

HARVEST TIME RESIDUES OF FANTAC AND BENFURACARB IN RICE AND SOIL

THESIS

SUBMITTED TO THE

G.B.Pant University of Agriculture & Technology,
Pantnagar- 263145, (U. S. Nagar) Uttaranchal, INDIA



By
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*IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
THE DEGREE OF*

Master of Science
(Agricultural Chemicals)

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Dedicated to

my mother for her dreams,

hopes and endless prayers.....

and to my father for

inciting faith and confidence in me.....

and my

beloved Family.....

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Certificate

This is to certify that the thesis entitled "**Harvest time residues of Fantac and Benfuracarb in rice and soil**" submitted in partial fulfillment of the requirement for the degree of Master of Science with major in **Agricultural Chemicals** of the college of Post Graduate studies, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of bonafide research carried out by **Renuka Pant Id. No. 32784**, under my supervision and no part of the thesis has been submitted for any other degree or diploma.

June 2007


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Certificate

We, the undersigned, members of the Advisory Committee of **Renuka Pant, Id. No. 32784**, a candidate for the degree of Master of Science with major in Agricultural Chemicals, agree that the thesis entitled "**Harvest time residues of Fantac and Benfuracarb in rice and soil**" may be submitted in partial fulfillment of the requirements for the degree.

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The economic scenario of India is dominated primarily by the agriculture sector. Nearly 64 percent of it is dependent on agriculture **(Singh, 2002)**. Agriculture uses 52 percent of the total insecticides used in India, of which rice crop alone accounts for 17 percent **(Shepard et al., 1993)**.

India is the largest technical grade manufacturer of pesticides among South Asian countries after Japan. The present production is about 80.5 thousand met. Pesticides are especially heavily used in cotton followed by rice, vegetables fruits and plantation crops, although emphasis is always on their judicious use.

The major amount of insecticides are used on rice, sugarcane, tea, root crops, vegetables, maize, wheat, groundnut, fruits, cotton and watermelon **(Gwo Chen Li. 2002)**.

Rice (*Oryza sativa*) is one of the most important staple food crop in the world. The slogan “Rice is life” is most appropriate for India as this crop plays vital role in our food security and is a means of livelihood for millions of rural household. Its is providing 43% of calorie requirement for more than 70% of Indian population. India has the largest acreage

under rice (44.6 m ha) and with production of about 90 million tones it is second to China (**The Hindu Survey of Indian Agriculture, 2005**).

Asian countries produce about 90 percent of the 576 million tons of rice grown worldwide in 2002. Typically, China and India together produce about 50 percent of the world's rice, and it is a significant agricultural crop in more than 50 other countries (**FAO**).

In India, rice consumes the largest amount of insecticides after cotton (**Pushpskumari et al., 2005**). The most common insects found on the rice are yellow stem borer, striped stem borer, pink stem borer, white stem borer, gall midge, brown plant hopper, white-backed plant hopper, green leaf hopper, leaf folder, cutworms, armyworms, scirpohaga species, leptocorisa, nymphula D; white -backed rice plant hoppers, rice leaf rollers, rice water weevils, grasshoppers, Stem borers and green leaf hoppers (**Singh, 2002**).

The harvest time residues of toxic chemicals need to be determined in the context of environment and health hazards (**Jasmine et al., 2005**). Several schedules for protecting rice/paddy involving chlorinated hydrocarbons, organophosphate, carbamates and synthetic pyrethroids have been used requiring very high as well as repeat doses (**Dikshit et al., 2005**).

Biostimulants are defined as “either natural or synthetic compounds that are applied directly to a target plant to alter its life processes or its structure to improve quality increase yields or facilitate harvesting **(Malik, 1999)**).

They have extensive application in modifying the physiological characters to improve crop productivity. They are used to balance the source and sink in plants.

The residue of biostimulant depends on their absorption and uptake by the treated leaf and subsequent translocation to different plant parts. Generally the residues of chemicals applied decrease in proportion to the time elapsed **(Bharathi et al., 2002)**.

One good example of biostimulant is Fantac which is a mixture of 5% N-acetyl thiazolidine carboxylic acid (L-cystine derivative) and 0.1% folic acid a is recommended for paddy crop. At recommended rates and in conjugation will well balanced fertilizer and pesticide programs. Fantac has shown to enhance crop yields both in quality and quantity **(Gupta et al., 1982)**.

Pesticide residues are a matter of great concern and basically impropportionate use of pesticides results in exclusive residues higher than permissible limits. These chemicals leave ill-effects on human and animal

health when the concentration increases beyond the safe tolerance limit **(Mahanthi et al., 2005)**.

Benfuracarb is a new carbamate insecticide developed by Otsuka chemical co; Ltd Japan. It controls a wide range of insects such as aphids, wireworms, corn rootworms, loopers, borers, and thrips in many different crops including cotton, corn, and beans **(Goto et al., 1983)**.

Current studies indicate that benfuracarb insecticide is effective in controlling Diamond back moth (DBM). The systemic activity of benfuracarb 5G is probably responsible for its insecticidal activity and residual effectiveness **(Takagi, 1989)**.

Analytical methodology for residue determinations has greatly advanced during the past 40 years. After application to target-area, agrochemicals and their possible degradation products and metabolites which get integrated into edible substances, biological system, water, sediment and air are called residues **(Regupathy, 2000)**.

To keep pace with increasing can be exploited and can prove to be economically beneficial. Thus, integrated approach to augment use of biostimulants and pesticides is the need of the hour **(Moore, 1979)**.

OBJECTIVES:

1. To develop a suitable method for extraction of benfuracarb and fantac from paddy and soil.
2. To perform recovery studies of benfuracarb and fantac in paddy and soil.
3. To determine the harvest time residues of benfuracarb and fantac in paddy and soil by HPLC.

Insecticides are agents of chemical or biological origin that control insects. Control may result from killing the insect or otherwise preventing it from engaging in behaviors deemed to be destructive (**Ware & Whitcare, 2004**). Carbamates tend to be more biodegradable and some are considerably less toxic to nontarget species (**Kuhr *et al.*, 1976**).

Benfuracarb is a new carbamate-insecticide developed by Otsuka Chemical Co; Ltd Japan. The chemical, toxicological and biological properties of benfuracarb (OK-174) are summarized by Japan. It has outstanding insecticidal activity against a number of pests and improved mammalian safety. In numerous field trials, it has been applied successfully as a soil and foliar insecticide and is effective against many pests including diabolic spp., Phyllotreta spp., Heteronychus spp., *Cydia pomonella* (L.), *Leptnotarsa decemlineata* (say), *Agriotes* spp., *Plutella xylostella* (L.), *Lissorhoptrus oryzophilus* Kuschel and *Aphis gossypii* Glov (**Goto *et al.*, 1983**).

History

First biologically active carbamates are Physostigmine found in legume *Physostigma venenosum*, common name is Calabar bean (growing in the Calabar province Nigeria along with the West Africa Coast line. The alkaloid present in the Calabar bean was Eserine (1863). In 1864, it was isolated & renamed Physostigmine. Biologically, *physostigmine* played a significant role in elucidating the mechanism of impulse transmission along the mammalian nervous system (**Kuhr et al., 1976**).

DISCOVERY AND DEVELOPMENT OF CARBAMATES

The first carbamate esters to exhibit insecticidal potential were derivatives of dithio carbamic acids investigated by E.J.Dupont de Nemours Company in 1931. The first bonafide carbamate insecticide was synthesized by Dr.Hans Gysin at the Geigys chemical company in Switzerland (**Kuhr et al., 1976**).

PROPERTIES

The insecticidal properties of the carbamate insecticide Benfuracarb were investigated. Benfuracarb, a sulfenylated derivative of carbofuran, proved to be a highly effective soil insecticide against a wide range of agricultural pests when applied as granules or as an emulsifiable concentrate. Under these conditions it was more effective (on a molar basis) than carbofuran.

Studies on the superior efficacy of benfuracarb were conducted using *Laodelphax striatellus* as a test insect. Benfuracarb was as effective as carbofuran by soil treatment in a closed system where no movement or loss of active ingredient in the soil occurred. Successive mobility studies in the soil indicated that the active ingredient in the benfuracarb treated soil was either mostly or completely immobile, and was retained for a longer period than was carbofuran. These properties of benfuracarb, combined with its excellent systemic action, probably lead to a greater ratio of absorption of the active ingredient than from carbofuran, thereby accounting for its greater insecticidal activity when applied to soil **(Osaki et al., 1992)**.

Benfuracarb and carbosulfan are sulfenylated pro-insecticides that are precursors of carbofuran. N-Sulfenylated pro-insecticides generally show lower mammalian toxicity, better residual insecticidal activity and lower phytotoxicity and are more lipophilic than their parent compounds. However, they also show less systemic activity in plants and are less stable on storage. Benfuracarb and carbosulfan are too lipophilic to be well translocated in plants; however, they are converted to carbofuran which is itself systemic. **(Cool, 1985)**.

Toxicity

This summary describes a number of toxicological studies conducted in the laboratory to assess the safety of the insecticide benfuracarb. Acute toxicity studies on mice, rats and dogs indicated that benfuracarb is of relatively low toxicity compared to existing carbamate insecticides. Benfuracarb was registered in Japan in October 1986 as a soil insecticide for the control of rice water weevil, thrips, and diamond back moth on vegetables and many other pests of major food crops grown in Japan. The tolerance levels of Benfuracarb in rice, fruits, vegetables and beans were set at 0.2, 0.5, 1.0 and 2.0 ppm respectively. It is concluded that Benfuracarb is safe in practical use when used as recommended (**toxicology overview, 1989**).

Uses

Benfuracarb is a contact and ingested insecticide. It is used to control insect pests in citrus, maize, rice, sugar beet and vegetables. It is active against Chrysomelidae, Elateridae, Aphididae, *Lissorhoptrus oyzophilus* and *Plutella xylostella*.

Umetsu et al. (1985) described the absorption and translocation of benfuracarb in bean and corn plants. The results revealed that benfuracarb was initially converted in and on the

plant into carbofuran, 2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl methylcarbamate, which was subsequently oxidized at the 3-position of the ring the N-methyl moiety.

Xue et al. (2003) studied on the translocation and degradation of benfuracarb in different soils in China, and leaching of benfuracarb in soils. Results indicated that the half-life of benfuracarb was 6.3-8.8 days in all the soils. Degradation of benfuracarb was slower under sterilized conditions compared with that under normal condition. When the soils were sterilized, the half-life of benfuracarb was 6.8-10.4 days in the three soils. Results suggested that chemical degradation had a greater effect than biodegradation. Within 30 days, benfuracarb was degraded fast and produced carbofuran and 3-hydroxycarbofuran.

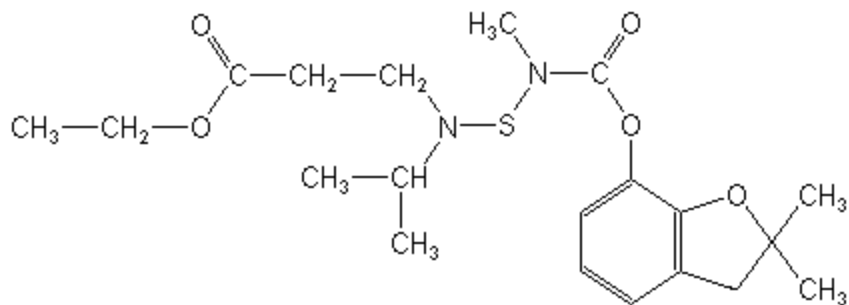
Xue et al. (2006) studied the adsorption and degradation of benfuracarb (0.05, 0.50 or 5.00 mg/litre) in sterilized or unsterilized Quaternary red earth, Fluvoaquic soil and tidal sand earth samples collected from Changsha, and Xiangying, Hunan, China. Benfuracarb degradation was slower in sterilized than in unsterilized soil samples, suggesting that chemical and biological degradation occurred. Within 30 days, benfuracarb degradation was very fast. Adsorption equilibrium was attained after

approximately 10h. The fast degradation of benfuracarb in soils (half life of 7.5-10.0days) may reduce the possibility of environmental contamination by this insecticide.

Physical and Chemical Properties:

Common name:	Benfuracarb
Chemical Name (IUPAC):	EthylN-[(2,3-dihydro2,2dirnethylbenzofuran 7-yl)oxycarbonyl(methyl)-aminothiol-N-isopropyl 1p-alaninate
Trade name:	Oncol, Furacon, OK 174,
Activity:	Benzofuranyl methylcarbamate insecticides.
CAS Registry No:	82560-54-1
Molecular formula:	$C_{20}H_{30}N_2O_5S$
Molecular weight:	410.5
Water solubility:	8 mg l^{-1} (20°C)
Vapour pressure:	2.7×10^{-5} Pa (20°C)
Physical state:	Pale yellow viscous oil
Specific gravity:	1.15 (g/ml)

Chemical structure:



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Mode of action

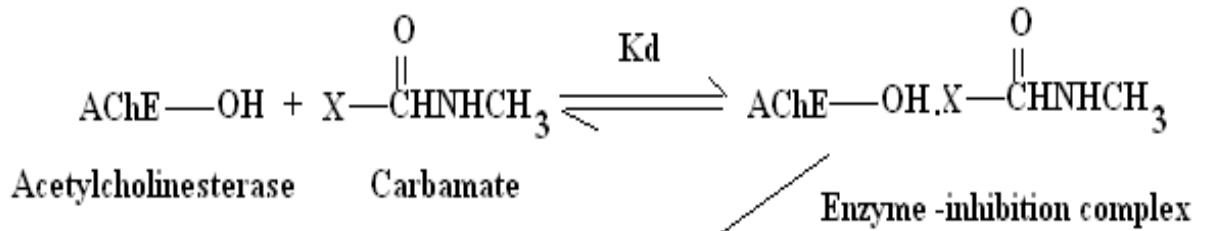
Carbamate insecticides are inhibitors of acetylcholine esterase. These compounds are rapidly detoxified and excreted so their risk to warm-blooded animals is less than the other agents. They are degraded rapidly in the environment so persistence is not a problem. There is, however, a danger to many useful insects, especially honeybees. Benfuracarb is a cholinesterase inhibitor. (<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/I/Insecticides.html#carbamates>).

The binding affinity of the N-methyl carbamates to the enzyme determines the Compound's ability and potency as a cholinesterase inhibitor. Anticholinesterase activity of N-methyl carbamates is a two-step process, the first being the formation of an enzyme inhibitor complex and the second being the carbamylation of the enzyme.

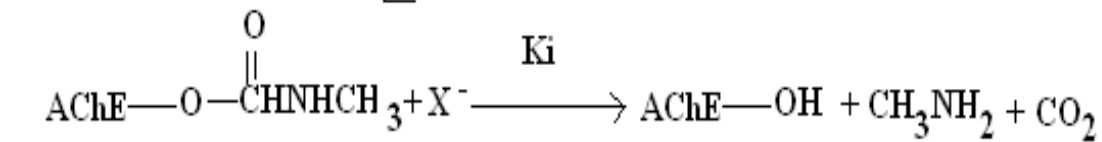
Metabolic pathway

Benfuracarb was developed as a pro-insecticide that utilizes the lability of the N-sulfenyl group to generate carbofuran. Benfuracarb is degraded in soil to carbofuran which is degraded by hydrolysis in flooded conditions. In plants and mammals, N-S bond cleavage occurs to form carbofuran which is subsequently hydrolysed and oxidised at the 3-position.

Step 1: Complex Formation



Step 2: Carbamylation



Carbamylated Acetylcholinesterase

Step 3: Enzyme Regeneration
Regenerated Acetylcholinesterase, methyl amine and CO₂

Key:-

K_d = is the equilibrium constant for the complex dissociating back to the reactants.

K_c = is the carbamylation rate constant (from complex to carbamylated enzyme) and is regarded as an estimate for the reactivity of the carbamate

K_i = the biomolecular rate constant for inhibition and is equal to k_c/K_d

AChE-OH = Acetylcholinesterase with the serine hydroxyl group.

X = is the leaving group
Chemical equation of N-methylcarbamate inhibition of AChE (**Fukuto, 1990**).

Studies

Deleu et al. (1995) studied the persistence of benfuracarb under controlled laboratory conditions. DT 50 value was found to depend on its physico chemical properties, environmental conditions and soil pH; Higher the soil humidity and temperature, the quicker the rates of dissipation.

Yasudomi et al. (1994); studied the insecticidal properties of benfuracarb against the brown rice planthopper, *Nilaparvata lugens* by different methods of application, i.e., topical, foliage, plant-base drench or granular application, parafilm test method and root dipping method. Benfuracarb, a sulfenylated derivative of carbofuran, exhibited relatively poor insecticidal activity against the pest by topical or foliage treatment, representing contact action. Soil treatment with Benfuracarb an aqueous solution, or in granular formulation, applied at the base of a potted rice plant, was at least as, or possibly more, effective than carbofuran because of the oral toxicity and systemic activity.

Mori et al. (1987) described the analytical procedures for the determination of benfuracarb and carbofuran residues in soil and water. The quantitative methods for soil samples involved extraction of residues using acetonitrile and dichloromethane followed by column cleanup using silica gel. Residues of Benfuracarb and carbofuran were detected by reversed-phase –high performance liquid chromatography using a Zorbax ODS column. Recoveries from fortified soil samples were 77-92% for Benfuracarb and 90-100% for carbofuran with a detection limit of 0.04 ppm for benfuracarb and 0.02 ppm for carbofuran. Recoveries from fortified water samples from paddy fields ranged from 94 to 96% for Benfuracarb and 94-97% for carbofuran with a detection limit of 0.001 ppm.

Meena et al. (1993) studied the efficacy of Oncol (benfuracarb), and Marshall (carbosulfan) in soil treatment at 1 & 2 kg/ha by seed soaking at 50 & 100 ppm on the development of *Meloidogyne incognita* and growth of Soyabean. The growth of soyabean was evaluated in a pot experiment. Greatest suppression of nematode numbers occurred at the highest doses, with Oncol being better as a soil treatment and Marshall better as a seed coating

Rao et al. (1987) determined the effectiveness of several insecticides as soil or seed dressings against the *Muscid Atherigona soccata* on sorghum. The highest effectiveness was obtained when seeds were treated with carbofuran at the same rate as oncol (benfuracarb) at 100 g/kg seed.

Bakheit et al. (2003) performed the residues of Benfuracarb on sweet melon the product was sprayed two times at the rate of 375 ml/fed (112.5.a.i./fed). Benfuracarb breaks down to carbofuran, which is subsequently metabolized to hydrolytic products in the form of plant conjugates.

Biostimulants

Biostimulants are organic materials which when applied in small quantities enhance plant growth development. They are also referred to, as positive plant growth regulators or metabolic enhancers. The scientific study of growth regulators began with Went's discovery of auxin activity (1928) and Kogl's structural elucidation (1934) of the first natural phytohormone, 3-Indolylacetic acid (**Draber, 1983**).

Biostimulants are used to modify a crop by changing the rate or pattern or both of its response to the internal and external factors that govern development, maturity and senescence or aging

as well as post harvest presentation. The regulation of biostimulant can be useful in numerous ways. Among others, it can (1) regulate the chemical composition of the plant and color of fruit (2) initiate or terminate the dormancy of seeds, buds and tubers (3) promote rooting and propagation (4) control plant or organ size (5) promote, delay or prevent flowering (6) induce or prevent leaf and fruit drop (8) influence mineral uptake from the soil (9) change the timing of crop development (10) increase plant resistance to pests (11) enhance plant resistance to such environmental factors as temperature, water and air pollution and (12) prevent post harvest spoilage **(Nickell, 1994)**.

Fantac

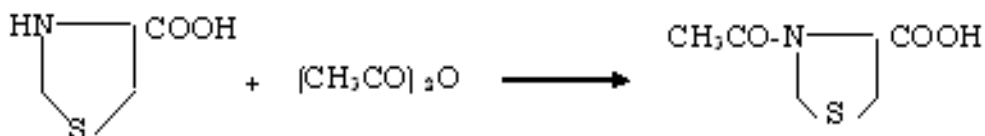
Fantac is a synthetic biostimulant which is a mixture of 5% N-acetyl thiazolidine 4-carboxylic acid and 0.1% folic acid. It enhances the plant metabolism and grants a better response under stressing conditions. It is quickly absorbed by the treated plant and, once inside, it is decomposed into L-cysteine, which has a bioactivator effect. It is manufactured by Montidison, Milan, Italy and its acute oral LD₅₀ is 4500 mg/kg for albino rates. The 'no effect' level in oral feeding studies in rats was 0.5 kg/day **(Thomas, 1982)**.

Formulation

Fantac's formulation type is a soluble concentrate. The formulation is normally applied in a established buffer solution with adjuvant, Bukovac, 2005 reported the importance of spray application and the role of spray additives in increasing the effectiveness of biostimulant. The spray application process is composed of number of interrelated components, from formulation of the active ingredient into a sprayable, bioactive solution (emulsion/suspension), to atomization, delivery, retention, and penetration into the plant tissue. Each of these events is critical to performances of the biostimulant. Also, each can be affected by spray additives, particularly adjuvants, which may be incorporated in the formulation of the active ingredient or added to the spray mixture.

Synthesis

Fantac is prepared by acetylation of 4-thiazolidine carboxylic acid with acetic anhydride (**Thomas, 1982**).



Mode of action

The favourable effect of Fantac on plant production is linked to well known role of L-cysteine and L-proline in the biochemical processes at the cell level. The advantages of providing ATCA (N-acetyl thiazolidine carboxylic acid) instead of L-cysteine lies in the fact that the plant cannot directly utilize this organic compound when applied by an external source. Penetration through root and leaves is often difficult and whenever, such compounds are absorbed they under improper translocation or metabolic degradation, that in practice, hinders their significant direct introduction into the normal nutritional pathways, ATCA, on the contrary, has its active sites adequately protected, so that it can cross the metabolic barrier in satisfactory quantities and slowly release the original amino acids into the cells. As a result of enzymatic role in many biochemical processes in the plant due to its thiol (- SH) group ATCA provides an antistress effect regulating osmotic equilibrium and enhancing mitochondrial oxidative phosphorylation under stressing conditions. (www.isagro.com).

Uses and effect

ATCA (Fantac) enhances the biochemical and physiological reserves of the plant by slowly releasing highly reactive thiol group

within the tissues. It is used to stimulate seed germination, increase plant growth, improve fruit rating and increase yield on wide range of crops including wheat corn, rice, sugar beet, potatoes, grapes strawberry and apples **(Thomas, 1982)**.

Ramteke and Somkuwar (2005) conducted a field experiment to study the effect of Fantac on grapes. Treatment comprised of Fantac @ 0.02, 0.05 and 0.1% sprayed at 45, 75 and 90 days after pruning (DAP). Clusters were also dipped twice at 102 and 112 DAP. Results revealed that Fantac increased the mean shoot length. The treatment with Fantac @ 0.05% with two foliar sprays at 45 and 75 DAP and bunch dipping once at 102 DAP was considered as the best and was effective in increasing the yield and improving berry characters.

Somasundaram et al. (1995) performed a field study to study the effect of foliar spray of Fantac on growth and leaf production of mulberry. The aqueous solution was sprayed once or twice in an irrigated mulberry garden. Results indicated that two aqueous sprays of Fantac at 0.05, 0.075 and 0.1% significantly increased the linear growth and number of leaves per plant as well as leaf yield over that obtained after a single spray and in the

control i.e. non sprayed treatment. Two aqueous sprays of 0.05% Fantac gave the highest net return.

Dubravec *et al.* (1995) conducted an experiment in which Fantac was applied twice 3 weeks after flowering and again, 10-15 days later. There was an enhancement of photosynthetic activity by it, produced a significant increase in apple yield and size. This has been shown to help maximum returns from fertilizer and other off farm investments, showing their place in sustainable agriculture system.

Ashfag *et al.* (1990) conducted an experiment to evaluate, the effect of Fantac on yield and growth behaviour of three pea cultivars (P-8, H-57 and green Feas). It was concluded that all treatments of Fantac enhanced the yield along with plant growth over control. Seed treatment plus foliar spray proved better, as regards to pods number per plant (21.83), grain per pod (5.87), pods weight per plant (71.42 gm), pod length (7.27 cm) and plant height (80.33 cm).

Environmental profile

Many toxicological studies were set up to evaluate the toxicity and the potential negative effects of the Fantac on man, animals and environment. The active ingredient did not evidenced any

negative effect in any of these toxicological studies. Application of Fantac not lead to any danger on growth, composition and circulation of blood, liver and reproductive organ in human beings and animals up till now there is no evidence of any teratogenic or mutagenic effect due to Fantac application. Trials carried on directly on human volunteers resulted in absolutely no harm to the persons in question. As a result of the above studies we can say that Fantac is safe for mammals, birds fish and insect (www.isagro.com).

The materials used and the methods followed in the present investigation are presented in this chapter.

3.1 Experimental Material

3.1.1

Chemicals	Source
Silver nitrate	E. Merck, India
Sodium dihydrogen ortho phosphate	E. Merck, India
Disodium hydrogen ortho phosphate	Spectrochem
Potassium chloride	E. Merck, India
Dichloromethane AR grade	Spectrochem,Pvt.Ltd. Mumbai
Methanol AR grade	Spectrochem,PVT.Ltd.Mumbai
Methanol HPLC grade	SpectrochemPVT.Ltd.Mumbai
Anhydrous Sodium Sulphate	E. Merck, India
Carbon tetra chloride	E. Merck, India
Activated Charcoal	Loba Chemie, India
Glass Wool	Loba Chemie, India

Florisil (100-200mesh)	E. Merck, India
Silica gel (100-200mesh)	E. Merck, India
n-hexane	E.Merck, India
Ethyl acetate	
Acetonitrile	E.Merck, India
Tert. Butanol	
Isooctane	
Sodium Chloride	E.Merck, India
Sodium dihydrogen orthophosphate	E.Merck, India

3.1.2 Instruments

1. Beckman HPLC (Model; 322) Liquid Chromatography coupled with a fixed wavelength
2. UV-VIS detector (λ_{\max} = 280nm for Benfuracarb)
(λ_{\max} = 210 nm for Fantac.)
3. Rotary vacuum evaporator (Buchi type).
4. Horizontal shaker.
5. Hot air oven.
6. Refrigerator (Voltas).
7. ph meter
8. Electronic balance.

3.1.3 Samples

Sample	Scientific name	Variety
1. Rice	Oryzae sativa	Pusa Basmati (Benfuracarb) Pant C-1 (Fantac)
2. Soil		

3.2 Experimental Site

The field experiments were conducted at G.B. Pant University of Agriculture and Technology Pantnagar, Uttarakhand situated at the foot hills of Shivalik range of Himalayas, i.e. 29°N latitude, 79.3°E longitude at an altitude of subtropical zone and represents Tarai climate.

The lab experiments were conducted in the Department of Chemistry, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar.

3.3 Field Experiment

3.3.1 The paddy crop (var.Pusa Basmati-1) was sowed on 17.6.2006 and the transplanting was done on 13.7.2006 at Crop Research Centre, Pantnagar.

3.3.2 The paddy crop (var. Pant C-1) was sowed on 19.6.2006 and transplanting was done on 20.7.2006 at Crop Research Centre, Pantnagar.

3.3.1.1 Insecticide treatment:-

A field trial of was conducted in randomized block design with three replications during the winter season of 2006-07. The plot size was 2.75m × 3.25m Interplant and inter row distance was 45 × 60cm.

Benfuracarb (Oncol 40 EC) was applied as a broadcast spray at the rate of 0.25 ppm and 0.50 ppm. There was a control treatment and three replications for each treatment. The treatment was done twice, on 12.09.06 and 29.09.06 respectively.

Fantac was also applied as a broadcast spray at the rate of 0.75 ppm and 1.50 ppm. Here too there were three replications for each treatment and also the control. The treatment was done twice, on 25.8.06 of and 10.9.06.

3.3.1.2 Sampling

The samples of paddy grain, straw, husk and soil treated with Benfuracarb were collected from each plot at the time of harvest on 08.11.06.

The samples of paddy grain, straw and soil treated with Fantac were collected from each plot at the time of harvest on 20.10.2006.

3.4 EXTRACTION OF BENFURACARB

3.4.1. Rice grain, husk and straw:

The well ground rice grain (50g), rice husk (25g) and chopped straw sample (25g) was extracted with AgNO₃ solution, pH 8.0 phosphate buffer solutions and methanol, for about half an hour. The filtrate was dried in the Rota-evaporator at a temperature < 50°C.

The extract was cleaned up by liquid partitioning with dichloromethane, and then it was passed through column packed with florisil (60-120 mesh) and silica gel (100-200 mesh size) respectively by using carbon tetra chloride, and a mixture of n-hexane: ethyl acetate. Filtrates were dried and the residue was redissolved in mobile phase [methanol: water, (75:25)] and filtered through 0.45 µm filter before subjecting to HPLC analysis.

3.4.2. Soil:

50g. air dried sample was extracted with AgNO₃ solution, pH 8.0 phosphate buffer solution and methanol, for about half an

hour. The filtrate was dried in the Rota-evaporator at a temperature < 50°C.

The extract was cleaned up by liquid partitioning with dichloromethane, and the residues were dissolved in carbon tetrachloride. The combined extract was cleaned up by column chromatography. The column was packed with florisil (60-120 mesh) and silica gel (100-200 mesh size) respectively by using CCl₄, and mixture of n-hexane: ethyl acetate. Filtrates were dried and the residue was redissolved in mobile phase and filtered through 0.45 µm filter before subjecting to HPLC analysis.

3.5 EXTRACTION OF FANTAC

3.5.1. Rice grain and straw:

The well ground rice grain (50g), rice husk (25g) and chopped straw samples (25g) were extracted with 50 mL methanol for one hour and filtered. The residue was re-extracted twice with methanol and filtered. The combined filtrate was concentrated in a rotary vacuum evaporator and taken in a 500 mL separatory funnel with addition of 25 mL isooctane/0.01% tertiary butanol in dichloromethane (1:4) was added and shaken vigorously for 30 seconds. After layer separation the organic phases were collected in a conical flask. The aqueous portion was again portioned with 25

mL isooctane /0.01% t-butanol in dichloro methane (1:4) and the organic phase was combined. It was then passed through anhydrous sodium sulphate for drying. Then it was evaporated to near dryness using a rotary evaporator and was dissolved in 2 mL of methanol- acetonitrile mixture.

3.5.2 Soil:

50 gram soil sample was mixed with 40 mL methanol in a wide mouthed conical flask and shaken for 30 min on a mechanical shaker and subsequently samples were filtered through a Buckner funnel and washed with 2 mL methanol. The combined filtertrate was transferred to a separatory funnel. To the separatory funnel containing extract 25 mL isooctane / 0.01% t-butanol in dichloro methane (1:4) was added and shaken vigorously for 30 seconds. After layer separation the organic phases were collected in a conical flask. The aqueous portion was again portioned with 25 mL isooctane / 0.01% t-butanol in dichloromethane (1:4) and the organic phases were combined. The combined extract was then passed through anhydrous sodium sulfate for drying. Then it was evaporated to near dryness using a rotary vacuum evaporator.

The methanol acetonitrile extract was cleaned up by column chromatography using silica gel (60-120 mesh) (absorbent),

dichloromethane, activated charcoal and anhydrous sodium sulfate. The column was eluted successively with methanol and acetonitrile mixture. The eluent was immediately evaporated to dryness in a rotary vacuum evaporator and the residue was reconstituted in 2 mL acetonitrile, sodium dihydrogen orthophosphate (6:94) pH 2.5 solution for final HPLC analysis.

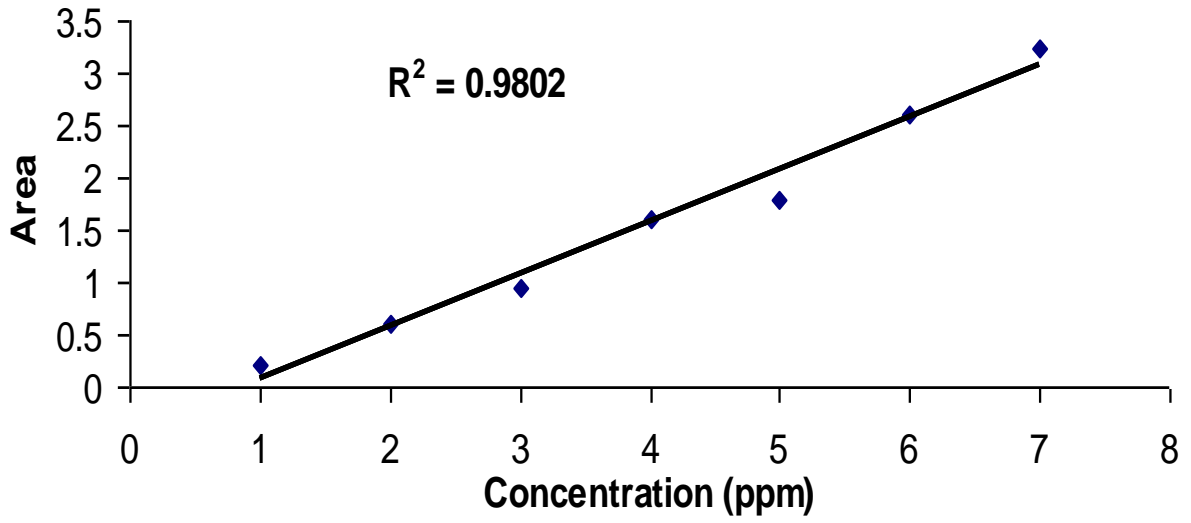
3.6.1 HPLC analysis of the Residue

The samples obtained from the above (3.4 & 3.5) were subjected to HPLC analysis. The amount of residue was studied by analyzing the sample by HPLC in following steps.

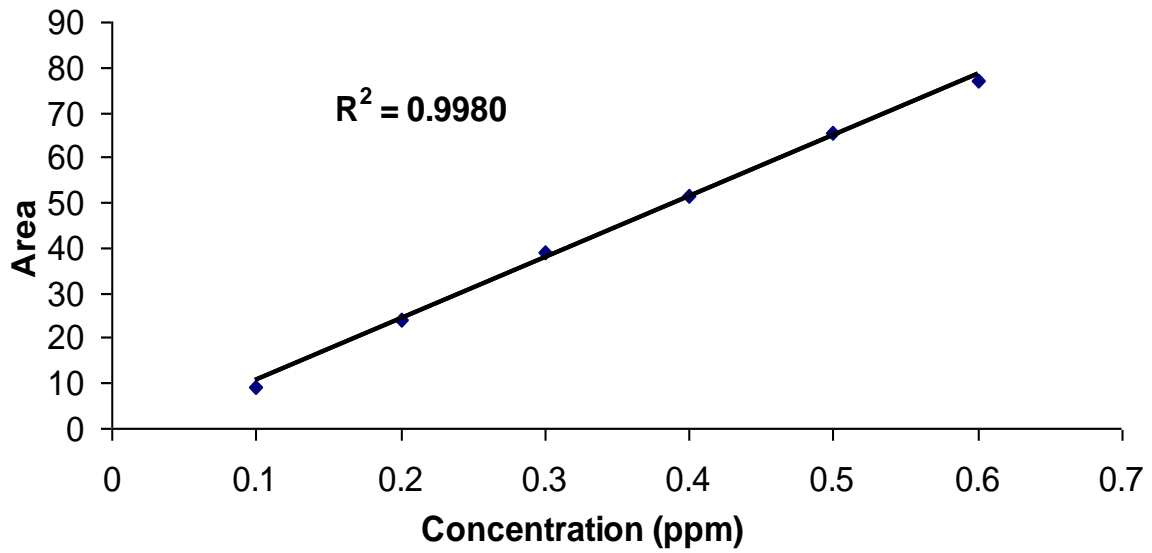
1. Standardization of conditions for standards of Benfuracarb and Fantac.
2. Injection of standards of Benfuracarb and Fantac.
3. Preparation of standard curve for this 200 ppm stock solution of analytical grade Benfuracarb and Fantac were prepared in methanol. Serial dilutions of varying concentrations ranging from 5 to 10 ppm were prepared by further dilution with methanol for Benfuracarb and acetonitrile- sodium dihydrogen orthophosphate (6: 94) pH 2.5 for Fantac.
4. The mobile phases were used acetonitrile- sodium dihydrogen orthophosphate (6: 94) pH 2.5 for Fantac and methanol: water

(75:25) for Benfuracarb. 10 μ l of the samples were injected for HPLC analysis.

5. Peak areas for each concentration were calculated and a calibration curves were plotted between peak area and concentration.



Calibration Curve of Benfuracrb (ppm)



Calibration Curve of Fantac (ppm)

3.6.2 HPLC conditions

Model	:	Beckman HPLC
Column	:	C-18 (25cm length × 0.4 mm)
Solvent	:	Acetonitrile- sodium dihydrogen orthophosphate (6: 94) pH 2.5 (Fantac) & methanol :Watar (75:25) (Benfuracarb).
Wavelength (λ_{\max})	:	280nm. (Benfuracarb) & 210nm. (Fantac).
Flow rate	:	1.5mL/min (Benfuracarb) 1mL/min (Fantac).
Retention time	:	10.2 min (Benfuracarb) and 10.5 min (Fantac).

3.7 Recovery Studies

Recovery studies were carried in order to establish the reliability of the analytical methods to know the efficiency of extraction and clean up steps for the present study.

3.7.1 for Benfuracarb

The rice grain, husk, straw and soil samples were fortified with 50 ppm analytical standard of Benfuracarb. The samples were

extracted and cleaned up following the procedure given in the proceeding sections (3.4).

Recovery percent was calculated as follows:

$$\text{Recovery \%} = \frac{\text{Concentration of Benfuracarb in fortified samples}}{\text{Concentration of analytical standard of Benfuracarb}} \times 100$$

3.7.1 for Fantac

The rice grain, straw and soil samples were fortified with 50 ppm analytical standard of Fantac. The samples were extracted and cleaned up following the procedure given in the proceeding sections (3.5).

Recovery percent was calculated as follows:

$$\text{Recovery \%} = \frac{\text{Concentration of Fantac in fortified samples}}{\text{Concentration of analytical standard of Fantac}} \times 100$$

The experimental results of the present investigation on the harvest time residue studies of Benfuracarb in rice grain, husk, straw and soil, and harvest time residue studies of fantac in rice grain, straw and soil are presented and discussed in this chapter.

4.1 Some general properties of soil samples

General properties of soil samples taken in the study are presented in Table 4.1

The soil was clay loam (0-15cm). The pH (1:2 soil water suspensions) was 7.44. The electrical conductivity (1:2 soil water suspension) was 0.283 dSm⁻¹ and the content of soil organic carbon was 1.18 percent.

4.2.1 Recoveries of Benfuracarb from rice grain, husk, straw and Soil

The values of percent recovery of Benfuracarb from fortified samples of rice grain, husk, straw and soil are depicted in Table 4.2.1. It is evident from the data presented in Table 4.2.1 that the recovery of Benfuracarb from soil samples varied from 87 to 92%. For rice grain, husk and straw the recovery of Benfuracarb varied from 92.5 to 94.5%, 92.6 to 95.3 % and 90.2 to 94.2%, respectively.

4.2.2 Recoveries of Fantac from rice grain, straw and Soil

The values of percent recovery of Fantac from fortified samples of rice grain, straw and soil are depicted in Table 4.2.2. It is evident from the data presented in Table 4.2.2 that the recovery of Fantac from soil samples varied from 85.6 to 88.4%. For rice grain and straw the recovery of Fantac varied from 89 to 92.2%, and 90.8 to 93 %, respectively.

West (1997) also reported that the recoveries of Benfuracarb and Fantac varied from 87 to 92% and 85.6 to 88.4% respectively in soil under laboratory conditions.

Table 4.1 Some general properties of soil surface (0-15 cm)

S. no.	Properties	
1.	Sand %	59.0
2.	Silt %	27.0
3.	Clay %	14.0
4.	Organic Carbon %	1.18
5.	pH	7.44
6.	Electric Conductivity (1:2dSm ⁻¹)	0.283

Table 4.2.1 Percent recovery of Benfuracarb from fortified samples of soil, rice grain, husk and straw

Sample	% Recovery	Mean
Soil	87	89
	88	
	92	
Rice grain	93	93.3
	94.5	
	92.5	
Rice husk	95.3	94.2
	94.8	
	92.6	
Rice straw	90.2	92.8
	94	
	94.2	

Table 4.2.2 Percent recovery of Fantac from fortified samples of soil, rice grain and straw

Sample	% Recovery	Mean
Soil	85.6	87.03
	87	
	88.4	
Rice grain	89	90.8
	91.2	
	92.2	
Rice straw	90.8	92
	92.2	
	93	

4.3.1 Harvest time residues of Benfuracarb in rice grain, husk, straw and soil

The harvest time residues of Benfuracarb in rice grain, husk, straw and soil were studied. Benfuracarb formulation (Oncol 40EC) was applied at the rate of 0.25 ppm and 0.50 ppm. The Benfuracarb residues were estimated in the harvested samples of rice grain, husk, straw and soil. (Table 4.3.1)

The results revealed that the residues of Benfuracarb were non detectable (<0.01ppm) for both the treatments. Therefore the use of this insecticide on rice does not pose any problem on human and animal health. The presence of Benfuracarb residue in soil was also below detectable limit and hence it is a safe from the environmental pollution point of view.

4.3.2 Harvest time residues of Fantac in rice grain, straw and soil

The harvest time residues of Fantac in rice grain, straw and soil were studied. Fantac formulation was applied at the rate of 0.75 ppm and 1.50 ppm. The Fantac residues were estimated in the harvested samples of rice grain, straw and soil and are represented in Table 4.3.2.

The results revealed that the residues of Fantac were non detectable (<0.01ppm) for both the treatments in rice grain, straw and soil. Here too, since the residues of Fantac were non detectable its usage can be considered safe for the environment as well as for animals and human beings.

Table 4.3.1 Harvest time residues of Benfuracarb(Insecticide) in rice grain, husk, straw and soil applied at 0.25 ppm and 0.50 ppm

Sample	Treatment rates	
	0.25 ppm	0.50 ppm
Rice grain	N.D.	N.D.
Rice husk	N.D.	N.D.
Rice straw	N.D.	N.D.
soil	N.D.	N.D.

(N.D. < 0.01 ppm)

Table 4.3.2 Harvest time residues of Fantac (biostimulant) in rice grain, straw and soil applied at 0.75 ppm and 1.50 ppm

Sample	Treatment rates	
	0.75 ppm	1.50 ppm
Rice grain	N.D.	N.D.
Rice straw	N.D.	N.D.
soil	N.D.	N.D.

(N.D. < 0.01 ppm)

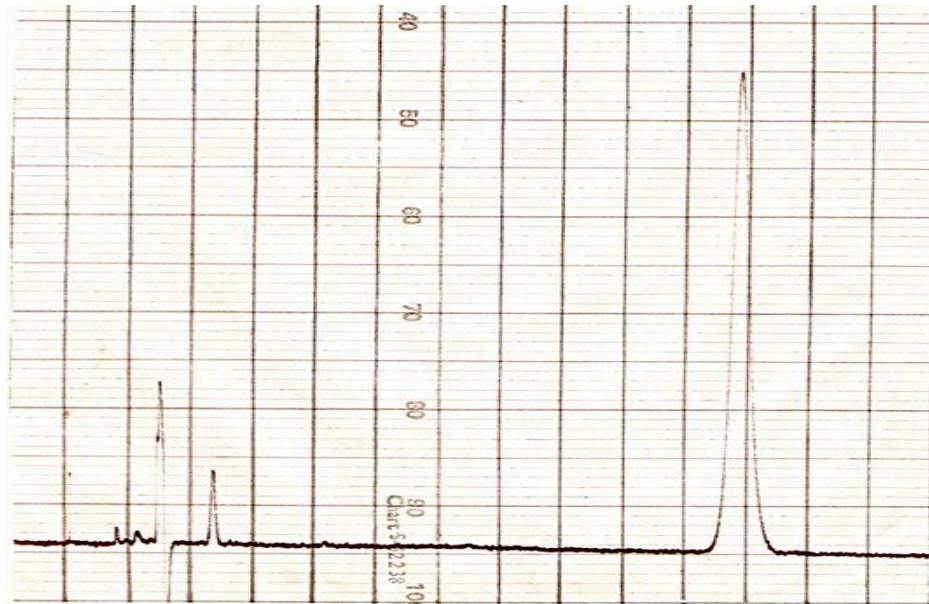


Figure 1a. Standard Chromatogram of Benfuracarb.

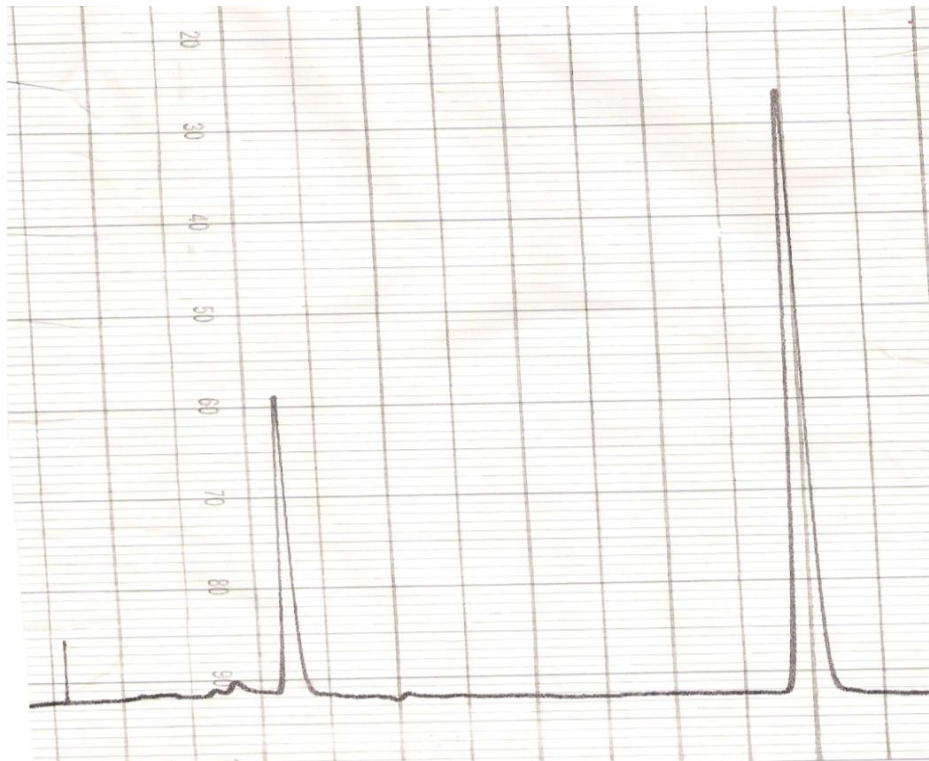


Figure 1b. Standard Chromatogram of Fantac.

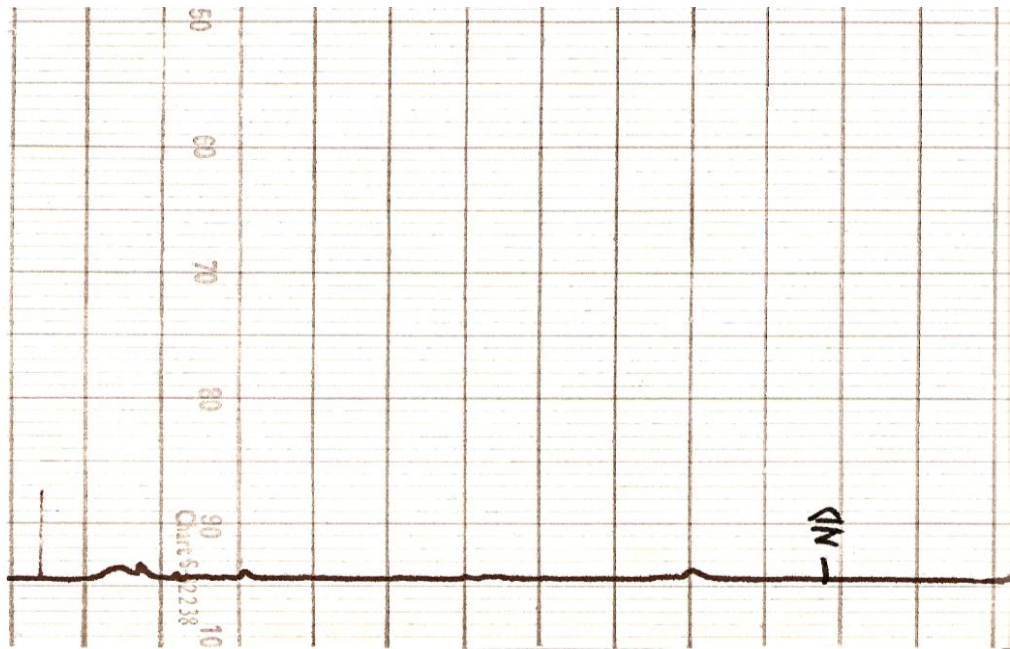


Figure 2a. Benfuracarb residues in Control sample of rice grain.



Figure 2b. Benfuracarb residues in Control sample of rice husk.



Figure 2c. Benfuracarb residues in Control sample of rice straw.



Figure 2d. Benfuracarb residues in Control sample of soil.



Figure 3a. Residues of Benfuracarb in rice grain @ 0.25 ppm.

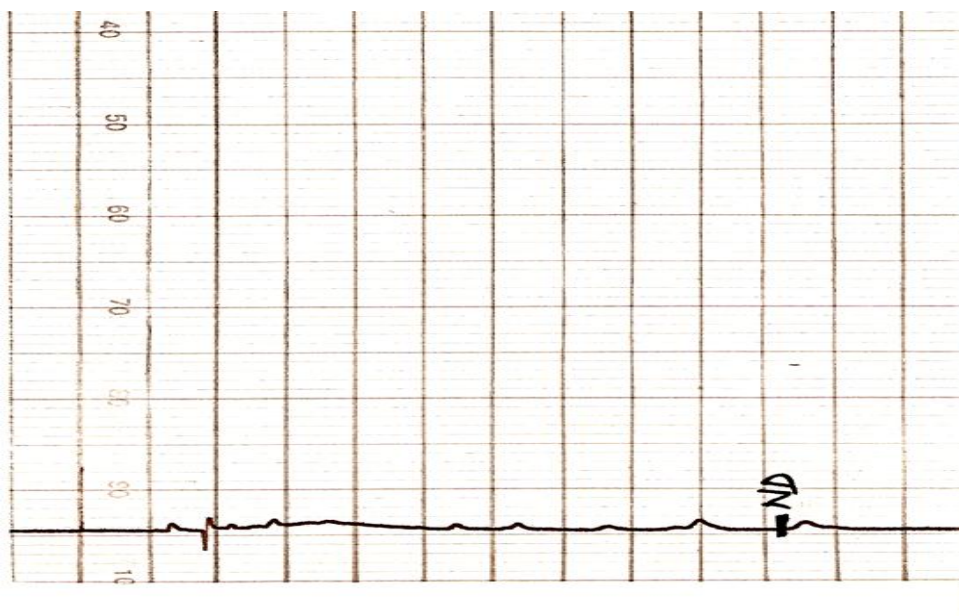


Figure 3b. Residues of Benfuracarb in rice husk @ 0.25 ppm.



Figure 3c. Residues of Benfuracarb in rice straw @ 0.25 ppm.



Figure 3d. Residues of Benfuracarb in soil @ 0.25 ppm.



Figure 4a. Residues of Benfuracarb in rice grain @ 0.50 ppm.



Figure 4b. Residues of Benfuracarb in rice husk @ 0.50 ppm.

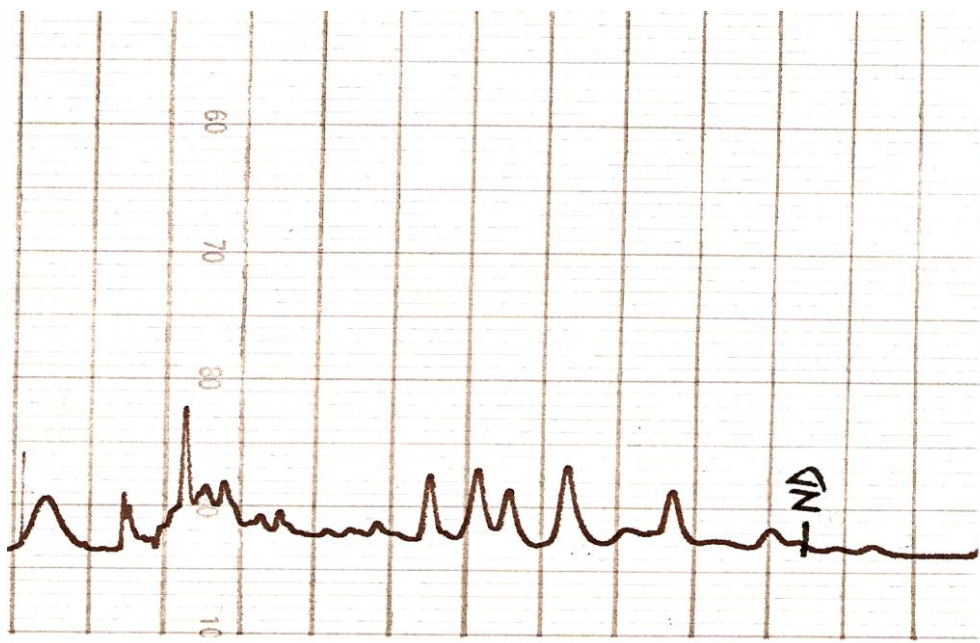


Figure 4c. Residues of Benfuracarb in rice straw @ 0.50 ppm.



Figure 4d. Residues of Benfuracarb in soil @ 0.50 ppm.



Figure 5a. Fantac residues in Control sample of rice grain.



Figure 5b. Fantac residues in Control sample of rice straw.

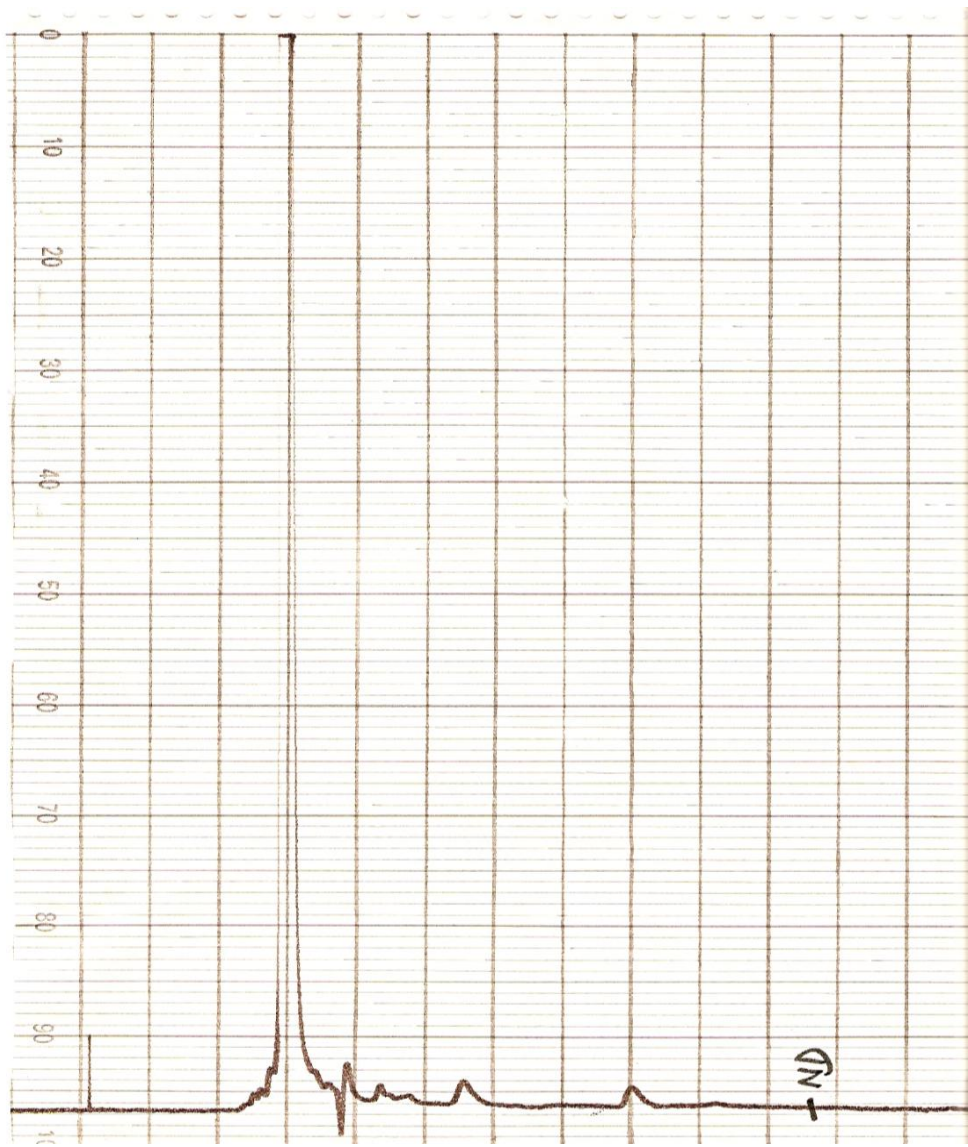


Figure 5c. Fantac residues in Control sample of soil.



Figure 6a. Residues of Fantac in soil @ 0.75 ppm.

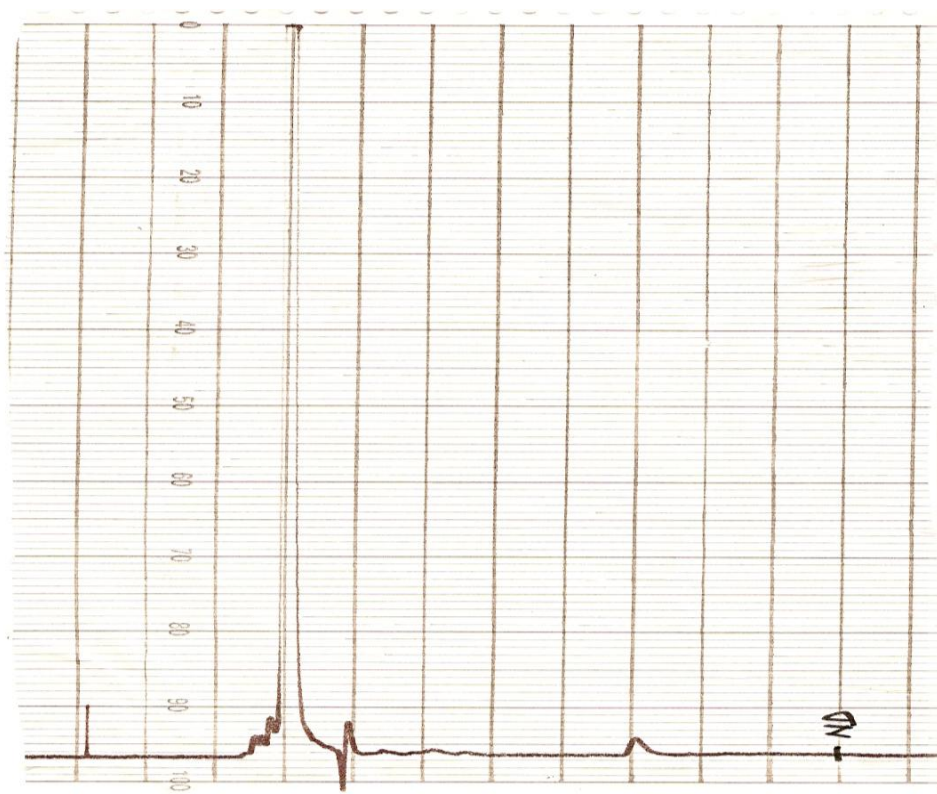


Figure 6b. Residues of Fantac in rice grain @ 0.75ppm.



Figure 6c. Residues of Fantac in rice straw @ 0.75ppm.



Figure 7a. Residues of Fantac in rice straw @ 1.50ppm



Figure 7b. Residues of Fantac in soil @1.50ppm.

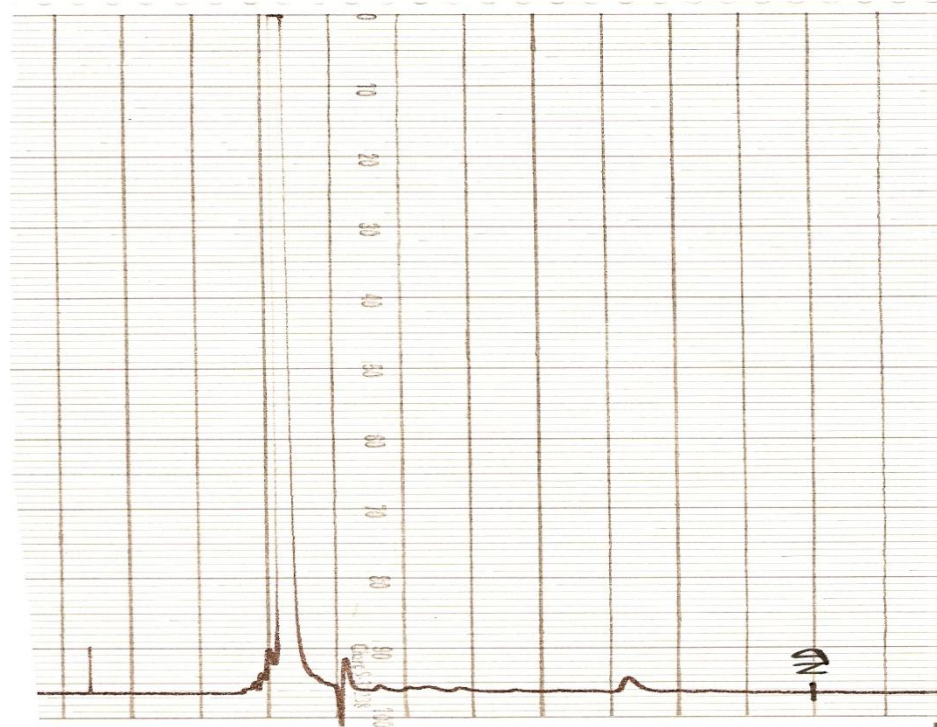


Figure 7c. Residues of Fantac in rice grain @1.50ppm.

A laboratory investigation was undertaken to determine the harvest time residues of Benfuracarb in rice grain, husk, straw and soil and that of Fantac in rice grain, straw and soil. The findings of the presented investigation are presented here in:

1] A simple method of extraction of Benfuracarb from rice grain, husk, straw and soil was developed involving extraction with methanol and further clean up procedure. The clean up procedure involved the partitioning of extract with dichloromethane followed by column clean up by using carbon tetrachloride and a mixture of n-hexane: ethyl acetate. The recoveries of Benfuracarb from rice grain, husk, straw and soil were in the range of 92.5 to 94.5%, 92.6 to 95.3%, 90.2 to 94.2% and 87 to 92% respectively.

2] A simple method of extraction of fantac from rice grain, straw and soil was developed which involved extraction with methanol followed by clean up of the extract. The extract was partitioned with isooctane/0.01%t-butanol in dichloromethane (1:4) followed by column clean up by methanol, acetonitrile mixture (1:1). The recoveries of Fantac from rice grain, straw and soil were in the range of 89 to 92.2%, 90.8 to 93% and 85.6 to 88.4% respectively.

3] The high percentage of recoveries indicate that the present methods are rapid and suitable method for estimating Benfuracarb and Fantac.

4] The analysis of the samples was done by High Performance Liquid Chromatography.

5] Benfuracarb formulation (Oncol 40EC) was applied at the rate of 0.25 ppm and 0.50 ppm and Fantac formulation was applied at the rate of 0.75 ppm and 1.50 ppm. The results revealed that the residues of Benfuracarb and Fantac were non detectable (<0.01ppm) for both the treatments. Thus, it can be concluded that both.

6] Benfuracarb and Fantac are safe from environmental and health points of view as their residues if present are in very low concentrations both in crop and soil the time of harvest.

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ABSTRACT

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Sem & Year: 1st, 2005-06

Topic: "Harvest time residues of Fantac and Benfuracarb in rice and soil"

Benfuracarb is a new carbamate insecticide which controls a wide range of insects such as aphids, wireworms, corn rootworms, loopers, borers, and thrips in many different crops including cotton, corn, and beans.

Fantac (biostimulant) is a mixture of 5% N-acetyl thiazolidine carboxylic acid (L-cystine derivative) and 0.1% folic acid. It is used to stimulate seed germination, increase plant growth, improve fruit rating and increase yield on wide range of crops including wheat corn, rice, sugar beet, potatoes, grapes strawberry and apples.

Harvest time residues of Benfuracarb and Fantac in paddy grain, husk, straw and soil were determined. Benfuracarb formulation (Oncol 40EC) was applied at the rate of 0.25 and 0.50 ppm and Fantac formulation was applied at the rate of 0.75 and 1.50 ppm. The analysis of the samples was done by high performance liquid chromatography. The results revealed that the residues of Benfuracarb and Fantac were non detectable (<0.01ppm) for both the treatments. Benfuracarb and Fantac are safe from environmental and health points of view as their residues if present are in very low concentrations both in crop and soil at the time of harvest.

(Bali Ram)
Advisor

(Renuka Pant)
Authoress