Materials and Methods
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All the field and laboratory experiments were conducted at Indian Agricultural Research Institute, New Delhi. The study was carried out during ruhi seasons of 2006-07 and 2007-08 selecting *Brassica oleracea* var. *botrytis* (L.) as main crop besides taking *Brassica oleracea* var. *capitata* (L.).

**General geographical and meteorological information of experimental site:**

The Indian Agricultural Research Institute is situated at 28° 35′ N latitude, 77° 12′ E longitude and at an altitude of about 227 m above the mean sea level of Arabian Sea. The climate is semi arid and sub-tropical type with extremes of weather conditions. Summers are hot and dry, May and June being hottest months with the maximum temperature ranging from 41 °C to 45 °C. Winters are severe with minimum temperature varying between 4 °C to 7 °C during January. The mean annual rainfall is around 740 mm, nearly 80% of which is received during July and September. The winter rains also common but their intensity, amounts and duration are uncertain. The mean maximum evaporation nature ranged between 12.4 to 16.8 mm during May - June and minimum between 2.2 to 2.3 during December-January. The mean wind speed varies from 0.7 to 16.1 km/hr during October to April.

During experiment period, the weekly mean meteorological data on maximum and minimum temperature, relative humidity (%), rainfall (mm), sunshine period and wind speed were obtained from the meteorological observatory of Nuclear Research Laboratory (NRL) of the Institute, New Delhi.

**Experimental details:**

**3.1. Determination of status of insect pests of cole crops, their natural enemies and current management practices:**

For determination of current status on the insect pests of cole crops, the study was conducted during 2006-08, in and around Delhi region. Four places viz., Najafgarh, Jarudankalan-I, Jarudakalan-II and Teekrikalan were selected within the surroundings of 10-15 km from IARI. In these places cauliflower occupies most of the cultivable fields as
the main crop during August to April. In each place, four plots measuring 10 m x 5 m were selected randomly and the data were collected at weekly interval till the harvest of crop between 8 am. Field scouts were conducted weekly between 8 am to 11:30 am. Observations were taken from five tagged plants replicated thrice per plots of each region. Besides this, interviews were also conducted every week with at least four farmers, to know about the ongoing plant protection measures and other agricultural practices.

3.2. Determination of seasonal incidence and distribution pattern of insect pest and their natural enemies:

The plots intended for collection of data on the incidence of pest and its parasitoid were kept free from insecticidal sprays. The larval counts of *P. xylostella* (L.) were recorded from twenty randomly selected plants each time in each cropping season. 4 total of 15 counts were taken during the crop growth period. In addition to this count, the braconid parasitoid *Cotesia plutellae* (Kurd.) cocoons on DBM were also recorded.

3.2.1. Spatial distribution of insect pests:

The pesticide free cauliflower crop was raised in an area of 5 m x 4m replicated four times during both the years. Twenty plants were randomly selected and then tagged for observations on major insect pest and their natural enemies. The observations were taken at weekly interval throughout the cropping season. The counts were first summarized in a frequency distribution. Initially the figures were placed in numerical order and then grouped into frequency classes. Number of plants that fall into a class was the class frequency (f). This way, the frequency simply recorded the number of sampling units (plants) containing the same number of insects.

The formula for the mean (X) of the sample containing ‘n’ sampling units can be applied as under calculated below

\[
X = \frac{\sum fx}{n}
\]
The variance ($S^2$) of the sample is given by

$$S^2 = \frac{\sum (fx^2) - [\sum (fx)^2 / n]}{n-1}$$

The study on the pattern of distribution of pest population was accomplished following statistical techniques:

**a. Variance-mean ratio ($S^2/x$):**

Mean ($X$) and variance ($S^2$) were worked out for each set of observations following usual statistical procedures. The distribution pattern was indicated random when $X = S^2$, aggregated if $X < S^2$ and regular if $X > S^2$.

**b. Negative binomial distribution (NBD):**

The variance of the population is usually greater than the mean ($S^2 > X$) and the population is clumped or aggregated. Negative binomial distribution is often suitable model for such situations (Bliss and Fisher, 1953).

The negative binomial is the mathematical counterpart of positive binomial and therefore, the probability series is given by expansion of $(q-p)^K$, where $p = m/k$ and $q = 1 + P$. The parameters of this distribution are mean $X$ and exponent $K$. The reciprocal of exponent “$K$” i.e. $1/K$ is a measure of the excess variance or clumping of the individuals in a population. As $1/K$ approaches 0 and $K$ approaches infinity, the distribution converges to the Poisson series ($S^2 = X$). Conversely, if clumping increases $1/K$ approaches infinity, $K$ approaches zero, the distribution converges to the logarithmic series (Fisher et al., 1943).

The exponent ($K$) which is also known as the dispersion parameter was calculated by the formula

$$K = \frac{X^2}{S^2 - X}$$
The values of K below 8 indicate negative binomial or aggregated distribution (Southwood, 1978).

c. **David and Moore’s index \( \frac{S^2}{X - 1} \):**

It is worked out by the equation \( \frac{S^2}{X - 1} \) when this value equals zero, the distribution refers to random, if less than zero it is regular and if it is more than zero then distribution is supposed to be contagious.

\[
\text{Index of Lexis} = \frac{S^2}{X}
\]

In case of random distribution, the value of this index is unity and the departure towards either indicates regularity or contagious nature, respectively.

d. **Charlier’s coefficient:**

\[
\text{Coefficient} = \frac{100 \frac{S^2}{X} - X}{X}
\]

In case of regular distribution the value of this coefficient will be imaginary, whereas at zero coefficients the distribution is said to be random and if it is significantly more than zero, then it refers to contagious nature.

e. **Lloyd index of mean crowding:**

\[
X^* = X + \left( \frac{S^2}{X} - 1 \right)
\]

Lloyd (1967) developed an index of mean crowding (X). The ratio of mean crowding to mean density is a suitable measure of patchiness. When mean crowding is regressed against mean density, the value of slope is more than one in case of contagious distributions at higher densities.

f. **Lloyd patchiness index:**

If the ratio of mean crowding (X) to mean \( X_1 \) was compared \( \frac{X}{X_1} = 1 + \frac{1}{K} \)

It can be seen that the reciprocal of K is the proportion by which mean crowding exceeds mean density.
Patchiness index describes how many times crowded individual is on the average, as it would be if the same population had a random distribution. The values of patchiness index < 1 indicate an aggregated distribution, if patchiness = 1 a random distribution and patchiness < 1 a regular distribution.

3.3 The details of field experiments:

The seedlings of Brassica oleracea var. botrytis (Cauliflower) variety hybrid suttind number SCH-3333 were transplanted on November 7 in rabi season 2006-07 and 2007-08. The field experimental statistical design was Randomized Block Design, each having plot size of 5 x 4 m (20 m²) with four replications. The plot to plot distance was kept as 0.5 m and 1 m between the blocks. Irrigation channels of 1.5 m wide were provided along the replication blocks. The total numbers of plots were 40 in 4 blocks thus comprising 10 plots in each block, all the plots under study were accommodated in a total area of 1045 m². Each plot had 6 rows, having row-to-row distance of 60 cm apart.

3.3.1 Efficacy of Bt var. aizawai and new insecticide on laboratory reared population of diamondback moth under natural condition:

The experimental field was exposed to artificial and natural infestation. Every ten plants of each replication were covered by mosquito nylon net (60 mesh) 2 x 3 x 2 m size to prevent the natural infestation. One strip of 5 cm x 1 cm Paraffin wax paper containing approx 20 eggs was stapled on each of randomly selected five plants. Nine insecticides were tested by single spray for their efficacy against lab population of P. xylostella during the rabi seasons of 2006-07. There were ten treatments which included Bt. var. aizawai @500 g, 1000 g, and 1500 g, August –5 @800 g, Indoxacarb 14.5 SC @ 50 g a.i. Spinosad 45 SC @ 50 g a.i. Fipronil 5 SC @ 75 g a.i. Cypermethrin 25 EC @ 60 g a.i, Micocyn-505 @ 450 g a.i and control (unsprayed). The details of treatments have been presented in table (23).

3.3.2. Comparison of simulated Bt-transgenic cauliflower with current insect pest management 2006-07 and 2007-08.
Three treatments comprising *Bt. var. aizawai* in different concentration viz., 500 gm (Model-I), 1000 gm (Model-II) and 1500 gm (Model-III) were sprayed five times at weekly interval to mimic *Bt.* transgenic cauliflower. The other treatments included August-5 @800 g (*Bt*-isolate), Indoxacarb 14.5 SC @ 50 g a.i. Spinosad 45 SC @ 50 g a.i. Fipronil 5 SC @ 75 g a.i. Cypermethrin 25 EC @ 60 g a.i. Mirecyn-505 (Chlorpyryphos 50 % + Cypermethrin 5 %) @ 450 g a.i and control (unsprayed).

a. Observations:

In both the seasons of year 2006-07 and 2007-08, the population counts of insect pests and their natural enemies were recorded on five randomly selected plants in each plot replicated four times, one day before spraying thus forming the pre-treatment counts subsequently. The treatment counts were taken after one, three, five, seven and fifteen days of each spray. The per cent reduction of pest population over control was calculated using modified Abott’s formula (Abbott, 1925) as by proposed Fleming and Retnakaran. (1985). To record yield data, the mature cauliflower were harvested from individual plots and weighed separately. The yield obtained in the net area of each treatment was recorded.

The formula for calculating per cent population reduction was applied as under

\[
\text{Percentage of population reduction} = \left(1 - \frac{\text{Post-treatment population in treatment}}{\text{Pre-treatment population in control}} \right) \times 100
\]

b. Agronomic practices

The experimental field was disc ploughed twice and leveled before transplanting of seedlings. The recommended fertilizer dose of (120 kg N, 80 kg P$_2$O$_5$ and 40 K$_2$O kg/ha) was applied in the form of Urea, Single Super Phosphate and Mureate of Potash respectively. Half the dose of Nitrogen, entire dose of Phosphorus and Potash were applied as basal dose and the remaining nitrogen was applied a month after planting. Seedling was planted with a spacing of 60 x 40 cm at the rate of one seedling per hill.
Four days after planting, gap filling was done. The plots were irrigated usually at 10-15 days interval. Hand weeding was done periodically to keep the field free from weeds.

c. Insecticidal requirement:

*Bt* var. *aizawai* and *Bt* -isolates (August-5, Almora) used in experiments were available as wettable powders while other insecticides were available in liquid form. For *Bt*-isolates, 0.1% triton-x was used and for remaining insecticides water was directly added to them to get desired concentration. The insecticidal requirement per liter of water was calculated using the formula given below:

\[
\text{Amount of insecticides used/ lit of water} = \frac{\text{Concentration required}}{\text{Per cent active ingredient}} \times 1000
\]

d. Timing and method of application:

In all treatments, spray was given at the time when larval population of DBM reached 9-12 larvae/plant under natural condition and 6-9 larvae/plant under protected condition, the pesticidal application was initiated using hydraulic knapsack sprayer in 500 liter water per hectare.

e. Statistical analysis: Field data

The data obtained on number of larvae/ plant was transformed to per cent reduction using Abbott’s formula then was subjected to statistical analysis employing Analysis of Variance (ANOVA) for comparison of treatment means as described by Snedecor and Cochran (1968) and Panse & Sukhatme (1978). Data recorded in per cent figures were transformed to *arc sine* values before subjecting it to statistical analysis. While studying the influence of abiotic factors on the incidence of DBM (*P. xylostella*) throughout the cropping season, correlation-matrix and multiple regression analysis were employed to observe the individual as well as cumulative effects of different weather factors viz., maximum and minimum temperatures (°C), relative humidity (%) during
morning hours (0700 hr) and afternoon (1400 hr), rainfall (mm), wind speed (km/hr) and sunshine period (hr/day).

3.4 Screening of Bt-cauliflower and Bt-cabbage germplasm against *P. xylostella* and *H. armigera*:

Sixty nine different germplasm of *Bt* cauliflower and cabbage were grown at greenhouse owned by Nunhems India Pvt. Ltd., Bilaspur, Gurgaon.

The experiment was conducted in a Completely Randomized Block Design with three replications. Thirty-day old seedlings with spacing of 60 x40 cm were transplanted during September, 2006. The cultural practices except plant protection measure were followed as per the crop production recommendation for horticultural crops (Anonymous. 1998).

When plants became 4-5 weeks old. about 30 eggs from the susceptible colony were placed on to each plant and after 6 days the per cent damage on leaves by larvae were assessed on the basis of grades and ratings as per given by screening of *Bt* cauliflower and cabbage germplasms against *P. xylostella* and *H. armigera* by Mishra *et al.* (1988).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Designation</th>
<th>Percent leaf damage score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Immune</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Highly resistant</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Moderately resistant</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Tolerant</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>Susceptible</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Highly susceptible</td>
<td>5</td>
</tr>
</tbody>
</table>

All the *Bt* germplasm were also for screened for *Helicoverpa armigera* through leaf bio-assay. Procedure described by Tabasmith and Cushing (1987). Leaf discs of 6 cm diameter were cut covering either side of midrib from 4-5 week old germplasm maintained in greenhouse. Leaf disc were placed slantingly so that the larvae could move and feed on either side. Ten neonates were released on each disc and container was covered with cap.
3.5. Laboratory studies:

3.5.1 Insect culture:

Field collected larvae of DBM were reared on cauliflower leaves at 27 ± 2 °C, 60 % R.H. with a photoperiod of 14:10 (LD). Aligarh and Hyderabad strain were maintained in the laboratory, without exposure to any Xenobiotics. Larvae of the field population used in bioassays were mostly F2 and F3 generation. Adequate precautions were taken to minimize the spread of viral disease in the culture through sterilization viz., by keeping all glassware in laminar flow chamber ten 30 minutes and cleaning with 70% alcohol. Pupae from the culture were placed in a plastic jars which were lined with filter paper. Adults were provided cotton swab of 10% honey solution enriched with one vitamin “E” capsule and 1% multi vitamin solution prepared in 100 ml of (10%) honey solution as food. Newly emerged adults at the sex ratio of 1:1 were kept in mating plastic jar and covered with muslin cloth. Fresh cauliflower leaves (for oviposition) and honey solution was regularly given to the adults. The top of jar was covered with a muslin cloth to avoid the escape of adults. Container was kept in a dark place to induce mating and egg laying. Oviposited leaves were taken out and replaced with fresh leaves.

3.5.2 Bioassays:

Cauliflower leaf disc method of bioassay was carried out in accordance with the description made by Tabashnik et al. (1987). Initially cauliflower leaves were washed with distilled water containing 0.1% Triton X-100, and then dried for about 1 hour. Cauliflower disc (5 cm diameter) were cut with a metal punch and then dipped in a test solution prepared with distilled water containing 0.1% Triton X-100 to facilitate uniform treatment with successive concentrations viz., 0.01, 0.1, 1.0, 2.0 and 2.5 ppm each. The leaves were dipped for 30 seconds in respective concentration of insecticides and leaf disc were placed slanting to dry the test solution for about 2 hour at room temperature. Then, two day old second instar larvae were released on each disc in an individual petriplate. At least 10 serial dilutions of each insecticide were used with 5 concentrations. Larvae were allowed to feed on the treated leaf disc for 72 hour at 28 °C before being checked for mortality against control. Bioassay with more than 10 per cent mortality in the control and heterogeneity on the basis of Chi-square test of significance were
discarded. The larval mortality, observed after 24, 48 and 72 hr of treatment, was corrected for control mortality with Abbott’s formula.

### 3.6 Bioassay procedure for Insect Growth Regulator:

Cauliflower leaves from the culture field were thoroughly washed and dried under shade for removing excess moisture. The leaves were then dipped for 30 seconds in respective concentration of insecticides and subsequently excess fluid was drained off. Third instars larvae were released on treated and untreated dry leaves in each of petriplates. The treatments were replicated thrice having 10 larvae per replication. Mortality was recorded after 24, 48-and 72 h of exposure. In order to calculate the LC$_{50}$ and EC$_{50}$ (Effective concentration for 50% cumulative pupal mortality after 48 hrs of feeding), the larvae treated with novaluron were transferred to fresh cauliflower leaves regularly. The observation on larval, prepupal and pupal mortality and malformations if any were recorded. LC$_{50}$ and EC$_{50}$ values were estimated by subjecting the data to probit analysis (Finney, 1971) using Maximum Liklywood Program (MLP) software. Control mortality less than 10% was corrected using Abbott’s formula.

### 3.7 Residue analysis:

#### 3.7.1 Sampling:

Samples of five curds were collected from each treated plot at different time interval viz., 5, 8 and 15 days after each spray. The samples from different replications were chopped and mixed thoroughly and a representative sample of 50 g was used for residue estimation.

#### 3.7.2 Determination of insecticidal residues:

Residues in/on cauliflower curds were determined at different intervals replicated twice. Residues of indoxacarb, mycocin (cypermethrin and chlorpyriphos), cypermethrin and fipronil were estimated by Gas liquid chromatography (GLC), whereas the residues of spinosad and imidacloprid were estimated by High Pressure Liquid chromatography (HPLC).
3.7.3 Preparation of Standards:

Stock solution of 1000 µg/ml of indoxacarb, chlorpyriphos, cypermethrin and fipronil were prepared in acetone and from the respective reference standards 99 % purity. Further dilutions were made in hexane. Standard solution of imidacloprid and spinosad were prepared in acetonitrile and further dilutions were also carried out with acetonitrile. The stock solutions were serially diluted to lower concentrations.

3.7.4 Sample processing for indoxacarb, mycocin (cypermethrin+chlorpyriphos), fipronil and cypermethrin:

**Year: 2006-07**

The seedling of hybrid variety (suttind no. SCH-3333) of *Brassica oleracea* var. *botrytis* (Cauliflower) was transplanted on November 7 in *rabi* season 2006-07 using Randomized Block Design, with each plot having size of 5 x 4 m with four replications. The plot to plot distance was kept as 0.5 m and 1 m between the blocks, irrigation channels of 1.5 m wide were provided along the replication blocks. The total number of plots was 40 in 4 blocks with 10 plots in each block; all the plots were accommodated in a total area of 1045 square meter. Each plot had 6 rows 60 cm of row to row distance. Over all, there were ten treatment viz., *Bt* var. *aizawai* @500 g, 1000 g, and 1500 g, august-5 @ 800 g, indoxacarb 14.5 SC @ 50 g ai/ha, spinosad 45 SC @ 50 g ai/ha, fipronil 5 SC @ 75 g ai/ha, cypermethrin 25 EC @ 60 g ai/ha, micocyn-505 @ 450 g ai/ha and control (unsprayed). The details of treatments are given Table 23.

**Year: 2007-08:**

During 2007-08, some treatments were altered from 2006-07 experiment due to heavy aphid infestation which is also considered as a major pest after DBM. Keeping this in view, additional treatments were taken to manage aphid population. Imidaclorpid, dimethoate, almora (*Bt*-isolate) were used as a replacement of *Bt* var. *aizawai* @500 g ai, micocyn and cypermethrin, besides these new treatments, indoxacarb, spinosad, august-5 (*Bt*-isolates), fipronil, model-I (*Bt* var. *aizawai*-1000 g), Model-II (*Bt* var. *aizawai*-1500 g ai) followed by control.

**Details on pesticide application:**
<table>
<thead>
<tr>
<th>Treatments</th>
<th>Name of insecticides and combinations</th>
<th>Trade name</th>
<th>Quantity of spray fluid used in g ai/ha</th>
<th>Sampling days</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006-07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>Spinosad</td>
<td>Tracer</td>
<td>50</td>
<td>5, 8 and 15</td>
</tr>
<tr>
<td>T3</td>
<td>Indoxacarb</td>
<td>Avaunt</td>
<td>50</td>
<td>5, 8 and 15</td>
</tr>
<tr>
<td>T4</td>
<td>Mycocin (Chlorpyriphos 50%+Cypermethrin 5%)</td>
<td>Mycocin</td>
<td>450</td>
<td>5, 8 and 15</td>
</tr>
<tr>
<td>T5</td>
<td>Cypermethrin</td>
<td>Colt</td>
<td>60</td>
<td>5, 8 and 15</td>
</tr>
<tr>
<td>T6</td>
<td>Fipronil</td>
<td>Regent</td>
<td>75</td>
<td>5, 8 and 15</td>
</tr>
<tr>
<td>T7</td>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2007-08</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>Spinosad</td>
<td>Tracer</td>
<td>50</td>
<td>5, 8 and 15</td>
</tr>
<tr>
<td>T2</td>
<td>Indoxacarb</td>
<td>Avaunt</td>
<td>50</td>
<td>5, 8 and 15</td>
</tr>
<tr>
<td>T3</td>
<td>Mycocin (Chlorpyriphos 50%+Cypermethrin 5%)</td>
<td>Mycocin</td>
<td>450</td>
<td>5, 8 and 15</td>
</tr>
<tr>
<td>T4</td>
<td>Cypermethrin</td>
<td>Colt</td>
<td>60</td>
<td>5, 8 and 15</td>
</tr>
<tr>
<td>T5</td>
<td>Fipronil</td>
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<td>75</td>
<td>5, 8 and 15</td>
</tr>
<tr>
<td>T6</td>
<td>Dimethoate</td>
<td>Rogar</td>
<td>187</td>
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<tr>
<td>T7</td>
<td>Imidacloprid</td>
<td>Confidor</td>
<td>25</td>
<td>5, 8 and 15</td>
</tr>
<tr>
<td>T7</td>
<td>Untreated control</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Seedling root dip treatment**

| T1         | Imidacloprid                         | Confidor   | 0.01%                                    | 30 DAT        |

3.7.5 Sample processing:

3.7.5.1 Indoxacarb, Mycocin (Cypermethrin and Chlorpyriphos), fipronil, and cypermethrin:

a. Extraction and clean up:

The cauliflower curd was cut into small pieces and representative 50g sample in duplicate was drawn. The sample was transferred to the jar of the mixer grinder and 100 ml distilled acetone was added. Further, it was macerated for three minutes and filtered through whatman filter paper No.1 using buchner funnel. The solid residue was transferred back to the jar and process of extraction was repeated twice using fresh 50 ml Acetone. The acetone extracts were combined and concentrated. The extract was quantitatively transferred to separatory funnel, diluted with 100 ml (10%) aqueous sodium chloride solution and partitioned thrice with dichloromethane (50 ml each).
dichloromethane phases were combined, passed through anhydrous sodium sulfate and concentrated to 1-2 ml using rotary evaporator.

The extracts were cleaned by Adsorption Column Chromatography using silica gel as absorbent. Cotton wool was plugged at the bottom and 50 ml hexane was added and then 10 g sodium sulphate, which was allowed to settle down. This was followed by the addition of silica gel (10 g) and anhydrous sodium sulfate at the top. The column was washed with 70 ml distilled hexane. The dichloromethane extract was concentrated to dryness, residue dissolved in 1-2 ml hexane and loaded on the column using small portions of hexane. For cypermethrin, spinosad, indoxacarb and mycocin the column was eluted with a mixture of hexane and acetone (9:1), while fipronil was eluted with a mixture of hexane and acetone (4:1).

b. Imidacloprid and Spinosad:

For this cauliflower curds were cut into small pieces and from 50g representative sample was taken into beakers. The samples were quantitatively transferred in to blender jar along with addition of 100ml acetone. The contents were macerated for 3 min and filtered through Buchner funnel. The solid residue was transferred back to the jar and extraction was repeated two more times with fresh 50 ml acetone. The extracts were concentrated to remove acetone. The remaining aqueous phase was diluted to about 200 ml by adding 5% aqueous sodium chloride solution and then made acidic by adding 2 ml concentrated hydrochloric acid. The acidic aqueous phase was portioned thrice with 50 ml n-hexane and the hexane phases were got discarded. The aqueous extract was then made alkaline by adding sodium hydroxide solution. The residues of imidacloprid were extracted by partitioning with dichloromethane (3 x 50 ml). The dichloromethane extracts were passed through anhydrous sodium sulphate and concentrated to dryness. The samples were passed through a short column of neutral alumina before their estimation. The residues were dissolved in 5 ml HPLC grade acetonitrile for HPLC analysis.

b. Estimation of residues:

c. Gas liquid chromatography:-

Residue of indoxacarb, cypermethrin, fipronil and mycocin (cypermethrin and chlorpyrifos) were analyzed on 5890 series II Gas Liquid Chromatograph (GLC)
equipped with electron capture detector using CP Sil 5 CB (25 m x 0.25 mm x 0.25 μm) column. The operating conditions were; Temperature detector-A 280 °C, Injector A-260 °C, Detector B-280 °C and Injector B-260 °C. The Column was programmed as 200 °C - 8 min increased @ 15 min to 280 °C. The carrier gas was highly purified nitrogen at a flow rate of 2 ml/minute. Under these conditions, the retention time of cypermethrin was 7.93 minute, fipronil 4.3 minute, cypermethrin-11 minute and chlorpyriphos 10.79 minute.

d. High pressure liquid chromatography:

Estimation of spinosad and imidacloripid residue was done through HPLC equipment.

e. Operational conditions

Mobile phase : Acetonitrile and water (70:30 v/v)
Flow rate : 0.5 ml/minute
Detector wavelength : 250 nm

f. Calculation of residue estimated in GLC and HPLC:

The quantitative and qualitative analyses were done by comparing the retention time and peak area points of the sample response with that of corresponding standards of 1 ppm of insecticide and 20 ppm of spinosad and imidacloripid. The calculation for residue estimation was done as under:

\[
\text{Residues} = \frac{\text{Area of the sample} \times \text{Standard injected (μg/ml)} \times \text{Total volume (ml)} \times \text{Standard volume (μl)} \times \text{Recovery factor}}{\text{Area of the standard} \times \text{Sample volume injected (μl)} \times \text{Sample weight (gm)}}
\]

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