MATERIALS & METHODS

3. MATERIALS AND METHODS

3.1 PARAMETERS CONSIDERED IN PESTS SAMPLING:

Any decision of pest management must be based on the economic ratio or benefit to cost, and this largely depends on the quantitative pest density.

The sampling procedure consisted of a constant number of sample for each sampling occasion. For each sampling programme, it was necessary to decide upon:

i) the size of the sampling unit used

ii) the number of sampling units taken, and

iii) the location (i.e. distribution) of that sampling unit.

3.1.1 The three different host environments:

i). Young plants in experimental garden: (Fig. 6i & 6ii)

Field studies were carried out within University campus (Siliguri, West Bengal) (26°4'N, 88°26'E and 126m amsl) .The area belongs to foothills of Darjeeling, plain topography, acidic soil (P^H 4.5 to 5.5), and an average annual rainfall of 350cm with minimum temperature of 12°C and maximum of 30°C in general. No significant annual climatic changes make conditions ideal for tea growing.

A small experimental plot (15m x 10m) was set up within

Fig. 6i. Four young cultivars (in 4 different blocks) in experimental garden (at the beginning of sampling).

Fig. 6ii. The same plantation after 2 years (at the end of sampling).

Fig.6i.

Fig.6ii.

Fig. 7. Some cultural practices followed:

a) Pegging for quick growth of young tea plant.

b) Mulching by water hyacinth in young plantation.

Fig. 7a.

Fig. 7b.

short distance from the research laboratory. Scheduled cultural operations starting from soil treatment, fertilizer application, plantation of shade-trees were carried out for tea plantation. The planting materials were collected from a clone house recognised by Tea Research Association, Tocklai. The plantation work was performed on 5th June, 1991 in four experimental blocks (5m x 3m) with proper spacing (71cm x 61cm; single hedge) and drainage. Each of the four experimental block comprised 30 plants of a single cultivar (i.e. any one of TV 1, TV 18, TV 25, TV 26). Pesticides were not used in any plot at the experimental site and weeds were controlled mechanically. Weather data were collected from the nearest meteriological observatory (4 km away) at Bagdogra airport.

ii). **Saplings in the nursery:** (Fig.8)

Part of the total plot was left for nursery raising. The size of the nursery was specified as 4.5 mt x 1.7 mt. An one side open (north side) overhead thatched bamboo shade with a slope to the south was erected to avoid direct sunlight falling on the young clones. The nursery was set up with cuttings of the above mentioned Tocklai varieties (TV) divided in four blocks in prepared soil within polythene packets. The solar intensity inside the nursery was compared with the outfield by using the illuminometer (Model 5200, Kyoritsu electrical instruments Ltd., Japan).

Fig. 8. A nursery with cutting from mother tea bushes.

Fig. 9. Sampling on mature tea bushes rarely protected from pests attack.

Fig. 8.

Fig. 9.

iii) **Mature tea bushes in established garden:** (Fig. 9)

To compare the population incidence in nursery and experimental plot with an established tea estate (50 years old bushes), sample of some common pests species (like red spiders, aphids and thrips) were considered. It may be mentioned that this established tea garden was poorly managed with little or no pesticide spraying.

3.1.2 The four young cultivars in experimental garden:

TV 1: (Fig. 10a)

It is one of the earliest clone released by 'Tocklai Experimental Station', Assam (India) in 1949. TV 1 is a standard clone, having high yield potential and high quality. It has a compact frame with acute branch angle (<50°). Leaves are erect, medium sized with pubescence on lower surface and sunken stomata. Surface matty in nature. Fairly draught tolerant. It is a hybrid, Assam x China in origin.

TV 18: (Fig. 10b)

It is of Cambod origin. More less of compact frame with glossy medium size leaf. Leaf axil with an angle of 50° to 70°. It has a high yield potential but of average quality. Leaf has pinkish pigmentation in the petiole.

Fig. 10. Part of shoot showing physical features of leaves of four cultivars selected in experimental garden:

a) TV 1 (Erect type) b) TV 18 (Semi-erect type)

c) TV 25 d) TV 26

(Horizontal type)

Fig.10a.

Fig.10b.

Fig.10c.

Fig.10d.

TV 25 & 26 (10c & 10d):

These are yield clones of Assam x Cambod origin. Morphologically both the clones are similar in nature, having compact frame and glossy medium sized leaf. Both the clones are fairly draught tolerant having high yield potential but of average quality. Leaf axil having angle > 70°.

3.1.3. Identifying characters for Pest species selected for study:

The pests of common occurence in terai were considered in present study were:

i) Aphids or Aphis (*Toxoptera aurantii* Boyer) (Hemiptera: Aphididae)

ii) Thrips (*Scirtothrips dorsalis* Hood) (Thysanoptera: Thripidae)

iii) Red spider mite (*Oligonychus coffeae* Nietner) (Acarina: (Tetranychidae) and

iv) Scarlet mite (*Brevipalpus phoenicis* Geijskes) (Acarina: Tenuipalpidae) particularly inside nursery.

The aphids:

The aphid (Fig. 11) (*T. aurantii*) is a polyphagous species attacking several economically important plants such as coffeae, tea and citrus and is recorded from all the tea

growing countries. Colonies of *T. aurantii* consist of dark brown alate and apterus adult females and nymphs.

It attacks the tender shoot, the undersurface of young leaves and the bud. As a result of the loss of sap, the leaves crinkled, curled and the growth of the shoot is retarded. They exude honey dews and are attended by small black ants.

The thrips:

Thrips prefer young leaves and buds (Fig. 12). The continuous feeding by adults and larvae causes lacerations which appear as two parallel streaks (Fig.13) when bud unfold in to leaves. The leaf surface become uneven and curled.

The red spider mites:

The red spider mite, *O. coffeae* occurs in almost all the tea growing countries in South-east Asia and Africa. *O. coffeae* is the largest of all tea mites and can be easily seen by the naked eye. They normally infest the upper surface of mature leaves, though in extreme cases they invade the lower surface as well as young leaves. The mites spin a web of silken threads on the leaf and protect themselves against adverse weather conditions.

They cause damage to the tissues of leaves thereby

Fig. 11. A twig heavily infested by aphids.

Fig. 12. Two leaves and a bud (strategic location of thrips).

Fig. 13. Laceration of the tissue by thrips damage leave two longitudinal streaks over the leaf.

Fig.11.

Fig.12.

Fig.13.

Fig. 14. *Polygonum orientale*, a common host plant of *Geocoris ochropterus*.

Fig. 15. *Leucus linifolia* used as vegetative food for rearing *G. ochropterus*, also harbour the predator.

Fig. 14.

Fig. 15.

reducing photosynthetic ability of the plants, resulting in yield reductions. Due to feeding leaves turn ruddy bronze.

The scarlet mites:

Scarlet mite attack both young and mature leaves and unlike red spider it abounds mostly on the undersurfaces of both young and mature leaves, specially along the mid rib and petiolar region.

Feeding results in brown discolouration of leaves, the affected petiole splits and dries up, causing premature fall of leaves and a broom stick like appearence of the bushes.

3.1.4 Methods followed in sampling of the pests:

In nursery and experimental blocks, an intensive sampling (48 hours interval) was done randomly in the respective habitat of occurrence of aphid, thrip and red spider mite pests all through the year. For ascertaining the population density of aphids and thrips, observations were confined to a fixed length i. e. 10cm from the apex of a twig of ten plants randomly choosen from each block, whereas for red spider mites in experimental field and scarlet mites in nursery, field counting was done by using a magnifying glass over the leaf chosen randomly. Direct counting method (Irwin *et al.*,1974) from the host foliage was

applied.

In established tea garden, a fortnightly sampling procedure (Muraleedharan *et al.*, 1988) was followed. To count the population of red spider mite, a mature leaf was plucked and pressed in between the fold of a filter paper so as to have impressions of red spider mites. For aphids and thrips, a young twig (two leaves and a bud) was plucked and kept in a plastic vial (5.5cm x 8.8cm). The collected specimen were brought to laboratory in polythene packet. For sampling, 50 bushes (Oomen, 1982) were selected at random during 1991-92 and 1992-93. All these samples were carefully transported under cool conditions to laboratory and counted under the binocular (Model Wild M38, Heerbrugg, Switzerland) microscope (Goodwin, 1991).

The aphids and thrips densities were expressed as number of individuals per twig, and for mites, it was the number of individuals per leaf considering both in upper and lower surface of maintenance leaves (Richards, 1940). Throughout the study, the sampling was done in late afternoon hours and the result was expressed on the basis of fortnightly mean.

The care was taken against dislodging of insects (Satchell & Mountford, 1962) and overlooking of small insects (Condrashoff, 1967) while handling the plant part during sampling.

3.1.5 Studies on natural rate of increase of the pests attacking young plantation:

Using monthly mean of pest population density, on young cultivars, two peaks were identified (Winter season and summer season peaks) for all the three pest species. The exponential phases of the peaks were analysed for statistical comparison and $\log_{10}$ transformed data were used. A regression line indicating the change of population with time was fitted for convenience of comparison of the natural growth rate of pest population on different varieties as well as seasons.

3.2 REARING OF THE PREDATOR *G. ochropterus*:

3.2.1 Field collection of *Geocoris*:

Several techniques like hand picking, sweeping, and pootering were applied for collecting *Geocoris* Darjeeling plains basin deliminated by the river Mahananda in the east and river Mechi on the west. Of the geocorines, *G. ochropterus* was found to be the most common species. In the early winter (1990) only a few immature stages of *G. ochropterus* could be collected from a common shurb, *Celosia cristata* L.. With the onset of spring and after first shower (7th April, 1991), *G. ochropterus* could be found in large

number on a road side weed, *Polygonum orientale* Wall (Polygonaceae) (Fig.14) associated with other prey species.

A leguminous plant *Crotalaria* spp. commonly itercrroped with tea plants for nitrogen fixation, was also found to harbour *Geocoris* bugs. Once the source of natural populaton was known the bug was regularly collected for experimentation and standarization of rearing techniques in laboratory and in experimental plots.

During monsoon (May to July) *Geocoris* was also found on maize crop infested with aphids. The association of nymphal instar of *G. ochropterus* was noted on young twigs of tea infested by aphids and from intersparced weeds (Labiate) of a managed tea estate (Sannyal tea estate, Matigara, Siliguri).

In late monsoon (July to September), the gecorine species was found by sweeping the weeds and grasses at large. The weed *Leucus indica* (Labiate) (Fig. 15) was found to harbour a thin population of the bug through the greater period of the year. Regular collection of the bug had to be done because of high rate of mortality of the species in the laboratory during the initial attempt to rear by several trial and errors. The weed *Polygonum orientale* was the chief source of *Geocoris* supply for experimentation.

For observing overwintering stage and duration,

Geocoris eggs, nymphs and adults were kept confined in field conditions.

3.2.2 Some preliminary trials on maintenance of laboratory culture:

Attempt was made to rear the omnivorous predator on a diet combination of green bean (*Phaseolous vulgaris* L.), sunflower seed, portion of vegetative part of a hemp weed, *Mikania*. Small glass tube with cotton wads (siphons) were used for provision of water. A parallel culture of *Tribolium* sp. was maintained to supply the animal food. To keep them healthy and viable, *Drosophila* was supplied as a supplementary food . Strict care was taken to avoid the fungal contamination of green bean, sunflower seed , dead grubs, and maintained a clean culture.

Attempt was also made to rear *Geocoris* on *corcyra* eggs supplemented with moisture from weed. A trial was also run to rear the *Geocoris* on artificial diet combination (sweetened condensed milk + water + honey, 2:1:2 by volume) as recommended by Mukherjee and Som Chowdhury (1971) for *Xylocoris flavipens* (Anthocoridae: Heteroptera) was undertaken.

3.2.3 Successful maintenance of laboratory culture:

a. Ant pupae as experimental food:

The ant pupae *(Oecophylla smaragdina)* were available from nature as well as from market (commercially available as fish bait). The availability of pupae throughout the year was ensured by preserving at -10^{o}c and RH 62% in air tight container. Such cold preserved food when brought to room temperature was found acceptable to *Geocoris* sp. *Oecophylla* was easily available during rainy season. Generally leaves of sal, mango were highly prefered as their nesting sites by the ant. The market price for the ant pupae was @ Rs. 60.00 (US $ 2 approx) per kg.

b. Rearing procedure:

Successful rearing could be done using commercially available mixture of ant *(Oecophylla smaragdina* Fabr.) egg, pupae, and early adults. A steady laboratory population of all stages of *Geocoris* could be maintained on this food for generations. For a consistent and a better result, the geocorines were fed with cold preserved pupae of ant supplemented with fresh twig of *Leucus linifolia* Spreng (Labiate) *ad libitum*. Culture was cleaned on every alternative days. The experimental individuals were reared separately to avoid cannibalism at 27 ± 1^{o}C, 80% RH and L:D of 12 hours each. All measurement on weight was taken by

using electric balance.

For determining fresh weight, nymphs were weighed soon after moult in a capped gelatin capsule. Oven drying of nymphs and adults were done at 60°C till constant weight was attained.

c. Experiment on fecundity:

Cotton wadding (Cohen& Debolt, 1983) was provided as oviposition site from where eggs were removed daily using a flat headed forceps. Egg laying by virgins were compared with that of females exposed to constant male company and fortinightly male company (for 48 hours) (10 replicates). Fecundity of virgin famale was used as control. The reason behind studing the reproduction under variable male company was to know the need of minimum male association of a female in laboratory for mass rearing and therefore economise on male rearing. Study on oviposition was continued till 50% of the population in culture died. Number of eggs laid, incubation period, percentage mortality, and female longevity were also observed.

3.2.4 Analysis of some dietary components of ant pupae:

A. Preparation of dry powder of pupae:

The pupae were shade dried for few hours and finally

oven dried at 50°c for 24hours. Later the pupae were ground to fine powder using pestal mortar and kept in sealed polythene packets in a desiccator over fused calcium. Biochemical estimations have been based on at least five replicates.

B. Total lipid (Ananthakrishnan, 1990a):

The extraction and estimation of total lipid from the dried ant powder was done by gravimetric method. 500 mg of the powder was ground with petroleum ether and filtered in a previously weighed glass tube. The resdiue was homogenized again with petroleum ether and filtered. Five such repetitions were done. The filtrate was placed in an oven at 50°c for complete evaporation. The test tubes were reweighed and the difference in weight gave an account of the lipid present in mg/g.

C. Total protein: (Lowry, *et al.*1951)

Dried ant powder was extracted for atleast 3 times using 5% Trichloroacetic acid (TCA) allowing sufficient time (8-12 hours). The filtrate was used for estimating the extracted protein following standard colorimetric method of Lowry *et al.*(1951). The protein amount was expressed in mg/g of weight of ant powder. BAS was used as standard.

D. Total carbohydrate: (Plummer, 1979)

TCA extracted solution (as above) was subject to treatment by $Ba(OH)_2$ and $ZnSo_4$ to precipitate the protein and avoid interference. Colometric method was used using Anthrone reagent for estimation. The carbohydrate amount was expressed in mg/g of wt. of ant powder. Glucose was used as standard.

E. Moisture estimation :

The amount of moisture in the pupae was determined by weighing fresh pupae and keeping those at $50^{o}c$ in an incubator till a constant weight was obtained. The difference between initial and final weight gave an estimate of the moisture content. It was expressed in percent.

3.2.5 Study on feeding behaviour:

Some aspects of feeding behaviour of nymphs & adults of *G.ochropterus* were studied. For this purpose, nymphs of five different instars, males and females were starved and kept only on water for 24 hours. In each category (nymphs and adults), individuals of same age after ecdysis were taken from a culture maintained on ant pupae and twigs of *Leucus linifolia* at 27 ± $1^{o}c$, 70% RH & L:D 12 : 12 hours. Thirty test individuals of each category were used for the study. A fresh individual of each category was released at a distance (6 cm) from source in a separate tube.

In studying the temporal pattern of feeding, two consequent probes were considered as significantly separate when punctuated by a gap or margin of at least one minute. The ingestion behaviour i.e. probing (sustained feeding) and pausing (= withdrawl of labium) was observed for each category during the first four probes occurring within the time limit of four hours after release. Due to significant reduction in the number of attending individuals and their probing duration, the above limits for the probes were specified. Cold-preserved ant pupae were used after bringing them to room temperature ($30\pm2^{o}c$) for the experiments.

Analyses of duration, and percentage of total feeding time in each category were done. percecent of total individuals of each category involved in the first four probes were recorded and statistically analysed.

3.2.6 Performance of *G. ochropterus* on different diet combinations:

An attempt was made to rear nymphs of *Geocoris* on *Leucus* twigs and tea (*Camellia* sp.) separately. In an other attempt, performnce of adults were evaluated on combinations like, ant pupae; *Leucus* twig + ant pupae; ant pupae + water in different set of experiments in laboratory. The objective behind such study was to know if there is any obligation to any one of these diet component and the extent to which the

predator can survive if the diet fall short of either of the animal/plant food.

3.3 PREY-PREDATOR INTERACTION:

3.3.1 Prey preference test: (Sanjayan & Ananthakrishnan, 1987)

The prey preference test was carried out using an 'X' shaped glass apparatus (Fig. 16)of diameter 2 cm. Tweenty test bugs of each category (early instars ,late instars, adult males, and adult famales) of the same age were kept starved for a period of 24 hours and provided with water to clear off their guts. Three tea leaves or twigs infested with sufficient number of one set of pest species i.e. aphids, thrips and red spider mites were placed in three limbs, of the apparatus. The fourth limb used as control, was with a leaf free of any pest. The test bugs were introduced through the control hole and the openings were plugged by a cotton wad. The number of bugs feeding in each limb was noted every 15 minutes to 2 hours. At the end of observations the bugs engaged in feeding were disturbed and the apparatus was rotated to eliminate conditioning, for a fresh observation. The experiment was repeated thrice with fresh batch of insects.

Fig. 16. Apparatus used for testing prey-preference by *G. ochropterus*.

Fig. 17. Device used to bring aphid infested twigs from field to laboratory, and glass chimneys used to monitor predation.

Fig. 18. Two selected pesticides with some apparatus used for laboratory study.

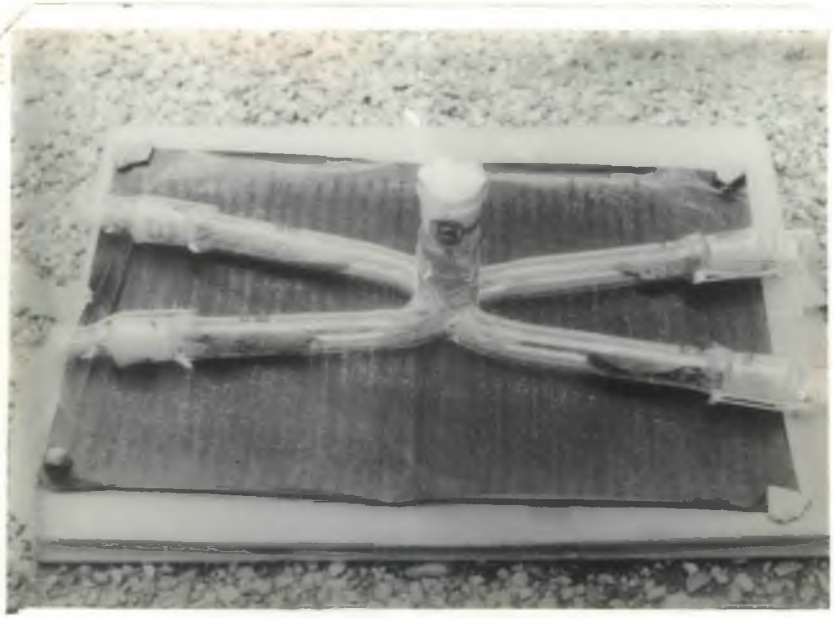

Fig.16.

Fig.17.

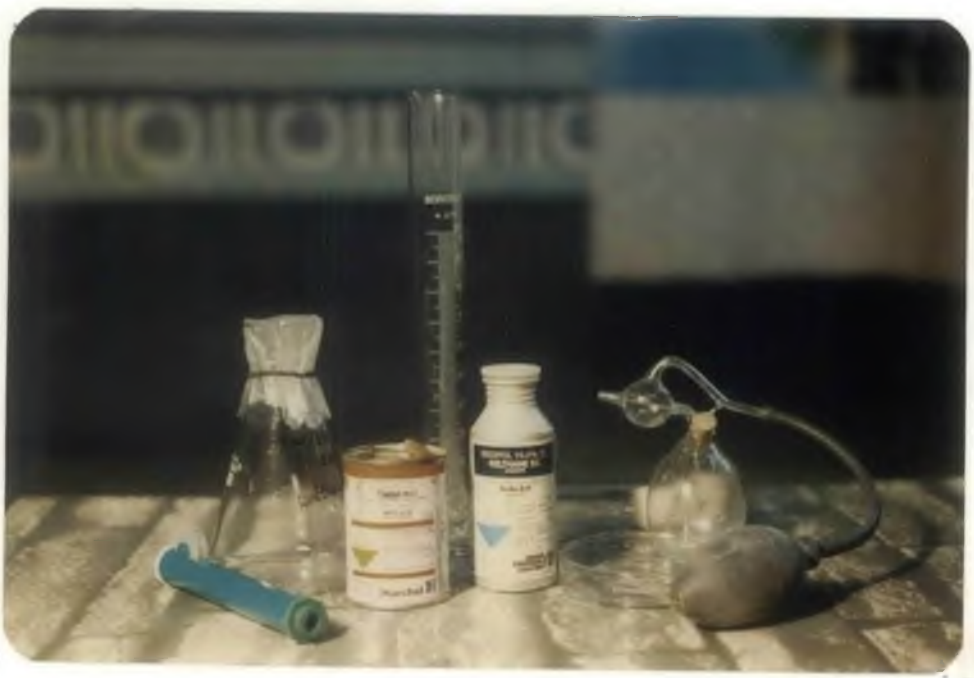

Fig.18.

3.3.2 Laboratory evaluation of predatory efficiency: (Grant & Shepard, 1985):

In laboratory evaluation of predatory efficiency aspests like 1) host preference, 2) searching abilities, 3) feeding behaviour 4) attack strategy, and 5) functional responses are observed.

predatory efficiency on aphids, thrips and mites:

The aphid infested twigs were collected from nearby tea estates and were immediately kept with water with their cut ends in water-filled conical flasks (100ml) (Fig. 16). After removing the gravid females and winged adults, the twigs were placed inside a glass chimney with a cloth covering the mouth and a split sponge plug at the base. Within each of these chimney, a newly hatched first instar was introduced to observe predation rate and stadial duration. The experiment was done taking 20 replications with adequate control in each case at 27 ± 1°c, 80 ± 10% RH and L:D of 12:12 hours.

The thrips and red spider mites were regularly collected from a tea estate (2 km away from laboratory). The infested leaves were brought to laboratory with special care. The eggs and nymphs of red spider mites were removed and *Geocoris* predation was observed on adult mites only. As for observation on thrips advanced larvae of thrips were provided as prey. Experiments with thrips and mites were conducted in

plastic container (5.5 x 4cm dia.) with a piece of soaked cotton swab inside the container to meet the water requirement of the predator and to maintain the turgidity of the leaf. In each of the plastic container, a newly hatched *Geocoris* nymph was introduced to observe the predation rate, stadia and post embryonic development. In every case the prey was provided in excess of predator's consumption capacity, and the number of prey consumption was monitored daily. The experiment were done taking 20 replicates with adequate control in each case at 27± 1°c , 80 ± 10% RH, and L:D of 12:12 hours.

3.3.3 Field-cage evaluation (Reed *et al.*, 1984):

Predator and prey were combined in one cage for a specified unit of time, after which the resulting prey mortality was determined. This tecnique provided quantitative assessment of predation on single pest species under field conditions, and it may be important in providing quantitative values for models that predict densities of pest population.

The experiment was conducted by caging adult *Geocoris* and aphid infested twigs in the field (Fig..19a) as well as in nursery (Fig. 19b & Fig. 19c). For conclusive results comparison with control were made.

Fig. 19. Different types of cages used in field trial:

a) Caging of young shoots with a portion of stem.

b) Caging in a small area inside nursery.

c) Caging over a larger area inside nursery.

Fig.19a.

Fig.19b.

Fig.19c.

3.4 PESTICIDES TOLERENCE OF *Geocoris ochropterus* (Hassan *et al.*, 1991):

3.4.1 The chosen pesticides:

1. Endosulfan (Thiodan 35% EC):

Chlorinated hydrocarbon, non-systemic, stomach insecticide and acaricide. The technical product is a brownish crystalline solid, practically insoluble in water, but moderately soluble in most organic solvents. It's a mixture of two isomers. It is stable to sunlight but subject to a slow hydrolysis to the alcohol and sulphur dioxide. It is compatible with non-alkaline pesticides. The acute LD_{50} for rat is 110 (55-220) mg/kg.

Effective against most crop, mites and some Hemiptera (aphids, mirids) and beetles.

2. Dicofol (Kelthane 18.5% EC):

Non systemic chlorinated hydrocarbon. Recommended for control of mites on a wide range of crops. Although residues in soil decrease rapidly, traces may remain for a year or more. Safe to bees.

The pure compound is white solid, practically insoluble in water, but soluble in most aliphatic and aromatic

solvents. It is hydrolysed by alkali but is compatible with all but highly alkaline pesticides. Wettable powder formulations are sensitive to solvents and surfactants, which may affect acaricidal activity and phytotoxicity. The acute oral LD_{50} for male rat is 809 ± 33 mg/kg.

Effective only against Acarina, kills eggs and all active stages of the mites.

The dosages of the pesticides applied in tea plantation are given below:

Group	Acaricides & insecticides (trade name)	Common name (active ingredient)	% a.i.	Recommended field conc. (% of product)
CH	Thiodan EC	Endosulfan	35	0.2
CH	Kelthane EC	Dicofol	18.5	0.5

The two selected pesticides i.e. thiodan and kelthane were 99% pure and were purchased from authorised dealer of the manufacturer viz. Hoechst India Limited, Bombay, and Indofil chemical company, Bombay respectively.

The chosen pesticides have been reported to be successfully applied on tea since 1982 by the 'Tocklai Experiental Station' and 'UPASI'(Tocklai wall chart for control of pests and dieases, October, 1967 and UPASI

recommendations in the "Planter's chornicle"- 15th Feb., 1968).

3.4.2 Guidelines followed:

The method to test the side effect of the above pesticides were according to the IOBC/WPRS (International Organization for Biological Control/ Western Palaearctic Regional Section) working group guidelines. The standard characteristic relevent to present study can be summerised as below:

1) Direct spray of predators (eggs, nymphs or adults),
2) Recommended concentration of pesticides,
3) Laboratory reared organisms of uniform age.
4) Water treated controls.
5) Adequate ventilation.
6) Mortality/reduction in beneficial capacity
7) Four evaluation categories: 1= harmless (<50%), 2= slightly harmful (50-79%), 3= moderately harmful (80-90%), 4= harmful (99%).

3.4.3 Collection and maintenance of predator as test material:

The nymphs (3rd instar) and adult *Geocoris ochropterus* were chosen for test and as such were collected from *Polygonum orietale* during April-June, 1993. The predators were reared on *Oecophylla smaragdina*.

While testing on each life stage, twenty five insect per concentration-treatment were used; each treatment was replicated four time. Each replicate included an untreated control. Five concentrations were tested for each pesticide.

3.4.4. Methods followed in experimentation:

The recommended dilution rate for application of thiodan was 1 part of insecticide in 400 parts of water by volume (400:1) while in case of kelthane the rate was 200:1 in tea (Banerjee, 1978). Use of doses higher than those recommended were necessary to find out LD_{50} value. The third instar nymphs and adults were spread on a glass plate and were directly sprayed with the pesticides. After drying in shade the nymphs or adults or eggs treated with each pesticide were kept separately in glass vials. The apparatus for spraying included a hand pump sprayer (Fig. 18) with uniform discharge rate of 0.5 ml per stroke. Each pesticide was mixed with distilled water to make up a stock solution which was diluted to the desired concentrations. The stock solution and it's diluted fractions were always prepared immediately before use. Treatments were made at about the same time each day.

The predators were fed with ant pupae and twig of *Leucus linifolia* at 27±1°c, 80% RH, and L:D 12:12 hours. Adults survived LD_{50} dosage levels of pesticides were paired

and observations on fecundity were noted upto 168 hours of post-treatment. Mortality was recorded after 48 hours of application.

3.4.5 Test on oral toxicity:

Experiments were designed to find the effect of pesticides as stomach poison. To study the acute exposure to contaminated prey, the big-eyed bug *(G. ochropterus)* was fed pesticide-treated pupae of *Oecophylla smaragdina*. Test concentration of the pesticide (1 ml) was dispensed on the pupae with a microlitre pipette. The treated materials were stirred in the above solution until thoroughly coated, then transferred to a petridish and allowed to dry. These were provided as food to third instars nymphs and adult females of *G. ochropterus*.

3.4.6 Calculations involved in pesticide experimentation:

In each case, the absolute number of mortality was corrected using ABBOTT formula:

$$\% \text{ mortality} = \frac{S-K}{100-K} \times 100$$

where S = % mortality of the treated group
and K = % mortality of the control group

Data on the juvenile mortality and the egg production per female on exposure to these pesticides were noted to obtain

the value of efficacy of a pesticide, using the following formula:

$$E = \frac{(100-M_t)\ R_t}{(100-M_c)\ R_c} \times 100$$

Where E = Total effect of the pesticide

M_t = Mortality of the treated group

M_c = Mortality of the control group

R_t = Average egg production per female of the treated group.

R_c = Average egg production per female of the control group.

Mortality was recorded after 48 hours and natural mortality was accounted for using ABBOTT'S formula (Abbott, 1925). The LD_{50} for each insecticide was calculated for 48 hours by log-probit analysis as has been applied by Walker and Turnipseed (1976) for other species of *Geocoris*. Data were analysed by using the probit procedure.LD_{50} were calculated by using the methods described by Busvine(1971).

3.5 STATISTICAL CALCULATIONS AND COMPUTER SOFTWARE USED :

The essential statistical calculations were done using standard methods available from Daniel (1974), Bailey (1985), Palanichamy and Manoharan,(1990) and calculator model Casio Fx 82C. Computer software was used for drawing graphics using

HPG package and statistical analysis were based on programmes on regression, ANOVA and Duncan's multiple range test (Duncan, 1955) and others. The programmes were developed by the University computer centre and Commerce department of the university.