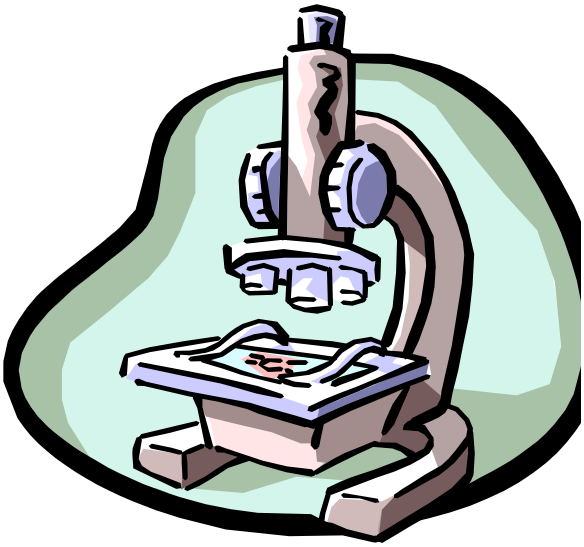
Chapter - 3



MATERIALS AND METHODS

CHAPTER 3 MATERIALS AND METHODS

3.1. Experimental site:

The field experiments were conducted at the Instructional farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India during summer, rainy and winter seasons of 2007-08 and 2008-2009. Population dynamics of key pests of cucurbitaceous vegetables were studied starting right from January 2007 and continued up to December 2008. The laboratory investigations were carried out in the Department of Agricultural Entomology.

3.2. Geographical location:

The terai agro-ecological region comprises northern parts of the state West Bengal lies between 25^{o} 57' to 27^{o} N latitude and 88^{o} 25' to 58^{o} 54' E longitude. The region is situated in the sub-himalayan plains comprising Kurseong, Kalimpong and Bhutan hills in the North, Bihar border in the West, Assam border in the East and Bangladesh in the South. The agro-ecological zone under consideration includes Siliguri and Islampur sub-divisions of Darjeeling and North Dinajpur district respectively, entire Jalpaiguri and Cooch Behar district. Out of 12,025 sq. km geographical area of the zone 8,567 sq. km. is brought under cultivation. Total geographical area of terai zone is about 13.5% of the state area which support about 9.7% of the state population. Rural population exceeds the average state figure which is more than 90%.

The Instructional Farm, where the experiments were undertaken, located at 26^{o}19' N latitude and 89^{o}23' E longitude at an altitude of 43 meter above the MSL.

3.3. Soils:

Soils of the region under consideration can be classified into two broad categories:

i. Old Himalayan Pediment Plains and

ii. Teesta Flood Plains formed mostly from the Himalayan detritus.

Soils of the region comprises dark brown top soil, 1-3 feet deep, which is in general, sandy loam in texture, acidic in reaction (pH 4.2-6.8), rich in raw humus content, porous, low water retention capacity, medium to high total nitrogen content, low to medium K content and poor secondary micronutrients.

3.4. Climate:

The terai region of West Bengal is characterized by typical humid climate with distinct features of high rainfall (annual average more than 3000 mm) and high relative humidity (average maximum 95% and average minimum 65%). Lions' share of the total rainfall (about 80%) received during rainy months from June to September. Maximum and minimum temperature varies from 24-33.2^{o}C respectively.

The weekly bright sunshine hours per day remain more or less steady from the beginning of the year up to early June, i.e. just before arrival of South-West monsoon. A sharp fall has been observed thereafter which continues up to early October. Average duration of bright sunny days is more than 8 hours during November to March. The whole area is humid and worm except having a short spell of winter during the months of December-February.

3.5. Materials required:

3.5.1. Plant materials:

i. Bitter gourd (*Momordica charantia* Linn.)

ii. Pumpkin (*Cucurbita pepo* Linn.)

3.5.2. Test insects:

i. Melon fruit fly *Bactrocera cucurbitae* (Coq.) (Diptera : Tephritidae)

ii. Epilachna beetle, *Henosepilachna vigintioctopunctata* Fab. (Coccinellidae: Coleoptera)

iii. Red pumpkin beetle, *Aulacophora foveicollis* (Lucas.), (Chrysomellidae: Coleoptera)

3.5.3. Lures and other chemicals:

i. Molasses

ii. Sugar

iii. Date palm juice

iv. Fermented date palm juice

v. Country liquor

vi. Neem cake

vii. Formaldehyde

ix. Bleaching powder

3.5.4. Chemicals and microbial:

Table-1: Chemicals and microbial required for conducting the investigation.

Chemical name	Common name/ Trade name	Manufacturer
4-(p-acetoxyphenyl)-2-butanone	Cue-lure	Ganesh Biocontrol Systems, Gujrat, India
Cypermethrin 10 EC	Ustad	United Phosphorus Ltd., India
Chlorpyriphos 20 EC	Dursban	Dow Agrosciences India Ltd.
Spinosad 45 SC	Tracer	Dow Agrosciences India Ltd.
Malathion 50 EC	Cythion	BASF India Ltd
Chlorpyriphos 50%+ Cypermethrin 5%	Nurel-D	Dow Agrosciences India Ltd.
Endosulfan 35EC	Thiodan	Aventis Crop Science India Ltd.
Metarhizium anisopliae 1.15% WP	Stanes Biomagic	T. Stanes and Co. Ltd.
Dichlorvos 76 EC	Nuvan	Syngenta India Ltd.

3.5.5. Equipments:

i. Texture analyzer (TA-XT plus, Stable Micro Systems, U. K.)

ii. Simple microscope (Olympus)

iii. Camera lucida

iv. Automiser

3.5.6. Miscellaneous items used:

In addition to the aforementioned materials, bucket trap (for melon fruit fly), sieve, brush, vernier scale, plastic jars, cloth, balance etc were used for the investigation.

3.6. Yield loss assessment due to infestation of key insect pests:

Studies were undertaken on avoidable yield loss due to insect pest infestation on selected cucurbits viz. bitter gourd and pumpkin during summer seasons of 2007-08 and 2008-09. In the experiment varieties used were ‘Green long’ of bitter gourd and ‘Bhumilakshmi’ of pumpkin. The crops were raised under recommended agronomic practices in 5m X 5m plots and the experiment was replicated six times by following completely randomized design (CRD).

Simple ‘t’ test procedure was followed for comparison between the yield of two plots in each of the six replications where the plants from one plot were protected

from insect-pests, diseases and both from insect-pests and diseases by applying insecticides, fungicides and both insecticides and fungicides. The plants of other plot were allowed to receive natural infestation. The treatment details were as follows:

T_1: Only insect-pest control (carbofuran 3G at 20 days of sowing followed by spraying with chlorpyriphos 20EC @ 2.00ml/l of water at fortnightly interval and poison baiting with molasses and spinosad)

T_2: Treated with only fungicide, (copper oxychloride (Blitox50WP @ 2gm/l of water)

T_3: T_1+T_2

T_4: Untreated (Control)

Carbofuran 3G was applied @ 0.8 kg. a. i. /ha after 20 days of sowing followed by spraying of chlorpyriphos 20EC @ 2ml/l of water at fortnightly interval and blitox @ 2gm/l of water was sprayed at fortnightly interval for T_1 and T_2 respectively. Whereas, six rounds of both insecticidal and fungicidal sprays were applied in T_3. Placement of poison bait with molasses along with the toxicant spinosad was started from flowering stage of the crop which was replenished at weekly interval. Observations on incidence of foliage feeding key pests *viz*. epilachna beetle and red pumpkin beetle were taken at weekly interval. At each harvest marketable and infested fruits by the melon fruit fly were recorded both on number and weight basis. The total yield/plot was calculated on cumulative basis and then converted in to kg/ha. Percentage of yield loss on number and weight basis were also deduced.

Data on pest population and percent fruit infestation both on number and weight basis among the different treatments over two successive years of investigation were pooled together and analysed statistically by following appropriate technique using INDOSTAT package of statistical analysis. Yield loss was assessed by pair 't' test between treated and untreated plots.

3.7. Determination of ETL of key pests of cucurbits:

Efficient management of most of the pests of agricultural importance depends mainly on the use of chemical insecticides. As the chemicals are hazardous to environment as well as human beings, their use should be judicious, need based and purely based upon ETL concept. The earlier concept of pest 'Pest control' aiming cent percent elimination of pests from the agricultural ecosystem was replaced by the term

'Pest management' that aims at reducing the pest population to a level that does not cause economic injury or economic loss. The idea as expressed by Pierce (1934) for assessment of insect damage and the initiation of control measures become one incentive for the development of a concept of economic injury level. The concept of economic injury level (EIL) was formally proposed by Stern *et al*. (1959). As per his version, economic threshold level (ETL) is nothing but the number of insect present per unit area (density or intensity) when management action should be taken to prevent the increasing pest population from reaching economic injury level.

3.7.1. Melon fruit fly, *Bactrocera cucurbitae* (Coq.) (Tephritidae: Diptera):

Nine farmers at nine different villages (Pundibari, Baudiardanga, Barorangras, Kathalbari, Jagyanarayanerkuthi, Jiranpur, Mowamari, Panishala, Bagroa) of Cooch Behar district of West Bengal were selected for growing bitter gourd and pumkin. Area of each field adjacent to each other was fixed at about 1 bigha. Three cuelure traps manufactured by Ganesh Biocontrol Systems, Gujrat, India were installed at flowering stage at each farmer's field that was treated as an individual replication. Trap to trap distance was kept as 30±5 m as suggested by Khan, 2002. All the fields were maintained without adoption of any management practices against melon fruit fly except routine agronomical activities. But the plots at University Instructional farm were kept under complete control measures so as to assess the cost of undertaking protection measure as well as to determine the extend of avoidable yield loss due to the fly infestation on different crops under consideration. The complete control measures include:

- Spot application of bait spraying (four sprays starting from flowering) using spinosad and mollasses at four spot (four pits) per field.
- Collection and burying of infested fruits and placement of cuelure trap @ 3 numbers per acre.

Trap catch of male adult melon fruit fly was recorded at weekly interval and then converted into catch per day. Observations on total number of fruits, number of infested fruits and yield were recorded at harvest. Percentage data were transformed into arcsine values for statistical analysis.

The EIL was computed based upon the procedure given by Stone and Pedigo (1972) and modified by Ogunlana and Pedigo (1974) using the following formula:

$$EIL = \frac{\text{Gain threshold}}{\text{Yield reduction per insect}}$$

$$\text{Gain threshold} = \frac{\text{Management cost (Rs./ha)}}{\text{Market value of the produce (Rs./q.)}} \quad \text{(q./ha)}$$

A correlation co-efficient 'r' between number of flies trapped/trap/day and percent infestation for each crops were worked out with the help of Microsoft Excel. A regression equation of Y = c – mx were then deduced where 'c' is the intercept; 'm' is the yield reduction per insect. Thus 'm' is the percent reduction in yield by increasing one male adult fly in the field. Management cost was determined based on the market price of spinosad, mollasses and cuelure traps required for undertaking protection measures per hectare of field including labour charges. Market value of the produce was determined according to the wholesale price of the respective vegetables prevailing at Cooch Behar district.

Substituting the values in the above formula, EIL was computed and economic threshold level (ETL) was set at 75% of EIL (Pedigo, 1991).

3.7.2. Epilachna beetle, *Henosepilachna vigintioctopunctata* (Fab.) (Coccinellidae: Coleoptera):

A field experiment was laid down in Completely Randomised Block Design (CRBD) during 2006-07 and 2007-08 with six treatments and three replications using the varieties 'Green Long', 'Bhumilakshmi' of bitter gourd and pumpkin respectively. The treatments were as follows:

T_1: Complete protection (Control)

T_2: Releasing 1 larva/plant

T_3: Releasing 2 larva/plant

T_4: Releasing 3 larva/plant

T_5: Releasing 4 larva/plant

T_6: Releasing 5 larva/plant

Seeds of the crop were sown at a distance of 2 metre from pit to pit. After one week of germination only one healthy plant per pit was maintained by thinning and

covered with nylon net. Second instar grubs of epilachna beetle at different level of density for the respective treatments were released in the cages. The cages were designed in such a way that they did not interrupt ventilation and aeration of the growing plants. Bottom edges of the cages were inserted into the soil in all sides to check escape or entry of the larvae. These cages were erected on bamboo sticks fixed in four corners. The larval stage of the beetle were released once at three weeks age of the plants and subsequently at fifteen days interval to maintain constant population till last harvest of the crops and in each time the pupal or pre-pupal stages of previously released larvae were taken out of the cages.

Yield data converted into quintal/ha and yield loss due to different treatments were derived by deducting the yield of respective treatments from the control (where no larvae was released). Value of yield loss was determined as per wholesale market price prevailing at Cooch Behar district at the time of harvesting. The ETL was computed based upon the procedure given by Stone and Pedigo (1972) and modified by Ogunlana and Pedigo (1974).

3.8. Biology of key pests of cucurbits:

3.8.1. Melon fruit fly, *Bactrocera cucurbitae* (Coq.) (Diptera: Tephritidae):

Studies on biology of *Bactrocera cucurbitae* was carried out in laboratory, Department of Agricultural Entomology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal during the year 2007-08. Infested fruits of bitter gourd and pumpkin were collected from the field and kept in rearing cage of size 2' X 2' X 2'. Within the cage, infested fruits were placed in a plastic tray containing 5 cm thick layer of sieved soil. After 6-7 days the decayed fruits were removed and the pupae were collected by sieving the soil. The individual pupae were kept in glass tube (2.5 cm diameter; 18 cm height). Freshly emerged adults (males and females) were then released in rearing cages. The semi-solid food of protinex powder was provided in the cage. A petridish containing a round piece of sponge dipped in 5% honey solution was also hung in the rearing cage. Fresh and tender fruits of bitter gourd and pumpkin were hung in the cages for egg laying. After egg laying, the eggs were detected by pecking the fruit below ovipositional puncture under simple microscope. Observations relating to the eggs were recorded from ten random samples (eggs) of five individuals of each fruits. The remaining eggs were transferred

Infested bitter gourd

Infested pumpkin

Eggs under simple microscope

Mature maggots

Puparium

Adult

Plate-1: Different life stages of melon fruit fly

in the glass tubes (2.5 cm diameter; 18 cm height) containing half of it with pulp of bitter gourd and pumpkin (as the case may be).

The pulps were prepared by crushing the fresh and tender peeled fruits of bitter gourd and pumpkin in a mixture-cum-grinder without adding water. After 5 days of placing the eggs in the tubes the larvae were transferred to a piece of fresh fruits of bitter gourd and pumpkin respectively.

The morphological parameters of egg, maggot, pre-pupal and pupal stages were recorded using stage and ocular micrometer. The adults were killed by using carbon tetrachloride (CCl_4) then pinned; wing stretched; dried and preserved. Ten specimens each of adult male and female were observed under microscope to study their morphological parameters. Incubation period, larval period, pre-pupal period, pupal period as well as larval and pupal mortality were recorded. Number of eggs laid by an individual female (fecundity), longevity and total life period of both male and female were also recorded.

3.8.2. Epilacna beetle, *Henosepilachna vigintioctopunctata* (Fab.) (Coccinellidae: Coleoptera):

For the selection and execution of any pest management, the basic requirement is to study biology of the pest on growth, development, longevity, fecundity and mortality in relation to the host plants (Hossain *et al.*, 2009). The effect of leaves of bitter gourd and pumpkin on growth and development of *Epilachna vigintioctopunctata* under laboratory condition (room temperature 30 ± 2^0C and relative humidity 85±5%) was studied during March to July of 2007 and 2008.

Pupae of epilachna beetle were collected from the cucurbit field. The pupae were kept in glass jar for emergence of adults. The mouth of the jar was securely tied with cotton cloth with the help of rubber band. Small plastic containers (6cm X 5cm) were used for collecting and dealing with the adults.

The adults so obtained were identified for their sexes at the beginning. A pair was released into a glass jar (12cm diameter X 18cm height) and its mouth was tied with cotton cloth. Fresh and healthy leaves of respective host plants viz. bitter gourd and pumpkin were provided to the adult daily in the morning. Uneaten leaves, faeces and frasses etc. were removed from the rearing jars at each time of providing food. Eggs laid by each female on the surface of leaves and longevity of adults were recorded.

Egg mass

Young grub

Mature grubs and pupa

Adult

Plate-2: Different life stages of epilachna beetle

The egg masses laid by the female were observed daily until they hatched. The first instar grubs immediately after hatching were transferred to petridishes (10 cm. diameter) with the help of a cotton brush to avoid any injury. Only one grub was reared in each dish on moistened blotting paper. They are fed with fresh and tender leaves of the respective hosts. During rearing, duration of different developmental stages were recorded till pupation. Five such petridishes were placed randomly on a table in the laboratory which constitutes five replications. Utmost care was taken during feeding of the grubs and cleaning of the rearing containers and maintaining proper hygienic condition. Pupae were kept undisturbed in the dishes till emergence of adults.

The optimum length and breadth of grubs in each instar, pupae as well as adults were measured by camera lucida. Data, thus obtained were analysed by using analysis of variance in CRD and mean values were separated by LSD (Least Significant Difference) at 5% level of significance. Paired "t" test was carried out for comparison of hosts in affecting biological parameters of the test insects.

3.9. Population dynamics of key pests of cucurbits:

With a view to study the population dynamics or seasonal fluctuation pattern of some key pests of cucurbitaceous vegetables as influenced by prevailing environmental conditions specifically by the important weather parameters, a commonly grown variety of bitter gourd *viz*. 'Green long' was grown during 2007 to 2008 and the crop was maintained in field round the year. The crop was sown four times in a year (3rd week of October, last week of January, 3rd week of April and 3rd week of July) and grown under usual agronomic practices having a spacing of 1.2 m X 1.2 m from pit to pit and row to row with a plot size of 10m X 10m with three replications. Thirty metre distance was maintained from one plot to another. No plant protection measures were adopted and the crop was left for natural insect pest infestation.

For melon fruit fly, Cuelure traps manufactured by Ganesh Biocontrol Systems, Gujrat, India, were hanged at about six feet above the ground level one at each plot. Each trap was considered as one replication. Cuelure impregnated "Melon block", was placed in each trap. The blocks were replenished at monthly interval and the trapped flies were removed and counted every week starting from 01.01.2007 to 31.12.2007 and again from 01.01.2008 to 31.12.2008. Mean number of flies caught

per trap per day were determined every year at weekly interval and average of both the years of study worked out.

In case of epilachna beetle and red pumpkin beetle observation were recorded on the basis of number of beetle(s) per pit (2 plants/pit) at seven days interval. During bearing stage, the fruits were harvested and separated into damaged and fresh ones. The damaged fruits thus obtained from consecutive harvesting were calculated into percentage damage in number and weight.

Meteorological observations like temperature, relative humidity, rainfall and daily sunshine hour were collected from the Meteorological Observatory of Central Tobacco Research Institute (CTRI), Indian Council of Agricultural Research (ICAR), Dinhata, Cooch Behar.

Data, thus obtained from the entire crop growing season in successive two years were analysed and presented graphically to determine the pattern of incidence of the key pests. Simple correlation between pest incidence and important weather parameters viz. temperature, relative humidity (RH), rainfall and sunshine hour of previous week were worked out to find the influence of environmental conditions on population dynamics of the pests. The data were also processed for multivariate regression models among the different abiotic factors and population fluctuation with an objective to see the actual role of weather factors on the population fluctuation. The whole analysis was done using INDOSTAT package of statistical analysis.

3.10. Relationship between melon fruit fly, *Bactrocera cucurbitae* (Coq.) density and percent fruit infestation:

Two important cucurbitaceous vegetables grown widely in West Bengal, viz. bitter gourd, *Momordica charantia* L. and pumpkin, *Cucurbita pepo* L. were selected for the present study. Four individual farmers of Cooch Behar district, West Bengal, growing these crops were selected. Each farmer had grown the crop in about 1 bigha (0.14 ha approximately) area and were advised to raise the crop as per recommended agronomic practices. In each field three cuelure traps manufactured by Ganesh Biocontrol Systems, Gujrat, India were installed at three different corners (individuals treated as one replication) of the field at an average height of 4 feet from the ground level during flowering stage for monitoring adult male fly population. The traps were observed for adult male capture at weekly interval.

Number of flies caught in a trap per week was counted and the daily capture calculated accordingly. Percent fruit infested by the fly were also determined on number basis as follows:

$$\% \text{ fruit infestation} = \frac{\text{Number of fruits infested}}{\text{Total number of fruits observed}} \times 100$$

In each time of recording observation the infested fruits were tagged to avoid recounting and continued up to ten consecutive weeks. Data thus obtained were statistically correlated with the help of appropriate statistical technique and presented. Subsequently from each field, three soil samples (pit size: 30cm x 30cm. x 12cm.) were collected randomly on the same date of recording fruit infestation. The number of pupa were counted by sieving the soil sample and subjected to analysis with Microsoft Office Excel-2003.

3.11. Behavioral characteristics of melon fruit fly:

3.11.1. Depth of pupation at varying soil type and moisture regime:

Bactrocera cucurbitae (Coq.) was obtained from infested pumpkin fruit grown at Instructional Farm, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India. The infested fruits were kept in rearing cage (20"x 20"x 20") on a plastic tray. The maggots were kept in close vigil for 3-4 days till they attained jumping stage (i.e. late third instar). At this stage they became matured which were collected for the purpose of pupation.

Soil was collected from the experimental fields at 0-15 cm depths from the surface. Before being placed in the containers, the soil was sieved through a 5 x 5 mm sieve and then dried in an oven for 48 hours at 100^0C for sterilization as described by Hou *et al*. (2006). Texture of soil was analyzed as sandy loam however; sand was collected to study its pupation behaviour. The pupation containers were made up of clear plastic, cylindrical in shape, 20 cm. in height and having a top opening of 9 cm. diameter. The top opening was covered and fastened with muslin cloth to prevent escaping of the larvae.

Both sand and sandy loam soils were wetted with tap water to get relative moisture gradients of 0, 20, 40, 60, 80 and 100%. The containers were then filled with the soils of respective moisture levels. Twenty five such larvae were released in each container having six moisture levels for pupation which were then marked accordingly the type of soil, their target moisture level and kept under laboratory conditions. This experiment was replicated thrice and carried out during March-April. After 48 hours of releasing the larvae, the distribution of pupation depth was checked. At first, the number of pupae on soil surface was recorded. Then the top soil was removed by scalpel in successive 2cm. layers. Each layer was sifted through a 2 x 2 mm sieve and tap water to separate the pupae from soil and the number of pupae in each layer was recorded. Data obtained in this way were statistically analysed with INDOSTAT package of statistical analysis.

3.11.2. Fly activity during the day:

In evaluating the fly activity as well as to determine the peaks in daily catch of adult male fly, 12 Cuelure traps were taken and observation were recorded from 01:00 to 24:00 hour of the day at 2 hours interval. Each trap were considered as single replication and used for three consecutive days. The experiment was laid out in randomized block design (RBD).

3.11.3. Impact of colour and height of placement of traps on fly catch:

The study was conducted at three adjacent farmers field of pumpkin each of which were about 1 bigha (0.14 acre approximately) area at Baudiadanga village, Cooch Behar, West Bengal, India. The Cuelure bucket traps of 11 cm. height and 8 cm. diameter with having four holes of 2 cm. diameter were stuck with different colours glossy papers. The plastic traps were used because they are cheap, more convenient than sticky traps and can be recycled. The treatments were as follows:

T_1: White trap

T_2: Orange trap

T_3: Green trap

T_4: Red trap

T_5: Blue trap

T_6: Yellow trap

T_7: Violet trap

T_8: Opaque trap

T_9: Transparent trap

In each field one trap of a particular colour totaling nine traps were installed and each field was treated as a replication. The study was conducted during 22nd to 26th April (Summer) and 23rd to 27th July (Rainy) of 2007 and 2008. Cuelure impregnated block (Mfg. Ganesh Bio-Control Systems, Gujrat, India) were hung from the roof of the bucket by a thread and cotton wicks soaked with 1 ml spinosad 45 SC. The traps thus prepared were randomly placed at an average height of 6 feet from the ground level all over the field. The observations were recorded in every 24 hours for five consecutive days and the caught flies were removed. Traps were rotated clockwise in different directions at each observation. The recorded data were averaged and daily catch/trap was calculated accordingly. Data, thus obtained, were subjected to appropriate statistical technique following INDOSTAT. Standard error of mean (SEM) and critical difference (CD) were calculated and presented in the table. Means were separated by the Least Significant Difference (LSD) option at 5% level.

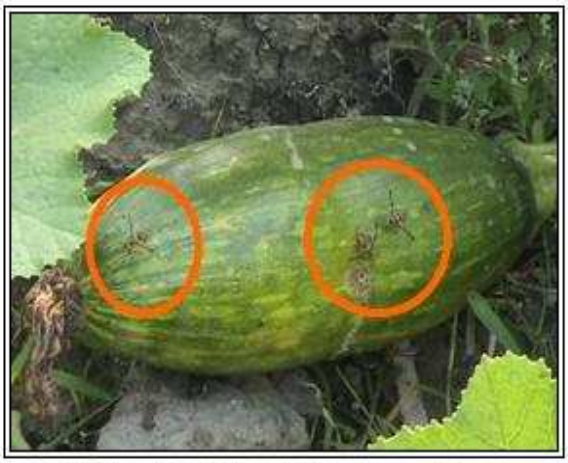
The yellow coloured cuelure traps were placed at different height from the ground level in the pumpkin field of about 1 bigha (0.14 acre approximately). The heights of trap placement were 2, 4, 6, 8, 10 and 12 feet respectively. Three traps were maintained at each level of heights, which constitute a replication. The sampling of melon flies were commenced during 15th to 19th April (summer) and 12th to 16th July (Rainy) of 2007 and 2008. The observations were recorded in every 24 hours for five consecutive days and the caught flies were removed and number of flies caught /trap/day was worked out.

Trap capture data were subjected to analysis of variance (ANOVA) by following appropriate statistical techniques using INDOSTAT package of statistical analysis.

3.11.4. Effect of depth of burying of infested fruit on fly eclosion:

To investigate the impact of burying fruits on adult fly eclosion, infested bitter gourd fruits were collected from the field and buried at 10, 20, 30, 40 and 50 cm. depths from soil surface 1 kg at each depth level. The buried fruits were carefully covered with mosquito net (3ft x 2ft x 3ft) so as to prevent escaping of adult fly in the environment. Adult flies after eclosion, caught in the net and counted. The experiment was replicated three times. Pupal harvest at each respective depth also recorded.

Melon fly visiting bitter gourd



Melon fly visiting pumpkin

Yellow cuelure trap

Orange cuelure trap

Transparent cuelure trap

Trapped male adults

Plate-3: Coloured traps evaluated in trapping melon fruit fly

3.12. Effect of bio-physical parameters of Bitter gourd cultivars on resistance to melon fruit fly:

Ten cultivars of bitter gourd including open pollinated, hybrids and local accessions were taken for the study. The test materials were planted on raised beds of 1.5 m width with a plant-to-plant spacing of 1.0 m during June 2007 (rainy season) and February 2008 (summer season). The experiment was replicated thrice and laid out in randomized block design (RBD). In each bed five plants were tagged randomly for recording observation. The rainy season crop fruited in August-September and the summer season crop during April-May. Recommended agronomic practices were adopted to raise the crop except chemical control of insect pests.

Fruits of marketable size were picked at 3 days intervals and brought to the laboratory for recording larval density per fruit and percent fruit infestation. Percent fruit infestation was calculated on number basis as follows:

$$\% \text{ fruit infestation} = \frac{\text{Number of fruits infested}}{\text{Total number of fruits observed}} \times 100$$

The infested fruits were cut open to count the number of maggots of melon fruit fly per fruit. Healthy fruits were used to observe morphological traits in the test cultivars. Observation on morphological traits of fruits was recorded from three randomly selected fruits from all the replicates. Vernier scale was used to measure length and diameter of the fruits, depth of ribs by scale, intensity of ribs (i.e. number of ribs/cm^2) determined by counting number of ribs per cm^2 area of fruit surface. Toughness of fruits was determined by using texture analyzer (Make: Stable Micro Systems, U. K.; Model: TA-XT plus).

3.13. Management of the key pests of cucurbit vegetables:

3.13.1. Soil application of different pesticides against melon fruit fly:

Maggots of the fly used for this experiment were recovered from infested pumpkin fruits collected from the field and kept in close vigil for 3-4 days till they attained jumping stage (i.e. late third instar). At this stage they became matured and ready to go for pupation. Soil was collected from the experimental fields from 0-15 cm depths from the surface. Before being placed in the containers, collected soil was

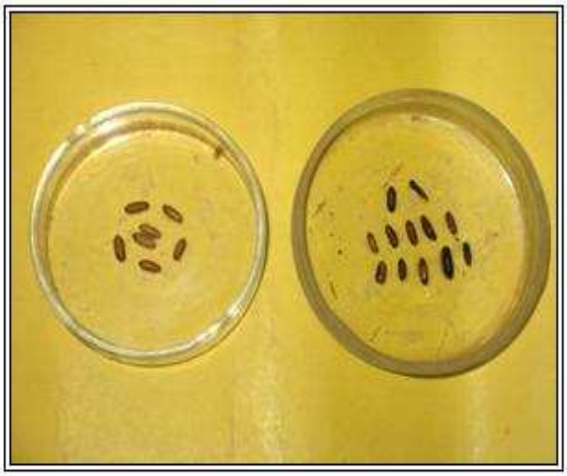
Mature maggots on treated soil

Kept for pupation

Harvesting of puparium

Separating puparium by seiving

Harvested puparium



Healthy and deformed puparium

Plate-4: Soil treatment with insecticides and their impact on melon fruit fly

sieved through a 5 x 5 mm sieve and then dried in an oven for 48 hours at 100^0C for sterilization as described by Hou *et al*. (2006). Texture of soil was analyzed as sandy loam. The pupation containers were made up of clear plastic, cylindrical in shape, 11 cm in height and having a top opening of 9 cm diameter. The top opening was covered and fastened with muslin cloth to prevent escaping of the larvae.

Insecticidal solution of cypermethrin, chlorpyriphos, DDVP, *Metarhizium*, formaldehyde, were prepared as per specified dosage mentioned in table and soil of each container treated with the help of an automiser. Neem cakes were grinded to make it dust, carbofuran 3G granules and bleaching powder applied in the soil of the container also as per the dosage specified in the table. In control, only tap water was applied by the automiser. Each treatment was replicated thrice. With a view to find out the residual toxicity of the toxic chemicals the third instar maggots at 'jumping stage' were released in the test container after 1, 3, 5 and 7 days of application of insecticides. In each treatment 20 mature maggots were released then covered with markin cloth and fastened with guarder.

After 2-3 days of larval release the soil was sieved with 350 mesh sieving tray under tap water. Dead larvae, pupa (both deformed and healthy) were collected and percentage of larval mortality, deformed pupa with respect to total larvae released was calculated. The healthy pupa were taken in a petridish and placed within a rearing cage for adult eclosion. The percentage of adult emergence was also worked out.

The data thus obtained were subjected to statistical analysis by appropriate techniques as per INDOSTAT and presented in the tables and figures.

3.13.2. Evaluation of indigenous bait materials in attracting melon fruit fly:

Some locally available fruit fly lures such as molasses, sugar, country liquor, fresh date palm juice and fermented date palm juice (locally known as '*taari'*) were tested as 'bait components' of the bait admixtures. Fifty gm molasses or sugar per 100 ml of water was added and the mixtures were stirred thoroughly whereas, twenty ml country liquor was used in 100 ml of water. In case of fresh and fermented date palm juice water was not added. Bait components thus prepared were utilized in preparing bait admixtures at the rate of 20 ml bait component + 0.5 ml spinosad 45 SC ('Tracer' manufactured by Dow Agrosciences) and were placed in small plastic containers (8 cm. diameters and 12 cm height).

Thus the treatments were as follows:

T_1: Mollasses + Spinosad

T_2: Sugar + Spinosad

T_3: Country liqour + Spinosad

T_4: Data palm juice + Spinosad

T_5: Date palm juice (fermented, locally known as "taari") + Spinosad

For each bait component, three traps were prepared which were taken as a replication. The traps in a replication were set at 10 m spacing in a row and each replication was 20 m away from one another. The experiment was conducted by following RBD. The observations on catch/trap were recorded daily at 06:00 hour, the numbers of flies trapped/trap were counted and the dead flies were removed after each counting.

Data, thus recorded were subjected to analysis of variance (ANOVA) by following appropriate statistical technique as per INDOSTAT package of statistical analysis.

3.13.3. Evaluation of suitable bait toxicant against melon fruit fly:

Fruit fly bucket traps were fabricated from plastic containers of 8 cm diameter and 12 cm height with four 2 cm hole diameter. Each of the bucket traps thus fabricated, were provided with a cotton wick (3 cm length and 1 cm diameter) soaked with 5% (a.i.) solution (vol:vol) of one of six liquid insecticides viz. cypermethrin 10 EC, chlorpyriphos 20 EC, spinosad 45 SC, malathion 50 EC, chlorpyriphos 50%+ cypermethrin 5%, dichlorvos 76 EC) and endosulfan 35EC. The traps were baited with a saw dust pellet impregnated with cuelure manufactured by Ganesh Bio-control System, Gujrat, India. For each bait toxicant, three traps were prepared which were considered as one replication. The traps in a replication were set at 10 m spacing in a row and each replication was 20 m away from one another. The observations on fly capture/trap were recorded daily at 06:00 hour, the number of flies trapped/trap counted and the dead flies, after counting were removed so as to avoid double counting.

3.13.4. Sanitary measures in managing melon fruit fly:

To investigate the impact of burying infested fruits in reducing adult fly eclosion, infested fruits were collected from the field and buried at 10, 20, 30, 40 and

50 cm. depths from soil surface respectively. The infested fruits were carefully covered with mosquito net (3ft X 2ft X 3ft) so as to prevent escaping of adult fly in the environment and kept for about two weeks. For bitter gourd 1 kg infested fruit and for pumpkin a whole infested fruit (2-4 kg weight) were used for each treatment and this was replicated thrice. After 10-12 days the adult flies started emerging which were caught in respective net and counted. In each case the pupal harvest at respective depths of burying (±5cm) were also recorded.

To determine the sanitation technique practiced with regard to fruit fly infested fruits (bitter gourd and pumpkin) the following treatments were compared:

T_1: Whole fruit on soil surface

T_2: Chopped fruits on soil surface

T_3: Chopped fruits treated with Spinosad 45 SC @1ml/2l of water

T_4: Chopped fruits treated with Spinosad 45 SC @1ml/2l of water

T_5: Whole fruits in polythene bag

The treatments were replicated four times. In each treatment the infested fruits were carefully covered with mosquito net (3ft X 2ft X 3ft) so as to prevent escaping of adult fly in the environment and kept for about two weeks. Edges of the net were also buried in soil to make it fly proof. Amount of infested fruits of bitter gourd was 1 kg and a whole infested fruit of pumpkin (2-4 kg weight) were used for each treatment. After 10-12 days the adult flies started emerging. The flies thus eclosed, caught in respective net and counted. In each case the pupal harvest at respective depths of burying (±5cm) were also recorded.

In case of pumpkin, the number of fly eclosed and pupal harvest were converted into number/kg infested fruit. The data thus recorded were subjected to ANOVA by using INDOSTAT package of statistical analysis and the treatments were compared with LSD (at 0.05).

3.13.5. Effect of *Metarhizium anisopliae* on different developmental stages of *Epilachna* beetle:

Effect of *Metarhizium anisopliae* on different developmental stages of *Epilachna vigintioctopunctata* (Fab.) and the adults of *Aulacophora foveicollis* (Lucas.) were evaluated. The experiments were conducted at the laboratory condition (temperature 30±2^{0}C and relative humidity 85±5%) during February to April of 2007-2008. The egg mass of epilachna beetle were collected from the cucurbit field and

reared in laboratory. A commercial preparation, **"**Stanes Biomagic", containing 1.15%WP *Metarhizium anisopliae* culture manufactured by T. Stanes and Company Ltd. was used as the source of green muscardine fungus.

Three doses viz. 4, 5, 6 gm per liter of water were sprayed topically with the help of automiser over the 1^{st}, 2^{nd}, 3^{rd} and 4^{th} instar grubs and 5-6 days old adult. The experiment was arranged in randomized block design (RBD) with three replications per treatment. For each replication 20 insects were used. The treated insects were transferred to petri dishes and fed with clean bitter gourd leaves.

The dead grubs/adults were counted and percentage mortality was calculated. The data thus obtained were then transformed by arc-sine transformation and statistically analysed by following standard statistical technique.

3.13.6. Integrated management of melon fruit fly:

The study was conducted at farmers' field of the district Cooch Behar, West Bengal during rainy season of the year 2009 on bitter gourd. There were eight treatments including control one, which were replicated thrice. Area of the plots was about 5 katha (360 sq m approx.). There were 24 plots in total and the plots were distributed in eight different villages keeping plot to plot distance not less than 100 m Each of the treatments was applied after flowering stage of the crop. The treatments were as follows:

T_1: Burying infested fruits

T_2: Bait spraying @ 15 days interval till final harvest (with mollasses and spinosad)

T_3: Soil treatment with chlorpyriphos20 EC @ 3ml/l at 15 days interval

T_4: Cuelure trap @ 12/ha

T_5: T_1+T_2

T_6: $T_1+T_2+T_3$

T_7: $T_1+T_2+T_3+T_4$

T_8: Control

The infested fruits as and when observed were buried in soil at more than 40 cm. depth. Bait spraying with molasses and spinosad was started after flowering of the crop and applied at fortnightly interval @ 20 spots /ha Soil treatment with chlorpyriphos and installation of cuelure trap were also undertaken after flowering. The observations on fruit infestation were recorded at fortnightly interval from each

treatment by counting healthy and infested fruits and percent infestation was derived. Data were subjected to statistical analysis under RBD design and the means were compared by LSD at 0.05 with INDOSTAT package of statistical analysis.