CHAPTER III

MATERIALS AND METHODS
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Periodical survey of the cabbage aphid, *Breviceoryne brassicae* (L.) and its natural enemies along with the host plants was conducted at different parts of Manipur (vide map) particularly at three different agro-climatic zones of Manipur, i.e., i) Imphal (c 785 m), ii) Kangpokpi (c 1,200 m) and iii) Mao (c 1,900 m) during 1989-92. The collected aphids were preserved in 70% alcohol and processed for permanent slide mounting by using chloral hydrate-phenol mixture. The stages of the natural enemies collected from the field were reared in the laboratory to get the respective adults. After identification all the insects were preserved in their respective forms in the Aphid Research Laboratory, Entomology Section, Department of Life Sciences, Manipur University.

The population trends of *B. brassicae* in relation to biotic and abiotic factors were studied on cabbage, *Brassica oleracea* L. (Local variety) at three sites, viz., i) Study site I (Imphal), ii) Study site II (Kangpokpi) and iii) Study site III (Mao) in Manipur consecutively for two cropping seasons during 1989-91. The sites are different both in terms of climate and altitude, where the cultivation practices of the crop were also different.

Studies were conducted at farmer’s field in Imphal and Mao and at the Agricultural farm, Kangpokpi. The experiment was
laid out in a randomised block design of each plot sized 4x2 sq.m. Spacing between the plants was 50 cm and 30 cm between the plots. The normal cultivation and agronomical practices of the crops were followed in all the places. No insecticidal treatments were applied during the whole period of the crop. In general, the crop was transplanted during the period from June to October in different agro-climatic conditions of Manipur. Fortnightly samples were taken from each replicate randomly, three leaves— one each randomly representing upper, middle and lower portions of each plant for population of the pest. Such sampling was specially recommended in case of cabbage (Church and Strickland, 1954) but similar sampling was convincingly followed presently in cauliflower and mustard also during the vegetative stage of the plants (Chandra and Kushwaha, 1986). Each sample consists of the total aphids or natural enemies present in three leaves per plant representing different stratum of the plant. However, with the initiation of flowering and sprouting, i.e., reproductive phases, sampling was done on 10 cm flowering twigs and three leaves per sprout. During sampling different morphic individuals of the pest, i.e., nymphs and adults (alate and apterous) were counted separately and calculated their percentage of composition.

**GROWTH RATE AND REPRODUCTIVE POTENTIAL**

Based on the data of the field population assessment of *B. brassicae*, the pooled data of both the cropping seasons of
the different study sites were processed to calculate the 'growth rate' and 'reproductive potential' by using the following formulae adopted by Odum (1971).

"Growth rate (GR) can be defined as the rate of change in the number of organisms per unit time at a particular instant".

"Reproductive potential (RP) can be defined as the rate of change in the number of organisms per unit time per individual at a particular instant".

\[ GR = \frac{dN}{dt}; \quad RP = \frac{dN}{dt} \cdot N \]

where, \( dN \) = change in number of insect,
\( dt \) = change in time (30 days),
\( N \) = initial number of insect.

The calculated values of GR and RP using the above formulae are provided in vide Table 3.

In addition to the pest, the stages of the natural enemies, viz., Syrphids, Coccinellids and Aphidiid parasites per unit sample were also recorded. The syrphid predators were identified at the Zoological Survey of India, Calcutta. The other insects were identified at the Aphid Research Laboratory, Department
of Life Sciences, Manipur University. Periodical meteorological data such as temperature, relative humidity and rainfall were also recorded from each experimental site to know the relationship between aphid population and abiotic factors. The correlations were worked out between the aphid population and both the biotic and abiotic factors. Correlation co-efficients were also calculated separately for each factors, because it was thought that different factors may be responsible for different fluctuations of the aphid population.

AERIAL POPULATION FLUCTUATION

Investigations on the population of alate aphid were made using YPT (Fig. 3) in the cabbage field at Imphal during 1992-93. Trap was developed by using aluminium sheet of 16x16x4 cm and painted with golden yellow synthetic enamel paint inside. The trap was placed at the equal height of the crop and altered as the crop grew up. One trap was employed in one plot of three replications. The trap was filled with 3/4 th of water always maintaining the level as maximum possible. The alate aphids trapped in YPT were collected by using camel hair brush for every alternate day and preserved in 70% alcohol. All the aphids were subsequently identified under the stereoscopic binocular microscope and the number of species were recorded in each case separately. The prevailing meteorological factors, such as, temperature, relative humidity and rainfall were recorded during
Fig. 3. Yellow Pan Trap in the experimental field of cabbage (local variety).
the period of investigation. The correlation between the aphid trapped and the weather factors was studied by subjecting the data to the correlation co-efficient.

APHID BIOLOGY

Life history of *B. brassicae* including development, reproductive phases, fecundity and longevity on cabbage (local variety) was conducted in three different seasons, i.e., a) Autumn (September-October), b) Winter (December-January) and c) Spring (March-April) in the laboratory by using the potted plants. The laboratory average temperature and relative humidity during the period of study were 24.25±0.18°C and 67.77±1.39% in autumn, 16.52±0.32°C and 65.91±1.41% in winter and 19.45±0.27°C and 63.35±1.41% in spring respectively. The life history of the aphid was also studied on six cruciferous host plants, viz., i) Cabbage-I, *Brassica oleracea* L. var. *capitata* Linn.; ii) Cabbage-II, *B. oleracea* L. (local variety); iii) Cauliflower, *B. oleracea* L. var. botrytis Linn.; iv) Knol-khol, *B. oleracea* L. var. gongylodes Linn.; v) Mustard, *B. juncea* (L.) Czern and Coss. and vi) Radish, *Raphanus sativus* L. in the laboratory (avg. temp. 16.33±0.21°C and 50.24±1.35% R.H.) in their respective potted plants. Freshly laid single instar aphid nymphs were obtained from the stock culture maintained in the laboratory and released on each respective potted plants. Rearing was developed providing the black paper cone around the leaf or shoot where the aphid
is feeding on (Fig. 4a,b). Detailed observations on different biological parameters, i.e., nymphaal duration, duration of reproductive phases and behaviours, fecundity, adult longevity were recorded carefully. All the treatments were provided identical environmental conditions. Ten replications were maintained for each host and observations on different biological parameters were made twice daily. Results were subjected to ANOVA to test the significance among the different sensons and the hosts to know the relationship between the different parameters of the life cycle.

EFFECT OF PHOTOPERIOD

The biology of the aphid was also conducted in the laboratory at three different photoschedules, viz., i) LD 8:16, ii) LD 12:12 and iii) LD 16:8 maintained separately in three different chambers at room condition (24±2°C and 70±5% R.H.). Care was taken to the extent possible to maintain the temperature and relative humidity constant. An artificial source of light (c 300 lux) was provided within the chamber, where light on/off systems were operated manually. Freshly laid aphid nymphs were obtained from the stock culture maintained in each chamber and released on respective potted plants of cabbage and exposed to different photoschedules. Each treatment was based on five replications. Detailed observations on various biological parameters were made daily. Results were analysed with suitable statistical methods.
Fig. 4(a&b) Rearing of *B. brassicae* on cabbage (local variety).
MORPHOLOGY

During the period of biological studies of aphid, laboratory culture of the aphid was also maintained seasonally on the respective potted plants to get various morphic individuals (nymphs, alate and apterous) of the aphid. Observations were made three times daily in order to know the successive stages of the aphid. Each and every stage of the aphid were collected separately and preserved in 70% alcohol. All the stages of aphid were processed for permanent slide by using Chloral hydrate-phenol mixture. Permanent slides were subjected to microscopic study with the help of ocular micrometer under stereoscopic binocular microscope for morphological variation, morphometric changes of the different parts and identification of instars. Observations were based on five replications and results were analysed with suitable statistical methods. For the identification purpose, suitable nymphal key characters were also prepared. The apterous oviparous females were also collected during the comparative biology on different hosts and processed for permanent slides to study the morphometric variations.

NATURAL ENEMIES

Developmental biology including larval voracity of common predators of this aphid was studied in the laboratory (avg. temp. 22.6-27.0°C and 52.4-76.5% R.H.). Some behavioural aspects of
these predators were also studied both in the field and the laboratory. Both sexes of the predators were collected from the *B. brassicae* infested cabbage field and allowed for mating and oviposition on the aphid infested leaves or plant twigs inserted in small bottles (25x40 mm) filled with Knop's solution which was enclosed by a glass chimney (10x4.5 cm) with their top covered with muslin cloth. A cotton swab soaked in 10% honey solution and a few flowering shoots of mustard were kept inside the chimney to provide food for syrphid flies. However, syrphids failed to lay eggs in the laboratory. Therefore, freshly laid eggs of syrphid were obtained from the potted plants kept outside the laboratory for individual rearing in the laboratory.

The eggs were picked up from among the aphid colonies and kept in petridishes (10x2 cm) separately for hatching. Observations were recorded on shape, size, colour, incubation period and hatchability of eggs. On hatching, the larvae of both the predators were reared in separate petridishes individually, which was provided with sufficient but counted number of aphids along with plant parts which was moistened with water soaked cotton. The plant parts along with the aphids were changed daily. Young predators larvae were provided with first or second instar nymphs of *B. brassicae* but as the predators matured, latter stages of the aphids were provided as food. Detailed observations on
larval voracity, rate of consumption, developmental periods and morphological changes of different stages were recorded daily. All the stages were preserved in 70% alcohol and measured the sizes with the help of slide calliper (Mitutoyo, Japan). Observations and calculations were based on ten replications. The newly hatched larvae were also reared in groups in petridishes with or without the aphid to observe their feeding behaviour and other related aspects. Other related observations were made simultaneously in the field also in order to observe or determine the various behavioural aspects and other ecological phenomena. Important morphological characters including shape, size and colour of the predator larvae were noted for field identification.

**CONTROL**

A laboratory trial was conducted to find out the most effective insecticide and botanical pesticides for the control of *B. brassicae*. For the purpose, six insecticides, viz., Carbaryl 0.2%, Dichlorvos 0.05%, Endosulfan 0.05%, Malathion 0.05%, Monocrotophos 0.05% and Quinalphos 0.05% representing different groups of insecticides and two botanical pesticides, viz., Achook 1.0% and Nimbecidene 0.03% were tested against the pest. The recommended concentrations of the insecticides and the botanical pesticides were prepared for the trial. The uniform sized fresh cabbage leaves were obtained from the unsprayed field and washed...
thoroughly with tap water. Each leaf was dipped completely in the respective insecticide and botanical pesticide solutions and dried in shade for 5 minutes. After drying the treated leaves were moistened with water soaked cotton and placed in Petridishes (10 cm dia.) and twenty healthy female aphids were released into each Petridish. A control was maintained simultaneously with water spray only. Three replications were maintained for each treatment including control. Observations were recorded on percent mortality of aphid after 24, 48 and 72 hr of the release.