DETERMINATION OF RESIDUES OF
CARBARYL AND ENDOSULFAN ON/IN
MANGO FRUITS

BY
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B. Sc. (Agri.)

A THESIS
SUBMITTED TO THE FACULTY OF AGRICULTURE,
Gujarat Agricultural University, Dantiwada,
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
For the Degree of
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AGRICULTURAL ENTOMOLOGY

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ABSTRACT

This study on the determination of residues of carbaryl and endosulfan on/in mango fruits is in partial fulfilment of the M.Sc. (Agri.) Course which VALAND VITHALBHAI MAGANBHAI has taken up under the direction of Dr. Shah A. H., Professor and Head, Department of Entomology, N. M. College of Agriculture, Navsari.

Chemical and micro bio-assay of residues of recommended insecticides, viz., carbaryl and endosulfan which were used at the concentration of 0.2 per cent and 0.075 per cent respectively, as schedule of spraying in 1976-77 and only one spraying in 1977-78 as a part of insect pest management programme in mango crop have been carried out.

In the chemical assay the residues of carbaryl on the mango fruits found immediately after the last spraying was 58.15 ppm in 1976-77, and the same after 3rd, 7th and 25th days were 37.22, 18.49 and 10.30 ppm respectively. Similarly, it was 52.56 ppm as initial deposit in 1977-78 and the same after 3rd, 7th and 19th day of one spraying were 38.00, 20.50 and 8.80 ppm respectively. Thus the reduction percentage of carbaryl residue found were 36.00, 50.32 and 65.71 per cent in 1976-77 and 27.70, 61.00 and 83.26 per cent in the year 1977-78 on 3rd, 7th and 25th/19th day after spraying of carbaryl respectively. The half life of carbaryl residues was 3.19 and 3.00 days in 1976-77 and 1977-78 respectively.
Washing of carbaryl-sprayed mango fruits had the initial residues of 42.52 ppm and 14.79, 13.61 and 8.82 ppm on 3rd, 7th and 25th day of spraying respectively in the year 1976-77. The same was 21.13 ppm in 1977-78 which thereafter reduced to 14.35, 13.62 ppm and below detectable level on 3rd, 7th and 19th day of spraying respectively. The reduction percentage thus due to washing treatment varied from 60 to 100 per cent within 25/19 days of spraying of carbaryl.

In residue determination by micro bio-assay method initial residue deposit of carbaryl on fruits was 76.00 ppm in 1976-77 and 58.56 ppm in the year 1977-78. The reduction percentages were 39.24, 100 and 100 per cent in 1976-77, but 36.17, 85.35 and 95.18 per cent in 1977-78 on 3rd, 7th and 25th/19th days, after spraying of carbaryl, respectively. The reduction percentage of carbaryl residue due to washing treatment varied from 35 to 96 per cent within three days and reached 100 per cent within seven days in both the years.

In the chemical assay method the initial residue deposit of endosulfan on the fruits found immediately after last spraying was 73.94 ppm and the same had reached to 15.52, 13.68 and 5.66 ppm after 3rd, 7th and 25th days respectively of spraying in the year 1976-77, while it was 9.75 ppm as initial deposit and was reached to 7.09, 6.25 and 2.53 ppm after 3rd, 7th and 19th day respectively of spraying for the year 1977-78. The reduction percentage of endosulfan residue thus were 79.01, 88.02 and 92.35 per cent in 1976-77 and 27.28, 35.90 and 74.5 per cent in the year 1977-78 for different time intervals observed.
The half life of endosulfan residue was 1.76 and 3.54 days in the year 1976-77 and 1977-78 respectively.

Washing of endosulfan-sprayed fruits showed the initial residue deposit of 24.51 ppm and reaching to 9.33, 3.24 and 2.53 ppm on 3rd, 7th and 25th days respectively in the year 1976-77, but initial endosulfan residue deposit of 4.12 ppm observed reached to 3.06, 1.47 and 0.30 ppm after 3rd, 7th and 19th day respectively in the year 1977-78. The reduction percentage thus due to washing treatment varied from 66 to 88 per cent within 25/19 days of spraying of endosulfan.

On the basis of residue analysis as stated above it is inferred that the time interval of 19/25 days should be observed between last spraying and the harvest in case of carbaryl, while in the case of endosulfan it should be more than 25 days if sequential spraying is done but where only one spraying is carried out then washing of fruits does help in reducing residue level of endosulfan below tolerance limit even after 7 days of treatment.
DECLARATION

This is to declare that the whole of the research work now submitted in this thesis as a partial fulfilment for the requirements for the degree of Master of Science (Agriculture) in Agricultural Entomology is the result of investigations done by the undersigned under the direct guidance and supervision of Dr. A. H. SHAH, Professor and Head, Department of Entomology, and no part of the work has been submitted for any other degree so far.

NAVSARI,
Dt. 29 August , 1978

(V. M. VALAND)

Countersigned by

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Professor and Head,
Department of Entomology,
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BIOGRAPHY

The author was born on February 1, 1942, at Baroda, District Baroda of Gujarat State (India). On completion of his primary education at his native place, Village Shihol, Taluka Petlad, District Kaira, he has gone through his S.S.C. Course of study at the then N.K. High School, Petlad, District Kaira, in the year 1960.

He received the degree of Bachelor of Science in Agriculture with second class through B.A. College of Agriculture, Anand, from Sardar Patel University, Vallabhbhai Vidyanagar, in 1965 and subsequently joined the Department of Agriculture, Gujarat State, in 1965. Being a transferred employee of the Gujarat Agricultural University since July, 1972, he was selected and deputed for higher training in Entomology leading to the degree of Master of Science (Agriculture) at N.M. College of Agriculture, Gujarat Agricultural University, Navsari Campus, in 1976.

The author's family includes his wife, Kusum, whom he married in 1965, three children, a son, Rashmikant, and two daughters, Pratima and Avnita.
ACKNOWLEDGEMENT

The author is fortunate in having Dr. A. H. Shah, M.Sc.(Ento.), Ph.D. (U.S.A.), Professor and Head, Department of Entomology, Gujarat Agricultural University, N. M. College of Agriculture, Navsari, as his advisor, to whom he is profoundly indebted for suggesting this problem, giving continuous guidance in carrying out this study and constant inspiration and painstaking help in preparing this manuscript.

He is thankful to the authorities of the Gujarat Agricultural University, Sardar Krishi Nagar, Dantiwada (District Banaskantha) for deputing and supporting him for this higher training in the subject of Agricultural Entomology leading to the degree of Master of Science (Agriculture).

He is grateful to Shri Mahavirbhai Joshi, the Director of Campus, Gujarat Agricultural University, Navsari, for allowing him to conduct the field spraying experimental trial on his own farm at village Changa, Taluka Gandevi, District Bulsar, in the year 1976-77. Thanks are also due to the Professor and Head, Department of Horticulture, N.M.College of Agriculture, Navsari, for sparing necessary number of mango trees at Navsari, for the research work in the year 1977-78.

He is also thankful to the Professor and Head, Department of Chemistry, N.M. College of Agriculture, Navsari, and the Irrigation Agronomist, Water Management and Soil Salinity Project, Gujarat Agricultural University, Navsari, for providing
necessary laboratory facilities to carry out the residual analytical work. Thanks are also due to the Principal, N. M. College of Agriculture, Navsari, for providing necessary facilities for the postgraduate research work.

He wishes to thank Dr. C. B. Patel, Associate Professor of Entomology, Shri V. T. Jose, a senior research fellow in the Department of Agricultural Entomology and Shri K.S.S. Pillai, Lecturer in English, for the help rendered during this work as well as in the preparation of this manuscript.

He is also grateful to the staff members of the Entomology Department, Plant Pathology Department, Chemistry Department and Horticulture Department for their help during the period of training.

He cannot forget his all other friends and colleagues who have directly or indirectly helped him during the period of this training.

Lastly he is greatly thankful to his parents and brothers for their bearing with great hardships to provide him all the tender care and necessary provisions to acquire the most needed under-graduate education prerequisite for higher training and also to his wife and children who sustained a lot due to him being comparatively unattentive to them during long period of time of this training.
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1. Average number of two spotted spider mite per five leaves of 16 mango trees before and after the fourth spraying of 0.2 per cent carbaryl and 0.075 per cent endosulfan in the experiment of insecticidal residue at village Changa, Taluka Gandevi, District Bulsar, in 1976-77. | 121 |
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1. Average number of two spotted spider mite per five leaves of mango trees before and after the 4th spraying of 0.2 per cent carbaryl and 0.075 per cent endosulfan ....... .. 121a
There are no two opinions that all insecticides are poisonous and consequently hazardous. This does not imply that we should stop their use for the welfare of mankind. This only calls for greater precautions and judicious use to guard against hazards due to immediate toxicity and environmental pollution. Even F.A.O. and W.H.O. have not categorically suggested any ban on the use of pesticides, but they have suggested a highly judicious use keeping in view Acceptable Daily Intake for human beings, which have been determined for a few pesticides. The conditions in a developing tropical country like India are basically different from those in advanced temperate countries, and climatic degradation of pesticides due to higher temperature conditions is faster in the tropical countries than in the latter.

Though an insecticide is effective in giving significant control of an insect pest, it cannot be recommended without finding out the safe period from the point of view of hazards to consumers. Bindra and Kalra (1973) have reviewed the work done in India on pesticide residues' safe period or waiting period. The time required for the insecticide residues
to come below the tolerance level is considered as the waiting period. Thus, if the waiting period is strictly observed for harvesting and consumption of the commodity, the treatments with insecticides are not hazardous.

No doubt the Insecticide Act will take care of the precautions needed for manufacture, formulation, storage, sale etc. of the pesticides, but their use should also be watched and regulated. Pest control practices should be based on recommendations of experts so as to have judicious use of the pesticides on crop plants, fruit trees etc. in various places of India under different environmental conditions. These recommendations should not be a static phenomenon but may be reviewed every year based on the findings of current scientific investigations and quickly made available to the operational staff for action in the field.

With the view stated above, it was thought necessary to investigate the status of pesticide on/in mango fruits after repeated use of insecticides in the mango plantations in South Gujarat. This is because in India mango is considered, from time immemorial, to be the king of all fruits and has relatively the same position in the tropics as enjoyed by apple in America and Europe.

In India, mango covers an area of about 7.5 lakh hectares, which is 61.2 per cent of the total estimated area
under fruits crops. Gujarat is well-known for her Alphonse (Hafus) variety of mango, mainly grown in Bulsar District.

The mango hopper is the most destructive insect pest among all the mango pests reported. It is found active throughout the year (Patel et al. 1973) and is responsible for lower yield because of its heavy infestation and damage during inflorescence and bud formation period. The nymphs are more destructive than the adults. On the recommendations of the Department of Agriculture and Gujarat Agricultural University farmers are nowadays using "Carbaryl" and "Endosulfan" in abundance on the mango trees right from the initiation of inflorescence till the harvesting of fruits as substitute for "Endrin" and "DDT" commonly used in the past. Sarup et al., (1965); Atwal (1976); Tandon et al., (1976) and Shah et al., (1978) also recommended the use of 0.1 to 0.2 per cent 'Carbaryl' and 0.03 to 0.07 per cent 'Endosulfan' three to six times at an interval of three to four weeks during the inflorescence and fruit formation period in the mango orchards.

Since Carbaryl and Endosulfan are the most common insecticides being used in the mango orchards of Gujarat, it was thought imperative to investigate, through this research, the status of residues of these two insecticides i.e., carbaryl and endosulfan in/on mango fruits due to their repeated use.
as a part of plant protection measures against mango hoppers under the South Gujarat conditions so as to fill the lacunae of information in our knowledge regarding their residue level as well as their waiting period. This will be of much use to organise plant protection practices not only based on the findings of an insecticide being effective and significant for control of an insect pest but also from the point of view of hazards to the consumers.
CHAPTER II

REVIEW OF LITERATURE

Pesticides have contributed a lot to the increased agricultural production by preventing losses caused by insects, pests and diseases in the developing countries. In order to accomplish their purpose, the pesticides must be lethal to pests, and should be less harmful to human being and livestock.

More stress is being given for the cultivation of fruits and vegetables as a part of the programme of balanced diet. To harvest a bumper crop, the use of pesticides is a must. The cultivators have no idea of health hazard of the residues of the insecticides because of their nonjudicious use. This is because of the fact that cultivators are not observing proper time interval between the use of the insecticides and harvest and as such the produce marketed are having pesticide residues beyond the tolerance limit.

As carbaryl and endosulfan are being considered safer among the insecticides because of their low mammalian toxicity, their use in India has amounted to 77 and 446 metric tonnes of active ingredient and 200 and 446 metric tonnes of active ingredient respectively during 1976 and 1977. Further, endosulfan is popular for its selective action towards the
pests and harmless to the predators, parasites and pollinators, while carbaryl is only slightly selective but safer for human beings, even though these insecticides were found hazardous because of their indiscriminate use and non-observance of waiting period between last spraying and harvest of produce for consumption. In view of the above facts the work on the estimation of insecticidal residues on harvested products in the agricultural crops is essential to have the idea regarding the safety of the use of various insecticides in agricultural production.

A review of the work done on the residual status of carbaryl and endosulfan insecticides in fruit and vegetable crops as well as their methods of estimation have been undertaken and is presented in brief below as (1) Methods of estimation of carbaryl and endosulfan, (2) Residue status of pesticides, i.e. carbaryl and endosulfan in fruits and vegetable crops.

2.1 Method Of Estimation Of Carbaryl and Endosulfan Residues

Different methods of estimation of carbaryl and endosulfan residues reported are mostly based on the principles of chemical assay and micro bio-assay by various workers. The review of the same is as follows:

2.1.1 Carbaryl.

Benson and Finocchiaro (1965) showed the rapid procedure for the estimation of carbaryl residues. They had
modified the official colorimetric method for carbaryl requiring no special equipment as well as requiring short time for analysis. Recoveries from eight crops at about 10 ppm levels ranged from 84 to 106 per cent.

Gajan et al., (1965) reported that carbaryl can be determined quantitatively in the form of a-naphthol by means of oscilliographic polarography. A characteristic peak for hydrolysis product of nitrosal carbaryl can be obtained which was a peak potential of 0.45 ± 0.5 V.Vs. as mercury pool reference electrode. Using modified clean up methods, as little as 0.2 ppm of carbaryl residues of certain fruits and vegetables can be determined. Recoveries from fortified crop attained 95.3 per cent ± 9.0 per cent at 0.2, 5.0 and 10.0 ppm levels.

Finocchiaro and Bensons (1965) described a thin layer chromatographic method for the semi-quantitative determination of carbaryl. Acceptable recoveries ranged from 0.05 to 0.5 ppm in case of nine crops including apples, black berries, carrots and leafy vegetables.

Chiba and Morley (1965) reported a procedure which gave a convenient screening range of residues of 0.1 to 0.3 ppm for carbaryl and its break-down products in lettuces and 0.03 ppm in tomatoes without clean up. By the use of a partitioning clean up method, even 50 ppb of carbaryl residue can be detected.
Nenov (1967) used enzymatic method based on the capability of insecticides to inhibit cholinesterase. The degree of inhibition of the cholinesterase activity corresponds within certain limits of the concentration of the insecticide in the solution. The cholinesterase activity is judged by the speed with which definite amount of the acid is liberated and the drop of pH and visual calorimetric comparison of a greenish yellow colour. Chloroform should be used as a solvent and extraction for the detection of carbaryl residues in the food products.

Butler and McDonough (1968) showed a method for the determination of residues of carbamate insecticides by using electron-capture gas chromatography based on the principles of hydrolysis of phenols as residues of carbaryl. This hydrolysed product with mobile MC-A.600 (benzo (b)-thein-4-yl methyl carbamate), and Nigara NIA-10242 (2, 3-dihydro-2, 2-dimethyl 7-benzofuranlymethyl carbamate) when heated with pyridine and trichloroacetyl chloride, produced trichloroacetate which were measured by electron capture gas chromatography. Hydrolysis and trichloroacetylation were performed without any intermediate washing step. The time of analysis was about one hour after clean up. Clean up procedures considered as a coagulation step followed by liquid chromatography on Florisil or liquid chromatography on a combination absorbent of alumina, florisil and Norit-A. The method was good to
determine residues in potatoes, sugarbeets, apples, and range grass. The lowest limit of sensitivity of this method was generally 0.01 to 0.10 ppm of residue.

Bogomolove (1968) studied a specific calorimetric procedure for estimating residual quantities of sevin in fruits, berry crops and canned food. The method is based on alkaline hydrolysis of sevin and calorimetric determination of the product by using a solution of n-nitrophenyl-diazonium. The estimation of sevin can be done in both the alkaline and acidic media. The sensitivity of the method comprised 0.05 mg/kg of product in an alkaline medium and 0.2 mg/kg of product in an acid one. The accuracy of the method is ± 5 to 10 per cent.

Falcheux and Lewis (1968) showed the channel layer chromatography used as a rapid clean up procedure for carbaryl residues before semi-quantitative determination by thin layer chromatography. With this procedure, apple, lettuce, cabbage, rough rice, cranberries, cucumbers, bell peppers, turnips (green and bottoms), corn meal, soya beans, hay, mustard greens, straw berries, sweet potato, and baby food could be successfully analysed for carbaryl even at 0.2 and 10 ppm.

Butler and Macdonough (1970) utilised the specific method for determining résidues of carbaryl by electron capture detection after derivative formation. They showed
that the residues of carbaryl can be determined by a specific procedure based on the preparation of two derivatives. Sample of bees, bee bread, bee-pollen and cow milk were extracted with chloroform and cleaned up by acetonitrile petroleum ether partitioning and then by liquid chromatography. Subsequent residues were hydrolysed to 1-naphthol and then part of which was trichloro-acetylated and part of which was brominated and acetylated. Both derivatives were then determined by electron capture gas chromatography, on the same column and at the same temperature. This procedure required little more time than the two analysis of a single derivative. Recovery from fortified samples of carbaryl at the level of 0.005-16.0 ppm ranged from 70 to 112 per cent.

Argauer et al. (1970) studied the extraction of carbaryl as 1-naphthol residues from fortified samples of honey bee, pollen, alfalfa and honey which were determined quantitatively by the strong florence associated with sodium salt of 1-naphthol. Recoveries averaged 95 per cent when both methanol and methylene chloride were used for extraction. A florisil column removed many fluorescent interference associated with plant extract. The colourless aqueous phase that resulted when the yellow methylene chloride eluant was extracted with 0.25 N sodium hydroxide was found which was suitable for measurement of the florescence of free 1-naphthol
present in the sample. For determining carbaryl the methylene chloride phase was evaporated near to dryness. Then carbaryl was hydrolysed by the addition of 0.25 N sodium hydroxide, the yellow colour was removed by the addition of petroleum ether and the fluorescence of the colourless aqueous phase was compared with standard. The aqueous phase was shaken with chloroacetic anhydride in benzene to form chloro acetylated 1-naphthol derivative for confirmation of the results by gas chromatography.

Tilden and Van-Middlelem (1970) studied the number of derivatives of N-methyl and N,N-dimethyl carbamate insecticide which were synthesized and evaluated as to their potential electron capturing properties in gas chromatography. One of the most promising derivatives, 4-bromo-N-methyl benzamide, was used for quantitative assessment by electron capture gas chromatography. Carbaryl residues were extracted from field treated spinach and chicory, sulphuric acid hydrolysis was used for converting carbaryl simultaneously to a methylamine salt which were extracted as water soluble products from the crop. Effective separation of the methylamine from the water soluble extract was accomplished by making the aqueous phase strongly alkaline. This phase was also helped to catalyse the coupling of the free amine with 4-bromo-benzol chloride. The lower limits of detection were 0.2 ppm for fortified crop extracts and the pure standards could be detected at the 20 picogram levels.
Vonesch et al., (1971) reported that rapid calorimetric method for determination of carbaryl in technical products and wettable formulations based on the reaction of carbaryl with diazolized-2-5-dichloroaniline. This reaction is sensitive to 0.1 microgram with a detection limit of 0.2 ppm. Recoveries of pure carbaryl were excellent and analysis of technical products was reproducible.

Argauer and Webb (1972) reported rapid analytical procedure for determining carbaryl on the leaves of bean and tomato plants. Small aliquots of methylene chloride extracts of treated leaves were placed in glass vials and the solvent was allowed to evaporate. Sodium hydroxide solution was then added to hydrolyse the carbaryl to 1-naphthol, which was leached out into the clear aqueous solution for measurement of the fluorescence intensity.

Ishii and Yamashita (1972) reported the residue analysis of carbamate insecticide by gas chromatographic determination of NAC (carbaryl) in tomatoes containing 0.06, 0.1 and 0.12 ppm. It gave 72, 94 and 74 per cent recoveries respectively. In cucumbers 0.1 and 0.2 ppm NAC gave 70 and 94 per cent recoveries respectively. In lettuce 0.05, 0.1 and 0.2 ppm NAC gave recoveries to an extent of 85, 81 and 92 per cent respectively. The limit of detection was 100 ng.
Murugesu (1974) described a simple, quick and reliable method for calorimetric estimation of residues of eight carbamate insecticides. The method was applicable to carbamates which on hydrolysis yield phenols with free para positions suitable for coupling with a diazo reagent to give coloured solutions. Optimum condition for the development and measurement of residues from the colour is required.

Rangaswamy and Majmudar (1974) reported a new calorimetric method for the estimation of carbaryl, 1-naphthol N-methyl carbamate, which is based on its reaction with diazolised O-toluidine. The absorption of the maximum red colour formed was measured at 520 nm. The method was sensitive to 0.1 microgram carbaryl per 20 gm. sample.

David and Dozis (1974) showed a reproducible direct method for simultaneous determination of small quantities (0.2 to 15 ng) of carbaryl and its hydrolysed product alfa-naphthol by gas liquid chromatography using electron capture detection. This is an improved one for the sensitivity over other direct methods of carbaryl determination described previously.

Ishii et al., (1974) studied the carbaryl recovery test with the help of the gas chromatography method. From carbaryl added (contained) in cucumber at the rate of 0.1 ppm, in tomatoes at the rate of 0.6 and 0.1 ppm and in lettuce at the rate of 0.1 and 0.2 ppm, the recoveries of carbaryl were
found to be 84.5, 84.7, 84.0, 76.0 and 84.5 per cent respectively.

Ernst et al., (1975) described a thin layer chromatographic method for recovering 90 to 94 per cent of carbaryl, Mesurol (4-C methylthio)-3-5 xylyl methyl carbamate) and propoxur from vegetables and fruits containing 0.5 to 2.0 ppm of the respective product.

Lee and Wang (1976) studied the effectiveness of the direct and dry film methods for the bio-assay of insecticidal residues on and in vegetable crops in laboratory tests in Tiwan. Musca domestica L. and Drosophila melongaster Meigen were the test insects, and carbaryl was the test insecticide for both the methods. The main advantage of the former method was its simple application whereas that of the latter method was its wider range of detection.

Kavadia et al., (1976) reported different methods viz., micro bio-assay, spectrophotometric and enzymatic for estimating pesticides residues in various substracts. The bio-assay method could estimate the residues varying from 80 to 95 per cent in most of the insecticides without any clean up. The spectrophotometric method, could show the residues from 80 to 99 per cent while enzymatic showed residues varying from 87 to 95 per cent. They have also suggested that the micro bio-assay and enzymatic methods were very easy, sensitive, less laborious and time
consuming, while spectrometric method required a large amount of chemicals and costly equipments. The micro bio-assay method will further help in estimating the toxic metabolites without additional cost.

Blass (1977) reported two methods for the determination of the residues of carbaryl. The first method involves the acylation of the unchanged compounds with pentafluorobromic or heptafluorobutyric anhydride after the addition of pyridine as a catalyst and measurement of the derivatives with an electron capture detector, the sensitivity of which was 0.03 ppm. The second method involved the phosphorylation of phenols in the compounds and the subsequent measurement of the phenyl carbamate derivatives by gas chromatography, using two types of flame detector, the sensitivity of which was 0.05 to 0.1 ppm.

2.1.2 Endosulfan.

Butler et al., (1962) showed a method for thiodan (endosulfan) residue determination from the plant material with n-hexane. The clean up procedure adopted was of the evaporation of the extract to dryness. The reaction with pyridine-alkali carried out in a single test tube, was sensitive to both isomers of technical thiodan and to thiodan diol. The method is sensitive to 5 microgram of thiodan, requires no special apparatus or reagents, and it is adoptable to routine laboratory work when a large number of samples are to be
analysed. It has been used for determining thiodan residue on sugar beet leaves, alfalfa, and strawberries and for thiodan vapour in air.

Maitlen et al., (1963) while using the method recommended by Butler et al., (1962) for estimation of thiodan, have modified the same. They stated that due to their procedure, there were lower blanks and less interference with modifications.

Greve and Wit (1971) showed a simple method for the identification of endosulfan by observing the GLC peak shifts brought about by diluted alcoholic alkali. The two isomers of endosulfan yielded a new product, the retention time of which was half the sum of the original isomers. The method was valid for amounts to the extent of 0.05 ng alfa plus beta isomers of endosulfan.

Kathpal and Dewan (1975) reported an improved clean up technique for the estimation of endosulfan from crops. The technique involved the use of Nuchar C-190 N as absorbent and a mixture of n-hexane and acetone (4 + 1) as the eluting solvent. The mean percentage recoveries of endosulfan (I & II) from plant tissue extract fortified at levels of 0.5 to 2.0 ppm varied from 86 to 102 per cent.
2.2 Residue Status of Pesticides In Fruits And Vegetables

Fruits and vegetables are considered to be most important supplementary food for balanced diet of human beings. Generally, only safer insecticides are recommended for the control of insect pests of fruits and vegetables because of their direct and immediate consumption after harvesting and even as raw material. The pesticide residue in vegetables will be reduced while washing and cooking whereas the residues present in fruits is not lost due to their direct and immediate consumption. Thus residue in fruits is harmful to human beings. Efforts have been made to review the work on residue status of carbaryl and endosulfan on/in fruits and vegetables in various countries including India.

2.2.1 Carbaryl.

2.2.1.1 Fruits

Johnson and Stansbury (1965) studied the carbaryl residue on various crops viz., spinach, lettuce, berry, apple and green bean and showed that the spinach group had an initial residue of 52 ppm but concentration was rapidly decreased and only 9 ppm remained after seven days having a half life of three days. Berry had a six fold decrease in residue over seven days having half life of two days.

Gutenmann and Lisk (1967) determined sevin residue to an extent of 0.1 ppm in an apple by electron gas chromatography. They showed that the residue on replicated apple
Zambonelli et al., (1970) showed that small traces of carbaryl residue could be determined 63 days after its spraying on vines.

Mitic Muzina et al., (1971) determined carbaryl residue in cherries, peach and apricot fruits treated for controlling fruit fly Rhagoletis cerasi. The degradation rate for carbaryl was very rapid, because of which it was found that even 10 and 15 days after the last application all residues in both fruits were below tolerance levels.

Polizu et al., (1971) reported that in peach, apple and plum trees, when sprayed with carbaryl 0.15 per cent during fruit maturation, the initial residue level was 10 to 15 ppm, 1.4 ppm and upto 1.5 ppm respectively. It was further found that the rate of residue loss varied with the rainfall but the spraying interval suggested was 21, 7 and 2 days for a tolerance level of 2 ppm respectively.

Similarly, initial deposits of 14.2, 25.2 and 33.1 ppm of carbaryl were found in each of the twelve bunches of grapes (variety Thompson seedless) when separately dipped in 1000, 2000, 3000 ppm of carbaryl for one minute. After 24 hours, residues declined by 17.7, 13.9 and 14.2 per cent respectively. Half of the residues declined within seven days after the treatment. The rate of dissipation was faster in lower concentration than in the higher ones. The lower concentration gave
a detectable residue up to 28 days which was 8.4 per cent, while the other two concentrations viz., 2000 and 3000 ppm gave detectable residue up to 35 and 40 days, which were 7.1 and 54 per cent respectively. The tolerance limit of carbaryl 10 ppm on grapes fixed by the FDA (U.S.A.) was reached after two days in 1000 ppm treated grapes, while in other two treatments carbaryl took ten days to reach the tolerance level. Thus ten days is the safe period for harvesting grapes after the treatment of carbaryl (Gupta et al., 1972).

Hussein and Gouhar (1972) stated that spraying of carbaryl 85 per cent W.P. at 150 gm. per 100 litres of water on pomegranate was giving good control for Virachola livia (Klug), provided the fruits were bagged with transparent papers after the treatment. However, residues were found to an extent of 57.43 ppm and 43.85 ppm after 14 days of treatment respectively in experiments conducted for two years.

Sundaram and Lecampte (1974) studied residue status of carbaryl when applied from an aircraft to a plantation of Pinus strobus L. near Ottawa, Canada, at the rate of 1 lb. toxicant per acre for the control of Pissodera strobi (peck). The samples of shoots from the treated and untreated trees were analysed for residues, one day after the treatment and at an interval of 90 days after the treatment. The carbaryl content in a shoot, one hour after spraying, was found to be
19.1 ppm, of which nearly 68 per cent was found on the surface of the shoots. The total residue decreased rapidly for the first few days and thereafter gradually until 59 days, when the residue level of only 1.1 ppm was found. The dissipation rate of the absorbed carbaryl residue was low, but the overall decrease in insecticide content was high with a half life of six days.

Nalbandyan (1975) studied the residue aspects in the grape vine, sprayed with sevin at the rate of 0.3 per cent for the control of several pests. The carbaryl residues were determined on grape surface and in the pulp 4, 5, 10, 15, 20 and 25 days after the treatment. The highest residue in the grape pulp was found on the 5th day but no residue was found after the 25th day of the treatment.

Kathpal et al.,(1976) studied the extent of carbaryl residues on grape berries at Udaipur. Two schedules each consisting of two application of 0.1 per cent and 0.2 per cent carbaryl, at fortnight intervals were tested. The initial deposit, as a result of the first spraying of 0.1 per cent spray was 22.0 ppm, which had dissipated to 1.24 ppm within 15 days after the application, but when the second spraying of the same concentration was made, the cumulative deposit was 22.2 ppm. In case of 0.2 per cent spray the residual deposit from the first and second spraying was 63.0 and 67.4
ppm respectively. Residual studies at different intervals after the treatment indicated that the residues from 0.1 per cent spray dose reached below tolerance level of 5 ppm within nine days, while in the case of 0.2 per cent treatment dose, the residue reached below the tolerance limit within 18 days after the application.

The second experiment was of having the treatment of dipping the grape berries in a 2000 ppm solution of carbaryl for two minutes. The initial deposit by this treatment was 188.03 ppm, which reached below the tolerance limit within 18 days. It was further found that the washing of the berries under running tap water for 30 seconds removed the carbaryl residue to an extent of 50 to 100 per cent, depending on the time elapsed after the treatment.

Saivaraj et al., (1976) studied the carbaryl residue in the grape berries at the time of harvest and found it to be ranging from 1.08 to 1.50 ppm for carbaryl with bio-assay and chemical assay respectively. It is thus cleared that the residue of carbaryl was 0.1 per cent in grape vine berries and was found to be below the tolerance limit.

Rajukkannu et al., (1977) studied the problem of residue of carbaryl in Muscat grape berries. The initial deposit was found to be 38.50 ppm of carbaryl when carbaryl was applied at the rate of 1.0 kg.a.i. per hectare. The residue
dissipated to the non-toxic level within five days. Hence, a waiting period of 5 days has been recommended for the safe consumption of grape berries when carbaryl is to be used for the pest of Muscat grape berries.

2.2.1.2 Vegetables.

Twelve days after soil treatment with 5 to 6 gm. granules of carbaryl per meter in the carrot crop, Stobwasser (1963) could found 0.4 to 0.5 ppm of carbaryl residue in carrots, but all residues disappeared from the carrots at the time of harvest.

Dewan et al., (1967) reported the carbaryl residue on marketable 'bhindi' fruit of 3 to 4 inch in size to be within the U.S. tolerance of 10 ppm. In the same findings it was reported that due to occurrence of rain, three days after application of 0.25 carbaryl WP on 'bhindi' fruits, no residue of carbaryl could be detected. Even fruits of the treated flowers had no residue of carbaryl.

Gandolfo et al., (1967) reported that there was much more residue of carbaryl when applied in dust formulation in comparison to spray formulation as found from the experiment of carbaryl application in lettuce six and twelve days before harvesting.

Elessawi and Et-Rafai (1967) conducted chemical analysis of the edible portion of untreated okra, cowpeas,
tomatoes and eggplants and found relatively low values of residues for apparent sevin. These crops under treatment of multiple application of sevin at the rate of 0.34 per cent under simulated commercial practices showed residues to be below the tolerance level even after two hours of the final application. Initial residues were much and persisted more in the case of okra and cowpeas in comparison to tomatoes and eggplants. Maximum carbaryl residue on okra, cowpeas, tomatoes and eggplants was 3.2, 5.03, 1.88 and 1.88 ppm respectively, three hours after the last application. These residues declined below 0.05 ppm within eight days in all the above vegetables.

Elkins et al., (1968) studied the carbaryl residues of green bean in commercial and home preparation. In the commercial preparation they reported the carbaryl residues to be on an average 7.6 ppm and 2.0 ppm in unwashed and washed preparation respectively, while unwashed and washed home preparation showed carbaryl residues to be on an average 11.0 and 5.3 ppm respectively, but no carbaryl residue was found in home canned green bean.

Cunat et al., (1968) reported the residue of sevin on tomatoes after nine days of treatment to be below U.S. and West German tolerance level.

Farrow et al., (1968) reported that there was no significant decrease in the carbaryl residue during different periods of storage of tomato fruits after harvest (fresh and
untreated fruit stored at 55°F for one, four and seven days). However, the residue apparently declined by 30 per cent during the canning operation.

Mann and Chopra (1969) showed the persistence of carbaryl in and on vegetable crops sprayed with 0.1, 0.2 and 0.4 per cent carbaryl applied four times at an interval of three weeks to cabbage and at the rate of 0.55, 1.1 and 2.2 lb/ac. applied to eggplants. By the first day after the final application 40-45 per cent and after one week 80 to 85 per cent carbaryl had disappeared. By the washing treatment there was a decrease of the carbaryl residue to a great extent but some penetration or translocation of the insecticide in both the crops was noticed. The rate of penetration was low, but somewhat greater for cabbage in comparison to the eggplant. The half life value of carbaryl was three days for cabbage and 3.2 days for eggplant.

Farrow et al. (1970) studied the residual level of carbaryl in broccoli canned commercially and in home cooking from the field of broccoli sprayed with carbaryl. The commercially canned preparation of broccoli indicated loss of almost all residues to an extent of 90 per cent. However, washing and home cooking indicated a loss of only 55 per cent of the residues.

Deshmukh and Rattanial (1970) estimated the carbaryl residue on brinjal leaves, stems and fruits both by means of
the bio-assay method and chemical assay method from the crop sprayed with 0.15 per cent carbaryl.

The initial deposit of carbaryl determined in leaves by the bio-assay method in August-September 1966 was 51.28 ppm, while in September-October 1966, it was 52.07 ppm. The chemical assay method showed that the initial deposits were 52.25 ppm in August-September 1966. The half life value of carbaryl on the leaves in August-September 1966 was 6.57 days, while in September-October 1966 it was 6.64 days.

Carbaryl initial residue deposits on the stem in September-October and October-November 1966 were 35.52 and 36.69 ppm respectively by the bio-assay method and by chemical assay it was 36.25 and 37.05 ppm respectively. The half life values were 6.005 and 6.19 days respectively.

Carbaryl initial residue deposits on brinjal fruits in September-October and October-November 1966 were 40.82 and 39.59 ppm respectively, with bio-assay, but in chemical assay it showed that the deposits were 41.50 and 40.25 ppm respectively. In October-November carbaryl residue on fruit was 20.58 ppm after five days of spray. Thus the authors recommended that an interval of 12 to 13 days should be observed between spraying and harvesting of fruits for the carbaryl treatment in brinjal crop.

Carlos et al., (1970) determined the effect of pinolene (B-Pinene polymer) to increase the initial deposit of carbaryl and the rate of decomposition of carbaryl on tomato leaves. The combination of treatment effectively extended (three fold increase) the period of carbaryl residue
on the foliage.

Lamb et al., (1970) showed that the washing process can remove 66 and 87 per cent of carbaryl residue from spinach treated with carbaryl, but the use of a detergent in washing water still increase the percentage removal of residue. Similarly, commercial water banching removed 96 to 97 per cent of carbaryl residue.

Deshmukh et al., (1972) studied the insecticidal residue status on tomato fruits when the crop was sprayed with carbaryl and endosulfan at the rate of 0.8 kg, and 0.42 litre/hectare. Fruits of marketable size were collected after the fifth spray and the residues were estimated. The initial deposit of carbaryl was 5.4 ppm and that of endosulfan 1.46 ppm. Both were below the tolerance limit fixed by the FDA of U.S.A.

The initial deposit and residue of carbaryl could be removed easily by washing and rubbing of the fruit's surface in comparison to that of endosulfan (i.e. 84 per cent and 39 per cent in case of carbaryl and endosulfan respectively).

Baicu et al., (1973) while testing the effectiveness and phytotoxicity of carbaryl with ultra low volume application at the rate of 0.25 per cent against Eurygaster and Aelia spp. on winter wheat and against the second, third and fourth larval instars of Leptinotara decemlineata (Say) on potato, reported that the residues left were below the tolerance limit at the time of harvest.
Sun (1974) studied the dissipation of carbaryl residue in vegetable crops viz., spinach, pigweed, leek sprayed at the rate of 0.5 and 1 kg. toxicant per hectare. The residue as determined with radioactive carbon (C\textsubscript{14}) and analysed by thin layer chromatography 0, 1, 2, 4 and 7 days after the treatment varied with the type and kind of vegetable as well as with the rate of application. Carbaryl was found less persistent and dissipation was faster at the room temperature than at 5°C. Washing with detergent water treatment removed more residues than that with plain water.

Srivastava et al., (1975) reported that carbaryl 0.25 and 0.50 per cent spray at the rate of 1500 litre/ha left initial deposit of 50.53 and 96.20 ppm of carbaryl on small fruits of okra (size 1.2 to 2.5 cm.), 27.20 and 58.03 ppm on medium sized fruits (3.8 to 5.0 cm.) and 17.53 and 40.20 ppm on normal marketable fruit (size 7.5 to 10 cm.) respectively.

Carbaryl residue dissipated faster during the first 2 to 3 days. Thus it was found that the residue from 0.25 per cent spray reached well below the tolerance limit of 10 ppm on normal marketable fruits, within 24 hours after the treatment.

The initial deposit from 0.25 per cent and 0.50 per cent carbaryl spray has been removed to the extent of 66.12 per cent and 69.55 per cent respectively by washing, 41.81 and 24.88 per cent respectively by open cooking, 81.75 and 48.5 per cent respectively by steam cooking and 79.24 and
77.01 per cent respectively by dehydration. The presence of carbaryl residue in okra seeds indicated that it has a mild systemic or translocation action.

Nath et al., (1975) reported that the initial deposit of carbaryl residue can be reduced to the extent of 66 and 70 per cent by washing, 42 and 25 per cent by open cooking, 82 and 49 per cent by steam cooking and 79 and 77 per cent by dehydration in the case of the treatment of crop with 0.25 and 0.5 per cent carbaryl respectively. They have further observed that in case of lower dose treatment, all the processes except open cooking could remove the residue below the tolerance limit of 10 ppm.

Srivastava and Kavadia (1976) tested residue status in vegetable crops with soil application of carbaryl 4 per cent granules at the rate of 15, 30 and 45 kg. a.i./ha. They found that for all treatment doses in clay loam and sandy loam soil the residue of carbaryl was below detectable level in carrot, radish and beetroot crops. It thus indicated that carbaryl was either not absorbed by root crops or the uptake was extremely feeble. It was also found that the uptake and translocation of carbaryl was less in all the three root crops planted in soil treated in the previous kharif season.

Rajukkanu et al., (1976) studied the residue of 0.1 per cent carbaryl spray on sweet potato for controlling Cylas formicarius (F) and found it to be below the official tolerance level.
Srivastava and Sharma (1976) showed that the treatment of carbaryl spraying at the rate of 0.2 and 0.3 per cent on cowpea pods and leaves could give initial residue level of 22.22 and 33.77 ppm and on pods, only 9.16 and 15.5 ppm respectively, three days after treatment, while in higher dosages it further reduced to 6.8 ppm within five days. Thus the residue on pods dissipated below the tolerance limit of 10 ppm within three days in lower dose treatment and within five days in higher dosage treatment.

Similarly, the cowpea leaves were having an initial deposit of 74.99 and 121.11 ppm. The residues were 9.86 and 13.61 ppm in seven days in 0.2 and 0.3 per cent spray treatment respectively. In higher dosages it further reduced to 2.3 ppm in nine days. Thus in case of residue in leaves it was shown that the dissipation below the tolerance limit of 10 ppm was found in seven days in lower dose application while it was nine days for higher dose application.

They also found that mild rain (10.8 mm) seven days after the treatment washed away the carbaryl residue to an extent of 72.17 and 75.17 per cent on pods and 81.97 and 82.85 per cent on leaves in both the dosages respectively. Washing treatment of the pods removed initial carbaryl residue by 77.50 and 83.82 per cent in both the dosages respectively, while in open cooking and steam cooking after the washing treatment, the pods have residue below detectable level in both dosages.

Kavadia and Srivastava (1976) reported the extent of
carbaryl residue on/in tomato fruits due to application of 0.25 per cent of carbaryl WP. The initial deposit of carbaryl residue was 6.5 to 7.7 ppm which reduced to an extent of 65 per cent in eleven days and completely dissipated in fifteen days. The half life of carbaryl determined was 2.48 to 3.71 days. The carbaryl reached the tolerance level of 5 ppm in five days. Equivalent waiting period was, therefore, suggested.

Rajukkannu et al., (1976) conducted an experiment for residue estimation of carbaryl and endosulfan on 'bhindi', tomato and brinjal due to spraying of carbaryl and endosulfan at the rate of 0.1 per cent and 0.07 per cent respectively done during the pre-flowering period and continued upto the harvesting.

They could show that the initial deposit of carbaryl residue was 15.92, 17.32 and 18.20 ppm, which reduced by 42.21, 48.60 and 50.54 per cent in respective fruits of bhindi, tomato and brinjal, but within three days carbaryl residue dissipated below the tolerance limit of 10 ppm in all fruits.

Endosulfan spray had left a high initial deposit, i.e. 2.73, 4.35 and 3.50 ppm in 'bhindi', tomato and brinjal respectively, and residues dissipated to 1.04, 1.20 and 1.60 ppm within three days, showing a reduction of 62.04, 74.11 and 54.20 per cent respectively.

Avasthi et al., (1977) showed that carbaryl, when used on cowpea at the rate of 6.25 kg/ha. during the life of the crop (i.e. 0.125 per cent as the first spray and 0.25 per
cent as second spray) gave an initial residue of 24.16 and 33.15 ppm respectively. The residue level reduced to about 17 to 20 per cent within one day. Washing of the pods by water brought about 90 per cent reduction in the initial residue level. It was thus below the tolerance limit of 5 ppm recommended by FAO/WHO (Anonymous 1975). They also observed that the boiling of pods in water could reduce 84 per cent of initial residue. It was more than 5 ppm in the case of 0.25 per cent treatment. Normal dissipation in both the treatments was at the rate of 4.4 days and 4.9 days RL 50 value (half life value of residue) and on the 10th day residue in both the treatments were above the tolerance limit. They further reported that the minimum waiting period i.e. T TOL (minimum time in days required for insecticide residues to reach the level of tolerance limit) values worked out to be about ten to thirteen days. The T BDL values (time in days required for the residues to reach below detectable level) corresponding to 0.25 ppm sensitivity of the method for the respective treatments were found to be 29.5 and 34.2 days respectively.

Initial residue from the second spraying of both the doses was 26.45 and 37.10 ppm respectively. Residues reduced to about 44 to 46 per cent after three days and 67.5 to 68.8 per cent after seven days. Statistical treatment of residue data led to conclude the RL 50 value of 4.90 and 5.6 days, T TOL values of 11.1 and 15.1 days and T BDL value of 32.4 and 39.6 days respectively for the lower and the higher concentration treatments.
2.2.2 Endosulfan.

2.2.2.1 Fruits.

Saunders et al., (1967) reported the residual effectiveness of endosulfan on cacao trunks for the control of *Xyleborus ferrugineus* at three places in Costa Rica during 1962-63. Sufficient endosulfan residue for beetle control was demonstrated on individual trees for 20 weeks after the treatment, but rain caused variation in the residue present in the trees.

Harrison et al., (1967) studied the residue on fruits and leaves of black-current bushes from commercial and trial plot conducted in England that had been sprayed with endosulfan for the presence of the two endosulfan isomer and one endosulfan sulphate. Total residue of these three compounds on the commercially grown fruits did not exceed one ppm. Considerably higher residue was found on fruit that had been deliberately over-sprayed.

Harrison et al., (1967) reported that the application of emulsified concentration of endosulfan on apple tree in England gave the most persistent deposit taking eleven weeks to fall below 5 per cent of the initial values. Endosulfan gave significant amount of ultra violet irradiation products like endosulfan sulphate.

Stenseth (1969), tested endosulfan applied to black-current bushes, and straw-berry plants and observed that there was in general less danger of harmful residue in strawberries than in black-currents. Endosulfan could be used on black-
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days interval before harvest.

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boysenberry plant sprayed with endosulfan (0.5 and 1.0 kg/
500 litre) and berries picked 3 to 14 days later, endosulfan
residue after ten days of spraying was less than 2 ppm, which
was the level permitted in South Africa.

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detected in coffee bean sample from experimental fields in
Brazil sprayed with 2 per cent solution of endosulfan at
various intervals. The sensitivity of the GLC method used
was about 0.01 ppm.

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with endosulfan (6 lb of thiodan-50 WP. per acre) and ethion
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dation as well as determination of insecticidal residue at
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with spraying of 0.1 per cent endosulfan at an interval of
a fortnight three times, for the control of fig midges,
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of 2 ppm.

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a fortnight three times, for the control of fig midges,
Udumbaria nainiensis Grover. They have found that the residue
in/on fig fruits resulting from two as well as three sprayings
of 0.1 per cent endosulfan dissipated below the tolerance
limit of 2 ppm within fifteen days and below detectable level within thirty days after the last application. It was, therefore, reported that the treatment was not likely to cause health hazards to the consumers.

Kathpal et al., (1977) determined the residue on ber (Zizyphus jujuba) fruits and leaves as a result of three sprayings of 0.1 per cent endosulfan at an interval of two weeks on the basis of the calorimetric technique. The endosulfan residue in/on ber fruits was found to be 2.14 and 5.14 ppm after one day of second and third spray respectively. The residue dissipated below the tolerance level of 2 ppm within five days of the application, while it reached below detectable level both in/on fruits and leaves within 21 days of the application. Washing of fruits with tap water for 4 to 5 minutes had removed the residue more or less completely.

2.2.2.2 Vegetables.

Leitao and Fernandes (1966) studied the fate of endosulfan which was applied to tomato crop at the rate of 52.5 and 105 ml a.i./ha. Residue on/in the fruits was determined at an interval of twentyeight days after the second and last treatments. After 28 days they estimated a residue of 0.015 and 0.03 ppm from the lower and higher concentration respectively. The reduction in the residue was rapid in the first four days and progressively slower after the period of four days. The final amount of residue found in the fruit skin ranged from 0.084 to 0.27 ppm, while those in the pulp was almost below 0.01 ppm.
Vail et al., (1967) studied on the retention of insecticide residue on the leaves of winter green vegetables in Southern California in 1961-63. Endosulfan was applied to cabbage, turnips, collards, lettuce, chard and table-beet infested by *Brevicorne brassicae* (L) or *Myzus persicae* (Sulz) in autumn and winter. The last application of insecticide was done at harvest time when the maximum temperature was below 70°F. The endosulfan residue on the leaves on assessment was often found to be more than 1 ppm during the weeks following the last application of the insecticide when the temperature was comparatively higher.

Hanoit (1973) studied the residue on glass house grown lettuces upto 34 days after the application of thiodan formulation as a powder (200 gm/acre), spray (20 gm/acre) or smoke (1 gm/m³), and on tomatoes, upto 13 days after the application of a thiodan smoke (1 or 2 gm/m³). In lettuce the residues were found within Belgian tolerance limits after 14, 28 and 31 days in all the three treatments, while in tomatoes the residues were negligible even after one day of the smoking treatment.

Nath et al., (1974) reported that the endosulfan spray of 0.1 per cent at the rate of 1500 litres per hectare provided the average initial deposit of 12.55, 10.35 and 6.98 ppm on small (1 to 3 cm), medium (3 to 7 cm) and normal marketable size (9 to 13 cm) of 'bhindi' fruits respectively. However, 0.2 per cent endosulfan spray gave the average initial deposits of 27.59 ppm on small, 18.62 ppm on medium and 15.10 ppm on
normal marketable size of fruits.

The endosulfan residue from both the application dosages dissipated more rapidly during the first two days as compared to the subsequent days. However, in none of the cases, the endosulfan residue reached below the tolerance limit of 2 ppm as prescribed by FAO (Anonymous, 1972) even up to six days after the treatment. The initial deposits from the application of 0.1 and 0.2 per cent endosulfan were reduced to the extent of 34.67 and 48.94 per cent due to washing, 26.22 and 23.58 per cent due to cooking, and 57.59 and 57.53 per cent respectively due to dehydration, but none of the three processes tried could bring the residue level below the tolerance limit. It was, therefore suggested that endosulfan spray should be avoided on 'bhindi' in order to save the consumer from health hazard.

Donald et al., (1974) studied the chemical changes of technical endosulfan incorporated into soil at the rate of 6.7 kg. per hectare. The alfa endosulfan decomposed fairly rapidly (50 per cent in ~60 days) with the simultaneous formation of equivalent amount of endosulfan sulphate which appeared to be relatively stable in soil. Beta endosulfan disappeared slowly (~ 50 per cent in 80 days) absorption of endosulfan from the soil was demonstrated in potato in the same season wherein application at the rate of 6.7 kg/ha of endosulfan could result in having 0.03 ppm of endosulfan sulphate, 0.06 ppm of beta-endosulfan and 0.01 ppm of alfa-endosulfan in the peel of potato, while 0.03 ppm of endosulfan
sulphate was in the pulp. Similarly, eight foliar sprays at the rate of 0.6 kg/ha each, resulted in having a residue of 0.01 ppm of endosulfan sulphate in the peel and pulp showing translocation through plants.

Jai-Sing and Deshmukh (1974) studied the residue level of endosulfan in/on okra fruits following the application of four sprays of 0.250 kg. endosulfan per hectare. Samples were taken on 0, 1st, 3rd, 5th, 7th, 10th and 15th days post treatment and analysed by means of gas liquid chromatography. The initial deposits of endosulfan was below the tolerance level of 2 ppm.

Kavadia et al., (1974) reported the half life of endosulfan in cauli-flower to be three days in head and four days in the leaves, when sprayed at the rate of 0.05 and 0.1 per cent. In case of recommended spray the rate of 0.05 per cent the residue fell below the tolerance level of 2 ppm within five days in the head and within ten days in the leaves.

Nath et al., (1975) reported that the initial residue could be reduced in fruits to the extent of 35 and 49 per cent by washing, 26 and 24 per cent by open cooking, 26.93 and 31.12 per cent by steam cooking and 58 and 57 per cent by dehydration processes respectively in the plot treated with 0.1 and 0.2 per cent of endosulfan spray. None of these processes could reach the residue level below the tolerance limit of 2 ppm in both the dosages.
Dixit et al., (1975) studied the various insecticidal schedules for okra crop with a view of estimating insecticidal residues on marketable fruits. Analysis of the fruits for the residue showed that the chemical could have the effective control of jassids for more than a week and that a waiting period of seven days was essential for the residue to be below the tolerance level.

Verma and Rattanmal (1976) showed the persistence of endosulfan in the cauliflower which was effective for the control of Lipaphis erysimae (Kalt) in field plot tests in New Delhi. The residue determined by bio-assay with Drosophila melanogaster (Mg) on leaves of three months old plants was determined after two applications (20 days interval of 0.05 per cent spray at the rate of 1100 litre/ha). The residue was found below the tolerance level of 2 ppm within seven days after the first application and eight days after the second application. Similarly, on application of 0.1 per cent spray did show the same behaviour in ten days after the application. The residue in curds following the second application at both the concentrations dropped below the tolerance level in four days. A waiting period of four days before consumption was considered necessary for the curds and eight and ten days for leaves when the crop is to be treated at 0.05 and 0.1 per cent.

Srivastava and Kavadia (1976) studied the extent of residue of 0.1 per cent endosulfan emulsion spray at the rate
of 1475 litre/ha on tomato fruits, grown in two different seasons. The residues were determined by spectrophotometric method and micro bio-assay with one-day old vinegar flies. The recoveries obtained by spectrophotometric and micro bio-assay methods were 85 and 93 per cent respectively. The initial deposit of above 8 ppm in the crops of two seasons was lost in seven days and fifteen days and it reached below the detectable level in twenty-five days. The half life was five to six days. The residue of endosulfan on tomato reached below the tolerance level of 2 ppm in seven days and hence a waiting period of seven days was recommended by them.

Biston et al., (1977) studied the residue status of endosulfan in tinned peas, bean, carrots, celery, salsify, asparagus and cherries prepared by seven different Belgian firms. On analysis result with the use of gas chromatography, none of the samples was found to have traces of endosulfan.
CHAPTER III

MATERIALS AND METHODS

Technical grades of carbaryl and endosulfan were used for preparing standard calibration curves (after mathematical extrapolation) using distilled acetone and n-hexane respectively as solvents. The commercial grade carbaryl 50 per cent W.P. was obtained from M/s. Paushak Limited, Baroda, and endosulfan 35 per cent EC was obtained from M/s. Hoechst Pharmaceuticals Limited, Bombay (Table 1). Both these insecticides were used for preparing standard dose mortality response curves for micro bio-assay.

3.1 Experimental Field Layout And Treatment

Sixteen mango trees were selected for each insecticide in 'Joshi Farm' at Changa village of Taluka Gandevi, District Bulsar in 1976-77 for the experiment work on estimation of insecticidal residue. The spraying on trees was carried out with the insecticidal solution of 0.2 per cent carbaryl and 0.075 per cent endosulfan as per treatments decided. Maruti Foot Sprayer with three action nozzle was used for spraying work. In all seven sprayings were done for each insecticide as follows:

<table>
<thead>
<tr>
<th>Serial No. of spray</th>
<th>Date of spraying the insecticide</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>27-1-1976</td>
</tr>
<tr>
<td>Second</td>
<td>18-12-1976</td>
</tr>
<tr>
<td>Third</td>
<td>8-1-1977</td>
</tr>
<tr>
<td>Fourth</td>
<td>3-2-1977</td>
</tr>
<tr>
<td>Fifth</td>
<td>26-2-1977</td>
</tr>
<tr>
<td>Sixth</td>
<td>26-3-1977</td>
</tr>
<tr>
<td>Seventh</td>
<td>27-4-1977</td>
</tr>
</tbody>
</table>
Similarly, an experiment work on estimation of insecticidal residue was undertaken in Horticultural Farm at N.M. College of Agriculture, Navsari, in the year 1977-78. In this experiment only one spraying was done on 26-4-1978 and the estimation of residue of insecticides was carried out.

3.2 Procedure For Preparation Of Standard Calibration Curve For Technical Carbaryl For Chemical Assay

The colorimetric method described by Benson and Finochiaro (1965) was used in the present investigations. The underlying principle is that carbaryl first hydrolysed with alcoholic potassium hydroxide (KOH) to 1-naphthol. This is when conjugated/treated with p-nitrobenzene diazonium floroborate produced an yellow colour. The intensity of the colour is then measured at 477 m-u wave length in Spectronic-20.

3.2.1 Reagents required.

(i) Acetone - Analytical grade redistilled.

(ii) Methylene Chloride (Dichloro methylene redistilled)

(iii) Coagulating solution
Dissolved one gm. ammonium chloride (NH₄Cl) in 400 ml. distilled water and pipetted one ml. of 85 per cent orthophosphoric acid (CH₃PO₄) in it.

(iv) Aqueous acetone solution (10% V.V.)
Ten millilitre of distilled acetone made into a volume of 100 ml. by adding distilled water in a 100 ml. volumetric flask.
### Table 1. Information regarding the insecticide dosage used

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of insecticide</th>
<th>Chemical name</th>
<th>Trade name and formulation</th>
<th>Dosage used per tree</th>
<th>Active ingredient</th>
<th>Source of availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbaryl</td>
<td>1-naphthyl-N-methyl Carbamate</td>
<td>Carbaryl 50 W.P.</td>
<td>10 litres (8 small trees) 20 gm</td>
<td>15 litres (8 big trees) 30 gm</td>
<td>Paushak Ltd., Alembic Road, Baroda.</td>
</tr>
<tr>
<td>2</td>
<td>Endosulfan</td>
<td>6,7,8,9,10, 10-hexachloro 1,5,5a,6,9, 9a, hexahydro-6,9,-methano-2,4,3 benzodio xathiepin-3 oxide</td>
<td>Thiodan 35 EC</td>
<td>10 litres (8 small trees) 7.50 ml</td>
<td>15 litres (8 big trees) 11.25 ml</td>
<td>Hoechst Pharmaceuticals Ltd., Bombay.</td>
</tr>
</tbody>
</table>
(v) **Chromogenic reagent**

25 ml. of absolute alcohol (Ethanol) was taken in a 50 ml. beaker. To this two ml. glacial acetic acid (HOAc) was added. The mixture was kept in a refrigerator for saturation. After 15 minutes 25 mg. of practical grade p-nitrobenzene diazonium fluoroborate was added and mixed by stirring with a glass rod for a few minutes. This mixture was then filtered and kept in an ice bath. This reagent was prepared freshly every time for chemical assay to be done with the use of Spectronic-20.

(vi) **Diethyl glycol solution (10% V/V.)**

Ten ml. diethyl glycol was taken into a 100 ml. volumetric flask and the volume was made up by adding methylene chloride in it.

(vii) **Alcoholic potassium hydroxide**

This was prepared by dissolving 56 gm. of KOH (potassium hydroxide) in ethanol (absolute alcohol) in a 1000 ml. volumetric flask and the volume made up by adding ethanol. The solution was filtered and stored for further use. The solution which turned yellow was discarded.

(viii) **Hyflo-super-cel (Absorbent)**

(ix) **Anhydrous sodium sulphate** (Na₂SO₄)

(x) **Technical grade Carbaryl**
3.2.2 Preparation of standard technical grade solution of carbaryl

(1) 100 mg. technical grade carbaryl was put into a 100 ml. volumetric flask and dissolved in acetone and made to its volume by addition of acetone which resulted in a 0.1 per cent solution. One millilitre of this solution thus contained 1000 microgram of technical grade carbaryl. This solution was designated as "Stage A" solution.

(2) Ten ml. of the above "Stage A" solution was taken in a 100 ml. volumetric flask and the volume was made up with acetone. This solution thus contained 100 microgram technical carbaryl per ml. of solution. This was marked as "Stage B" solution.

(3) Ten ml. of "Stage B" solution was again taken in a 100 ml. volumetric flask and the volume was made up with acetone. This was marked as "Stage C" solution. One millilitre of this solution thus contained 10 microgram of technical grade carbaryl. This "Stage C" solution was used for preparing standard calibration curve for chemical assay.

3.2.3 Procedure for preparation of standard calibration curve for estimation of carbaryl.

(1) 0, 1, 2, 4, 6, 8, 10 and 12 ml. standard carbaryl solution ("Stage C") was pipetted into 25 ml. volumetric flasks respectively, and separately. Then the volume was made up by adding 10% V.V. acetone solution.

(2) 5 ml. of each of the solution was pipetted into 50 ml. beaker separately.
Fig. 1 - STANDARD CALIBRATION CURVE FOR CARBARYL BY CHEMICAL ASSAY.
(3) Two ml. of previously prepared alcoholic potassium hydroxide (KOH) solution was added in the above solution and mixed well by continuous swirling for three minutes.

(4) One ml. of glacial acetic acid was added into it.

(5) Finally, one ml. of chromogenic reagent solution was added at the time of taking the reading in Spectronic-20.

(6) The above mixture was then allowed to stand for exactly two minutes and the absorbance of yellow colour developed was measured at 477 mμ in Spectronic-20 against distilled water.

(7) The standard calibration curve (Figure 1) was drawn by using insecticide (microgram) on 'X' axis and absorbance on 'Y' axis. In order to obtain a straight line the regression equation was worked out and absorbance for each of 10, 20, 40, 60 and 80 microgram of carbaryl was calculated and shown in Table 2. From standard calibrated curve prepared by chemical assay minimum amount of 10 microgram of carbaryl can be determined from the unknown sample.

Table 2. Absorbance corresponding to various quantities of carbaryl technical grade

<table>
<thead>
<tr>
<th>Sn. No.</th>
<th>Amount of technical carbaryl (microgram)</th>
<th>Net average absorbance at 477 mμ</th>
<th>Absorbance as worked out from regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.03</td>
<td>0.011</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0.06</td>
<td>0.059</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>0.13</td>
<td>0.115</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>0.22</td>
<td>0.251</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>0.38</td>
<td>0.347</td>
</tr>
</tbody>
</table>
3.3 Chemical Assay For Carbaryl Residue Estimation From The Mango Fruits

Two mango fruits were plucked randomly from each mango trees by using a harvesting stick, and immediately transferred into the polythene bags duly tagged and perforated for aeration. The harvesting of fruits were done for carbaryl residue estimation of the experiment of the year 1976-77 at the below mentioned time interval after last spraying of insecticides:

(1) Immediately after the last spraying;
(2) Three days after the last spraying;
(3) Seven days after the last spraying;
(4) At the time of harvesting of fruits (25 days after last spraying)

In the year 1977-78, the trees of the experiment were sprayed once with 0.2 per cent carbaryl and the harvesting of fruits were done for carbaryl residue estimation at the below mentioned time interval after spraying of insecticide:

(1) Immediately after the spraying;
(2) Three days after the spraying;
(3) Seven days after the spraying;
(4) At the time of harvesting of fruits (19 days after the spraying)

3.3.1 Method of estimation of the carbaryl residues on the mango fruits.

(1) One mango fruit from each sample was weighed and put in a one litre glass jar and added 100 ml. dichloromethylene.

(2) The mango fruit was washed thoroughly by swirling with a glass rod for 10 to 15 minutes, and then it was taken out of the jar.
(3) The aliquot was filtered through a funnel over anhydrous sodium sulphate (Na$_2$SO$_4$).

(4) The aliquot was kept on a hot water bath and the volume was made 60 ml. It was then transferred into a labelled air-tight bottle and preserved in a refrigerator until further use.

(5) Out of the 60 ml. aliquot, 10 ml. was used for micro bio-assay and 50 ml. for chemical assay.

3.3.2 Method of estimation of the carbaryl residue in the mango fruits.

(1) One mango fruit from each sample was washed thoroughly with tap water for one minute, and allowed to dry. It was then cut and chopped to a size of 0.5 to 1 cm. with a knife.

(2) 50 gm. of chopped fruit from each mango samples with control was put into a labelled wide mouthed 250 ml. conical flask separately and 100 ml. dichloromethylene was added to it.

(3) The flasks were covered with polythene sheet on the mouth duly tightened with rubber bands. These flasks were put on a motorised electrical shaker for one hour.

(4) The extract was then filtered through a funnel plugged with cotton-wool, anhydrous sodium sulphate and a thin layer of hydrosuper-cel over sodium sulphate.

(5) The aliquot was kept on a hot water bath and the volume was made 60 ml. The aliquot was then transferred into a labelled air-tight bottle, and preserved in a refrigerator until further use.
3.3.3 Clean up procedure.

(1) 50 ml. of the above filtrate (3.3.1(5)) was taken in a beaker and then one ml. of diethyl glycol solution (10% V.V.) was added to it.

(2) The beaker was then placed over a hot water bath and evaporated the solution to dryness.

(3) Three millilitre of analytical grade redistilled acetone was added to the beaker and then swirled to have the residue to dissolve in acetone. Fifteen millilitre of coagulating solution was then added into the above beaker. Afterwards this mixture was swirled for some time and allowed to stand for 20 minutes for coagulation.

(4) The coagulated mixture was then filtered through a Whatman filter paper No.42 into a 25 ml. volumetric flask.

(5) Due care was taken to wash the funnel, filter paper and beaker with aqueous acetone solution (10% V.V.). The mixture was collected in the above 25 ml. volumetric flask. The volume of the said filtered mixture and wash was made upto 25 ml. with the addition of aqueous acetone solution (10% V.V.).

3.3.4 Determination of carbaryl by chemical assay method

(1) Five millilitre of the above aliquot was pipetted into a 50 ml. beaker and 2 ml. of freshly prepared alcoholic potassium hydroxide (KOH) solution was added. The solution was swirled for three minutes in order to mix it well.
(2) One millilitre of glacial acetic acid and one millilitre of chromogenic reagent were added in the above beaker (previously prepared) and swirled for three minutes for proper mixing.

(3) After two minutes the absorbance of yellow colour was measured at 477 m u using Spectronic-20.

3.4 Microbio-assay For Carbaryl Residue Estimation From The Mango Fruits

(1) Ten millilitre of aliquot, which was preserved in the refrigerator as stated vide 3.3.1(5) and 3.3.2(6), was used in preparing two replications using 5 ml. solution in each for micro bio-assay.

(2) Thus, 2.5 ml. of the solution was pipetted into each plate of petri dish duly marked as A₁ and A₂ for replication one and two respectively (Plate 1).

(3) The petri dishes were swirled gently in order to spread the solution uniformly, and the solvent was allowed to evaporate completely to dryness.

(4) After the drying of the solution in dishes, a small lump of media was placed in each petri dish as a food.

(5) Twenty anaesthetised male Drosophila melanogaster Meigen flies (one day old) were released into each pair of petri dishes.

(6) The mortality count was taken at an interval of one hour up to eight hours. During the experiment the room temperature was kept constant, i.e. 26°C ± 1°C.
Plate 1. Petri dishes used for micro bio-assay method
(7) The amount of residues of carbaryl in sample was estimated on the basis of five hours mortality count with reference to standard dosage mortality response regression curve.

3.4.1 Rearing technique of Drosophila

The culture of vinegar fly (*Drosophila melanogaster* Meigen), was obtained from the Division of Entomology, Indian Agricultural Research Institute, New Delhi, on 4th November 1976 and maintained in the laboratory of the Entomology Section at N.M. College of Agriculture, Navsari. The flies were reared on artificial diet suggested by Lewis (1960).

3.4.2 Composition of artificial diet.

(1) Agar agar : 7 gm
(2) Yeast powder : 7.5 gm
(3) Maize flour : 50 gm
(4) Jaggery : 44 gm
(5) Propionic acid : 1.7 ml
(6) Distilled water : 500 ml

3.4.3 Method of preparation of the diet.

The weighed quantity of 7.0 gm agar agar powder was added into 200 ml. distilled water, with constant stirring and then allowed to settle down. 7.5 gm of yeast, 50 gm of maize flour and 44 gm jaggery were added in the remaining 300 ml. distilled water and boiled for three to five minutes in order to dissolve them. To this solution the agar agar solution was added slowly and mixed by stirring. It was then boiled for 10 to 15 minutes for thorough
mixing. 1.7 ml. of propionic acid was added with the help of a pipette and stirred well to mix it. The mixture was again boiled for two to three minutes, and finally poured into sterilized glass jars.

Ten jars having a size of 10.2 cm. diameter and 13.5 cm. height were used. Fifty to sixty ml. of the medium was poured in these jars. This was allowed to cool at room temperature, and thereafter these jars were covered with polythene sheet duly perforated and tied with rubber band. These jars were preserved in refrigerator for further use.

3.4.4 Method for rearing Drosophila

Ten to fifteen pairs of three to four days old flies were introduced in glass jars covered with polythene sheets. Pin holes were made in the polythene sheet for aeration. These jars were kept at 26°C ± 1°C in a controlled temperature cabinet (Plate 2). A second set of glass jars were prepared similarly after an interval of four days for continuous maintenance of culture. The flies were allowed to oviposit, for 5 days and then discarded from the jar. The flies which emerged after twelve days were also discarded. Those flies which emerged within 24 hours thereafter were used for the experiment till three days consecutively. The flies which emerged after fifteen days were also not used for the experiment.

The transfer of flies from rearing jars into 10 x 2.5 cm. specimen tubes were done through the use of a funnel whose stem
Plate 2. Controlled temperature cabinet
passed through a rubber cork fitted in the mouth of the tube in such a way that the stem inside would not touch the sides of the tube and remained 2 cm. above the bottom. On the top of the polythene sheet of the rearing jar the funnel was inverted (Plate 3). The rubber band was then removed quickly and polythene sheet remaining in between the mouth of the jar and inverted funnel was removed slowly. The whole apparatus was toppled so as to have the jar in inverted position over the funnel. The funnel and jar were being held in one hand, and the flies were tapped at the top and sides of the jar (Plate 4) so as to force them to go in the tube attached to the funnel.

The flies in the specimen tube were tapped and collected at the bottom. The specimen tube containing flies was removed from the stem of funnel (Plate 5). The cork containing the funnel was then replaced with a cork having a hole of 0.2 cm. diameter in the center for aeration, duly plugged with cotton-wool. Six drops of chloroform were dripped on the cotton plug with the help of a dropper. The specimen tube was held in the horizontal position and rolled slowly so as to avoid sticking of flies in the bottom. The flies were in a coma within 2 to 2.5 minutes, and were taken out immediately into the counting plate to avoid an over dose of anaesthesia.

Only male flies were used for the bio-assay work because they are highly susceptible and less tolerant to the insecticides as compared to the females. The male flies were identified by their small size and distinct black spot on the tip of their abdomen. The counting was done quickly so as to avoid the escape of the flies due to the sudden recovery from the coma.
Plate 3. Funnel inverted on the jar and polythene sheet removed

Plate 4. Funnel with jar tapped for
Plate 5. Specimen tube containing flies
3.4.5 Procedure For Preparation Of Standard Dose Mortality Response Curve For Carbaryl by Micro Bio-assay Method

Two gm. of 50 per cent carbaryl wettable powder was taken in a 1000 ml. volumetric flask and dissolved in acetone and then the volume was made up by addition of acetone. Thus a 0.1 per cent solution was prepared and was marked as "Stage A" solution. One millilitre of this solution contained 1000 microgram of carbaryl technical.

One millilitre of the above "Stage A" solution was pipetted into a 1000 ml. volumetric flask containing a few ml. of acetone and then made up the volume by addition of acetone. Thus a "Stage B" solution thus containing one microgram of carbaryl technical per one millilitre was prepared. It was used for preparing the standard dosage mortality response curve for carbaryl.

The residue film of the insecticide was prepared in both the dishes of the paired petri dishes of 10 cm. diameter. Five pairs of petri plates were used into which 1 ml., 2 ml., 3 ml., 4 ml. and 5 ml. respectively of "Stage B" solution was pipetted in such a way that half of the quantity was put in the lower plate and the other half put in the top cover plate. A control petri plate was also maintained where in 1 ml. acetone per dish was taken. The solution pipetted in petri plates was spread uniformly by gently swirling the dishes and was then allowed to dry. A small lump of the media was also placed in each petri dish as food after evaporation of solution.

Twenty anaesthetised male Drosophila flies were introduced into each of the above prepared paired petri dishes. The mortality
counts were then taken at an interval of one hour up to eight hours. About fifty per cent of the flies have died within five hours.

From the data of mortality due to response of different quantities of insecticides tested in controlled condition a standard dosage mortality response regression curve was drawn (Figure 2) as per Sun and Sun (1952). From this standard dosage mortality response curve a minimum amount of one microgram of carbaryl can be determined.

This standard dosage mortality response regression curve was utilised to assess the residue of carbaryl on/in the mango fruits of the experiment.

3.5 Procedure For Preparation Of Standard Calibration Curve For Technical Endosulfan For Chemical Assay

The calorimetric method employed for endosulfan residue analysis was essentially the same as employed by Butler et al., (1962) and modified by Mailten et al., (1963). The clean up technique employed was that modified by Kathpal and Dewan (1975).

3.5.1 Reagents required.

(1) n-Hexane - Analytical grade (redistilled)
(2) Endosulfan - Technical grade.
(3) Pyridine solution 96 per cent in distilled water

Pyridine was purified by refluxing over potassium hydroxide (KOH) for one hour. 50 gm. KOH was taken in a one litre distillation flask. 700 ml. pyridine was added to this and heated for one hour. The above
Carbaryl concentration in microgram

Fig. 2 - STANDARD DOSAGE MORTALITY RESPONSE CURVE FOR CARBARYL ON/IN THE MANGO FRUITS BY MICRO BIO-ASSAY
mixture was then cooled and decanted into a separate cleaned flask and distilled again. Then a 96 per cent pyridine solution was made by the addition of distilled water at the rate of 4 per cent in the above redistilled pure pyridine according to the quantities of pyridine.

(4) **Mineral oil (refined)**

(5) **Methanolic sodium hydroxide (NaOH) 0.025 N.**

This was prepared by dissolving 100 mg. of analar grade sodium hydroxide in 100 ml. of analar methanol.

(6) **Pyridine methanolic sodium hydroxide reagent**

It was prepared by adding 10 ml. of 0.025 N methanolic sodium hydroxide into 50 ml. of the 96 per cent pyridine solution. This reagent is stable for only 6 hours and as such it was to be prepared fresh every time.

3.5.2 **Apparatus required**

1. **Stopper test tube**
   19/22 standard taper outer joint test tube 12.5 cm/mg.

2. **Oil bath assembly**
   Oil bath provided with cover having provision of holes for holding thermometer and stopper test tubes.

3. **Electric water bath**

4. **Spectronic-20**

5. **Vacuum pump**

6. **Multi-folder evaporator**
3.5.3 Preparation of standard technical grade solution of endosulfan

(1) One gram technical grade endosulfan was taken in a 100 ml. volumetric flask and slowly dissolved with n-hexane and the volume was made up so as to have a one per cent solution. Thus one millilitre of this solution contained 10,000 microgram endosulfan. The solution was designated as "Stage A" solution.

(2) Ten millilitre of stock solution "Stage A" was pipetted into a 100 ml. volumetric flask and the volume was made up with n-hexane. Thus one millilitre of this solution contained 1000 microgram of endosulfan and was designated as "Stage B" solution.

(3) Again one millilitre of the "Stage B" solution was pipetted into a 100 ml. volumetric flask and the volume was made up with n-hexane. Thus one millilitre of this solution contained 10 microgram endosulfan and was designated as "Stage C" solution. This solution was used for preparing standard calibration curve for chemical assay.

3.5.4 Procedure for preparation of standard calibration curve for estimation of endosulfan by chemical assay

(1) 0, 1, 2, 3, 4 and 5 ml. of the above "Stage C" solution of endosulfan was pipetted into glass stoppered test tubes separately. Then the volume was made up to 5 ml. in each test tube by the addition of distilled n-hexane.

(2) One drop of mineral oil was then added into each test tube with the help of a dropper.
(3) The test tubes were then put into a hot water bath at 40° to 50°C so as to evaporate the solution to dryness.

(4) Finally, 7.5 ml. of freshly prepared methanolic sodium hydroxide pyridine reagent was pipetted into each of the above test tubes and then stoppered. The stoppers were duly tied with rubber bands to avoid reopening of the stopper due to gas formation in the tubes.

(5) These test tubes were then immersed in an oil bath assembly up to solution level at 100 ± 2°C for four minutes.

(6) The tubes were then removed from the oil bath and the outer surface was cleaned with the help of cloth.

(7) The tubes were then kept in ice water for about a minute for cooling, and the stoppers were removed from the test tubes.

(8) Absorbance of colour complex (Pink) was measured in Spectronic-20 at 520 mμ against distilled water. The absorbance readings were taken within 10 to 15 minutes after cooling of the test tubes.

The standard calibration curve (Figure 3) was prepared by plotting quantities of insecticide (microgram) on 'X' axis and absorbance on 'Y' axis and the regression equation has been worked out on the basis of which the absorbance as worked out from the regression equation for 10, 20, 30, 40 and 50 microgram of insecticide and shown in Table 3. From this standard dosage mortality response curve a minimum amount of 0.1 microgram of endosulfan can be determined.
Fig. 3 - STANDARD CALIBRATION CURVE FOR ENDSULFAN BY CHEMICAL ASSAY.
Fig. 3 - STANDARD CALIBRATION CURVE FOR ENDSULFAN BY CHEMICAL ASSAY.
Table 3. Absorbance corresponding to various quantities of endosulfan technical grade

<table>
<thead>
<tr>
<th>Sy. No.</th>
<th>Amount of technical endosulfan (microgram)</th>
<th>Net average absorbance at 520 mÅ</th>
<th>Absorbance as worked out from regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.07</td>
<td>0.068</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0.12</td>
<td>0.121</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>0.18</td>
<td>0.174</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>0.21</td>
<td>0.227</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>0.29</td>
<td>0.280</td>
</tr>
</tbody>
</table>

3.6 Chemical Assay For Endosulfan Residue Estimation Form The Mango Fruits

The samples of the mango fruits were collected for the estimation of endosulfan residue in the same manner as described in carbaryl residue estimation (3.3).

3.6.1 Method of estimation of endosulfan residue on the mango fruits.

(1) One mango fruit was weighed and put in one litre beaker and 100 ml. n-hexane was added.

(2) The mango fruit was washed thoroughly by swirling with a glass rod for 10 to 15 minutes and then taken out from the beaker.

(3) The filtered aliquot was then concentrated to 50 ml. by way of keeping on water bath. This solution was then transferred into a labelled bottle air-tight being made and preserved in a refrigerator until further use.
(4) Out of the 50 ml. aliquot, 10 ml. solution was used for micro bio-assay and 40 ml. was used for chemical assay.

3.6.2 Reagents required.

(1) **Acetone** (redistilled)
(2) **n-Hexane** (redistilled)
(3) **Decolourizing Carbon** – Nuchar-190 N
(4) **Isopropanol** – Analar grade
(5) **Sodium sulphate** (Na$_2$SO$_4$)

3.6.3 Apparatus required.

(1) Electric water bath
(2) Blender
(3) Vacuum pump with accessories
(4) Separating funnel
(5) Buchner funnel
(6) Stoppered test tubes
(7) Oil bath assembly
(8) Spectronic-20

3.6.4 Method of estimation of the endosulfan residue in the mango fruits

(1) One mango fruit was taken from each sample and washed for about a minute with ordinary tap water and allowed to dry. It was then cut and chopped to a size of 0.5 to 1 cm. with a knife.

(2) 40 gram of the chopped fruit material was put into a Braun Maltimix MX-32 blender and blended for 1.5 minutes with the use of 80 ml. of n-hexane-isopropanol mixture (2 : 1).
(3) The blended material was then filtered through a Buchaner funnel by using a suction pump. It was then washed three times with 25 ml. 5 per cent aqueous sodium sulphate solution ($Na_2SO_4$) to remove isopropanol.

(4) The filtrate obtained from the suction flask was transferred into a separating funnel and shaken vigorously for 2 to 3 minutes as described by Jones and Riddick (1952) and then allowed to stand for 5 to 10 minutes for separating n-hexane layer and water layer. The upper layer was taken out and preserved after discarding the lower aqueous layer.

(5) Water traces were removed by passing n-hexane extract through 2 to 3 cm. column of anhydrous sodium sulphate.

(6) The aliquot was kept on a hot water bath and concentrated to a volume of 50 ml. The concentrated solution was then transferred into a labelled air-tight bottle and preserved in a refrigerator until further use.

(7) Out of 50 ml. aliquot, 10 ml. was used for micro bio-assay and 40 ml. was used for chemical assay.

3.6.5 Clean up procedure

(1) 40 ml. of the above aliquot was taken in a beaker and 10 ml. of acetone was added to it.

(2) Then one gram decolourizing carbon Nuchar C-190N was added and the beaker was shaken for about a minute.

(3) After shaking, the solution was allowed to stand as such for two minutes to settle the absorbant.

(4) The supernatant solution was then filtered through anhydrous sodium sulphate.
(5) 15 ml. of fresh solvent mixture of n-hexane and acetone (80 : 20) was added in the above empty beaker and shaken for about half a minute. It was then allowed to stand for two minutes and again the supernatant solution was filtered through the same funnel containing anhydrous sodium sulphate. This procedure was repeated for three times.

(6) Finally, the beaker and anhydrous sodium sulphate layer in the funnel were rinsed with 20 ml. of solvent mixture (n-hexane and acetone 80 : 20).

(7) The filtrate solution was then kept on a hot water bath and the solution was concentrated to a quantity of 5 ml. in the beaker. This solution was then used for the chemical assay.

3.6.6 Determination of endosulfan by chemical assay method

The above 5 ml. of filtrate solution was transferred into a stoppered test tube and the beaker was washed with n-hexane.

Further procedure followed was the same as that of the method of estimation of endosulfan described in the procedure for preparation of standard calibration curve by chemical assay (3.5.4).

3.7 Recovery Test of Carbaryl by Chemical Assay Method

The recovery experiments were done by adding and fortifying 10 ppm of carbaryl on/in mango fruits and thereafter carbaryl residue was estimated as per chemical assay method.
for the experiment. The results of recoveries of carbaryl indicated that to an extent of $100.3$ and $98$ per cent carbaryl can be recovered by the procedure adopted for residue analysis on/in fruits by chemical assay.

Bensons and Finacchiaro (1965) also reported that the recoveries ranging from $84$ to $106$ per cent from eight different crops to which $10$ ppm level of carbaryl solution was added. Similarly, Gajan et al., (1965) also reported carbaryl recoveries from fortified fruits and vegetable crops to be $95.3 \pm 9.0$ per cent at $0.2$, $5.0$ and $10.0$ ppm level of carbaryl, whereas Ishii and Yamashika (1972) reported that residue analysis of carbaryl (NAC) in tomato containing $0.06$, $0.1$ and $0.12$ ppm giving recoveries to an extent of $72$, $94$ and $74$ per cent respectively, but the same in cucumber having $0.1$ and $0.2$ ppm NAC gave $70$ and $94$ per cent recoveries respectively, in lettuce having $0.05$, $0.1$ and $0.2$ ppm NAC gave respectively $85$, $87$ and $92$ per cent recoveries. Thus the nature of fruits and vegetables also are having effect in recoveries of insecticidal residue. Still, however, the present finding did have concurrence with the one report by Benson and Finacchiaro (1965) and Gajan et al., 1965 as far as method of analysis was concerned.

3.8 Recovery Test of Endosulfan by Chemical Assay Method

The recovery experiments were done by adding and fortifying $10$ ppm of endosulfan on/in mango fruits and thereafter endosulfan residues were estimated as per chemical assay followed
for the experiment. The results of recovery of endosulfan indicated that to an extent of 66.00 and 47.20 per cent endosulfan can be recovered, by the procedure adopted for residue analysis on/in fruits by chemical assay.

Kathpal and Dewan (1975) reported the recoveries ranging from 86 to 102 per cent from plant tissue extracts fortified at the level of 0.5 to 2 ppm of endosulfan (I & II) whereas Srivastava and Kavadia (1976) reported the residue to an extent of 0.1 per cent of endosulfan. The recoveries obtained by them with the spectrometric and bio-assay methods were 85 and 93 per cent respectively. Thus the nature of fruits and vegetables were also having effect in recoveries of insecticidal residues. The present finding did not have any concurrence with the above reports of Kathpal and Dewan (1965) as well as Srivastava and Kavadia (1976) as far as recoveries are concerned, even though the methods of analysis were the same. This difference may be due to differential adhering characteristics of endosulfan with different fruits and vegetables.
CHAPTER IV

RESULTS AND DISCUSSION

4.1 Carbaryl Residue

4.1.1 Determination of carbaryl residue on mango fruits by chemical assay.

Seven carbaryl sprayings were done at the rate of 0.2 per cent on the mango trees in the experiment conducted in 1976-77. The results obtained for carbaryl residue on the mango fruits by chemical assay are presented in Table 4. These data indicated that the initial deposit of carbaryl residue on fruits found immediately after last spraying was 58.16 ppm and the residue after 3rd, 7th and 25th day of last spraying of carbaryl was 37.22, 18.49 and 10.30 ppm respectively. Thus the per cent reduction of carbaryl residue were 36.00, 50.32 and 65.71 per cent after 3rd, 7th and 25th day of last spraying. Gunther and Blinn (1955) as well as Hoskin (1961) have suggested the unit of half life value of insecticidal residue for comparison and thus the half life value of carbaryl residue on mango fruits worked out was 3.19.

Similarly, in the experiment conducted in 1977-78 also the mango trees were sprayed only once with 0.2 per cent carbaryl. The estimation of carbaryl residue was then done and the results obtained by the chemical assay are presented in Table 5. The initial deposit of carbaryl residue on fruits
Table 4. Residue of carbaryl on/in mango fruits due to the treatment of 0.2 per cent carbaryl spraying in 1976-77 by chemical assay

<table>
<thead>
<tr>
<th>Stage of sampling</th>
<th>On the fruit</th>
<th>In the fruits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Residue in ppm</td>
<td>Percentage of reduction</td>
</tr>
<tr>
<td>(1) Immediately after the last spraying</td>
<td>58.16</td>
<td>-</td>
</tr>
<tr>
<td>(2) 3 days after the last spraying</td>
<td>37.22</td>
<td>36.00</td>
</tr>
<tr>
<td>(3) 7 days after the last spraying</td>
<td>18.49</td>
<td>50.32</td>
</tr>
<tr>
<td>(4) At the time of harvesting (25 days after the last spraying)</td>
<td>10.30</td>
<td>65.71</td>
</tr>
</tbody>
</table>
was 52.56 ppm while the residue was 38.00, 20.50 and 8.80 ppm after 3rd, 7th and 19th day of spraying respectively (Table 5). These reduction on residue basis were 27.70, 61.00 and 83.26 per cent after 3rd, 7th and 19th day respectively of the spraying and the half life value for carbaryl residue on the mango fruits was 3.00 days.

From the above results of both the years it can be seen that there was not much difference in the initial deposit of carbaryl as well as residue reduction due to weathering action on the mango fruits arising due to seven sequential sprayings and one spraying of carbaryl done in the year 1976-77 and 1977-78 respectively. This behaviour indicated that there was no cumulative effect seen in the residue due to sequential sprayings. But it can be further seen that the residue at the time of harvest remained below the tolerance limit prescribed, in the year 1977-78.

4.1.2 Determination of carbaryl residue in mango fruits by chemical assay

One minute washing of the carbaryl sprayed mango fruit with tap water, resulted in considerable removal of initial carbaryl residue as can be seen from Table 4. The initial carbaryl residue deposit after washing treatment in the fruits as determined by chemical assay was 42.53 ppm, and the same after 3rd, 7th and 25th day of last spraying of carbaryl was 14.79, 13.61 and 8.82 ppm respectively in the year 1976-77. Similarly, in the year 1977-78 it was 21.13 ppm as initial residue deposit, while it was 14.35, 13.62 ppm and BDL (Below
<table>
<thead>
<tr>
<th>Stage of sampling</th>
<th>On the fruit</th>
<th>In the fruits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Residue in ppm</td>
<td>Percentage of reduction</td>
</tr>
<tr>
<td>(1) Immediately after the spraying</td>
<td>52.56</td>
<td>-</td>
</tr>
<tr>
<td>(2) 3 days after the spraying</td>
<td>38.00</td>
<td>27.70</td>
</tr>
<tr>
<td>(3) 7 days after the spraying</td>
<td>20.50</td>
<td>61.00</td>
</tr>
<tr>
<td>(4) At the time of harvesting (19 days after the spraying)</td>
<td>8.80</td>
<td>83.26</td>
</tr>
</tbody>
</table>

BDL: Below detectable level.
Detectable Level) after 3rd, 7th and 19th day respectively of one spraying of carbaryl. The reduction percentage of carbaryl residue due to washing treatment were thus 60.26, 26.39 and 14.37 per cent in 1976-77, but 54.31, 33.56 and 100 per cent in 1977-78 on 3rd, 7th and 25th/19th day after spraying of insecticide respectively (Table 4 and Table 5).

From the above result it can be seen that there was a difference in initial deposit of carbaryl as well as residue reduction due to weathering action on mango fruits arising due to seven sprayings and one spraying of carbaryl done in the year 1976-77 and 1977-78 respectively. This behaviour indicated that even though there was a cumulative effect due to sequential seven sprayings and binding as well as translocation of insecticide in fruits, the carbaryl residue remained below tolerance limit at the time of harvest of fruits. Data of both the years showed that a safe interval of 19 to 25 days should be observed for harvesting of mango fruits after spraying of carbaryl for the plant protection measure against the mango pests.

4.1.3 Determination of carbaryl residue on mango fruits by micro bio-assay

Seven carbaryl sprayings were done at the rate of 0.2 per cent on mango trees in the experiment conducted in 1976-77. The work of analysis of insecticidal residue on fruits was done using micro bio-assay method. The results obtained are represented in Table 6. The initial deposit of carbaryl residue found immediately after last spraying was 76.00 ppm and the residue after 3 days of spraying of carbaryl was 46.18 ppm.
Table 6. Residue of carbaryl on/in mango fruits due to treatment of 0.2 per cent carbaryl spray in 1976-77 by micro bio-assay

<table>
<thead>
<tr>
<th>Stage of sampling</th>
<th>On the fruits</th>
<th>In the fruits</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Residue in ppm</td>
<td>% Reduction</td>
<td>Residue in ppm after washing</td>
</tr>
<tr>
<td>(1) Immediately after the last spraying</td>
<td>76.00</td>
<td>-</td>
<td>49.22</td>
</tr>
<tr>
<td>(2) 3rd day after the last spraying</td>
<td>46.18</td>
<td>39.24</td>
<td>1.54</td>
</tr>
<tr>
<td>(3) 7th day after the last spraying</td>
<td>BDL</td>
<td>100.00</td>
<td>BDL</td>
</tr>
<tr>
<td>(4) At the time of harvesting (25 days after the last spraying)</td>
<td>BDL</td>
<td>100.00</td>
<td>BDL</td>
</tr>
</tbody>
</table>

DDL: Below Detectable Level.
while they were below detectable level in both the cases, i.e. after 7th and 25th days of last spraying. Thus the percentages of reduction of carbaryl residue were 39.24, 100 and 100 per cent after 3rd, 7th and 25th day of last spraying of carbaryl.

Similarly, the mango trees were sprayed once with 0.2 per cent carbaryl in the year 1977-78 and the estimation of carbaryl residue was done at the respective time intervals and the data obtained by micro bio-assay method are presented in Table 7. It can be seen that the initial carbaryl residue deposit was 58.56 ppm while carbaryl residues were 37.38, 8.58 and 2.82 ppm after 3rd, 7th and 19th day of spraying respectively. These reductions on residue basis were 36.17, 85.35 and 95.18 per cent after 3rd, 7th and 19th day respectively after one spraying (Table 7).

From the above results it can be seen that there was some difference in the initial deposit of carbaryl as well as residue reduction due to weathering action on mango fruits arising due to seven sprayings in the year 1976-77 and one spraying of carbaryl in the year 1977-78. This behaviour indicated that there was cumulative effect in case of carbaryl residue on mango fruits due to sequential seven sprayings of carbaryl in comparison to one spraying of carbaryl in mango orchards.

4.1.4 Determination of carbaryl residue in mango fruits by micro bio-assay.

One minute washing of the carbaryl sprayed mango fruit with tap water resulted in considerable removal of the initial carbaryl residue as can be seen from Table 6 and 7. The initial
Table 7. Residue of carbaryl on/in mango fruits due to treatment of 0.2 per cent carbaryl spray in 1977-78 by micro bio-assay

<table>
<thead>
<tr>
<th>Stage of sampling</th>
<th>Residue on the fruits</th>
<th>Residue in the fruits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Residue in ppm of</td>
<td>Percentage of reduction in ppm after washing</td>
</tr>
<tr>
<td>(1) Immediately after the spraying</td>
<td>58.56</td>
<td>-</td>
</tr>
<tr>
<td>(2) 3rd day after the spraying</td>
<td>37.38</td>
<td>36.1%</td>
</tr>
<tr>
<td>(3) 7th day after the spraying</td>
<td>8.58</td>
<td>85.35</td>
</tr>
<tr>
<td>(4) At the time of harvesting (19 days after the spraying)</td>
<td>2.82</td>
<td>95.18</td>
</tr>
</tbody>
</table>

BDL : Below Detectable Level.
carbaryl residue deposit during 1976-77 was 49.22 ppm and 1.54 ppm was detected after 3rd day of spraying of carbaryl and below detectable level of carbaryl residue after 7th and 25th day of spraying of carbaryl. Similarly, it was 6.00 ppm as initial carbaryl deposit and 1.44 ppm after 3rd day of one spraying of carbaryl and thereafter residue remained as below detectable level on 7th and 19th day after one spraying of carbaryl (Table 7). The reduction percentages of carbaryl residue due to washing treatment were thus 96.66, 100.00 and 100.00 per cent in 1976-77 and 96.15, 100.00 and 100.00 per cent in 1977-78 after 3, 7 and 25/19 days respectively of spraying in both the years (Table 6 and 7).

From the above results it can be seen that there was more difference in the initial residue deposit of carbaryl between two treatments but in the dissipation of residue, there was no difference as can be seen from the data of 3rd, 7th and 25th/19th day after seven sequential sprayings in 1976-77 or one spraying of carbaryl in 1977-78. This behaviour indicated that there was a cumulative effect in the initial deposit due to sequential sprayings in comparison to one spraying, but the degradation rate remained the same for both cases to bring the carbaryl residue below tolerance limit.

It is evident from the determination of carbaryl residue on the fruits by chemical assay that the phenomenon of dissipation trend was more or less similar and it was such that it reached above tolerance level in 1976-77 but below the prescribed tolerance limit of 10 ppm of FAO/WHO recommendations (Anonymous 1972), i.e.
10.30 and 8.80 ppm in 1976-77 and 1977-78 respectively. The percentage of reduction in residue had more or less similar trend i.e. 36.00, 50.32 and 65.71 per cent in 1976-77 and 27.70, 61.00 and 83.26 per cent in 1977-78, three, seven and twentyfive/nineteen days respectively after carbaryl treatment (Table 4 and 5). The half life value of carbaryl were also more or less similar in both the years, i.e. 3.19 days for 1976-77 and 3.00 days for 1977-78 (Table 4 and 5). The initial carbaryl residue deposit in mango fruits after washing, i.e. 42.53 ppm in 1976-77 was higher in comparison to that in 1977-78 i.e. 21.17 ppm. But the carbaryl residue thereafter remained more or less same in quantities on 3rd and 7th day after spraying of carbaryl. Thus, similar trend in dissipation value, i.e. 14.79 and 13.61 ppm in 1976-77 as well as 14.35 and 13.62 ppm in 1977-78 was observed. In both the years carbaryl residue did not reach below tolerance limit of 10 ppm at the time of harvesting of mango fruits. Thus washing of fruits did have the reduction effect in insecticidal residue, but the data (Table 4 and 5) indicated that the effect of persistency was more in 1976-77 because of sequential sprayings in comparison to 1977-78 where only one spraying was done. Still, however, a safe interval of 19/25 days or more were required to reach the carbaryl residue below tolerance limit (Table 4 and Table 5).

Estimation of residue by micro bio-assay method (Table 6) also indicated more or less same trend for both the years and it reached below the tolerance limit of 10 ppm at the time of 7th day after spraying of the mango fruits, i.e. below detectable level and 8.85 ppm in 1976-77 and 1977-78 respectively. The percentage of reduction in residue showed more or less similar
10.30 and 8.80 ppm in 1976-77 and 1977-78 respectively. The percentage of reduction in residue had more or less similar trend i.e. 36.00, 50.32 and 65.71 per cent in 1976-77 and 27.70, 61.00 and 83.26 per cent in 1977-78, three, seven and twentyfive/nineteen days respectively after carbaryl treatment (Table 4 and 5). The half life value of carbaryl were also more or less similar in both the years, i.e. 3.19 days for 1976-77 and 3.00 days for 1977-78 (Table 4 and 5). The initial carbaryl residue deposit in mango fruits after washing, i.e. 42.53 ppm in 1976-77 was higher in comparison to that in 1977-78 i.e. 21.17 ppm. But the carbaryl residue thereafter remained more or less same in quantities on 3rd and 7th day after spraying of carbaryl. Thus, similar trend in dissipation value, i.e. 14.79 and 13.61 ppm in 1976-77 as well as 14.35 and 13.62 ppm in 1977-78 was observed. In both the years carbaryl residue did not reach below tolerance limit of 10 ppm at the time of harvesting of mango fruits. Thus washing of fruits did have the reduction effect in insecticidal residue, but the data (Table 4 and 5) indicated that the effect of persistency was more in 1976-77 because of sequential sprayings in comparison to 1977-78 where only one spraying was done. Still, however, a safe interval of 19/25 days or more were required to reach the carbaryl, residue below tolerance limit (Table 4 and Table 5).

Estimation of residue by micro bio-assay method (Table 6) also indicated more or less same trend for both the years and it reached below the tolerance limit of 10 ppm at the time of 7th day after spraying of the mango fruits, i.e. below detectable level and 8.85 ppm in 1976-77 and 1977-78 respectively. The percentage of reduction in residue showed more or less similar
trend, i.e. 39.24, 100.00 and 100.00 per cent in 1976-77 and
36.17, 85.35 and 95.18 per cent in 1977-78 for 3rd, 7th and 25th/
19th day respectively after treatment (Table 6 and 7). The initial
carbaryl residue in mango fruits after washing was 49.22 ppm in
1976-77 and was much higher in comparison to 6.00 ppm in 1977-78.
The carbaryl residue thereafter showed similar trend in dissipation
value, i.e. 1.54 and 1.44 ppm on 3rd day and below detectable level
on 7th and 25th/19th day after spraying in 1976-77 and 1977-78
respectively. Thus within 7 days and 25/19 days after spraying
of mango fruits, carbaryl residue reached below the detectable
level and as such washing of fruits did have a reduction effect
in the status of insecticide residue on fruits. But the data
(Table 6 and 7) indicated that the effect of persistency was more
in 1976-77 in comparison to 1977-78. This was due to cumulative
effect of residue gathered because of seven sprayings in 1976-77
in comparison to only one spraying of carbaryl in 1977-78.

4.1.5 Carbaryl residue on the fruits.

From the results it is clear that the residue of carbaryl
dissipated to a good amount both due to time intervals as well
as washing of fruits. The reduction per cent of carbaryl residue
after three, seven and twentyfive/nineteen days after spraying
was 36.00, 50.32 and 65.70 and 27.70, 61.00 and 83.26 per cent
respectively for 1976-77 and 1977-78 (Table 4 and 5). There are
some reports on the rapid dissipation of carbaryl residue within
a few days. Dewan et al., (1967) reported 100 per cent reduction
of carbaryl deposit on okra fruits within three days after treat-
ment. Srivastava et al., (1975) reported 92.53 per cent reduction
of carbaryl residue that arose after 2 to 3 days of 0.25 per cent
spray of carbaryl on okra fruits. But, Avasthi et al., (1977) reported that initial carbaryl residue of 26.45 and 37.10 ppm respectively that arose due to spray of carbaryl at the rate of 0.125 and 0.25 per cent reduced to 44 to 46 per cent after 3 days and 67.5 to 68.8 per cent after 7 days respectively. Thus the results of the present findings were in concurrence for the percent reduction of carbaryl residue reported by Dewan et al., (1967), Srivastava et al., (1975) and Avasthi et al., (1977).

Similarly, the reports of Johnson and Stansbury (1965) gave evidences for the dissipation of carbaryl residue in spinach below the tolerance level in 7 days, i.e. the initial residue of 52.00 ppm declined to 9 ppm in 7 days. They have also determined the half life of carbaryl residue as 3 days. But the present finding that carbaryl residue dissipated below tolerance level after 25/19 days of spraying was in contradiction to one reported by Johnson and Stansbury (1965) because of crop difference. Still, however, it was worthwhile to observe that the trend of the half life values of 3.19 and 3.00 days (Table 4 and 5) obtained in the present study were more or less similar to that reported by Johnson and Stansbury (1965).

The residue apparently reached below the tolerance level by 25 days or 19 days of the treatment were in concurrence to those of Polizu et al., (1971), Kathpal et al., (1976) and Gupta et al., (1972). Polizu et al., (1971) reported that the residue of carbaryl reached below the tolerance level in peach, apple and plum upto 21 days of the treatment when carbaryl was used at a rate of 0.15 per cent. Gupta et al., (1972) reported
that carbaryl residues were below the tolerance level within 2 days but were detectable up to 28 days when carbaryl was used at a rate of 0.001 per cent (i.e. 1000 ppm). A similar trend of dissipation of carbaryl residue to reach below tolerance level in 18 days with single spray of 0.2 per cent carbaryl on grape berries was reported by Kathpal et al., (1976). It was in fact obvious from the above results that the residue of carbaryl dissipated below the tolerance level after 25/19 days of spraying, i.e. at the time of harvest of fruits, and indicated that safe interval of at least 19 to 25 days should be observed for harvesting of the mango fruits after spraying of carbaryl.

4.1.6 Carbaryl residue after washing the fruits.

In order to determine the effect of washing on residue of carbaryl, the mango fruits were washed for one minute with tap water. This process resulted in having an initial residue of 42.53 ppm from 58.16 ppm and 21.13 ppm from 52.56 ppm in the years 1976-77 and 1977-78 respectively (Table 4 and 5). Thus the reduction of carbaryl residue due to washing ranged from 26 to 60 per cent in the present findings. Srivastava et al., (1975) reported that the residue reduced immediately after spraying to 5.94 from 17.53 ppm and to 12.24 ppm from 40.20 ppm due to washing when sprayed at the rate of 0.2 and 0.5 per cent of carbaryl respectively. Kathpal et al., (1976) also found that the washing of berries under running tap water for 30 seconds removed the carbaryl residue to an extent of 50 to 100 per cent depending on the time elapsed after the treatment. The reports of various workers also showed that carbaryl residue reduced to a range of
80 to 90 per cent due to washing of various crops viz., tomato, bhindi, cowpea and eggplants (Deshmukh et al., 1972; Nath et al., 1975; Srivastava and Sharma, 1976 and Avasthi et al., 1977). Thus the results of present findings are in concurrence with the above reported works.

Kathpal et al., (1976) as well as Mann and Chopra (1969) reported about the possibility of a cumulative effect of several sprayings for high residue in initial deposit and the same fact was observed in the present findings for the year 1976-77.

In nutshell, the washing did help in reducing the residue level to great extent in the present findings but results indicated that the residue level did not reach below tolerance level after washing treatment even after three and seven days after spraying but required a period of 25/19 days after spraying. Thus it indicated that the habit of washing fruits should be followed as a recommended practice in daily consumption of fruits of mango to avoid any human hazard.

4.2 Endosulfan Residue

4.2.1 Determination of endosulfan residue on mango fruits by chemical assay.

Seven endosulfan sprayings were done at the rate of 0.075 per cent on mango trees in the experiment conducted in 1976-77. The result obtained for endosulfan residue on mango fruits are presented in Table 8. These data indicated that the initial deposit of endosulfan residue on fruits found immediately after last spraying was 73.94 ppm, and the residue after 3rd, 7th and 25th day of last spraying of endosulfan was 15.52, 15.68
and 5.66 ppm respectively. Thus the percentage of reduction of endosulfan residue was 79.01, 88.02 and 92.35 per cent after 3rd, 7th and 25th day of spraying of endosulfan respectively. Gunther and Blinn (1955) as well as Hoskin (1961) have suggested the unit of half life value of insecticidal residue for comparison and thus the half life value of endosulfan residue on mango fruits worked out to be 1.76 days.

Similarly, the mango trees were sprayed once with 0.075 per cent endosulfan in the experiment conducted in 1977-78. The estimation of endosulfan residue was then done and the results obtained are presented in Table 9. The initial deposit of endosulfan residue on fruits was 9.75 ppm while the residue was 7.09, 6.25 and 2.53 ppm after 3rd, 7th and 19th day of spraying respectively (Table 9). The reduction of residue basis were 27.28, 35.90 and 74.05 per cent after 3rd, 7th and 19th day respectively after spraying with half life value for endosulfan residue on mango fruits to be 3.54 days.

From the above results of both the years, it can be seen that there was much difference in initial deposit of endosulfan arising due to seven sprayings and one spraying of endosulfan done in the year 1976-77 and 1977-78 respectively. But the trend of dissipation remained the same for both the years. This behaviour indicated that there was cumulative effect due to sequential sprayings. It can be further seen that the residue at the time of harvest not reached below tolerance limit of 2 ppm prescribed by FAO/WHO (Anonymous 1972) in the year 1976-77 and 1977-78.
<table>
<thead>
<tr>
<th>Stage of sampling</th>
<th>Residue on the fruits (ppm)</th>
<th>Percentage of reduction</th>
<th>Half life in days</th>
<th>Residue in the fruits (ppm) after washing</th>
<th>Percentage of reduction due to washing</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Immediately after the last spraying</td>
<td>73.94</td>
<td>-</td>
<td>-</td>
<td>24.51</td>
<td>49.43</td>
</tr>
<tr>
<td>(2) 3 days after the last spraying</td>
<td>15.52</td>
<td>79.01</td>
<td>-</td>
<td>9.33</td>
<td>66.85</td>
</tr>
<tr>
<td>(3) 7 days after the last spraying</td>
<td>13.68</td>
<td>88.02</td>
<td>1.76</td>
<td>3.24</td>
<td>75.32</td>
</tr>
<tr>
<td>(4) At the time of harvesting (25 days after the last spraying)</td>
<td>5.66</td>
<td>92.35</td>
<td>-</td>
<td>2.53</td>
<td>54.82</td>
</tr>
</tbody>
</table>
4.2.2 Determination of endosulfan residue in mango fruits by chemical assay.

One minute washing of the endosulfan sprayed mango fruits with tap water, resulted in considerable removal of initial endosulfan residue as can be seen from Table 8. The initial residue deposit after washing treatment in the fruits was 24.51 ppm and the same after 3rd, 7th and 25th day of last spraying of endosulfan were 9.33, 3.24 and 2.53 ppm respectively in the year 1976-77. Similarly, it was 4.12 ppm as initial residue deposit while 3.06, 1.47 and 0.30 ppm after 3rd, 7th and 19th day respectively of only one spraying of endosulfan in the year 1977-78. The reduction percentage of endosulfan residue due to washing treatment was thus 66.85, 75.32 and 54.88 per cent in 1976-77, but 56.84, 76.84 and 88.14 per cent in 1977-78, on 3rd, 7th and 25th/19th day after spraying of insecticide respectively (Table 8 and 9).

From the above result it can be seen that there was difference in initial deposit of endosulfan as well as residue reduction due to weathering action in mango fruits arising due to seven sprayings and one spraying of endosulfan done in the year 1976-77 and 1977-78 respectively. This behaviour indicated that even though there was a cumulative effect due to sequential seven sprays and binding as well as translocation of insecticide in the fruits but the endosulfan residue remained below tolerance limit at the time of harvesting of fruits due to washing treatment. Data of both the years thus showed that safe interval of 19 to 25 days and treatment of washing of fruits should be observed for harvesting of mango fruits when spraying of endosulfan is to be done for the plant protection measure against the mango hoppers in mango orchards to save consumers from human hazard.
Table 9. Residue of endosulfan on/in mango fruits due to the treatment of 0.075 per cent endosulfan spraying in 1977-78 by chemical assay

<table>
<thead>
<tr>
<th>Stage of sampling</th>
<th>Residue in ppm</th>
<th>Percentage of reduction</th>
<th>Half life in days</th>
<th>Residue in ppm after washing</th>
<th>Percentage of reduction due to washing</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Immediately after the spraying</td>
<td>9.75</td>
<td>-</td>
<td>4.12</td>
<td>57.74</td>
<td></td>
</tr>
<tr>
<td>(2) 3 days after the spraying</td>
<td>7.09</td>
<td>27.28</td>
<td>3.06</td>
<td>56.84</td>
<td></td>
</tr>
<tr>
<td>(3) 7 days after the spraying</td>
<td>6.25</td>
<td>35.90</td>
<td>3.54</td>
<td>76.48</td>
<td></td>
</tr>
<tr>
<td>(4) At the time of harvesting (19 days after the spraying)</td>
<td>2.53</td>
<td>74.05</td>
<td>0.30</td>
<td>88.14</td>
<td></td>
</tr>
</tbody>
</table>
4.2.3 **Endosulfan residue on the mango fruits.**

The initial residue of endosulfan was found to be 73.94 ppm in the year 1976-77 (Table 8), while in the following year the residue was only 9.75 ppm (Table 9). The vast variation in the initial residual level was due to the cumulative effect of seven sprays of endosulfan in the previous year in comparison to a single spray in the later year. But the same difference was not observed in the rate of dissipation of residue as the reduction per cent was not varying as of the initial deposit in the next interval such as 3, 7 and 25/19 days after spraying. The percentage reduction of endosulfan residue on 3rd, 7th and 25th/19th day were 79.01, 88.02 and 92.35 in the year 1976-77 and were 27.28, 35.90 and 74.05 in the year 1977-78 respectively (Table 8 and 9). Leitao and Fernandes (1966) reported that the endosulfan sprayed at the rate of 52.5 and 105 ml. a.i./ha in tomato fruits showed a rapid reduction of its residues in the first 4 days. Nath et al., (1974) also reported that the residue dissipated more rapidly in the first two days after the application of endosulfan in both the concentrations, i.e. 0.1 and 0.2 per cent in okra fruits. These findings are relatively in contradiction to the present study where the percentage reduction is only 79.01 and 27.28 for both the years. Benoit (1973) found that the endosulfan residue reached below the tolerance level in 34 days in lettuce. Srivastava et al., (1976) reported that endosulfan spray on fig fruits at 0.1 per cent after 3 sprays, took 30 days to reach below the tolerance residue level. It is thus evident from the above reports that the endosulfan may
take such a long period of time of 30 days for reaching below the tolerance level prescribed. Therefore the endosulfan residue of 5.66 ppm and 2.53 ppm, i.e. above tolerance limit, found after 25/19 days for both the years were in concurrence to those reported by Benoit (1973) and Srivastava et al., (1976) as far as period of observation is concerned.

Further, the half life of the endosulfan for the period of 25 days covered by the seven sequential spraying of endosulfan in the year 1976-77 worked out to be 1.76 days, whereas the same for an unequal period i.e. 19 days after single spraying of endosulfan in the year 1977-78 was found to be 3.54 days. One of the explanations that can be offered for such a phenomenon is that the oxidative reduction and hydrolytic bio-degradation factors could become weakly operative in dissipating the residues in the year 1977-78 in comparison to that in the year 1976-77.

The second explanation that can be offered for such surprising phenomenon is that the difference in climatic conditions prevailing in the area of two places where the experiments have been carried out.

4.2.4 Endosulfan residue after washing mango fruits.

In order to determine the inner residue of endosulfan on fruits, the mango fruits were washed for one minute with tap water. This process resulted in an initial reduction of residue, i.e. 24.51 ppm from 73.94 ppm and 4.12 ppm from 9.75 ppm in the year 1976-77 and 1977-78 respectively (Table 8 and 9).
The dissipation of the endosulfan residue attained below the tolerance level after 25 days in 1976-77 and after 7 days in the year 1977-78 due to washing. The reduction percentage of endosulfan residue are coinciding with the results of both the years, as well as those reported by Nath et al., (1974) and Nath et al., (1975). Further, Kathpal et al., (1977) found that in case of 0.1 per cent endosulfan spray on ber fruits the residue was below the tolerance level within 5 days after washing the fruits for 4 to 5 minutes with tap water. But in the present findings it was only after 25 days when sequential sprayings were done in the year 1976-77 while it was even after 7 days when one spraying was done in 1977-78.

The half life value found to be more or less similar in both the years, i.e. 1.76 days in 1976-77 and 3.54 in the year 1977-78. Srivastava and Kavadia (1976) also reported the half life of endosulfan as 5 to 6 days. This reference gives an indication that the degradation of endosulfan to its half life value may be possible upto 6 days.

These findings could reveal that the safe interval of 25 days are required to be observed for harvesting as well as washing of mango fruits with water before the consumption, when the orchard is to be sprayed for more than one spray of endosulfan.
CHAPTER V

SUMMARY AND CONCLUSIONS

Chemical and micro bio-assay of residues of recommended insecticides viz., carbaryl and endosulfan on/in mango fruits had been carried out. Seven sequential sprayings of carbaryl at the rate of 0.2 per cent and endosulfan at the rate of 0.075 per cent in the form of schedule of plant protection work were done in the experiment conducted in the year 1976-77. Thereafter the samples of fruits were harvested and duly analysed for the status of insecticidal residues at different time intervals, i.e. immediate, three, seven and twentyfive days after the last spraying. Similarly, to know the effect of washing, the samples of fruits were harvested and duly analysed after washing treatment for the status of insecticidal residues at the same time for the interval as stated above. Further, to know the difference of sequential sprayings and one spraying of insecticide on the status of insecticidal residue on/in mango fruits, an experiment was conducted in the year 1977-78, where only one spray of carbaryl at the rate of 0.2 per cent and endosulfan at the rate of 0.075 per cent have been carried out when fruits were half matured. Thereafter sample of fruits were harvested and duly analysed for the residue content as such and after washing treatment for different time intervals, i.e. immediate, three, seven and nineteen days after spraying.
In case of carbaryl both the methods, i.e. chemical as well as micro bio-assay were used for determination of residue while for endosulfan only chemical assay was used for the determination of residue.

5.1 Carbaryl Residue

5.1.1 Chemical assay: In chemical assay the average initial residue deposit of carbaryl on the fruits immediately after last spraying was 58.16 ppm and residues after 3rd, 7th and 25th days of last spraying of carbaryl was 37.22, 18.49 and 10.30 ppm respectively. Thus the percentage of reductions of carbaryl residue were 36.00, 50.32, and 65.71 per cent after 3rd, 7th and 25th days of last spraying respectively having the half life of carbaryl residue on the fruits to be 3.19 days for the year 1976-77. Similarly, the initial residue deposit of carbaryl was 52.56 ppm while it was 38.00, 20.50 and 8.80 ppm respectively after one spraying on 3rd, 7th and 19th days of insecticide with the result that the reduction percentages of carbaryl residue were 27.70, 61.00 and 83.26 per cent respectively of the initial residue having the half life value of carbaryl residue on the fruits to be 3.00 days for the year 1977-78.

The initial deposit of carbaryl after one minute washing of the carbaryl sprayed mango fruits with tap water was 42.53 ppm and the same has dissipated with the result that 14.79, 13.61 and 8.82 ppm were found on 3rd, 7th and 25th days respectively after last spraying while for the year 1977-78 the initial residue deposit of carbaryl was 21.13 ppm and thereafter it was 14.35, 13.62 ppm and below detectable level on 3rd, 7th and 19th days.
respectively after one spraying of carbaryl. The reduction percentages of carbaryl residue due to washing treatment were thus 60.26, 26.39 and 14.37 per cent in 1976-77 and 54.31, 33.56 and 100 per cent in 1977-78 for different time intervals reported as above.

5.1.2 Micro bio-assay: In micro bio-assay the initial residue deposit of carbaryl found immediately after last spraying was 76.00 ppm and the same on 3rd, days of spraying was 46.18 ppm while it was below detectable level after 7 and 25 days of last spraying. Thus the percentages of reduction of carbaryl residue were 39.24, 100.00 and 100.00 per cent on 3rd, 7th and 25th days respectively of last spraying of carbaryl for the year 1976-77. But the initial residue deposit of carbaryl was 58.56 ppm which has dissipated and found to be 37.38, 8.58 and 2.82 ppm respectively after one spraying in the year 1977-78. Thus reductions on residue basis were 36.17, 85.35 and 95.18 per cent after 3rd, 7th and 19th days respectively.

The initial residue deposit of carbaryl due to one minute washing of the carbaryl sprayed mango fruits with tap water was 49.22 ppm but the residue on 3rd day was 1.54 ppm and below detectable level on 7th and 25th days of spraying of carbaryl in the year 1976-77, while the initial carbaryl residue after washing treatment was 6.00 ppm and thereafter it was 1.44 ppm on 3rd day and below detectable level on 7th and 19th days respectively of one spraying of carbaryl in the year 1977-78. The reduction percentages of carbaryl residue due to washing treatment were thus 96.66, 100.00 and 100.00 per cent in the year 1976-77 and 96.15, 100.00 and 100.00 per cent in the year 1977-78 after
3, 7 and 25/19 days respectively after spraying of insecticide.

5.2 Endosulfan Residue

5.2.1 **Chemical assay:** The initial residue deposit of endosulfan on the mango fruits immediately after last spraying was 73.94 ppm and residue after 3rd, 7th and 25th days of last spraying of endosulfan were 15.52, 13.68 and 5.66 ppm respectively. Thus the percentages of reduction of endosulfan residue were 79.01, 88.02 and 92.35 per cent after 3rd, 7th and 25th days respectively of the last spraying of insecticide with the half life of endosulfan residue on the fruits to be 1.76 days for the year 1976-77. Similarly, for the year 1977-78, the initial residue deposit of endosulfan was 9.75 ppm and thereafter they were 7.09, 6.25 and 2.53 ppm on 3rd, 7th and 19th days respectively after one spraying of insecticide. The reduction percentages of endosulfan residue thus were 27.28, 35.90 and 74.05 per cent after three, seven and nineteen days respectively of spraying, with the half life value of endosulfan to be 3.54 days.

The initial residue deposit of endosulfan after one minute washing of insecticidal sprayed mango fruits with tap water was 24.51 ppm and it has dissipated thereafter with the result that the same on 3rd, 7th and 25th days of spraying were 9.33, 3.24 and 2.53 ppm respectively in the year 1976-77, while in the year 1977-78 due to washing the fruits, the initial residue of endosulfan was 4.12 ppm and afterwards they were 3.06, 1.70 and 0.30 ppm on 3rd, 7th and 19th days respectively of once spraying of endosulfan. The reduction percentages of endosulfan residue due
to washing were thus 66.85, 75.32 and 54.82 per cent in 1976-77 and 56.84, 76.48 and 88.14 per cent in 1977-78 on 3rd, 7th and 25th/19th days respectively after spraying of endosulfan.

5.3 Conclusion and Recommendation

It could be inferred from the above results of both the years that the carbaryl residue remained below tolerance limit at the time of harvesting of fruits and therefore the safe interval of 19 to 25 days should be observed for harvesting of mango fruits after spraying of carbaryl as found in chemical assay. Washing of the mango fruits with the tap water for one minute did reduce carbaryl residue but it could reach below tolerance level only after twentyfive days of last spraying.

Similarly, it can be inferred from the results of estimation of endosulfan residues for both the years that endosulfan residue could not remain below tolerance limit of 2 ppm at the time of harvest of fruits i.e. 25/19 days after spraying but the treatment of washing with tap water did show the reduction of residue in the year 1977-78 only with the result that the residue was below tolerance level after 19 days when spraying was done once in the experiment whereas it was not so in the case of sequential sprayings followed in the experiment of 1976-77. Therefore the fruits cannot be used for human consumption without washing atleast for 19 days when one spraying of endosulfan is done in the mango orchard, while fruits cannot be even used after washing when sequential spraying is being practiced as a method of plant protection measures. Thus the report of Benoit (1973) and Srivastava (1976) as well as the
result of the present findings of requirement of time interval of more than 30 and 25 days respectively are in concurrence for the above inference.

Further, from the results of carbaryl and endosulfan residue estimation by chemical assay it can be seen that for the plant protection schedule for mango orchard, the use of endosulfan can be made only during the period of flowering and thereafter the schedule should consist of the use of carbaryl during the period of fruit formation and ripening. This practice, if followed, will not only benefit the cultivators but also the consumers. The reason for this is that endosulfan is considered safer to bees as pollinators in mango orchard in comparison to carbaryl and its use will favour good fertilization in the orchard, while the use of carbaryl in the later stage will benefit the consumers because its residue was found below tolerance level at the time of harvesting and consumption, because the safe interval between last spraying and harvesting of fruits can be observed. Based on the results of the above, the inference and recommendation will be that the judicious use of insecticide is being required to be practised by the cultivators, and further the involvement of human hazard can be avoided if the habit of observing safe interval between spraying of carbaryl and harvesting as well as washing of fruits before consumption as food by the consumers are practised.
REFERENCES


Stobwasser, H. 1943. Carbaryl has been used as such for the control of carrot maggot in Germany. Z. pflanzenschutz., 70:459.


* Original not referred.
APPÉNDICES
APPENDIX "A" TABLES
### Appendix Table 1. Residue of Carbaryl/Endosulfan on mango fruits by chemical assay in the year 1976-77

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stage of sampling</th>
<th>$R_I$</th>
<th>$R_{II}$</th>
<th>$R_{III}$</th>
<th>$R_{IV}$</th>
<th>Total</th>
<th>Mean</th>
<th>Half life value in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbaryl (0.2 per cent)</td>
<td>0</td>
<td>50.50</td>
<td>56.12</td>
<td>66.75</td>
<td>59.26</td>
<td>232.63</td>
<td>58.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>36.75</td>
<td>37.38</td>
<td>35.50</td>
<td>39.25</td>
<td>148.88</td>
<td>37.22</td>
<td>3.19</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>21.01</td>
<td>17.64</td>
<td>19.02</td>
<td>16.27</td>
<td>73.94</td>
<td>18.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>13.01</td>
<td>14.26</td>
<td>14.26</td>
<td>0.00</td>
<td>41.53</td>
<td>10.30</td>
<td></td>
</tr>
<tr>
<td>Endosulfan (0.075 per cent)</td>
<td>0</td>
<td>71.69</td>
<td>92.93</td>
<td>118.86</td>
<td>12.26</td>
<td>295.74</td>
<td>73.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8.49</td>
<td>16.99</td>
<td>30.01</td>
<td>6.60</td>
<td>62.09</td>
<td>15.52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>8.49</td>
<td>17.93</td>
<td>19.81</td>
<td>8.49</td>
<td>54.72</td>
<td>13.68</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>4.72</td>
<td>5.66</td>
<td>7.55</td>
<td>4.72</td>
<td>22.65</td>
<td>5.66</td>
<td></td>
</tr>
</tbody>
</table>
Appendix Table 2. Residue of Carbaryl/Endosulfan in mango fruits by chemical assay in the year 1976-77

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stage of sampling</th>
<th>$R_1$</th>
<th>$R_{II}$</th>
<th>$R_{III}$</th>
<th>$R_{IV}$</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbaryl</td>
<td>0</td>
<td>39.88</td>
<td>41.75</td>
<td>44.26</td>
<td>44.26</td>
<td>170.15</td>
<td>42.53</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15.77</td>
<td>14.76</td>
<td>14.26</td>
<td>14.26</td>
<td>59.05</td>
<td>14.79</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>11.76</td>
<td>11.76</td>
<td>11.76</td>
<td>0.00</td>
<td>35.28</td>
<td>8.82</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>0</td>
<td>17.85</td>
<td>29.25</td>
<td>36.79</td>
<td>14.15</td>
<td>98.04</td>
<td>24.51</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.66</td>
<td>10.42</td>
<td>18.40</td>
<td>2.83</td>
<td>37.31</td>
<td>9.33</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2.83</td>
<td>3.45</td>
<td>3.86</td>
<td>2.83</td>
<td>12.97</td>
<td>3.24</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>2.45</td>
<td>5.19</td>
<td>2.40</td>
<td>0.09</td>
<td>10.13</td>
<td>2.53</td>
</tr>
</tbody>
</table>
Appendix Table 3. Residue of Carbaryl/Endosulfan on mango fruits by chemical assay in the year 1977-78

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stage of sampling</th>
<th>R_I</th>
<th>R_II</th>
<th>R_III</th>
<th>R_IV</th>
<th>Total</th>
<th>Mean</th>
<th>Half life value in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbaryl (0.2 per cent)</td>
<td>0</td>
<td>51.76</td>
<td>54.25</td>
<td>54.25</td>
<td>51.56</td>
<td>211.82</td>
<td>52.56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>34.25</td>
<td>34.25</td>
<td>44.26</td>
<td>39.25</td>
<td>152.01</td>
<td>38.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>19.25</td>
<td>21.76</td>
<td>21.76</td>
<td>19.25</td>
<td>82.02</td>
<td>20.50</td>
<td>3.00</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>11.75</td>
<td>11.75</td>
<td>0.00</td>
<td>11.75</td>
<td>35.25</td>
<td>8.80</td>
<td></td>
</tr>
<tr>
<td>Endosulfan (0.075 per cent)</td>
<td>0</td>
<td>8.8997</td>
<td>12.2982</td>
<td>9.7493</td>
<td>8.0501</td>
<td>38.9973</td>
<td>9.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>5.9260</td>
<td>5.5106</td>
<td>7.6382</td>
<td>5.9260</td>
<td>25.0088</td>
<td>6.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>1.6779</td>
<td>2.5276</td>
<td>3.3772</td>
<td>2.5276</td>
<td>10.1103</td>
<td>2.53</td>
<td></td>
</tr>
</tbody>
</table>
Appendix Table 4. Residue of Carbaryl/Endosulfan in mango fruits by chemical assay in the year 1977-78

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stage of sampling</th>
<th>$R_I$</th>
<th>$R_{II}$</th>
<th>$R_{III}$</th>
<th>$R_{IV}$</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbaryl (0.2 per cent)</td>
<td>0</td>
<td>21.76</td>
<td>21.76</td>
<td>19.25</td>
<td>21.76</td>
<td>84.53</td>
<td>21.13</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>BDL</td>
</tr>
<tr>
<td>Endosulfan (0.075 per cent)</td>
<td>0</td>
<td>5.0765</td>
<td>3.8020</td>
<td>4.2268</td>
<td>3.3772</td>
<td>16.4825</td>
<td>4.12</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.6516</td>
<td>1.6774</td>
<td>3.3772</td>
<td>2.5276</td>
<td>12.2343</td>
<td>3.06</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1.6779</td>
<td>0.8284</td>
<td>2.1028</td>
<td>1.2532</td>
<td>5.8623</td>
<td>1.47</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>0.2018</td>
<td>Nil</td>
<td>0.4036</td>
<td>Nil</td>
<td>0.6054</td>
<td>0.30</td>
</tr>
</tbody>
</table>

BDL: Below detectable level
Appendix Table 5. Residue of Carbaryl on/in mango fruits by micro bio-assay in the year 1976-77

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stage of sampling</th>
<th>R_I</th>
<th>R_H</th>
<th>R_III</th>
<th>R_IV</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbaryl</td>
<td>0</td>
<td>49.47</td>
<td>68.79</td>
<td>91.86</td>
<td>93.84</td>
<td>303.96</td>
<td>76.00</td>
</tr>
<tr>
<td>(0.2 per cent)</td>
<td>3</td>
<td>16.98</td>
<td>40.20</td>
<td>22.08</td>
<td>105.48</td>
<td>184.74</td>
<td>46.18</td>
</tr>
<tr>
<td>(on the fruits)</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>BDL</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>BDL</td>
</tr>
</tbody>
</table>

| Carbaryl       | 0                 | 38.19| 45.00| 39.06 | 74.64| 196.89 | 49.22|
| (0.2 per cent) | 3                 | 2.66 | 1.22 | 1.92  | 0.36 | 6.16   | 1.54 |
| (in the fruits)| 7                 | -    | -    | -     | -    | -      | BDL  |
|                | 25                | -    | -    | -     | -    | -      | BDL  |

BDL : Below detectable level.
Appendix Table 6. Residue of Carbaryl on/in mango fruits by micro bio-assay in the year 1977-78

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stage of sampling</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbaryl (0.2 per cent)</td>
<td>0</td>
<td>74.64</td>
<td>47.52</td>
<td>50.88</td>
<td>61.08</td>
<td>234.12</td>
<td>58.56</td>
</tr>
<tr>
<td>(on the fruits)</td>
<td>3</td>
<td>37.32</td>
<td>40.80</td>
<td>40.80</td>
<td>30.48</td>
<td>149.40</td>
<td>37.38</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>17.04</td>
<td>3.48</td>
<td>10.20</td>
<td>3.48</td>
<td>34.20</td>
<td>8.58</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>6.84</td>
<td>3.42</td>
<td>0.72</td>
<td>0.36</td>
<td>11.34</td>
<td>2.82</td>
</tr>
<tr>
<td>Carbaryl (0.2 per cent)</td>
<td>0</td>
<td>3.42</td>
<td>3.48</td>
<td>6.84</td>
<td>10.20</td>
<td>23.94</td>
<td>6.00</td>
</tr>
<tr>
<td>(in the fruits)</td>
<td>3</td>
<td>0.36</td>
<td>0.36</td>
<td>3.42</td>
<td>0.36</td>
<td>4.50</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>BDL</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>BDL</td>
</tr>
</tbody>
</table>
APPENDIX "B"

EFFECT OF CHEMICAL CONTROL OF
THE MANGO HOPPER ON POPULATION
OF TWO SPOTTED SPIDER MITE IN
THE MANGO TREES
EFFECT OF CHEMICAL CONTROL OF
THE MANGO HOPPER ON POPULATION
OF TWO SPOTTED SPIDER MITE IN
THE MANGO TREES

Before the introduction of DDT and other synthetic
toxicants for pest control practices, the phytophagous mites
were injurious sporadically, but not as severe as they became
after the extensive use of pesticides. Often the outbreaks of
mites are attributed to the use of a chlorinated hydrocarbon,
DDT and others. A hypothesis prevails from 1960 onwards that
certain pesticides are stimulus to enhance the fecundity of
mites either directly or indirectly which may cause mite out­
breaks in that spots. The increase in reproductive potential
goes for the direct stimulus and indirectly to the metabolic
changes in the host plant which provides an improved nourishment
to the mites. In the acarological literature there are evidences
as well as contradictory results. Works of Loecher's (1958)
and Sciferts (1961) indicated that DDT stimulated increased
reproduction when applied directly on the mites, whereas the
works of Attiah (1964) and Boudreaux (1958) as well as Saini
and Cutkomp (1966) was contradictory to the above view. There
are evidences for the increase of mite population indirectly
through pesticide-induced improvement in nutrition or growth
factors in the leaves. (Chaboussou 1961, 1963a, 1963b, 1965;
Fleschner 1952 and 1958; Huke 1953; Huffakker et al., 1950;
Klostermayer and Rasmussen 1953; Michelbacher 1959; Michelbacher
et al., 1952; Rodriguez et al., 1957; Rodriguez et al., 1960
and Saini et al., 1960).
Chaboussou (1965) presented extensive investigations on the effects of pesticides such as DDT, carbaryl, diazinon and parathion, on leaf chemistry in relation to nutritional requirements of several spider mites. In some cases Chaboussou found that a lower dosage of a material resulted in greater increase than a higher dosage and increase in population was observed when carbaryl was compared to DDT. He stated that both the materials might be inhibiting the predators.

The work of Oatman (1965) gave evidences for considerable increase in the population of the European red mite, *Panonychus ulmi* (Koch) and two spotted spider mite, *Tetranychus telarius* L. in apple after the application of carbaryl. The work of Gunthart and W. Vogch (1965) also indicated significant increase in the population of *Panonychus ulmi* (Koch) on fruit trees due to application of carbaryl, while endosulfan gave reduction in the population. Further investigations of Chaboussou (1966) revealed increased fecundity, prolonged adult life and short period of adult emergence and reproduction due to the effect of carbaryl application. These may lead to increase in the reproduction rate of the mites. However, Harries (1966) did not support the hypothesis but agreed with the destruction of the predatory mite, *Typhlodromus* spp. In northern Egypt, in fruit trees, the mite population increased with respect to the use of carbaryl (Febetti 1966). The report of Rasmy (1971) gave evidences for the increase of *Tetranychus urticae* in cotton with the use of carbaryl.
as a spray. Dittrich et al., (1974) ascertained the increased egg-laying capacity of the female of *Tetranychus urticae* as a result of the carbaryl application.

In the light of the works of the above mentioned scientists, it is worthwhile to visualize the mite excesses observed in cotton, sorghum, fruit trees and vegetables which might have developed after the constant use of certain insecticides such as carbaryl, DDT and other chlorinated hydrocarbon pesticides.

Preliminary efforts were thus thought over to make out the effect of application of carbaryl and endosulfan on the population of *Tetranychus telarius* L. in the mango trees, because Shah et al., (1975) have also reported increased population of sorghum mite, *Olygonychus indicus* first due to application of carbaryl and endrin for the control of stem borers in sorghum. For this the number of two spotted spider mites present in the leaves of the mango trees was recorded after fourth spraying of carbaryl and endosulfan in a trial conducted at the village Changa of Gandevi taluka in the district of Bulsar for the estimation of the residue of insecticides deposited in the mango fruits due to plant protection measures against mango hoppers. The criteria for taking samples was by counting the number of mites present in five leaves per tree. Sixteen trees were selected for each treatment. The observations recorded are presented in Appendix Table 1 and Appendix Figure 1. The average number of mites present in sixteen trees at the time of the fourth spraying was 1735, 252 and 61 in carbaryl, endosulfan and control treatment respectively (Appendix Table 1). After 72 hours of the fourth spraying the mite population
declined a little in the case of carbaryl (1540 mites) and increased slightly in the case of endosulfan (274 mites) which is not of considerable importance.

Thus it is evident from the appendix table 1 that the population of red spider mites in the mango trees sprayed continuously with carbaryl increased invariably as compared to trees treated with endosulfan and control. The present finding is in concurrence with the report by Rasmy (1971), Chaboussou (1966); Oatman (1965), Fabietti (1966) and Dittrich et al., (1974) for increased population of *Tetranychus* spp mites due to carbaryl application.

The initial vast difference of population is assumed to be attributed to the pesticide increased reproductive potential - hypothesis put forward by various workers or due to the hypothesis of destruction of predators by the pesticides which is generally accepted as the reason for mite outbreaks. If a combined effect of both the increased fecundity and destruction of predators occur as well, then it is less likely to build up the population of predators in time to check the damage and therefore such a chemical, i.e. carbaryl, under plant protection practice would perhaps amount to mite epidemics in the area.
## APPENDIX TABLE 1

Average Number of Two Spotted Spider Mite per Five Leaves of 16 Mango Trees Before and After the Fourth Spraying of 0.2 per cent Carbaryl and 0.075 per cent Endosulfan in the Experiment of Insecticidal Residue at Village Changa, Taluka Gandevi, District Bulsar in 1976-77

<table>
<thead>
<tr>
<th>Name of treatment</th>
<th>Average number of two spotted spider mite per five leaves of 16 mango trees</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before the fourth spraying</td>
</tr>
<tr>
<td>Carbaryl (0.2 per cent)</td>
<td>1735</td>
</tr>
<tr>
<td>Endosulfan (0.075 per cent)</td>
<td>252</td>
</tr>
<tr>
<td>Control</td>
<td>61</td>
</tr>
</tbody>
</table>
Appendix Fig. 1 - AVERAGE NUMBER OF TWO SPOTTED SPIDER MITE PER FIVE LEAVES OF MANGO TREES BEFORE AND AFTER THE 4TH SPRAYING OF 0.2 PER CENT CARBARYL AND 0.075 PER CENT ENDOSULFAN.
REFERENCE


* Original not referred.