MATERIALS AND METHODS

All the solvents viz. benzene, petroleum ether (60-80°C) chloroform, methanol etc that were used for the preparation of the compounds, were purified and dried according to the methods described in Vogel's Practical Organic Chemistry[234]. All the melting points given here are uncorrected.

Analyses of the compounds for carbon, hydrogen and nitrogen were carried out at Regional Sophisticated Instrumentation Centre, Chandigarh University, Punjab.

Tin was estimated gravimetrically by conversion to stannic oxide and finally to volatile stannic iodide, essentially by the method of Van der Kerk and Luijten[235].

The infrared spectra have been taken in the range between 4000-500 cm^{-1} for the compounds using Beckmann IR-20 infrared spectrophotometer.

1) Preparation of starting materials

i) bis (Tricyclohexyltin) oxide

bis (Tricyclohexyltin) oxide was recovered from 'Plictran 50 WP' by dissolving it in chloroform, filtered. Filtrate was concentrated and allowed to stand overnight. Fine white powdery mass precipitated, which was dried in air.

M.P. 189°C (Lit M.P. 190-195°C)

ii) Oxalyl bis-N-phenyl hydroxamic acid

This was prepared by the method of Choudhury et al[236] by the reaction of oxalyl chloride with β-phenyl hydroxylamine in

cold diethyl ether in presence of pyridine. This was crystallised from rectified spirit.

M.P.$^{180°C}_{\wedge}$ (Lit.[236] M.P. 180°C).

iii) Succinyl bis-N-phenyl hydroxamic acid.

This was prepared by the method of Ghosh et al[237] by the reaction of appropriate acid chloride with β-phenyl hydroxylamine in cold ether in presence of pyridine. This was recrystallised from rectified spirit.

M.P. 174°C (Lit.[237] M.P. 174°C).

iv) Glutaryl bis-N-phenyl hydroxamic acid.

This was prepared by the method of Dutta[238] by the reaction of glutaryl chloride with β-phenyl hydroxylamine in cold ether in presence of pyridine. The white compound was recrystallised from rectified spirit.

M.P. 165°C (Lit.[238] M.P. 165°C).

2) Preparation of Organotin Compounds

(i) bis (Tricyclohexyl tin) succinyl bis-N-phenyl hydroxamate[238]

bis (Tricyclohexyltin) oxide (2.5 g) and succinyl bis-N-phenyl hydroxamic acid (1 g) were taken in 120 ml benzene and refluxed for six hours using Dean and Stark Water separator. The yellow coloured solution was filtered and concentrated over water bath. Shining white crystals appeared from petroleum-ether-chloroform mixture and was purified from the same solvent. Dried in vacuum.

M.P. 142°C (Lit.[238] M.P. 142°C).

(ii) bis (Tricyclohexyltin) glutaryl bis-N-phenyl hydroxamate[238]

bis (Tricyclohexyltin) oxide (1.4 g) and glutaryl bis-N-phenyl hydroxamic acid (400 mg) were taken in 150 ml benzene and refluxed for six hours using Dean and Stark Water Separator. The yellow coloured solution was filtered, concentrated and methanol was added. White amorphous compound that appeared was further crystallised from chloroform-methanol mixture. Dried in vacuum.

M.P. 148°C (Lit.[238] M.P. 148°C).

(iii) Dicyclohexyltin Oxalyl bis-N-phenyl hydroxamate[238]

bis (Tricyclohexyltin) oxide (600 mg) and oxalyl bis-N-phenyl hydroxamic acid (500 mg) were taken in 150 ml benzene and refluxed for six hours. using Dean and Stark Water Separator. The yellow coloured solution was filtered and concentrated over water-bath. White shining crystals precipitated. It was recrystallised from chloroform-methanol mixture.

M.P. 220°C (Lit.[238] M.P. 220°C).

(iv) Tricyclohexyltin-N-hydroxy phthalimide[239]

bis (tricyclohexyltin) hydroxide (770 mg) was dissolved in 150 ml benzene and N-hydroxy-phthalimide (326.3 mg) was added to the solution, with vigorous stirring. It was then refluxed for four hours using a Dean and Stark Water Separator. Deep orange coloured solution obtained which was filtered and concentrated over a water-bath. Filtrate was left overnight. Yellow crystals separated. This was then recrystallised from chloroform-methanol mixture.

M.P. 195°C.

(v) Tricyclohexyltin N-hydroxy Succinimide[239]

bis (tricyclohexyltin) oxide (770 mg) was dissolved in benzene and N-hydroxy succinimide (230 mg) was added to this solution with vigorous stirring. The mixture was then refluxed for 5 hrs with Dean and Stark Water Separator. The solution was filtered, concentrated over a water bath and kept overnight. White precipitate appeared. It was recrystallised from chloroform-methanol mixture and dried in vacuum.

M.P. 150°C

(vi) Tricyclohexyltin diphenyl Carbazone[233]

bis (tricyclohexyl tin) oxide (1.1 g) and diphenyl carbazone (480 mg) were taken in benzene (1:1) and refluxed for four hours using a Dean and Stark Water Separator. The deep red solution was filtered and concentrated over a water bath. Red powdery mass appeared. This was recrystallised from chloroform-methanol mixture.

M.P. 130°C (Lit.[233] M.P. 130°C).

(vii) Tricyclohexyltin oxine

bis (tricyclohexyltin) oxide (760 mg) and oxine (290 mg) was dissolved in benzene and refluxed for five hours using Dean and Stark Water Separator. The deep yellow solution was concentrated over a water bath and left overnight. Shining yellow crystals appeared which was further crystallised from chloroform-methanol mixture. Filtered and dried in vacuum.

M.P. 90°C.

% Analysis for $C_{27}H_{39}ONSn$

Found	C	63.56	H	7.52	N	2.51	Sn 23.36
Calculated	C	63.28	H	7.61	N	2.73	Sn 23.24

(viii) Tricyclohexyltin phthalimide

bis (tricyclohexyltin) oxide (385 mg) and 147 mg of phthalimide were refluxed in benzene using a Dean and Stark Water Separator, for five hours. The solution was filtered and concentrated over a water bath. Methanol was added, fine white shining crystals appeared. It was further purified from chloroform-methanol mixture. Dried in vacuum.

M.P. 165°C

% Analysis for $C_{26}H_{37}O_2NSn$

Found	C	60.72	H	7.77	N	2.65	Sn 23.19
Calculated	C	60.70	H	7.19	N	2.72	Sn 23.15

'Plictran 50 WP' which was supplied by Dow and M & T Chemicals, U.S.A. was used as such.

3) Determination of acaricidal activity

(i) Collection of specimens

The mite infested leaves or plant parts were brought to the laboratory in polythene bags after tightly closing the mouth of the bag with a rubber band.

(ii) Mounting for microscopical examination

Before examining under microscope, high degree of transparency in the mite is needed. This was done by placing the

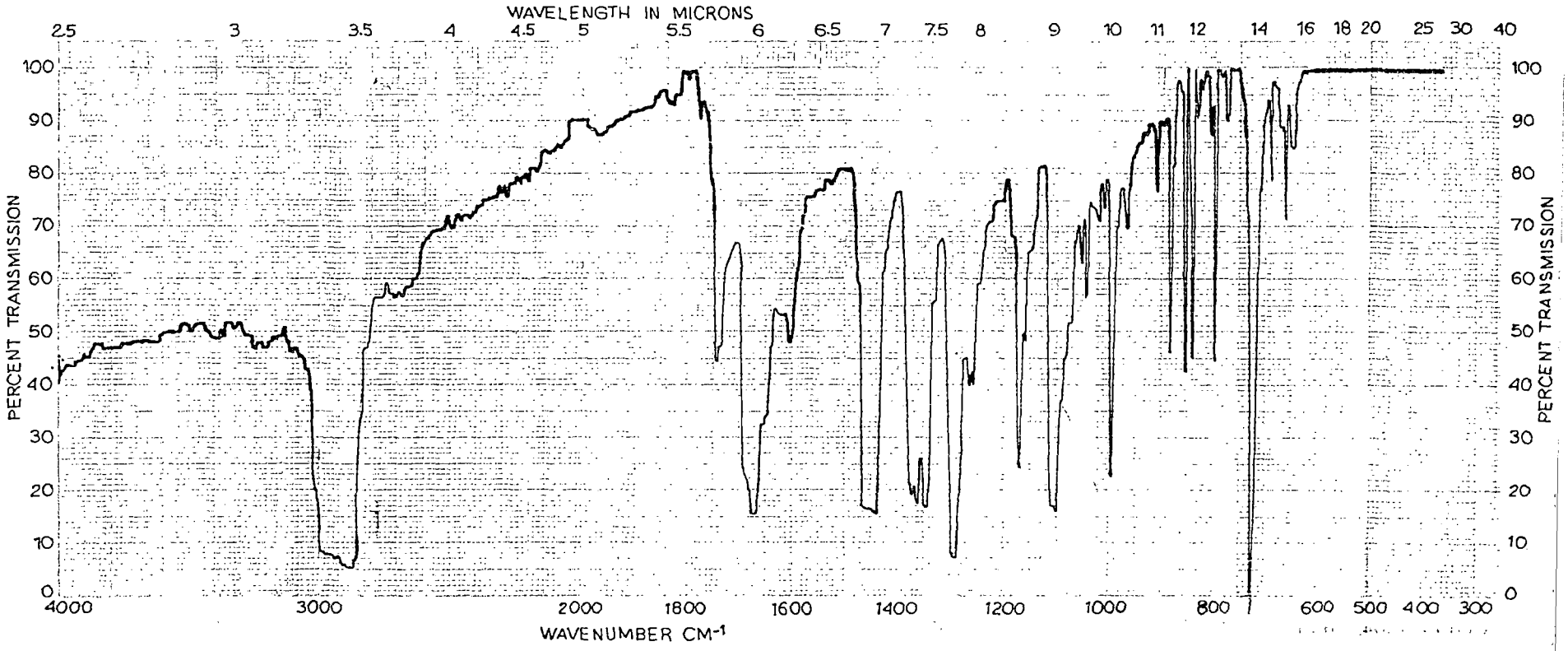

Fig. 7 IR spectrum of Tricyclohexyltin phthalimide.

specimen to be examined on a slide and putting a drop of lactic acid over it. The slide was then gently warmed for a few second, which reduced the normal opacity of mites, appendages extended. It was then carefully examined under the microscope.

(iii) Identification

Specimens were identified as per the identifying characters as suggested by Gupta (1985)[280] especially for sub-families, tribes and genera and the following genera were identified:

a) Tetranychus telarius
b) Oligonychus coffae
c) Brevipalpus obovatus
d) Petrobia harti (vide - Appendix-I)

(iv) Rearing

After proper identification of the particular specimen, it was reared in the laboratory in large numbers for experimental purpose. Rearing was done by two methods. These were cultured in bean seedlings, kept in pots. The adult mites were picked up from the infested leaves or plant parts and transferred to the leaves of potted seedlings or the infested plant parts were kept on top of the seedlings. When the detached part dried up the mites migrated to the leaves of the potted seedlings. Petroleum jelly was applied around the base of the stem to prevent the escape of the mites.

The other method used were keeping exercised leaves of the host plant in petridish (15 cm diameter) over a cotton pad super saturated with water. Leaf was periodically changed and water was

added daily to maintain a thin film of water at the margin of leaf to prevent the escape of mites. Mites were transferred on to the leaves by picking up with a fine brush moistened with water.

(v) Preparation of the test-solutions

The acaricidal compound was formulated as emulsion using polyoxyethylene sorbitan mono-oleate (Tween-80) as emulsifer and benzene or acetone as solubiliser. Finally distilled water added to obtain required stock solution. Stock solution was further diluted to the desired concentrations and used for the different tests described below:

(vi) Slide-dip method for contact toxicity assessment

This method was originated by Voss[241] improved by Dittrich[242] Adult females of known ages were used for the screening. This was done by collecting eggs over 4-5 hours and rearing adults of a new generation which appeared 6-7 days later, at temperature ranging from 27°C to 29°C.

For the test, microscope slide was covered with a strip of double -sided scotch-tape and twenty adult females were stuck on to the tape, on the dorsal side, in two rows of ten. The prepared slides were dipped, for 5 seconds in serial concentrations of the compound being tested. Slides were drained, by placing on edge for 15 minutes, at room temperature. Mortality counts were made after 24, 48 and 72 hours. Mites not showing movement of appendages when touched by a fine brush were recorded as dead.

Three tests were conducted at each dosage level. The treated slides were placed on the top of a moist cotton-pad in petri-dishes, which contained water to maintain the humidity. For the control set, slides were dipped in distilled water for the same length of time.

(vii) Method for assaying stomach toxicity

Experiment was conducted with the compounds at desired concentrations according to the method of Das (1987)[157], Goyal and Bath (1965)[144]. Each concentration was replicated thrice, there being twenty starved adult mites in each set. Fresh leaves of the host plant of the mites under examination were placed in petri-dish over moist cotton pad and sprayed with the compound with the help of an atomiser. The adult mites were released immediately after spraying and mortality counts were recorded after 24, 48 and 72 hours.

(viii) Method of assaying contact plus stomach toxicity

The method followed here was that of Mansour and Plant[139] (1979). The mites were released on fresh leaves and sprayed with the different concentration of the compound. Each test was replicated thrice and mortality counts were made after 24, 48 and 72 hours.

Determination of LC_{95} and LC_{50}

The LC_{50} [lethal concentration for 50% mortality] and LC_{95} ($\mu g/ml$) were calculated by least square regression analysis using a Computer (H.C.L. PC BB AT).

Method for assaying ovicidal activity

To find out the ovicidal properties of the acaricides, the method followed was that of Subramanium (1977)[217], Puttaswami and Giraddi[220], Gupta et. al. (1977)[243]. The gravid female mites from laboratory bred pure culture were allowed to lay eggs on excised fresh leaves of bean in case of T. telarius and tea leaves in case of O. coffae. The leaves were kept afresh by placing them on a wet cotton swab in a petri-dish. 30 eggs were retained in each leaf and the rest were removed along with the mobile stages. The leaves with the eggs were sprayed with the compound at different concentrations with an atomiser to have an uniform coverage. Tap water was sprayed on eggs kept as controls. Each treatment was replicated thrice and observation were made on the hatching of eggs and the number hatched were recorded from the 4th day of the treatment upto 6 days, as most of the eggs of the control set hatched within that Period. Mean of three replication was calculated and the percent mortality of the eggs was calculated by using the following formula given by Abbott[244]

$$P_T = \frac{P_o - P_c}{100 - P_c} \times 100$$

where, P_T = corrected mortality, P_o = observed mortality and P_c = control mortality.