FATE OF CARBOFURAN APPLIED TO THE SOIL BASIN OF BLACK PEPPER

By

BETTY BASTIN

THESIS

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Department of Soil Science and Agricultural Chemistry
COLLEGE OF HORTICULTURE
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DECLARATION

I hereby declare that the thesis entitled '**Fate of carbofuran applied to the soil basin of black pepper**' is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship, associateship or other similar title, of any other University or Society.

Vellanikkara

Betty Bastin
BETTY BASTIN

Dr.P.A.Wahid
Associate Dean

College of Agriculture
Nileshwar
July, 1996

CERTIFICATE

Certified that the thesis entitled '**Fate of carbofuran applied to the soil basin of black pepper**' is a record of research work done independently by **Smt.Betty Bastin**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Dr.P.A. Wahid
Chairman
Advisory Committee

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Smt.Betty Bastin**, a candidate for the degree of **Doctor of Philosophy in Agriculture** with major in **Soil Science and Agricultural Chemistry**, agree that the thesis entitled '**Fate of carbofuran applied to the soil basin of black pepper**' may be submitted by Smt.Betty Bastin in partial fulfilment of the requirement, for the degree.

Dr.P.A.Wahid
Associate Dean
College of Agriculture
Nileshwar
(Chairman)

Dr.A.I.Jose
Associate Dean
College of Horticulture
Vellanikkara
(Member)

Dr.C.C.Abraham
Former Associate Dean
College of Horticulture
Vellanikkara
(Member)

Dr.V.K.Venugopal
Associate Professor
Dept. of Soil Science & Agrl. Chemistry
College of Agriculture
Vellayani
(Member)

Dr.D.Sita Rama Rao
Associate Professor
Dept. of Agricultural Entomology
College of Horticulture
Vellanikkara
(Member)

EXTERNAL EXAMININER

DEDICATED TO MY MOTHER

Susanna

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INTRODUCTION

INTRODUCTION

Black pepper (*Piper nigrum* L.) is a prestigeous spice crop of Kerala. Though a number of spices are produced in Kerala, black pepper (Plate 1) as 'The king of spices' has more export value. It is native to Western Ghats of our country. Kerala contributes 85 per cent of the total production in the country (Spices Board, 1995). Pepper is produced in Kerala mainly by small and marginal farmers unlike in other pepper producing countries of the world. It performs well in laterite soils of Kerala. Idukki and Wayanad districts account for the major share of pepper cultivation in the state (FIB, 1995).

Black pepper production in the state is badly affected by pests and diseases. Damage due to plant parasitic nematodes is a serious problem in black pepper gardens. The nematodes are the primary incitants of slow decline of black pepper (Ramana and Eapen, 1995). Many workers had attributed the disorder to the infestation primarily by the root knot nematode, *Meloidogyne incognita* and burrowing nematode, *Radopholus similis* (Butler, 1906; Anandaraj *et al.*, 1991; Ramana *et al.*, 1992). Though the magnitude of crop loss due to nematode infestation is not available the problem is rampant in all black pepper growing areas of Kerala and Karnataka (Ramana, 1991). *Trophotylenchulus piperis*, a new semiendoparasitic nematode was also reported to infest black pepper in Kerala (Mohandas *et al.*, 1985). Sheela and Venkitesan (1990) and Anandaraj *et al.* (1994) attributed 'slow wilt' (slow decline) disease in Kerala to fungal-nematode complex. Infected plants exhibit initial yellowing of lower leaves which progresses upwards

Plate 1. A black pepper plant (var. Panniyoor-1)

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during later stages. The vines exhibit root knots and necrosis at varying degrees on main roots and branches. Affected vines die gradually after the appearance of external symptoms. Disinfectants and nematicides like methyl bromide and dazomet used in early times have now been replaced with insecticides like carbofuran and aldicarb which are also efficient nematicides at relatively higher levels of application. In Kerala, carbofuran (Furadan) is recommended for application to the soil basin of black pepper vine to control nematodes (KAU, 1995).

Being an export oriented crop, great care is to be exercised in black pepper cultivation to keep the pesticide residues in the crop below the permissible limits. Presence of pesticide residues in the produce is totally unacceptable in international market. Rejection or detention of the exported produce from our country due to the presence of insecticide residues had occurred in the past (Spices Board, 1995). In the present day agriculture when more and more farmers are switching over to high yielding varieties, there is a concomitant hike in the use of pesticide also. Carbofuran being systemic in nature, is absorbed by the plants readily from the soil following its application. A knowledge of absorption and distribution of the insecticide in the plant system and in the produce is all the more significant from the point of view of its acceptability for human consumption.

Soil-applied carbofuran is retained in the soil depending on the soil characteristics. It is also likely to be leached downwards during rains and may find way to ground water sources. This may, in turn, pose pollution problems depending on the residue levels. The bio-availability of the insecticide may also be determined by soil components and their properties. Obviously a knowledge of the behaviour of the insecticide in soil, its persistence and degradation is essential for evolving efficient pesticide management strategy in black pepper gardens. The present study is undertaken with the following objectives.

a) To study sorption of carbofuran in soils.

b) To understand degradation and persistence of carbofuran in soils.

c) To study the influence of organic matter on bio-availability of the applied pesticide.

d) To study the leaching of applied carbofuran in soil.

e) To study absorption of carbofuran by the vine and terminal residue levels in the berry under field conditions.

f) To study the phytotonic effects, if any, of carbofuran on the growth of the crop.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Literature on adsorption, mobility, degradation and persistence of carbofuran in soil, its absorption and metabolism in crops and also the phytotonic effects of the insecticide is reviewed in this section. Related aspects and certain other important pesticides are also referred to in contexts.

2.1 Adsorption

The three most important factors that control the fate and behaviour of a pesticide in a soil environment are the adsorption and desorption processes, leaching and movement and degradation (Haque, 1974).

Adsorption influences almost all the reactions of a pesticide in soil. As a result of adsorption, the added toxicant loses its bioactivity. Adsorption restricts the movement and hence prevents the chemical from reaching its target ie., the pest. Adsorption of a compound leads to its longer persistence in soil, thus creating problems of pollution (Bailey and White, 1964 and 1970; Hamaker and Thompson, 1972; Rajukkannu, 1978).

Freed and Haque (1973) found that adsorption was highly correlated with cation exchange capacity and organic matter content. Garg (1982) in his study of adsorption of carbofuran in different soils, *viz.*., alluvial, red, laterite, black and forest soils, found that the amount of insecticide translocated into the seedlings from different soils depended on the adsorption and degradation capacities of soil. The uptake of carbofuran from different soils was in the following order red > laterite > forest > alluvial > black. The amount of carbofuran adsorbed in the soil was related to clay content and organic carbon status. Organic matter has been considered as an important factor influencing the adsorption of carbofuran in soil. Soluble salt content of soil and pH were negatively correlated with carbofuran adsorption (Honora, 1981). Singhal *et al.* (1988) evaluated the influence of particle size and other physico-chemical properties of the soils on the adsorption of carbofuran. The influence of the particle size on adsorption of carbofuran was investigated by mixing the insecticide with the soil sample at 50 μg/g soil . They observed that the recovery of carbofuran after one day, seven days and fourteen days was directly proportional to the particle size and inversely proportional to the duration of contact. An investigation was conducted at the College of Agriculture, Vellayani by Moossa (1994) using undisturbed soil columns to study the movement and sorption of carbofuran in soils with varying physico-chemical properties at different intervals. Regression model of soil properties on the carbofuran content showed significant regression coefficient only for organic matter and clay content at all the intervals. Organic matter exerted a positive influence while clay content had negative effect.

The adsorption of pesticides in soil have been described by several models. Of these Freundlich isotherm equation (Furmidge and Osgerby, 1967; Bailey *et al.*, 1968; Chopra *et al.*, 1970; Mithyantha, 1973; Rajukkannu, 1978), Langmuir equation (Weber, 1966; Nearpass, 1969) and BET (Goring, 1967) equations are more widely used.

According to Giles *et al.* (1960) the particular type of adsorption isotherm originates due to specific interaction between the adsorbate molecules and the adsorption sites. They classified adsorption isotherms into four basic types *viz.*., S, L, C and H types. The S type is the common when the solid has a high affinity for the solvent. The L type is the common isotherm which occurs when the solid has a high affinity for the adsorbate. The constant partition or the C type isotherm is common when new sites become available as the solute is adsorbed from the solution. The H type is rare and occurs only when the solute has a very high affinity for the adsorbent. Rajukkannu (1978) found that almost in all the soils and their clay fractions studied, adsorption isotherm for carbofuran was of L type which indicated that the soils as well as their clay fractions possessed a high affinity for adsorption of carbofuran. Singh *et al.* (1990) studied the sorption and desorption characteristics of carbofuran in four soils collected from rice growing areas in India. They reported organic matter as the most important factor which determined the rate of sorption-desorption of carbofuran. Freundlich constant, K, and specific organic sorption of carbofuran in soil were small due to its low octanolwater partition coefficient. The desorption rate decreased with increase in organic matter content of the soil.

According to Achik *et al.* (1991b), carbofuran is a molecule which tends to desorb and remain in solution. The estimated values for desorption coefficient of carbofuran were always significantly higher than the estimated values for adsorption coefficient, irrespective of the soil. This indicates that carbofuran is not strongly adsorbed and shows a tendency to desorb whatever may be the soil type. Kumari and Singh (1992) determined the adsorption of carbofuran on forest loam and black silt loam soils collected from different parts of India. The result showed that the amount of carbofuran adsorbed was high in forest loam soils which had a lower pH and higher organic matter content. The adsorption isotherms in these soils conformed to Freundlich's adsorption model.

2.2 Mobility in soil

Movement of chemicals through soil is important because of the potential pollution hazard that may be caused when they reach the water table. The degree of leaching of an insecticide from soil is correlated with the water solubility, but it is also modified by the capacity of insecticide for adsorption on soil fractions (Edwards, 1973).

Helling *et al.* (1971) reported that organic matter, pH, clay, cation exchange capacity and field moisture capacity are the major factors that influence the behaviour of pesticides in soil.

Many workers studying the movement of a wide variety of pesticides have reported that pesticides are leached to a greater degree in the light textured soils than in heavier textured soils (Gantz, 1960; Harris, 1964; Kearney *et al.*, 1965). Hartley (1961) reported that percolating water is the principal means of movement of the relatively volatile pesticides and found that diffusion in soil is important only for short distance movement of the chemicals. Fuhremann and Lichtenstein (1980) observed that phorate and carbofuran with high water solubility were more mobile than insecticides with low water solubilities *viz.*., DDT, lindane, fonofos and parathion.

Felsot and Wilson (1980) found that carbofuran was highly mobile in agricultural soils and reported to have a direct relationship between adsorption of insecticide and its mobility in soils. As a general rule, Rao *et al.* (1985) suggested that pesticides with solubilities greater than 10 mg L^{-1} and half-lives greater than 50 days show the highest potential for leaching and they included carbofuran under this category.

According to Moreale and Bladen (1982), elution curves obtained by a method using undisturbed soil columns (1 m deep) showed the leaching characteristics of carbofuran likely to contaminate the ground water and resulted in real contamination hazards. Moreale and Bladen (1983) observed that due to increasing utilization of carbofuran, relatively high water solubility, persistence in soils of low pH (less than 5.5) and low adsorption by soil colloids, the migration of this chemical to relatively deep water table can occur. Residual amounts were fairly high and the possibility of water table contamination depends primarily on the volume of water present (dilution effect).

Copin *et al.* (1985) in a field trial studied the leaching loss of carbofuran in corn field by soil analysis and observed that no residue was detected at a depth greater than 10 cm. Residue of carbofuran in the top layer was smaller and decreased to undetectable limit within three months. Kumari *et al.* (1988) studied the carbofuran movement in soil during water infiltration and found that larger amount of water was needed for leaching carbofuran beyond 152 cm in clay loam soil than silt loam soil. Lee *et al.* (1990) studied the fate of carbofuran in soil and found that carbofuran is highly mobile in soils and it moved to a depth of 70 cm under natural precipitation and evaporation conditions in 135 days after application and the concentration throughout the profile was < 0.02 mg kg^{-1} soil.

Rajukkannu and Sree Ramulu (1984) conducted leaching studies with carbofuran in five types of soils using soils columns and classified carbofuran as "mobile" (class IV) in red, black and alluvial soils while in laterite soils, it was ranked "moderately mobile" (class III). Leaching of carbofuran was very rapid in red, black and alluvial soils and the leachate contained 55, 66.5 and 41.5 per cent of the added carbofuran. In laterite soil, a major portion of the added carbofuran was retained in the first column section (0-10 cm) and the movement was not detected beyond the third column section (20-30 cm).

Vasudevan (1985) studied the vertical movement of carbofuran in red, black, alluvial and laterite soils using ^{14}C-carbonyl labelled carbofuran. It was high in 0 to 5 cm soil depth in all the four soils and its content decreased with increasing soil depth. In red, black and alluvial soils, the activity of ^{14}C-carbofuran was recorded even beyond 15 cm depth while in laterite soil no appreciable amount was recorded. The mobility of ^{14}C carbofuran was in the decreasing order from red, alluvial, black and laterite soils.

Radder *et al.* (1989) conducted an experiment to determine the leaching loss, movement and distribution of carbofuran in soil columns and showed that leaching loss of carbofuran in black soil increased with time up to 72 h, whereas in red and laterite soils, the increase was only upto 48 h and then decreased. In saline alkali soil, there was no leaching until 12 h due to the dispersed condition of the soil, later the leaching increased till 72 h. The quantity of carbofuran lost by leaching during 72 h period in four soils followed the order red soil > laterite soil > black soil > saline soil. The quantity of carbofuran retained in the soil decreased with increase in depth.

Naitam and Sukhani (1990) studied leaching of carbofuran and isofenphos by the use of glass tubes as soil columns. Columns were filled with soil to a height of 50 cm. Insecticide was placed at 5 cm depth from top. Water was added to the extent of field capacity level. It was found that the vertical movement of carbofuran was faster than isofenphos. This was attributed to the increased solubility of carbofuran (600 ppm) than isofenphos. The quantity of the applied carbofuran recovered from 10-20 cm layer was 24.9 per cent and 15.3 per cent in 20-30 cm layer in the 14th day. Moossa (1994) studied the movement and sorption of carbofuran in Ultisol, Inceptisol, Entisol and Mollisol using undisturbed soil columns. Distribution of carbofuran in vertical columns at 1, 5, 10, 20, 40 and 80 days after application showed significant differences with depth, but between soils, differences were observed only on the first, 40th and 80th day. Initial distribution of carbofuran was high in the Entisol of Kayamkulam and Inceptisol of Chengannur but in later observations, the Entisol of Kuttanad, the Mollisol of Nedumangad and the Ultisol of Malappuram recorded higher values than the first two, probably, due to higher adsorption and poor mobility.

Somasundaram and Coats (1990) studied the mobility of pesticide using soil thin layer chromatography technique. They found that mobility of carbofuran was less in clay loam and silty clay loam compared to sandy loam and loamy sand. From the soil properties, they concluded that the low mobility in clay loam and silty loam soil was due to its low pH, high organic matter and high clay content compared to sandy loam and loamy sand soils.

Somasundaram *et al.* (1991) conducted a detailed study of the mobility of carbofuran and its hydrolytic product carbofuran phenol using soil tlc technique. They have shown that carbofuran phenol is less mobile than carbofuran and soil pH does not have much influence on the mobility of carbofuran. But CEC, organic matter, clay content and water holding capacity have got an inverse relationship with mobility. Of the above factors organic matter and CEC were the best predictors for the mobility of carbofuran.

2.3 Degradation and persistence in soil

Decomposition of pesticide proceeds chemically by hydrolysis of the solvated molecules or from microbiologically and photochemically induced degradation. Microbial degradation appears to be the most common pathway among these alternatives (Pionke and Chesters, 1973). Getzin (1973) reported that chemical hydrolysis in alkaline soil was the most important mechanism of carbofuran breakdown, but in acid and neutral soils both chemical and microbial processes were involved.

Sethunathan (1972) reported that pesticides which persist for longer periods under nonflooded conditions were degraded faster under flooded soil conditions. Venkateswarlu *et al.* (1977) reported that rapid degradation of carbofuran occurred in soils under flooded conditions than under nonflooded conditions.

Williams *et al.* (1976) have reported that the microbial degradation rate of carbofuran was 40 times greater in non-sterile soils compared to sterile soils. Kandasamy *et al.* (1977) found that *Helminthosporium* sp. showed greater ability to degrade carbofuran than *Trichoderma viride* and *Aspergillus niger* and it degraded completely within three weeks. All the three isolates formed hydroxycarbofuran as intermediate. Rajukkannu (1978) reported that carbofuran degradation was faster in black soils and slow in laterite soils.

Read (1983) inferred that carbofuran added to soil samples from fields previously treated with insecticide for soil insect control broke down much faster than in soils from fields with no history of carbofuran use. The rate of carbofuran degradation in soils with a history of carbofuran treatment was compared with similar soils with no previous carbofuran treatment. Degradation estimates were based on bioassay with *Diabrotica virgifera* and release of $^{14}CO_2$ from ^{14}C-carbofuran. Buhler *et al.* (1992) studied the uptake of carbofuran by musk melon plants growing in enhanced (soil in which a pesticide is rapidly degraded by population of microorganisms previously exposed to the pesticide) and non-enhanced soils. Both estimates of degradation showed rapid loss of carbofuran occurring in soils with previous history of application as opposed to a much slower rate of degradation in non history soils. Plant uptake of carbofuran measured by bioassay with *Acalymma vittatum* and residue analysis by gas chromatography was dependent upon the concentration of the insecticide in soil. The control of *A. vittatum* on plants growing in soils with history of previous application of carbofuran was reduced compared with plants growing in non enhanced soil.

Arunachalam and Lakshmanan (1990) studied the decomposition of labelled carbofuran under four conditions *viz.*. sterile flooded (SF), sterile non-flooded (SNF), non sterile flooded (NSF) and nonsterile nonflooded (NSNF) and observed that in SF and SNF soil more than 75 per cent of the added carbofuran was recovered as residue after 60 days. In NSF and NSNF soil more than 75 per cent of the added carbofuran was metabolised during the same period with 3-ketocarbofuran and carbofuran 7 phenol as major metabolites during incubation.

Yu *et al.* (1974) studied the degradation of carbofuran in a model ecosystem and were able to detect the metabolites carbofuran phenol, 3-hydroxy-carbofuran, 3-ketocarbofuran, 3-hydroxycarbofuran phenol and N-hydroxy methyl carbofuran. Greenhalgh and Relangur (1982) observed that when carbofuran was applied to humic mesosol, 3-hydroxycarbofuran and 3-ketocarbofuran were detected as transformation products. They also observed that 3-hydroxy carbofuran attained maximum value in 16-36 days. Brahmaprakash and Sethunathan (1985) reported that degradation of carbofuran occurred in soils mainly by hydrolysis and 3-hydroxycarbofuran was found to be the chief metabolite in soil.

Investigation by Caro *et al.* (1973) in small water-sheds revealed that the disappearance of carbofuran from the soil followed first order kinetics with a half-life ranging from 46 to 177 days. 3-hydroxycarbofuran, which constituted about 5 to 10 per cent also had a similar removal rate. According to Caro *et al.* (1976) carbofuran disappeared from soil by apparent first order kinetics and the half life values ranged from 145 to 434 days depending on soil pH, moisture level and temperature. Rajukkannu and Sree Ramulu (1981) studied the degradation of carbofuran in four soils under field capacity and flooded moisture levels and found that there was an exponential decrease in the concentration of carbofuran whose dose at initial application was 100 ppm and the process of disappearance obeyed the first order kinetics.

Garg and Agnihotri (1984) studied degradation rate of carbofuran in five soils and observed that half-life period of carbofuran showed wide variation between different soils. In the case of laterite soils, half-life period was 69 days compared to 8 days in black soil. Detailed investigations were made to study the degradation and persistence of carbofuran in soils and sorghum plant using ^{14}C- labelled material (Vasudevan, 1985). Four major soil groups of Tamil Nadu were used for the study *viz..*, red (Alfisol), black (Vertisol), alluvial (Vertisol) and laterite

(Ultisol). He found that the degradation of carbofuran in black and red soils was dominated by chemical hydrolysis. In the alluvial and laterite soils, the carbofuran degradation was largely attributed to microbial degradation. The-half life values for non-sterile red, black, alluvial and laterite soils were 18.3, 13.7, 15.7 and 38.4 days respectively. The half-life values for sterile red, black, alluvial and laterite soils were 22.1, 14.9, 97.3 and 114.5 days respectively.

Kearney *et al.* (1969) have defined persistence as the time required to reduce the pesticidal concentration in soils by 75 to 100 per cent of the amount initially added.

Edwards (1964; 1967) in his exhaustive reviews on the persistence of insecticides in the soil, observed differences among various soil types with respect to the persistence of applied insecticides. According to him organic matter, pH and clay content are the important soil properties affecting pesticide persistence. Employing bioassay techniques, Harris (1969) found that carbofuran was detectable upto 16 weeks after application in sandy loam soil. He also noticed the persistence of this carbamate insecticide for a longer period in muck soil as compared to sandy loam soil. Gupta and Devan (1974) under Indian conditions observed much shorter persistence for carbofuran in soils. The residue persisted for 35 days when 0.4 to 1.33 kg ai ha^{-1} was applied to soil. Honora (1981) studied the adsorption and degradation of carbofuran in soils. She found that the residues of carbofuran persisted in soil up to 60 days in detectable amounts. Sahu and Agnihotri (1983) in a study carried out in Delhi, showed that granular formulation of carbofuran applied as a side dressing @ 0.6 kg ai ha^{-1} to maize crop during kharif persisted in the soil for about 30 days in 1975 and 45 days in 1976.

According to Getzin (1973) persistence of the insecticide was influenced by soil sterilization and pH. Carbofuran degraded 7-10 times faster in alkaline soils (pH 7-9) than in acid and neutral soils (pH 4.3-6.8). Das *et al.* (1975) found that carbofuran persisted more in laterite and black soils of Kerala as shown by the mortality of *Aphis craccivora* released on cowpea (*Vigna unguiculata*) grown in the treated soils. The persistence of carbofuran was higher at field capacity level than under flooded conditions (Rajukkannu, 1978). Sethunathan *et al.* (1980) in their study on the persistence of carbofuran in 4 soils *viz.*., alluvial, laterite, pokkali and kari to control the build up of brown plant hopper (*Nilaparvata lugens* Stal) found that rapid degradation of carbofuran occurred in soils under flooded condition than under nonflooded condition. Carbofuran disappeared rapidly between 20 and 40 days after flooding in most soils. Rajukkannu and Sree Ramulu (1981) studied the persistence of carbofuran and found that it persisted for a longer period in soils at field capacity moisture level than under flooded condition. Laterite soils recorded a longer persistence while the black soils registered the least. The half-life value of carbofuran at field capacity ranged from 22 to 47 days and were highest in laterite soils followed in the decreasing order in alluvial, red and black soils. Gorder *et al.* (1982) conducted both field and laboratory studies on the persistence of soil-applied carbofuran. They identified soil moisture as the most important factor in carbofuran persistence. They did not observe carbofuran residue at a depth of more than 7.5 cm, 22 weeks after application.

Getzin and Shanks (1990) measured the persistence of carbofuran in Pacific North West soils using ^{14}C-labelled material. The half-life was > 2 weeks in all cases and > 15 weeks in 5 soils. The carbofuran decay curve always possessed an initial lag phase where soil mixing enhanced insecticide decline.

The persistence of carbofuran in soil and water samples collected from different rice growing areas in Philippines was studied under laboratory conditions by Siddaramappa and Seiber (1979). They found that degradation in soil was associated with microbial activities. Application of carbofuran to a depth of about 3 cm from the surface of flooded soil reduced the concentrations in standing water but increased the persistence in soil.

Field and laboratory tests carried out on the persistence of granules of carbofuran applied at 1.12 or 2.24 kg ai/ha in 5 soils with different histories of insecticide use and control of *Diabrotica virgifera*, high percentages of control in field plots coincided with recoveries of larger amounts of carbofuran about one month after insecticide application. Carbofuran persistence was shortest during field and laboratory experiments in those soils characterized by both histories of carbofuran use and rootworm control problems. Rapid dissipation of carbofuran under field conditions was attributed to inherent soil properties such as adapted microbial population or differences in rainfall (Felsot *et al.*, 1982).

2.4 Absorption and metabolism in crop

Dorough (1968) studied the metabolism of carbofuran in bean plants. Hydrolysis, oxidation and conjugation were observed as the three most important steps in the metabolic pathway. Major metabolites detected in bean plant were 3-hydroxycarbofuran and carbofuran-7-hydroxy phenol. Metcalf *et al.* (1968) studied the metabolism of carbofuran in plant (cotton) and mammals. They found that carbofuran was metabolised by hydroxylation to 3-hydroxy carbofuran which was subsequently oxidised to 3-ketocarbofuran. The phenols were present either in the free state or as conjugates, principally the glucosides in the various biological systems. Garg (1982) found that the amount of carbofuran translocated into the sorghum seedlings from different soils depended on the adsorption and degradation capacities of soil. The uptake of carbofuran from different soils was in the following order: red > laterite > forest > alluvial > black. Vasudevan (1985) observed the presence of the parent compound ^{14}C-carbofuran and its metabolites, 3-hydroxy and 3-ketocarbofuran and two unknown metabolites in soil and in roots and shoots of sorghum plants through tlc analysis when ^{14}C labelled carbofuran was applied to soils. In the organic phase extracted from the sorghum shoots, 3-hydroxycarbofuran formed the major share of the total radioactivity in all the sampling periods. The autoradiography of the two week old sorghum plants registered larger concentration of ^{14}C-carbofuran in the leaves than in roots, stem or leaf sheath. Higher concentration was observed in leaf margins than in the central lamina. He also reported that the per cent activity of ^{14}C-carbofuran in shoots of sorghum raised from red soil was the highest followed by alluvial, black and laterite soils. Higher availability of carbofuran in red soil was responsible for higher per cent radioactivity in sorghum plants grown in it. The chemical degradation of carbofuran due to high soil pH, in black soil caused less accumulation of the insecticide in plant parts. In laterite soil, slow and steady release of soil adsorbed carbofuran into soil solution resulted in the higher accumulated ^{14}C-carbofuran from 20th day to the harvest stage.

Ashworth and Sheets (1972) reported that the carbofuran which had been absorbed in tobacco plants through the roots or through the petioles of isolated leaves was metabolised with a half-life of about four days by progressive hydroxylation and oxidation to 3-ketocarbofuran. All these carbamates were hydrolysed to their corresponding phenols and were conjugated as their glycosides. 3-hydroxycarbofuran and its glycosides were the major metabolites found in the carbofuran treated tobacco plants. Huynh *et al.* (1975) studied the metabolism of carbofuran in rice plants in sunlight and in laboratory conditions at different temperatures and light intensities. Carbofuran phenol, 3-hydroxycarbofuran, 3-ketocarbofuran and seven unknown metabolites were detected by thin layer chromatography. Sonobe *et al.* (1983) estimated the carbofuran residues from root crops when ^{14}C-carbofuran was added to the soil. The treated crops were harvested at 5, 10 and 15 days post application and composited. Carbofuran was the major residue in potatoes and radishes. The angelic ester of 3-hydroxycarbofuran, carbofuran and 3-hydroxycarbofuran were the main residues in carrots. Rouchard *et al.* (1990) observed that carbofuran applied to soils at the time of sowing was absorbed more intensively and for longer periods by cauliflower, brussels sprout and sugarbeet. In the foliage, carbofuran was metabolised rapidly to 3-hydroxycarbofuran.

Caro *et al.* (1973) reported that up to 0.14 per cent of applied carbofuran was accumulated in maize, a major portion being 3-hydroxycarbofuran and it decreased sharply before harvest. Residues were mostly in leaves and the carbamate content in the yield was below the tolerance limit. Talekar *et al.* (1977) observed that after soil treatment of labelled ^{14}C-carbofuran and ^{14}C-phorate, both the insecticides were readily translocated to the leaves of two week old seedling of both soybean and mung bean. Measurable but insignificant amounts of radiocarbon were present in the seeds of plants grown in treated soil. Ali (1978) reported that the translocation and persistence of carbofuran was much greater in rice leaves than in stems and as a result there was increased mortality among the hoppers when fed on the leaves than on the stems. In a comparative study of the persistence, movement and metabolism of six insecticides in soils and plants, Fuhremann and Lichtenstein (1980) found that most of the carbofuran residues were recovered from the oat leaves in the form of carbofuran and 3-hydroxycarbofuran. Two soil types *viz.*., loamy and sandy were used. In the case of more water soluble insecticides like phorate and carbofuran the largest portion of the total plant residues were in the oat tops whereas it was in the roots in the case of DDT and lindane treated systems. Sahu and Agnihotri (1983) reported that the residues of carbofuran were concentrated mainly in the leaves and they were not detectable in the grain samples taken at green cob stages or at harvest in maize.

Ashworth and Sheets (1970) studied the absorption and translocation of ^{14}C-labelled carbofuran in one month old tobacco plants. It was readily absorbed from the nutrient solution and was completely translocated to the leaves without accumulation in the root. Autoradiography of the freeze-dried plants indicated that accumulation was more in older leaves than in the region of terminal bud. In the case of leaves, labelled carbofuran was not readily absorbed from the nutrient solution and even when it was absorbed, it was not translocated from treated leaf to other parts of the plants.

Pree and Saunders (1974) observed that carbofuran phenol, 3-keto-carbofuran phenol and two unknown metabolites were present in the foliage of mugho pine (*Pinus mugho*) in addition to 3-hydroxycarbofuran. An increase in 3-keto phenol in the treated plants was accompanied by decreased levels of 3-hydroxycarbofuran. Application of carbofuran at the rate of 2 g tree^{-1} produced a peak concentration of 283 ppm, with the application of the insecticide at 1 and 0.4 g tree^{-1} produced 106 ppm and 36 ppm respectively. The presence of the toxicant was detected within a week and its concentration increased for 60-90 days before declining. Active residues persisted for at least two years. Rajukkannu *et al.* (1983) found that the application of carbofuran granules on the top frond of coconut to control rhinoceros beetle had left residues in the tender coconut water and copra more than the tolerance limit on the 11th day but the level got reduced on 23rd day well below the tolerance limit and based on this result, a waiting period of three weeks was suggested by them for the safe consumption of coconut water and copra. Vasconellos *et al.* (1983) reported that in course of tests in oranges (*Citrus sinensis*) in Rio-de-Janeiro to control insect pests by soil application of granular preparation containing 5 per cent carbofuran at 400, 300, 200 and 100 g tree^{-1}, respectively, orange fruits analysed at 90 days after treatment contained carbofuran residues of 0.13, 0.12, 0.10 and less than 0.05 ppm respectively. Beevi *et al.*(1991) reported that the residues of carbofuran in cucumber were not detectable when applied at the time of sowing. But when applied at the flowering stage, maximum residues were seen on the third day after the treatment, the values being 2.88 and 3.38 mg kg^{-1} respectively for 0.5 and 1.5 kg ai ha^{-1}. The residues reached below the maximum residue limit in 23 days. Abraham (1993) conducted a study about the chemodynamics of carbofuran in cucumber plants. Insecticide was applied at two doses *viz..*, 0.5 and 1.5 kg ai ha^{-1} at three occasions *viz..*, sowing, vining and flowering stage of the crop. The concentrations of carbofuran, 3-hydroxy-carbofuran and 3-ketocarbofuran in the vine, leaf and fruit reached almost nondetectable levels by 28th day after treatment.

2.5 Phytotonic effects of carbofuran

Carbofuran 3G as growth stimulant was reported by Apple (1971), Balasubramaniyam and Morachan (1981) and Nayeem and Bapat (1976).

Mueke *et al.* (1978) were of the view that the phytotonic effect of carbofuran and other insecticides in alfalfa were indirectly due to the better control of aphids. Salam (1989) reported increased growth of rice plants by application of carbofuran granules and the growth increase was expressed in terms of litter production, leaf area index, root production and grain yield.

Patel and Srivastava (1990) in their studies on the phytotonic effect of insecticides on cowpea and green gram found that in the case of cowpea, maximum weight of plant, weight of root and maximum height of plant were recorded for the carbofuran treated plants. In the case of green gram, maximum plant height was observed in the case of carbofuran treated plants. Carbofuran increased the number of trifoliate leaves and length of tap root. Phytotonic effect of carbofuran in sorghum cultivars *viz..*, CSH 9, CSV 10, GJ 35, CSV 1 and CSH 1 was studied by Patel and Sukhani (1990). The results indicated that the different plant growth parameters like number of leaves/plant, seedling weight and shoot length at 10 days of germination and parameters like number of leaves plant^{-1}, plant height and dry fodder yield at harvest were significantly higher in carbofuran-treated plots than in untreated plots. The days to 50 per cent panicle emergence decreased considerably resulting in early flowering in treated plots. Phytotonic effect was evident from the early maturity and higher fodder yield of plants which received carbofuran.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

An investigation was taken up in order to study the fate of carbofuran applied to the soil basins of black pepper. This study involved four experiments which were conducted at the Radiotracer Laboratory, College of Horticulture, Vellanikkara during the period from 1993-1995.

3.1 Carbofuran and its metabolites

3.1.1 Technical grade carbofuran and its metabolites

Technical grade carbofuran used for this study was 75 per cent pure. Analytical grade 3-hydroxycarbofuran (99% purity) and 3-ketocarbofuran (99% purity) produced by FMC Corporation, Middle port, New York were also used. The important characteristics and properties of carbofuran are given in Table 1. The chemical configurations of carbofuran and its metabolites are given in Fig.1. The toxicity of the chemical and its metabolites are presented in Table 2.

3.1.2 Carbofuran - commercial grade

In field experiments the formulated product of carbofuran *viz.*., Furadan 3G (Rallis India Limited, Bombay) containing 3 per cent active ingredient was used for the study.

3.1.3 Labelled carbofuran

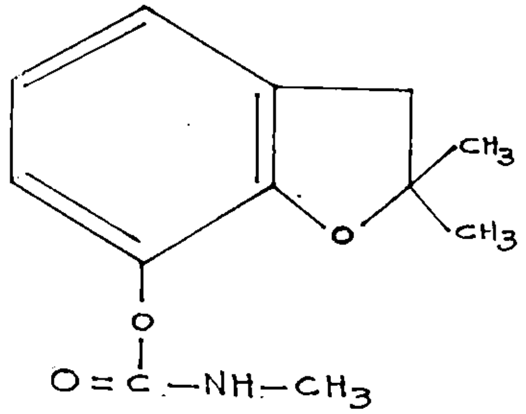
Carbonyl labelled ^{14}C-carbofuran with a specific activity of 492.1 MBq $mmol^{-1}$ (13.3 mCi $mmol^{-1}$), a product of FMC Corporation, Middle Port, New York was used in studies employing labelled carbofuran. The insecticide was

Table 1. Important characteristics and other relevant information on carbofuran

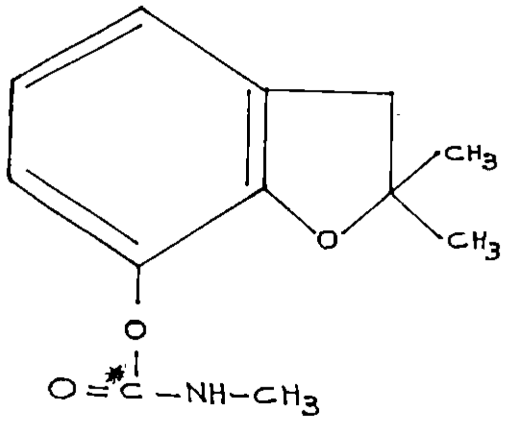
Chemical name	2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methyl carbamate
Chemical formula	$C_{12}H_{15}NO_3$
Molecular weight	221.26
Physical state	White, crystalline solid
Melting point	153° to 154°C
Relative density	1.180
Vapour pressure	2×10^{-5} mm Hg (33°C)
Water solubility	700 mg L^{-1} (25°C)
Odor threshold	Odorless to slightly phenolic
Trade name	Furadan, Curaterr and Yaltox
Uses	Used as insecticide, acaricide and nematicide in several crops
Formulation	Granules, dispersible liquid
Application mode	Soil incorporated granules or can be applied as foliar spray

Kuhr and Dorough, 1976; Mac Rae, 1989; Wauchope *et al.*, 1992.

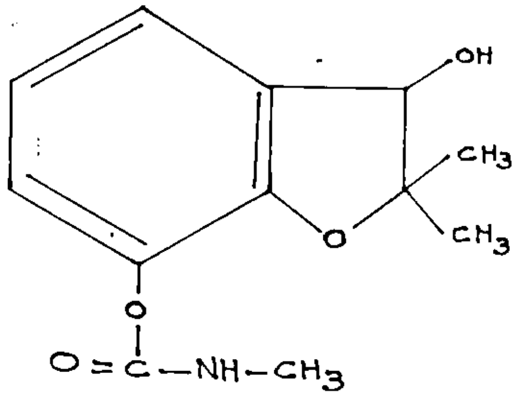
Fig.1. Chemical structures of carbofuran and its chief metabolites



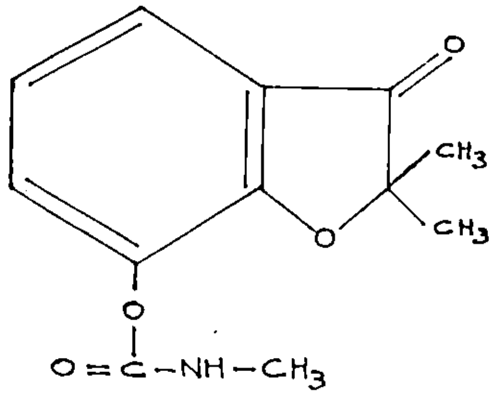
Carbofuran
(2,3-Dihydro-2,2-dimethyl-7-benzofuranyl N-methyl carbamate)



Side chain labelled carbofuran
*Labelled carbon



3-Hydroxycarbofuran
(2,3-Dihydro-2,2-dimethyl-3-hydroxy-7-benzofuranyl N-methyl carbamate)



3-ketocarbofuran (3-oxo-carbofuran)
(2,3-Dihydro-2,2-dimethyl-3-oxo-7-benzofuranyl N-Methyl carbamate)

Table 2. Toxicity (acute) of carbofuran and its chief metabolites

Compound	Oral LD_{50} (mg kg^{-1})	References
Carbofuran	11.0	Fuhremann and Lichtenstein, 1980
3-hydroxycarbofuran	17.9	Schoenig, 1967
3-ketocarbofuran	69.0	Schoenig, 1967

dissolved in benzene and stored in a refrigerator until used. Before use, radioactive purity of ^{14}C-carbofuran was determined by thin layer chromatography. The radiochemical purity of the compound was found to be 96 per cent.

Aqueous solution of labelled carbofuran was prepared by taking required quantity of the benzene solution of the insecticide in a beaker, evaporating off the solvent at room temperature and redissolving the residue in required quantity of distilled water.

3.2 Soils

In all studies, laterite soil (Ultisol) was used as it is the major soil type growing black pepper in Kerala State. The soils were collected from farmers' fields with no previous history of carbofuran application. Surface soil samples (0-15 cm depth) were collected from two major black pepper growing tracts of Kerala State namely, Wayanad and Kannur. The soils designated as S_1 and S_2 were collected from Kannur and those designated as S_3 and S_4 were from Wayanad. Soil samples designated as S_5, S_6 and S_7 were collected in bulk from three different locations of Wayanad district. The particulars of the locations from which the soil samples were collected are given in Table 3.

3.2.1 Processing of soil samples

The soils were brought to the laboratory. Gravel and stones were removed from them and were air dried in shade. The soils were then gently ground with a wooden hammer to break the clods and passed through 2-mm sieve. The sieved soils were stored in polythene containers until use.

Table 3. Details of the soil sampling locations

Soil No.	Location	District
S_1	Kalladi	Kannur
S_2	Kannur	,,
S_3	Manjappara	Wayanad
S_4	Nenmeni	,,
S_5	Manjappara	,,
S_6	Kuppamudi	,,
S_7	Jayagiri	,,

3.2.2 Physico-chemical characteristics

The soils S_1, S_2, S_3 and S_4 were analysed for physico-chemical characteristics *viz*.., mechanical separates, pH, organic carbon, CEC and free iron oxides. The analytical methods employed are given (Table 4). The organic matter status of the soils S_5, S_6 and S_7 were also determined.

3.3 Experiment 1. Sorption of carbofuran in laterite soils

3.3.1 Kinetics of carbofuran adsorption

Soils from Kannur (S_1) and Wayanad (S_3) were selected for this study (Table 3). One gram portions of soils taken in centrifuge tubes (20 x 100 mm) were treated with 5 mL of aqueous solution of ^{14}C-carbofuran (specific activity 1074 dpm μg^{-1}). The centrifuge tubes with contents were shaken for 30 min, 1.5 h, 2.5 h and 4 h on a wrist action shaker to allow sorption. Sufficient number of replicates were maintained to permit withdrawal of duplicate tubes at each interval for each soil. After shaking, the tubes with soil suspension were centrifuged at 10,000 rpm for 5 min. After centrifugation, 1 mL of the supernatant was transferred to a scintillation counting vial and mixed with 20 mL liquid scintillator (PPO, 4 g; POPOP 0.2 g; methanol 100 mL; Ethylene glycol 20 mL and 1,4-dioxane to make up to 1.0 L). The vials were counted in a liquid scintillation counter.

3.3.2 Sorption isotherm

The surface soils from Kannur, S_1 and S_2 were used in this study. These soils differed in their organic matter content (Table 5). The organic matter contents were 4.21 and 1.82 per cent respectively for S_1 and S_2. Two gram portions of each soil taken in a series of 20 x 100 mm centrifuges tubes were mixed with 0.2, 0.4,

Table 4. Analytical methods employed for the determination of the physico-chemical characteristics of soils

Soil property	Method of extraction	Method of estimation	Instrument used	References
Mechanical analysis	International Pipette method			Piper (1942)
pH (H_2O)	1:2.5 soil-water suspension		pH meter	Jackson (1973)
Organic carbon	Walkley and Black method			Jackson (1973)
Cation exchange capacity	Saturating soil with 1$\underline{N}$ KCl and replacing with 1$\underline{N}$ NH_4Cl	Estimating K content	EEL flame photometer	Jackson (1973)
Free iron oxides	Dithionite-citrate-bicarbonate method	Colorimetry by orthophenanthroline method	Spectronic 20 spectro photometer	Mehra and Jackson (1960)

Table 5. Physico-chemical characteristics of the soils used in the study on sorption of carbofuran

Soil no.	pH	Organic matter (%)	Free iron oxide (%)	Cation exchange capacity (cmol(+) kg^{-1})	Mechanical composition (%)		
					Sand	Silt	Clay
S_1	5.3	4.21	0.23	13.87	41.99	20.75	37.26
S_2	5.3	1.82	0.46	8.87	57.36	7.75	34.89
S_3	5.1	0.96	2.98	10.08	75.27	6.18	18.55
S_4	5.3	1.09	3.92	10.94	50.97	15.08	33.95

0.6, 0.8 and 1 mL of aqueous solution of ^{14}C-carbofuran solution (specific activity 1074 dpm μg^{-1}) in distilled water. Distilled water was added to each of the centrifuge tubes so that the total volume of solution came to 10 ml. The tubes were stoppered tightly and shaken for 30 minutes. After shaking, the tubes were centrifuged for 5 minutes at 10,000 rpm. The radioactivity of the supernatant solution was then found out by the same procedure given in the previous Section 3.3.1. The equilibrium concentration of carbofuran (ng mL^{-1}) and the quantity of carbofuran adsorbed (ng g^{-1} soil) (Swanson and Dutt, 1973) were computed.

3.4 Experiment 2. Persistence of carbofuran in soil

For this study, technical grade carbofuran (non-radioactive) was used. Degradation and persistence of carbofuran were studied in two soils of Kannur (S_1 and S_2) and Wayanad (S_3 and S_4).

Twenty gram portions of each soil were transferred into a number of 20 x 100 mm size glass tubes. Freshly prepared aqueous solution of carbofuran was added to soil in each tube to give 140 μg of carbofuran $tube^{-1}$ (i.e., 7 μg carbofuran g^{-1} soil). The concentration of the solution was so chosen that the volume of the solution added was sufficient to bring the soil moisture content to 60 per cent WHC.

The procedure adopted for addition of carbofuran solution to soils is as follows. The quantity of soil (20 g) was divided into three equal portions. One portion was transferred into the tube followed by the addition of one-third of the required quantity of carbofuran solution. The tube with its contents was then rolled to bring about effective mixing of the solution with soil. The same procedure was repeated for the addition of each of the remaining two portions of the soil and carbofuran solutions. After addition of the solution, the glass tubes were plugged with nonabsorbent cotton and incubated at room temperature for varying intervals.

The moisture regime of the soil-insecticide system was maintained at 60 per cent water holding capacity throughout the experimental period. This was done as follows. Tubes with the same quantity of soil were incubated after adding the same quantity of water to maintain 60 per cent water holding capacity. These tubes were weighed at regular intervals and the difference in weights of tubes with soil between two intervals was considered to be due to water loss. This was made up by adding equivalent quantity of water to each tube to maintain the moisture regime at 60 per cent water holding capacity.

Sufficient number of replicates were maintained for each soil so as to allow the withdrawal of two replicates at different intervals of time following incubation. Samples were withdrawn at intervals of 0, 5, 15, 20, 25, 30, 45, 60 and 75 days after application of the insecticide.

At the end of each interval, the contents of the tube were extracted to quantitate the pesticide and its metabolites as given in Section 3.7. From the data obtained, kinetics of disappearance of the parent molecule from the soil was evaluated mathematically.

3.5 Experiment 3. Absorption of soil-applied carbofuran by pot-grown black pepper vine

Three soils collected from Wayanad district namely, S_5, S_6 and S_7 (Table 3) were used for this study. ^{14}C-carbofuran was used in this experiment.

3.5.1 Details of the experiment

Design : CRD

Soils used : 3 (S_5, S_6 and S_7)

Levels of carbofuran application : 3 (L_0 : 0; L_1 : 1.0; L_2 : 2 kg ai ha^{-1})

Number of replications : 4

Total number of samples : 36

Black pepper variety : Panniyur-1

Date of start of experiment : 26-8-1994

Date of application of carbofuran : 19-10-1994

Treatment combinations : S_5L_0
S_5L_1
S_5L_2
S_6L_0
S_6L_1
S_6L_2
S_7L_0
S_7L_1
S_7L_2

The soils selected had organic matter contents of 1.28 (S_5), 2.45 (S_6) and 3.09 per cent (S_7). Sufficient number of pots were filled with 1 kg of air-dried 2-mm sieved soil. One rooted pepper cutting was planted to each pot. The plants were allowed to establish for a month. Pots were irrigated at regular intervals so that moisture regime of soils was maintained at 60 per cent water holding capacity. About one month after establishment, freshly prepared aqueous solution of ^{14}C-labelled carbofuran (specific activity 1074 dpm μg^{-1}) was applied. The prepared carbofuran solution had a concentration of 48 $\mu g\ ml^{-1}$. Ten milli litres of this solution were applied to pots receiving carbofuran at the rate of 1 kg ai ha^{-1} and 20 mL to pots receiving carbofuran at the rate of 2 kg ai ha^{-1} as one time application. A control pot with vine but without carbofuran application was also maintained in each replication. Plant protection measures were adopted whenever needed. Fertilizers were applied @ 100 kg N, 50 kg P_2O_5 and 100 kg K_2O ha^{-1} in the form of urea, superphosphate and muriate of potash respectively. Urea was applied in two equal splits whereas the other two fertilizers were given as single dose. The plants were trailed and supported on thick coir rope (Plate 2).

The plants were harvested at the end of 3 months of growth. The stem portion was cut at 1 cm above the soil level. The stem and leaf portions of each plant were separated and fresh weights of these parts were recorded. The stem and leaf portions were then extracted separately for determining the insecticide residue and metabolites by the methods given in Section 3.8.

The roots were carefully removed from the soil, washed thoroughly with tap water and dried in oven at 70°C for the determination of dry weights and radio activity content. The growth characteristics of the control plants were compared with those of the insecticide - treated vines for studying the phytotonic effects, if any, due to insecticide application.

Plate 2. A general view of the pot culture experiment with black pepper vine

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3.5.2 Autoradiography of thin layer chromatogram of leaf extracts

The organic extracts of leaf samples from all the replications for the same soil and for same level of carbofuran application were combined, evaporated and volume made up to 1 mL (concentration) with methanol and stored in small vials. Thus for 3 soils, possessing different organic matter contents and two levels of carbofuran application, there were six samples. This was done to get a measurable quantity of radioactivity as the radioactivity of the single plant was less. A suitable volume (0.1 mL) of each of the six extracts was spotted on tlc plates along with standard carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran. The plates were developed with ether : hexane (3:1) solvent system for a distance of 15 cm and air dried. The plates were then exposed to X-ray films of proper size for 3 months and later developed. The standard compounds on the plates were identified by adopting the method described by Archer (1976). The silica gel areas on the plates corresponding to authentic compounds (carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran) were scraped into 15 mL of scintillation liquid (PPO, 4 g; POPOP, 0.2 g; methanol, 100 mL; ethylene glycol, 20 mL and 1, 4 dioxane to make up to 1.0 L) taken in 20 mL scintillation vial and radioactivity was determined in a liquid scintillation counter (Pharmacia LKB, Wallac OY, Finland).

The ^{14}C activity of the concentrated extracts of the six leaf samples was also determined using the liquid scintillation counter by taking 0.1 mL extract of each in 15 mL scintillation cocktail taken in scintillation counting vial.

3.5.3 Autoradiography of black pepper vine

An eight month old bush pepper plant of variety Panniyur-I with 2 mature spikes and 4-5 leaves was used in this study. The plant was uprooted carefully and roots were washed thoroughly in clean water. The surfaces of the leaves, stem, root and berry were wiped dry using cotton. It was then placed in a long test tube in such a way that the roots were immersed completely in aqueous solution of ^{14}C-carbofuran (specific activity 1074 dpm μg^{-1}) contained in it. The mouth of the tube was plugged with non-absorbent cotton and the whole system was kept in sunlight for six hours. Distilled water was added to the tube at intervals so that solution in the tubes remained at the original level. The plant was taken out and the roots were cut off. The shoot portion was then wiped dry and mounted on a thick paper with the help of adhesive. The specimen was kept pressed for four days and after this period it was exposed to an X-ray film (Agfa-Geevaert India Ltd.) for two months. The X-ray film was developed to obtain the autoradiograph of the plant. The film was developed using a commercial X-ray film developer solution (Agfa-Geevaert India Ltd.). The developed film was photographed by placing it against an illuminated white background.

The radioactivity of the different parts of the specimen used for autoradiography was measured using biological oxidiser. Each plant part was separated, weighed to get dry matter content before its radioactivity was measured.

3.6 Experiment 4. Mobility of carbofuran in the soil basins of black pepper vine and absorption of the insecticide by the vine

This study was conducted with field-grown black pepper vine in KADP farm attached to College of Horticulture. The soil was acidic laterite. Furadan (granule) was used in this experiment. The physico-chemical characteristics of the soil are given in Table 18.

3.6.1 Experimental details

Variety	: Panniyur-1
Age	: 5 years
Number of treatments	: 2

T_1 - Single application of carbofuran at the onset of SW monsoon @ 1 g ai plant^{-1}

T_2 - Application of carbofuran in 2 equal splits @ 1 g ai plant^{-1} at the onset of SW monsoon and NE monsoons

Number of replications	: 4
Standard on which pepper vines were trailed	: *Garuga pinnata*
Date of start of experiment	: 28-6-1994

3.6.2 Application of carbofuran to the soil basins

The basins of the pepper plants were cleared of weeds. A circular area was marked around each plant at a radius of 30 cm from the base of the plant. Surface soil was removed to a depth of 2 to 3 cm from the entire area of the basin and kept as a raised edge towards the boundary.

The required quantity of Furadan 3G was weighed and mixed with equal volume of sand. This mixture was uniformly applied over the entire circular area marked around each plant at the onset of SW monsoon for treatment T_1 and at the onset of both SW and NE monsoons for treatment T_2. The applied area was immediately covered with the soil removed from it. At the time of application of carbofuran during SW monsoon, the vines were in full bloom.

3.6.3 Collection of soil samples

Soil samples were collected from the carbofuran applied area up to a depth of 60 cm at an interval of 10 cm using soil auger. This was done as follows. Three points were first located at and around the plant within the circular applied area (30 cm radius). From each point, sample was taken to a depth of 0-10 cm. All the 3 samples were then mixed together to get a composite sample. The same procedure was repeated up to a depth of 60 cm. The composite soil samples collected for each 10 cm depth were sieved through 2 mm sieve and kept ready for extraction. Another portion of the sample was used for soil moisture estimation and the contents of residues were expressed as $\mu g\ g^{-1}$ soil on moisture free basis. Soil samples were taken at intervals of 0, 10, 30 and 60 days after application of carbofuran for T_1 treatment. The sampling intervals were 0, 11, 21 and 31 days after application of carbofuran at the onset of NE monsoon in treatment T_2. The holes formed as a result of taking soil samples were immediately filled with fresh uncontaminated soil and these points were not considered for future sampling.

3.6.4 Collection of leaf samples

Recently matured leaves from fruiting branches (laterals) were collected. Leaves were collected from all around the plant and from upper, middle and lower portions. The samples were mixed together to get a representative one for each vine. This was used for residue extraction. The samples were collected at intervals of 10, 20, 30, 45, 60, 76 and 91 days after application of insecticide in the SW monsoon

(T_1) and at intervals of 6, 11, 21, 31, 51 and 60 days after application of insecticide in NE monsoon (T_2).

3.6.5 Collection of berry

The spike which had been in the emerging stage at the time of carbofuran application in June had come to maturity towards December. The mature spikes from all sides of the plant and from the upper middle and lower portions of the vine were collected. The spike samples so collected were mixed together to get a representative sample for each vine. Spikes for the 2 treatments were collected at 51, 60 and 76 days after application of carbofuran in NE monsoon season. The berries were carefully detached from the rachis prior to extraction.

3.7 Analysis of soil for carbofuran and its metabolites

3.7.1 Extraction of soil samples

The extraction of soil samples was done as per the method outlined by Venkateswarlu *et al.* (1977). Twenty gram portions of the previously sieved fresh soil were transferred to flat bottomed conical flasks. Fifty milli litre portions of chloroform-diethyl ether (1:1) mixture were added and the contents of the flask were shaken on a wrist action shaker for 30 minutes. The solvent mixture was carefully transferred to centrifuge tubes and centrifuged for 10 minutes at 7000 rpm. After centrifugation, the supernatant liquid was poured to a 250 mL beaker. This was repeated two more times with 40 mL of chloroform-ether (1:1) mixture and the combined extract was allowed to evaporate. The residues in the beaker were dissolved in 2 mL methanol and stored at 4°C in small vials for estimation after separation by thin layer chromatography (tlc).

3.7.2 Estimation by thin layer chromatography (tlc)

The residues extracted by organic solvent were separated by tlc (Siddaramappa *et al.*, 1979). Thirty grams of silica gel G taken in flat bottomed flasks were shaken with 60 mL of distilled water for two minutes and the slurry was spread on glass plates of 20 cm x 20 cm size by means of an applicator (Camag, Germany) to provide 300 μ thick silica gel layer. The plates were allowed to set for three hours at room temperature and then activated by keeping in an air oven maintained at 100°C for an hour and stored.

The methanol extracts were spotted on silica gel coated plates along with authentic compounds of carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran. The plates were developed with ether-hexane (3:1) solvent system for a distance of 15 cm and then air dried. The authentic compounds were detected with a chromogenic spray consisting of 2N NaOH in absolute methyl alcohol followed by a spray reagent composed of 5 mg paranitrobenzene-diazonium tetra fluoroborate in 25 mL absolute methyl alcohol plus 25 mL diethyl ether. The spots were pink in colour against a white back ground (Archer, 1976). Silica gel areas opposite to authentic compounds were scraped off into centrifuge tubes carefully. Carbofuran compounds in the silica gel was allowed to react with 1.25 mL of 0.3 per cent sodium nitrate solution, 1.25 mL of 0.2 per cent sulfanilic acid in 1N HCl and 2.5 mL 4N NaOH in boiling water bath for 20 minutes. After cooling, silica gel was removed by centrifugation at 5000 rpm and the colour intensity of the supernatant was measured at 490 nm in a Spectronic 20 spectrophotometer (Bausch and Lomb, USA). From the absorbance values obtained, the concentration of the residues and the metabolites were calculated based on the standard curve prepared using standard compounds. In the case of pot culture experiment using ^{14}C-labelled carbofuran radioassay of extract was done to estimate the residue contents.

3.7.2.1 Preparation of standard curve

A primary stock solution of 1000 μg mL^{-1} carbofuran in methanol was prepared. From this 0, 10, 20, 30, 40, 50, 60, 80 and 100 μL were spotted separately on silica gel coated plates along with standard carbofuran. Carbofuran content of the spots was estimated following the method described in 3.7.2. The spots gave final concentrations of 0, 2, 4, 6, 8, 10, 12, 16 and 20 μg mL^{-1} respectively. The absorbance of these standard concentrations were plotted on a graph paper so as to get a straight line.

In the case of 3-hydroxycarbofuran and 3-ketocarbofuran primary stock solutions of 500 μg mL^{-1} were prepared using analytical grade 3-hydroxycarbofuran and 3-ketocarbofuran. using the above stock solutions, 0, 20, 40, 80 and 100 μL were spotted on silica coated plates separately so as to give 0, 2, 4, 8 and 10 μg mL^{-1}. The absorbance of these standards were plotted on a graph paper to get the calibration graph.

3.8 Analysis of pesticide residues in plant samples

3.8.1 Extraction of shoot and estimation of carbofuran residues

The stem and leaf portions were extracted as per the method outlined by Siddaramappa and Watanabe (1979). Twenty five grams each of the fresh stem and leaf portions were weighed out into 250 mL flat bottomed flasks after chopping into bits. The plant parts were digested with 150 mL of 0.25<u>N</u> HCl for 1 hour while refluxing. Cooled to room temperature and filtered through glass wool. The walls of the condensor and filter cake were rinsed with additional portions of 0.25N HCl. Sodium chloride 5 g was added to the filtrate to check formation of emulsion. The above filtrate was transferred to a 500 mL separating funnel and added aqueous sodium lauryl sulphate solution. This was done to break the emulsions present if any. Forty millilitres of methylene chloride (dichloromethane) were added to the separating funnel and shaken for 2 minutes. Then the lower layer of methylene chloride was collected in a 250 mL beaker by passing through a funnel containing anhydrous sodium sulphate and activated charcoal over cotton to remove moisture and coloured substances present. The extraction was repeated with 40 mL each of methylene chloride twice and the lower methylene chloride layer was collected into the beaker. The combined extract was dried, residue redissolved and made up to 3 mL with methanol and stored in small bottles at 4°C in refrigerator until use.

The carbofuran residues of these samples were estimated by tlc method described under Section 3.7.2.

3.8.2 Extraction of berry and estimation of residues

The fresh berries immediately after harvest were used for extraction. Prior to extraction, the berries were smashed in a mixer. Fifty grams of smashed berries from each treatment were hydrolysed with 0.25N HCl and extracted in methylene chloride as described in Section 3.8.1.

3.8.3 Radioassay

The extraction of carbofuran residues from plants (stem and leaves) with 0.25 N HCl and then with methylene chloride was essentially the same as described earlier (section 3.8.1). The methylene chloride extract was evaporated off to dryness, and the residues were again dissolved in 3 mL methanol. A suitable aliquot (0.1 mL) of methanol extract was mixed with 15 mL of liquid scintillator (PPO, 4g; POPOP, 0.2g; methanol 100 mL; ethylene glycol, 20 mL and 1,4-dioxane to make up to 1.0 L) and measured the radioactivity in a liquid scintillation counter (Pharmacia LKB Wallac OY, Finland).

Radioactivity of the aqueous layer after extraction of the HCl digests of both soil and plant was negligible and hence not reported.

3.8.4 Estimation of bound residues (unextractable) in the shoot and root

The residues of stem and leaf obtained after filtration of HCl digest of the radioactive plant samples of Section 3.5.1 were used for this estimation. The residues were carefully removed from the funnel and dried at 40°C in an oven. For the determination of the unextractable plant bound (^{14}C) carbofuran residues, 0.1 to 0.2 g dried stem and leaves were combusted in a biological sample oxidiser (0X 500 R.J. Harvey Instrument Corporation, New Jersey) and the $^{14}CO_2$ evolved by combustion of the crop residues was trapped in 15 mL of Cocktail solution (M/s. Harvey Carbon-14 Cocktail) in a scintillation vial. The radioactivity trapped was determined in a liquid scintillation system (Pharmacia LKB Wallac OY, Finland). The operating conditions of the biological material oxidiser were : N_2 flow rate, 350 mL min^{-1}; O_2 flow rate, 350 mL min^{-1}; the combustion zone temperature, 900°C and combustion time, 2 min.

The roots were not subjected to HCl and solvent extraction. The oven dried (70°C) roots (0.1-0.2 g) were analysed directly in the biological oxidiser as explained above and the radioactivity was determined.

3.9 Recovery of carbofuran from soil and plant samples

Fresh, uncontaminated soil, leaf and berry samples were used for estimating efficiency of extraction and recovery of the residues.

Spiking or fortification of the samples were done by adding 10 $\mu g\ g^{-1}$ of carbofuran and hydroxycarbofuran separately into conical flasks containing soil or chopped plant material and keeping overnight. After this the residues were estimated adopting the methods described earlier for residue analysis of the soil, leaf and berry samples.

3.10 Statistical analysis

The data relating to experiment 3 was statistically analysed applying the analysis of variance for completely randomised design. In view of the vide variability of the radioactive counts (dpm) the data were subjected to log transformation after converting the dpm values to ng of carbofuran.

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

Sorption of carbofuran and its leaching in soil, its persistence and degradation in soil, absorption of soil-applied carbofuran by pot-grown and field-grown black pepper vines and its translocation in the plant system have been studied. The results are presented and discussed in this section.

4.1 Sorption of carbofuran in laterite soil

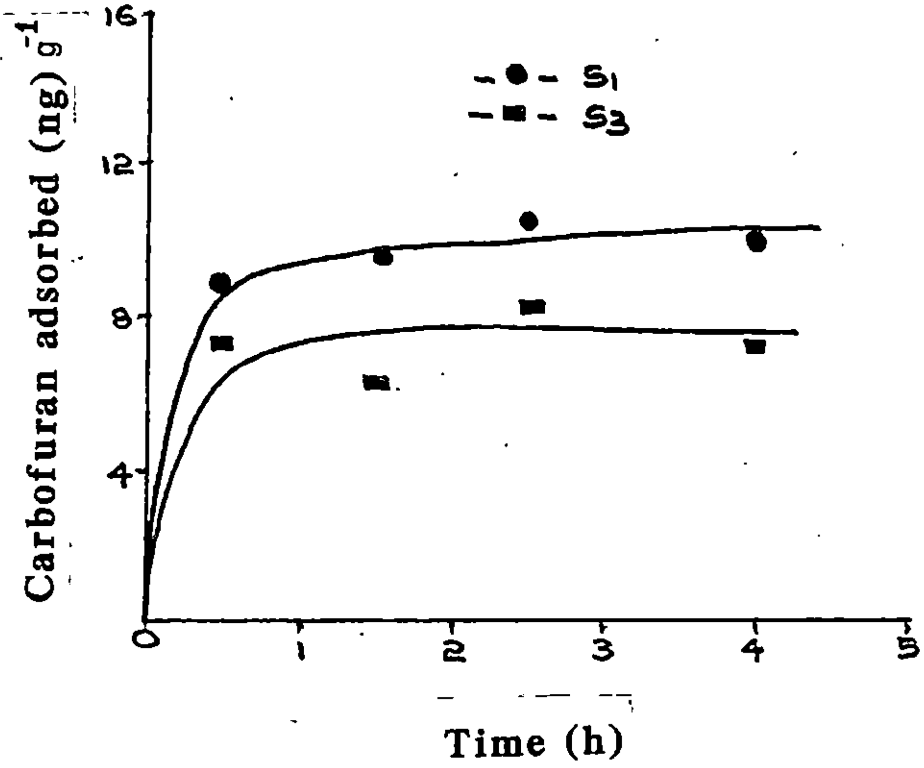
4.1.1 Kinetics of carbofuran adsorption

Kinetics of carbofuran adsorption was studied in two soils namely, S_1 and S_3 with widely varying organic matter contents (Fig. 2). S_1 had an organic matter content of 4.21 and S_3 had an organic matter content of 0.96 per cent (Table 5). Irrespective of the organic matter contents, the rate of adsorption was more or less the same. The sorption was nearly complete within about half an hour; beyond which there was only marginal increase in adsorption. Out of the 94.5 ng applied, S_1 adsorbed 8.93 and S_3 adsorbed 7.20 ng g^{-1} soil within 30 min. At the end of 4 h, S_1 adsorbed 10.42 and S_3 adsorbed 7.25 ng of the applied pesticide. The quantities of carbofuran adsorbed by these soils at the end of four hour equilibration period amounted to 11.02 and 7.67 per cent respectively.

4.1.2 Adsorption isotherm

The sorption pattern of carbofuran was examined in two soils S_1 and S_2. The data generated from the experiment were examined for their goodness of fit

Fig.2. Kinetics of carbofuran sorption in soil



with the Freundlich adsorption equation,

$$x/m = KC^n$$

where x is the quantity in ng of insecticide adsorbed on soil mass m in g; C is the concentration of insecticide in the equilibrium solution (ng mL^{-1}) and K and n are constants. Taking logarithm, the equation may be written as

$$\log \frac{x}{m} = \log K + n \log C$$

The adsorption isotherms of carbofuran for the two soils are presented in Fig.3. The Freundlich's adsorption isotherm constants are given in Table 6. These constants are computed by subjecting the sorption data to statistical method of least squares. Very high coefficients of determination (R^2) were obtained for the two soils indicating a very good fit of the data to the exponential Freundlich equation. The R^2 values were 0.88 and 0.96 for S_1 and S_2 which accounted for 88 and 96 per cent variations respectively in these soils.

Many workers have used Freundlich equation for describing sorption of several pesticides in soils (Hance, 1965, Bailey *et al.*, 1968; Chopra *et al.*, 1970; Mithyantha, 1973; Rajukkannu, 1978; Wahid and Sethunathan, 1978; Kumari and Singh, 1992). This model has been successfully used to describe the sorption of carbofuran also (Jamet and Piedallu, 1975; Singh *et al.*, 1990; Achik *et al.*, 1991a; Kumari and Singh, 1992). The results of the present study also corroborate this. The Freundlich constant, K, is a measure of the extent of adsorption on a comparable scale as it provides the quantity of pesticide adsorbed at an equilibrium concentration of 1 ng mL^{-1} in this case. Comparison of K values indicated that adsorption was

Fig.3. Freundlich isotherm for the adsorption of carbofuran in laterite soil

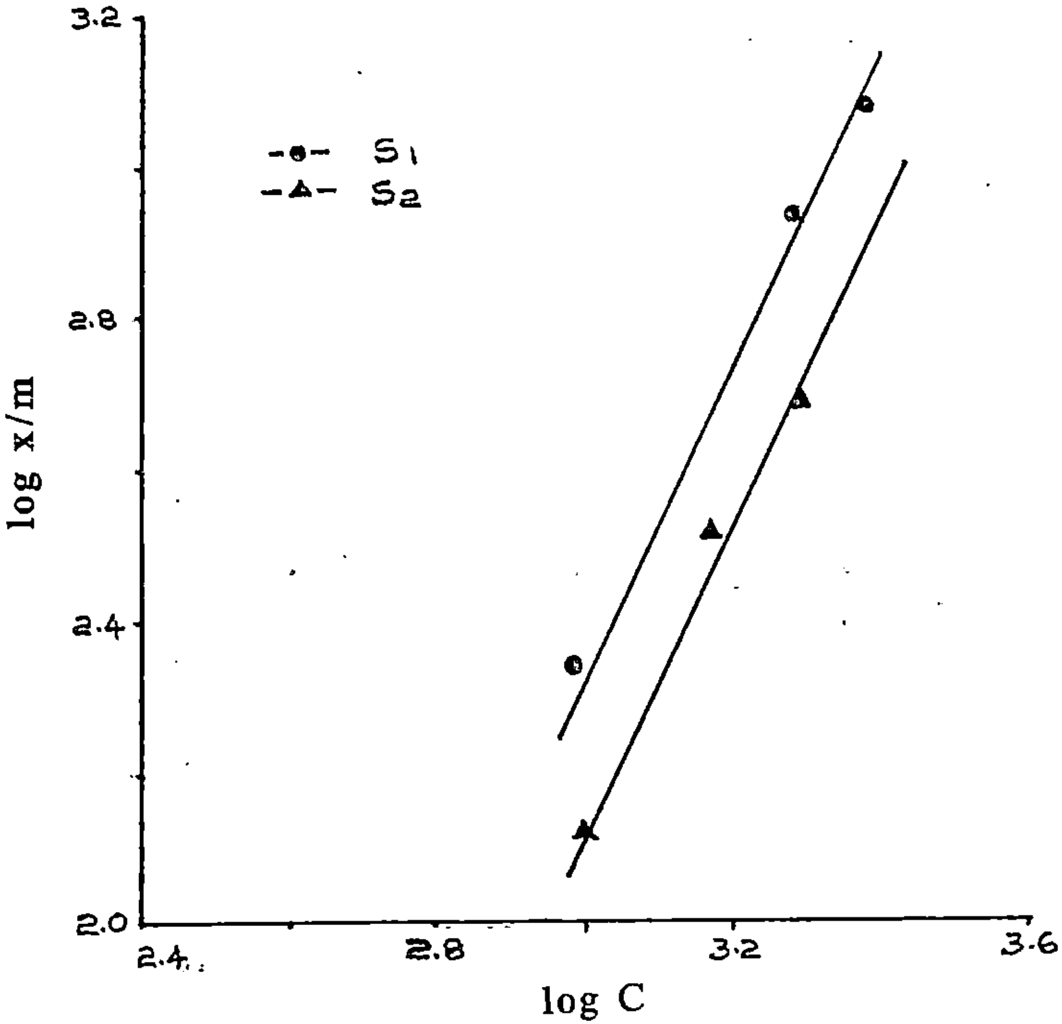


Table 6. Freundlich isotherm constants for the adsorption of carbofuran in laterite soils

Soil no.	K	n	R^2
S_1	2.51×10^{-4}	1.98	0.88
S_2	1.174×10^{-4}	2.02	0.96

more in S_1 than in S_2. The values of n for the two soils are very much similar and hence the isotherms run almost parallel to each other (Fig.3).

The variations observed in the magnitude of adsorption by the two soils appears to be due to the differences in their organic matter content (Table 5). A comparison of the sorption data presented in Fig.2 as well as that presented in Fig.3 support this. S_1 had higher organic matter content than S_3 and hence it adsorbed more carbofuran than S_3 (Fig.2). Similarly S_1 registered greater adsorption of the pesticide than S_2 whose organic matter status was less than that of S_1 (Fig.3). The influence of soil organic matter content on the sorption of several pesticides is well documented (Weber, 1970; Mithyantha, 1973; Rajukkannu, 1978; Wahid and Sethunathan, 1978; Honora, 1981). In the case of carbofuran also, a few reports are available on the importance of organic matter in enhancing its sorption in soil (Jamet and Piedallu, 1975; Felsot and Wilson, 1980; Singh *et al.*, 1990). It may however be pointed out that the differences in the quantities of carbofuran adsorbed by the soils are very small (Figs. 2 and 3). The magnitude of adsorption may also be compared based on K values (Table 6). In either case, only marginal differences were seen in the amounts of carbofuran adsorbed despite a substantial difference in the organic matter content of the soils. Achik *et al.* (1991a) reported K values of 0.478 and 0.629 $\mu g\ g^{-1}$ soil for carbofuran adsorption in a loamy clay soil and a clay soil respectively at an equilibrium concentration of 1 $\mu g\ g^{-1}$. These soils had 1.6 and 2.5 per cent organic carbon respectively. Comparing the extent of adsorption of three pesticides viz., methyl parathion, fenitrothion and carbofuran, Singh *et al.* (1990) observed that lowest adsorption in soil occurred for carbofuran compared to the other two pesticides. This was also the case with soils rich in organic matter. Very low adsorption of carbofuran in soil as observed in the present study as well as by others (Singh *et al.*, 1990; Achik et al., 1991b) could be due to the high water solubility (700 ppm) of the pesticide (Somasundaram *et al.*, 1991; Windholz *et al.*, 1983). For neutral organic molecules, strong inverse relationship between water solubility and extent of soil sorption had been demonstrated (Somasundaram *et al.*, 1991). Besides water solubility, the octanol/water partitioning coefficient (Kow) was also found to be highly correlated with sorption of pesticides in soil (Singh *et al.*, 1990; Somasundaram *et al.*, 1991). Higher the Kow values, lower will be the sorption of the chemical in the soil. The Kow value of carbofuran is 207 as against 5754 for a relatively non-polar compound parathion. The latter is adsorbed in soil to a greater extent. Wahid and Sethunathan (1978) reported Freundlich K values of parathion sorption as 12.3 and 38.0 μg at an equilibrium concentration of 1 μg mL^{-1} in soils with 1.62 and 2.88 per cent organic matter respectively. Although organic matter has been implicated in the sorption of carbofuran in soils as observed in this study as well as by previous workers (Edwards, 1964; Rajukkannu, 1978; Honora, 1981) the magnitude of its influence is not considerable as is reflected in the difference in the quantities adsorbed by soils with low and high organic matter contents. Carbofuran being more polar, it may not have affinity for adsorption on hydrophobic sites such as lipids and waxes found in the soil organic constituent. Conversely, it may have to compete with water molecules for sorption sites in the soil. Such an explanation can be advanced from the fact that adsorption of less polar compounds on soils takes place mainly on the hydrophobic sites for which water molecules do not compete (Walker and Crawford, 1968).

4.2 Persistence and degradation of carbofuran in soil

Persistence and degradation of carbofuran in four soils *viz.*, S_1, S_2, S_3 and S_4 were studied in laboratory incubation experiments. The organic matter and clay contents of these soils are presented in Table 5. The quantity of carbofuran applied to the soil was 140 µg. The amounts of carbofuran remaining in the soil at different intervals of incubation were determined. The data are presented in Table 7 and the degradation kinetics is illustrated in Figs. 4 to 7. The initial recovery of the applied carbofuran (time zero) was found to be 95 per cent. It is evident from the figure that the applied carbofuran remained as such up to 20 days in S_1 and 30 days in S_2, S_3 and S_4. Reduction in the quantity of carbofuran remaining in the soil was seen from subsequent intervals. In S_1, carbofuran residue decreased to 2.50 $\mu g\ g^{-1}$ on 75th day of incubation. This would mean that following the commencement of degradation, it took nearly 55 days for attaining this level. In the other 3 soils *viz.*, S_2, S_3 and S_4 the residue levels at the end of the experiment were 2.29, 2.32 and 3.50 $\mu g\ g^{-1}$, respectively.

Decomposition of carbofuran was reported to proceed both by chemical and microbiological means. Getzin (1973) found that chemical hydrolysis was the most important mechanism of degradation in alkaline soils whereas in both acid and neutral soils, chemical as well as microbial processes were involved. Williams *et al.* (1976) and Munnecke *et al.* (1982) also reported microbial degradation to be an important mode of carbofuran breakdown in soil especially in non-alkaline soils. The soils under study are acidic in reaction with pH ranging from 5.1

Table 7. Quantities of carbofuran and hydroxycarbofuran ($\mu g\ g^{-1}$) remaining in the soils at different intervals of incubation

Soil no.	Days after application of carbofuran										
	Carbofuran									3-Hydroxy carbofuran	
	0	5	15	20	25	30	45	60	75	45	60
S_1	6.67	6.17	6.34	6.34	6.00	4.67	3.43	3.50	2.50	1.65	3.57
S_2	6.56	6.56	6.83	6.84	6.79	6.75	6.13	2.57	2.29	0.86	4.22
S_3	6.58	6.84	6.17	6.33	6.50	6.50	3.07	2.32	NA	1.65	2.93
S_4	6.50	6.75	6.75	6.33	6.58	6.83	5.56	NA	3.50	2.77	2.57

NA - Not analysed

4.

Fig.4. Kinetics of carbofuran degradation in soil S_1

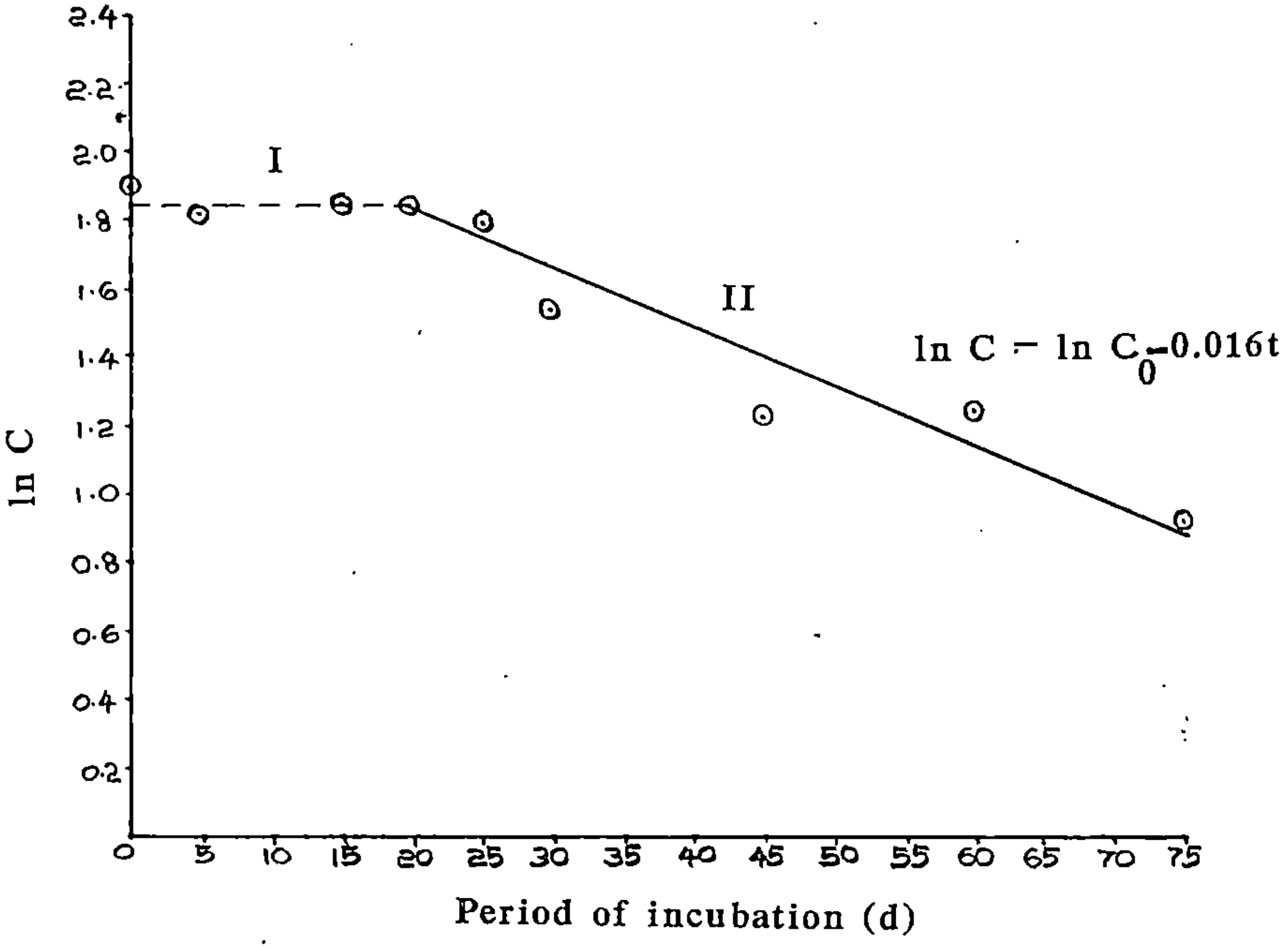


Fig.5. Kinetics of carbofuran degradation in soil S_2

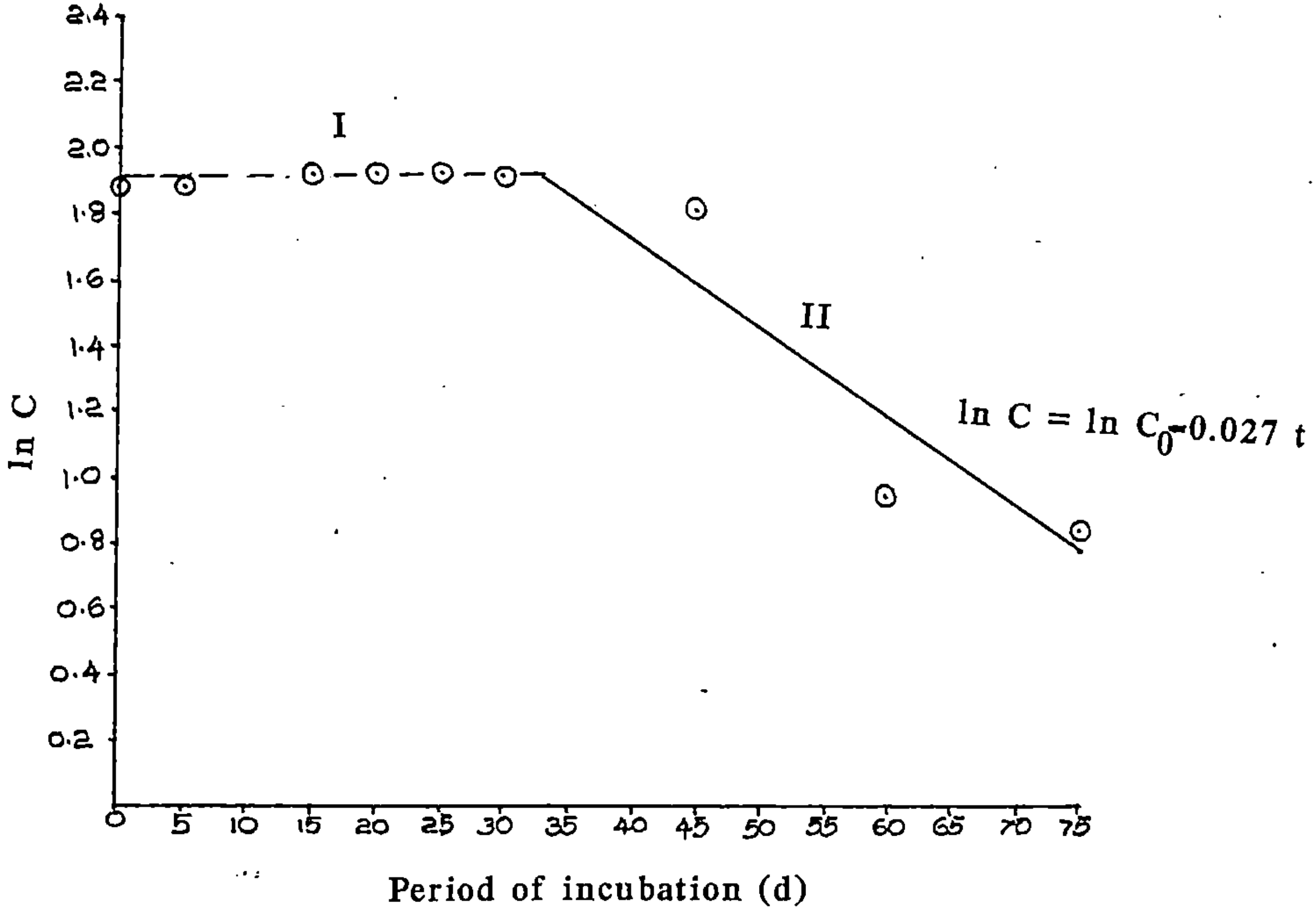


Fig.6. Kinetics of carbofuran degradation in soil S_3

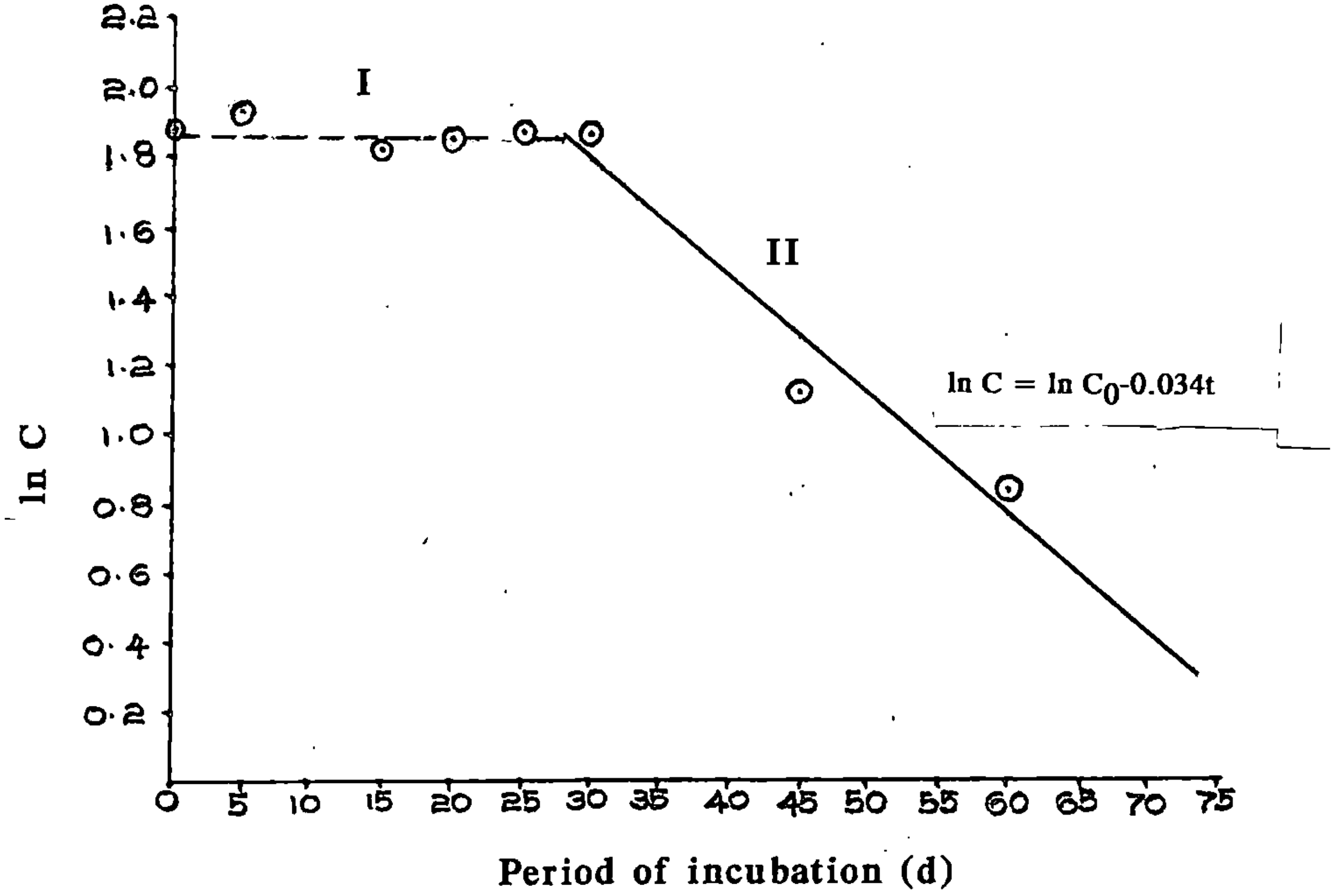
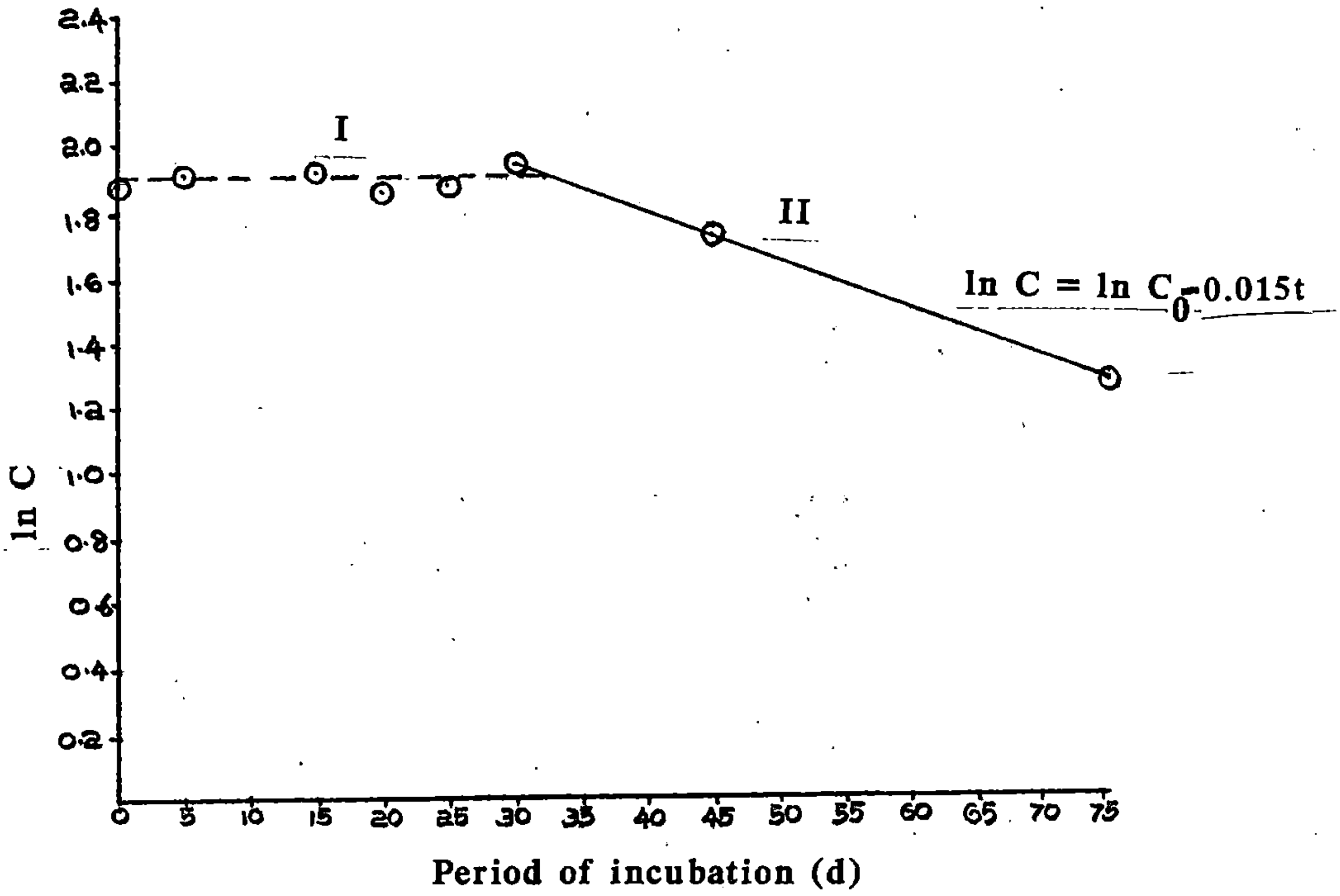


Fig.7. Kinetics of carbofuran degradation in soil S_4



to 5.3 (Table 4). Therefore, the degradation of carbofuran in these soils could be mediated by both chemical and microbial agents. Nevertheless the mechanism of degradation could be mainly microbial as could be deduced from the initial lag period. It may be noted that in all the soils, there was a long lag period extending up to 20 to 30 days before the commencement of the degradation of the pesticide. An initial lag phase before decomposition was also observed by Felsot *et al.* (1985) when carbofuran was applied to soil for the first time. Suett (1986) had also reported long lag periods before the decomposition of carbofuran under similar situations. Getzin and Shanks (1990) observed that carbofuran decay curve possessed an initial lag phase, where soil mixing enhanced insecticide decline. Moossa (1994) detected the degradation product of carbofuran after a period of 80 days following its application to soil. There are also reports of rapid degradation of carbofuran in soils which have a previous history of carbofuran application (Felsot *et al.*, 1982). This was attributed to the presence of micro organisms adapted to the soil contaminated with the insecticide (Read, 1983). Since the soils used in the present study did not have a history of carbofuran application, it would be reasonable to assume that organisms capable of degrading the compound are not active in these soils and hence a long lag period observed in this study is justified.

The data generated from the study are not directly amenable to kinetic modelling due to the presence of the lag period. In order to determine the magnitude of persistence following commencement of degradation, the plot of the data for each soil were split into two regions to represent the initial lag period (Region-I) and the degradation phase (Region-II). The data for Region-II were taken to study the kinetics of degradation. For this purpose, the data were tested for correspondence to the first order kinetic equation of the form:

$$C = C_0 e^{-kt}$$

where C is concentration of the insecticide ($\mu g\ g^{-1}$) remaining at time, t (days), C_0 is the initial concentration of the insecticide ($\mu g\ g^{-1}$) and k is degradation rate constant (d^{-1}). The first order rate constants describing the degradation of carbofuran for the four soils under study are given in Table 8. Very high coefficients of determination have been obtained for the use of first order rate equation for describing the degradation of the pesticide. This indicates that the degradation of carbofuran in the four soils tested followed first order kinetics and therefore it is possible to work out half-life for Region-II. The half-lives of the pesticides in these soils were worked out using the relationship,

$$t_{1/2} = \frac{0.693}{k}$$

The data are given in Table 8. The data revealed that the half-lives for the soils varied from 20.38 to 46.20 days.

Only one metabolite *viz.* 3-hydroxycarbofuran was detected. This compound was detected on 45th and 60th days of incubation (Table 7). The concentration of 3-hydroxycarbofuran ranged from 0.86 to 2.77 $\mu g\ g^{-1}$ soil on 45th day of incubation and from 2.93 to 4.22 $\mu g\ g^{-1}$ soil on 60th day.

The results revealed that parent compound, carbofuran, constituted the major portion of residues and 3-hydroxycarbofuran, the major metabolite. These results are in conformity with the findings of Lee *et al.* (1991) who found that of the total residues present in soils, the concentration of carbofuran was the highest

Table 8. First order rate constants for the degradation of carbofuran in laterite soils

Soil no.	K (d^{-1})	Half-life (d)	R^2
S_1	0.016	43.31	0.93
S_2	0.027	25.67	0.86
S_3	0.034	20.38	0.92
S_4	0.015	46.20	0.98

(69-74%) followed by that of 3-ketocarbofuran phenol (19-40%) and 3-hydroxycarbofuran (0.93-1.28%). The occurrence of carbofuran as the major residue in soil was also reported by Caro *et al.* (1973), Vasudevan (1985) and Singh and Kalra (1990). Among the metabolites, 3-hydroxycarbofuran ranked first in terms of its production followed by 3-ketocarbofuran and some other unknown metabolites (Vasudevan, 1985). Metcalf *et al.* (1968) reported five metabolites as the products of carbofuran degradation in soil. These were 7-phenol, 3-keto-7-phenol, 3-ketocarbofuran, 3-hydroxy-7-phenol and 3-hydroxy carbofuran. Although the metabolites of carbofuran are not as toxic as carbofuran (LD_{50}, 11 mg kg^{-1}) they still retain appreciable toxicity. The compounds, 3-hydroxycarbofuran and 3-ketocarbofuran have LD_{50} values of 17.9 and 69.0 mg kg^{-1} (Schoenig, 1967). The minor metabolites like 3-keto-7-phenol, 3-hydroxy-7-phenol and 7-phenol have comparatively higher LD_{50} values and are less toxic.

Several other workers have also found 3-ketocarbofuran and 3-hydroxycarbofuran as the major metabolites of carbofuran degradation in soil (Fuhremann and Lichtenstein, 1980; Lee *et al.*, 1991). The occurrence of these metabolites, especially 3-hydroxycarbofuran, the predominant metabolite observed in the present study which is relatively more toxic than to the other metabolites, assumes importance from the point of view of control of soil-dwelling nematodes using carbofuran.

4.3 . Absorption of soil-applied carbofuran by black pepper

Absorption of soil-applied carbofuran by black pepper vine was studied in pot culture. ^{14}C-labelled carbofuran was used in this study. There were three levels of carbofuran *viz.*, 0, 1 and 2 kg ai ha^{-1} and three soils namely, S_5, S_6 and

S_7. The specific activity of the applied carbofuran was 1074 dpm μg^{-1}. The actual quantities of carbofuran applied per pot (1 kg soil) were 480 μg and 960 μg for the lower (1 kg ai ha^{-1}) and higher (2 kg ai ha^{-1}) doses of application respectively. The radioactivity data (dpm $plant^{-1}$) relating to the absorption of carbofuran by the vine were first converted to ng $plant^{-1}$ based on the specific activity of the pesticide used. Since considerable variations were observed in the dpm values, the data were log transformed for normalisation prior to statistical analysis of variance (ANOVA).

4.3.1 Efficiency of extraction of carbofuran from plant samples

The extraction of the residues from plant samples namely leaf and stem was done by refluxing the samples with HCl (Siddaramappa *et al.*, 1979). This method was reported to yield 85-90 per cent recoveries of carbofuran (Ramanand *et al.*, 1988). The efficiency of extraction is generally evaluated based on the recovery of the compound following spiking the plant sample with known amounts of carbofuran. In such a case, the spiked pesticide has an independent existence as it is not incorporated into the organic matrix of the plant material. In the present study, the recovery of ^{14}C- labelled pesticide was determined in the HCl extract of the plant sample as well as in the residue left after extraction. The latter was done using a biological oxidiser. A comparison of the recoveries of radioactivity in HCl extract and in the plant residue left after HCl extraction is given in Table 9. Results indicated that the extraction of plant samples with HCl could not recover the plant radioactivity completely. In the case of stem, HCl extraction yielded about 12 per cent of the radioactivity contained in it. About 88 per cent of the radioactivity was still retained in the residues of stem samples after extraction which could be recovered following combustion in a biological oxidiser. The recovery of radio-

Table 9. Radioactivity recovered (%) from plant samples following HCl extraction and combustion of plant residues after extraction

Levels of carbofuran applied (kg ha^{-1})	Stem		Leaf	
	HCl extract	Combustion of plant residue	HCl extract	Combustion of plant residue
1	12.90	87.10	26.34	73.70
2	11.70	88.30	30.90	69.10

activity in the HCl extract of leaf was much more than from stem. The recovery ranged from 26.34 to 30.90 per cent while combustion of the leaf residue left after HCl extraction yielded recoveries from 69.10 to 73.70 per cent. The difference in the extent of recoveries between the two levels of applied carbofuran was, however, only marginal.

These results are suggestive of the inadequacy of the method of determining the efficiency of extraction of pesticide residues in plant samples following spiking or fortification of the samples with known quantities of the chemical. This method is generally adopted for determining the extraction efficiencies of pesticides in soil and plant samples (Cook *et al.*, 1969, Siddaramappa *et al.*, 1979, Lee and West Cott, 1983). The results of the present study also indicated differential efficiencies of extractions depending on the type of samples. The extraction efficiency is better for leaf than for stem samples as could be deduced from the fraction of the total radioactivity extracted from these plant parts (Tables 10 and 11). In view of the fact that major portion of the radioactivity was retained in the plant residues even after HCl extraction, it may be suggested that recoveries and efficiencies of extraction may be best estimated employing radiolabelled compounds in conjunction with combustion methods as followed in the present study.

4.3.2 Radioactivity recovered from different plant parts

The log-transformed data generated from the pot culture experiment as well as the retransformed values expressed as ng carbofuran equivalent plant^{-1} and as dpm plant^{-1} are given in parentheses in Tables 10, 11, 13 and 14.

Table 10. Radioactivity due to ^{14}C-label recovered in the methylene chloride extracts of the HCl digest

Level of carbofuran applied (kg ha^{-1})	Leaf	Stem
1	3.46 +(4300) ++(4619)	2.77 (719) (772)
2	3.92 (12395) (13312)	3.07 (1429) (1534)
SE±	0.082	0.062
CD (0:05)	0.24	0.18

\+ Denote log transformed data retransformed in ng carbofuran equivalent plant^{-1}

\+\+ Denote retransformed data in dpm plant^{-1}

Table 11. Radioactivity due to ^{14}C-label recovered from the combustion of plant residues left after HCl digestion

Level of carbofuran applied (kg ha^{-1})	Leaf	Stem
1	3.99 +(12028) ++(12918)	3.59 (458.8) (5217)
2	4.34 (27767) (29822)	3.89 (10788) (11586)
SE±	0.048	0.047
CD (0.05)	0.14	0.14

\+ Denote log transformed data retransformed in ng carbofuran equivalent $plant^{-1}$

++ Denote retransformed data in dpm $plant^{-1}$

4.3.2.1 Radioactivity in the solvent extracts of plant

The results indicated that the solvent extractable ^{14}C in the HCl digest of the leaves was more than that of the stem (Table 10). With increasing level of applied carbofuran, the extracted ^{14}C label also increased. The carbofuran-derived ^{14}C contents in the leaf were 4619 and 13312 dpm plant^{-1} for the lower and higher levels of application respectively. The radiocarbon contents of the stem for these two levels of application were 772 and 1534 dpm plant^{-1}. The increase in radioactivity with increase in the applied level of the chemical was about three times in the case of leaf and about twice in the case of stem. Talekar *et al.* (1977) reported that among plant parts, leaves had the highest concentration of radiocarbon derived from ^{14}C-labelled carbofuran applied to mung bean and soybean. Siddaramappa *et al.* (1979) obtained similar results in rice and they attributed it to the rapid upward movement of the insecticide following absorption by the roots. Sahu and Agnihotri (1983) and Vasudevan (1985) observed that residues of carbofuran accumulated mainly in leaf in maize and sorghum. The high water solubility (700 ppm) of carbofuran and its metabolites, 3-hydroxy and 3-keto carbofuran and consequent rapid translocation through the transpiration stream was responsible for the accumulation of insecticide in leaves (Vasudevan. 1985).

4.3.2.2 Radioactivity in the plant residues left after HCl digestion (bound residues)

As already mentioned, the recovery of radioactivity from the plant tissues by HCl digestion was not complete as the combustion of residues left after HCl digestion in a biological oxidiser yielded much more radioactivity than that could be extracted chemically (Table 9). The presence of non-extractable residues from ^{14}C-carbofuran has been reported by a few workers who used combustion methods using biological oxidiser (Fuhremann and Lichtenstein, 1980; Vasudevan, 1985; Somasundaram *et al.*, 1989; Lee *et al.*, 1991) for determining the bound residues. The radioactivity recovered from combustion of plant residues left after HCl digestion also indicated slightly larger values for leaf than for stem (Table 11). This variation is negligible as compared to that of HCl extraction (Table 10). In this case also greater recovery was observed at the higher dose of carbofuran application. In the leaf, the radioactivity increased from 12918 to 29822 dpm plant^{-1} as the level of carbofuran application increased from 1 to 2 kg ai ha^{-1}. For the stem, the corresponding values were 5217 and 11586 dpm plant^{-1}, respectively.

Pesticide residues absorbed and translocated in plant tissues may be present in the form of free residues, extractable conjugates bound to natural compounds of plants and unextractable or bound residues incorporated into the plant constituents. According to Dorough (1976), residues of pesticide remaining in the cropmarc after exhaustive solvent extraction can be considered to be bound. Khan *et al.* (1984) reported that when ^{14}C-carbofuran was applied to radishes and edible portions were exhaustively extracted with solvents after 21 days of application, the amount of bound residues was 92.6 per cent. Lee *et al.* (1991) reported an extraction efficiency of 52 per cent for HCl extraction of carbofuran residues indicating a high percentage of nonextractable residues. The results of the present study also indicated a greater portion of carbofuran-derived ^{14}C in the bound form (plant residues left after HCl extraction) in the stem (about 88 per cent) and in the leaf (about 70 per cent) (Table 9). Vasudevan (1985) opined that the bound residue might be due to the incorporation of ^{14}C carbofuran degradation products into insoluble lignin and cellulose fraction. Although interest in bound and conjugated residues has been increasing, relatively little information is available at present to evaluate the hazards they may pose. Information on the chemical forms and quantities of bound residues which may be present in foods is necessary for evaluation of the risk of toxicity, if any, arising from these compounds.

4.3.2.3 Radioactivity recovered from shoot

The combined radioactivity recovered from the HCl extracts following methylene chloride extraction and the combustion of residues of the acid digestion of stem and leaf portions constituted the radioactivity content of the shoot. The partitioning of the total radioactivity in stem and leaf as percentage of total radioactivity of shoot is presented in Table 12. The percentage of radioactivity in the leaves ranged from 72.6 to 75.9 at the lower level of carbofuran application. At the higher level of carbofuran application the values ranged between 75.4 to 80.7. In stem, the radioactivity recovered ranged from 24.1 to 27.4 per cent at the lower level of carbofuran application and from 19.3 to 24.6 per cent at the higher level of application.

4.3.2.4 Radioactivity recovered from roots

Roots were not subjected to hydrochloric acid digestion and extraction with dichloromethane but were directly combusted in the biological oxidiser as the radioactivity content was very less (Table 13). The ^{14}C contents of roots obtained for carbofuran application rates of 1 and 2 kg ai ha^{-1} were 2710 and 4541 dpm plant^{-1} respectively. Lee *et al.* (1991) also reported very low radioactivity in the roots of rice seedlings grown in soils labelled with ^{14}C-carbofuran. Ashworth and Sheets (1972) stated that carbofuran did not accumulate in tobacco roots when grown in culture solutions containing ^{14}C-carbofuran. Moreover, carbofuran in the

Table 12. Percentage radioactivity recovered from the leaf and stem following HCl extraction and combustion of residues of HCl digest

Level of carbofuran applied (kg ha^{-1})	Leaf			Stem		
	S_5	S_6	S_7	S_5	S_6	S_7
1	73.9	75.9	72.6	26.0	24.1	27.4
2	75.8	75.4	80.7	24.2	24.6	19.3

Note: S_5, S_6 and S_7 denote soil numbers

Table 13. Radioactivity recovered from the combustion of the roots of black pepper vine

Level of carbofuran applied (kg ai ha^{-1})	Radioactivity in roots
1	3.27 +(2533) ++(2710)
2	3.55 (4244) (4541)
SEm±	0.042
CD (0.05)	0.13

\+ Denotes log transformed data retransformed in ng carbofuran equivalent plant^{-1}
++ Denote retransformed data in dpm plant^{-1}

root declined to negligible levels as the compound was depleted in the culture solution.

4.3.2.5 Total recovery of radioactivity in the plant

The total recovery of ^{14}C label in the plant which includes solvent extracted ^{14}C from the acid digests of leaf and stem, the amount of ^{14}C derived from combustion of residues of stem and leaf left after HCl digestion and also the radioactivity recovered from combustion of roots indicated that the quantity of ^{14}C at higher level of carbofuran application was nearly double of that recovered from lower level of carbofuran application; the values being 26225 and 60814 dpm plant^{-1} for 1 and 2 kg ai ha^{-1} respectively (Table 14).

4.3.3 Influence of soil organic matter on carbofuran absorption

The data presented in Table 14 also indicate the relative uptake of carbofuran from the three soils. The differential absorption of carbofuran by the vine from the three soils is very much evident from the data. The soil properties could have influenced the absorption of the insecticide. Among the three laterite soils, S_5 had the lowest content of organic matter (1.28%); S_6 had 2.45 per cent and S_7 had the highest (3.09%) organic matter content. The results indicated that the quantities of ^{14}C recovered from plants grown in these soils varied significantly. The radioactivity recovered from plants grown on S_5 was the highest (76127 dpm plant^{-1}) and that grown on S_7 was the lowest (23048 dpm plant^{-1}). The absorption from S_6 came in between (31383 dpm plant^{-1}). Since the absorption decreased with increasing organic matter content of the soil, it may be deduced that this parameter had influenced the absorption of the insecticide from the soil.

Table 14. Effect of organic matter on the absorption of soil applied ^{14}C-carbofuran by black pepper vine

Levels of carbofuran applied (kg ai ha^{-1})	S_5	S_6	S_7	Means
1	4.58	4.39	3.99	4.32
	+(37767)	(24888)	(10599)	(24418)
	++(40562)	(26730)	(11383)	(26225)
2	5.01	4.51	4.46	4.66
	(103997)	(33553)	(3232)	(56624)
	(111693)	(36036)	(34712)	(60814)
Means	4.79	4.45	4.22	
	(70882)	(29220)	(21460)	
	(76127)	(31383)	(23048)	

SEm± for organic matter levels : 0.053
SEm± for carbofuran levels : 0.043

CD (0.05) for comparison of levels of organic matter : 0.16
CD (0.05) for comparison of levels of carbofuran : 0.13

\+ Denote log transformed data retransformed in ng carbofuran equivalent plant^{-1}
++ Denote retransformed data in dpm plant $^{-1}$

S_5, S_6 and S_7 denote soil numbers

The sorption of carbofuran was found to increase with increasing organic matter (Figs. 2 and 3), suggesting that the concentration of the insecticide in the soil solution could be decreased. Consequently it may be expected that the bioavailability of carbofuran may be decreased with increasing organic matter content of the soil and as a result, its absorption by the plant also diminishes. These findings are in agreement with the findings of Fuhremann and Lichtenstein (1980) who reported that in soils with low organic matter contents, insecticide residues were more mobile and hence more susceptible to plant uptake than in soils with more organic matter. Rajukkannu (1978) described organic matter as a more potential adsorber of carbofuran than inorganic components. This fact also supported the lower uptake of carbofuran by plants grown in soils with high organic matter contents. It is also possible that with increasing organic matter content, the bound residues could also be increased rendering the insecticide unavailable to the plant. Lee *et al.* (1991) reported that soil bound residues of ^{14}C activity from labelled carbofuran in fulvic and humic acid fractions as well as in humin.

These results relating to the magnitude of bio-availability of the insecticide, its concentration in soil solution and its absorption by the vine from soils differing in organic matter content, are important from the practical point of view. It is to be expected from these results that the pesticide concentration in the soil solution and its availability to the vine as well as its biocidal action on the target pests could be reduced in soils rich in organic matter. Generally, black pepper vine is grown not only in homesteads but also on plantation scale in recently cleared forest areas. In such cases, the soils being unexploited and virgin, may have high organic matter status. The current recommendation of carbofuran as a nematicide in black pepper gardens does not take into account the organic matter content of the soil.

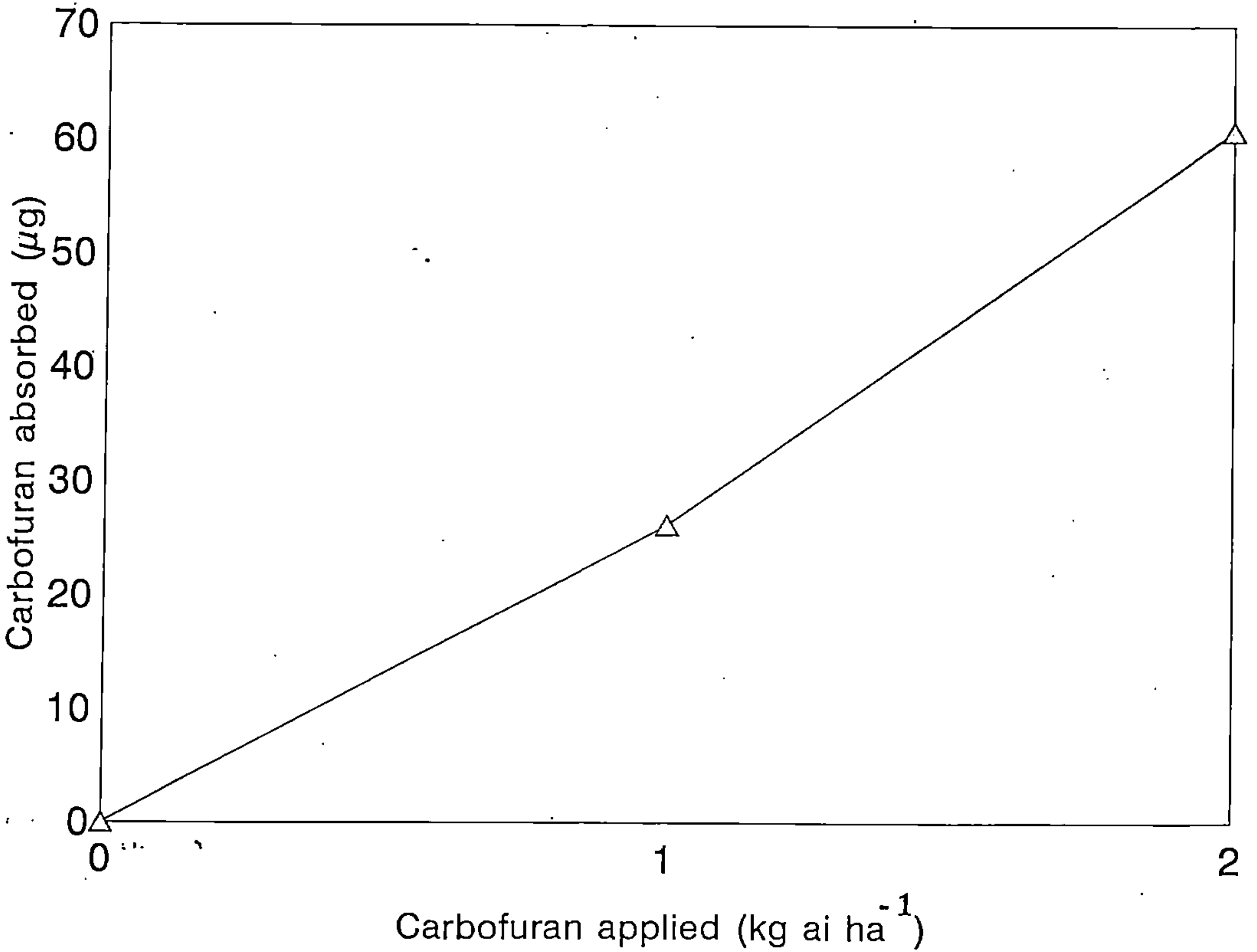
Based on the results obtained from this study, it may be stated that discriminatory application of carbofuran is required depending on the organic matter content of the soil for obtaining effective control of nematodes. In other words soils with less organic matter may need less quantity of carbofuran as compared to a soil with high organic matter content for achieving effective control of the pest. More studies are, however, needed to assess the pesticidal effect of carbofuran in relation to organic matter content of soil.

It is assumed that the original source of ^{14}C label in the plant system is the labelled carbofuran absorbed by it. It is based on this assumption that the absolute radioactivity data (dpm values) have been converted to carbofuran equivalents in ng plant^{-1} using the specific activity of the applied carbofuran and it is the data on carbofuran equivalents that are given in Tables 10, 11, 13 and 14. Going by the above reasoning it may be concluded that with increasing level of applied carbofuran from 1 to 2 kg ai ha^{-1} the black pepper vine absorbed 26.2 and 60.8 μg carbofuran from each pot (Fig. 8). The quantities of carbofuran equivalent to 1 and 2 kg ai ha^{-1} are 0.5 and 1 mg pot^{-1}. This would mean that the plant absorbed 5 per cent and 6 per cent of the applied carbofuran at the two rates of application respectively indicating that the absorption was more or less proportional to the applied quantity.

4.3.4 Autoradiography of thin layer chromatogram

Since the radioactivity in the solvent extracts of the different samples generated from this experiment was too low, they were pooled. The extracts of leaves were pooled for each soil and for each level to form separate samples. The pooled extracts were concentrated to 1 mL by evaporation at room temperature. The

Fig.8. Absorption of ^{14}C-labelled carbofuran by black pepper vine as influenced by the level of soil application



concentrated solution was used for tlc to identify the metabolites, if any. In the case of stem extracts this was not done since the radioactivity was still less.

The thin layer chromatograms were autoradiographed (Plate 3). Besides carbofuran, one metabolite *viz.*, 3-hydroxycarbofuran could also be detected in the autoradiogram . The partitioning of the radioactivity between the parent molecule and hydroxycarbofuran is given in Table 15. The data indicated that a greater part of ^{14}C label was accounted for by the metabolite hydroxycarbofuran compared to the parent molecule carbofuran. On an average, about 22 to 30 per cent of the radioactivity was due to hydroxycarbofuran and about 15 to 20 per cent due to carbofuran. These percentages were worked out with respect to the radioactivity contained in the extract used for tlc. The above results are in close agreement with the finding of Lee and West Cott (1983) who reported that in rapeseed plants, 3-hydroxycarbofuran was found in relatively higher amounts compared to carbofuran and 3-ketocarbofuran. This could be due to the production of 3-hydroxycarbofuran in plants presumably by hydroxylation at the benzylic carbon giving rise to 3-hydroxycarbofuran which subsequently could have conjugated, hydrolysed or oxidised to 3-ketocarbofuran. Fuhremann and Lichtenstein (1980) also found that the major constituent of organic soluble ^{14}C-carbofuran residues recovered in oat tops was 3-hydroxycarbofuran. Knaak *et al.* (1970) was of the opinion that rapeseed plants conjugated 3-hydroxycarbofuran thus slowing its degradation. The abundance of 3-hydroxycarbofuran as observed in the present study can be explained thus. Also 3-ketocarbofuran was not detected in the organic extracts of leaf. Ashworth and Sheets (1972) identified several carbofuran metabolites *viz.*, carbofuran phenol, 3-ketocarbofuran phenol, 3-ketocarbofuran, 3-hydroxycarbofuran phenol, 3-hydroxycarbofuran and certain unknown glycosidic

Plate 3. Thin layer autoradiogram of the extract of black pepper leaf showing ^{14}C-carbofuran and its metabolite, 3-hydroxycarbofuran

a) Carbofuran b) Hydroxycarbofuran

171189

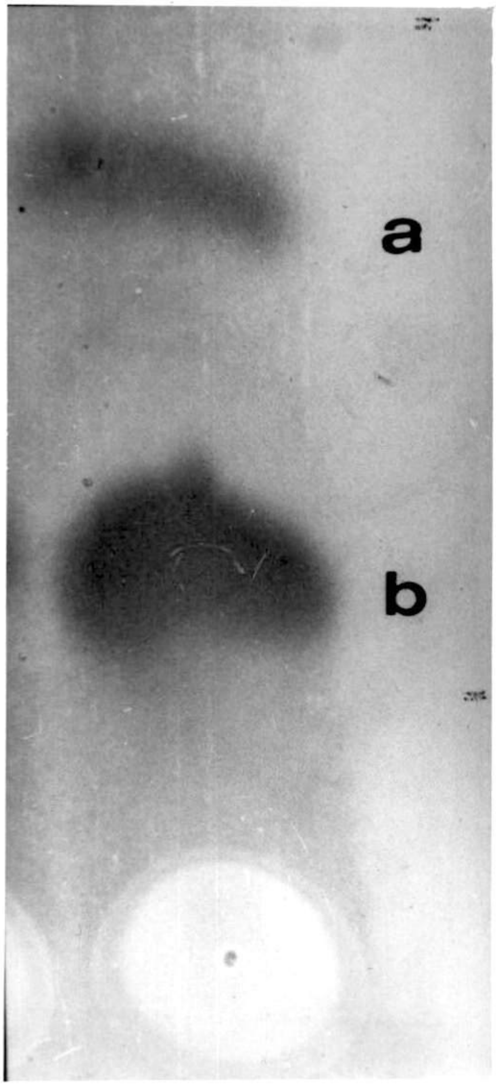


Table 15. Partitioning of the ^{14}C-label (dpm) in black pepper leaf following chemical extraction

Levels of carbofuran applied (kg ha^{-1})	Carbofuran			3-hydroxycarbofuran		
	S_5	S_6	S_7	S_5	S_6	S_7
1	443 (15.0)	324 (19.8)	NA	741 (25.1)	492.3 (30.1)	95.7 (22.4)
2	198.4 (19.0)	324.6 (18.9)	345.9 (17.5)	2396 (22.9)	508.4 (29.6)	467.9 (23.7)

Parentheses indicate percentages; S_5, S_6 and S_7 indicate soil numbers
NA : Not analysed

conjugates in tobacco plants. But the contents of 3-ketocarbofuran was very less and they concluded that the 3-ketocarbofuran produced was readily converted into its corresponding phenol and conjugated as glycosides. According to Metcalf *et al.* (1968) 3-keto carbofuran is hydrolytically unstable and it did not accumulate in large quantities in plants. They also reported that the very low levels of 3-ketocarbofuran in plants could have resulted from its conversion to corresponding phenol followed by conjugation.

As is evident from Table 15 only 40 to 50 per cent radioactivity was explained by carbofuran and its metabolite hydroxycarbofuran. The remaining 50 per cent of the radioactivity is unaccountable. The unaccounted radioactivity could be due to incorporation of radioactivity to other substances which were not separated by tlc and that carried by unknown compounds located at origin and/or distributed on the tlc plates (Ashworth and Sheets, 1972).

4.3.5 Autoradiography of black pepper vine

An attempt was made in the present study to use bush pepper for studying the distribution pattern of absorbed ^{14}C-carbofuran through autoradiography. Bush pepper is produced by rooting of the fruit bearing laterals. Generally the runner shoots are used for vegetative propagation of the vine. Since the vine takes two or more years to come to flowering and because two years' growth period would produce a bulky and voluminous plant, this plant material cannot be used for autoradiographic purpose. On the other hand, bush pepper (Plate 4) developed from the lateral shoot, is not a vine and comes to bearing within a short period. In view of its bushy nature, it can be conveniently used for autoradiographic purpose. In the present study such a bush pepper plant was allowed to absorb ^{14}C-carbofuran from

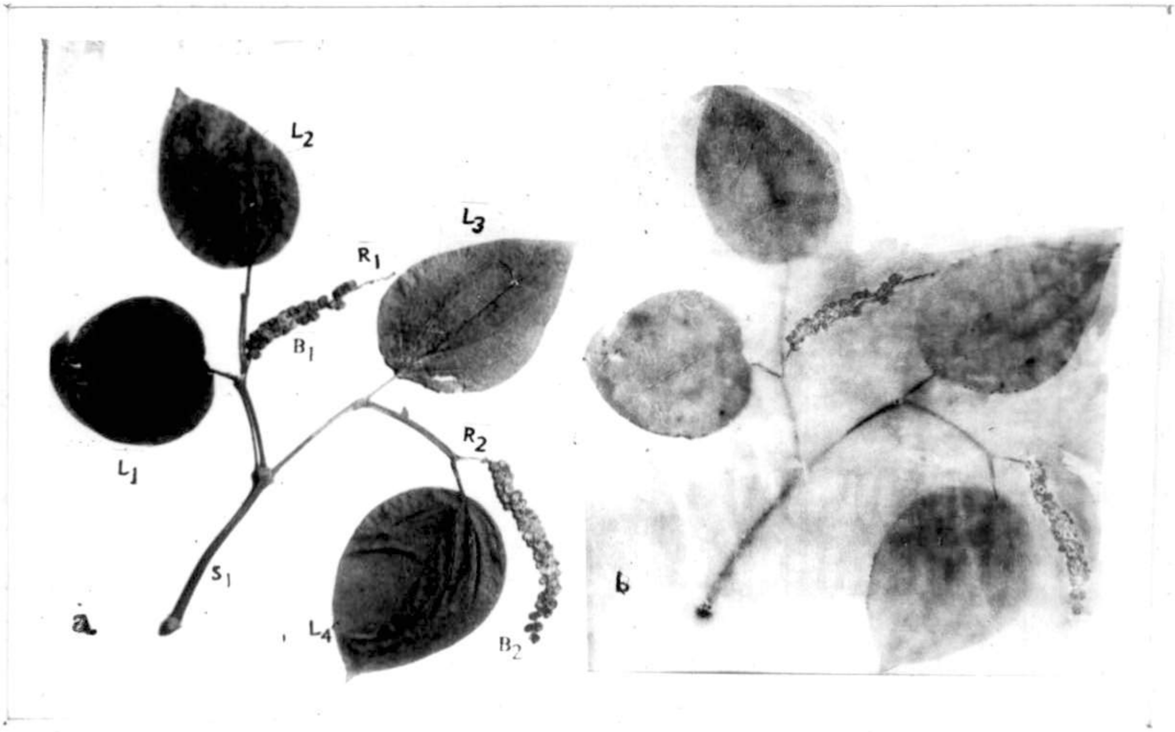
Plate 4. A bush pepper plant (var. Panniyoor-1)

an aqueous solution for 6 hours in a green house. The autoradiograph of the labelled plant showing the distribution of the ^{14}C-label is given in Plate 5 and the actual counts obtained for the various plant parts are given in Table 16. The data indicated that substantial absorption has occurred during the period of 6 h. The absorbed carbofuran was mainly translocated to the leaf and to a lesser extent to the berries also. The leaves contained 19.51 to 24.43 per cent of the total radioactivity in the plant. The stem also contained considerable radioactivity (40.86%). The percentage radioactivity distribution in the berries B_1 and B_2 were 0.95 and 0.85 respectively. Corresponding values for rachis were 0.72 and 0.54. A comparison of these data with those given in Tables 10 and 11 relating to the absorption of carbofuran by plants grown in pots revealed discrepancy in the relative distribution of carbofuran-derived radioactivity. While ^{14}C content was more in leaf than in stem in the case of pot-grown plants, it was just the reverse in the case of autoradiographed plants. This discrepancy may be due to the differences in the residence times of the absorbed insecticide in the plant system. Whereas the period of absorption and translocation was only 6 hours in the case of plants used for autoradiography, it was 60 days for the plants grown in pots. Consequently it could be expected that the absorbed ^{14}C-carbofuran in the case of autoradiographed plant was still in its transit and the stem showed a higher concentration of the radiolabel. In the case of the plant grown in pot, sufficient time has elapsed allowing a longer distribution period for the absorbed chemical. Further, in these plants, carbofuran had also undergone degradation and other metabolic changes and as a result redistribution of the compounds carrying ^{14}C-label might also have occurred. The data relating to the plants under pot culture should be viewed from this angle and must be considered to yield a more reasonable picture of the distribution while the autoradiographed plant

Plate 5. Autoradiograph showing the distribution of ^{14}C-carbofuran in bush pepper plant following its absorption through root

a) Photograph of the plant

b) Autoradiograph of the plant showing the distribution of absorbed ^{14}C - carbofuran


L2
L3
R1
B1
L1
R2
S1
a
L4
B2
b

123

Table 16. Partitioning of the absorbed ^{14}C-label from the applied ^{14}C-carbofuran in the autoradiographed bush pepper plant

Plant part	Part no.	Dry weight (mg)	Total counts (dpm)	Per cent distribution
Leaf	L_1	521.34	52283	24.43
	L_2	491.06	41749	19.51
	L_3	768.74	41865	19.57
	L_4	648.21	31105	14.54
Berry	B_1	444.50	2023	0.95
	B_2	668.97	1828	0.85
Rachis	R_1	86.02	1550	0.72
	R_2	80.07	1165	0.54
Stem	S_1	111.23	87426	40.86

yields the translocation and distribution of carbofuran immediately after its absorption by the root. One of the important results obtained from the autoradiography is that the absorbed carbofuran is readily translocated and accumulated in the berry which is the edible part of the plant.

4.3.6 Phytotonic effect of carbofuran on growth of black pepper

The plants used in pot culture experiment were used to assess the phytotonic effect due to carbofuran application. The growth of plant at the two levels *viz.*, L_1 (1 kg ai ha^{-1}) and L_2 (2 kg ai ha^{-1}) of soil application of carbofuran were compared with the growth of plants without carbofuran application (L_0). The dry weights of treated plants and controls are presented in Table 17. The data revealed that biomass production in carbofuran treated plants did not differ significantly from the control plants indicating that there is no phytotonic effect of carbofuran on black pepper.

4.4 Mobility of carbofuran in soil

This experiment was conducted to assess the mobility of carbofuran applied to the soil basin of black pepper vine in the field. The rate of application was the one recommended by the Kerala Agricultural University, *viz.*, 1 g ai $vine^{-1}$ applied during the South West and North East monsoons (KAU, 1995). The physico-chemical characteristics of the soil are presented in Table 18. There were two treatments which were designated as T_1 and T_2. In T_1, 1 g ai of carbofuran was applied to the soil basins of each vine at the onset of SW monsoon whereas in T_2, carbofuran was applied at the same rate at the onset of SW monsoon as well as at the onset of NE monsoon.

Table 17. Effect of soil-applied carbofuran on the growth of black pepper vine

Soil	Levels of carbofuran applied (kg ha^{-1})	Dry weight (g)				
		Stem	Leaf	Root	Shoot	Total
S_1	0	1.643	3.203	1.083	4.847	5.930
	1	1.825	3.023	0.597	4.848	5.830
	2	2.020	3.212	0.670	5.233	5.517
CD for comparison with control		NS	NS	NS	NS	NS
S_2	0	2.057	3.533	1.460	5.590	7.050
	1	2.143	3.834	0.685	5.985	6.670
	2	1.912	3.163	0.570	5.075	5.645
CD for comparison with control		NS	NS	0.319	NS	NS
S_3	0	1.697	3.373	1.283	5.070	6.353
	1	2.050	3.585	0.525	5.635	6.160
	2	1.757	3.337	0.570	5.045	5.665
CD for comparison with control		NS	NS	0.305	NS	NS

S_1, S_2 and S_3 indicate soil numbers

NS - Not significant
CD - ($P < 0.05$)

Table 18. Physico-chemical characteristics of the soil at the field experiment site

Depth of soil layer (cm)	pH	Organic carbon (%)	Cation exchange capacity (cmol(+) kg^{-1})	Free iron oxides (%)	Mechanical composition (%)	
0-10	6.4	2.30	10.71	5.49		
10-20	6.0	0.85	8.07	5.66		
20-30	4.9	0.83	8.05	5.95	Sand	62.48
30-40	5.8	0.79	6.75	5.50	Silt	11.66
40-50	5.4	0.75	6.89	6.44	Clay	25.86
50-60	6.3	0.67	6.09	6.53		

The data relating to the concentration of carbofuran remaining in the soil at different intervals following its application on 28-6-'94 are given in Table 19. Along with these data the cumulative rainfall received during each interval are also given. The experimental floor received a total rainfall of 3801.4 mm. The results indicated that the concentration of carbofuran on oven-dry soil basis declined sharply in the surface 10 cm soil layer within 10 days from 28.59 to 11.42 $\mu g\ g^{-1}$. Further decrease was more gradual and on 30th day the concentration had decreased to 1.85 $\mu g\ g^{-1}$. Although soils were analysed at day 76 and beyond, the concentrations were too low to be detected. In the 10-20 cm soil layer, though the initial concentration was high it remained more or less steady at 30 and 60 days, the values being 4.44 and 4.04 $\mu g\ g^{-1}$ respectively. In still lower layers viz. 20-30 and 30-40 cm, the residue levels decreased especially on the 30th and 60th day of application. In the 40-50 and 50-60 cm layers the concentrations ranged between 3.44 and 7.01 $\mu g\ g^{-1}$ and between 4.87 and 5.20 $\mu g\ g^{-1}$ respectively. These two layers showed considerable fluctuations and as such no general trend could be observed. Studies on the mobility of pesticides in the field are often beset with the problem of large variability. Studies conducted by Mojasevic et al (1992) on the mobility of several pesticides including carbofuran in soil also showed wide variations in the residue levels at lower depths primarily because of the low pesticide concentrations in the soil strata. Almost all the soil layers showed a gradual decline in the concentrations by 60th day. The data showed that considerable amount of leaching of carbofuran had taken place and as a result the chemical had reached 50 to 60 cm soil depths or even beyond it. This may be attributed to the high water solubility of carbofuran and relatively low adsorption on the soil.

Table 19. Dynamics of carbofuran content ($\mu g\ g^{-1}$) of soil following its application at the onset of SW monsoon

Soil depth (cm)	Days after carbofuran application			
	0	10	30	60
0-10	28.59	11.42	3.23	1.85
10-20		6.73	4.44	4.04
20-30		8.75	4.19	3.75
30-40		4.60	3.57	3.84
40-50		7.01	3.44	5.20
50-60		5.20	5.06	4.87
Rainfall (mm)		218.20	952.10	3801.40

The rainfall data indicated that during the period following application, the experimental floor received 3801.4 mm rainfall. Rainfall received during the initial 10 days was 218.2 mm. The sharp decline in the carbofuran concentration of the soil basin within the first 10 days could be due to intense leaching of the insecticide due to the heavy rainfall. Further leaching was not found to be corresponding to the amount of rainfall received. With decreasing concentration in the soil, the magnitude of leaching may also be expected to decrease in spite of the increase in the rainfall. Although 3801.4 mm rainfall was received in 60 days, the applied carbofuran was not totally leached away by the rainwater from the root zone of the vine. The most active root zone of the vine was reported to be lying within a soil column of 30 cm radius and 60 cm depth around the vine (Sankar *et al.*, 1988). The results indicated that the root zone soil retained carbofuran in the concentration range of about 3 to 5 $\mu g\ g^{-1}$ during 60 days following its application and this region is not totally depleted of the pesticide in spite of the heavy rains during this period.

4.4.2 Mobility of carbofuran consequent to application in NE monsoon

The data relating to the concentrations of carbofuran remaining in the different soil layers in the root zone of black pepper following second split application at the onset of NE monsoon are given in Table 20. The total amount of rainfall received during NE monsoon was 401.1 mm. The application was done on 11-10-'94. The concentrations of carbofuran in different soil layers were monitored for 31 days following its application. It may be mentioned that carbofuran residues due to the previous application in the SW monsoon season was negligible just prior to the application in NE monsoon and hence the concentration obtained on zero day

Table 20. Dynamics of carbofuran content ($\mu g.g^{-1}$) of soil following its application at the onset of SW and NE monsoons

Soil depth (cm)	Days after carbofuran application			
	0	11	21	31
0-10	25.81	4.51	4.71	4.99
10-20		3.99	3.78	4.46
20-30		3.87	3.26	3.76
30-40		3.58	2.84	4.96
40-50		2.65	2.05	3.60
50-60		3.19	NA	NA
Rainfall (mm)		110.40	275.80	401.10

(just after application) was only due to the current application. Immediately after application (0 day), the 0-10 cm soil layer had a concentration of 25.8 μg g^{-1} which decreased sharply to 4.51 μg g^{-1} on day 11. This layer maintained somewhat a steady concentration for a month, the values being 4.71 and 4.99 μg g^{-1} on day 21 and day 31 respectively. The initial sharp decrease may be attributed to both leaching and also degradation due to adapted microbial population consequent to previous application in SW monsoon. The carbofuran content in the 10-20 and 20-30 cm layers did not show much decrease. But in still lower layers (30-40 and 40-50 cm) the residues showed a slight decrease, though the deepest layer showed highly irregular values for the residue content.

In both SW and NE monsoon seasons considerable leaching of the applied carbofuran had occurred. A comparison of the data for the first 30 days after application indicated slightly greater retention of the insecticide in the root zone during the NE monsoon than in the SW monsoon. This is expected because of the higher rainfall received in the SW monsoon season than for the same period during NE monsoon, the values being 952.1 and 401.1 mm in the SW and NE monsoons respectively.

4.4.3 Mobility of hydroxycarbofuran

4.4.3.1 Mobility during SW monsoon season

Except for zero day of application, hydroxycarbofuran was detected in the soil at all subsequent sampling intervals during SW monsoon (Table 21). Consequent to application of the insecticide at the onset of SW monsoon period on 28-6-'94 the content of carbofuran on the 10th day was 6.80 μg g^{-1} in the surface layer (0-10 cm). The value decreased to 6.74 μg g^{-1} on the 30th day

Table 21. Dynamics of hydroxycarbofuran content ($\mu g\ g^{-1}$) of soil after application of carbofuran at the onset of SW monsoon

Soil depth (cm)	Days after carbofuran application		
	10	30	60
0-10	6.80	6.74	3.83
10-20	2.53	2.91	4.41
20-30	4.42	4.91	5.13
30-40	2.09	3.12	1.76
40-50	3.61	5.49	1.37
50-60	3.17	4.78	1.41
Rainfall (mm)	218.20	952.10	3801.40

and decreased sharply to 3.83 $\mu g\ g^{-1}$ on the 60th day. In all the sub-surface layers (20-60 cm), the concentration of 3-hydroxycarbofuran varied without any regular pattern. The concentration ranged from 4.42 to 5.13 $\mu g\ g^{-1}$ in 20-30 cm layer and from 1.76 to 3.12 $\mu g\ g^{-1}$ in the 30-40 cm layer. The corresponding values for the still lower layers ie. 40-50 cm and 50-60 cm are 1.37 to 5.49 $\mu g\ g^{-1}$ and 1.41 to 4.78 $\mu g\ g^{-1}$ respectively. The concentrations on 60th day for all the lower three layers were less than that on the 10th day due to leaching during heavy rains. The concentrations of hydroxycarbofuran in the soil during SW monsoon were lower than the corresponding values for carbofuran during the same period. This is an indication that the major portion of residues in soil is the parent compound carbofuran itself (Fuhremann and Lichtenstein, 1980; Vasudevan, 1985; Singh and Kalra, 1990).

4.4.3.2 Mobility of hydroxycarbofuran in NE monsoon

The second split of carbofuran was applied on 11-10-'94 at the onset of NE monsoon. The data relating to hydroxycarbofuran levels in soil are given in Table 22. The surface 0-10 cm layer showed an initial increase from 3.25 to 4.15 $\mu g\ g^{-1}$ which decreased later to 2.10 $\mu g\ g^{-1}$ by 31st day. The two adjacent lower layers (10-20 and 20-30 cm) also showed a similar trend. The 30-40 and 40-50 cm layers showed an initial decrease followed by an increase. The content decreased from 2.73 to 2.26 $\mu g\ g^{-1}$ on 21st day and later increased to 2.51 $\mu g\ g^{-1}$ in the 30-40 cm layer. In the 40-50 cm layer there was an initial decrease from 2.14 to 1.44 $\mu g\ g^{-1}$ and then an increase to 3.03 $\mu g\ g^{-1}$. The lower-most layer (50-60 cm) showed comparatively low values for the 11th and 21st day of sampling, the values being 1.76 and 1.58 $\mu g\ g^{-1}$ respectively. With increasing depth

Table 22. Dynamics of hydroxycarbofuran content ($\mu g\ g^{-1}$) of soil after application of carbofuran at the onset on SW and NE monsoons

Soil depth (cm)	Days after carbofuran application		
	11	21	31
0-10	3.25	4.15	2.10
10-20	3.17	4.34	3.71
20-30	2.67	3.83	3.29
30-40	2.73	2.26	2.51
40-50	2.14	1.44	3.03
50-60	1.76	1.58	3.29
Rainfall (mm)	110.40	175.80	401.40

(50-60 cm) a decrease was noticed on the 11th and 21st day after application of carbofuran suggesting the leaching of the metabolite from the upper layers to the lower layer. In this period also, the concentrations of hydroxycarbofuran was lower than that of carbofuran, which indicates that carbofuran is the major residue in the soil. Also 3-hydroxycarbofuran contents obtained in this period was less than that of SW application which might be due to the heavy rainfall obtained during that season. The rainfall received were 952.1 and 401.4 mm respectively for the SW monsoon and NE monsoon periods for a duration of about 30 days.

In contrast to the laboratory experiment, in which a long lag period was observed prior to the commencement of degradation of carbofuran, rapid degradation of the insecticide occurred in the field condition with not so much as a lag period. In soils retreated with carbofuran as well as in cases with a history of the insecticide application, shortening or lack of a lag period was reported (Felsot *et al.*, 1985; Suett, 1986). However in the present study the field did not have a history of carbofuran application. Yet, degradation had occurred within a short period (10-11 days) after its application. It was even likely that the degradation had occurred much earlier than 10 and 11 days following its application in the SW and NE monsoons because samples were not collected at still shorter intervals in this study. The rapid degradation of carbofuran in the field soils could also be due to the greater microbial population present in the root zone of the vine.

The large variability observed in the concentration of the residues in the field soil at different depths indicated that a regular pattern in the mobility is difficult to observe in the field. The results of the study however proved that carbofuran and its metabolites would be rapidly carried downwards with rain water.

Leaching coupled with the degradation of the chemical were responsible for the decrease in soil concentration of the pesticide with the time. Heavy leaching of carbofuran from the root zone of the vine is likely to cause contamination of ground water sources since the pesticide is generally applied at a higher dose for the control of nematode. Moreale and Bladen (1982) observed that due to increasing utilization of carbofuran, relatively high water solubility and persistence in soils of low pH (less than 5.5), the migration of this chemical to relatively deep water table can occur. The results of the present study indicated that probability for leaching to ground water sources is more.

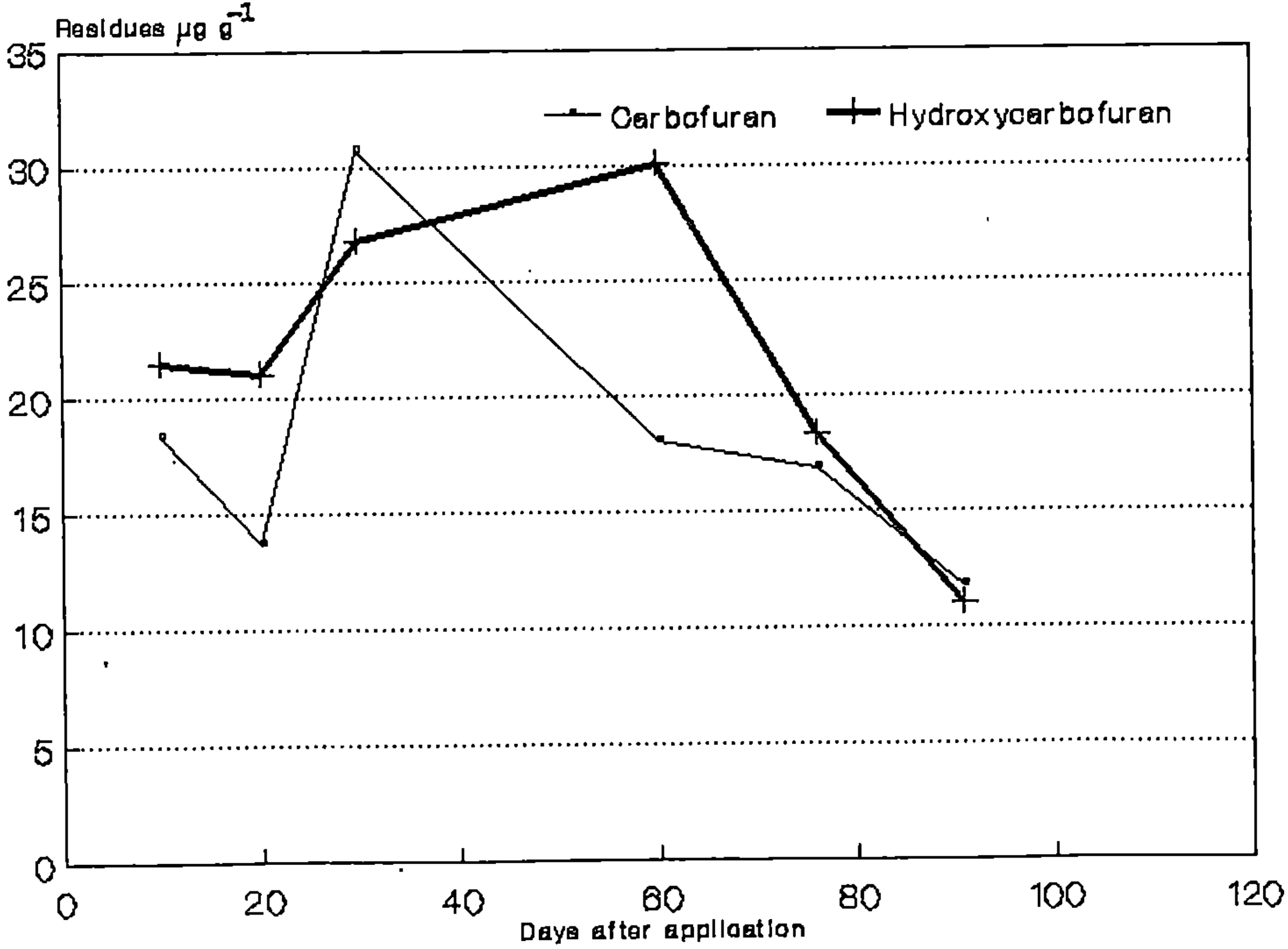
4.4.4 Absorption of carbofuran

4.4.4.1 Residues in foliage

The data on the contents of carbofuran as well as its metabolite 3-hydroxycarbofuran on fresh weight basis are illustrated in Fig.9. Though attempts were made to determine 3-ketocarbofuran, due to its very low content, estimations were restricted to the major metabolite, 3-hydroxycarbofuran and the parent compound carbofuran.

In treatment T_1 (SW monsoon application) the foliar concentration of carbofuran showed a tendency to increase during the first 30 days after its application. Thereafter there was a gradual decrease. The highest concentration observed was 30.72 $\mu g\ g^{-1}$ at day 30. Although the concentration had started decreasing beyond 30 days even on 91st day, considerable amount of carbofuran was observed in the foliage (11.76 $\mu g\ g^{-1}$). As regards its metabolite 3-hydroxycarbofuran, its concentration also increased with time up to 60 days and then declined. The peak value observed was 30.08 ppm on 60th day. The lowest value of 11 ppm was

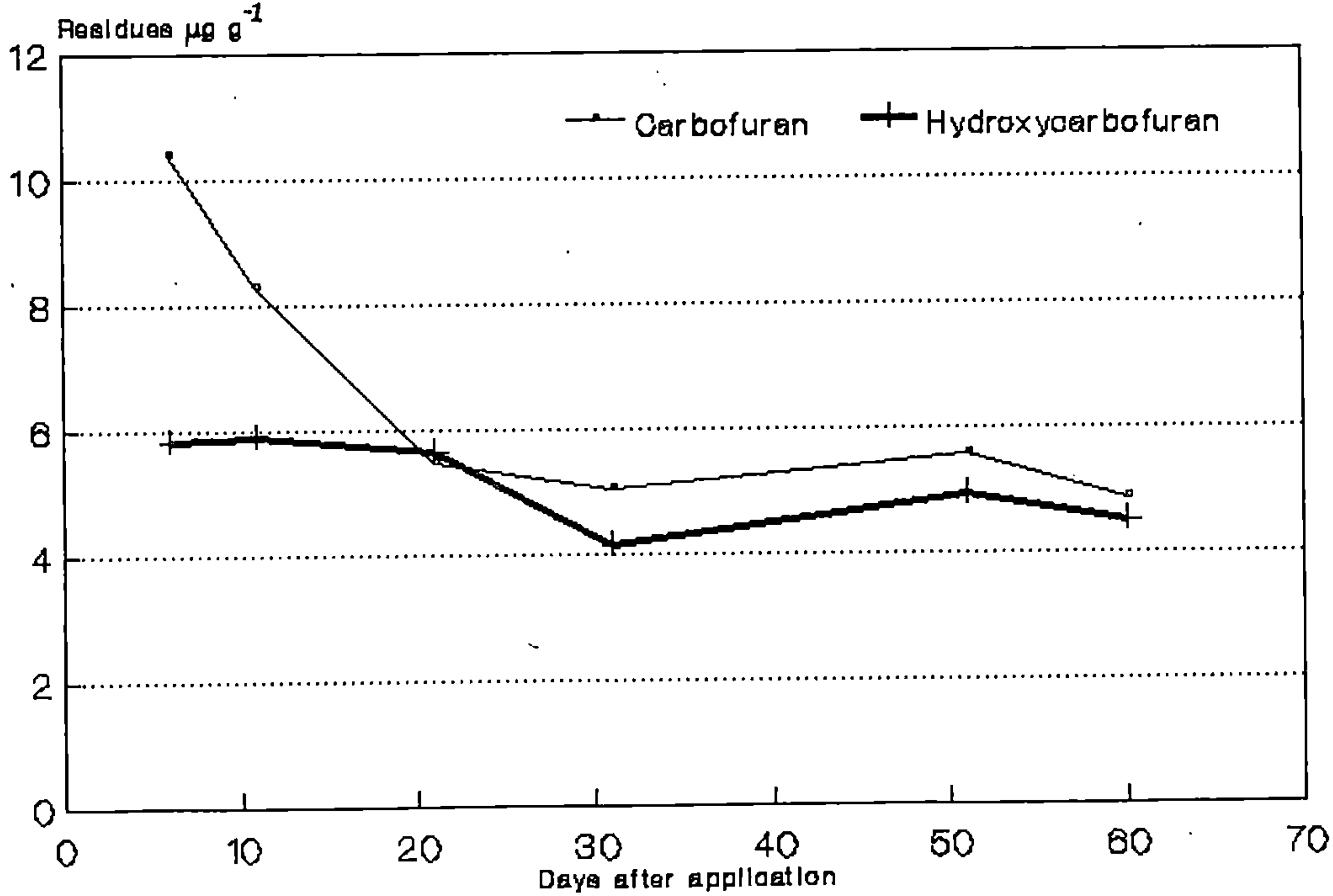
Fig.9. Residue levels of carbofuran and hydroxycarbofuran in the foliage of black pepper following the application of carbofuran at the onset of SW monsoon



observed on the last sampling day ie. 91st day after application. Pree and Saunders (1974) observed the presence of many metabolites namely carbofuran phenol, 3-ketocarbofuran phenol and two unknown metabolites in the foliage of mugho pine grown in carbofuran treated soils. The reduction of residue levels with time might have resulted from dilution by plant growth and volatalization from plant tissue surfaces (Archer *et al.*, 1977). Siddaramappa and Watanabe (1979) and Ferreira and Seiber (1981) had suggested the possibility of vapour loss of systemically applied carbofuran from rice plants in guttation fluids collected on rice leaf blades. Also the leaf surface residues produced by guttation might have physically removed by washing off processes during rainfall.

The data on foliar concentrations of carbofuran and its metabolite 3-hydroxycarbofuran following second split application of carbofuran in the NE monsoon season are illustrated in Fig.10. The data indicated that the levels of carbofuran showed a decreasing trend during the period from the 6th day of application (10.39 $\mu g\ g^{-1}$) to 31st day of application (5.03 $\mu g\ g^{-1}$). The residue decreased to 4.84 ppm by 60th day of application. The concentration of the metabolite (3-0H carbofuran) remained more or less steady (5.9 $\mu g\ g^{-1}$) during the first three weeks following the application and then decreased. The concentration obtained on the 60th day was 4.50 ppm. The data on foliar content of residues indicated that considerable absorption of carbofuran had occurred from the first split of carbofuran application at the onset of SW monsoon. The depletion of carbofuran from the root zone during the SW monsoon may also be due to the crop removal, besides leaching and degradation. It is also interesting to note that there was rapid degradation of carbofuran in the leaves as evidenced from the greater concentration of its metabolite 3-hydroxycarbofuran. It is also noteworthy that only one

Fig.10. Residue levels of carbofuran and hydroxycarbofuran in the foliage of black pepper following the application of carbofuran at the onset of SW and NE monsoons



metabolite *viz.*, 3-hydroxy carbofuran occurred in significant amounts in the leaf tissues. This observation corroborates with the findings of Ashworth and Sheets (1972), Sahu and Agnihotri (1983), Vasudevan (1985).

The data for the NE monsoon indicated that not much absorption of carbofuran from the second split application had occurred during this period. This was deduced from the absence of a hike in the concentration of insecticide in the leaf following the second split application.

4.4.4.2 Carbofuran residues in berry

The insecticide residue level in the berry, the edible part was monitored at three pickings. The first picking was done on 1-12-1994 (156th day of application of the first split of carbofuran), second picking on 165th day and the third picking on 181st day. With respect to the second split application, the three periods of harvest corresponded to 51, 60 and 76 days respectively. The data relating to the carbofuran residues in green berry on fresh weight basis are presented in Table 23 and Fig.11. The residue levels tended to decrease with delay in the harvest. Irrespective of whether the vine received only one split (1 g ai plant^{-1}) or two splits (2 g ai plant^{-1}) the residue levels remained more or less the same. The content for treatment T_1 (SW application) were 5.72, 4.41 and 2.90 $\mu g\ g^{-1}$ respectively for the first, second and third pickings. As in the case of parent compound, its metabolite 3-hydroxycarbofuran also showed a decreasing trend with delay in harvest. The hydroxycarbofuran contents due to T_1 treatment were 9.01, 6.42 and 5.53 $\mu g\ g^{-1}$ respectively for the first, second and third picking.

Table 23. Residue levels of carbofuran and its metabolite in berry after application of carbofuran at the onset of SW monsoon

Stage of harvest	Days after application	Carbofuran ($\mu g\ g^{-1}$)	Hydroxycarbofuran ($\mu g\ g^{-1}$)
First picking	156	5.72	9.01
Second picking	165	4.41	6.42
Third picking	181	2.90	5.53

Fig.11. Residue levels of carbofuran and its metabolite in berry after application of carbofuran at the onset of SW monsoon

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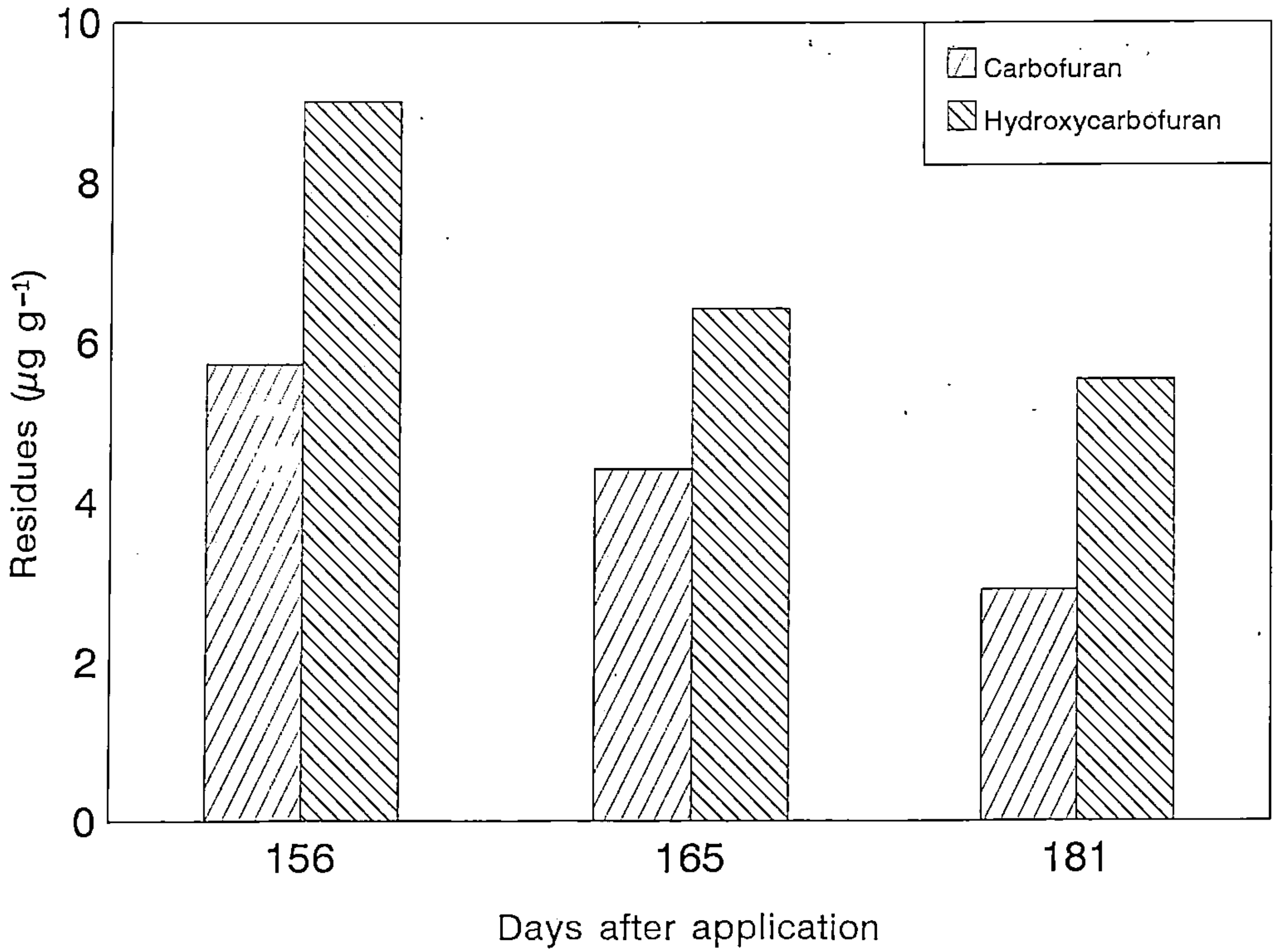


90

The concentration of carbofuran for treatment T_2 were 4.38, 3.70 and 2.47 $\mu g\ g^{-1}$ respectively for the first, second and third pickings (Table 24 and Fig.12). The values for hydroxycarbofuran were 12.80, 3.40 and 6.86 $\mu g\ g^{-1}$. The concentration of hydroxycarbofuran was also more than that of parent compound carbofuran in the berry as in the case of leaves. This might be due to the transport of the hydroxycarbofuran, the major metabolite produced in the leaves to the berry along with the transport of food materials. The decrease in content of residues in berry with time must be due to degradation or dilution by increase in mass of berry due to growth of tissues.

The results of the field experiment proved that carbofuran accumulated in the green berries. When carbofuran was applied to the soil basins of pepper vines at the rate of 1 g ai plant^{-1} at the onset of both SW and NE monsoons, at the time of harvest (third picking) the fresh berries contained 2.47 and 6.86 $\mu g\ g^{-1}$ of carbofuran and hydroxy carbofuran respectively on fresh weight basis. Though the amount of insecticide added (2 g ai plant^{-1}) to the soil basins is providing sufficient concentration in soil to kill nematodes (Kenneth, 1990), it is accumulating in the berries. Several importing countries had set up stringent regulations regarding the content of residues in food produce. In the case of vegetable crops the PFA tolerance level is 0.10 ppm. Germans have kept a tolerance level of 0.05 ppm in dried pepper.

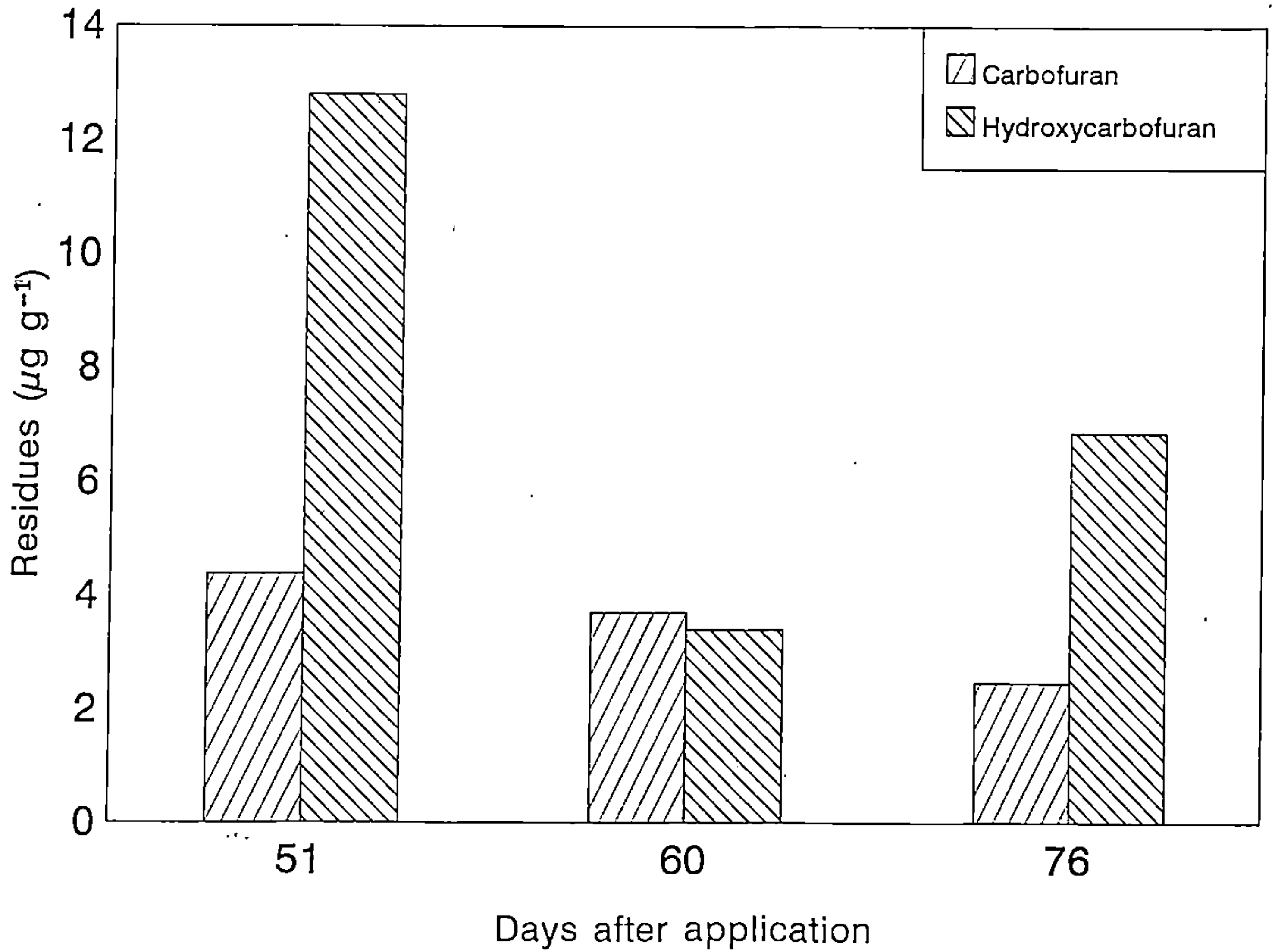
Though the insecticide residues accumulate in the berries, several methods of processing lead to a reduction in its content. The common household practice of drying the berries itself reduces the content of residues to some extent. Recently, dipping the fresh berries in boiling water has been recommended (KAU,

Table 24. Residue levels of carbofuran and its metabolite in berry after application of carbofuran at the onset of SW and NE monsoons

Stage of harvest	Days after application in NE monsoon	Carbofuran ($\mu g\ g^{-1}$)	Hydroxycarbofuran ($\mu g\ g^{-1}$)
First picking	51 (156)	4.38	12.80
Second picking	60 (165)	3.70	3.40
Third picking	76 (181)	2.47	6.86

Parentheses indicate days after application in SW monsoon

Fig.12. Residue levels of carbofuran and its metabolite in berry after application of carbofuran at the onset of SW and NE monsoons



1995). This practice reduces the residue content considerably. Since the residue level in the berries is more than the acceptable level there is an immediate need to examine whether the quantity of carbofuran applied to the vine could be reduced without affecting the nematicidal effect or a non-systemic nematicide has to be used in pepper gardens.

The results also indicated that early harvest of the berries will lead to greater accumulation of carbofuran residues in the produce from treated plants. The increasing use of immature berries for culinary purposes is more harmful due to greater accumulation of pesticides.

SUMMARY

SUMMARY

A study on the fate of carbofuran applied to the soil basin of black pepper (*Piper nigrum* L.) was conducted during 1992-1995 at the College of Horticulture, Vellanikkara. The main objectives of the investigation were to study the adsorption kinetics of carbofuran in laterite soil, to study the persistence of the pesticide in the soil and to study the influence of organic matter on the bioavailability of insecticide. The mobility of carbofuran under field conditions was also studied. An attempt was also made to assess the effect of carbofuran application to soil on the growth of black pepper vine. The soil at the experimental site was laterite (Ultisol). The variety of black pepper used for the study was the high yielding variety Panniyoor-1. The salient findings of the study are summarised below.

1. Adsorption of carbofuran in soil was found to be explained by Freundlich adsorption equation. The Freundlich constant K increased with increasing organic matter content. However the rate of adsorption of carbofuran in soils was not found to be influenced by organic matter content of the soil. The sorption was found to be complete within half an hour beyond which the rate of adsorption increased only marginally.

2. An initial lag period of 20-30 days was observed before the commencement of degradation of carbofuran in soils when studied under laboratory conditions. Only one metabolite namely, 3-hydroxycarbofuran was detected in soil as a result of carbofuran degradation. The half life period after the lag phase ranged between 20.38 and 46.20 days for the different soils.

3. The crop marc left after HCl extraction retained significant amounts of radioactivity which could be recovered following combustion. This indicates presence of bound residues even after the extraction indirectly suggesting that spiking or fortification of samples with known quantities of insecticide to determine extraction efficiency of solvents would be misleading.

4. Among the plant parts extracted, leaf was found to accumulate largest quantity of radioactivity than stem of the black pepper vines grown in soil treated with carbofuran. Radioactivity in the roots was low.

5. The absorption of carbofuran by the vine was more from soils with low organic matter contents than from soils with relatively more organic matter indicating the low bio-availability in high organic matter soils and vice versa. This points to the need for discriminatory application of carbofuran for achieving expected pesticidal effect in soils depending on the organic matter status of soil.

6. Autoradiography of the thin layer chromatography of the leaf extracts of black pepper vines grown in soils in which carbofuran was applied showed the presence of parent compound carbofuran and only one metabolite ie. 3-hydroxycarbofuran. The carbofuran derived activity was more for the metabolite 3-hydroxycarbofuran (22-30%) than for carbofuran itself (15-20%).

7. Autoradiography of the bush pepper plant which absorbed ^{14}C carbofuran for 6 h in sunlight indicated that carbofuran was readily translocated to the leaves, berries and rachis. The stem, contained appreciable quantity of the insecticide during this short term absorption.

8. Phytotonic effect due to carbofuran application was not observed in black pepper.

9. The foliar concentrations of carbofuran and hydroxycarbofuran in vines grown on carbofuran-treated soil showed an increase during the initial sampling followed by a decrease.

10. In foliage the metabolite 3-hydroxycarbofuran was found to be the chief residue as compared to carbofuran.

11. The carbofuran residues in berries decreased with increase in delay in harvesting. The fresh berries contained 5.72 $\mu g\ g^{-1}$ of carbofuran during the first picking (156 days after application) in single application during South West monsoon. The values corresponding to second picking (165 days after application) and third picking (181 days after application) were 4.41 and 2.90 $\mu g\ g^{-1}$.

    The fresh berries contained 4.38, 3.70 and 2.47 $\mu g\ g^{-1}$ of carbofuran for the first, second and third picking consequent to two split applications in SW and NE monsoons. It is suggested therefore that the harvesting of berries from carbofuran-treated vines may be delayed as far as possible to bring the residue levels to the minimum.

12. In berries hydroxycarbofuran accumulated in amounts greater than carbofuran.

13. Studies on the mobility of carbofuran in the field revealed that carbofuran and its degradation product, 3-hydroxycarbofuran, were susceptible to heavy leaching during SW and NE monsoon seasons. The leaching was relatively

more in SW monsoon than in NE monsoon due to heavier rainfall during SW monsoon.

14. The presence of hydroxycarbofuran in the field soil at 10 days after application at the onset of SW monsoon is an indication that degradation starts early under field conditions. The lag period under field conditions is less than that of observed for in laboratory experiment.

15. The parent compound carbofuran was found to be the major residue present in the soil under field conditions.

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* Originals not seen

APPENDICES

APPENDIX - I

ANOVA for ^{14}C recovered in the methylene chloride extracts of HCl digest of stem

Source	Degrees of freedom	Sum of squares	Mean square	F value
Organic matter	2	1.009	0.504	10.94**
Carbofuran	1	0.553	0.553	12.00**
Interaction	2	0.001	0.000	0.01
Error	18	0.829	0.046	
Total	23	2.392		

** Significant at $P < 0.01$

APPENDIX - II

ANOVA for ^{14}C recovered from the combustion of plant residues left after HCl digestion of stem

Source	Degree of freedom	Sum of squares	Mean square	F value
Organic matter	2	2.061	1.030	39.08**
Carbofuran	1	0.551	0.551	20.90**
Interaction	2	0.282	0.141	5.35*
Error	18	0.475	0.026	
Total	23	3.368		

** Significant at $P < 0.01$
* Significant at $P < 0.05$

APPENDIX - III

ANOVA for ^{14}C recovered in the methylene chloride extracts of HCl digest of leaf

Source	Degree of freedom	Sum of squares	Mean square	F value
Organic matter	2	2.728	1.364	17.00**
Carbofuran	1	1.273	1.273	15.87**
Interaction	2	0.173	0.086	1.08
Error	18	1.445	0.080	
Total	23	5.619		

** Significant at $P < 0.01$

APPENDIX - IV

ANOVA for ^{14}C recovered from the combustion of plant residues left after HCl digestion of leaf

Source	Degrees of freedom	Sum of squares	Mean square	F value
Organic matter	2	1.371	0.685	24.75**
Carbofuran	1	0.751	0.751	27.12**
Interaction	2	0.561	0.280	10.12**
Error	18	0.499	0.028	
Total	23	3.182		

** Significant at $P < 0.01$

APPENDIX-V

ANOVA for ^{14}C recovered from the combustion of roots (oven dried at 70°C)

Source	Degrees of freedom	Sum of square	Mean square	F value
Organic matter	2	0.091	0.045	2.11
Carbofuran	1	0.475	0.475	22.06*
Interaction	2	1.971	0.986	45.82**
Error	18	0.387	0.022	
Total	23	2.924		

** Significant at $P < 0.01$

* Signifcant at $P < 0.05$

APPENDIX - VI

ANOVA for the effect of organic matter on the absorption of soil-applied ^{14}C-carbofuran by black pepper vine

Source	Degrees of freedom	Sum of squares	Mean square	F value
Organic matter	2	1.322	0.661	29.60**
Carbofuran	1	0.688	0.688	30.83**
Interaction	2	0.153	0.076	3.42
Error	18			
Total	23	2.565		

** Significant at $P < 0.01$

APPENDIX-VII

Recovery of carbofuran and 3-hydroxycarbofuran from soil and plant

Name of chemical compound	Soil		Leaf		Berry	
	Added concentration ($\mu g\ g^{-1}$)	Recovery (%)	Added concentration ($\mu g\ g^{-1}$)	Recovery (%)	Added concentration ($\mu g\ g^{-1}$)	Recovery (%)
Carbofuran	10	77.0	10	82.0	10	86.0
3-hydroxycarbofuran	10	84.2	10	84.0	10	92.0

APPENDIX - VIII

Dates of application of carbofuran in field and dates of harvest of berry

Date	Operation done
28-6-'94	Application of carbofuran at the onset of SW monsoon
11-10-'94	Application of carbofuran at the onset of NE monsoon
1-12-'94	Harvesting started (First picking)
10-12-'94	Second picking
26-12-'94	Third picking

APPENDIX - IX

Yield from black pepper vines treated with carbofuran

Sl.no. of plants	Weight of green berries (kg)	
	SW application	SW + NE application
1	1.015	3.533
2	1.464	3.300
3	0.110	0.890
4	0.150	0.090
Mean yield	0.685	1.953

APPENDIX - X

Weather data for the experimental period
(from 28-6-94 to 25-12-95)

Month and date	Rainfall (mm)	Temperature (°C)		Relative humidity (%)		Evaporation (mm)
		Maximum	Minimum	FN	AN	
1	2	3	4	5	6	7
June						
28-6-'94	8.6	30.5	23.0	98	75	3.4
29-6-'94	47.0	30.5	22.8	96	95	3.0
30-6-'94	83.6	25.8	22.0	98	69	1.4
July						
1-7-'94	9.4	30.0	22.5	95	66	2.9
2-7-'94	6.2	30.2	23.0	96	86	4.2
3-7-'94	17.4	28.6	22.4	98	87	2.8
4-7-'94	17.8	27.2	22.2	98	78	2.4
5-7-'94	5.6	30.2	22.0	93	68	4.2
6-7-'94	0.4	31.0	23.5	93	95	3.8
7-7-'94	22.2	31.2	23.8	98	83	2.4
8-7-'94	7.4	29.7	23.0	98	77	2.3
9-7-'94	14.8	29.5	22.2	96	71	3.0
10-7-'94	30.4	30.4	22.0	98	80	3.4
11-7-'94	40.2	29.6	22.0	96	98	3.2
12-7-'94	83.0	27.8	21.2	98	89	2.1
13-7-'94	30.1	29.5	23.5	96	83	3.4
14-7-'94	83.2	29.2	21.2	96	98	2.3
15-7-'94	29.3	25.5	22.0	96	94	2.1

Contd.

Appendix-X. Continued

1	2	3	4	5	6	7
16-7-'94	47.6	28.0	22.4	96	98	2.5
17-7-'94	40.8	30.4	21.0	95	95	3.8
18-7-'94	64.0	27.0	22.0	98	81	2.2
19-7-'94	19.2	28.5	23.0	98	81	2.3
20-7-'94	44.8	28.0	20.8	96	83	2.8
21-7-'94	70.4	28.4	22.0	98	81	1.9
22-7-'94	6.9	29.0	22.4	96	70	2.6
23-7-'94	26.0	30.2	22.4	98	76	3.2
24-7-'94	0.5	28.8	23.0	88	76	1.9
25-7-'94	19.8	29.8	23.7	98	90	2.9
26-7-'94	7.6	28.0	23.0	96	89	3.2
27-7-'94	67.4	25.5	23.0	96	98	3.1
28-7-'94	60.4	25.6	22.0	98	93	2.0
29-7-'94	37.6	26.5	21.8	96	96	3.2
30-7-'94	65.0	25.6	22.0	98	93	2.2
31-7-'94	26.2	26.5	22.5	96	95	1.8
August						
1-8-'94	51.6	28.2	22.0	96	75	2.4
2-8-'94	106.2	29.4	22.3	96	98	2.0
3-8-'94	191.2	24.5	22.0	98	98	2.0
4-8-'94	237.6	27.5	21.8	98	72	2.2
5-8-'94	261.7	29.9	22.2	98	83	3.5
6-8-'94	263.3	30.0	22.5	92	73	3.0

Contd.

Appendix-X. continued

1	2	3	4	5	6	7
7-8-'94	268.3	30.4	23.4	92	73	2.3
8-8-'94	269.3	30.4	25.0	93	67	3.0
9-9-'94	-	31.8	24.2	95	70	4.0
10-8-'94	-	31.0	24.0	95	70	3.3
11-8-'94	-	30.0	23.0	92	76	2.8
12-8-'94	294.1	29.5	23.4	95	78	2.6
13-8-'94	296.1	30.8	22.5	98	74	3.4
14-8-'94	-	32.6	23.5	98	63	3.6
15-8-'94	-	32.5	23.2	95	65	2.6
16-8-'94	-	32.8	24.0	90	62	4.3
17-8-'94	307.9	32.0	23.0	96	66	3.4
18-8-'94	9.8	29.0	22.0	96	76	2.6
19-8-'94	22.4	27.6	22.2	96	80	2.4
20-8-'94	18.2	29.5	22.4	96	70	2.5
21-8-'94	0.6	30.0	23.0	96	75	3.0
22-8-'94	41.0	29.5	22.6	95	77	3.2
23-8-'94	4.0	30.2	23.0	96	71	3.6
24-8-'94	0.6	30.6	22.1	98	71	3.8
26-8-'94	4.0	31.2	23.0	85	68	2.6
26-8-'94	12.2	30.0	22.5	96	71	3.0
27-8-'94	11.6	30.4	22.9	91	72	2.7
28-8-'94	15.1	30.0	21.6	97	80	3.3

Contd.

Appendix-X. Continued

1	2	3	4	5	6	7
29-8-'94	12.2	30.5	22.5	95	71	3.2
30-8-'94	21.6	30.4	22.4	98	81	3.3
31-8-'94	28.0	28.4	23.0	98	89	2.0
September						
1-9-'94	79.4	30.2	22.4	98	98	3.4
2-9-'94	50.9	27.6	23.0	96	74	2.9
3-9-'94	4.4	30.4	22.4	96	69	3.8
4-9-'94	9.4	31.2	23.8	98	92	3.0
5-9-'94	35.8	29.6	22.4	98	87	3.6
6-9-'94	27.4	27.2	22.5	96	69	2.6
7-9-'94	22.2	30.8	22.3	96	68	2.9
8-9-'94	-	31.1	24.0	95	61	3.3
9-9-'94	-	32.0	24.4	92	62	4.4
10-9-'94	-	32.0	24.0	89	67	4.3
11-9-'94	-	32.0	23.6	93	62	4.0
12-9-'94	-	32.8	23.8	93	58	4.0
13-9-'94	-	32.8	23.5	96	60	4.3
14-9-'94	-	32.5	23.6	93	61	3.5
15-9-'94	8.2	32.1	23.0	96	67	3.6
16-9-'94	-	31.2	22.0	92	65	3.4
17-9-'94	-	31.4	23.0	95	50	3.9
18-9-'95	-	32.0	21.6	90	57	3.5
19-9-'94	1.2	31.2	22.0	91	57	4.3

Contd.

Appendix-X. Continued

1	2	3	4	5	6	7
20-9-'94	-	31.8	21.0	84	52	4.4
21-9-'94	-	32.5	23.0	84	51	4.4
22-9-'94	-	33.0	24.0	84	61	4.6
23-9-'94	-	33.2	23.6	90	58	4.0
24-9-'94	-	32.9	24.0	91	57	3.4
25-9-'94	-	33.2	24.2	93	55	3.8
26-9-'94	-	33.0	23.0	89	62	4.5
27-9-'94	0.2	33.0	24.2	85	64	4.0
28-9-'94	-	33.3	24.5	86	61	4.3
29-9-'94	0.6	33.2	33.2	88	52	3.8
30-9-'94	0.8	34.0	23.5	95	62	3.6
October						
1-10-'94	4.4	34.0	23.5	90	70	3.2
2-10-'94	15.6	32.6	22.5	95	66	3.5
3-10-'94	1.0	32.1	24.1	92	71	3.6
4-10-'94	14.0	32.0	23.0	98	81	3.0
5-10-'94	14.2	29.2	24.4	98	71	2.6
6-10-'94	0.6	30.2	22.0	96	63	2.2
7-10-'94	2.2	31.8	23.5	87	78	3.4
8-10-'94	3.6	30.6	22.2	88	61	3.3
9-10-'94	-	33.0	24.0	89	65	3.8
10-10-'94	26.8	34.0	22.4	95	63	3.6

Contd.

Appendix-X. Continued

1	2	3	4	5	6	7
11-10-'94	-	32.8	23.0	85	72	4.2
12-10-'94	20.2	32.8	22.2	87	62	3.2
13-10-'94	2.0	32.4	22.6	95	64	3.4
14-10-'94	-	32.4	23.0	90	67	4.0
15-10-'94	5.7	31.8	22.4	96	68	3.3
16-10-'94	5.4	31.0	22.0	96	80	2.9
17-10-'94	48.4	30.5	22.0	96	85	3.0
18-10-'94	7.2	30.2	21.6	96	51	2.8
19-10-'94	3.8	33.2	23.0	88	77	3.7
20-10-'94	17.7	32.6	22.6	95	60	2.5
21-10-'94	-	32.8	23.0	92	59	2.6
22-10-'94	10.4	34.0	22.4	90	71	3.7
23-10-'94	21.4	34.4	22.5	95	64	2.8
24-10-'94	0.4	33.2	23.8	95	74	3.0
25-10-'94	0.4	33.0	23.3	90	65	2.8
26-10-'94	9.5	33.0	22.5	92	66	3.0
27-10-'94	55.3	32.4	22.0	96	68	2.8
28-10-'94	22.8	32.4	23.0	98	79	2.6
29-10-'94	4.6	30.0	23.0	84	59	2.8
30-10-'94	31.0	33.8	22.0	95	68	2.8
31-10-'94	9.6	31.6	21.4	88	67	3.0
November						
1-11-'94	12.0	32.5	22.0	95	87	2.8
2-11-'94	4.8	31.0	23.0	92	58	2.0

Contd.

Appendix-X. Continued

1	2	3	4	5	6	7
3-11-'94	4.4	33.0	23.0	96	73	2.2
4-11-'94	1.9	31.6	22.8	84	63	2.4
5-11-'94	23.6	31.5	22.9	76	75	2.6
6-11-'94	78.0	32.0	22.6	83	84	2.0
7-11-'94	-	30.0	22.8	84	49	4.4
8-11-'94	-	32.0	24.0	77	57	5.6
9-11-'94	0.6	32.0	24.8	76	60	6.2
10-11-'94	-	32.0	22.4	80	65	5.6
11-11-'94	-	29.0	20.4	73	60	5.4
12-11-'94	-	30.2	24.0	82	62	5.2
13-11-'94	-	29.4	24.0	84	63	4.2
14-11-'94	-	32.0	23.8	87	58	4.1
15-11-'94	-	31.6	24.5	73	53	4.6
16-11-'94	-	32.0	25.0	69	57	7.0
17-11-'94	-	32.0	24.4	69	56	6.2
18-11-'94	-	32.0	25.0	72	47	6.2
19-11-'94	-	32.5	24.9	70	55	5.9
20-11-'94	-	32.0	25.0	66	56	6.4
21-11-'94	-	30.7	23.5	80	59	4.2
22-11-'94	-	31.0	24.0	75	55	5.6
23-11-'94	-	33.0	23.8	76	51	5.0
24-11-'94	-	33.0	23.8	55	42	4.5
25-11-'94	-	33.0	23.7	62	47	5.2

Contd.

Appendix-X. Continued

1	2	3	4	5	5	7
26-11-'94	-	33.0	21.5	75	49	5.6
27-11-'94	-	32.6	21.8	73	49	3.4
28-11-'94	-	32.4	22.0	74	56	4.0
29-11-'94	-	31.4	22.9	72	40	5.4
30-11-'94	-	33.0	22.1	73	46	4.0
December						
1-12-'94	-	34.5	22.0	84	56	4.0
2-12-'94	-	33.2	22.5	69	49	3.7
3-12-'94	-	32.5	22.0	69	50	4.3
4-12-'94	-	31.8	22.0	72	45	5.6
5-12-'94	-	32.3	22.5	59	42	5.2
6-12-'94	-	32.0	21.6	59	39	5.0
7-12-'94	-	31.4	22.1	67	42	5.1
8-12-'94	-	31.5	22.1	74	41	5.7
9-12-'94	-	32.0	18.6	74	40	4.1
10-12-'94	-	33.0	19.0	72	45	4.1
11-12-'94	-	32.2	20.2	70	48	4.1
12-12-'94	-	32.0	21.0	71	33	5.3
13-12-'94	-	32.0	19.8	74	31	6.0
14-12-'94	-	31.0	19.5	67	39	6.6
15-12-'94	-	32.3	20.0	69	42	5.3
16-12-'94	-	32.3	21.0	72	46	6.2
17-12-'94	-	31.5	22.2	77	54	3.8
18-12-'94	-	32.0	23.0	77	49	5.0

Contd.

Appendix-X. Continued

1	2	3	4	5	6	7
19-12-'94	-	32.8	25.0	68	50	5.5
20-12-'94	-	33.0	25.0	78	48	6.0
21-12-'94	-	33.4	25.5	82	54	7.0
22-12-'94	-	31.0	24.0	74	48	6.6
23-12-'94	-	31.8	24.0	67	45	6.5
24-12-'94	-	32.2	24.0	63	46	6.8
25-12-'94	-	30.8	23.0	66	45	5.8

FATE OF CARBOFURAN APPLIED TO THE SOIL BASIN OF BLACK PEPPER

BY

BETTY BASTIN

ABSTRACT OF THE THESIS

submitted in partial fulfilment of
the requirements for the degree

DOCTOR OF PHILOSOPHY IN AGRICULTURE

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Kerala Agricultural University

Department of Soil Science and Agricultural Chemistry
COLLEGE OF HORTICULTURE
Vellanikkara - Thrissur
KERALA

ABSTRACT

An investigation on the fate of carbofuran applied to the soil basin of black pepper (*Piper nigrum* L.) was conducted during 1992-1995 at the College of Horticulture, Vellanikkara. Laterite soils collected from the predominant pepper growing tracts of Kannur and Wayanad of Kerala state were used for the study. A field study was also conducted at the KADP farm attached to the College of Horticulture, Vellanikkara.

The sorption of carbofuran in laterite soils and its persistence, influence of organic matter on the bio-availability of carbofuran, mobility of the insecticide in soil under field conditions and the effect of carbofuran on the growth of black pepper were assessed. The main findings of the study are given below.

The sorption of insecticide in soil was found to be explained by Freundlich adsorption equation. The rates of sorption of carbofuran was not influenced by the organic matter contents.

An initial lag period was observed prior to carbofuran degradation in soil under laboratory conditions. Only one metabolite (3-hydroxycarbofuran) was detected as the product of degradation.

The total content of ^{14}C derived radioactivity was high in plants grown in soils with lowest organic matter content and vice versa. Hence, bioavailability of the insecticide is less in soils with high organic matter contents. So a discriminatory application of carbofuran is required depending on organic matter content of the soil. Thus for effective control of the pest in a soil with high organic matter content more

quantity of carbofuran has to be applied than a soil with low content of organic matter.

The residues of ^{14}C-carbofuran absorbed plants left after HCl extraction, retained significant amounts of radioactivity which could be recovered following combustion. This indicated that determination of extraction efficiency by spiking or fortification of the samples with known quantities of insecticides would lead to overestimation of the recovery.

The main metabolite of the carbofuran in the leaves was 3-hydroxycarbofuran.

Autoradiography of the bush pepper plants which was allowed to absorb ^{14}C-carbofuran for 6 h showed that carbofuran was absorbed and translocated to the leaves and berry.

Both carbofuran and hydroxycarbofuran were leached to the lower layers of soil (0-60 cm) during SW and NE monsoons. The content of hydroxycarbofuran was less than carbofuran in soils.

In the foliage and berries carbofuran and the metabolite, 3-hydroxycarbofuran accumulated. The content of hydroxycarbofuran was greater than that of carbofuran. As the harvesting time was delayed the content of residues also decreased. The early harvesting and use of immature berries may lead to more concentration of pesticide in the produce. This will lead to chances of more residue build-up in the human system.