CHAPTER -II

MATERIAL AND METHODS

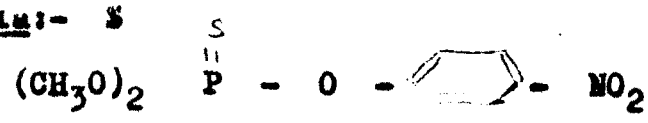
II -o- MATERIALS AND METHODS -o-

(A) About the pesticides used in the present investigation :-

(1) Metacid - 50 (Methyl parathion)

Chemical name :- O, O - dimethyl O - (P-nitrophenyl)

Structural formula:-



Empherical Name:- $C_8 H_{10} NO_5 P_5$

Molecular Weight:- 263.2

Solubility :- 55 to 60 ppm in water at 25°C, readily soluable in most organic solvents, less soluble in petroleum ether and mineral oils.

Stability:- Relatively stable at Ph 1to7 undergoes fast decomposition at Ph 8to9.

Formulations:- Metacid -50 (50% M/W emulsifibre concentrate of methyl parathion).

Biological properties:- Metacid is an insecticide with a very broad spectrum of activity. It kills nearly all sucking and biting pests. Metacid acts as a contact, stomach and breathing poison. It is notable for its fast killing action. As the active ingradients penetrates into the plant tissue, it controls both concealed and mining pests. Metacid displays good plant tolerance and therefore can be used in nearly all crops.

Uses and recommendation :- Metacid is widely used to control aphids, jassids, thripe, mites, leat rollers, Cut worms and crickets on crops like cotton, pome, grapes, citrus fruits and vegetables.

Toxicity :- Oral toxicity- LD50 male rats - 14.0 Mg/Kg
Dermal toxicity - LD50 male and female rats - 67.0 Mg/Kg
(In xylene) active ingradient applied to dorsal skin not removed.

Inhalation toxicity :- LD50 male rats 200 Mg/m^3
1 nour exposure.

Antidate :- Two Mg of atropine sulpnate by intravenus route.

The above mentioned organophosphorus insecticide is used to study their effect on physiology of oil seed plants like safflower (Carthamus tinctorius). This organophosphorus insecticide is widely used as an insecticide as a spray and the residual effect of this pesticide is known to remain in the environment for a long time. (Deshpande and Swamy, 1987).

(B) The plant used for the Experiment is Carthamus tinctorius (safflower), seeds are used for the experiment obtained from Kharedi Vikari Sangh, Satara.

(C) Procedure :-

The seeds of carthamus tinctorius are sown in the earthen pots. The pots are filled with soil mixed with fertilizer in the proportion 3:1 well decomposed farm yard manure is used. Then in this pot seeds are sowned. Then every alternate day the pots watered. It has been observed that after 2 month the plants get infested by aphids. The aphids are black to dark brown in colour. Aphids form colonies on the plants. After a week of full infestation, the healthy and infested plants are analysed for the organic constituents like chlorophyll content, Nitrogen content, carbohydrate content, polyphenol content and growth parameter like, leaf area, leaf moisture, plant height, and Biomass etc. Then by random sampling method the healthy and infested leaves are taken cleaned well and dried in an oven at 80°C till to obtain the constant weight. This oven dried material is used for the analysis of inorganic constituents such as Na, K, Ca, Mg, Mn, Cu, Fe, Zn, Cl etc. The plant which are infested by apnids are sprayed with organophosphorus insecticidse. Such as methyl parathion in such a way that 1st pot sprayed with higher concentration of insecticide 2nd pot with recommended dose and 3rd pot with below recommended dose and the concentrations used for this are 0.1%, 0.05%,0.025% respectively. After 10 days of treatment, the plants are analysed as above for growth parameters organic and inorganic constituents. The results are compared as the effect of insecticides on growth parameters, organic and inorganic constituents is treated and control plants.

(D) Growth Parameters-

1) Leaf area :- Take atleast 10 leaves and measure the leaf length and leaf breadth and calculate the leaf area by using

the formula:-

Formula for leaf area = L x B x f

Where, L = Leaf Length, B = Leaf Breadth

and f = Factor.

2) Height :- Measure the height of the plants which are sprayed with insecticide and before sprayed insecticide.

3) Biomass :- Take the plant and take the weight of the plant. This weight of the plants sprayed insecticide and before sprayed insecticide is the Biomass. Biomass can calculated by using change weight method after drying the plant.

4) Leat moisture :- Take the leaves of the insecticide sprayed plant and before sprayed insecticide plant. Then take the weight of these leaves and record it. This is fresh weight and these leaves are taken in the oven after dried therd leaves again take the weight. This is dry weight of the leaves.

Formula :- Leat moisture = Fresh weight - dry weight.

5) Chlorophyll :- The chlorophylls were estimated by method of Arnon (1949).

(a) Extraction :- Take 0.5 gm of fresh plant material was homogenised in morter with pestle along with a pinch of $MgCO_3$ ($MgCO_3$ is added to perfect mg in the nucleams of chlorophyll molecule). Chlorophylls were extracted in about 40ml 80% acetone. The extract was filtered through what mann No.1 filter paper using buckness funnel under sucession (suction). The residue on filter paper was thoroughly washed twice with 15 of aliquotes of (small amount) 80% acetone. Collecting the washing and the filtrate together in the same flask volume of the chlorophyll extract was recorded (100 ml.)

(b) Reading Absorbance for chlorophyll :-

Absorbance of chlorophyll extract was read on 663nm and 645nm on the spectrophotometer using 80% acetone as blank. From these absorbance readings at using the formulas chl. a, b calculated.

Chl. 'a' = 'x' = 12.7 A663 - 2.69 A645

Chl. 'b' = 'y' = 22.9 A645 - 4.68 A663

Total Chl. (a+b) = 'z' = 8.02 A663 + 20.2 A645

$$\text{Chl\% } 100 = \frac{X/Y/Z \times \text{Vol. of ext.} \times 100}{1000 \times \text{wt. of plant material.}}$$

or total Chl. in mg/100-1 g.

(6) Nitrogen :- For the estimation of nitrogen Nessler's method given by Hawk et al is used.

(A) Digestion of plant material :- 0.5 gms. of the leaves are cut into small pieces. After washing and drying the plant material, these pieces are transfered to 300 ml kjeldahl flask. 5ml of H_2SO_4 (1:1) and a pinch or microsalt is added to the flask. Microsalt acts as a catalyst during the digestion of plant material, to avoid bumping 3-4 small glass pieces are also added to the flask. The flask is heated on a low flame. Till the material dissolved completely and then on a strong flame heating is continoused. Till the extract changes from brown black to yellow and finally to a colourless extract. Then the flask is cooled to room temperature and the contents are transferred to a 100ml with volumetric flask kjeldahl flask is rinse 3-4 time with

D.W. Then the extract is filtered through whatmann No.1 filter paper and this filtrate used for the estimation of Nitrogen.

(b) Part II :- Estimation of Nitrogen :- Estimation of Nitrogen is carried out in Nessler's tube which are arranged.

(7) Estimation of polyphenols from Healthy and infected plant material.

(a) Proportion of plant extract :- Take 2gms. of Healthy and infected plant material and crush it in 80% acetone by using morter and pestle, filter the extract through whatmann No.1 filter paper by using Buckner funnel. Collect the filtrate and measure the volume (adjust the volume to 100 ml with 80% acetone)

(b) Estimation :- Take 2ml. plant extract in Nessler's tube and to it 10ml 20% Na_2So_3. The Adjust the volume 35ml mark with D.W. Then add it to 2ml folin Dennis reagent and make the final volume 50ml mark with D.W. Shake the tubes throughly well to get uniform colour. Prepare standards by using 1ml, 2ml, 3ml, 4ml tannic acid and follow the above procedure for the development of the colour. Blank contains all ingradients except std. tannic acid. Measure the absorbance at 660nm on spectrophotometer against Blank. Calculate the polyphenols by using standard curve of tannic acid and substituting the values in the formula given as below.

$$\text{Polyphenols in \%} = \frac{0.1 \times \text{graph value} \times \text{Total vol. of extract} \times 100}{\text{Wt. of plant material} \times \text{extract used} \times 1000}$$

(8) Estimation of carbohydrates (Starch).

(a) Extraction :- 2 gms of fresh and mature green leaves of the plant and also infected leaves crushed in 80% ethanol with a pinch of acid free sand. The extract was filtered through Buckner's funnel using whatmann No.1 filter paper. The residue on the paper was washed 3 to 4 times with 80% alcohol. This residue was transfered to a conical flask containing 50ml distilled water and 5ml conc. HCl. The flask was stoppered by using a cotton plug and was hydrolised at 15lb pressure for about half an hour. After cooling the flask the content or the extract was neutralised by using sodium carbonate. Then filtered through whatmann no.1 filter paper.the filter paper washed once with distilled water and the volume of the filtrate was measured. This filtrate was used for the estimation of strach.

(b) Estimation of Strach :- A series of test tube was prepared as given in the observation table. First tube is blank containing no sugar solution. While the next 5 tubes contain standard glucose in various quantities (0.1, 0.2, 0.3, 0.4, 0.5 ml) Last 2 tubes were having filtrate prepared for the starch estimation and the tubes were boiled on watner bath for 10 min. Then 1ml. nelson's Arsenomolybdate reagent was added to each tube and the final volume was adjusted to 10ml with D.W. The blue colour developed was measured by taking absorbance at 560nm on a colorimeter. A graph of absorbance Vs std. glucose concentration

was drawn using absorbance reading for glucose from the graph. The ml of glucose or sugar present in the filtrate taken for estimation was calculated. From this the amount of sugar i.e. starch present in the total volume of the filtrate and then the percentage of starch was calculated.

Estimation of reducing sugars from the plants:-

Procedure :- Preparation of plant extract :-

2gms of green and matured leaves and infested leaves of safflower were crushed in 80% ethanol. Extract was filtered through Buckner's funnel using whatmann No.1 filter paper. The residue on the filter paper was washed 3 to 4 times with 80% alcohol. The filtrate was collected and kept for condensation on a waterbath. After condensing to 3 to 5 ml the evaporating dish was removed. About 4gms of a mixture of potassium oxalate and lead acetate (1:1) were added to the filtrate. It was mixed thoroughly and about 50ml destilled water were added to the mixture. Then it was filtered through whatmann No.1 filter paper. The filter paper was washed with alcohol and volume of the filtrate was recorded. This filtrate is used for the estimation of reducing sugars.

Estimation of Sugars :- In 10ml marked test tube standard glucose is taken in different amounts. In two such tubes plant extract is taken 1ml alkaline cu-tartarate is added to all the tubes along with a blank tube containing no sugar solution. The tubes are boiled in a water bath for 10 minutes. After cooling tubes to room temperature, 1ml Nelson's Arsenomolybdate

reagent is added to each tube. The volume of each tube is adjusted to 10ml with distilled water, the dark blue colour developed. Then take the reading at absorbance 560nm on a colorimeter. A graph of standard solution Vs absorbance at 560nm is plotted. From this graph the ml of glucose present in the plant extract is calculated by using the extract reading.

(8) Inorganic Constituents:-

I) Preparation of acid digest (Extract):- The leaf material of random sampling was taken, cleaned well in distilled water and dried at 80°C in an oven till constant weight obtained. This oven dried material was taken for the estimation of different inorganic elements by following the method of Toth et al (1948).

0.5g of oven dried powdered material was transferred to a 150ml beaker to which 20ml conc. HNO_3 were added. The beaker was covered with watchglass and kept till the primary reaction subside. It was then subjected to slow heating to dissolve solid practicles completely. After cooling to room temperature, 10ml of 60%. Perchloric acid were added and mixed thoroughly. It was then heated strongly and vigorously until a clean and colorless solution reduced to about 2-3ml. While heating the liquid was not allowed to dry. It was then cooled and transferred quantitatively to a 100ml volumetric flask and volume was made to 100ml with distilled water and kept overnight. Next day it was filtered through a dry whatmann filter paper No.44 (ashless)

and the filtrate was used for the estimation of different inorganic elements.

(II) Estimation of sodium and potassium :-

Sodium and potassium were estimated flame photometrically following the standard procedure. The standard solutions of known concentrations in parts per million (PPM) of Na^+ in Nacl (1 to 10 PPM) and K+ in Kcl (1 to 50 PPM) were used for calibration curves. From these calibration curves the unknown concentrations of Na^+ and K^+ in the acid digest samples were calculated.

(III) Estimation of calcium Magnesium Iron, Copper, Zinc and Manganese:-

The acid digest extract was used to estimate Ca^{2+}, Mg^{2+} Fe^{3+}, Cu^{2+}, Zn^{2+}, and Mn^{2+} elements on atomic absorption spectrophotometer (Perkin - Elmer Model - 3030) using acetylene air flame. The light source employed was hollow cathode lamp. The concentration of Ca, Mg, Fe, Cu, Zn, and Mn were read at 422.7nm 285.2nm, 248.3nm, 324.8nm, 213.9nm and 279.5nm respectively (Perkin - Elmer, 1973).

(IV) Estimation of Chlorides :-

For estimation of chlorides, method described by Imamul Huq and Larher (1983) with slight modification was used. Estimation was done using chapmen and pratt's (1961) method. The chlorides were extracted in boiling distilled water. After

cooling the extract was filtered through a layer of cheese cloth. The filtrate was collected in 25ml volumetric flask and final volume was made with distilled water. From this 10ml of extract was taken for titration against standardised 0.05 N $AgNo_3$. A few drops of 25% acetic acid solution were added to the filtrate until the pH of the solution was 6 to 7. Then a few drops of 1% potassium chromate solution were added and titrated against standardised 0.05 N $AgNo_3$ (Dissolve 8.5 g AR grade $AgNo_3$ in 1000 ml distilled water) until the first permanent reddish brown colour appears and noted the burette readings.

(1 ml of 0.05 N $AgNo_3$ = 1.775 mg Cl^-)

Effect of pesticide study :-

After spray the insecticide on plant:-

(a) Physical properties :- The physical properties like leaf area, height, biomass, leaf moisture were studied by the procedure as given above in effect of aphid infestation study.

(b) Organic constituents :- The organic constituents like chlorophyll, Nitrogen, polyphenols, carbonydrates estimated by the procedure as given above in effect of aphid infestation study.

(c) Inorganic Constituents :- The inorganic constituents like Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Cu^{2+}, Mn^{2+}, Zn^{2+} and Cl^- from the dry leaves from control and methyl papathion sprayed plants were done according to the procedure described in earlier section of Aphid infestation studies.