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Absorption of soil-applied carbofuran by black pepper (*Piper nigrum* L.) plants in laterite soil

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Abstract

The effect of organic matter on availability of soil-applied carbofuran and its absorption by black pepper (*Piper nigrum*) plants were studied in a pot culture experiment employing ^{14}C-labelled carbofuran. Absorption of carbofuran from soil was inversely related to the organic matter content of the soil. This was attributed to the enhanced degradation of the insecticide with increasing soil organic matter resulting in its reduced availability, rather than its adsorption in the soil. The absorbed carbofuran was mainly translocated to the leaf (64.0%) and stem (33.5%) and to a lesser extent to the berries (1.5%). The results also indicated that carbofuran had no phytotonic effect on black pepper plants but retarded root production.

Key words: black pepper, carbofuran, organic matter, *Piper nigrum*, residues.

Introduction

A widespread problem which limits black pepper (*Piper nigrum* L.) productivity in Kerala in India is slow decline disease caused by plant parasitic nematodes (Anandaraj *et al.* 1991; Ramana & Eapen 1995), although nutrient deficiencies (Wahid *et al.* 1982) and fungi (Sheela & Venkitesan 1990) were also implicated. Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methyl carbamate), a systemic insecticide, has been recommended for the control of the nematodes (KAU 1996). Information on the absorption of this chemical and its residues in black pepper plants is not available in literature. Organic matter can affect the bio-availability of carbofuran through its influence on adsorption in the soil (Rajukkannu 1978). Application of carbofuran to soil has also resulted in increased growth of many crop plants revealing its phytotonic effect (Balasubramaniyan & Morachan 1981; Patel & Sukhani 1990). Black pepper is grown extensively in laterite soil (which covers more than 60% of the land area in Kerala) of wide-ranging characteristics, from organic matter-rich recently cleared forest soils to highly denuded organic matter-depleted soils. This study was therefore taken up to assess the influence of organic matter on the absorption of soil-applied carbofuran by black pepper plants and its impact on the residue level in the plant. The phytotonic effect of the applied chemical, if any, on the growth of plants was also examined.

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Materials and methods

Laterite soil (Ultisol) was collected in bulk from three locations of Wayanad, a major black pepper growing District in Kerala, for the pot culture experiment. The soils selected had organic matter contents of 1.28% (S_1), 2.45% (S_2) and 3.09% (S_3). Pots were filled with 1 kg of air-dried, 2-mm sieved soil and planted with rooted black pepper (var. Panniyur-1) cuttings (1 plant pot^{-1}). They were kept in greenhouse and were irrigated at regular intervals to maintain moisture at 60% water holding capacity (WHC). After 1 month of establishment, the plants were treated with carbofuran as described below. Technical grade carbofuran (75% pure) and carbonyl labelled ^{14}C-carbofuran (specific activity 492.2 MBq $mmol^{-1}$, product of FMC Corporation, Middle Port, New York, purity 96%), were used for the study. Analytical grade 3-hydroxy carbofuran and 3 ketocarbofuran (both 99% purity) supplied by the same company were used for identification of metabolites of carbofuran. Carbofuran was applied to the soil in the pots at two levels, namely, 1 kg and 2 kg ai ha^{-1}. There were nine treatments being the combinations of three soils (S_1, S_2 and S_3) and three carbofuran levels. Each treatment was replicated four times and thus there were 36 pots arranged in a completely randomised design. Black pepper plants without carbofuran application served as control.

Aqueous solution of technical grade carbofuran (48 $\mu g\ ml^{-1}$) was mixed with ^{14}C-carbofuran to give a final specific activity of 64.44 kBq μg^{-1}. Required quantities (10 ml and 20 ml pot^{-1} for 1 kg and 2 kg ai ha^{-1}, respectively) of this solution were applied to the respective pots. The quantities of carbofuran applied through the solution were 0.5 mg and 1.0 mg pot^{-1}, respectively, for these treatments.

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. These parts were digested wtih HCl and extracted separately with methanol for determining the insecticide residue and metabolites (Siddaramappa & Watanabe 1979). Carbofuran residues were separated by thin layer chromatography (Siddaramappa *et al.* 1979) and the radioactivity of the separated components were measured in a liquid scintillation system (LKB-Wallac, Finland). The residual stem and leaf materials left after HCl digestion and solvent extraction were dried at 40°C and combusted in a biological oxidizer (Harvey Instrument Corporation, USA). The $^{14}CO_2$ produced was trapped in a liquid scintillation cocktail supplied by the same firm. The operating conditions of the biological oxidizer were: N_2 flow rate: 350 ml min^{-1}, O_2 flow rate: 350 ml min^{-1}, combustion zone temperature: 900°C and combustion time: 2 min.

The roots were carefully removed from the soil, washed thoroughly with tap water and dried in an oven at 70°C. The oven-dried roots (0.1–0.2 g) were combusted directly in the biological oxidizer for radioactivity measurements. In view of the wide variability in radioactive counts, the data were subjected to log transformation prior to statistical analysis.

An 8 month-old bush pepper plant (var. Panniyur-1) with spikes and 4–5 leaves was used for studying the relative distribution of carbofuran in the plant system through autoradiography. The plant was uprooted carefully and washed thoroughly. After drying the plant by pressing between absorbent paper sheets, the root portion of the plant was immersed in a solution containing ^{14}C-carbofuran (specific activity, 64.44 kBq μg^{-1}) in a long test tube. The mouth of the tube was plugged with non-absorbent cotton and the whole system was kept in the sun for 6 h. The roots were then removed and the shoot portion with spikes was used for autoradiography. Radioactivity of different parts of the dried plant (70°C) was also determined after combustion of each part in a biological oxidizer.

Results and discussion

Distribution of carbofuran in black pepper plants

Measurement of radioactivity in different parts of black pepper plants indicated that the concentration of labelled compounds was higher in leaves followed by stem and roots (Fig. 1). The black pepper plants absorbed 5.1% and 5.8% of carbofuran from the applied levels of 1 kg and 2 kg ai ha^{-1}, respectively. About 70% of the total radioactivity was contributed by the leaves and the stem and root portions accounted for 22% and 8% radioactivity, respectively.

Total radioactivity of the stem and leaf portions were computed as sum of the solvent extractable and unextractable portions of the absorbed radioactivity. The aqueous layer following solvent extraction of the HCl digest was practically devoid of radioactivity. Irrespective of the level of applied carbofuran the extractable portion of the residue in the stem was less than 13%, whereas more than 87% was accounted for by the bound residues (Fig. 2). In the case of leaf also about 70% of the absorbed radiolabel was unextractable by the solvent. These results suggest that extraction of carbofuran residues by the solvent would be an underestimate of the quantity present in the plant system. Since the bound residues (unextractable by solvent but determined through combustion) cannot be partitioned into the parent molecule and its metabolites, it is not possible to estimate the actual quantity of the carbofuran residues in the plant. Partitioning of the solvent extractable radiolabeled compounds in the leaf indicated that 19% to 23% of the radioactivity was due to carbofuran residues, 24% to 27% due to hydroxycarbofuran and 51% to 57% by unknown compounds. There was not much difference between the applied levels of carbofuran in this regard. The presence of unextractable residues from ^{14}C-carbofuran was also reported by other workers (Vasudevan 1985; Lee *et al.* 1991). The bound residues may be due to the incorporation of the degradation products of ^{14}C-carbofuran into insoluble lignin and cellulose fractions.

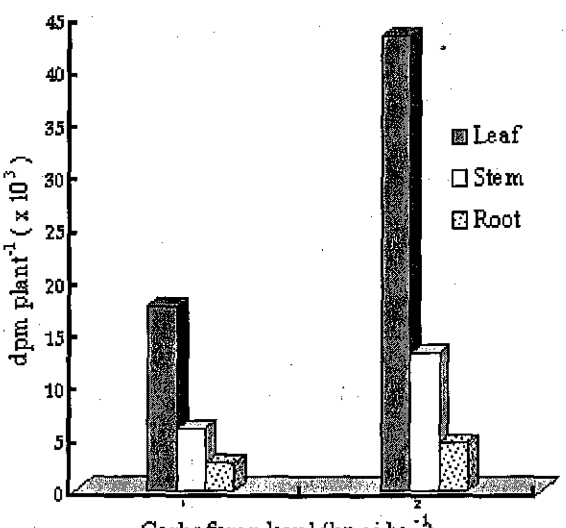

Fig. 1. Distribution of ^{14}C-carbofuran metabolites in black pepper plants

Absorption of carbofuran in relation to organic matter content

Absorption of ^{14}C-carbofuran by black pepper plants growing on the three soils are given in Table 1. Total radioactivity includes radioactivity in the HCl digest and in bound residues (radioactivity retained in the residual material after HCl digestion) in the leaf and stem portions and the total radioactivity in the root portion (determined by combustion). The data indicated differential absorption of carbofuran by the plant depending on the organic matter content of the soil. The plant absorbed highest quantity of carbofuran from the soil with lowest organic matter content and vice versa. Organic matter can influence the bioavailability of an applied chemical through differential sorption-desorption patterns (Fuhremann & Lichtenstein 1980). In the case of carbofuran, soil sorption due to organic matter may not be a deciding factor because irrespective of the organic matter content, quantities of carbofuran existing in soil solutions were very high indicating greater bioavailability (Singh *et al.* 1990). On the other hand, organic matter may enhance degradation of the applied carbofuran because of the greater microbial activity in organic matter-rich soils. This would mean that greater the organic matter content of the soil, greater will be the biodegradation of applied carbofuran

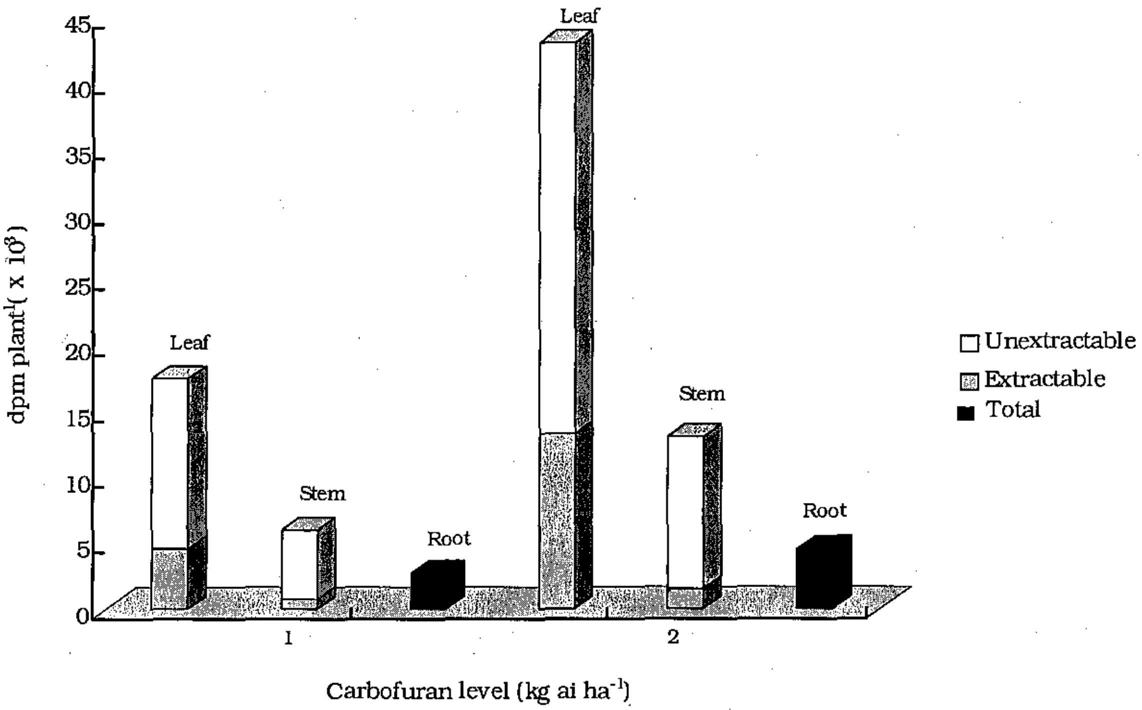

Fig. 2. Radioactivity recovered from black pepper plant tissues

and lesser the quantity of carbofuran available to the plant. The results of Lee *et al.* (1991) indicating that ^{14}C label from applied carbofuran could be detected in fulvic and humic acid fractions and in humin as bound residue also supports this view. It is evident from these results that the nematicide concentration in the soil solutions and its availability to the plant as well as its biocidal action could be reduced in soils rich in organic matter.

Autoradiography of bush pepper plant

The absorbed carbofuran was mainly translocated to the leaves and stems and to a lesser extent to the berries. The leaves contained 64.0% of the total radioactivity in the plant. The stems also contained considerable radioactivity (33.5%). Berries accounted for 1.5% and rachis 1.0% radioactivity. Nevertheless, greater care is to be exercised in bringing down the residue levels in the berry, which is used for human consumption.

Phytotonic effect of carbofuran

Black pepper plants used in pot culture experiments were used to assess the phytotonic effect, if any, due to carbofuran application. Growth of the plants at the two levels of application of carbofuran were compared with

Table 1. Absorption of applied ^{14}C-carbofuran (log dpm) by black pepper plants as influenced by soil organic matter

Carbofuran level (kg ai ha^{-1})	Soil organic matter content			Mean
	S_1	S_2	S_3	
1	4.58	4.39	3.99	4.32
2	5.01	4.51	4.46	4.66
Mean	4.80	4.45	4.23	
CD (P=0.05) Soils 0.16;		Carbofuran levels 0.13;		Interaction NS

S_1=1.28%; S_2=2.45%; S_3=3.09%

Table 2. Effect of soil-applied carbofuran on growth of black pepper plants

Soil organic matter content	Biomass production (g plant^{-1})											
	Root				Shoot				Total			
	Carbofuran level (kg ai ha^{-1})				Carbofuran level (kg ai ha^{-1})				Carbofuran level (kg ai ha^{-1})			
	0	1	2	Mean	0	1	2	Mean	0	1	2	Mean
S_1 (1.28%)	1.083	0.597	0.670	0.783	4.847	4.848	5.233	4.976	5.930	5.445	5.903	5.759
S_2 (2.45%)	1.460	0.685	0.570	0.905	5.590	5.977	5.075	5.547	7.050	6.662	5.645	6.452
S_3 (3.09%)	1.283	0.525	0.570	0.792	5.070	5.635	5.094	5.266	6.353	6.160	5.664	6.059
Mean	1.275	0.602	0.603		5.169	5.487	5.134		6.444	6.089	5.737	
CV%				31.49				21.60				21.27
CD (P=0.05) Carbofuran level				0.210				NS				NS
Soil type				NS				NS				NS
Soil type x carbofuran level				NS				NS				NS

that of plants without carbofuran application. The biomass production of black pepper plants with and without carbofuran application showed that the overall growth of the plant remained unaffected (Table 2). However, a comparison of shoot and root production indicated a significant reduction in root growth consequent to carbofuran application. Root growth decreased to the extent of 50% compared to that in the control plants. In some other plants such as rice, cowpea and sorghum, phytotonic effect of carbofuran was observed (Salam 1989; Patel & Sukhani 1990; Patel & Srivastava 1990). According to Mueke *et al.* (1978), the phytotonic effect of carbofuran in alfalfa was due to better control of aphids. In the present study, application of carbofuran was found to retard root production probably because the chemical exerted phytotoxic effect at the applied concentration.

References

Anandaraj M, Ramachandran N & Sarma Y R 1991 Epidemiology of foot rot disease of black pepper (*Piper nigrum* L.) in India. In: Sarma Y R & Premkumar T (Eds.) Diseases of Black Pepper (pp. 113–115). National Research Centre for Spices, Calicut.

Balasubramaniyan P & Morachan Y B 1981 Effect of carbofuran on the growth and yield components and yield of low land rice (IET 1444). Madras Agric. J. 68: 252–255.

Fuhremann T W & Lichtenstein E P 1980 A comparative study of the persistence, movement and metabolism of six ^{14}C insecticides in soils and plants. J. Agric. Food Chem. 28 : 446–452.

(KAU) Kerala Agricultural University 1996 Package of Practices Recommendations - Crops 1996. Directorate of Extension, Kerala Agricultural University, Mannuthy, Thrissur.

Lee J K, Kyung K S & Wheeler W B 1991 Rice plant uptake of fresh and aged residues of carbofuran from soil. J. Agric. Food Chem. 39 : 588–593.

Mueke J M, Manglitz G R & Kehr W R 1978 Pea aphid: Interaction of insecticides and alfalfa varieties. J. Econ. Ent. 71 : 61–65.

Patel G M & Sukhani T R 1990 Phytotonic effect of carbofuran on growth and dry fodder yield of sorghum cultivars. Indian J. Ent. 52 : 529–533.

Patel M B & Srivastava K P 1990 Phytotonic effect of insecticides on cowpea, *Vigna unguiculata* (Linn.) and green gram, *Vigna radiata* (Linn.). Indian J. Ent. 52 : 583–588.

Rajukkannu K 1978 Studies on the dynamics of carbofuran in soils and certain vegetable crops. PhD Thesis, Tamil Nadu Agricultural University, Coimbatore.

Ramana K V & Eapen S J 1995 Parasitic nematodes and their management in major spices. J. Spices Aromatic Crops 4 : 1–16.

Salam M A 1989 Reaction of IR-20 rice to carbofuran and urea. Int. Rice Res. Newsl. 14 : 32.

Sheela M S & Venkitesan T S 1990 Interaction between *Meloidogyne incognita* and the fungus *Fusarium sp.* on black pepper vine (*Piper nigrum* L.). Indian J. Nematol. 20 : 184–188.

Siddaramappa R & Watanabe I 1979 Evidence for vapour loss of ^{14}C-carbofuran from rice plants. Bull. Environ. Contam. Toxicol. 23 : 544–551.

Siddaramappa R, Tirol A & Watanabe I 1979 Persistence in soil and absorption and movement of carbofuran in rice plants. J. Pesticide Sci. 4 : 473–479.

Singh N, Wahid P A, Murty M V R & Sethunathan N 1990 Sorption-desorption of methyl parathion, fenitrothion and carbofuran in soils. J. Environ. Sci. Health Part B 25 : 713–728.

Vasudevan P 1985 Investigation on the interactions of ^{14}C labelled and non radio labelled carbofuran in Tamil Nadu soils and sorghum plant. PhD Thesis, Tamil Nadu Agricultural University, Coimbatore.

Wahid P A, Kamalam N V & Venugopal V K 1982 Mineral nutrition of slow wilt affected black pepper (*Piper nigrum*). J. Plantn. Crops 10 : 21–25.