-0.016	0	0	0
$\Delta T_5/\Delta D$	0.009	0.110	-0.057	0.004	-0.009	-0.009	0

5.4.10. 3-hydroxy carbofuran residue in the leaves

Residue of 3-hydroxy carbofuran was present in all the leaves at both level of application (Fig-15 and fig-16).

Fig-16: Residue of 3-hydroxy carbofuran in 1st to 4th leaves of banana on single dose application (T_4)

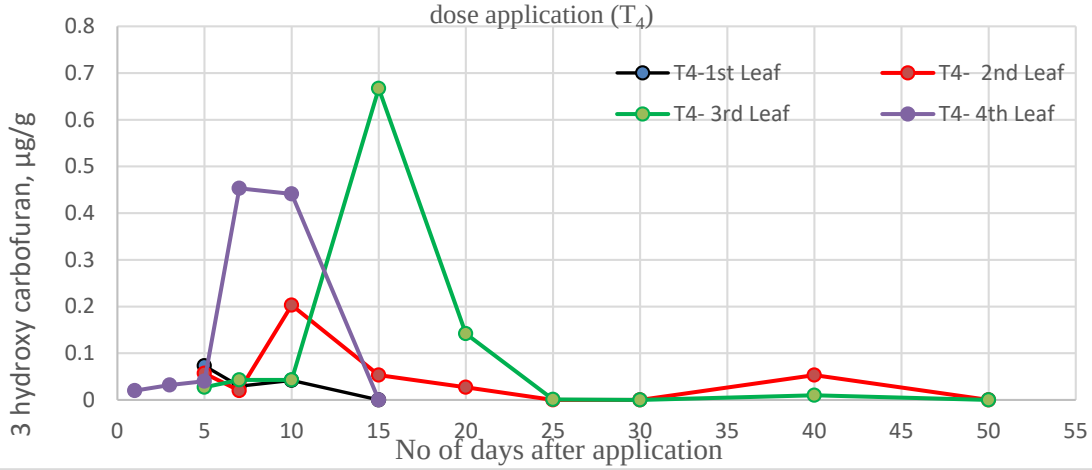
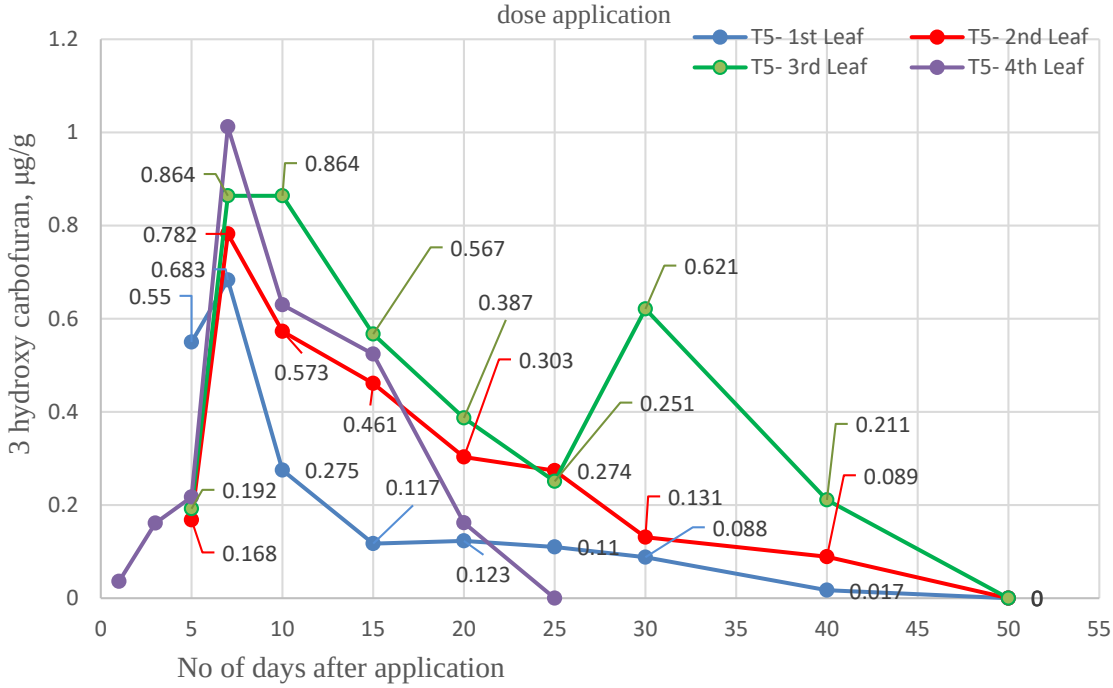


Fig-17: Residue of 3 hydroxy carbofuran in 1st to 4th leaves of banana on double dose application

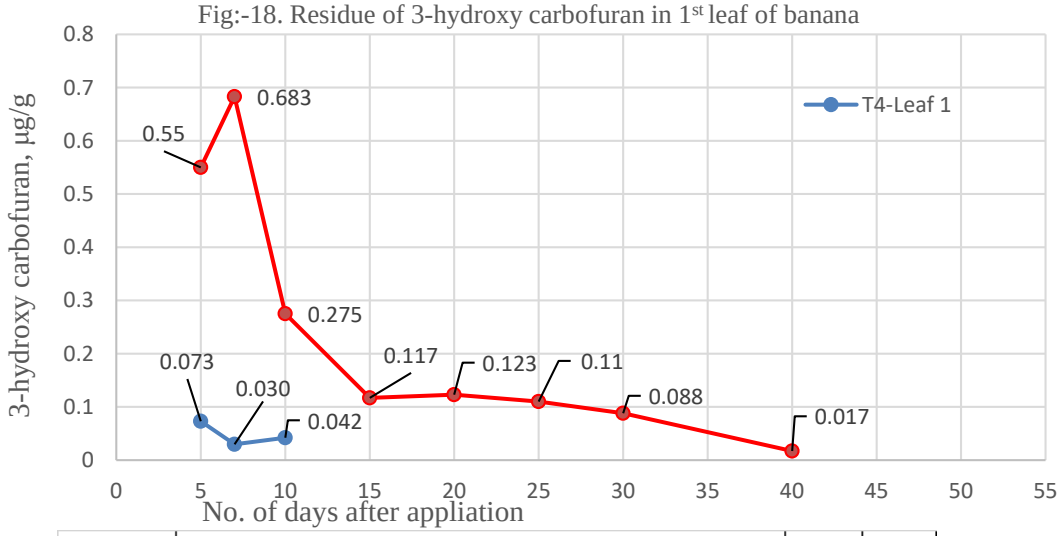


Residues of 3-hydroxy carbofuran in the 1st leaf at T_5 level of application was 7.5, 23.1 and 6.5 times higher on day 5th, 7th and 10th respectively, than that present in T_4 level of application (fig-17). The TPD ($\Delta T_4/\Delta D$) for T_4 level in this leaf was negative on day 5th, however followed by a positive value on day 7, then dissipating to BDL on day 15. $\Delta T_5/\Delta D$ for T_5 level in this leaf was initially positive on 5th and then on 15th indicating accretion on these periods and was negative on other days of observation up to day 30 before attaining the BDL on day 50th. This may be due to the differential adsorption pattern of 3-hydroxy carbofuran molecule both in soil and corm as a sink for the banana plant for absorbed nutrients and molecules from the soil. However, at this rate of dissipation of day 30th, 3-hydroxy carbofuran would have completely dissipated on 42.42 days in the first leaf.

Residues of 3-hydroxy carbofuran in the 2nd leaf at T_5 level of application was 2.9, 39.1, 2.8, 8.7, 11.2 and 1.7 times higher on day 5, 7 and 10, respectively, than that was present in the first leaf at T_4 level of application (fig-18). The TPD ($\Delta T_4/\Delta D$) for T_4 level in this leaf was negative on day 5, however followed by a positive value on day 7. On day 10, 15 and 20 it was negative and on day 25th and 30th residue was at BDL. However, on day 40 $\Delta T_4/\Delta D$ value was positive (0.005 µg/day) with residue of 0.053 µg. At T_4 level 3-hydroxy carbofuran residue in this leaf was presumed to be present up to 50.6 days though it was BDL on day 50th. $\Delta T_5/\Delta D$ for T_5 level of application in 2nd leaf was positive with a value of 0.307 µg/day compared to the

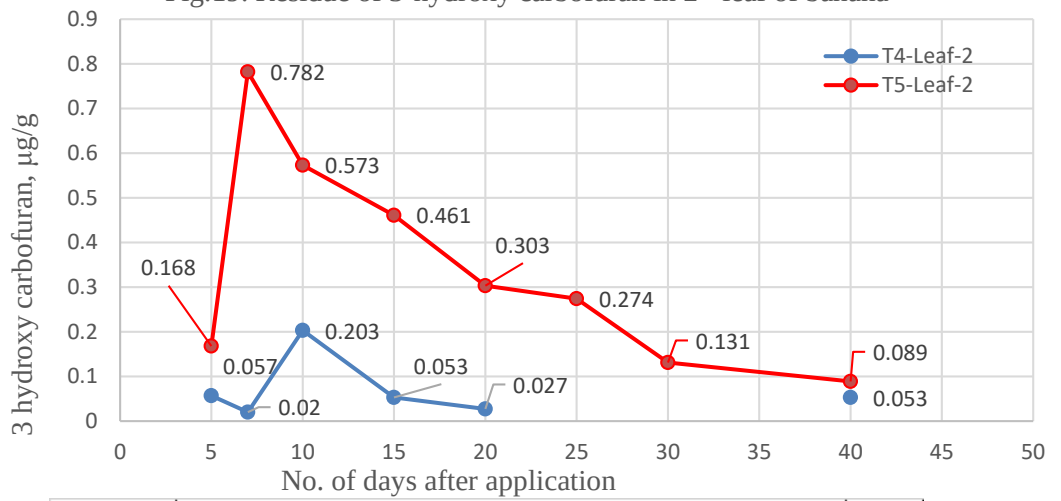
BDL in day 3rd, indicating substantial accretion on these periods and then it was negative on other days of observation up to day 40 before attaining the BDL on day 50.

Fig:-18. Residue of 3-hydroxy carbofuran in 1st leaf of banana



Parameter	Days after application									
	3	5	7	10	15	20	25	30	40	50
T_5 / T4		7.5	23.1	6.5						
$\Delta T_4 / \Delta D$	0.0365	-0.02	0.0041	-0.008						
$\Delta T_5 / \Delta D$	0.275	0.067	-0.136	-0.032	0.001	-0.003	-0.004	-0.007	-0.002	

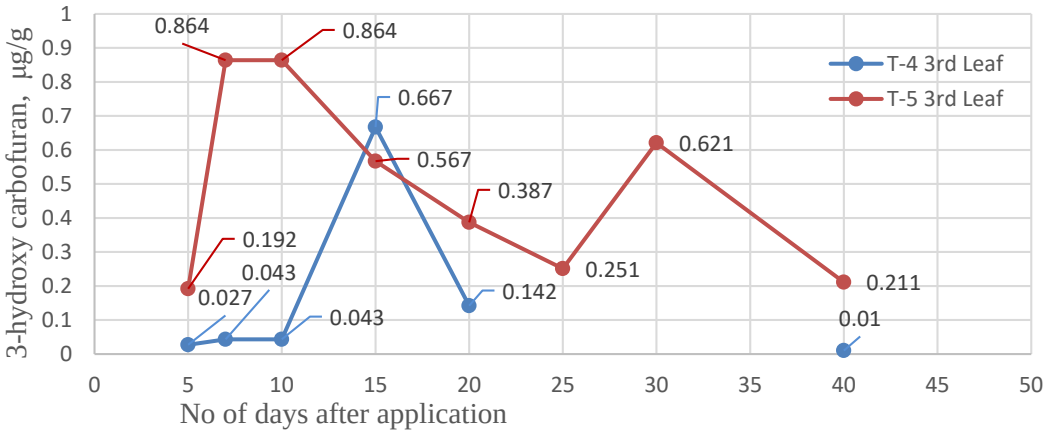
Fig.19. Residue of 3-hydroxy carbofuran in 2nd leaf of banana



Parameter	Days after application									
	3	5	7	10	15	20	25	30	40	50
T_5 / T4		2.9	39.1	2.8	8.7	11.2			1.7	
$\Delta T_4 / \Delta D$	0.029	-0.019	0.06	-0.03	-0.01	-0.01		0.005	-0.01	
$\Delta T_5 / \Delta D$	0.084	0.307	-0.1	-0.02	-0.03	-0.01	-0.03	-0.004	-0.01	

Residues of 3-hydroxy carbofuran in the 3rd leaf at T_5 level of application was 7.11, 20.1, 20.1,2.73 and 21.1 times higher on day 5, 7 and 10, 20 and 40 days respectively than that was present in the first leaf at T_4 level of application (fig-19). On day 15th, it is noted that residue of 3-hydroxy carbofuran at T_5 level was lower than that of T_4 level. $\Delta T_5/\Delta D$ for T_5 level of application in 3rd leaf was positive with a value of 0.34 and 0.074 µg/day compared to the BDL level on day 5 and 25 day respectively, indicating substantial accretion on these periods. It was negative on other days of observation up to day 40 before attaining the BDL by 50th day. It is noticeable that at T_5 level of application, residue of 3-hydroxy carbofuran in the 3rd leaf should have dissipated completely by 45.15 days.

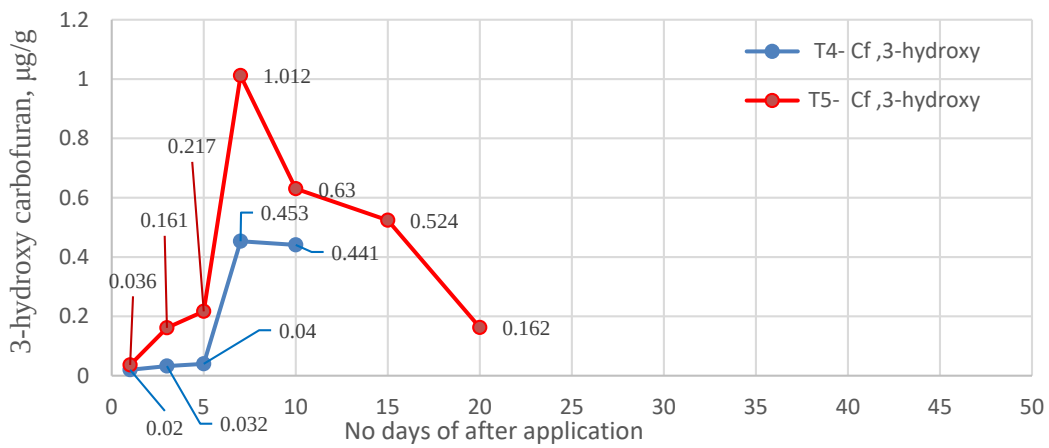
Fig. 20. Residue of 3 hydroxy carbofuran in 3rd leaf of banana



Parameter	Days after application									
	3	5	7	10	15	20	25	30	40	50
T_5 / T4		7.111	20.09	20.09	0.85	2.725			21.1	
$\Delta T_4/\Delta D$	0.014	0.008		0.125	-0.105	-0.028		0.001	-0.001	
$\Delta T_5/\Delta D$	0.096	0.336		-0.059	-0.036	-0.027	0.074	-0.041	-0.021	

In the 4th leaf, residues of 3-hydroxy carbofuran at T_5 level of application was 1.8, 5.03, 5.43, 2.234 and 1.43 times higher on 1st, 3rd, 5th, 7th and 10th days respectively than that was present in the first leaf at T_4 level of application (fig-20). It is noticeable that at both levels translocation to leaf has started occurring on 2nd hour of application itself which has not been observed in case of other metabolites and on leaves. Also, $\Delta T_4/\Delta D$ and $\Delta T_5/\Delta D$ recorded a positive value continuously from 0th to 5th day. 3-hydroxy carbofuran at T_4 level of application in the 4th leaf would have dissipated fully on 15.01 days and the same at T_5 level on 25.4 days.

Fig. 21: Residue of 3-hydroxy carbofuran in 4th leaf of banana



Parameter	Days after application							
	0	1	3	5	7	10	15	20
T_5 / T4		1.8	5.03	5.43	2.234	1.429		
$\Delta T_4 / \Delta D$	0.020	0.006	0.004	0.207	-0.004	-0.088		
$\Delta T_5 / \Delta D$	0.036	0.063	0.028	0.398	-0.127	-0.021	-0.072	-0.032

It can be seen that transformation of total carbosulfan and their metabolites in different leaves were also in the order L_3> L_4> L_2> L_1 for both level of application as it was observed in the case of residue of carbosulfan molecule. (Fig-21, Fig-22 and Fig-4). In T_4 highest level of total carbosulfan residue was noticed in all leaves on day 10th of application. At T_5 level, highest total carbosulfan residue was noticed on 10th day of application in 2nd, 3rd and 4th leaves, whereas in 1st leaf it was on day 1.

Fig. 22 Mean residue of total carbosulfan in 1st to 4th leaf of banana at T_4

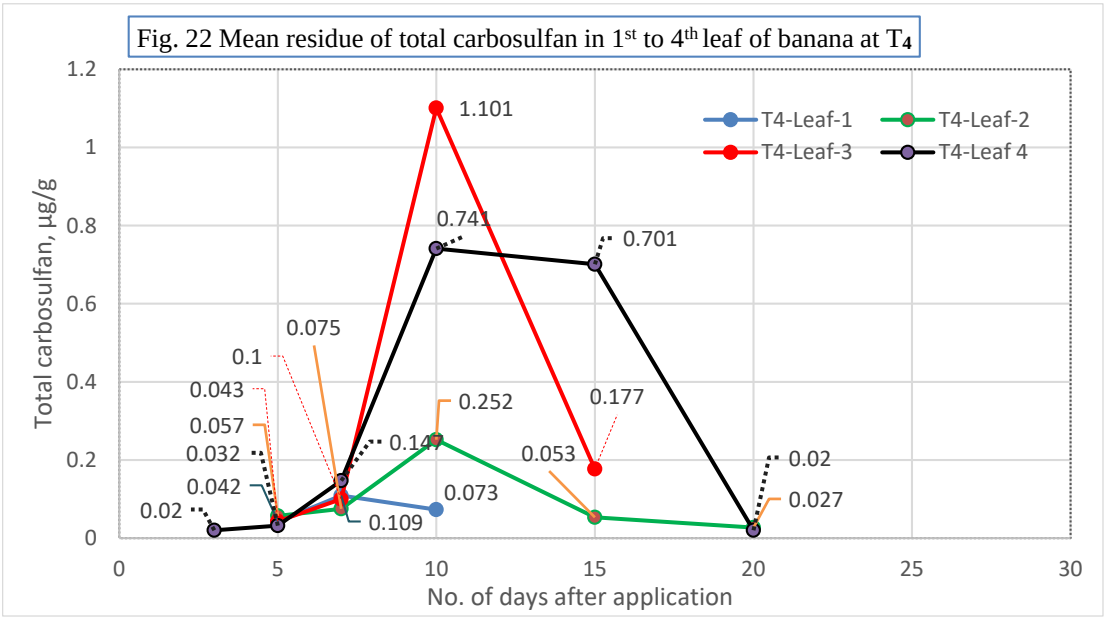
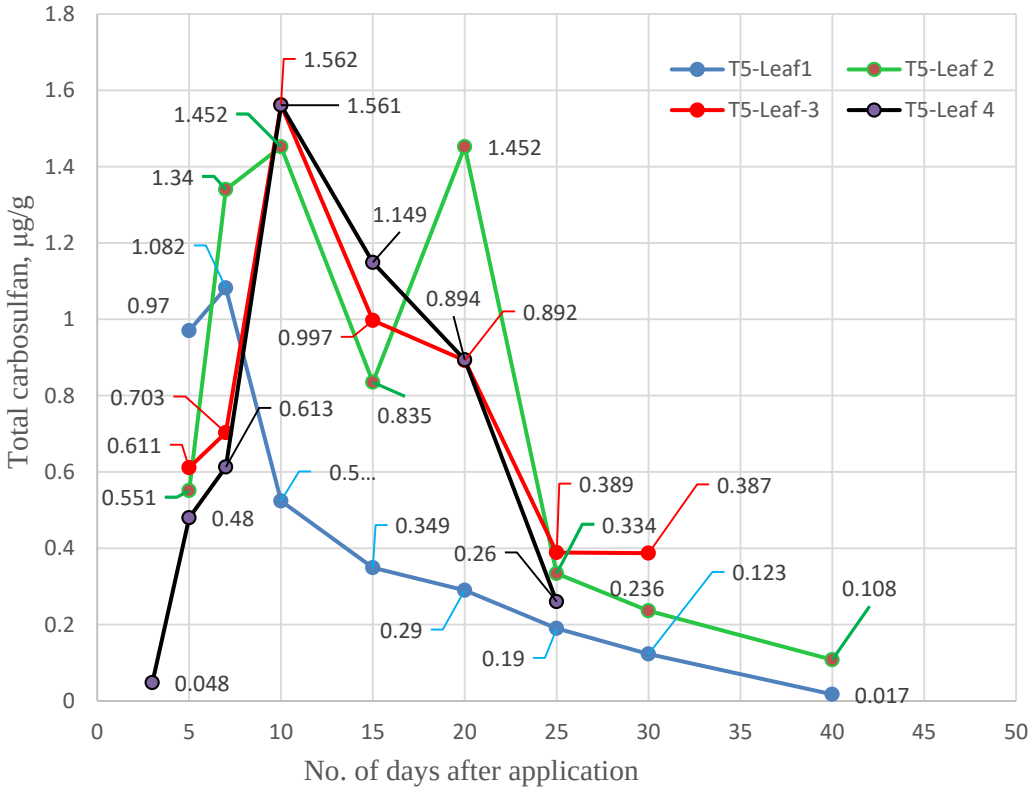


Fig. 23 Mean residue of total carbosulfan and its metabolites in 1st to 4th leaf of banana at T_5



5.5 Effect of treatments on the biometric, yield and harvest observation over the period of study

The treatments with fipronil and carbosulfan in banana have no significant effect over the parameters such as length of 4th leaf, plant height, plant girth, number of active leaves and length/breadth ratio of leaf (Result chapter para 4.9, table-42 to 47). However, the treatments had significant influence on breadth of index leaf, blossom bud, bunch, pseudo stem weight, and corm weights. Breadth of leaf at T_2 level of application was significantly higher than all treatment up to 20th week and later at 22nd week and on harvest it was significantly higher than T_1, T_3, and T_4 remaining on par with T_5.

Weight of bunch at T_2 level of application was higher than other treatment and was significantly higher than other treatments and was in the order T_2> T_3> T_4> T_1> T_5. Least bunch weight was recorded by the T_5 level, presumably be due to a slight growth inhibitory action of carbosulfan at higher level of application. A growth deterrent action was observed in tissue culture banana plants at a higher level of application in a pre-evaluation experiment conducted together with this experiment. Literature of the effect of insecticide on the growth of banana are

scanty. However, Triques, *et al* 2021 observed a reduction in the seed germination and root elongation (which were not significant) in *Raphanus sativus* and *Allium cepa* over control when applied with 0, 0.52, 1.04, 2.08, 4.16 and 8.32 mg of fipronil per kg dry weight of soil.

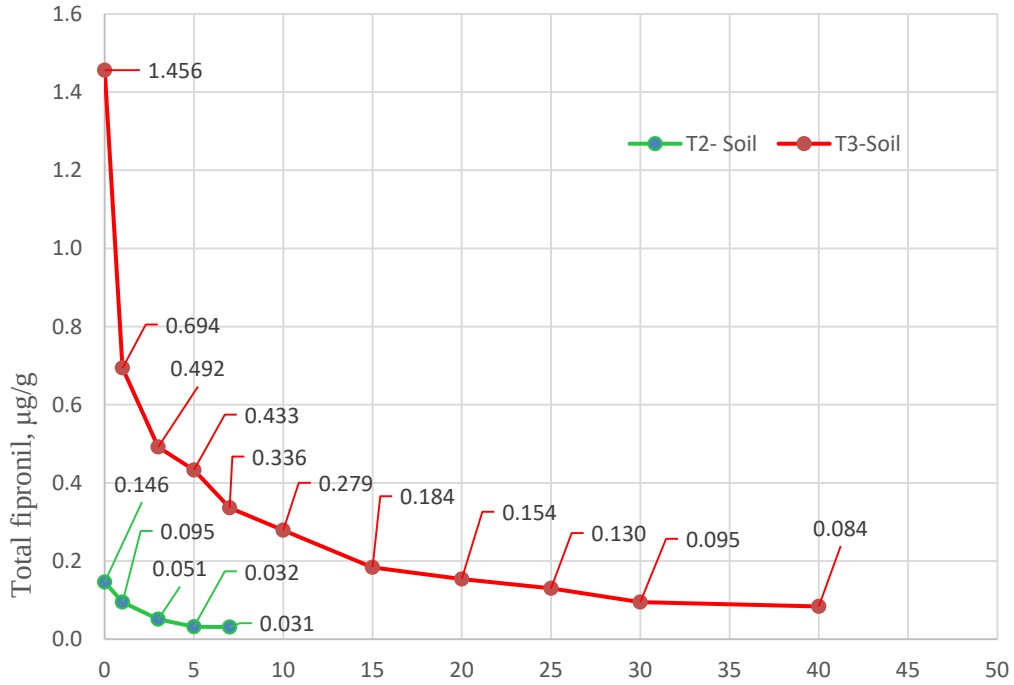
5.6 Fipronil and its metabolites in soil

The residues of fipronil, fipronil desulfinyl, fipronil sulphide and fipronil sulfone (as metabolites of fipronil) are presented in table 35 and the mean values in the table 37 and Fig-23. The data revealed that mean total of metabolites of fipronil were persistent up to 40 days in the soil at T_3 level of application and at T_2 i.e., at normal recommended rate it persisted only up to 7 days. The T_3/T_2 ratio (fig-23) was highest (13.53) on day 5 followed by 7^{th},0^{th}, 3^{rd} and 1^{st} day of application. The TPD for T_2 and T_3 ($\Delta T_2/\Delta D$ and $\Delta T_3/\Delta D$ respectively) were constantly negative till complete dissipation of the molecule. Following $\Delta T_2/\Delta D$ of day 5, it is clear that at T_2 level of application all the molecules would have dissipated completely only day 69^{th} day, however it can be noted that it was BDL on day 10 onwards continuously. On extending the same to T_3 level of application ($\Delta T_3/\Delta D$ at rate of day 40^{th}), it would have persisted till 50.5 days and it was at BDL on day 50.

Fipronil desulfinyl metabolite was not detected in rhizosphere soil for both T_2 and T_3. Fipronil sulfide was also not detected in rhizosphere soil at normal rate of application in the soil (T_2). However, it was detected up to 3^{rd} day in soil treated at double the rate of application (T_3). The maximum concentration observed was on 0^{th} day at 0.328 ppm level. In T_2, fipronil molecule remained as such in the rhizosphere soil from the time of application to 3^{rd} day dissipating from 0.121 μg g^{-1} on 0^{th} day to 0.051 μg g^{-1} and finally reaching BDL on 5^{th} day. The pattern of dissipation of fipronil at double the level of application in soil was different from that in T_2. In T_3, double rate of application has resulted in a longer persistence of fipronil molecule till 40^{th} day (0.084 μg g^{-1}), recording the highest residue on day zero (0.0866 μg g^{-1}) and dissipated to BDL on 50^{th} day. Fipronil sulphone metabolite, was detected right from 0^{th} to 7^{th} day samples, except for 3^{rd} day sample. The maximum residue value was recorded on 1^{st} day (0.036 μg g^{-1}). Fipronil sulfone residues were highest on day zero (0.261 μg g^{-1}) which was absent on 30^{th} day, but presenting a residue of 0.071 μg g^{-1} on 25^{th} day. However in between, on day 10^{th} fipronil

sulfone was at below detectable limit again registering 0.067 µg g^{-1} on 15thday suggesting a differential rate release of adsorbed parent molecule getting transformed to metabolites.

Fig-24: Mean residue of total fipronil in soil



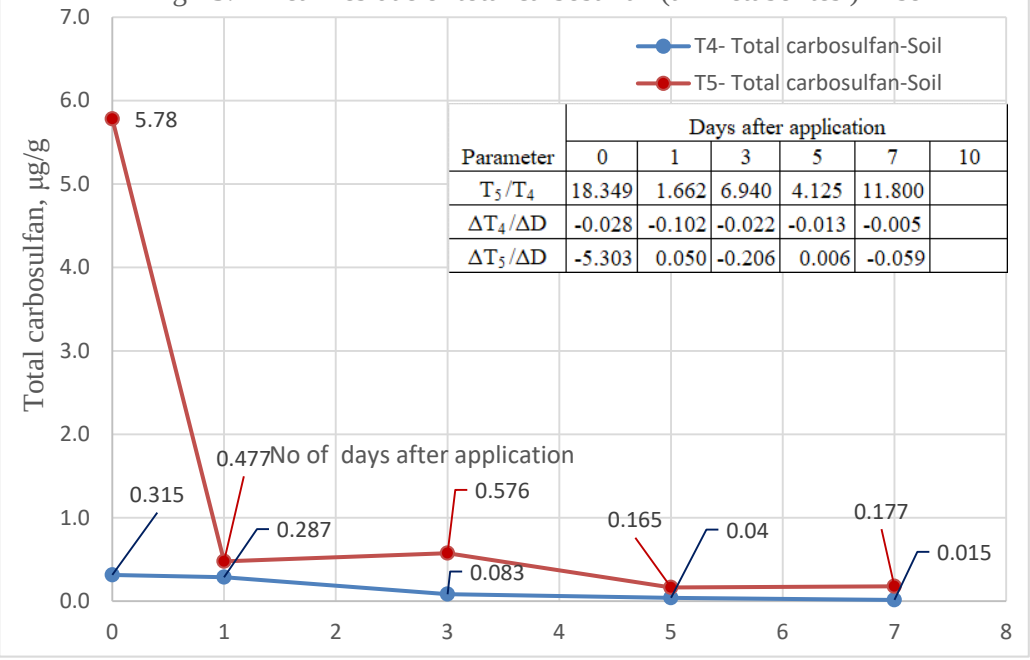
Parameter	Days after application											
	0	1	3	5	7	10	15	20	25	30	40	50
T_3/T_2	9.973	7.305	9.647	13.531	10.839							
$\Delta T_2/\Delta D$	-0.051	-0.022	-0.010	-0.001	-0.010							
$\Delta T_3/\Delta D$	-0.762	-0.101	-0.030	-0.049	-0.019	-0.019	-0.006	-0.005	-0.007	-0.001	-0.008	

5.7 Carbosulfan and its metabolites in soil

Total carbosulfan residue in the soil was noticeable in samples of 0th day i.e., samples 2 hours after application it was 18.35 times higher in T5 level than that of T_4 (fig-23). However, the drastic reduction of T_5 level of 5.78 µg g^{-1} on 0th day to 0.477 µg g^{-1} on 1st day was indication of either absorption by the rhizome or adsorption by organic-clay surface in the soil matrix. It persisted in the soil only up to day 7 of application at both levels. $\Delta T_4/\Delta D$ was negative throughout the period of presence of residue in the soil whereas the $\Delta T_5/\Delta D$ was fluctuating on alternate day of observation form 0th day to7thday. Similar fluctuating transformation per day (TPD) of positive (accretion) and negative (dissipation rate) value on every alternate day of observation was noticed in the 2nd leaf for 3-keto carbofuran at T_5 level though it was absent in 2nd leaf at T_4 level of application. The comparatively low level of

carbosulfan in the soil at T_5 level on day 1st may be attributed to inherent sampling error possibilities coupled with absorption by the rhizome of the plant when present in excess in soil solution over the adsorption potential of soil OMCC.

Fig-25: - Mean residue of total carbosulfan (all metabolites) in soil



Parameter	Days after application					
	0	1	3	5	7	10
T_5/T_4	18.349	1.662	6.940	4.125	11.800	
$\Delta T_4/\Delta D$	-0.028	-0.102	-0.022	-0.013	-0.005	
$\Delta T_5/\Delta D$	-5.303	0.050	-0.206	0.006	-0.059	

In soil the residue of metabolite of carbosulfan viz., carbofuran was absent at T_4 level, whereas at T_5 level it was present only on 0th day. Even on 0th day, 3-keto carbofuran was absent in soil at both T_4 and T_5 level (fig-24).

The mean 3 hydroxy carbosulfan content in soil on day 1 (1.949 µg/g) was 21 times higher than that on 0th day (0.095 µg g^{-1}) (fig-25). Unlike ΔT4/ΔD where it remained negative the period of application for T_4 application, $\Delta T_5/\Delta D$ was positive for day one (0.075 µg/day) due to higher content on day 3 than on day 1indicating gradual dissipation in the soil.

On day 1st, the mean carbosulfan content at T_5 level in soil was 17 times (3.804 µg/g) greater than that at T_4 level (0.22 µg g^{-1}) (fig-26). As in the case of 3 hydroxy carbosulfan, a similar trend in dissipation rate as indicated by $\Delta T_4/\Delta D$ and $\Delta T_5/\Delta D$ values of carbosulfan molecule, was noticed.

5.8 Effect of fipronil and carbosulfan on soil enzymes

Soil urease activity on 10th day (table 24) was significantly influenced by the treatment at double doses as evidenced from a lower urease activity in these soils when compared with T_2 (normal dose of application). The urease activity in T_3 was lower than T_2 and

that in T_5 was lower than T_4 as well as in control. However, dehydrogenase activity and acid phosphatase activity were not significantly influenced by the treatments.

Fig-26: Mean residue of carbosulfan and its metabolites in soil

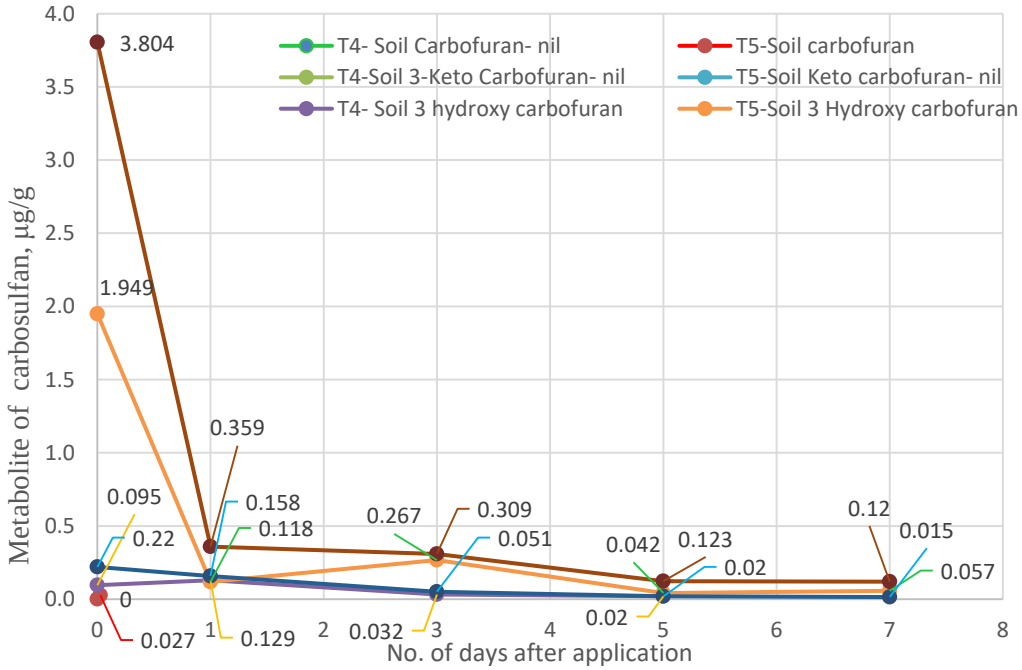
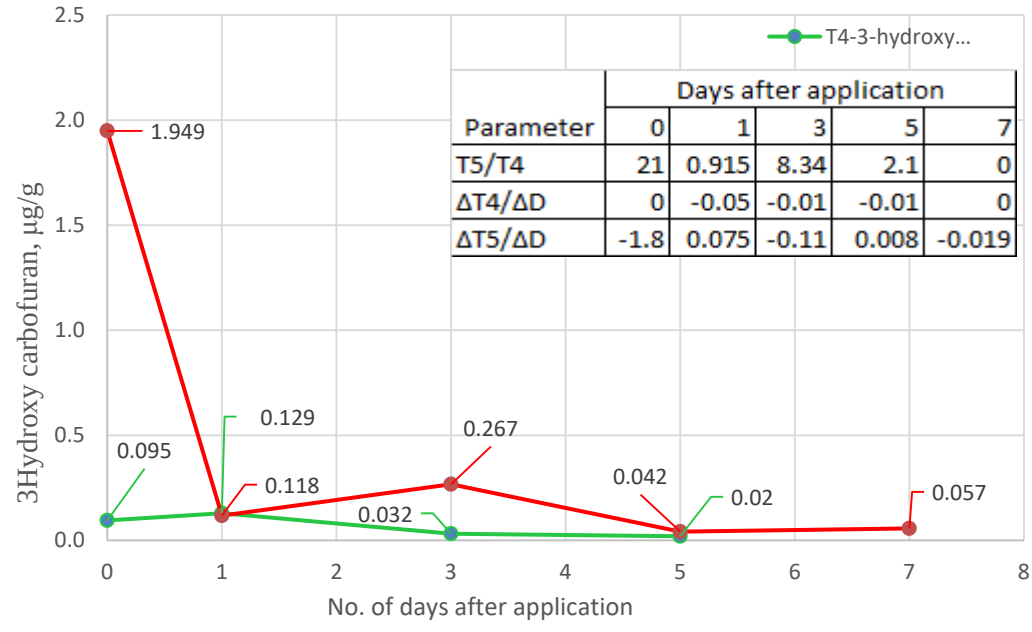
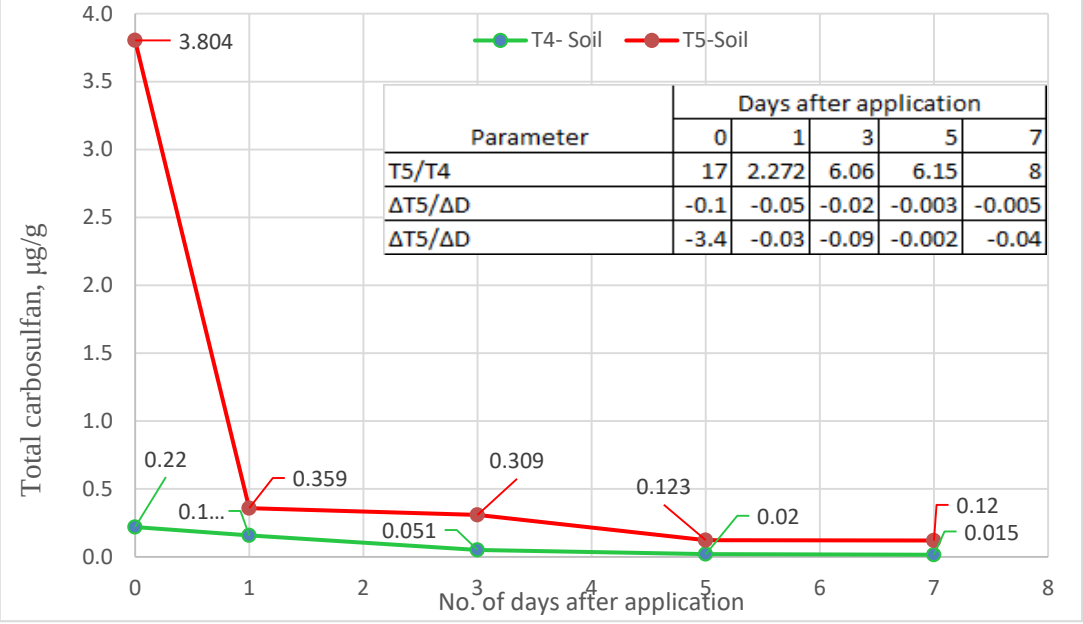


Fig-27: Mean residue of 3-hydroxy carbofuran in soil



Parameter	Days after application				
	0	1	3	5	7
T5/T4	21	0.915	8.34	2.1	0
ΔT4/ΔD	0	-0.05	-0.01	-0.01	0
ΔT5/ΔD	-1.8	0.075	-0.11	0.008	-0.019

Fig-28 Mean residue of carbosulfan molecule in the soil



Parameter	Days after application				
	0	1	3	5	7
T5/T4	17	2.272	6.06	6.15	8
ΔT5/ΔD	-0.1	-0.05	-0.02	-0.003	-0.005
ΔT5/ΔD	-3.4	-0.03	-0.09	-0.002	-0.04

5.9 Effect of fipronil and carbosulfan on soil microorganisms

It was observed that population of actinomycetes, bacteria and fungi population were higher in T_1, after 10 days of application in soil. Though the application of the chemicals as per treatment schedule on 60th day of planting (i.e., 10 days after 2nd application) did not affect the population of actinomycetes significantly on 10th day of after application (table 39), actinomycetes population were highest in T_1-control (1.23 x 1.0E+05 cfu g^{-1}) and least in T_4 (1.17 x 1.0E+05 cfu g^{-1}). The data revealed that the population of actinomycetes were not significantly affected by either fipronil or carbosulfan application, both at single and double doses.

A significant antagonistic influence of both these compounds on the population of bacteria and fungi, where the control samples of soil recorded maximum population (table 26). The bacterial population in T_1 was significantly higher than all other treatments. Among the treatments, carbosulfan had more inhibitory influence on bacterial population and maximum inhibition was observed in T_4 followed by T_5, T_3 and T_2. Maximum inhibition of fungal population was observed in treatment with fipronil wherein maximum inhibition was observed in T_2 followed by T_3, T_4 and T_5 and the population of fungi in the treatment were found to be on par.

5.10 Additional soil application of treatments after bunch emergence

It is evident from the table that no residue of carbosulfan and fipronil and their metabolites were present in banana at harvest due to additional application of treatments just after bunch emergence in various harvested parts (table 38).

5.11 Pseudo-stem injection at five times the recommended dose on bunch emergence stage.

It is observed that carbosulfan residue persisted in the bunch pulp on 15^{th} day of emergence after application of five times the recommended dose as injection into the pseudo stem (0.0128 μg g^{-1}) and dissipated to BDL in the sample harvested after 30 days of bunching. Residues of fipronil and its metabolites were not detected in the samples. The residue of fipronil and carbosulfan were not detected in flower bud, flower bract alone, bunch pulp and in the peel.

6. SUMMARY

A study entitled “Dissipation and distribution of fipronil, carbosulfan and their metabolites in banana var. Nendran (AAB) and soil” was conducted at College of Agriculture, Vellayani, with an objective to evaluate the dissipation, metabolism and persistence of fipronil and carbosulfan in banana plant and its parts, grown under red loam soils and to the study impact of these compounds on soil organisms of red loam soils of Vellayani. The study was conducted during August 2016 to April 2017. Laboratory experiments were also conducted to decide the appropriate methodology of extraction and clean up using suitable techniques for the residue estimation of fipronil, carbosulfan and their metabolites from various samples types. The summary of the results are as follows

1. The soil under field experiment is sandy loam in texture with, moderately acidic with a bulk density 1.24 Mg m^{-3}, pH of 5.9, organic carbon content of 1.5 per cent, available P and K were high (recording 196.1, 358.4 kg ha^{-1} respectively), extractable Ca and Mg were 393 (sufficient) and 90.3 (deficient) mg kg^{-1}respectively. The available S was 11.70 mg kg^{-1} and HCl extractable essential micronutrients viz., Fe, Zn, Mn, Cu were in sufficient range (217.8, 3.8, 11.4, 1.5 mg kg^{-1}) and hot water extractable B content was deficient with a value of 0.4 mg kg^{-1}. During the growth period only 189 mm rainfall was received and cumulative evapotranspiration was as high as 869.9 mm necessitating irrigation on alternate days except the rainy days.
2. The experiment for residue estimation of fipronil and its metabolites from various samples were standardized by using control samples spiked with pure analytical standards of fipronil desulfinyl, fipronil, fipronil sulphide and fipronil sulfone. The order of appearance of peak for these compounds with retention time was fipronil desulfinyl (3.08 min), fipronil (3.17 min), fipronil sulphide (3.28 min) and fipronil sulfone (3.43 min).
3. Mean of method validation parameters namely percentage recovery, RSD value, linearity and limit of quantitation (LOQ) with matrix match for different samples types such as banana leaves, pseudo-stem, bunch finger, flower and corm for the two

methods tried for extraction and clean-up of residues of pesticides (conventional and QuEChERS method) for fipronil and its metabolites in "LC-MS/MS–(UPLC Waters Aquity+ AB Sciex API 3200 triple quadrupole mass analyser revealed that QuEChER's method has better percentage recovery (accuracy) range of 80.0-119.9 per cent and precision of estimation (RSD percentage range) of 0.4 – 20, linearity range of 0.01 to 1.00 ppm and LOQ of 0.01 ppm for all the molecules in different matrices. Hence QuEChERS method was found to be better for the extraction of the molecules of fipronil and its metabolites from all sample types collected from banana plants.

4. For extraction and estimation of residues of fipronil and its metabolites from soil too, QuEChERS method has superior percentage recovery (accuracy) range of 80.1-97.1 per cent, precision of estimation (RSD percentage range) of 1.0 -18.7 per cent, linearity range of 0.01 to 1.00 ppm and LOQ of 0.01 ppm.
5. Method validation for extraction and cleanup carbosulfan and its metabolites from banana leaves, pseudo-stem, bunch finger, flower and corm as per QuEChER's method followed by estimation in "LC-MS/MS–(UPLC Waters Aquity + AB Sciex API 3200 triple quadrupole mass analyser is found to have more acceptable values for percentage recovery (accuracy) range of 80.1-97.1 per cent, precision of estimation (RSD percentage range) of 0.4 - 19.9 percent, linearity range of 0.01 to 1.00 ppm and LOQ of 0.01 ppm, when compared with those obtained through conventional methods.
6. Residue estimation of carbofuran and its metabolites from soil extracted and cleaned up through QuEChERS method too has been found to have better values of percentage recovery (accuracy) range of 80.1-97.1 per cent, precision of estimation (RSD percentage range) of 1.0 - 18.7 percent, linearity range of 0.01 to 1.00 ppm and LOQ of 0.01 ppm.
7. It was found that fipronil and its toxic metabolites viz., fipronil desulfinyl, fipronil sulphide and fipronil sulfone did not get translocated into 1^{st}, 2^{nd} and 3^{rd} leaves of banana at various intervals of sampling till 50^{th} days after application of 3 doses of insecticides as basal, 60 days and 150 days after planting.

8. Even at double the recommended dose of application, fipronil insecticide did not result in residues in leaves and were below the detection limit in all samples collected from 1st three leaves during the different sampling days.
9. In the fourth leaf, 0.034 μg g^{-1} of residue of fipronil molecule was noted only on 40th day, indicating a very low level of translocation of this chemical into the foliage when applied in soil.
10. No residue of carbosulfan molecule was detected in T_4 and T_5 during the sampling period in the 1st, 2nd, 3rd and 4th leaf of banana at both the levels of application.
11. In the 1st leaf when applied at recommended dose (T_4), residue of carbofuran molecule (metabolite of carbosulfan) was present only on the 7th day at 0.0797 μg g^{-1}. At double the recommended dose (T_5), carbofuran residues were detected from 5th day onwards at a concentration of 0.388 ppm and on day 7th it was (T_5) 3.3 times higher than T_4 which subsequently got dissipated to BDL on 40th day.
12. The residues of carbofuran 3-keto could not be detected in 1st leaf even on 50th day of application at the recommended rate (T_4). However, at double dose (T_5) though the residues were BDL up to 3rd day, it was 0.033 μg g^{-1} on 5th day which further increased to 0.131 μg g^{-1} on the 7th day. On 15th day 0.0123 μg g^{-1} was detected which further dissipated to BDL on 20th day.
13. The residues of Carbofuran-3 hydroxy was detected only on 5th, 7th, and 10th day of application at T_4 level. At T_5 level, up to 3rd day the residue was BDL, while the samples collected from 5th to 40th day were detected with residues. Here again the peak level of residue was noted on the 7th day (0.683 μg g^{-1}) and dissipated to lowest level of 0.017 μg g^{-1} on the 40th day and dissipated to BDL by 50th day.
14. In the 1st leaf, the total carbosulfan (including metabolites) content was highest on day 7th at both levels and was BDL on day 15th and 50th for T_4 and T_5 levels, respectively.
15. Carbofuran in the 2nd leaf persisted only on 7th and 10th day at T_4 level and its residues were present in leaves from 5th to 40th day. Metabolite, 3 keto carbofuran was absent in 1st leaf at T_4 level. However, it was present in the leaves from 5th to 25th day,

whereas 3 hydroxy carbofuran at T_4 and T_5 levels were present from 5th to 20th day and 5th to 40th day, respectively.

16. Carbofuran residues in the 3rd leaves on T_4 levels were detected on 5th, 15th with a peak value of 0.362 μg g^{-1} and 20th and dissipated to BDL on day 25th. At T_5 level the same was present from 5th to 40th day (with peak on 7th and 10th day) and dissipated to BDL by day 50. In the 3rd leaf, 3 keto carbofuran was present only on 15th and 20th day at T_4 level however, was detected continuously from 10th to 40th day for T_5 level before dropping down to DBL on day 50. A similar trend was shown by hydroxy carbofuran in the 3rd leaf at both the levels. Residues of carbosulfan molecule was not detected at both levels of application in 3rd leaf too.

17. Sum of all the metabolites of carbosulfan in the 3rd leaf was seen only from 5th to 20th day at T_4 level and was found on 5th to 40th day and was BDL on day 50 recording a peak value on day 15th and 7th for T_4 and T_5 levels respectively.

18. At T_4 level of application, in the 4th leaf, carbofuran residues persisted on 5th, 7th and 10th days, with a peak value (0.453 μg g^{-1}) on day 7. For T_5 level, the residues persisted from 1st day to 20th day of application, with the peak value of 0.452 μg g^{-1} observed on 10th day. Carbofuran-3- keto residue at T_4, was present in the 4th leaf, only on 7th and 10th day (0.075 and 0.080 μg g^{-1}). However, in T_5 its residues were recovered from day 5 to 20 only, among which peak was noted on 7th day (0.238 μg g^{-1}). Carbofuran-3 hydroxy residues in 4th leaf at T_4 level of application were detected on the 1st day and persisted till day 10 and at T_5 level it was present from day 1 to day 20. At both levels of treatment maximum of residues carbofuran-3 hydroxy were recorded on 7th day on application. In 4th leaf too, carbosulfan residue was absent throughout the period of observation.

19. Mean carbosulfan (sum of all the metabolites) residue in the 4th leaf was seen only from 1st to 20th day at T_4 level and it was found on 1st to 40th day and was BDL on day 50. In 4th leaf peak residue was noted on day 7th at both levels of application.

20. The presence of carbosulfan in the 1st to 4th leaves till day 20th and subsequent dissipation pattern prediction for BDL in 22.5 day indicated that, leaves of banana

applied with carbosulfan is not safe for use within 23 days of application for serving or food packing (as commonly practiced in many households of Kerala).

21. In soil, Fipronil desulfonyl was not detected at both the levels of application. Fipronil sulphide residue, though not found in T_4 level of application, was present in the soil on with a peak residue on 0^{th} day and dissipated to BDL on day 10. Residue of fipronil molecule in the soil at T_4 level was found till 3^{rd} day and recorded a peak value at 2 hours of application (on 0^{th} day). Again, at T_5 level maximum concentration was observed on 0^{th} day and it persisted till day 40. Fipronil sulfone molecule resided in the soil till 7^{th} and 25^{th} day with peaks on 1^{st} and 0^{th} day respectively at T_4 and T_5 levels of application. The mean fipronil content (sum of all the metabolites) persisted through 7^{th} and 40^{th} days of application in soil at T_4 and T_5 respectively indicating that higher levels of application can lead to accumulation and persistence for a longer period.

22. Carbofuran and 3 keto carbofuran residues were not detected at T_4 level of application in the soil. At T_5 level these followed similar trend except for the presence of carbofuran on 0^{th} day at 0.027 μg g^{-1}. The residues of 3-hydroxy carbofuran and carbosulfan at T_4 and T_5 levels of application persisted till 5^{th} and 7^{th} day respectively from the date of application. Mean of carbosulfan in the soil at 0^{th} hour of application registered the peak (5.78 μg g^{-1}) persisted till day 7 with a residue of 0.177 μg g^{-1}.

23. As compared with T_4 level, urease activity on day 10 was significantly and negatively influenced by higher level of application (T_5) as evinced from a significantly lower value. Lower values of urease activity in T_1 indicate a significantly higher level of activity of heterotrophic microorganism in soils with fipronil and carbosulfan at recommended dose. However, dehydrogenase activity and acid phosphatase activity were not significantly influenced by the treatments.

24. Based on the observations, it can be concluded that application of carbosulfan and fipronil as per package of practice recommendation will not result in a development of residue in the harvested food products such as fruit, (both raw and ripe), flower bud and inner core of the pseudostem.

References

Aajoud, A., Raveton, M., Aouadi, H., Tissut, M. and Ravanel, P. 2006. Uptake and xylem transport of fipronil in sunflower. *J. Agric. Food Chem. 54*(14), pp.5055-5060.

Adrasha S. K., Kalleshwaraswamy C. M., Pavithra H. B., and Sharanabasappa. 2015. Field evaluation of selected insecticides against areca nut white grub, *Leucopholis lepidophora* (Blanchard) (Coleoptera: Scarabaeidae). *Pest Manag. Horti. Ecosys*. 21 (1): 60-64pp.

Ahmadi, K., Abdollahzadeh, Y., Asadollahzadeh, M., Hemmati, A., Tavakoli, H. and Torkaman, R. 2015. Chemometric assisted ultrasound leaching-solid phase extraction followed by dispersive-solidification liquid–liquid microextraction for determination of organophosphorus pesticides in soil samples. *Talanta*. *137*:167-173.

Akubor, P.I., Obio, S.O., Nwadomere, K.A. and E. Obiomah. 2003. Production and quality evaluation of banana wine. *Plant Foods Hum. Nutr.* 58: 1–6.

Amde, M., Tan, Z.Q., Liu, R. and Liu, J.F. 2015. Nanofluid of zinc oxide nanoparticles in ionic liquid for single drop liquid microextraction of fungicides in environmental waters prior to high performance liquid chromatographic analysis. *J. Chromatogr. A. 1395*: 7-15.

Anastassiades, M., Lehotay, S.J., Štajnbaher, D. and Schenck, F.J. 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. AOAC. Int. 86*(2): 412-431pp.

Anastassiades, M., Scherbaum, E., Tasdelen, B., and Stajnbaher, D. 2007. Recent developments in QuEChERS methodology for pesticide multiresidue analysis. In: Ohkawa HM, Hisashi; Lee, Philip W (eds) Pesticide chemistry: Crop protection, public health, environmental safety. Wiley-VCH Verlag GmbH & Co: KGaA. 439-458pp.

Araujo, L., Troconis, M.E., Cubillán, D., Mercado, J., Villa, N. and. Prieto, A. 2013. Single drop microextraction and gas chromatography–mass spectrometry for the determination of diflufenican, mepanipyrim, fipronil, and pretilachlor in water samples. *Environ. Monit. Assess.* 185: 10225–10233pp.

Aravinna, A G P., J A Liyanage, J.A., Mubarak, A.M., Wickramaratne. S and Liyanage, S. 2006. [on-line] Monitoring of widely used pesticides in surface water reservoirs and shallow wells in Walawe area. 126.B. Proceedings of the 62nd Annual Session-2006, Part 1 – Abstracts. Sri Lanka Association for the Advance of Science. https://www.researchgate.net/profile/A_G_Aravinna2/publication/256519023_Monitoring_of_widely_used_pesticides_in_surface_water_reservoirs_and_shallow_wells_in_Walawe_area/links/02e7e5233331918eb8000000.pdf. [20 Aug. 2020]

Babar, K. S., Pawar, K. S., Chennegowda, S.C., Aware, P.K., Ravindran, S.S and Parimi, S. 2016. Efficacy of insecticides on adults and soil inhabiting maggots of cotton flower bud midge (*Dasineura gossypii* Fletcher). *J. Farm Sci.* 29(4): 461-465pp.

Bhan, B and Kanwar, R.S. 2012. Compatibility of carbosulfan and Azotobacter chroococcum asseed treatment for management of *Heteroder aavenae* in Wheat. *Ann. Pl. Protec. Sci*. 20 (2): 456-458pp.

Bhattacherjee, A.K. 2013. Persistence behaviour of imidacloprid and carbosulfan in mango (Mangifera indica L.). *Bull. Environ. Contamination and toxicol. 90*(2): 233-237.

Beevi, S. N., Mathew, T.B., George, T., Nair, P.K., Rajith, R., and Xavier, G. 2015. Persistence and dissipation of granular premix broad spectrum systemic fungicides trifloxystrobin and tebuconazole on banana (Musa spp) and soil. Proceedings of national symposium on pest management in horticulture ecosystem. 128 p.

Beevi S.N., Paul. A., George.T., Mathew. T. M., Kumar. N. P., Xavier. G, Kumar. G.T.P., Rajith. R, Prathibha. K.R. and Kumar S.V. 2014. Pesticide. Residues in Soils under Cardamom Cultivation in Kerala, India. Pesticide Research Journal Vol. 26(1): 35-41.

Bobe, A., Coste, C.M. and Cooper, J.F. 1997. Factors influencing the adsorption of fipronil on soils. *J. Agric. Food Chem. 45*(12), pp.4861-4865.

Bobe[1], A., Cooper, J.F., Coste, C.M. and Muller, M.A. 1998. Behaviour of fipronil in soil under Sahelian Plain field conditions. *Pesticide Sci. 52*(3), pp.275-281.

Bobe[2], A., Meallier, P., Cooper, J.F. and Coste, C.M., 1998. Kinetics and mechanisms of abiotic degradation of fipronil (hydrolysis and photolysis). *J. Agric. Food Chem. 46*(7): 2834-2839.

Bonmatin, J.M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E.A., and Noome, D.A. 2015. Environmental fate and exposure; neonicotinoids and fipronil. *Environ. Sci. Pollut. Res. 22*(1) 35-67.

Bouyoucos, G.J., 1951. A recalibration of the hydrometer method for making mechanical analysis of soils 1. *Agron. J. 43* (9). 434-438pp.

Broadbent, F.E., Hill, G. N. And K. B. Tyler. 1958. Transformations and Movement of Urea in Soils. *Soil Sci. Soc. Am. J.* 22 (4). 303-307pp.

Bruzzoniti, M.C., Checchini, L., Carlo, R.M.D., Orlandini, S., Rivoira, L., and Bubba. M. D. 2014. QuEChERS sample preparation for the determination of pesticides and other organic residues in environmental matrices: a critical review. *Anal. Bioanal Chem.* 406: 4089-4116.

Burris, E., Leonard, B. R., Martin, S. H., White, C. A., Graves, J. B., Shaw, R. and Scott, W. P. 1994. Fipronil: Evaluation of Soil and Foliar Treatments for Control of Thrips, Aphids, Plant Bugs and Boll Weevils. *Proc. Beltwide Cotton Conf. USA*. 2: 838–844pp.

Cabras, P., Angioni, A., Garau, V.L., Pirisi, F.M., Cabitza, F., Pala, M., and Farris, G.A. 2001. Fenhexamid residues in grapes and wine. *Food Additives & Contaminants*, *18*(7), pp.625-629.

Casida l. E. Jr., Klein, d. A., and Thomas. S 1964. Soil dehydrogenase activity. *Soil sci*. 98 (6) p371-376.

Cessna, A.J., Larney, F.J., Kerr, L.A., and Bullock, M.S. 2006. Transport of trifluralin on wind-eroded sediment. *Canadian J. Soil Sci. 86*(3): 545-554.

Cessna, A.J., Wolf, T.M., Stephenson, G.R., and Brown, R.B. 2005. Pesticide movement to field margins: routes, impacts and mitigation. *Field boundary habitats: implications for weed. Insect and Disease Management*. 1: 69-112.

Chai, T., Zhao, H., Yang, S., Wang, M., and Qiu, J. 2015. Different transformation of carbosulfan to its higher toxic metabolites in pakchoi (Brassica campestris ssp.) and cucumber (*Cucumis sativus* L.) after field application. *Int. J. Environ. Anal. Chem*. 95(12): 1124-1133.

Chaudhary, V and Singh, A. 2016. Assessment of pesticide residues in commonly used vegetables in Meerut district, western Uttar Pradesh. *India. Prog. Agric*. 16 (2): 248-250.

Christopher, H.J., Robert, H.E., and. Robert, Z.M. Robert M. 2001. Pesticide Bio transformation in Plants and Microorganisms. Similarities and Divergences. American Chemical Society. 4p.

Cole, L.M., Roush, R.T. and Casida, J.E., 1995. Drosophila GABA-gated chloride channel: modified [3H] EBOB binding site associated with Ala→ Ser or Gly mutants of Rdl subunit. *Life sciences*, *56*(10), pp.757-765.

Colliot, F., Kukorowski, K.A., Hawkins, D.W. and Roberts, D.A., 1992. Fipronil: a new soil and foliar broad spectrum insecticide. *Brighton Crop Prot. Conf. Pests Dis*. 1 29–34pp.

Cole, L.M., Nicholson, R.A. and Casida, J.E., 1993. Action of phenylpyrazole insecticides at the GABA-gated chloride channel. *Pesticide Biochemistry and Physiology*, *46*(1), pp.47-54.

Costa, A.I., Queiroz, M.E., Neves, A.A., Sousa, F.A.D., and Zambolim, L. 2015. Determination of pesticides in lettuce using solid–liquid extraction with low temperature partitioning. *Food Chem*. 181: 64-71.

Curbelo, M. A. G., Borges, J. H., T. M. B., and Delgado, M. A. R. 2013. Determination of organophosphorus pesticides and metabolites in cereal-based baby foods and wheat flour by means of ultrasound-assisted extraction and hollow-fiber liquid-phase microextraction prior to gas chromatography with nitrogen phosphorus detection. *J. Chromatogr. A.* 1313: 166-174.

Defang, H.X.F. 1989. The degradation of carbofuran after its repeated application to rice soil. Rural Eco-Environment. 02.

Deka, S.C., Barman, N., and Baruah, A.A.L.H. 2004. Monitoring of pesticide residues in honey samples of Jorhat district, Assam (India). *Pestic. Res J.* 16(1): 83-85.

Dhanya, M.S., George, T and Kumar, S.V. 2014. Persistence of carbosulfan formulations in laterite and coastal alluviul soils of Kerala. *Pesticide Res. J*. 28(2): 210-214pp.

Dobozy, V.A. 2000. Pesticide residues in food 2000: Fipronil (Addendum). *Environmental Protection Agency, Washington DC.*

Dutta, D., Gopal, M. and Niwas, R. 2008. Persistence of Fipronil and its Metabolites in Cabbage and Soil. *Pesticide Research Journal* Vol. 20 (1): 117-120

FAO. 1997. [online] Pesticide Residues in Food. Report 1997. FAO Plant Production and Protection Paper. 145. http://www.fao.org/3/w8141e/w8141e0i.htm#TopOfPage. [26 Aug. 2020].

FAO. 2021. [online] http://www.fao.org/faostat/en/#search/banana [26 July. 2021]

FAO-CODEX[a]. 2020. [on-line]. Pesticides Database Search. 96-Carbofuran. http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/pesticide-detail/en/?p_id=96. [20 Aug. 2020].

FAO-CODEX[b]. 2020. [on-line] Pesticide database search. 202-Fipronil. http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/pesticide-detail/en/?p_id=145. [07 Oct. 2020].

Farajzadeh, M.A. and Mogaddam, M. R. A. 2016. Acid–base reaction-based dispersive liquid–liquid microextraction method for extraction of three classes of pesticides from fruit juice samples. *J. of Chromatogr. A.* 1431: 8-16.

Fent, K. 2004. Ecotoxicological effects at contaminated sites. Toxicology. 205 (3): 223-240.

Ferreira, R.C., Papini, S., and Andréa, M. M. D. 2015. Bioavailability and influence of 14C-carbofuran on Eisenia andrei avoidance, growth and reproduction in treated natural tropical soils. *J. Environ. Sci. Health.* Part B. 50(4): 266-274.

Garg, N., Bhattacherjee, A.K., and Jyotsna. 2018. Bacterial degradation of imidacloprid and carbosulfan under *in-vitro* conditions in mango (*Mangifera indica*) — a preliminary study. *Cur. Horti.* 6(2): 23–26pp.

Geng, Y., Jiang, L., Zhang, Y., He, Y., Wang, L., Peng, Y., Wang, Y., Liu, X., and Xu, Y. 2018. Assessment of the dissipation, pre-harvest interval and dietary risk of carbosulfan, dimethoate, and their relevant metabolites in greenhouse cucumber (*Cucumis sativus* L.) *Pest Manag. Sci*. 74 (7): 1654-1663.

GOK [Government of Kerala]. 2007. *Bench mark soils of Kerala.* Soil Survey Organisation. Department of Agriculture (S.C. Unit). 623p.

GOK [Government of Kerala]. 2019. *Agricultural Statistics 2017-18*. Department of Economics and Statistics. 225p.

Gomaz, K. A. and Gomaz. A. A. 1984. *Statistical proceedings for agricultural research* (2nd Ed.). John Wiley and Sons, Inc. Singapore. 262p

Gunasekara, A.S., Truong, T., Goh, K.S., Spurlock, F. and Tjeerdema, R.S., 2007. Environmental fate and toxicology of fipronil. *J. Pestic. Sci*. 0706180001.

Gupta R P and Dakshinamoorthy C. 1980. *Procedures for Physical Analysis of Soil and Collection of Agrometeorological Data*. Indian Agricultural Research Institute, New Delhi, 293p.

Hainzl, D. and Casida, J.E., 1996. Fipronil insecticide: novel photochemical desulfinylation with retention of neurotoxicity. *Proceedings of the National Academy of Sciences*. 93(23): 12764-12767.

Hainzl, D., Cole, L.M. and Casida, J.E., 1998. Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. *Chem. Res. Toxicol.* 11(12): 1529-1535.

Hakme, E., Lozano, A., Uclés, S., Amadeo, R., and Alba, F. 2018. Further improvements in pesticide residue analysis in food by applying gas chromatography triple quadrupole mass spectrometry (GC-QqQ-MS/MS) technologies. *Anal. Bioanal. Chem*. 410:5491–5506pp.

Holder, P. J., Jones, A., Tyler C. R., and Cresswell, J. E. 2018. Fipronil pesticide as a suspect in historical mass mortalities of honey bees. *Proc. Natl. Acad. Sci.* U.S.A. 115: 13033–13038.

Indiastat, 2018. [on-line]. India Position in Production of Banana in the World. 2018. https://www.indiastat.com/table/agriculture-data/2/global-comparison-with-india-of-banana/472674/1341069/data.aspx. [05 Nov. 2020].

Hao, G. and Yang, G., 2013. Pest control: Risks of biochemical pesticides. *Sci.* 342: 799-799.

Iesce, M.R., Greca, M.D, Cermola, F., Rubino, M., Marina Isidori, M., and Pascarella, L. 2006. Transformation and ecotoxicity of carbamic pesticides in water. *Environ. Sci. Pollut. Res.* 13 (2) 105 – 109pp.

Inao, K., Iwafune, T., Horio, T., and Kitayama, I. 2018. Behavior of isoprothiolane and fipronil in paddy water, soil, and rice plants after nursery-box or submerged applications. *J. Pestic. Sci.* 43(2): 132–141.

Jackson, D., Cornell, C.B., Luukinen, B., Buhl, K. and Stone, D., 2009. Fipronil technical fact sheet. National Pesticide Information Center, Oregon State University Extension Services.

Jackson, M.L. 1958. *Soil Chemical Analysis*. Prentice Hall of India Private Limited. New Delhi.

JMPR, [Joint meeting of FAO-WHO Pesticide Residue], 2000. Pesticide Residues in Food Report of the 2000 FAO/WHO Joint Meeting of Experts. pp242.

Johnson. L.F. and Curl. E.A. 1972 - *Methods for research on the ecology of soil* borne plant pathogens. Burgess Publishing Company, Minneapolis. 247p.

Juan, E. P., Grande, B. C., Otero, R. R. and Gándara, J. S. 2006. The dissipation rates of cyprodinil, fludioxonil, procymidone and vinclozoline during storage of grape juice. *Food Control*. 17 (12): 1012-1017.

Kamel, F. and Hoppin, J.A., 2004. Association of pesticide exposure with neurologic dysfunction and disease. *Environ. Health Perspectives*. 112(9).950-958.

Karaismailoglu, M.C., 2017. Assessments on the potential genotoxic effects of fipronil insecticide on Allium cepa somatic cells. *Caryologia*, *70*(4): 378-384pp.

KAU [Kerala Agricultural University]. 2011. *Package of Practices Recommendations: Crops* (14th Ed.). Kerala Agricultural University. Thrissur. 360p.

KAU [Kerala Agricultural University]. 2015. Development of Technologies including alternatives for Banned Pesticides for the Management of Pests and Diseases of Major Crops in Kerala. RKVY project report. Kerala Agricultural University, 61-63pp.

KAU [Kerala Agricultural University]. 2016. *Package of Practices Recommendations: Crops* (14th Ed.). Kerala Agricultural University. Thrissur. 360p.

Kiefer, K., Müller, A., Singer, H., and Hollender, J. 2019. New relevant pesticide transformation products in groundwater detected using target and suspect screening for agricultural and urban micropollutants with LC-HRMS. Water Research. 165:114972.

Kim, I.S., Ryu. J.Y., Hur, H.G., Gu, M.B., Sang Don Kim, S.D., and Jae Han Shim, J.H. 2004. Sphingomonas sp. Strain SB5 Degrades Carbofuran to a New Metabolite by Hydrolysis at the Furanyl Ring. J. Agric. Food Chem. 52(8); 2309–2314pp.

Kumar, R., Niwas, R., Gopal, M., Mukherjee, I. and Devakumar, C., 2015. Groundwater Ubiquity Score (GUS) based assessment of leaching potential of some pesticides. *Annals of Plant Protection Sciences, 23*(1), pp.144-147.

Kumar, R., and Singh, B. 2013. Persistence and Metabolism of Fipronil in Rice (Oryza sativa Linnaeus) Field. *Bull. Environ. Contam. Toxicol.* 90: 482–488pp.

Kuchi, V. S., Kabir, J., Bouri, F. K., Gupta, R and Dhua, R.S. (2017). Quality Characterization Of Banana Varieties Grown Under West Bengal Conditions. *Int. Nat. J. Sci. Res*. 7. 253-258.

Lehotay, S.J., De, K.A., Hiemstra, M., and Bodegraven P.V. 2005. Validation of a fast and easy method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. *J. AOAC. Int.* 88(2): 595-614pp.

Li, Y. and Yuan, T., 2008. Detection of pyrethroid pesticide residues in concentrate juice. *Chinese Journal of Health Laboratory Technology, 18*, pp.1774-1775.

Li, X., Ma, W., Li, H., Zhang, Q and Ma, Z. 2020. Determination of residual fipronil and its metabolites in food samples: A review. *Trends in Food Sci. Tech*. 97: 185-195pp.

Lykken, L. (1963). Important considerations in collecting and preparing crop samples for residue analysis. In: Gunther F.A. (eds) Residue Reviews / Rückstands-Berichte. Reviews of Environmental Contamination and Toxicology (Continuation of Residue Reviews), vol 3. Springer, New York, NY. https://doi.org/10.1007/978-1-4615-8377-6_2.

Massoumi A., and Cornfield A.H. 1963. A rapid method for determining sulphur in water extracts of soils. *Analyst.* 88. 321.2

Martin, J. P. 1950. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. *Soil Sci*. 69: 215-232pp.

Martinez, L.C., Plata-Rueda, A., Zanuncio, J.C. and Serrao, J.E., 2014. Comparative toxicity of six insecticides on the rhinoceros beetle (Coleoptera: Scarabaeidae). *Florida Entomologist, 97*(3), pp.1056-1062.

Merlinkamala, I. and Kennedy, J.S. 2017. Dissipation dynamics and risk assessment of carbosulfan in brinjal (Solanum melongena L.) fruits. J. *of Entomol. Zool Stud*. 5(5): 1353-1357pp.

Merlinkamala, I. and Chandrasekaran,S. 2015. Degradation and Persistence of Carbosulfan 25 EC in black, red and alluvial soils. *J. Agric. and Vete. Sci*. (IOSR-JAVS) e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 8, Issue 6 Ver. I (Jun. 2015), pp12-14.

Moffat, A.S., 1993. New chemicals seek to outwit insect pests. *Sci. 261*(5121): 550-552pp.

Mohapatra, D., Mishra, S and Sutar, N. 2010. Banana and its by-product utilisation: an overview. J. Sci. Ind. Res. 69: 323-329.

Moore, M.T. and Kröger, R. 2010. Effect of Three Insecticides and Two Herbicides on Rice (*Oryza sativa*) Seedling Germination and Growth. *Arch Environ Contam Toxicol* **59,** 574–581 (20).

Mortensen, S.R., Holmsen, J.D and Weltje, L. 2015. Fipronil should not be categorized as a Bsystemic insecticide: a reply to Gibbons *et al*. (2015). *Environ. Sci. Pollut. Res.* 22:17253–17254pp.

Narayana, C. K. 2015. *Banana and Plantains Post Harvest Management, Storage, Ripening and Processing*. New India Publishing Agency. Pitam Pura, New Delhi. PIN. 110034. E-ISBN: 9789351244127

Nengming, C., Junying, Y., Dianyan, L; Jia. X; Xuemei Z; Tang Siyu, T., and Yong, C. 2019. Determination of carbosulfan and its metabolites in field celery by gas chromatography-tandem mass spectrometry.Journal of Southern Agriculture, Vol. 50, No. 8, pp. 1822-1828.

Ngim, K.K. and Crosby, D.G. 2000. Abiotic processes influencing fipronil and desthiofipronil dissipation in California, USA, rice fields. *Environmental Toxicology and Chemistry: An International Journal*, *20*(5), pp.972-977.

Nimisha, A.M., and Nisha, MS. 2019. Effect of biofumigation for the management of nematodes in banana. *J. Entomol. and Zool. Stud*. 2019; 7(5): 951-956pp.

Omeroglu, P.Y., Boyacioglu, D., Ambrus, Á., Karaali, A., and Saner, S. An Overview on Steps of Pesticide Residue Analysis and Contribution of the Individual Steps to the Measurement Uncertainty. Food Anal. Methods 5, 1469–1480 (2012).

Ostmark, H.E. 1974. Economic insect pests of bananas. *Annu. Rev. Entomol*. 19 (1). 161–176. https://doi.org/10.1146/annurev.en.19. 010174.001113

Park, B.J., Kwon, O.K., Kim, J.K., Kim, J.B., Kim, J.H., Yoon, S.K., Shim, J.H. and Hong, M.G., 2009. Characteristics of pesticide runoff and persistence on agricultural watersheds in korea. *Korean Journal of Environmental Agriculture*, *28*(2), pp.194-201.

Pascual, J and Schneider, C. 2019. A speculative claim of mass mortalities of honeybee colonies caused by fipronil in France is not supported by published field data. Proceedings of the National Academy of Sciences of the United States of America. 116 (31). 15339-15340pp.

Paul, A., Beevi, S.N., Mathew, T.B., George, T., Prathibha, R., Neethu, S.K. and Mithra, I.V., 2015. Pesticide residues in soils of banana in Kerala. *Journal of Insect Science (Ludhiana)*, *28*(1), pp.68-71.

Pimentel, D., Acquay, H., Biltonen, M., Rice, P., Silva, M., Nelson, J., Lipner, V., Giordano, S., Horowitz, A. and D'amore, M., 1992. Environmental and economic costs of pesticide use. *Bioscience*. 42(10): 750-760.

Porto, A. L. M., Melgar. G. Z., Kasemodel, M. C. and Nitschke, M. 2011. Biodegradation of Pesticides In *Pesticidesin the Modern World - Pesticides Use and Management* Edited by Dr. Margarita Stoytcheva. ISBN 978-953-307-459-7

GOI$_1$ [Plant protection quarantine and storage-Government of India]. 2020. Consumption of chemical pesticides in various states/UTS during 2014-15 to 2019-20. http://ppqs.gov.in/sites/default/files/statewise_consumption_chempesticides_0.xls . Accessed on 31/08/2020 at 13.39hrs.

GOI$_2$ [on-line]. (Plant Protection, Quarantine and Storage. Government of India). 2020. Consumption of chemical pesticides in various states/UTS during 2014-15 to 2019-20.
http://ppqs.gov.in/sites/default/files/pesticidewise_consumptionimported_1.xls accessed on 31/8/2020 13.01 hrs.

PPQS-GOI$_3$. 2020. [on-line]. (Plant Protection, Quarantine and Storage. Government of India). Major uses of pesticides (Registered under the Insecticides Act, 1968) up to -30/06/2020, based on certificate issued. Central Insecticide Board and Registration Committee. Government of India. http://ppqs.gov.in/sites/default/files/major_use_of_pesticides_insecticides.pdf. [03 Sep. 2020].

PubChem[1]. 2005. [online]. Fipronil. National Library of Medicine, National Center for Biotechnology Information. Compound summary. https://pubchem.ncbi.nlm.nih.gov/compound/3352. [26 Aug. 2020].

PubChem[2]. 2006. [online]. Fipronil sulfide. National Library of Medicine, National Center for Biotechnology Information. Compound summary. https://pubchem.ncbi.nlm.nih.gov/compound/9953940. [26 Aug. 2020].

PubChem[3]. 2007. [on-line]. Fipronil-desulfinyl. National Library of Medicine, National Center for Biotechnology Information. Compound summary. https://pubchem.ncbi.nlm.nih.gov/compound/22673275. [26 Aug. 2020].

PubChem[4]. 2005. [on-line]. Fipronil sulfone. National Library of Medicine, National Center for Biotechnology Information. Compound summary. https://pubchem.ncbi.nlm.nih.gov/compound/3078139#section=USDA-Pesticide-Data-Program. [26 Aug. 2020].

PubChem[5]. 2005. [on-line]. Carbosulfan. National Library of Medicine, National Center for Biotechnology Information. Compound summary. https://pubchem.ncbi.nlm.nih.gov/compound/41384. [26 Aug. 2020].

PubChem[6]. 2005. [on-line]. Carbofuran. National Library of Medicine, National Center for Biotechnology Information. Compound summary. https://pubchem.ncbi.nlm.nih.gov/compound/2566. [26 Aug. 2020].

PubChem[7]. 2005. [on-line]. 3-Hydroxycarbofuran. National Library of Medicine, National Center for Biotechnology Information. Compound summary. https://pubchem.ncbi.nlm.nih.gov/compound/ 27975. [26 Aug. 2020].

PubChem[8]. 2005. [on-line]. 3-Ketocarbofuran. National Library of Medicine, National Center for Biotechnology Information. Compound summary. https://pubchem.ncbi.nlm.nih.gov/compound/ 27999. [26 Aug. 2020].

Pushpangadan, P., Kaur, J., and Sharma, J. 1989. Plantain or edible banana (*Musa X Paradisica Var – Sapiemtum*) some lesser-known folk uses in India. *Ancient Sci. Life*. IX (1): 20-24.

Qin, Z.Q., Deng, Z.Y., Huang, D.F., Huang, W.H., Wei, J.J., Yan, M. X., and Lin, S. H. 2013. Field efficacy of insecticides on sugarcane borers in Guangxi, China. *Proc. Int. Soc. Sugarcane Technol*. 28.

Rai A. B., Jaydeep, H. and Kodandaram, M.H. 2014. Emerging insect pest problems in vegetable crops and their management in India: An appraisal. *Pest Manag. Hortic. Ecosyst.* 20 (2): 113-122.

Rajeswaran, J., Santharam, G., Chandrasekaran, S., Jayakumar, R. and Kuttalam, S., 2005. Harvest Time Residues of Carbosulfan in Cotton Seed, Lint Oil and Soil. *Annals Plant Protec. Sci.*13(1): 145-148.

Ramı´rez, R.F., Esquivel, L.E.B., Martı´nez, F.D.B., Acosta, I.L., and Pe´rez, M.D.O. 2012. Development and validation of an analytical method to determine fipronil and its degradation products in soil samples. *Bull. Environ. Contam. Toxicol.* 89: 744–750.

Rashid, M., Baghdadi, A., Sheikhi, A., Pourian, H.R. and Gazavi, M., 2010. Compatibility of Metarhizium anisopliae (ascomycota: Hypocreales) with several insectisides. *Journal of Plant Protection Research*, 50(1), pp. 22-27.

Ratra, G.S. and Casida, J.E., 2001**[a]**. GABA receptor subunit composition relative to insecticide potency and selectivity. *Toxicology letters*, *122*(3), pp.215-222.

Ratra, G.S., Kamita, S.G. and Casida, J.E., 2001**[b]**. Role of human GABAA receptor β3 subunit in insecticide toxicity. *Toxicology and applied pharmacology*, *172*(3), pp.233-240.

Rejczak, T. and Tuzimski, T. 2017. QuEChERS-based extraction with dispersive solid phase extraction clean-up using PSA and ZrO2-based sorbents for determination of pesticides in bovine milk samples by HPLC-DAD. *Food Chem.* 217: 225-233pp.

Robinson, J.C. and Saúco, V.G., 2010. *Bananas and plantains* (2nd Ed.). Cabi (vol 19)

Rouchaud, J., Gustin, F., Steene, F.V.D., Pelerents, C., Vanparys, L., Michel, D.T., Seutin, E., Gillet, J.,Benoit, F., and Ceustermans, N. 1990. Comparative soil and plant metabolism of carbosulfan, furathiocarb and carbofuran in brussels sprouts, cauliflower and sugar beet crops. *Toxicol. Environ. Chem.* 25 (2-3): 109-124.

Sahoo, N. Sethunathan and P.K. Sahoo. 1998. Microbial degradation of carbosulfan by carbosulfan - and carbofuran- retreated rice soil suspension. *J. Environ. Sci. Health*. B33 (4), 369-379.

Saini, S., Sushil and Kumari, B. 2015. Persistence of Fipronil and Effect of Processing on Fipronil plus its Metabolite's Residues in Chilli (C*apsicum annum linn*.). *Pestic. Res. J.* 27(1): 88-95.

Samsidar, A., Siddiquee, S. and Shaarani, S.M., 2018. A review of extraction, analytical and advanced methods for determination of pesticides in environment and foodstuffs. *Trends. Food Sci. Techno.* 71: 188-201.

Sannino, A., Bolzoni, L., and Bandini, M. 2004. Application of liquid chromatography with electrospray tandem mass spectrometry to the determination of a new generation of pesticides in processed fruits and vegetables. *J. Chromatogr. A.* 1036(2): 161-169.

SANTE. [online]. 2016. SANTE/11945/2015. Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. [26 Aug. 2020].

Santos, G.G., and Forrer, K. 2011. Avoidance behaviour of *Eisenia fetida* to carbofuran, chlorpyrifos, mancozeb and metamidophos in natural soils from the highlands of Colombia. *Chemosphere*. 84. 651–656.

Schrijver, A.D., and Mot, R.D. 1999. Degradation of pesticides by actinomycetes. *Crit. Rev. Microbiol.* 25(2): 85-119pp.

Seiber, J. N. 2011. Sustainability and Agricultural and Food Chemistry. *J. Agric. Food Chem.* 59 .1. p1–21.

Shamsipur, M., Yazdanfar, N., and Ghambarian, M. 2016. Combination of solid-phase extraction with dispersive liquid–liquid microextraction followed by GC–MS for determination of pesticide residues from water, milk, honey and fruit juice. *Food Chem*. 204: 289-297.

Sharma, K. K., 2013. *Pesticide residue analysis manual*. Directorate of Information and Publications of Agriculture, Indian Council of Agricultural Research. New Delhi.

Shuai, X., Chen, J., and Ray, C. 2011. Adsorption, transport and degradation of fipronil termiticide in three Hawaii soils. Pest Manag. Sci. 68:731-739.

Siji, S. and Nandini P.V. 2017. Chemical and nutrient composition of selected banana varieties of Kerala. *Int. J. Adv. Engi. Manag. Sci.* 3(4): 401.

Sims, J.T. and Johnson, G.V. (1991). Micronutrient Soil Tests. In Micronutrients in Agriculture, J.J. Mortvedt (Ed.).

Silva, P.M.C.S.D., Pathirane, A. and Gestel, C.A.M.V. 2009. Influence of temperature and soil type on the toxicity of three pesticides to *Eisenia andrei*. Chemosphere. 2009, 76, 1410–1415.

Singh, A[1]., Srivastava, A., and Srivastava, P.C. 2015. Sorption kinetics of fipronil on soils. *Bull. Environ. Contamination and toxicol. 93*(6): 758-763

Singh, L.M[2]., and Mahanta, B. 2013. Effect of carbosulfan, *Glomus fasciculatum*, *Trichoderma harzianum* and vermicompost alone and combination in management of *Meloidogyne incognita* on Green gram. *Ann. Pl. Protec. Sci*. 21 (1): 154-156pp.

Singh, N.V[3]., Singh, S., and Kumar, J. 2015. Efficacy of different insecticidal seed treatment against termite in wheat (*Triticum aestivum* L.) crop. *J. Exp. Zool*. 18 (1): 453-456.

Soler, C., Hamilton, B., Furey, A., James, K.J., Manes, J., and Pico, Y. 2006. Optimization of LC–MS/MS using triple quadrupole mass analyzer for the simultaneous analysis of carbosulfan and its main metabolites in oranges. *Analytica chimica acta*. 571(1): 1-11.

Songa, E.A. and Okonkwo, J.O., 2016. Recent approaches to improving selectivity and sensitivity of enzyme-based biosensors for organophosphorus pesticides: A review. *Talanta*, 155: 289-304.

Steichen, J.C., Rocheleau, T.A., Aronstein, K. and Roush, R.T., 1993. A single-amino acid substitution in a gamma-aminobutyric acid subtype A receptor locus is associated with cyclodiene insecticide resistance in Drosophila populations. *Proceedings of the National Academy of Sciences*, *90*(5), pp.1957-1961.

Stevens, M.M., Fox, K. M, Coombes, N. E and Lewin, L A. 1999. Effect of fipronil seed treatments on the germination and early growth of rice. Pest Management Sci. 55 (5) 517-523.

Subbaraya, U., Lutaladio, N. and Baudoin, W.O., 2006. Farmers' knowledge of wild Musa in India. *Food and Agriculture Organization of the United Nations*, pp.33-36.

Tabatabai, M. A. and Bremer. 1982. Sulphur, In: Page, A. I., Miller, R. H. and Keeney, D. R. (eds), *Methods of Soil Analysis Part 2. Chemical and microbiological Properties* (2nd Ed.). American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin, USA, pp 501-534.

Timonin. M. I. 1940. The interaction of higher plants and soil micro-organisms: i. Microbial population of rhizosphere of seedlings of certain cultivated plants. *Can. J. Res*. 18c (7): 307-317.

Tingle, C.C.D., Rother, J.A., Dewhurst, C.F., Lauer, S. and King, W.J., 2003. Fipronil: environmental fate, ecotoxicology, and human health concerns. In *Reviews of environmental contamination and toxicology*. Springer, New York, NY. 1-66pp.

Tiryaki, O., and Temur, C. 2010. The fate of pesticide in the environment. *J. Biol. Environ. Sci*, *4*(10), pp.29-38.

Thuyet, D.Q., Watanabe, H. and Ok, J., 2013. Effect of pH on the degradation of imidacloprid and fipronil in paddy water. *J. Pestic. Sci.* 12-080pp.

Tomlin, C.D.S., 1994. The pesticide manual: A World compendium (11th Ed.). British Crop Protection Council. Blackwell Scientific Publications. UK

Tomlin, C. D. S., 2006. The Pesticide Manual, A World Compendium, 14th ed.; British Crop Protection Council: Hampshire, England, pp 462-464.

Tomlin, C.D.S., 2009. *The pesticide manual: A World compendium* (15th edition). British Crop Production Council. UK.

Toth, S.J., and Buhler, W.G. 2009. Environmental effects of pesticides. Department of Entomology and Horticultural Science. North Carolina State University.

Trevisan, M.J., Baptista, G.C.D., Trevizan, L.R.P. and Papa, G., 2004. Residues of carbosulfan and its carbofuran metabolites and 3-hydroxy-carbofuran in oranges. *Revista Brasileira de Fruticultura*. 26(2): 230-233.

Triques, M. C., Oliveira, D., Goulart, B. V., Montagner, C. C., Espíndola, E. L. G. and Oliveira, V. B. D. M. 2021. Assessing single effects of sugarcane pesticides fipronil and 2,4-D on plants and soil organisms. Ecotoxicology and Environmental Safety. 208. 111622.

Unger, T.A., Andrew, W. 1996. *Pesticide synthesis handbook*. (8th ed.). Noyees publishing. Norwich. 511p.

USEPA [United States Environmental Protection Agency]. 1996. New pesticide fact sheet – fipronil. EPA 737-F-96-005. Office of Pesticide programs. Washington DC. 1-10pp.

USEPA [United States Environmental Protection Agency]. 2009. Fipronil. Human Health Risk Assessment petition to support and maintain the established rice grain tolerance for imported rice. EPA 737-F-96-005. Office of Pesticide programs. Washington DC, 2-38pp.

USGS. 2003. NWQA Program: Fipronil and degradation products in the rice-producing areas of the Mermentau River Basin. Louisiana, February-September 2000. USGS Fact Sheet FS-010-03.

Varghese, T.S., Tripathy, V. & Kumar, J. 2016. Dissipation of Imidacloprid on White Psyllium (*Plantago ovata*) Under Semi Arid Conditions. *Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci.* **88,** 547–551 (2018).

Verma, A., Srivastava, A., Chauhan, S.S and Srivastava, P.C. 2014. Effect of sunlight and ultraviolet light on dissipation of fipronil insecticide in two soils and effect of pH on its persistence in aqueous medium. *Air, Soil and Water Res.* 7: 69–73pp.

Vijayan, A.P. 2000. Carbofuran residues in banana. Ph.D. thesis, Kerala Agricultural University, Thrissur, 57-150pp.

Wang[a], Y. L., You, L. Q., Mei, Y. W., Liu, J. P., and He, L. J. 2016. Benzyl Functionalized Ionic Liquid as New Extraction Solvent of Dispersive Liquid-liquid Microextraction for Enrichment of Organophosphorus Pesticides and Aromatic Compounds. Chinese Journal of Analytical Chemistry. Volume 44, Issue 6, 2016, pp 942-949.

Wang[b], X., Martínez, M. A., Wu, Q., Ares, I., Larrañaga, M. R. M., Arturo, A., and, Yuan, Z. 2016 Fipronil insecticide toxicology: oxidative stress and metabolism, Critical Reviews in Toxicology, 46:10, 876-899.

Ware, G. W. 2000. *The Pesticide Book*. 5th ed. Thomson Publications. Fresno, CA. pp. 69

Watanabe, E., Miyake, S., Baba, K. Eun, H and Endo, S. 2006. Immunoassay for acetamiprid detection: application to residue analysis and comparison with liquid chromatography. *Anal Bioanal Chem.* 386. 1441–1448pp.

Widawsky, D., Rozelle, S., Jin, S. and Huang, J., 1998. Pesticide productivity, host-plant resistance and productivity in China. *Agric. Econ.* 19(1-2): 203-217.

WHO [World Health Organization]. 1997. *Pesticide Residues in Food, 1997*: Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues, *Lyons, France 22 Sept.-1 Oct. 1997* (Vol. 145). Food & Agriculture Org[on-line]. Available: https://apps.who.int/iris/handle/10665/42130

Wu, L., Hu, M., Li, Z., Song, Y., Yu, C., Zhang, H., Yu, A., Ma, Q. and Wang, Z., 2016. Dynamic microwave-assisted extraction combined with continuous-flow microextraction for determination of pesticides in vegetables. *Food chem. 192*: 596-602pp.

Xavier, G., Chandran, M., George, T., Beevi, S.N., Mathew, T.B., Paul, A., Arimboor, R., Vijayasree, V., Pradeepkumar, G.T. and Rajith, R., 2014. Persistence and effect of processing on reduction of fipronil and its metabolites in chilli pepper (*Capsicum annum* L.) fruits. *Environ. Monitoring and Assess. 186*(9): 5429-5437pp.

Li, W.Y., Li-Qin, Y.O.U., Yu-Wen, M.E.I., Jian-Ping, L.I.U. and Li-Jun, H.E., 2016. Benzyl functionalized ionic liquid as new extraction solvent of dispersive liquid-liquid

microextraction for enrichment of organophosphorus pesticides and aromatic compounds. *Chinese J. Anal. Chem.* 44(6): 942-949.

Ying, G.G. and Kookana, R.S., 2001. Sorption of fipronil and its metabolites on soils from South Australia. *J. Environ. Sci. Health.* Part B, *36*(5): 545-558.

Ying, G.G. and Kookana, R.S., 2006. Persistence and movement of fipronil termiticide with under-slab and trenching treatments. *Environ. Toxicol. Chem.: An Int. J. 25*(8), 2045-2050.

Yu, Z., Meng, M., Ya-jun., Yan-ping, L., Zheng, F., Ye, Y. and Chao-hua, Z. 2013. Leaching patterns of fipronil in 3 kinds of soil in Hainan province, China. Afr. J. Agric. Res. 8(17): 1725-1730pp.

Yusa, V., Coscollà, C., Mellouki, W., Pastor, A. and De La Guardia, M., 2009. Sampling and analysis of pesticides in ambient air. *J. Chromatogr. A.* 1216(15): 2972-2983.

Zhao, X., Yeh, J.Z., Salgado, V.L. and Narahashi, T., 2005. Sulfone metabolite of fipronil blocks γ-aminobutyric acid-and glutamate-activated chloride channels in mammalian and insect neurons. *J. pharmacol. Exp. Ther.* 314(1): 363-373.

Zheng,Y., Liu, H., Yang, Z., Ji. S., Wang. J., Pang. P, Lili Feng. L., Wang, H., Wub and Yang, W. 2015. An acetylcholinesterase inhibition biosensor based on a reduced graphene oxide/silver nanocluster/chitosan nanocomposite for detection of organophosphorus pesticides. Anal. 15. (7): 6213-6219.

Zheng, W., Park, J.A., Abd El-Aty, A.M., Kim, S.K., Cho, S.H., Choi, J.M., Yi, H., Cho, S.M., Ramadan, A., Jeong, J.H. and Shim, J.H., 2018. Development and validation of modified QuEChERS method coupled with LC–MS/MS for simultaneous determination of cymiazole, fipronil, coumaphos, fluvalinate, amitraz, and its metabolite in various types of honey and royal jelly. *Journal of chromatography B.*1072(1): 60-69.

Zheng, Y., Liu, Z., Jing, Y., Li, J. and Zhan, H., 2015. An acetylcholinesterase biosensor based on ionic liquid functionalized graphene–gelatin-modified electrode for sensitive detection of pesticides. *Sensors and Actuators B: Chem.* 210:389-397pp.

Zhu, G., Wu, H., Guo, J., and Kimaro, F.M. 2004. Microbial degradation of fipronil in clay loam soil. *Water, Air, and Soil Pollution*. 15*3*(1-4): 35-44.

APPENDIX I

Table i. Effect of fipronil and carbosulfan on soil microorganism at 10th day of application.

Treatment details	Soil microorganisms, mean (cfu g^{-1})		
	Actinomycetes (serial dilution 10^2)	Bacterial (serial dilution 10^5)	Fungi (serial dilution 10^3)
T1 Control	1.23 x 1.0E+04	1.3 x 1.0E+07	5.3 x 1.0E+04
T2- 30 mg ai fipronil / plant	1.21 x 1.0E+04	7.1 x 1.0E+06	2.3 x 1.0E+04
T3- 60 mg ai fipronil/plant	1.20 x 1.0E+04	2.1 x 1.0E+06	2.4 x 1.0E+04
T4- 400 mg ai Carbosulfan/plant	1.17 x 1.0E+04	1.5 x 1.0E+06	2.5 x 1.0E+04
T8- 800 mg ai Carbosulfan/plant	1.19 x 1.0E+04	2.3 x 1.0E+06	2.8 x 1.0E+04

Dissipation and distribution of fipronil, carbosulfan and their metabolites in banana var. Nendran (AAB) and soil.

by

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Abstract of the

THESIS

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ABSTRACT

The study entitled “Dissipation and distribution of fipronil and carbosulfan and their metabolites in banana (*Musa* spp) and soil” was carried out at the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani during the period from August 2016 to April 2017. The objective was to assess the dissipation, metabolism and persistence of fipronil and carbosulfan in banana, cv. Nendran (AAB), grown under red loam soils (AEU 8-southern laterites) of College of Agriculture, Vellayani, and its impact on soil organisms.

Banana variety Nendran, grown as per package of practices recommendations, KAU in randomized block design at the Instructional Farm, Vellayani, with five treatments viz., T1- absolute control (No application of carbosulfan and or fipronil), T2- recommended practice of 30 mg ai of fipronil per plant per application, applied 3 times on 0, 60 and 150 days of planting, T3 – double dose of T2, T4- recommended practice of 400 mg ai of carbosulfan per plant per application, applied 3 times on 0, 60 and 150 days of planting and T5- double the dose of T4. Samples viz, soil, leaves, fingers bunches and flower bud, central core of pseudo-stem and corm were collected and analyzed for residue at definite time intervals.

The residue estimation of the target pesticide molecules and their toxic metabolites were performed by adopting standard extraction and clean up procedure viz., conventional acetone extraction followed by hexane partitioning as well as by QuEChERS method. Method validation was conducted by spiking with analytical standards from which recovery, (accuracy 70-120% of spiked values), standard deviation of recoveries, RSD value (below 20%), linearity (calibration curve), for the adopted method were worked out and compared for compliance. Acetonitrile extracted samples were analyzed using “Waters Acuity UPLC

system" with suitable column and then subjecting the effluent to triple quadrupole API 3200 MS/MS system equipped with electrospray ionization interface (ESI) operating in required mode as per the molecule. Extraction using QuEChERS method gave satisfactory values for validation parameters and hence adopted for the studies.

The presence of carbosulfan in the 1st to 4th leaves till day 20th and subsequent dissipation pattern prediction for BDL in 22.5 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 many households of Kerala).

Sample matrices revealed the presence of metabolites each for fipronil and carbosulfan with variation from below detectable limit (BDL) to a highest content of 3.804 µg/g carbosulfan at 2 hours of application in the soil. 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 did not register any detectable level of fipronil or carbosulfan and their metabolites and even with an additional application of treatment on the day of bunching also did not register any detectable level of fipronil or carbosulfan and their metabolites.

Residue of fipronil and their toxic metabolites in the first, second and third leaves of banana on penultimate day of completion of pre-bunching application was found to be below the detectable levels throughout the period of sampling and this may be attributed to low absorption, fast metabolism and mobility. However, on 40th day the fipronil was detected in the 4th leaf to the extent of 0.034 µg g^{-1} and was not detectable on 50th day.

Metabolites of Carbosulfan residue existed in the first, second and third leaves between 5th and 20th day and it dissipated to below detectable limit on 40th day of application. The content of residue under treatment T_5 was distinctly higher than T_4 during these periods and dissipated to BDL on 25th day.

In soil, the fipronil though dissipated to BDL before 50th day, persisted from 2 hours of application till 40th day. However, carbosulfan and its metabolites were early to dissipate to BDL on 7th day of completion of application. Sample in experiment with 5 times the recommended dose of application as injection into pseudostem at the time of bunch emergence also did not record any residue above detectable level in the flower bud, flower bract alone, bunch on 15th day of emergence, bunch on 30th day of emergence, peel, bunch on harvest and pseudostem.

Soil urease activity on 10th day was significantly influenced by the treatment where T_1, T_3 and T_5 are significantly lower than T_2 and T_4. However, dehydrogenase activity and acid phosphatase activity were not significantly influenced by the treatment. Bacterial population was higher in T_1. Treatments were found to significantly influence the weight of blossom bud on dehorning, pseudostem, bunches and corm. Other biometric parameters were not influenced by the treatment.

The above results show complete dissipation of fipronil and carbosulfan to safe limits in soil and banana leaf, when applied as per package of practices recommendation for banana cultivation (fipronil 30 mg a.i. and carbosulfan 400 mg a.i. per plant applied thrice viz., on 0, 60 and 150 days of planting), within 50 and 23 days of application. Also, the application of fipronil and carbosulfan as per the above dose in no way results in the accumulation of residue on any of the edible plant parts of banana and hence it is safe for human consumption.

സംഗ്രഹം

1. വെള്ളായണി കോളേജിലെ സോയിൽ സയൻസ് ആൻഡ് അഗ്രികൾച്ചറൽ കെമിസ്ട്രി വിഭാഗത്തിൽ, 2016 ഓഗസ്റ്റ് മുതൽ 2017 ഏപ്രിൽ വരെയുള്ള കാലയളവിൽ "വാഴയിലും (*മൂസ എസ്പിപി.*) മണ്ണിലും, ഫിപ്രോണിൽ, കാർബോസൾഫാൻ അവയുടെ ഉപാപചയ രാസത്വരകങ്ങൾ (അവശിഷ്ടാംശം) എന്നിവയ്ക്ക് സ്വാഭാവികമാറ്റം സംഭവിച്ച്, അളവിൽ കുറഞ്ഞില്ലാതാകുന്ന കാലഗണയെ സംബന്ധിച്ച് പഠനം നടത്തുകയുണ്ടായി.

2. ചുവന്ന പശിമരാശി മണ്ണിൽ (എഇയു 8-തെക്കൻ ലാറ്ററൈറ്റുകൾ) വളർത്തിയ, വാഴ *സിവി. നേന്ത്രൻ* (എഎബി) കൃഷിയിൽ ഫിപ്രോണിലിന്റെയും കാർബോസൾഫാന്റെയും വ്യാപനം, ഉപാപചയം, സ്ഥിരത എന്നിവ വിലയിരുത്തുക, മണ്ണിലെ ജീവികളിൽ അവയുടെ സ്വാധീനം എന്തൊക്കെയാണ് എന്നതായിരുന്നു പഠന ലക്ഷ്യം.

3. കെഎയൂ വിളപരിപാലന ശുപാർശകൾക്കനുസൃതമായി വെള്ളായണിയിലെ ഇൻസ്ട്രക്ഷണൽ ഫാമിൽ ക്രമരഹിത ബ്ലോക്ക് രൂപകൽപ്പനയിൽ (ആർബിഡി) വളർത്തിയ നേന്ത്രൻ വാഴകളിൽ, അഞ്ച് വിവിധ രീതികളിൽ പരിചരണം രീതികളിൽ, അതായത് T_1, സമ്പൂർണ്ണ നിയന്ത്രണം (കാർബോസൾഫാനും, ഫിപ്രോണിലും ഇല്ലാതെ), T_2,. ശുപാർശ ചെയ്ത അളവായ ഒരു ചെടിക്ക് 30-മില്ലി ഗ്രാം എ.ഐ ഫിപ്രോനിൽ, നടീലിന്റെ 0, 60, 150 ദിവസങ്ങളിൽ 3 തവണ എന്ന ക്രമത്തിലും, T_3, മേൽ T_2 ന്റെ ഇരട്ട ഡോസ്, T_4, ഒരു ചെടിക്ക് 400 മില്ലിഗ്രാം എ.ഐ കാർബോസൾഫാൻ നടീലിന്റെ 0, 60, 150 ദിവസങ്ങളിൽ 3 തവണ എന്ന T_5 മേൽ T_4 ന്റെ ഇരട്ടി ഡോസും നൽകി പരിപാലിച്ചു . മണ്ണ്, ഇലകൾ, വാഴക്കായ്, കൂമ്പ്, പഴം, പഴത്തോൽ, പിണ്ഡി, മാണം എന്നീ വിവിധ സാമ്പിളുകൾ നിശ്ചിത ഇടവേളകളിൽ ശേഖരിച്ച്, കീടനാശിനി അവശിഷ്ടാംശം രാസപരിശോധന നടത്തി വിശകലനം ചെയ്തു.

4. അംഗീകൃതമായ വേർതിരിച്ചെടുക്കൽ മാനദണ്ഡങ്ങൾ ഉപയോഗിച്ച്, അസറ്റോനൈട്രിലിൽ വേർതിരിച്ചെടുത്ത് മെഥനോളിൽ സൂക്ഷ്മീകരിച്ച സാമ്പിളുകളും "വാട്ടർസ് അക്വിറ്റി യുപിഎൽസി സിസ്റ്റം" എന്ന സൂക്ഷ്മ-തൻമാത്രാ-പരിശോധന ഉപകരണത്തിൽ (അനുയോജ്യമായ നിര (കോളം) ഉപയോഗിച്ച്) ട്രിപ്പിൾ ക്വാഡ്രൂപോൾ എപിഐ 3200 എംഎസ്/എംഎസ് ഉപകരണ-സംവിധാനത്തിൽ വിശ്ലേഷണം ചെയ്തു. **"ക്യുയൂഇസി-ഇആർഎസ്"** എക്സ്ട്രാക്ഷൻ സാധൂകരണ രീതികൾക്ക് തൃപ്തികരമായ ഫലം നൽകിയതിനാൽ, ടി രീതി തുടർ പരീക്ഷണ സാംപിൾ പഠനത്തിനായി സ്വീകരിച്ചു.

5. മണ്ണിൽ ചേർത്തത് മുതൽ 20 ദിവസം വരെ, 1 മുതൽ 4 വരെ ഇലകളിൽ കാർബോസൾഫാന്റെ സാന്നിധ്യവും 22.5 ദിവസത്തിനുള്ളിൽ കണ്ടെത്താവുന്ന പരിധിയ്ക്ക താഴെ (ബിഡിഎൽ) ആകാനുള്ള സാദ്ധ്യത, ശുപാർശിത അളവിൽ കീടനാശിനി മണ്ണിൽ ചേർത്ത വാഴയിൽ നിന്നുള്ള ഇലകൾ, വിളമ്പുന്നതിനോ ഭക്ഷണ പാക്കിംഗിനോ 23 ദിവസത്തിനുള്ളിൽ ഉപയോഗിക്കുന്നത് സുരക്ഷിതമല്ല എന്നാണ് സൂചിപ്പിക്കുന്നത്.

6. ഫിപ്രോണിൽ / കാർബോസൾഫാൻ എന്നീ കീടനാശിനികൾ മണ്ണിൽ പ്രയോഗിച്ച് 2 മണിക്കൂറിനുള്ളിൽ തന്നെ മണ്ണിൽ അവയുടെ അവഷിഷ്ടങ്ങൾ, ബിഡിഎൽ മുതൽ 3.804 മൈക്രോ ഗ്രാം / ഗ്രാം എന്ന ഉയർന്ന തോതിൽ സാന്നിധ്യം രേഖപ്പെടുത്തി. എന്നാൽ വാഴക്കായ്, കൂമ്പ്, കൂമ്പില മാത്രം, കുലച്ച് 15, 30 ദിവസങ്ങളിലെ കായ്, പുറംതൊലി, വിളവെടുപ്പ് സമയത്തെ കായ്, പഴം, വാഴപിണ്ഡി, വാഴമാണം എന്നിവയിൽ ഫിപ്രോണിൽ, കാർബോസൾഫാൻ, എന്നിവയുടെ സാന്നിദ്ധ്യം കണ്ടെത്താനായില്ല.

7. വാഴയുടെ 1,2,3 ഇലകളിലെ ഫിപ്രോണിലിന്റെ അവശിഷ്ടങ്ങളും സാമ്പിൾ ചെയ്യുന്ന കാലയളവിലുടനീളം ബിഡിഎൽ ആയിരുന്നു. ഇത് കുറഞ്ഞ ആഗിരണം, വേഗത്തിലുള്ള മെറ്റബോളിസം, ചലനാത്മകത എന്നിവ കാരണമാകാം. എന്നിരുന്നാലും, 40-ാം ദിവസം, 4-ാം ഇലയിൽ 0.034 മൈക്രോ ഗ്രാം / ഗ്രാം എന്ന തുച്ഛമായ അളവിൽ ഫിപ്രോനിൽ കണ്ടെത്തി. 50-ാം ദിവസം അത് കണ്ടെത്താനായില്ല.

8. കാർബോസൾഫാൻ അവശിഷ്ടങ്ങൾ, 5 മുതൽ 20-ാം ദിവസം വരെ 1, 2, 3, ഇലകളിൽ നിലനിന്നിരുന്നു. ഇത് പ്രയോഗത്തിന്റെ 40-ാം ദിവസം ബിഡിഎല്ലിനു താഴെയായി. ഈ കാലയളവിൽ റ്റി5-ൽ ഉള്ള അവശിഷ്ടം റ്റി4-നേക്കാൾ കൂടുതലായിരുന്നു. 25-ാം ദിവസം ബിഡിഎലാകുകയും ചെയ്തു. മണ്ണിൽ, ഫിപ്രോണൈൽ 50-ാം ദിവസത്തിനുമുമ്പ് ബിഡിഎല്ലിലേക്ക് മറയുന്നുണ്ടെങ്കിലും, മണ്ണിൽ ചേർത്ത് 2 മണിക്കൂർ മുതൽ നാൽപ്പതാം ദിവസം വരെ നിലനിൽക്കുന്നു. എന്നിരുന്നാലും, കാർബോസൾഫാനും അവശിഷ്ടം മണ്ണിൽ ചേർത്ത് 7-ാം ദിവസം ബിഡിഎലായി.

9. കുലവരുന്ന സമയത്ത് സ്യൂഡോസ്റ്റെമിലേക്ക് ശുപാർശ ചെയ്ത അളവിന്റെ 5 മടങ്ങ് കുത്തിവയ്ക്കുന്നത് പോലെ പരീക്ഷണത്തിലും കൂമ്പ്, കൂമ്പ് ഇതൾ ബ്രാക്റ്റ് മാത്രം), കുലച്ച് 15, 30 ദിവസങ്ങളിലെ കായ്, പുറംതൊലി, വിളവെടുപ്പ് സമയത്തെ കായ്, പഴം, വാഴപിണ്ഡി, വാഴമാണം എന്നിവയിൽ ഫിപ്രോണിൽ, കാർബോസൾഫാൻ എന്നിവയുടെ അവശിഷ്ടം കണ്ടെത്താനായില്ല.

10. റ്റി1, റ്റി3, റ്റി5 എന്നിവയിൽ, റ്റി2, റ്റി4 പരീക്ഷണ ഘടകങ്ങളെകാൾ 10-ാം ദിവസത്തെ മണ്ണ് സാംപിളിലെ യൂറിയെസ് പ്രവർത്തനം കുറവായിരുന്നു. എന്നിരുന്നാലും, പരീക്ഷണ ഘടകങ്ങൾ, ഡീഹൈഡ്രജനേസ്, ആസിഡ് ഫോസ്ഫേറ്റേസ് പ്രവർത്തനത്തെ സാരമായി സ്വാധീനിച്ചില്ല. റ്റി-1 ൽ ബാക്ടീരിയ ജനസംഖ്യ കൂടുതലായിരുന്നു. പരീക്ഷണ ഘടകങ്ങൾ പുഷ്പ മുകുളം, സ്യൂഡോസ്റ്റം, കുലകൾ, കോം എന്നിവയുടെ ഭാരത്തെ ഗണ്യമായി സ്വാധീനിക്കുന്നതായി കണ്ടെത്തി.

11. വാഴകൃഷിക്ക് ശുപാർശ ചെയ്യുന്ന പാക്കേജ് പ്രകാരം ഫിപ്രോണിലും കാർബോസൾഫാനും പ്രയോഗിക്കുമ്പോൾ, 50, 23 ദിവസത്തിനുള്ളിൽ മണ്ണിലും, വാഴയിലയിലും സുരക്ഷിതമായ പരിധിയിലേയ്ക്ക് എത്തുന്നതായി മുകളിലുള്ള ഫലങ്ങളിൽ നിന്നും മനസിലാക്കാം. കൂടാതെ, മേൽപ്പറഞ്ഞ ഡോസ് അനുസരിച്ച് ഫിപ്രോണിലും കാർബോസൾഫാനും മണ്ണിൽ പ്രയോഗിക്കുന്നത് മൂലം അവയുടെ അവശിഷ്ടം ഒരു തരത്തിലും വാഴയുടെ ഭക്ഷ്യയോഗ്യമായ സസ്യഭാഗങ്ങളിൽ അടിഞ്ഞുകൂടുന്നില്ല, എന്നതിനാൽ അവ മനുഷ്യഉപഭോഗത്തിന് സുരക്ഷിതമാണ്.