MANAGEMENT OF COTTON WHITEFLY *BEMISIA TABACI* (GENN.) AND BOLLWORMS *PECTINOPHORA GOSSYPIELLA* (SAUND.) AND *HELICOVERPA ARMIGERA* (HUB.) IN THE PUNJAB, PAKISTAN

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IN THE NAME OF ALLAH

THE MOST GRACIOUS AND

BENEFICENT

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	i
LIST OF FIGURES	vi
ABSTRACT	x
GENERAL INTRODUCTION	xvi

CHAPTER-I

COTTON WHITEFLY *BEMISIA TABACI* (GENNADIUS)

(Homoptera : Aleyrodidae)

INTRODUCTION	1
REVIEW OF LITERATURE	3
METHODS AND MATERIALS	9
RESULTS	14
Activity of whitefly adults during day	14
Efficiency of two type of traps for monitoring whitefly adults	14
Host range	15
Crops	21
Fruit and forest plants	22
Ornamental plants	23
Weeds	24
Carry over	86
Population trends on cotton	86
Host preference for oviposition and survival	87
Development period	92
Vertical distribution of whitefly	93
Control Measures	96
Chemical control	96
(a) Efficacy of two formulations and three volumes of application	96
(b) Effect of insecticide on whitefly	96
(c) Effect of insecticide on parasitoids	99
(d) Evaluation of some non-conventional and conventional chemicals against whitefly	100
Biological control	103
(a) Parasitism	103
(b) Effect of conservation of natural enemies on whitefly infestation	104
(c) Predators	104
(d) Efficiency of *Chrysoperla carnea* in the field	108
(e) Predators population on alternate host plants	108
DISCUSSION AND CONCLUSIONS	111
REFERENCES	114

CHAPTER-II

PINK BOLLWORM *PECTINOPHORA GOSSYPIELLA* (SAUNDS)
{Lepidoptera : Gelechiidae}

INTRODUCTION 125
REVIEW OF LITERATURE 127
METHODS AND MATERIALS 137
RESULTS 145
Adults activity 145
Oviposition 145
Period of infestation on squares 153
Oviposition performance for squares of different ages 153
Larval feeding 154
Effect of rainfall on infestation 156
Effect of pink bollworm attack on squares on development of bolls 157
Effect of pink bollworm attack on number of seeds and lint/boll 160
Boll age preference to pink bollworm attack 160
Pupation 161
Carry over sources 163
Ginning waste 163
(a) Pink bollworm larval mortality in the ginning waste 163
(b) Emergence of moth in ginning waste in heaps of different sizes 164
(c) Emergence of moths from ginning waste spread in the field 165
Crop residue 166
Effect of irrigation on survival of pink bollworm larvae 166
Effect of ploughing on survival of pink bollworm larvae 167
Effect of destruction of sticks on pink bollworm larvae 168
Effect of ginning on mortality in pink bollworm larvae 169
Left-over bolls on cotton sticks 170
(a) Pattern of emergence of *P.gossypiella* 171
Ratoon cotton 173
Alternate host plants 174
Control measures 175
Chemical control 175
Biological control 176
Cultural control 177
Use of sex pheromones 177
(a) Efficiency of different types of traps 178
(b) Mass trapping 179
(c) Feasibility of pink bollworm control with communication disruption 181
DISCUSSION AND CONCLUSIONS 188
REFERENCES 192

CHAPTER-III

AMERICAN BOLLWORM *HELICOVERPA* ARMIGERA (HAB) {Lepidoptera : Noctuidae}

INTRODUCTION 205
REVIEW OF LITERATURE 207
METHODS AND MATERIALS 213
RESULTS 217
Adults activity 217
Oviposition behaviour of *Helicoverpa armigera* on cotton 217
Distribution of *H. armigera* on cotton plant 221
Biology 225
Number of generations 225
Host suitability 226
Alternate hosts and population dynamics 227
Infestation of *H. armigera* on various crops before cotton cultivation 233
Distribution of *H. armigera* larvae on cotton plant 233
Pupation 237
Diapause 238
Life tables of *H. armigera* on cotton 242
Life table of *H. armigera* of the first generation 242
(a) Egg mortality 242
(b) Larval mortality 243
(c) Pupal mortality 244
Life table of *H. armigera* of the second generation 244
(a) Egg mortality 244
(b) Larval mortality 245
(c) Pupal mortality 245
Life table of *H. armigera* of the third generation 245
(a) Egg mortality 245
(b) Larval mortality 246
(c) Pupal mortality 246
Generation increase rate 250
Control Measures 251
Chemical control 251
(a) Efficacy of insecticides on eggs of *H. armigera* 251
(b) Efficacy of four insecticides on larvae of *H. armigera* 255
(c) Evaluation of some insecticides against *H. armigera* 256
Biological control 257
Feeding efficiency of *Chrysoperla carnea* on *H. armigera* 258
Cultural control 259
DISCUSSION AND CONCLUSIONS 260
REFERENCES 263

LIST OF FIGURES

Fig. No.	Figure Title	Page
1	Hourly catches of whitefly adults on yellow sticky traps based on mean number during July at Central Cotton Research Institute, Multan.	16
2	Hourly catches of whitefly adults on yellow sticky traps based on mean number during August at Central Cotton Research Institute, Multan.	17
3	Hourly catches of whitefly adults on yellow sticky traps based on mean number during September at Central Cotton Research Institute, Multan.	18
4	Hourly catches of whitefly adults on yellow sticky traps based on mean number during October at Central Cotton Research Institute, Multan.	19
5	Mean number of whitefly adults caught/trap/day with two trap designs during July-October at Multan	20
6	Population trends of *Bemisia tabaci* on *Mentha viridis* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	27
7	Population trends of *Bemisia tabaci* on *Glycine max* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	28
8	Population trends of *Bemisia tabaci* on *Solanum melongena* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	29
9	Population trends of *Bemisia tabaci* on *Corchorus capsularis* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	30
10	Population trends of *Bemisia tabaci* on *Cucurbita pepo var. melopepo* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	31
11	Population trends of *Bemisia tabaci* on *Cucumis melo var. phut* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	32
12	Population trends of *Bemisia tabaci* on *Mentha viridis* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	33
13	Population trends of *Bemisia tabaci* on *Cucumis melo* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	34
14	Population trends of *Bemisia tabaci* on *Ipomoea batatas* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	35
15	Population trends of *Bemisia tabaci* on *Sasame indicum* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	36
16	Population trends of *Bemisia tabaci* on *Citrullus lanatus* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	37
17	Population trends of *Bemisia tabaci* on *Vigna radiata* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	38
18	Population trends of *Bemisia tabaci* on *Capsicum frutescens* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	39
19	Population trends of *Bemisia tabaci* on *Abelmoschus escultantus* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	40
20	Population trends of *Bemisia tabaci* on *Cucumis sativus* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	41
21	Population trends of *Bemisia tabaci* on *Broussonetia papyrifera* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	42

22	Population trends of *Bemisia tabaci* on *Punica granatum* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	43
23	Population trends of *Bemisia tabaci* on *Morus alba* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	44
24	Population trends of *Bemisia tabaci* on *Bauhinia purpurea* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	45
25	Population trends of *Bemisia tabaci* on *Albizzia lebbek* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	46
26	Population trends of *Bemisia tabaci* on *Psidium guajava* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	47
27	Population trends of *Bemisia tabaci* on *Gardenia jasminoides* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	48
28	Population trends of *Bemisia tabaci* on *Chrysanthemum morifolium* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	49
29	Population trends of *Bemisia tabaci* on *Ipomoea cairiea* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	50
30	Population trends of *Bemisia tabaci* on *Tagetes erecta* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	51
31	Population trends of *Bemisia tabaci* on *Ageratum conyzoides* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	52
32	Population trends of *Bemisia tabaci* on *Gelianthus annuus* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	53
33	Population trends of *Bemisia tabaci* on *Hibiscus rosa-sinensis* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	54
34	Population trends of *Bemisia tabaci* on *Lantana camara* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	55
35	Population trends of *Bemisia tabaci* on *Gomephrena globosa* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	56
36	Population trends of *Bemisia tabaci* on *Solanum melongena* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	57
37	Population trends of *Bemisia tabaci* on *Euphorbia pulcherrima* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	58
38	Population trends of *Bemisia tabaci* on *Ipomoea tricolor* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	59
39	Population trends of *Bemisia tabaci* on *Hibiscus mutabilis* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	60
40	Population trends of *Bemisia tabaci* on *Corchorus triloailanis* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	61
41	Population trends of *Bemisia tabaci* on *Euphorbia hirta* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	62
42	Population trends of *Bemisia tabaci* on *Phyllanthus pilulifera* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	63
43	Population trends of *Bemisia tabaci* on *Conyza bonariensis* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	64
44	Population trends of *Bemisia tabaci* on *Convolulus arvensis* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	65

45	Population trends of *Bemisia tabaci* on *Eclipta alba* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	66
46	Population trends of *Bemisia tabaci* on *Malvastrum tricuspidatum* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	67
47	Population trends of *Bemisia tabaci* on *Solanum xanthocarpum* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	68
48	Population trends of *Bemisia tabaci* on *Achyranthes aspera* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	69
49	Population trends of *Bemisia tabaci* on *Polygonum sp.* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	70
50	Population trends of *Bemisia tabaci* on *Citru7llus tetragona* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	71
51	Population trends of *Bemisia tabaci* on *Xanthium strumarium* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	72
52	Population trends of *Bemisia tabaci* on *Physalis alkakengi* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	73
53	Population trends of *Bemisia tabaci* on *Solanum nigrum* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96	74
54	Mean number of *Bemisia tabaci* adults on cotton during 1994	88
55	Mean number of *Bemisia tabaci* adults on cotton during 1995	89
56	Mean number of *Bemisia tabaci* adults on cotton during 1996	90
57	Mortality of *Bemisia tabaci* nymnphs with some conventional and non conventional chemicals at different intervals.	101
58	Mortality of *Bemisia tabaci* adults with some conventional and non conventional chemicals at different intervals.	102
59	Whitefly population (line) and total parasitism (bar) on it on cotton where farmer used pesticides at Khokhran.	105
60	Population trend of whitefly and its parasitoids on cotton where natural enemies were preserved at Khokran	106
61	Relative abundance of predators in cotton at Khokhran	107
62	Population level of *Bemisia tabaci* and *Chrysoperla carnea* in the field	109
1	Average number of *Pectinophora gossypiella* male moths caught/trap/week at Multan during 1994	146
2	Average number of *Pectinophora gossypiella* male moths caught/trap/week at Multan during 1995	147
3	Average number of *Pectinophora gossypiella* male moths caught/trap/week at Multan during 1996	148
4	Average number of *Pectinophora gossypiella* male moths caught/trap/fortnight at Khanewal during 1994	149
5	Average number of *Pectinophora gossypiella* male moths caught/trap/fortnight at Khanewal during 1995	150
6	Average number of *Pectinophora gossypiella* male moths caught/trap/fortnight at Khanewal during 1996	151
7	*Pectinophora gossypiella* infestation and rainfall during 1994 and 1995	158
8	Average number of *Pectinophora gossypiella* male moths caught / trap / week in the insecticide and PBW Rope treated plots during 1996	182

9	Infestation of *Pectinophora gossypiella* in the plots treated with PBW Rope and insecticide during 1991	183
10	Mean number of male moths of *Pectinophora gossypiella* caught in plots with T-bag (average of small and large) PBW rope and insecticide during 1992	185
11	Infestation of *Pectinophora gossypiella* in the plots treated with T-bag (average of small and large) PBW rope and insecticides during 1992	186
1	Number of moth catches of *Helicoverpa armigera*/week/light trap at Multan during 1994	218
2	Number of moth catches of *Helicoverpa armigera*/week/light trap at Multan during 1995	219
3	Number of moth catches of *Helicoverpa armigera*/week/light trap at Multan during 1996	220
4	Incidence of *Helicoverpa armigera* on major host plants.	228
5	Incidence of *Helicoverpa armigera* on minor host plants.	229
6	Infestation of *Helicoverpa armigera* on different host plants at different locations	234
7	a) Plastic tray used for rearing and pupation of *H. armivera* larvae b) Plastic tray and cups used for the emergence of adults of *H. armigera*	239
8	Emergence of *Helicoverpa armigera* adults from pupae that formed in trays from field collected larvae from October to January at Multan	240
9	Emergence of *Helicoverpa armigera* adults from field collected pupae during different months and kept in plastic trays in the field	242
10	Mortality of *Helicoverpa armigera* larvae with insect growth regulators and insecticides	258

ABSTRACT

WHITEFLY (*Bemisia tabaci)*

On Cotton *Bemisia tabaci* adults were most active from 0600 to 0700 hours in July, from 0600 to 0800 hours in August, between 0800 to 1000 hours in September and from 0900 to 1000 hours in October.

During the present survey whitefly was recored from 145 plant species in Multan and Khanewal Districts. These included 41 crops, 41 ornamentals, 45 weeds and 18 fruit and forest trees. Based on the rate of infestation and duration of feeding, hosts have been categorised as major, minor and incidental hosts. In the crops 15 in ornamentals 13 in fruit and forest trees six and in weed 14 plant species were regarded as major host others were minor or incidental.

Of the 145 host plants *B. tabaci* was found on 114 hosts during winter. These include 32 crops, 31 ornamentals, 16 fruit and forest trees and 35 weeds. After winter maximum population was found on *Vigna radiata, V. cylindrica, Glycine max, Lens culinaris, Helianthus annuus, Solanum melongena, Cucurbita pepo var. melopepo, Cucumis melo, Citrullus lanatus var. listulosus, C. melo var.* phut from crops, *Althaea rosea, Chrysanthemum morifolium, Lantana camara* and *Hibicus mutabilis* among ornamentals, *Euphorbia prostata, Citrullus tetragona, Xanthium strumarium, Malvastrum tricuspidatum, Solanum nigrum* and *Achyranthes aspera* from weeds, *Morus alba and Albizzia lebbek* from fruit and forest trees. These hosts seem to play an important role for its shifting and build up on cotton.

Maximum oviposition took place on *S. melongena* followed by *G. hirsutum , X. strumarium,. L. camara, A. aspera and C. tetragona.* The most suitable host for

development was *S. melongena* followed by *L. camara, C. tetragona, G. hirsutum, A. aspera and X. strumarium* in descending order.

In studies on vertically distribution maximum whitefly nymphs and pupae were found on leaves on node number nine in July and as the plant grew it shifted upwards or downwards and was maximum on leaves of node ten in August on the main stem. On side branches population trends were similar to on the main stem. Within the branch leaves close to main stem were more susceptible than the young leaves.

Monocrotophos and lambda-cyhaluthrin gave higher mortality with high volume of application than with low and ultra low volumes. Monocrotophos proved better than lambda-cyhaluthrin. Acetamiprid and diafenthiuron gave highest mortality than monocrotophos and lambda-cyhaluthrin. From the non-conventional insecticide biosal (neem extract) gave higher mortality than the *Eruca sativa* oil + detergent or *Eruca sativa* oil + neem soap or detergent alone.

Encarsia species and *Eretmocerus* species were common parasitoids of whitefly nymphs and pupae. Among the alternate host plants maximum parasitism was on *A. rosea, A. aspera, Ipomoea batatas, X. strumarium* and *S. melongena.* On cotton its maximum parasitism was 40% in the plot where natural enemies were conserved compared with 5% where insecticide were used.

The important predators associated with the whitefly infestation on all the plants were coccinellids, *Chrysoperla carnea, Orius sp., Geocoris* and unidentified species of spiders. From these coccinellids, *C. carnea* and spiders played important role in regulating whitefly infestation.

PINK BOLLWORM (*Pectinophora gossypiella)*

The pink bollworm adults continued to emerge throughout the year. It completed four generations and the larvae of the fifth generation entered diapause in the bolls or soil in early November. Larvae of fifth generation have long and short diapause. In the sex pheromone baited traps maximum adults from diapausing population were caught during March-April but continued to emerge up to August.

Females laid eggs singly on all plarts of the plant but maximum eggs were laid on squares and bolls. When bolls were not present, maximum eggs were laid on squares. They prefer less than half-grown squares (1-10-days old). Fecundity of females developed from diapausing larvae, an average was 75 and those developed from the active generations was 125. Infested squares turned into rosette flowers. Most of these were shed. Eight to ten percent bolls that developed from rosette flowers were not normal and shrivelled on one side. Number of seeds and weight of lint produced from rosette were less than from bolls developed from healthy flowers. Fourteen to twentyeight days old bolls were most susceptible.

Carry over sources of the pink bollworm such as alternate hosts, ratoon cotton, left over bolls and survival of pink bollworm larvae in the soil, double seeds and ginning waste etc., were studied. There was no major alternate hosts and very negligible infestation was found on *Althaea rosea* and *Hibiscus rosa-sinensis.* However, low infestation was found on ratoon cotton. From diapausing larvae in the soil emergence was completed by May. Afterwards larvae did not survive when temperatures were above 40°C. In ginning waste most of the double seeds were crushed.

The ginning waste when put in the soil increased organic matter and nitrogen in the soil thus improving its fertility. Left over bolls were the only source of carry over. All the larvae were killed in the bolls on the upper part of the sticks kept horizontally in the conventional way in big heap. However, 5% larvae survived on the lower part of heap. Larval mortality was 100% when sticks were kept in small bundles in the upright position (vertically).

Pyrethroids proved better than all doses of naturalyte. Residual effect of these chemicals lasted for less than 5-days. The number of larvae in the bolls was higher after 12 days than after 7 days of spary. This indicated that eggs laid 4-5 days after spray hatched and entered the bolls.

AMRICAN BOLLWORM *(Helicoverpa armigera)*

H. armigera remained active almost throughout the year. Number of moth caught was lower during January and February than June to August with a peak in April. The females laid eggs on all parts of the plant including terminal buds, leaves, fruiting parts and internodes. About 70-80% eggs were found on the upper part of the plant followed by middle and bottom parts. At low infestation rate 71% eggs were found on the main stem but at medium and high levels of infestation 51-54% eggs were found on side branches. Minimum eggs were found on fruiting parts at all levels of infestation. On main stem more eggs were laid on leaves up to seventh node, highest being on first and second fully developed leaves. On the side branches maximum eggs were laid on first fully expanded leaves and the oviposition decreased towards the main stem. More eggs were found on squares near the tips of branchs. For oviposition squares were preferred to flowers and bolls.

Development period and survival varied with the host and temperature. It took 24.5 days on *Trifolium aestivum* and 34.0 days on *Glycine max*. Under forced feeding it could developed even on *Mangifera indica* which is not a recorded host.

It was recorded from 61 plant species belonging to 56 genera of 20 families including 31 crops, 14 ornamentals and 16 weed plants. Based on level of infestation and duration of feeding host were categorized as 19 major and 42 minor. Maximum infestation on major hosts was found from March to June. At 27±3°C it completed development in 24-30 days on cotton in the laboratory. It had 7-8 generations in a year.

The larvae were recorded from all parts of the cotton plant. Maximum number of young larvae was on top, half-grown on the middle and full-grown on the lower parts of the plant. Maximum young larvae were found on squares followed by vegetative parts. Half-grown larvae preferred flowers and full-grown bolls. No young larva was found on bolls and no full-grown larva was found on vegetative parts.

Pupation took place on or in the soil at different depths. Maximum larvae pupated at 2.5 to 5.0 cm below the soil. In sandy soils pupation took place 12.5 to 17.5 cm below the soil. The pupal period was 6-8 days during summer and increased 65 datys in larvae that pupated in October and November, up to 70 days in December and 70-90 days in January. From the field collected pupae in October adults emerged up to after 150 days.

First generation life table was prepared in June-July when crop was 30 to 55 days old. Key egg mortality factors during this period were desiccation (42.1%), dislodging (31.5%) and predation (12.5%). Parasitism remained extremely low being 1.9%. Important mortality factors in the first and second instar larvae were dropping

and predation. In the third instar missing and predation of larvae by chewing insects and birds was 24.1% followed by predation and parasitism. In the fourth, fifth and sixth instars, predation followed by parasitism were main mortality factors. Maximum pupal mortality was due to decaying because of flood irrigation (15.3%), predation by ants, interculturing and desiccation (26.8%). In the second generation during August-September loss was 70.8% and in the third generation during October-November 60.0%. Larval and pupal mortality factors were almost the same. However, because of high humidity diseases also caused larval mortality. Pupal mortality due to decaying and other factors was almost the same and caused 56% reduction. After harvest of cotton irrigation, ploughing and planking gave considerable mortality in pupae.

Methomyl, profenofos and thiodicarb were effective insecticides on eggs and larvae of *H. armigera*.

High parasitism was recorded on *Cicer arietinum* and *Carthamus oxycantha* where insecticide were not used in rain-fed areas. In the main cotton belt parasitism was extremely low because of use of insecticides.

Chrysoperla carnea is very efficient predator and consumed on an average 241.6 eggs in its life span. It completed its development on 191.2 first instar larvae or 147.3 second instar or 92.0 third instar larvae of *H. armigera*.

GENERAL INTRODUCTION

Cotton plays an important role in the economy of Pakistan. It accounts for about 60% of its export earnings and over 55% of domestic edible oil production. It provides raw materials to over 400 textile mills, 1,035 ginning factories and 5,000 oil expellers. As a result millions of people are employed in cotton based industries.

Pakistan ranks fourth in cotton production and third as exporter of raw cotton in the world. The production decreased from 12.8 million bales im 1993 to 9.0 million bales in 1996. The major reasons for low production were the flare up of cotton leaf curl virus and increase in attack of cotton whitefly, *Bemisia tabaci* (Genn.); African bollworm, *Helicoverpa armigera* (Hub.) and pink bollworm, *Pectinophora gossypiella* (Saund.).

As reported by Shabbir (1973) about 168 insect pests attack cotton crop. Among these about a dozen are economically important. However, at present *B. tabaci, H. armigera* and *P. gossypiella* are the most destructive and cause maximum loss to cotton crop. The other sucking pests and bollworms including cotton jassid, *Amrasca devastans* (Dist.); thrips, *Thrip tabaci* (Lind); aphids, *Aphis gossypii* (Glov.); two spotted mites, *Tetranychus urticae* (Koch.), spotted bollworms, *Earias vittella* (F.) and spiny bollworm, *E. insulana* (Boisd.) are sporadic in nature. The black headed cricket, *Gryllus bimaculatus* (Deg.) is localized pest in the D.G. Khan and D.I. Khan districts. white ant, *Microtermes obesii* (Hol.); *Spodoptera litura* (Fb.) and Cutworms, *Agrotis* spp. cause considerable damage when appear in epedemic form.

Because of lack of basic information on biology including phenology, carry over sources, and shifting of pests from alternate hosts to cotton, their control is becoming uneconomical.

Studies have been carried out on three major cotton pests including cotton whitefly, pink bollworm and African bollworm. Results of these studies have been described in three chapters. For control of whitefly efficacy of chemical and biological control methods; for the pink bollworm use of sex pheromone, chemical and cultural control measures and for *H. armigera* chemical, biological and cultural control measures was studied. The results of these studies are reported in the respective chapters.

CHAPTER-I

COTTON WHITEFLY

BEMISIA TABACI (GENNADIUS)

(HOMOPTERA : ALEYRODIDAE)

INTRODUCTION

Cotton whitefly *Bemisia tabaci* (Gennadius) (Homoptera : Aleyrodidae) is highly polyphagous and has been reported from more than 500 host plants from different parts of the world. (Greathead, 1986). It has been referred as the cassava whitefly, sweet potato whitefly, tobacco whitefly and cotton whitefly (Mound, 1963). It has been reported most frequently as an economic pest of crops in the old and new world between 52ºN and 28ºS, having economic importance in most parts of the tropics including Asia, Africa, America, Europe, USSR, Australia and the Pacific Islands (Anon., 1986). In 16 out of 27 cotton producing countries, *B. tabaci* has been reported as a major pest from mid to late cotton growing season (Anon., 1989). In the undivided Punjab, American cotton varieties failed completely during 1919 and 1926 and partially in 1921, 1923 and 1927 because of whitefly attack (Hussain and Trehan, 1933). It causes damage in three ways, by direct feeding which results in reduction of vigour of plant, shedding of immature fruiting parts and lint contamination with honeydew and associated fungi. It acts as vector of 70 diseases to commercial crops and weeds in different parts of the world (Muniyappa, 1980). Adult life, fecundity, development rate and period varies depending on the environmental factors such as temperature, relative humidity and host plant species. Light intensity and adults attraction are positively correlated and the leaf colour plays an important role in host plant selection. However, the oviposition is influenced by the number of hairs on the leaves (Duffey, 1986).

Pesticide worth seven billion rupees are imported every year. Almost 80% of the pesticides are used on cotton. Their intensive and indiscriminate use has created high level of resistance in whitefly resulting in serious pest control failure (Ahmad,

1996). In Pakistan its main damage to cotton is because it transmits leaf curl virus and loss runs in millions of rupees in the recent years. Cotton production decreased from 13 million bales in 1991 to 9.2 million bales in 1992 and 8.0 million bales in 1993.

To develop effective management strategy for its control, studies on adult activity, biology, host-range and population dynamics on alternate hosts and on cotton, oviposition preference, development period and survival, chemical control and role of natural enemies were carried out from 1993 to 1996. The results of these studies are presented here.

REVIEW OF LITERATURE

It is highly polyphagous and a large number of host plants have been reported by various authors. Avidov and Harpaz (1969) reported 52 plant species as hosts in Israel, Chang (1969) 87 in Taiwan, Azab *et al.* (1971) 172 in Egypt, Gameel (1972) 115 in Sudan. However, Mound and Halsey (1978) reported 315 plant species and Greathead (1986) more than 500 host plant species worldwide as its hosts.

In areas now in Pakistan, Hussain and Trehan (1933) reported 44 plant species as hosts of *B. tabaci.* Later on Husain *et al.* (1936) added two species, Rehman (1940) four and Ahmad and Harwood (1973) added three more species to this list. Inayatullah *et al.* (1985) reported 104 plant species belonging to 80 families. More recently Khan *et al.* (1985) recorded this species from 105 plants including crops, vegetables, ornamentals, fruit and forest plants and weeds from various parts of Pakistan.

Biology of *B. tabaci* has been studied by a number of workers in different countries. In Israel Avidov (1956), in Egypt Azab *et al.* (1971), Bedford (1936), El-Halaly *et al.* (1977), in India Hussain *et al.* (1936), Trehan (1944) and Misra and Lamba (1929), in Pakistan Naqvi (1973), in the USA Gerling *et al.* (1982) and Butler *et al.* (1986).

Development of *B. tabaci* varies greatly on different host plant species. Time required for its development from egg to adult at 26.1°C was minimum on sweet potato (16 days) and maximum on carrot (38 days). Thirty percent less time was required for *B. tabaci* to complete its life cycle on squash, alfalfa, egg plant, cucumber and lettuce than on carrot. On sugar beet eggs were laid but nymphs did not complete development (Coudriet *et al.*, 1985). Rate of development is positively correlated with temperature

(Azab *et al.*, 1971; Butler *et al.*, 1983). The lower and upper thresholds are 11 and 33°C and optimum temperature for growth is 28°C. development from egg to adult on cotton at this temperature is completed in 20 days (Gerling *et al.*, 1986).

B. tabaci adults are small in size measuring 1.1 to 1.2 mm in length, males are slightly smaller than females. Emergence mainly occurs between 0800-1200 hours through a T-shaped fissure on dorsal surfaces of the pupae and takes 5-15 minutes. The rate of emergence is highest during first four hours after sunrise (Hussain and Trehan, 1933; Azab *et al.*, 1971; Butler *et al.*, 1983 and 1986; Musuna, 1986). Besides sexual reproduction, parthenogenic reproduction has also been noticed and from unfertilized eggs only males were produced (Hussain and Trehan, 1933). Male female adults sex ratio was 1:1 to 1:6 (Azab *et al.*, 1971; Butler *et al.*, 1983). Mating generally occurred from 8.00 to 4.00 PM (Butler *et al.*, 1986).

Adult longevity is affected by the environmental factors, mainly temperature and relative humidity as well as host plant species. Under laboratory conditions males lived for 4 and females for 11.7 days during summer when mean temperature was 28.5°C and for 13.7 and 57.3 days during winter when mean temperature was 14.2°C on sweet potato (Azab *et al.*, 1971). On egg plant males lived for 3 and females for 17 days during summer and 34 and 55.3 days during winter (Avidov, 1956). On cotton males lived for 13.2 and female for 61.5 days during October to December (Gameel, 1978) while 6 and 18.5 days on *Vigna cylindrica* (Lobia) during summer when temperatures were between 29-33°C (Khalifa and El-Khidir, 1964).

Pre-oviposition period was 3.3 days at 28°C and 4.9 days at 14°C under laboratory conditions. In the field and insectory it ranged from 1 to 8 days at 21.7°C to 26.5°C and 22 days at 13.8°C (Avidov, 1956; Avidov and Harpaz, 1969). Oviposition

threshold temperature determined by Avidov (1956) was 14°C. However, Hussain and Trehan (1933) reported that females did not lay eggs below 22.8°C under field conditions.

Temperature, relative humidity and host plant affected the fecundity of the pest (Horowitz, 1983). Maximum number of eggs were laid during the first week of adult life. Fecundity ranged from 28 to 42 (Hussain and Trehan, 1933), 72 at 26.7°C and 81 at 32.2°C (Butler *et al.*, 1983) but Avidov and Harpaz (1969) reported 50 eggs, Azab *et al.* (1971) 48 to 394 and Avidov (1956) 300 eggs per female.

After emergence adults migrate from older leaves to young leaves for oviposition during morning hours (Musuna, 1986). Vertical distribution on host plant depens on the structure of the plant (Trehan, 1944; Ohenesorage *et al.*, 1980; Von Arx, 1982; Von Arx *et al.*, 1984; Ohenesorage and Rappe, 1986). Adults show two types of migration, short distance within plant canopy (Avidov, 1956; Ohenesorage *et al.*, 1980) and long distance for host selection generally controlled by air current and drift (Verma, 1963). Migration usually took place between 0600 to 0900 hours (Gerling and Horowitz, 1984). Bellows *et al.* (1988) reported positive correlation between temperature and whitefly adult trap catches but not with humidity. Correlation between light intensity and adult attraction of both sexes was reported by El-Halaly *et al.* (1981a and b). Leaf colour plays significant role in host-plant selection and adults are strongly attracted to greenish-yellow (Hussain and Trehan, 1940) or yellow (Ahmad and Harwood, 1973; El-Halaly *et al.*, 1981a and b; Berlinger, 1980 and 1986 and Sharaf, 1982). Selection for oviposition is strongly influenced by leaf trichome (Duffey, 1986). Young apical cotton leaves were not used for oviposition and feeding because of the high hair density (Mound, 1965). However, Abbasi (1980) reported that nymphal

feeding on old leaves have a detrimental effect on fecundity. Effect of the thickness of leaf cuticle was reported by Walker (1985 and 1987), sugar and nitrogen contents by Noldus *et al.* (1986); Joyce (1958); Hussain *et al.* (1936) and Berlinger *et al.* (1983).

Variation within populations have been reported by a number of authors (Burban *et al.*, 1992; Brown, 1991 and 1992). Haman and Salguero (1987) reported that whitefly population in an area is not identical and a part of population shows differences in host preference, duration of life stages and even in disease transmission. For example in Puerto Rico two races of *B. tabaci* have been identified. The Jatropha race was associated exclusively with *Jatropha gossypifolia* but could not feed and reproduce on most of other hosts (Bird, 1957). On the other hand, *Sida* race, failed to breed on *J. gossypifolia* but colonizes successfully on other hosts (Bird and Sanchez, 1971 and Bird *et al.*, 1972). Similarly in Brazil *B. tabaci* does not feed and breed on cassava and *Manihot esculenta* whereas these plants served as its major hosts in Africa (Costa and Russell, 1975). This host association was the exclusive criterion for differentiating races or biotypes (Brown, 1992). However, Singer (1986) and Thompson (1988) reported that relationship between host selection for oviposition site and fecundity, growth, and survival play key role in the evolution of biotypes. Only those plants and plant parts are selected for oviposition, which are most suitable for nymphal development (Hassal and Southwood 1978). Selection is made by visual (Prokopy and Owens, 1983) and olfactory senses (Visser, 1986 and 1987).

Life tables for *B. tabaci* on cotton have been prepared by Horowitz *et al.* (1984) who concluded that mortality in *B. tabaci* is highest due to abiotic factors during crawler and young nymphal stages.

A number of reasons for problems and often failure in controlling whitefly with insecticides have been reported by various authors. Waxy covering on the nymphs and pupae may be responsible to provide protection to nymphs and pupae (Jhonson *et al.*, 1982), while location of all the stages on under surface of the leaves, rapid reproduction rate and wide range of hosts within and between cropping seasons (Matthews *et al.*, 1995). As a result of elimination of natural enemies with insecticides may even aggregate the problem as reported in Sudan (Joyce, 1955 and Eveleens, 1983); in America (Kraemer, 1966 and Miller, 1986) and in Turkey (Sengonca, 1975).

Greathead and Bennett (1981) and Gerling (1985) reported natural enemies of whitefly from different parts of the world. Muhyuddin *et al.* (1989) listed 11 aphelinid from Pakistan. These include *Encarsia adrianae* Lopez Avila, *E. cibcenses* Lopez Avila, *E. formosa* Gohan, *E.longifasciata* Subba Rao, *E. lutea* (Masi), *E. mohyuddini* Shafee and Rizvi, *E. partenopea* (Masi), *E. shafeei* Hayat, *Eretmocerus aligarhensis* Khan and Shafee, *E. corni* Haldman *and E. mundus* Mercet. They also studied biology and population dynamics of some species. *Encarsia formosa* has been used for biological control of *Trialeurodes vaporariorum* (Westwood) in green houses in Belgium, Canada (DeBach, 1964), Belgaria, Denmark (Hansen, 1985), Finland, France, Italy, Irish Republic (Anon., 1980), Netherlands (Lenteren and Hulspas-Jordaan, 1983), Spain (Casadevall *et al.*, 1979), Switzerland (Freuler *et al.*, 1980), USSR (Beglyarov *et al.*, 1980), Tasmania and South Australia (Wilson, 1960).

Life cycle and ecology of some of the parasitoids were also reported by Clausen and Berry (1932) and Webber (1897) in the USA, Hussain and Trehan (1933) in India, Avidov (1956) in Israel, Gerling (1967) in California, Azab *et al.* (1970) in Egypt and Gameel (1969) in Sudan.

Over 20 species of predators including many general predators like *Chrysoperla spp.*, coccinellids, mirid bugs and mites were recorded associated with whitefly from different parts of the world (Gerling, 1985). Mohyuddin *et al.* (1989) reported *Brumoids suturalis* (F.), *Catana parcesetosa* (Sic.), *Oenopia sauzeti* Muls., *Scymnus sp., nubilus* Muls. (Coccinellidae) and *Chrysopa carnea* Steph. (Chrysopidae) as predators from Pakistan.

Because of indiscriminate and excessive use of insecticides against whitefly, it developed resistance to a number insecticides in different part of world. In early 1980s resistance to dimethoate, monocrotophos and DDT was reported for the first time in Sudan (Dittrich and Ernst, 1983). Resistant to both adults and nymphs to dimethoate, endosulfan, methomyl, amitraz and their combinations of endosulfan was reported by Ahmad *et al.* (1987). Adults resistance to chlorfenvinphos, dimethoate and combinations of endosulfan was reported by Abdeldaffie *et al.* (1987). In the USA adults of *B. tabaci* were resistant to organophosphates and pyrethroids (Horowitz *et al.*, 1988; Probhaker *et al.*, 1985, 1988 and 1989), resistance to these two groups and carbamate was reported by Bryne *et al.* (1992). A high level of resistance in whitefly has been recorded to organophosphates (OPs) like dimethoate, methamidophos and monocrotophos, and to pyrethroids like cypermethrin and deltamethrin in Pakistan (Ahmad, 1996). Cahill *et al.* (1995) tested eleven strains of *B. tabaci* from 7 countries including Pakistan and concluded that level of resistance and biotype were not directly related but the role of acetylcholinesterase sensitivity and general esterase activity in resistance to OPs and pyrethroids may be responsible. Great variations between and within *B. tabaci* population collected from Sudan Turkey and Guetemala were reported by Dittrich *et al.* (1990a and b). Horowitz *et al.* (1994) suggested that area wise insecticide resistance management strategy can reduce its resistance level.

METHODS AND MATERIALS

Adult activity

To determine most active time of whitefly adults during the day, five yellow plastic traps measuring 24 sq. cm coated with stickum were fixed on bamboo sticks at crop canopy height. Hourly whitefly adult catches from sunrise to sunset were recorded daily from July to October. Hourly catches for fifteen days of each month were added and total for each hour was calculated.

Host range

To determine the host range of *B. tabaci* in cotton growing areas of southern Punjab, all available plant species were examined throughout the year for alternate hosts from 1993 to 1996. Sample size varied from 10-150 leaves depending on the size and nature of plant species. Nymphal/pupal population on leaves was counted under binocular microscope and calculated on 100 cm^2 basis. Based on the level of infestation and duration of attack, plant species have been categorized as major, minor or incidental hosts. The plant species on which whitefly was recorded for three to twelve months regarded as major hosts. Plants on which low level of infestation was recorded for three to ten months regarded as minor hosts. Host on which whitefly was recorded for one or two months were termed as incidental.

Population trend on cotton

Population trends of *B. tabaci* on cotton were studied at three sites in the same locality during 1994-1996. The distance between the sampling points was 400m. From one site 15 leaves from 15 randomly selected plants were examined alternatively one

each from top, middle and base at weekly intervals. Means of three points were calculated.

Host preference for oviposition, development and rate of survival

Preference for oviposition and suitability for development of whitefly on six hosts was studied under field conditions when day temperature was 30-35°C and night temperature was 15-22°C. Six plants including *Gossypium hirsutum, Solanum melongena, Lantana camara, Xanthium strumarium, Achyranthes aspera* and *Citrullus tetragona* planted in pots separately were placed in a cage, measuring 110 x 90 x 85 cm in a circle. Total leaves on each plant were counted and ten pairs of two-day-old whitefly adults per leaf, were released in the centre of the cage to give equal chance of host selection for oviposition. This was replicated three times. After 24 hours, adults were removed from the cages and the number of eggs laid on each plant was counted and to make leaf area uniform calculated on 10 sq. cm area. Hatching of eggs was recorded 6 days after oviposition and subsequent observations were made at weekly intervals till the nymphs completed the development.

Suitability of 22 plant species reported as hosts, for development was compared during October when day temperature was 30-35°C and night temperature was 15-22°C. Cotton was kept as control. These included two vegetables, eight ornamentals, two fruit and forest trees and ten weeds. Five pairs of two-day-old laboratory reared whitefly adults were released in microcages fixed on the leaves on potted plants in five replicates for 24 hours. Number of eggs laid on each plant species was recorded. Observations on the development of eggs, nymphs and pupae were recorded after every third day. Development percentage data of immature stages was calculated.

To determine whether or not development period differs on different host plants development of *B. tabaci* was studied on 16 plant species. These included weeds, vegetables and ornamental plants. The plants grown in pots were kept at 27±3°C in the laboratory. Two-day-old, five pairs of the whitefly reared in the laboratory were released in microcages fixed on the leaves on these plants in five replicates. Adults were removed after 24 hours. Observations on incubation, nymphal and pupal development periods were recorded daily.

Vertical distribution

Vertical distribution of nymphal population of *B. tabaci* was studied on unsprayed cotton cultivar CIM-70. The crop was sown in the last week of May in 1992 and 1993. Plant to plant distance was 22 cm and row to row 75cm. Second and third instar nymphs and pupae were counted on all the parts on main stems and side branches of five plants from top to base during July and August. First expanded leaf was considered as node one.

Control measures

Efficacy of two insecticides from different chemical groups (pyrethroid) karate, and (organophosphate) nuvacron in ULV formulation and two volumes of application of E.C. formulations of both was determined. The treatments with insecticide and volumes were as follows:

	Volumes	Insecticides
i)	Untreated check	Karate 2.5EC @ 21g a.i./ha
ii)	High volume (250 lit/ha)	Karate 0.8ULV @ 20g a.i./ha
iii)	Low volume (25 lit/ha)	Nuvacron 40WSC @ 500g a.i./ha
iv)	Ultra low volume (2.5 lit/ha)	Nuvacron 40WSC @ 500g a.i./ha +1250ml vegetabale oil

Four plots of cotton cultivar CIM-109 were sown on 10.6.90, each measuring 13.0 x 12.2m in four replicates. Knap Sack sprayer was used with volume of application as given above. One plot was kept as control. Four applications were made from August to September in each plot. Whitefly adults were counted from ten randomly selected leaves, from top, middle and base of each plant at interval of 48 hours, I week and 2 weeks after each spray. Nymphal population on these leaves was counted with 20 x magnifying lens. To study mortality and effect of insecticide on parasitoids, leaves from each treatment, with maximum population of whitefly were brought to the laboratory. Nymphs and pupae were counted and the leaves were kept in one lb glass jars for emergence of adults and the parastoids. Survival/mortality of whitefly and parasitoids was calculated on the basis of emergence one week after each sampling.

Efficacy of various botanical insecticides such as *Eruca sativa* oil alone and in combination with detergents (Zip) and neem soap (neem extract), biosal 0.32 EC. and two insecticides including, acetamiprid 20SP (mospilan) and diafenthiuron 500 SC (polo) were studied for control of whitefly. Commercial cultivar, CIM-240 was sown on 06.06.1996 in plots measuring 9 x 7.5m in three replicates. Untreated check was kept as control for comparison. Crop was sprayed four times at weekly intervals during peak period of pest attack. Nymphs and adults of whitefly were recorded from 15 randomly selected leaves, taken from top, middle and bottom of the plant after 24 hours, 72 hours and one week of each spray.

To study parasitism in *B. tabaci,* leaves of different host plants infested with the whitefly were examined throughout the year. Pieces of leaves having red eyed

nymphs/pupae were cut and kept in gelatin capsules for emergence of the parasitoids. Predators associated with whitefly infestation on all the plants were recorded.

Efficacy of Yellow Rim Glass (YRG) imported trap from the U.S.A. and locally made, Yellow Sticky trap (YS) was compared for attracting whitefly adults during 1996-97. Five traps of each design were installed vertically at crop canopy, at a distance of 100 m from trap to trap. Whitefly catches were counted hourly from dawn to dusk daily from July to October. Monthly population was calculated.

RESULTS

Activity of whitefly adults during day

To determine activity of whitefly *Bemisia tabaci* (Gennadius) adults during day, five yellow plastic traps coated with stickum (Ca. 24 sq. cm) were fixed on bamboo sticks at crop canopy. Whitefly adults catches from sunrise to sunset were recorded daily at hourly intervals from July to October.

Mean number of adults caught at hourly intervals for 15 days is presented in **Fig.1-4.** During July whitefly adults became active just after sunrise and maximum adults were caught from 0600 to 0700 hours and then dropped gradually till 1400 hours. Catches again increased and highest number of whitefly adults were trapped between 1700 and 1800 hours before sunset **(Fig.1).** During August maximum adults were caught between 0600 to 0800 hours **(Fig.2)** while in September between 0800 to 1000 hours **(Fig. 3)** after which catches decreased gradually. In October when morning temperatures became fairly low as compared with July-September whitefly adults resumed activity slightly late and maximum adults were captured from 0900 to 1100 hours and their activity ceased an hour earlier than during July to September **(Fig. 4).** This indicated that light intensity as well as temperature affects catching of the whitefly adults.

Efficiency of two type of traps for monitoring whitefly adults

Two types of traps, imported yellow rim glass trap (YRG) and other locally made yellow sticky trap (YS) were compared for their efficiency for attracting whitefly adults during 1996-97. Five traps of each design were installed vertically above crop

canopy, at a 100 m distance from one trap to an other. Number of whitefly caught from dawn to dusk were counted daily from July to October.

Yellow sticky traps caught significantly more whitefly adults as compared with yellow rim glass traps, indicating that yellow sticky trap is superior to yellow rim glass trap (**Fig. 5**).

Host range

The whitefly *B. tabaci* has been reported from more than 500 plant species from different parts of the world (Greathead, 1986). Host range of *B. tabaci* in cotton growing areas in southern Punjab was studied. Out of a total of 206 plant species examined during 1993-96, *B. tabaci* was recorded from 145 plant species in Multan and Khanewal Districts. The list of the hosts on which *B. tabaci* was not found is given in **Table 1.** The list of plant species on which *B. tabaci* was recorded and period of activity are given in **Tables 2-6.** These included crops, ornamentals, weeds and fruit and forest trees.

Population trends of the whitefly were studied on 145 plant species. Based on the level of infestation and duration of attack, plant species have been categorized as major, minor or incidental hosts. Population trends on 48 major host plants are shown in (**Fig. 6-53**). The data on population trends of minor host plants are given in **Tables 7-10**. The plants on which whitefly was found for one or two months only have been given in the text.

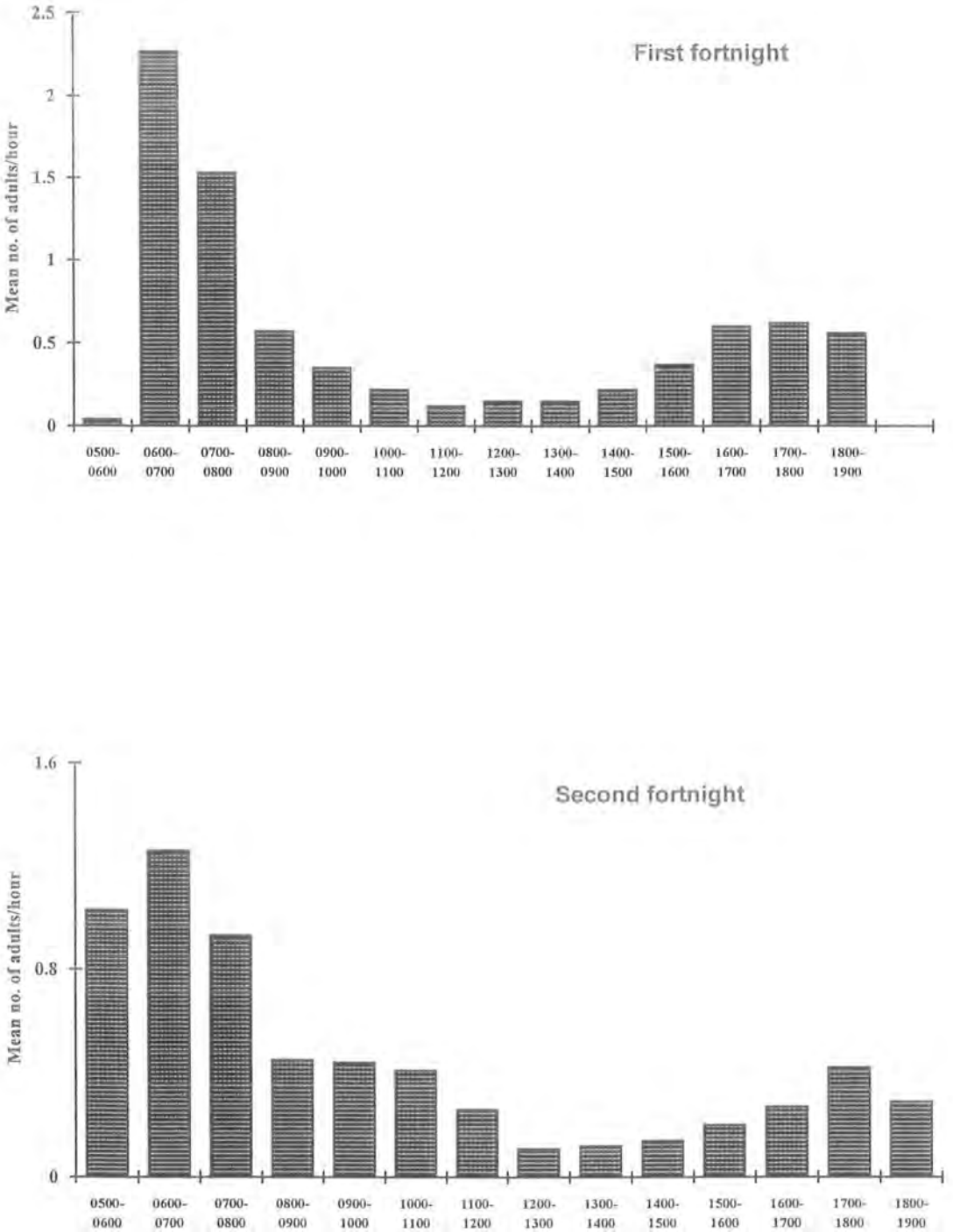


Fig.1 Hourly catches of whitefly adults on yellow sticky traps based on mean number during July at Central Cotton Research Institute, Multan.

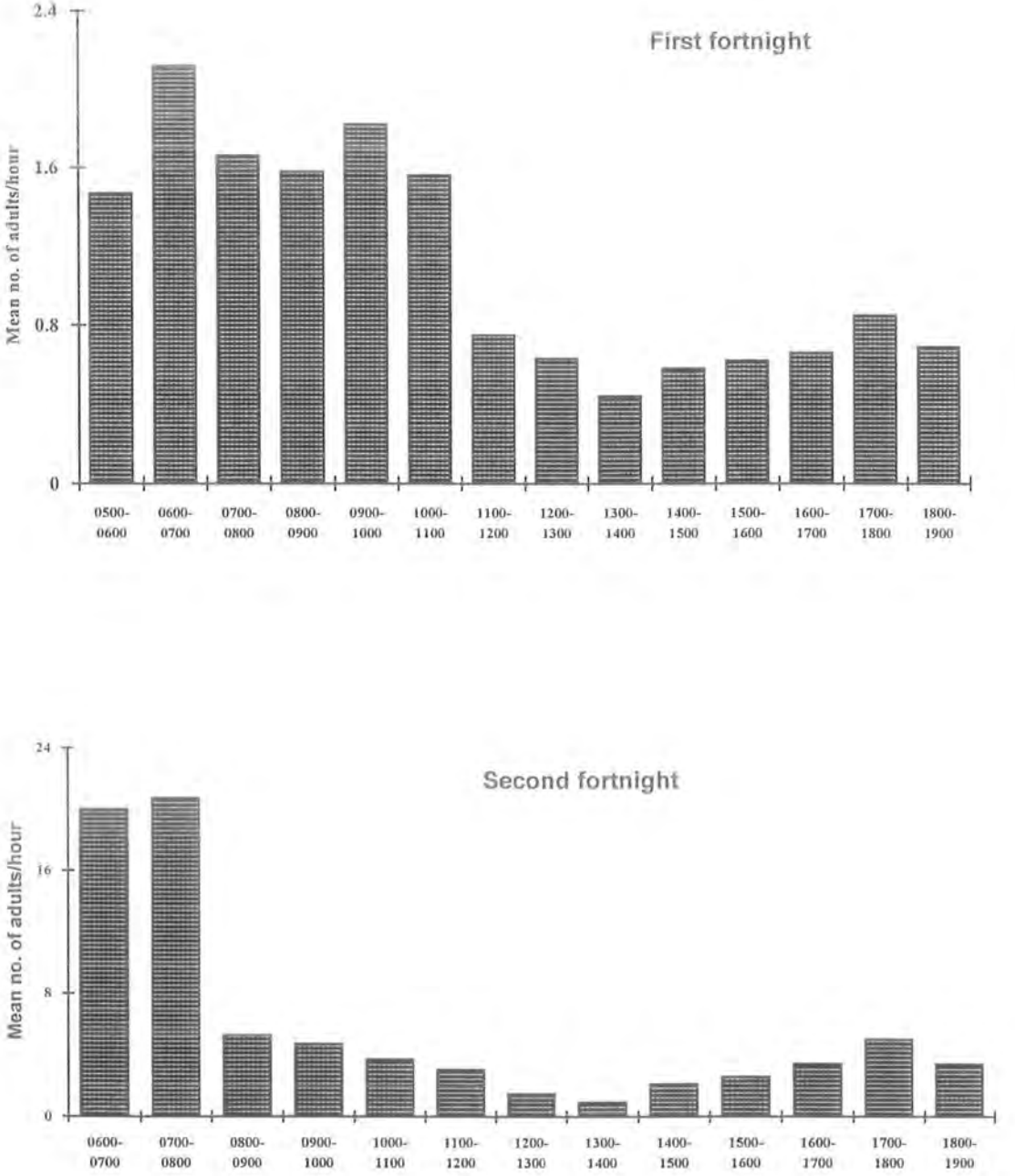
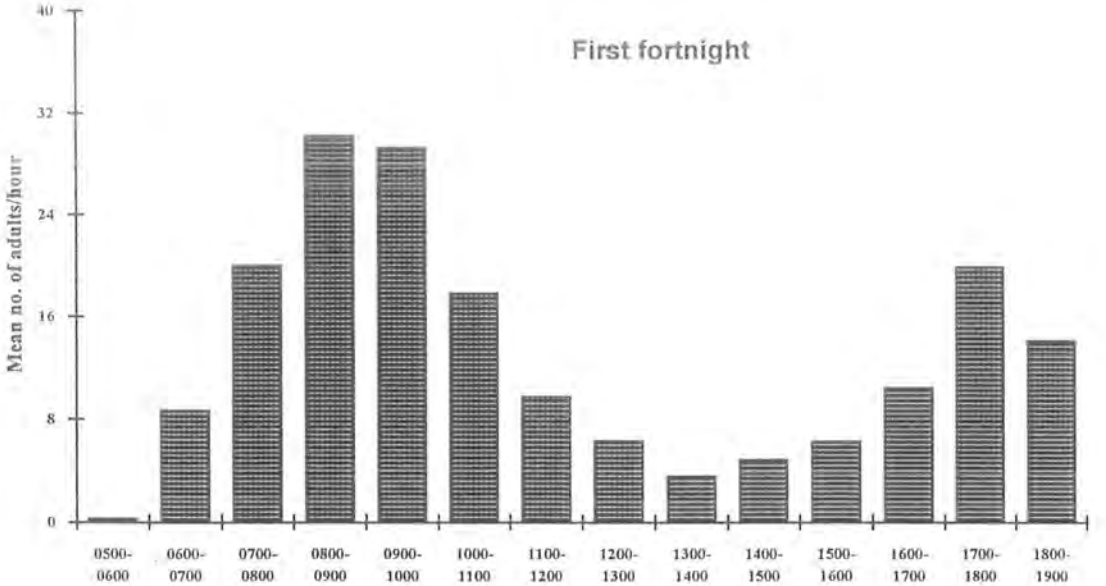


Fig.2 Hourly catches of whitefly adults on yellow sticky traps based on mean number during August at Central Cotton Research Institute, Multan.



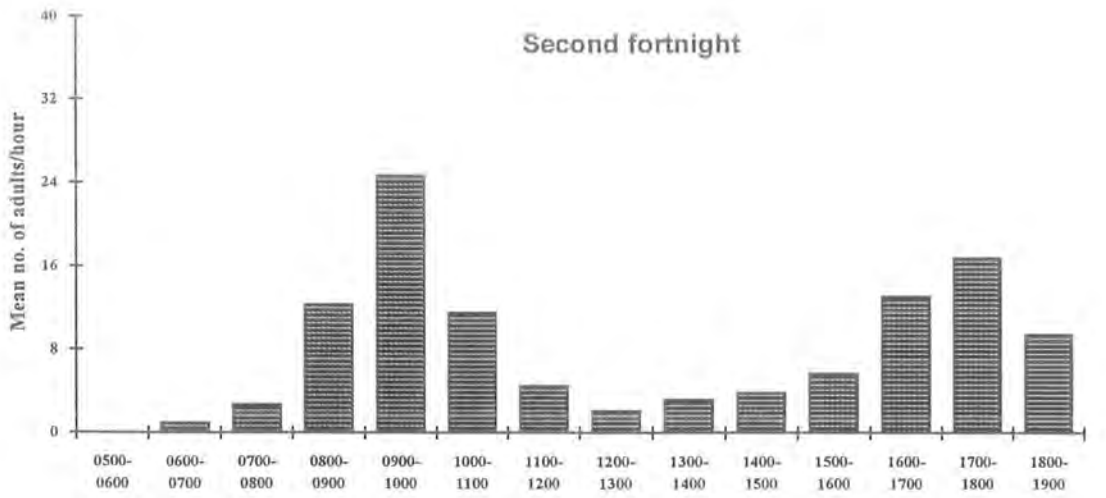


Fig.3 Hourly catches of whitefly adults on yellow sticky traps based on mean number during September at Central Cotton Research Institute,Multan

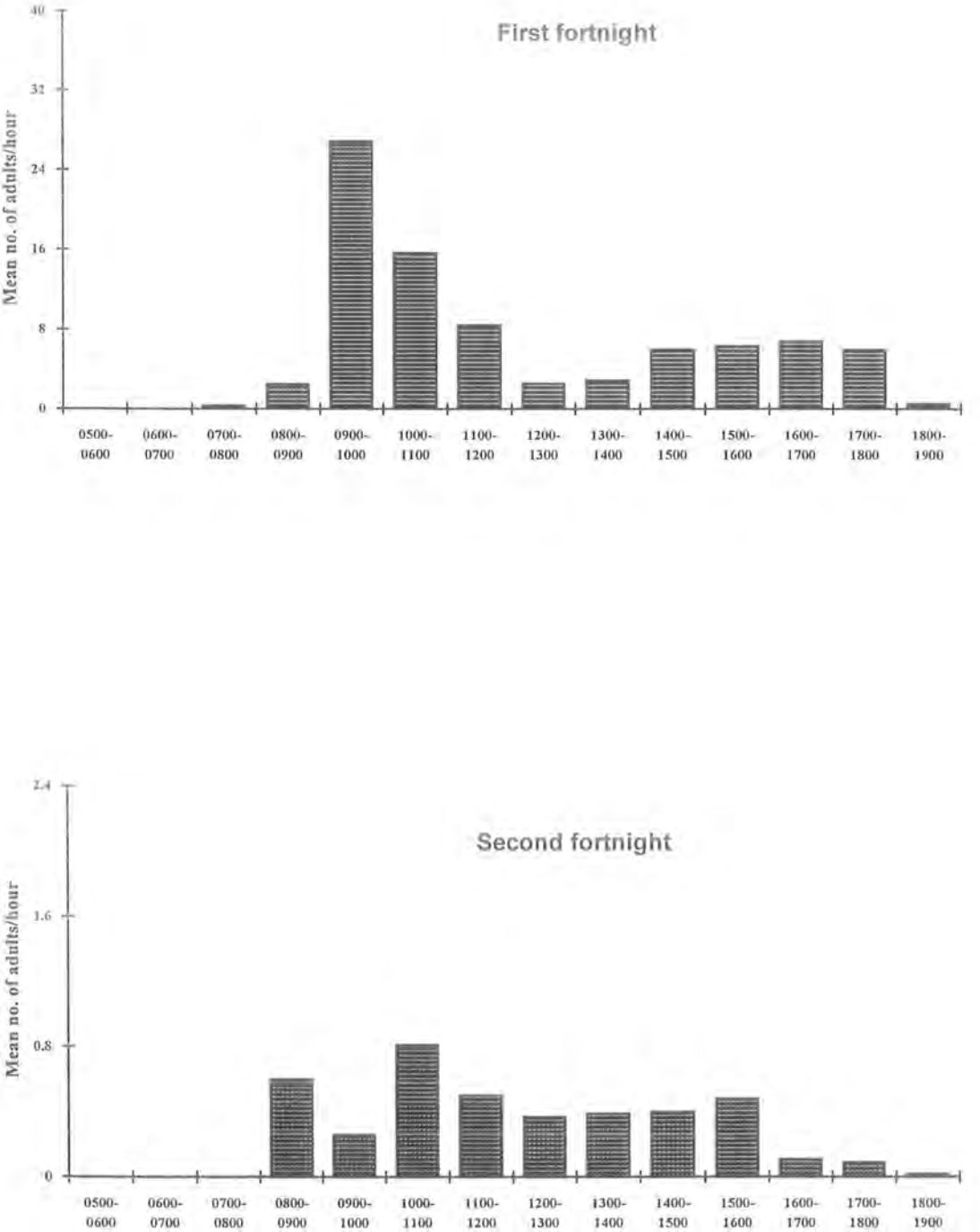


Fig.4 Hourly catches of whitefly adults on yellow sticky traps based on mean number during October at Central Cotton Research Institute, Multan.

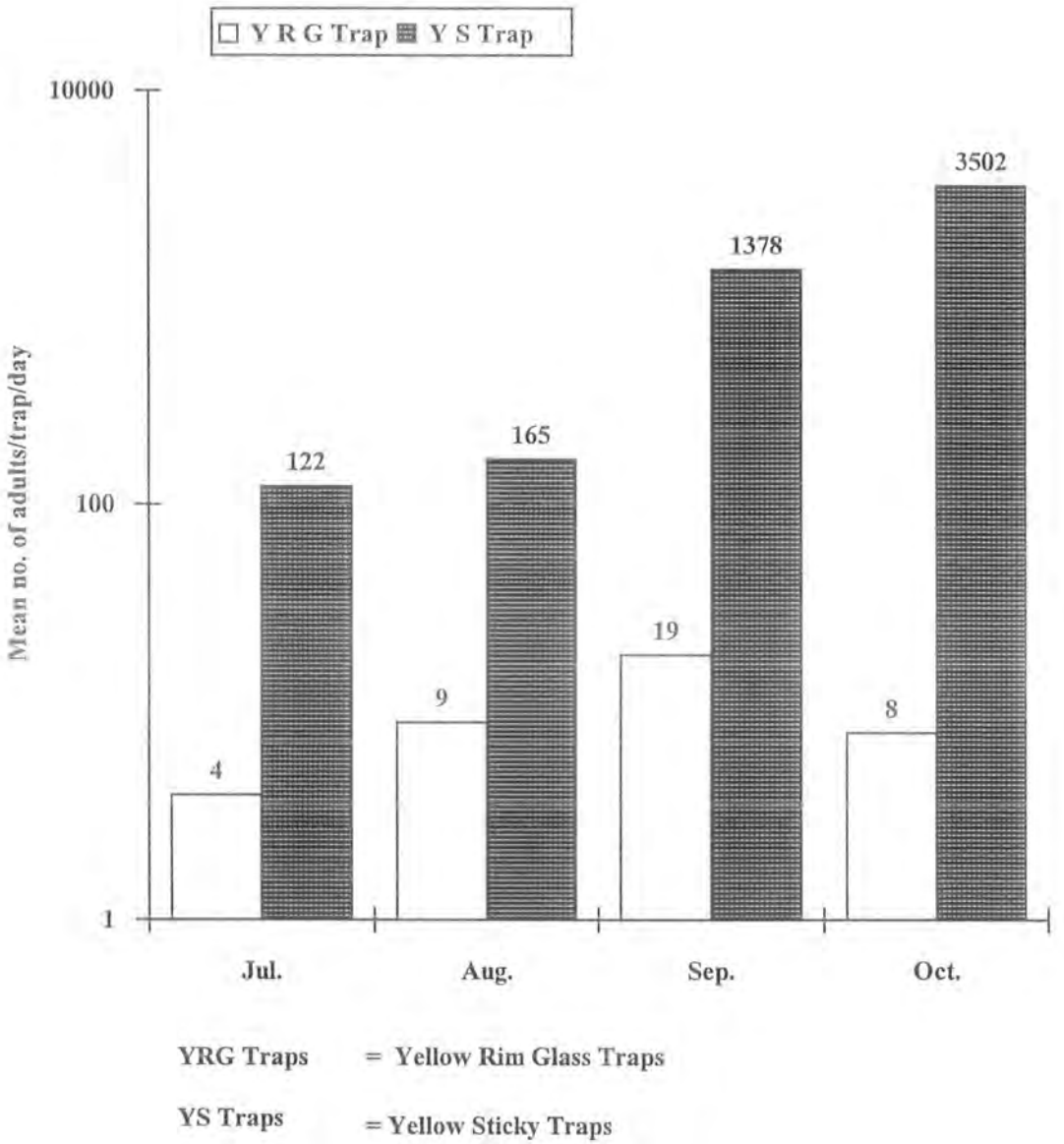


Fig.5 Mean number of whitefly adults caught /trap/day with two trap designs during July- October at Multan

Crops

Population levels of whitefly based on 100 cm^2 leaf area on 15 major hosts from crops and vegetables are presented in **Fig.6-20**. On *Mentha viridis* it was present throughout the year with a peak in September (**Fig.6**), on *Glycine max* it was present from March to May with a peak in May (**Fig.7**) and on *Solanum melongena* throughout the year maximum in September (**Fig.8**). On *Corchorus capsularis* it was present from May to November with a peak in August (**Fig.9**), on *Cucurbita pepo* var. *melo pepo* its population was found from October to May with peak in October (**Fig.10**), on *Cucumis melo* var. phut it was available from May to October with maximum population in October (**Fig.11**), on *Citrullus lanatus* var. *fistulosus* it was present from April to November highest population in April (**Fig.12**) and on *Cucumis melo* it was available from March to December with a peak in August (**Fig.13**). On *Ipomoea batatas* it remained active throughout the year with maximum population in October (**Fig.14**), on *Sesame indicum* it was found from June to October with highest in June (**Fig.15**), on *C. lanatus* it was available from March to November with a peak in July (**Fig.16**) and on *Vigna radiata* it was present from March to December with highest population in August (**Fig.17**). On *Capsicum frutescens* it was recorded throughout the year with highest population in October (**Fig.18**), on *Abelmoschus esculentus* it was present from March to December with a peak in October (**Fig.19**) and on *Cucumis sativus* it was available from February to May with maximum population in May (**Fig.20**)

The host plants on which whitefly was recorded in small numbers are regarded as minor hosts. Out of 26 minor hosts, it was found on 19 hosts for three to ten months during the year (**Table 7**). Highest population per 100 cm^2 was on *Raphanus sativus* in September (17.0), on *Brassica campestris* in September (15.0), on *Sesbania sesban* in September (12.0), on *Benincasa hispida* in September (11.5), on *Cucumis melo var.*

utilissma in May (7.4), on *Solanum turberosum* in October (5.5), on *Oleracea var. botrytis* in September (3.8), on *Helianthus annuus* in April (3.2), on *Cymopsis tetragonoloba* in August (2.4), on *Lycopersicumesculentum* in October (2.0), on *Lufa cylindrica* in September (1.9), on *Vigna cylindrica* in June (1.5), on *Hibiscus cannabinus* in June (1.3), on *Spinacea oleracea* in September (0.8), on *Momordica charantia* in September (0.7), on *Cucurbita pepo* and *Lagenaria siceraria* in April (0.6), on *Colocasia esculenta* in June (0.4) and on *Brassica rapa* in October(0.2).

On seven plants whitefly was recorded occasionally for one or two months only. On *Raphanus* sp. var. Red Radish it was in May (0.2) and in October (2.5). On *Daucus carota* it was in September (0.7) and in October (1.2), on *Trifolium alexandrium* in October (5.9) and in November (1.2), on *Beta vulgaris* in October (0.2) and in November (0.1), on *Pisum sativum* in October (1.5) and in November (0.2), on *Trigonella foenom-graceum* in October (9.0) and on *Lens culinaris* in March (0.01) and in April (0.02).

Fruit and forest plants

Whitefly infestation was recorded from 18 fruit and forest trees. These include 6 major and 12 minor hosts. The population trends on 6 major hosts are presented in **Fig. 21 to 26.** On *Broussonetia papyrifera* it was present from April to January with a peak in October **(Fig.21)** and on *Punica granatum* it was found from May to January with highest population in September **(Fig.22)**. On *Morus alba* it remained active throughout the year with maximum population in September **(Fig.23)**, on *Bauhinia purpurea* it was found throughout the year with a peak in June **(Fig.24)**, on *Albizzia lebbek* it was available throughout the year with a peak in July **(Fig.25)** and on *Psidium guajava* throughout the year with maximum population in October **(Fig.26)**.

Of the remaining 12 minor hosts whitefly was found for 3-10 months (**Table 8**). Highest population was found on *Ehretia laevis* (6.4) in October, on *Eucalyptus citriodora* in September (5.2), on *Moringa oleifera* in September (3.9), on *Acacia nilotica* in October (1.4), on *Morus laevigata* in Agusut (1.3), on *Leucaena leucophylle* in October (1.1), on *Ficus carica* in October (0.8), on *Salmalia malabarica* in December (0.8), on *Cassia fistula* in September (0.6), on *Vitis vinifera* in August (0.5), on *Pongamia pinnata* in August (0.4) and on unidentified species A in September (0.3).

Ornamental plants

This group included 41 plant species as hosts of whitefly. Out of these 13 were major, 26 minor and five incidental hosts. Population trends on major hosts are presented in **Fig.27-39**. On *Gardenia jasminoides* it was present throughout the year with a peak in September (**Fig.27**), on *Chrysanthemum morifolium* it was found throughout the year with highest population in September (**Fig.28**), on *Ipomoea cairiea* it was available from May to February with maximum population in September (**Fig.29**), on *Tagetes erecta* it was found from May to March with highest population in October (**Fig.30**) and on *Ageratum conyzoides* it was available throughout the year with peak population in September (**Fig.31**). On *Helianthus annuus* (ornamental) it was found throughout the year with highest population in September (**Fig.32**), on *Hibiscus rosa-sinensis* it was available throughout the year with a peak in October (**Fig.33**), on *Lantana camara* it was found throughout the year with highest population in September (**Fig.34**), on *Gomphrena globosa* it was found from June to November with a peak in October (**Fig.35**), on *S. melongena* (ornamental variety) it was present from April to February with highest population in August (**Fig.36**), on *Euphorbia pulcherrima* it was available throughout the year with maximum population in September (**Fig.37**), on *Ipomoea tricolor* it was found throughout the year with a peak in September (**Fig.38**)

and on *Hibiscus mutabilis* it was present throughout the year with highest population in October (**Fig.39**).

On 22 minor hosts population fluctuated at low level for 3-10 months (**Table 9**). Maximum individuals per 100 cm^2 were found on *Campsis undulata* in September (167.3), on *Althea rosea* in October (39.3), on *Ruellia tuberosa* in October (11.4), on *Tecoma stans* in September (9.9), on *Lathyrus odorantus* in September (9.9), on *Erythrina suberosa* in July (9.1), on *Cestrum nocturnum* in October (8.8), on *Antigonon leptopus* in September (6.9), on *Impatiens balsamina* in September (5.4), on *Celosia argentia* var. *cristata* in October (4.6), on *Ipomoea carnea* in September (3.9), on *Vitex agnus-costus* in September (3.2), on *Lawsonia inermis* in September (2.9), on *Hamelia patens* in June (2.5), on *Ocimum basilicum* in October (1.9), on *Gerbera Jamesonii* in October (1.9), on *Elettaria cardamum* in July (1.8), on *Ipomoea purpurea* in September (1.1), on *Verbena hybrida* in May (0.6), on *J. multiflorum* in June and September (0.5) on *Bougainvillea glabra* in September (0.4) and on *Argyrelia speciosa* in January (0.1).

On the six occasional hosts it was found on, unidentified species (Gul-Mukhi) in February (0.5) and in March (0.03), on *Buddieja paniculata* in September (0.7), on *Mathola incaria* in October (19.5) and November (26.6), on *Tropaeolum majus* in October (13.8) and November (0.2), on *Ceniraria hybrida* in December (0.2) and on *Jasminum rigidum* in October (0.3); for one or two months only.

Weeds

Whitefly infestation was found on 45 weeds. Of these 14 were major hosts. Population trends of these are presented in **Fig. 40-53**. On *Corchorus triloailanis* it was present from May to November with a peak in September (**Fig.40**), on *Euphorbia hirta*

it was present from May to February with highest population in September (**Fig.41**), on *Phyllanthus pilulifera* it was found from April to December with maximum population in September (**Fig.42**) and on *Conyza bonariensis* it was found throughout the year with a peak in November (**Fig.43**), on *Convolvulus arvensis* it was available throughout the year with highest population in October (**Fig.44**), on *Eclipta alba* it was present throughout the year with maximum population in October (**Fig.45**), on *Malvastrum tricuspidatum* it was active throughout the year with a peak in September (**Fig.46**). On *Solanum xanthocarpum* its popualtion was available throughout the year with highest popuation in September (**Fig.47**), on *Achyranthes aspera* it was present throughout the year with a peak in October (**Fig.48**). On *Polygonum sp.* it was present from April to February with highest population in September (**Fig.49**), on *Citrullus tetragona* it was active from April to December with a peak in September (**Fig.50**), on *Xanthium strumarium* it was available from April to January with highest population in October (**Fig.51**), on *Physalis alkakengi* it was available from April to February with a peak in October (**Fig.52**) and *Solanum nigrum* it was active throughout the year with a peak in October (**Fig.53**).

Whitefly infestation on 26 minor hosts was found for three to ten months. Population data of minor hosts are presented in **Table 10**. Highest number per 100 cm^2 was present on *unidentified weed-4* in September (148), on *Euphorbia prostrata* in October (30.4), on *E. helioscopia* in October (30.4), on *unidentified weed-1* in September (30.0), on *Digera arvensis* in October (29.0), on *Datura metel* in September (28.0), on *Cassia absus* in September (25.0), on *Verbena officianalis* in September (14.3), on *Trianthema portulacastrum* in December (9.1), on *Rhynchosia* sp. in September (9.0), on *Withania somnifera* in September (8.0), on *Tribulus terrestris* in

September (8.0), on *Cleome viscosa* in October (6.1), on *unidentified weed-3* in July (6.0), on *Unidentified weed-2* in June (5.5), on *Nicotiana plumbaginifolia* in October (5.2), on *Amarantus viridis* in October (4.4), on *Mentha longifolia* in June (3.5), on *Chenopodium murale* in November (1.9), on *Melilotus indica* in November (1.2), on *Cnicus arvensis* in October(1.2), on *Malva parviflora* in May (1.0), on *Abutilon indicum* in November (0.9), on *Ipomoea aquatica* in August (0.8), on *C. album* in October (0.6) and *Rumex dentatus* in October (0.6).

Among the 5 occasional hosts, it was present on *Heliotropium euphorium* in April (1.4) and in June (7.0), on *Corchorus olitorius* in September (0.2) and October (1.0), on *unidentified weed-5* in October (0.2) and November (0.5), on *Leuces cephalates* in September (4.7) and October (6.3) and on *Anagallis avensis* in December (0.9).

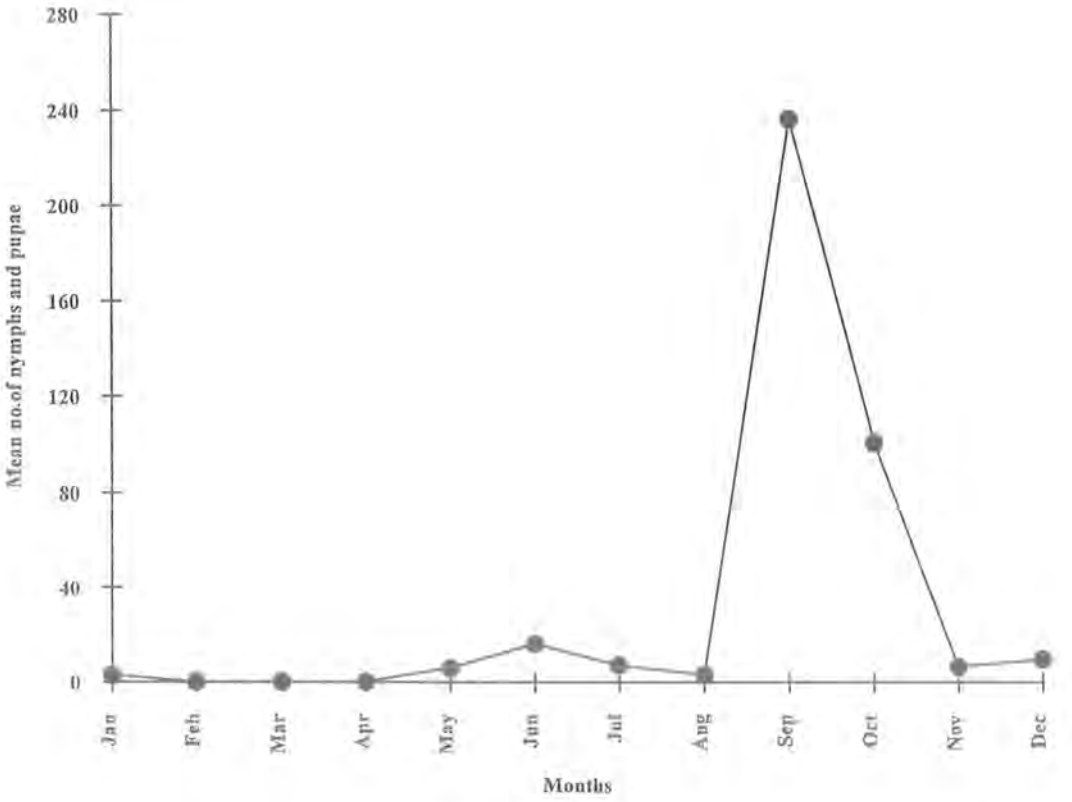


Fig.6 Population trends of *Bemisia tabaci* on *Mentha viridis* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96.

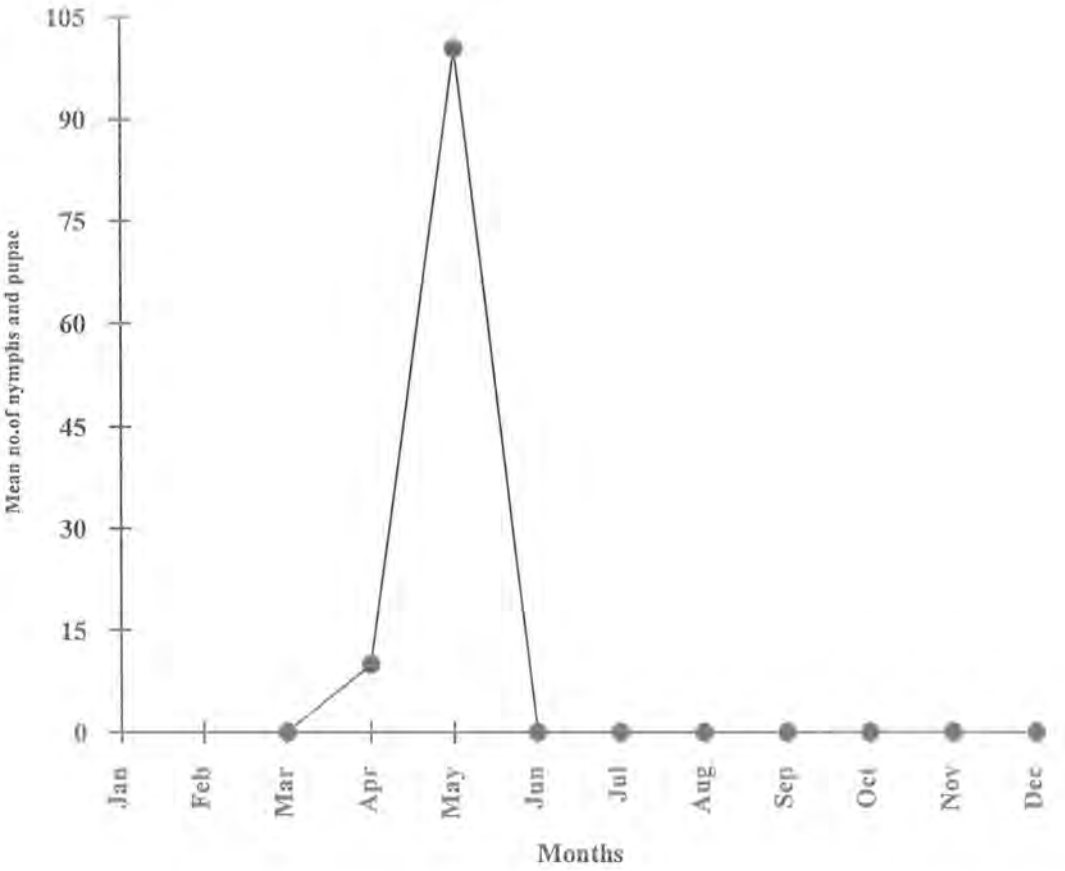


Fig.7 Population trends of *Bemisia tabaci* on *Glycine max* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96.

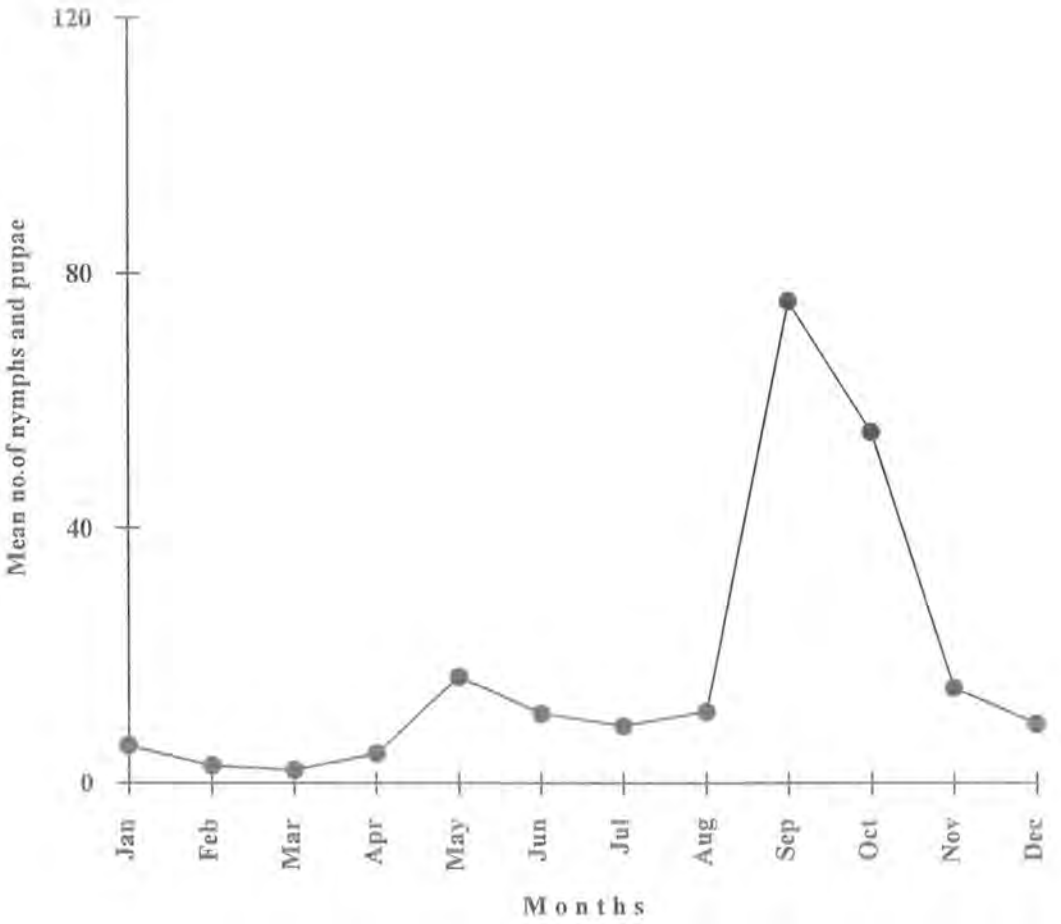


Fig. 8 Population trends of *Bemisia tabaci* on *Solanum melongena* based on mean number of nymphs and pupae/100cm^2 leaf area during 1993-96.

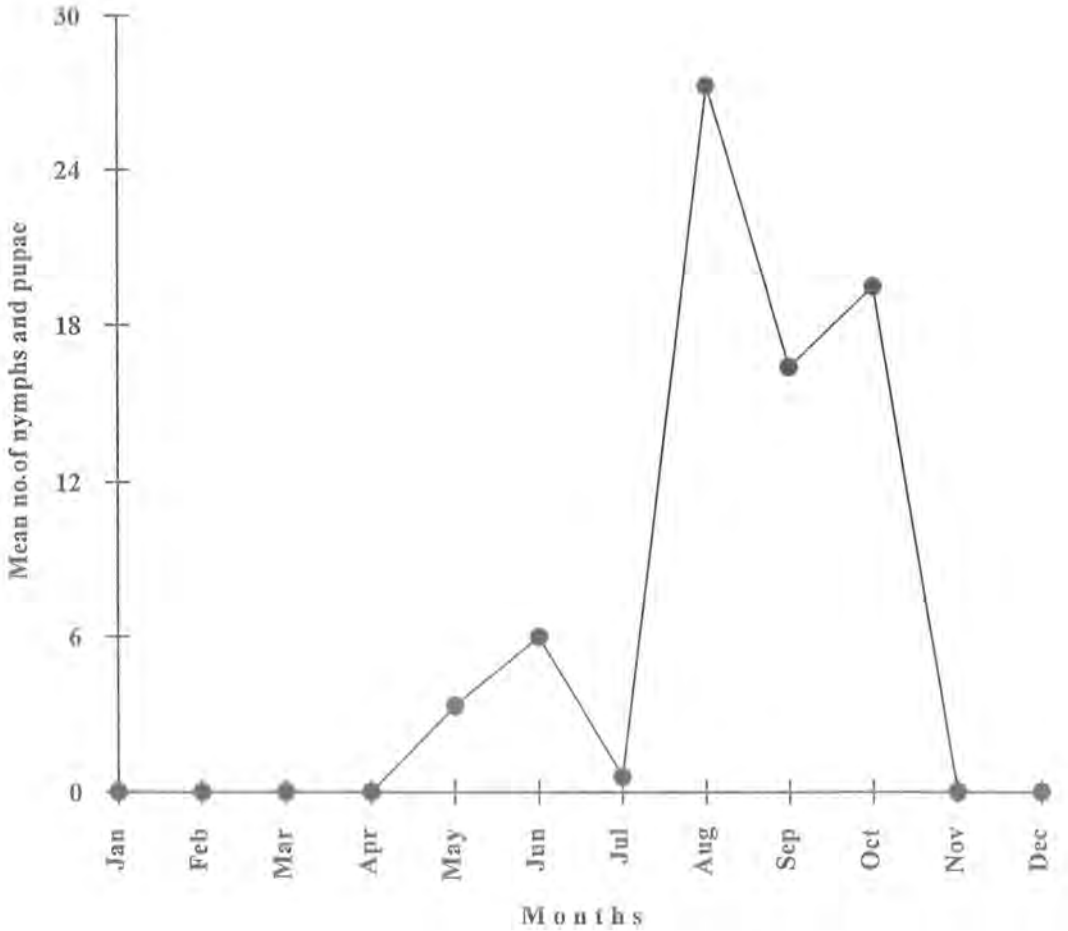


Fig.9 Population trends of *Bemisia tabaci* on *Corchorus capsularis* based on mean number of nymphs and pupae/100cm^2 leaf area during 1993-96.

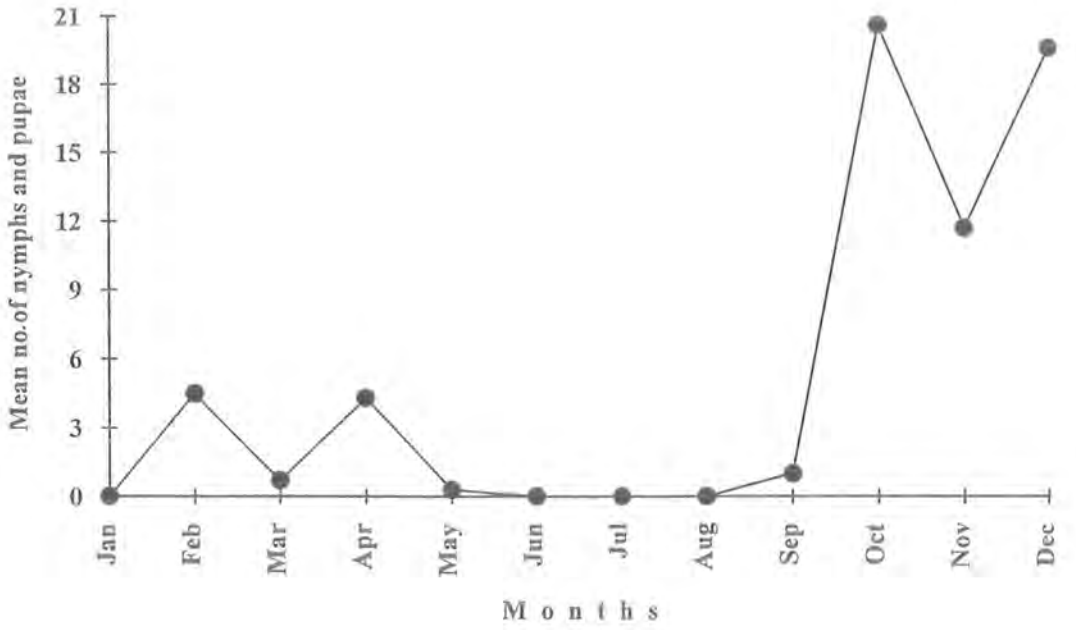


Fig.10 Population trends of *Bemisia tabaci* on *Cucurbita pepo* var *melopepo* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96.

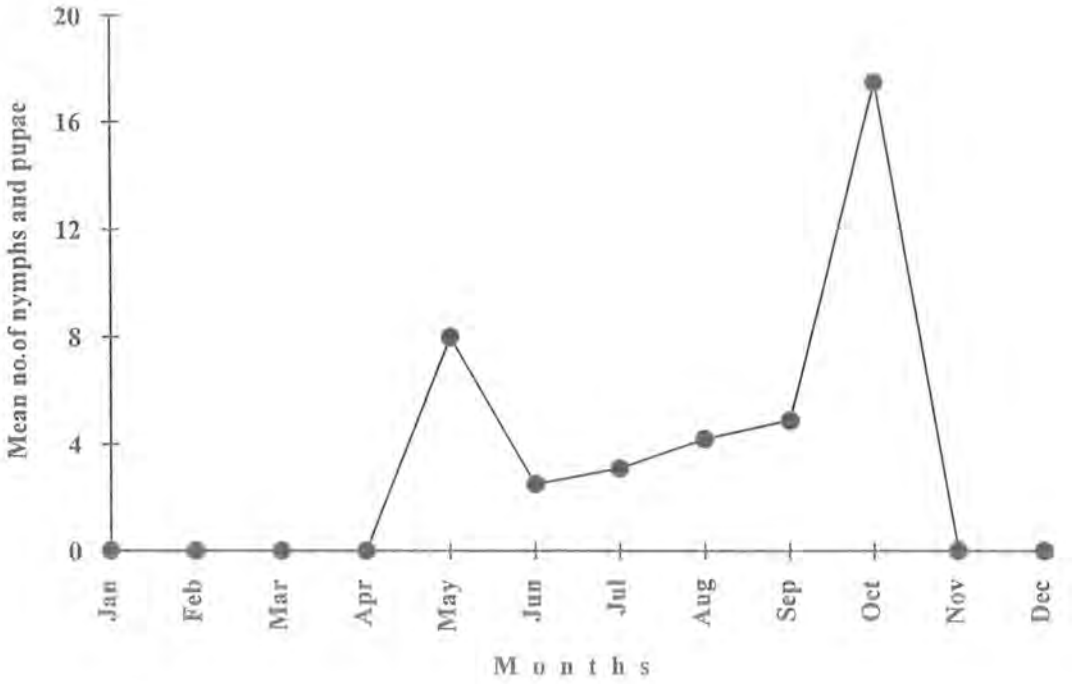


Fig.11 Population trends of *Bemisia tabaci* on *Cucumis melo* var. phut based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96.

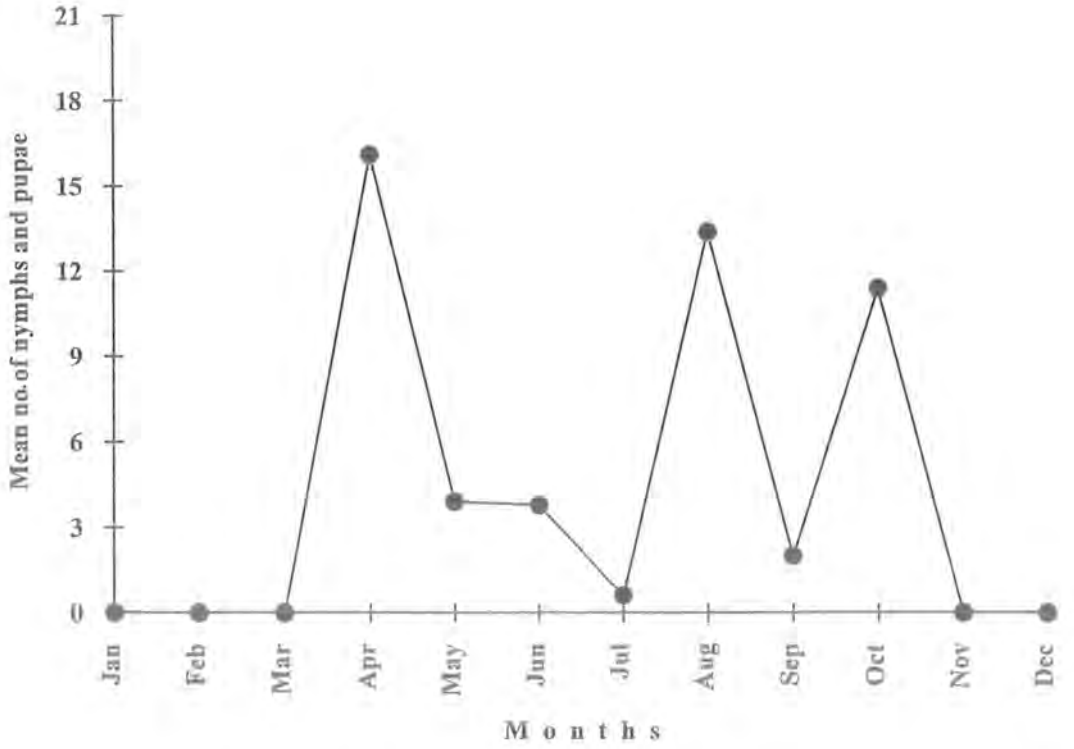


Fig.12 Population trends of *Bemisia tabaci* on *Citrullus lanatus* var. *fistulosus* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96.

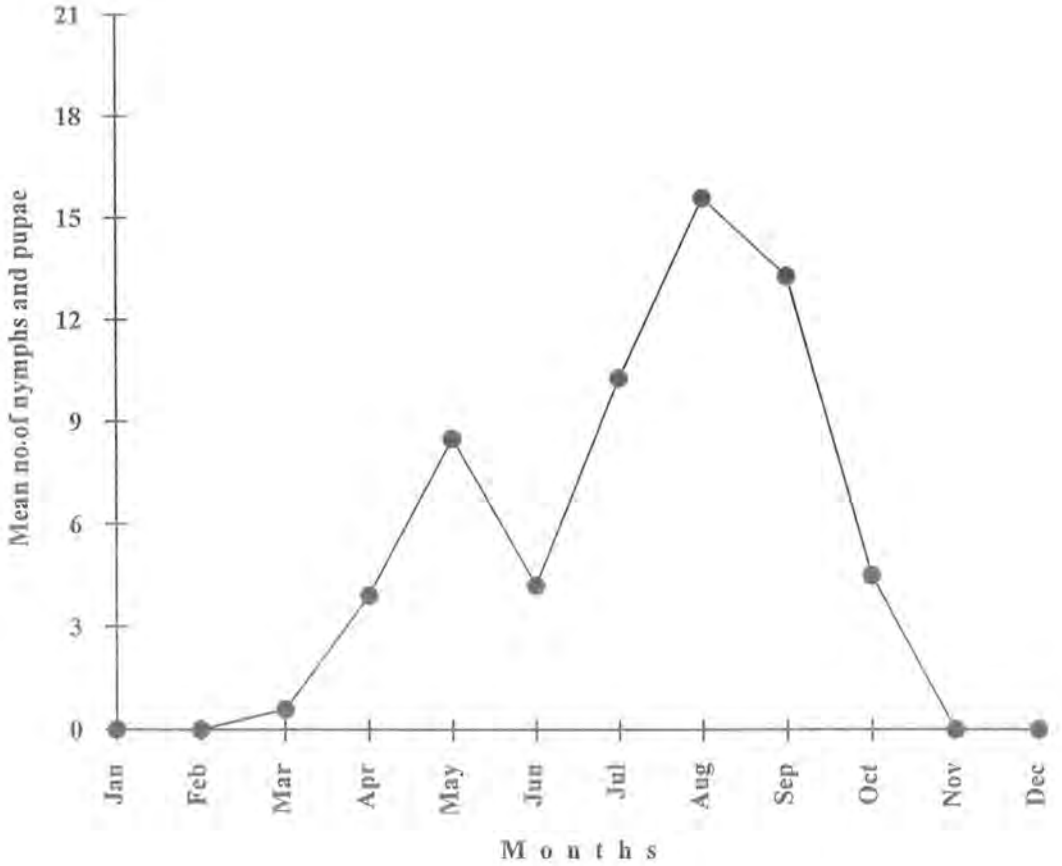


Fig.13 Population trends of *Bemisia tabaci* on *Cucumis melo* based on mean number of nymphs and pupae/ 100cm^2 leaf area during 1993-96.

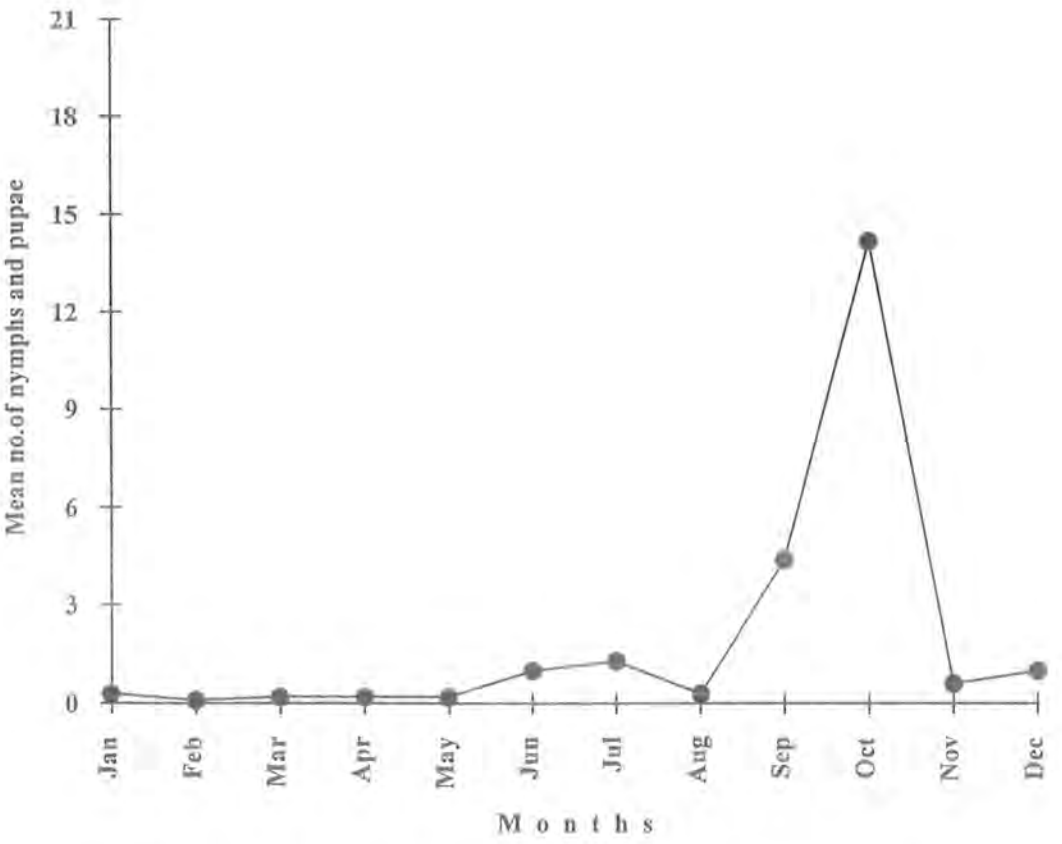


Fig.14 Population trends of *Bemisia tabaci* on *Ipomoea batatas* based on mean number of nymphs and pupae 100 cm^2 leaf area during 1993-96.

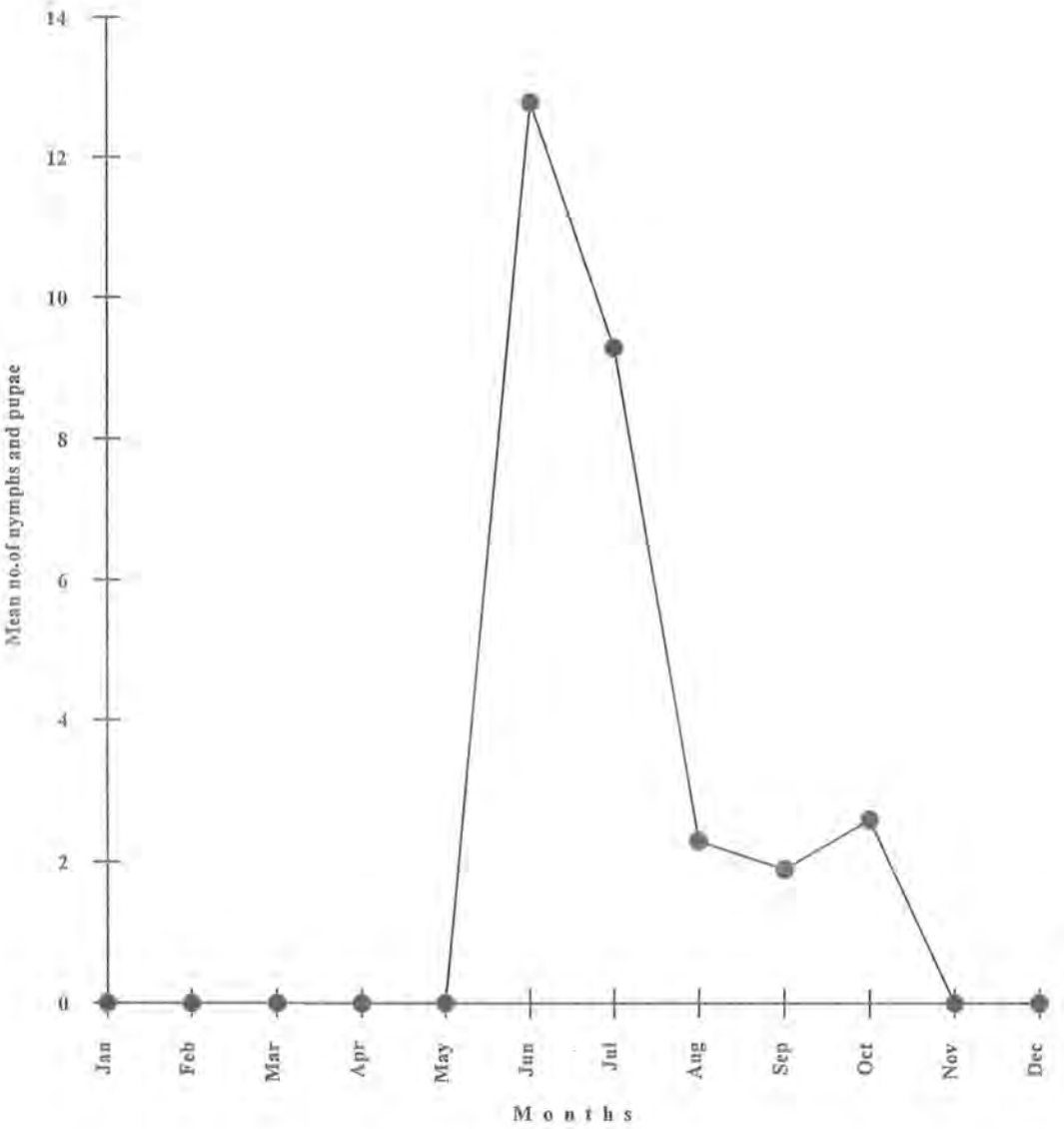


Fig.15 Population trends of *Bemisia tabaci* on *Sasame indicum* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96.

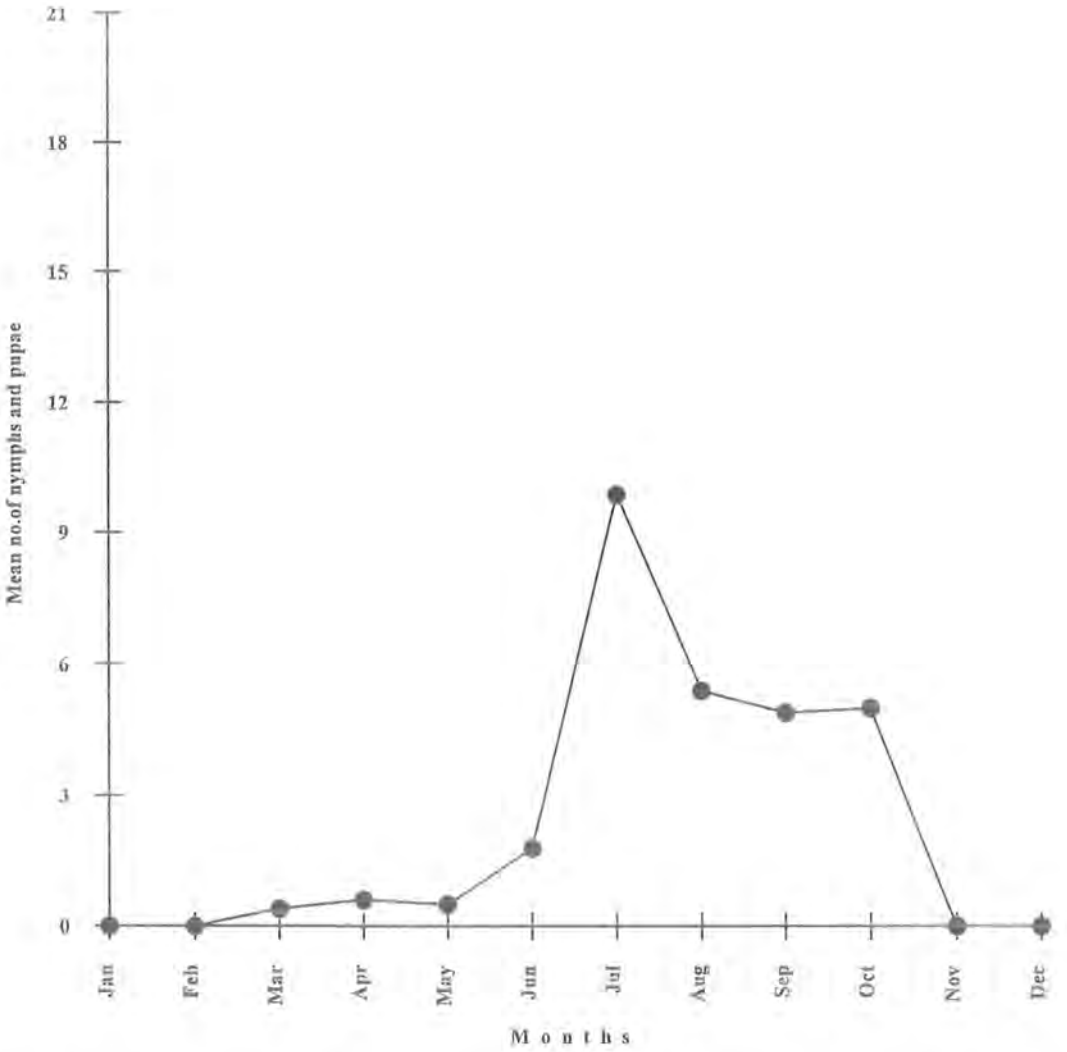


Fig.16 Population trends of *Bemisia tabaci* on *Citrullus lanatus* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96.

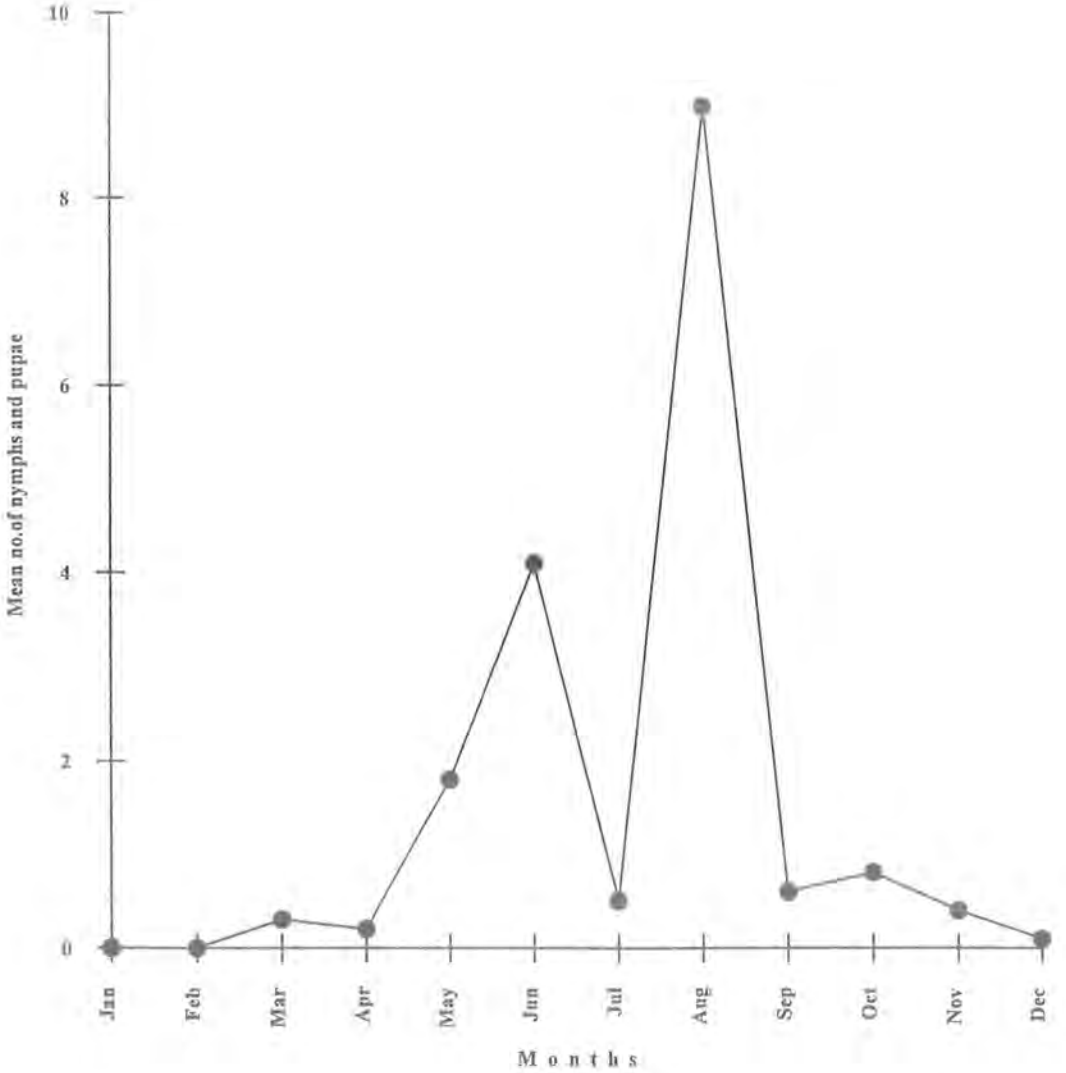


Fig.17 Population trends of *Bemisia tabaci* on *Vigna radiata* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96.

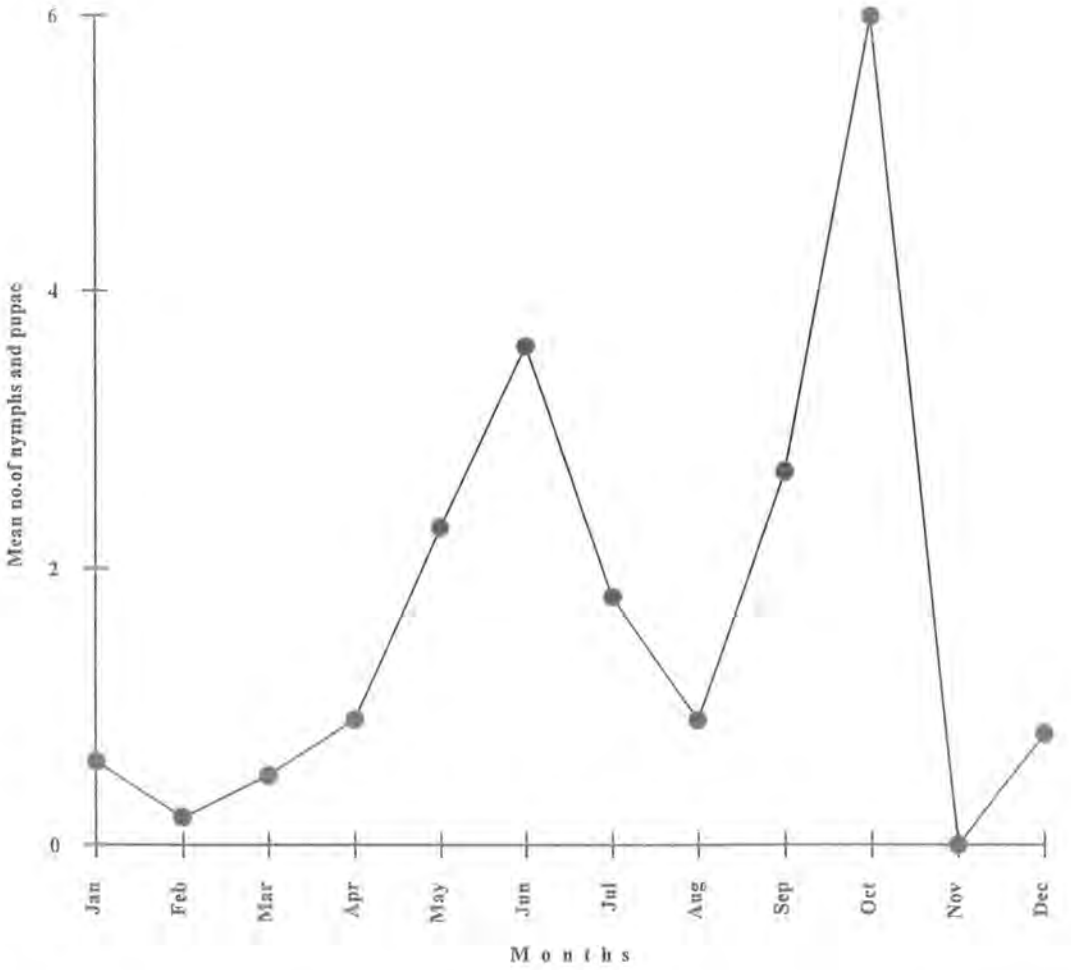


Fig.18 Population trends of *Bemisia tabaci* on *Capsicum frutescens* based on mean number of nymphs and pupae/100 cm2 leaf area during 1993-96.

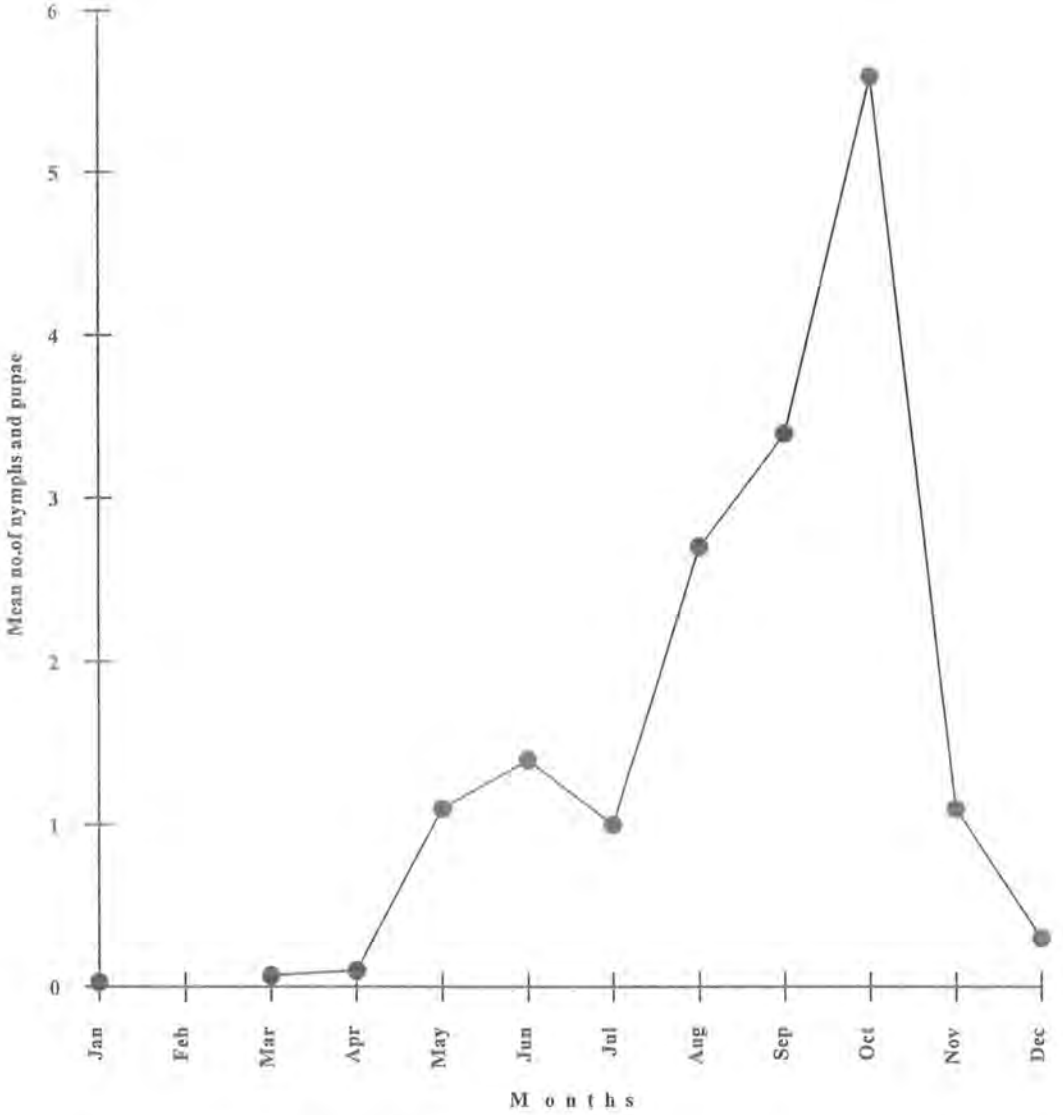


Fig.19 Population trends of *Bemisia tabaci* on *Abelmoschus esculantus* based on mean number of nymphs and pupae/100 cm^2 leaf areaduring 1993-96.

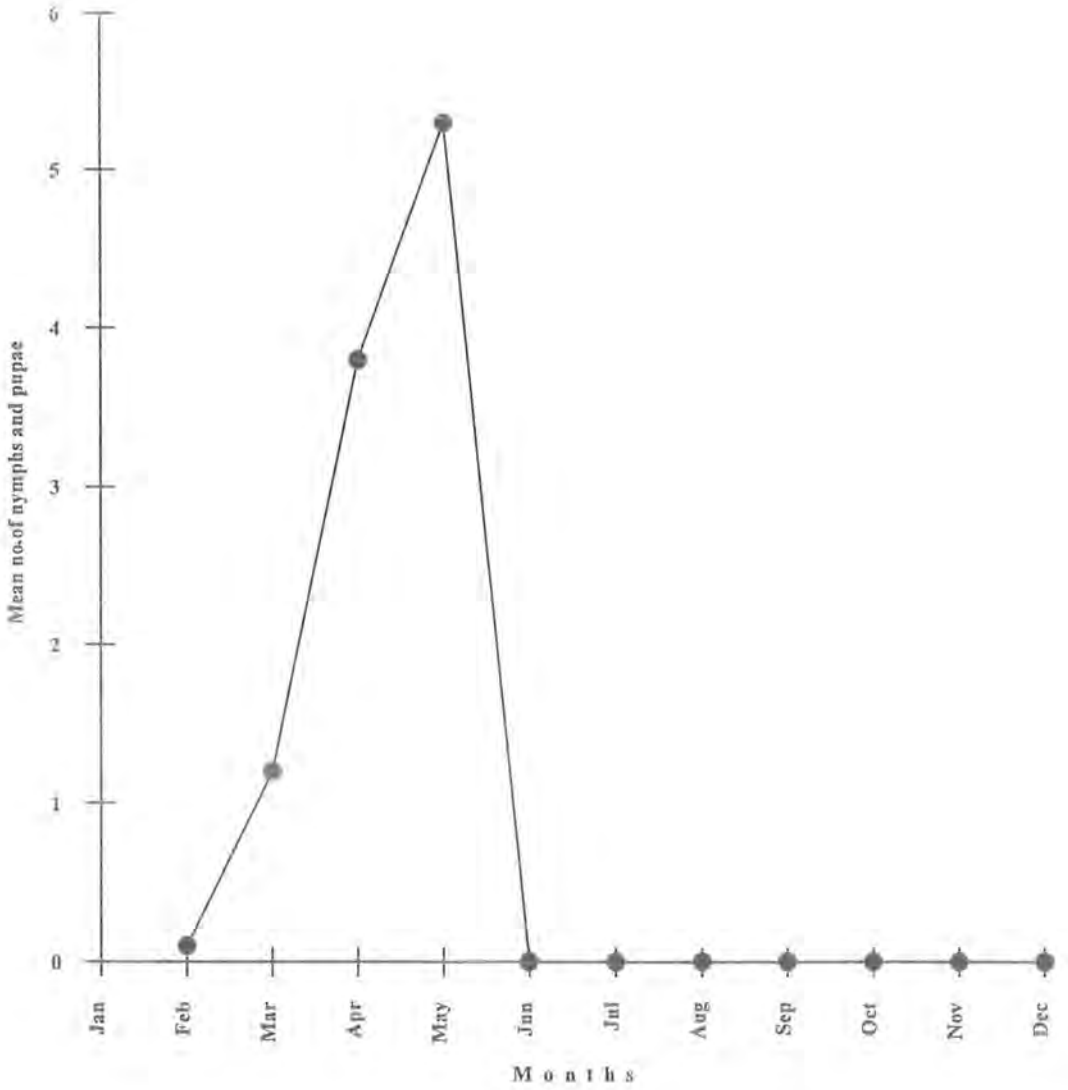


Fig.20 Population trends of *Bemisia tabaci* on *Cucumis sativus* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96.

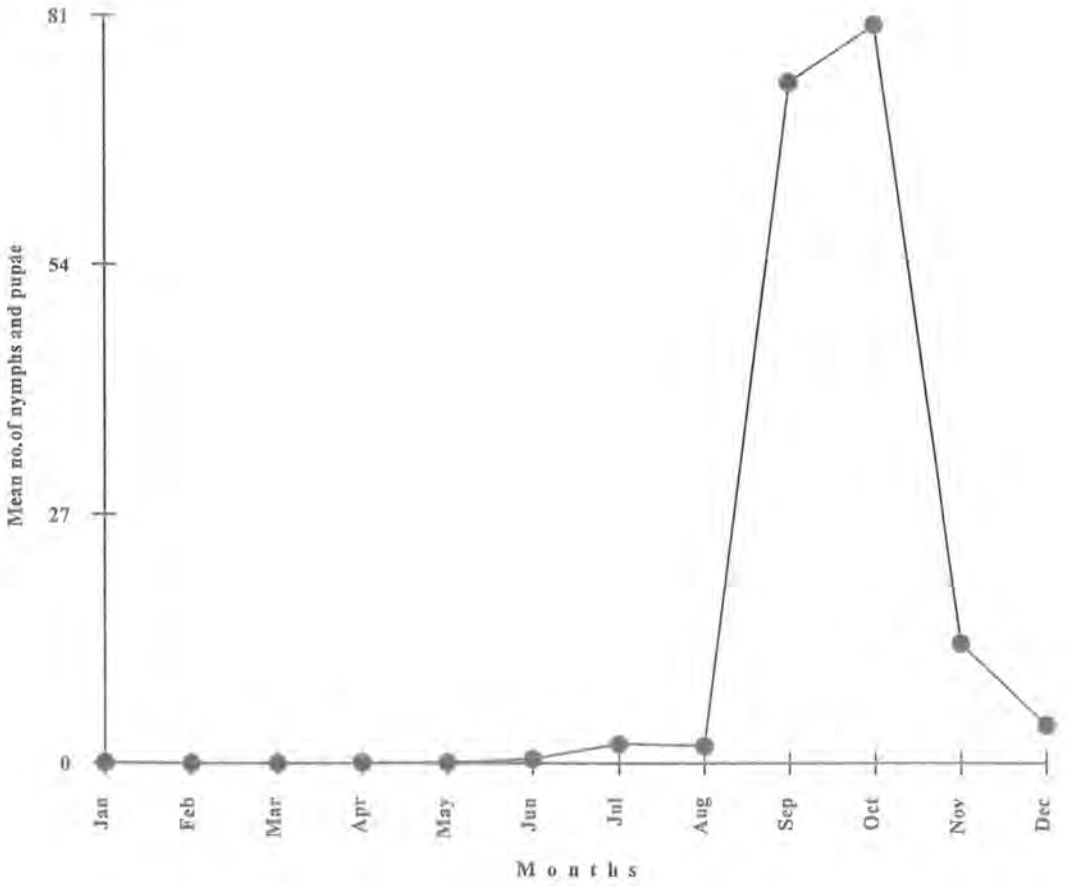


Fig.21 Population trends of *Bemisia tabaci* on *Broussonetia papyrifera* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-1996

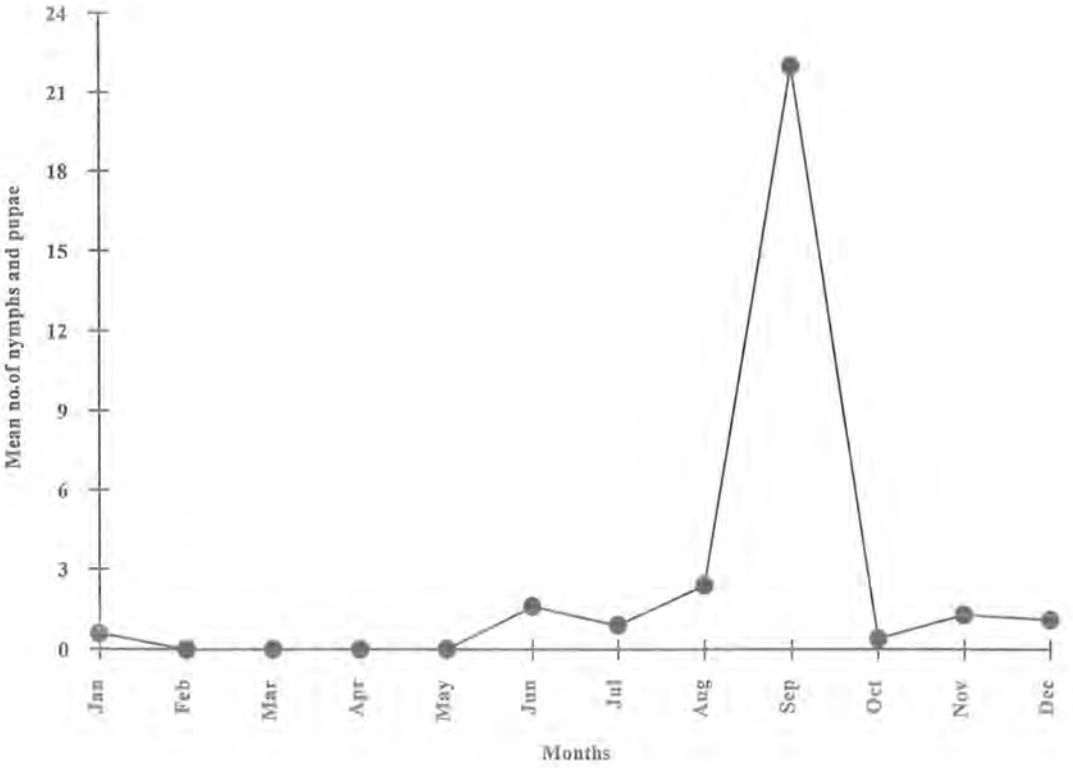


Fig.22 Population trends of *Bemisia tabaci* on *Punica granatum* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-1996

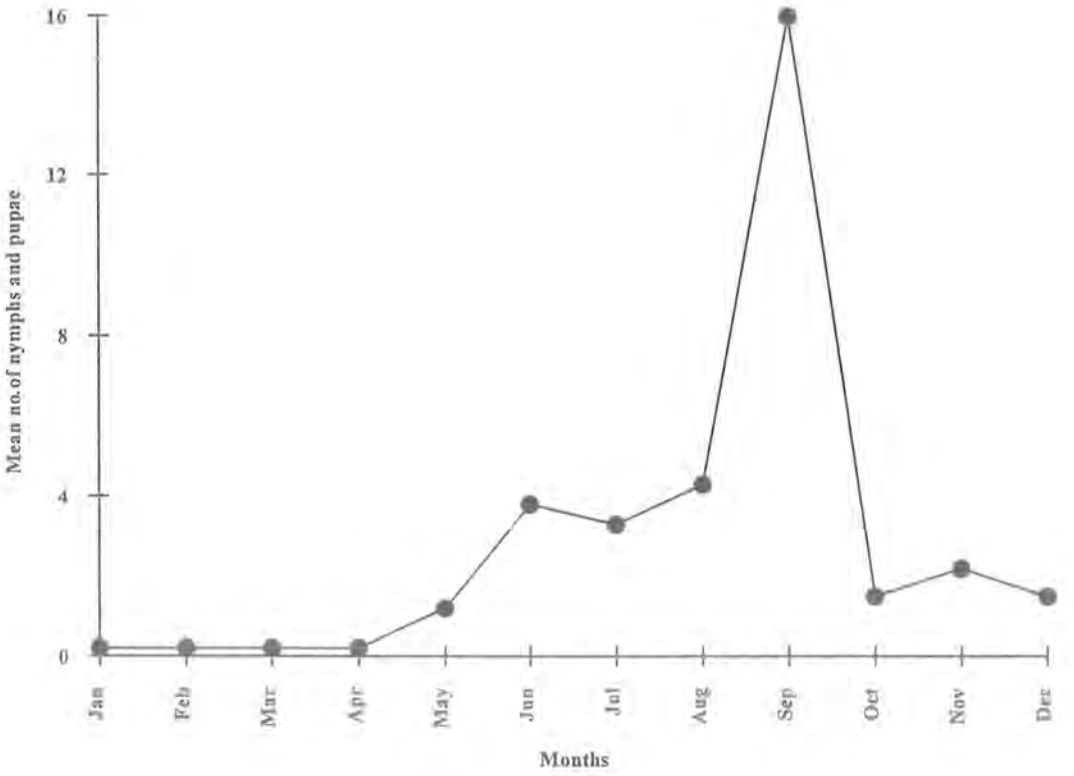


Fig.23 Population trends of *Bemisia tabaci* on *Morus alba* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-1996

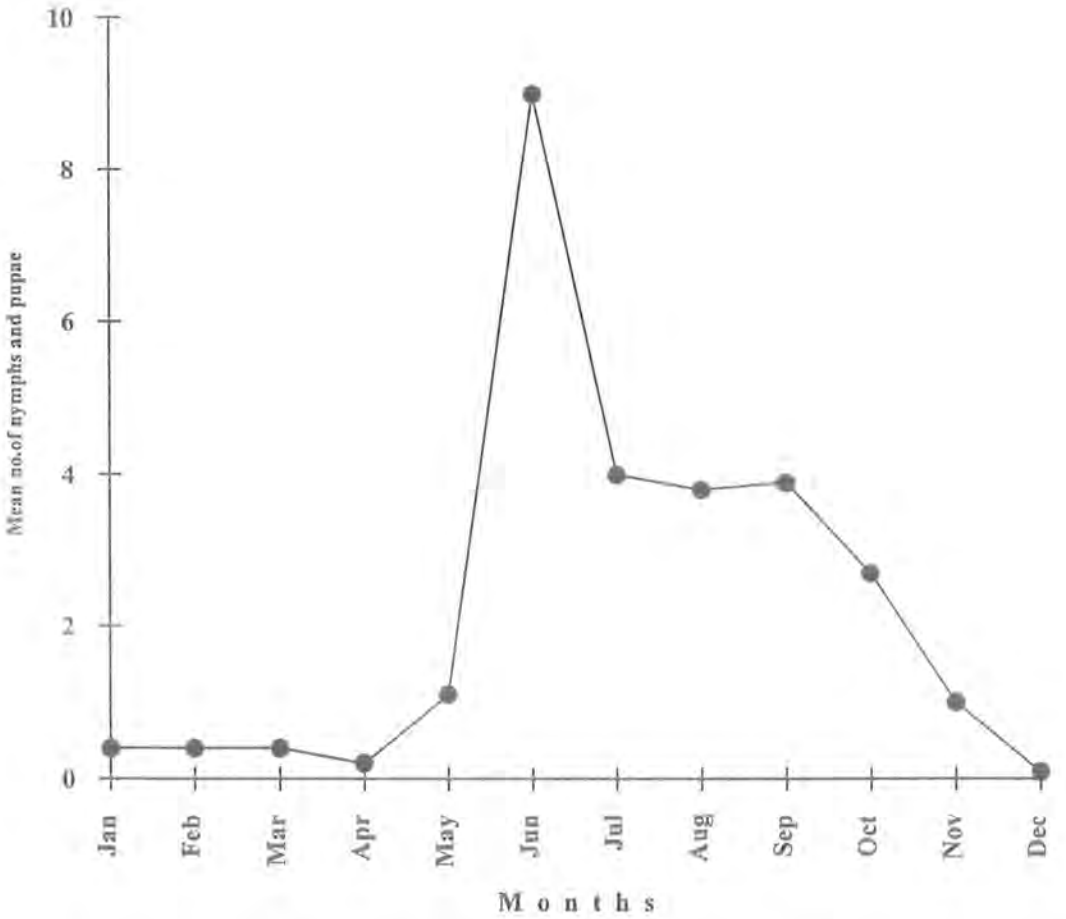


Fig.24 Population trends of *Bemisia tabaci* on *Bauhinia purpurea* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-1996

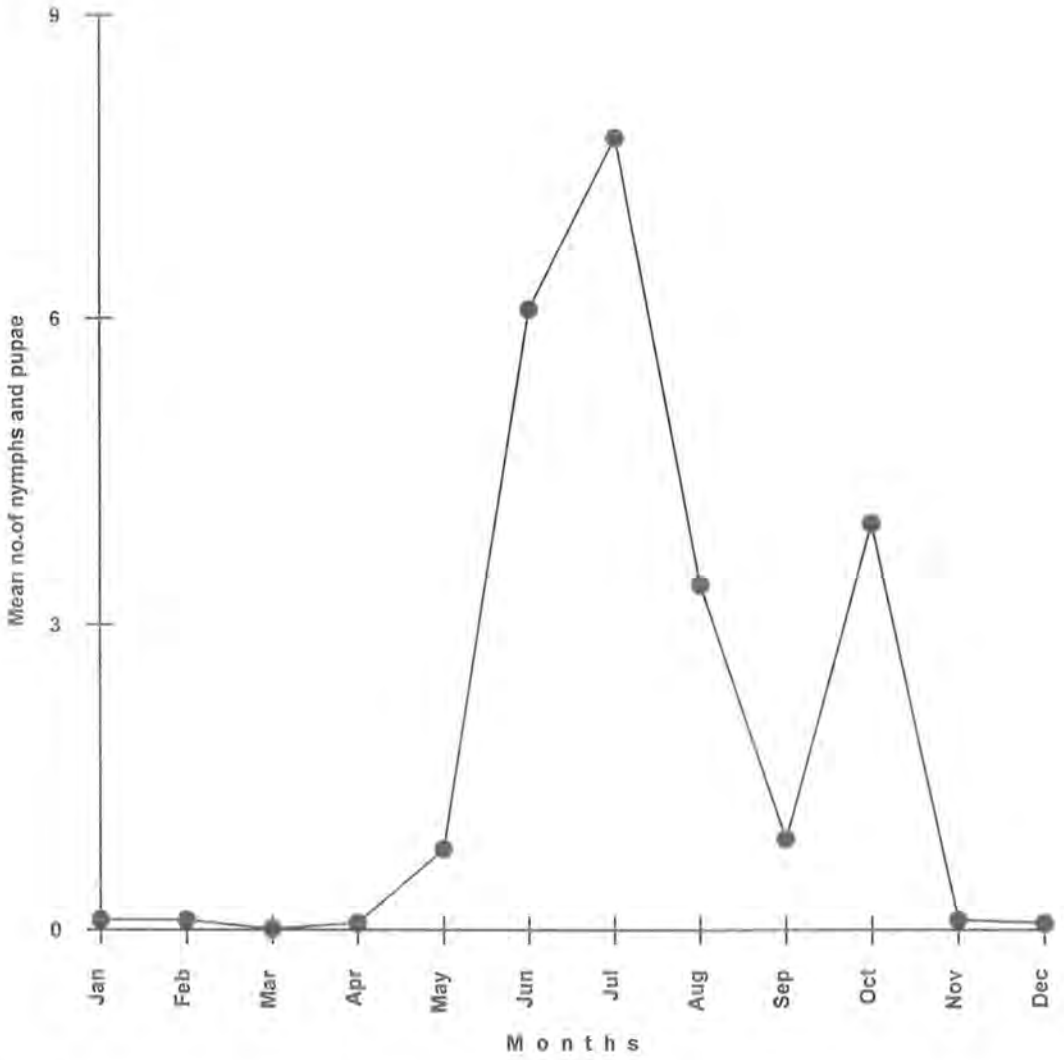


Fig.25 Population trends of *Bemisia tabaci* on *Albizzia lebbek* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-1996

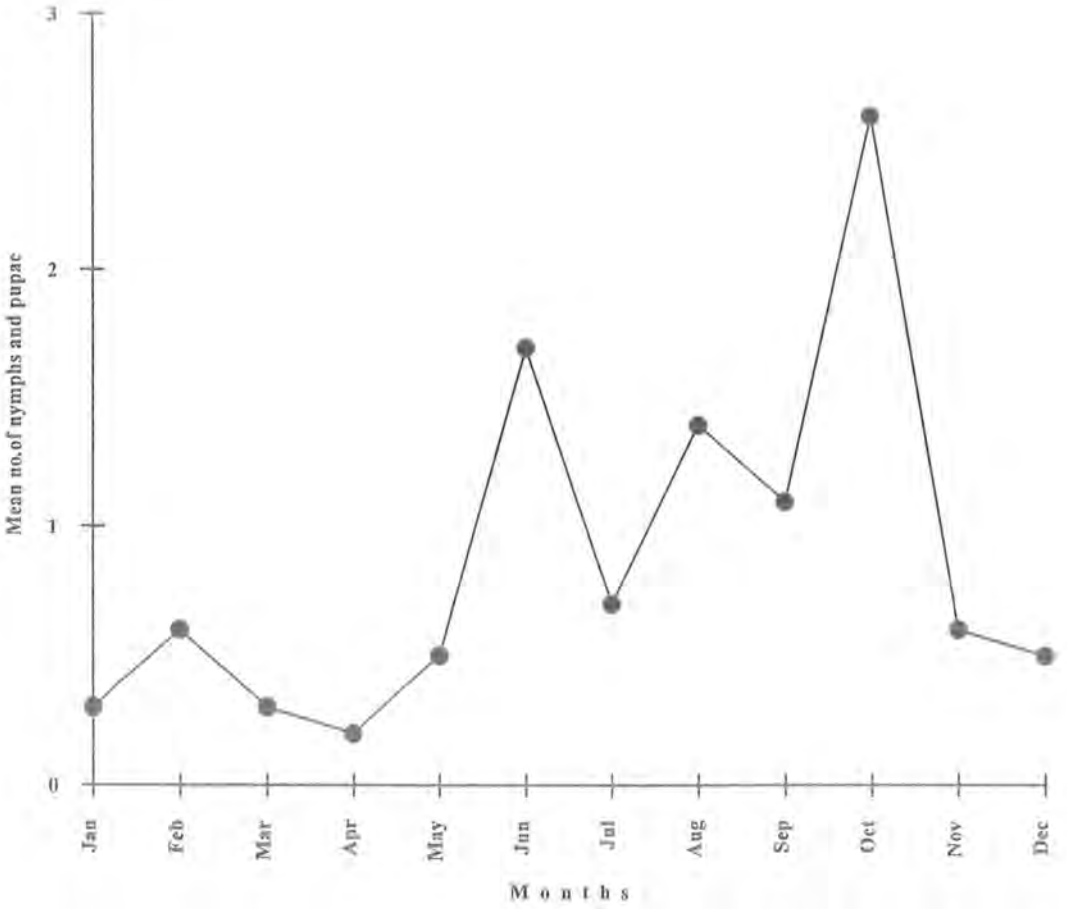


Fig.26 Population trends of *Bemisia tabaci* on *Psidium guajava* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-1996

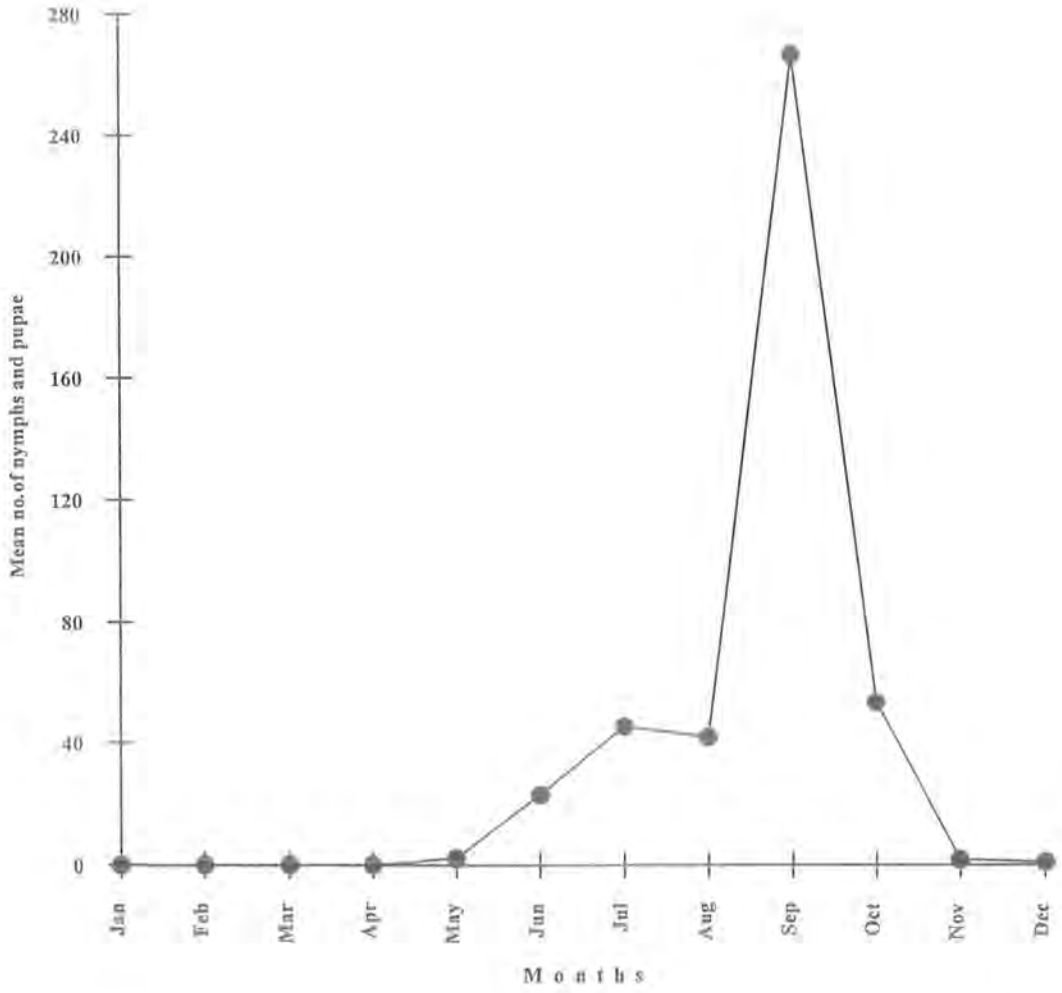


Fig.27 Population trends of *Bemisia tabaci* on *Gardenia jasminoides* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96

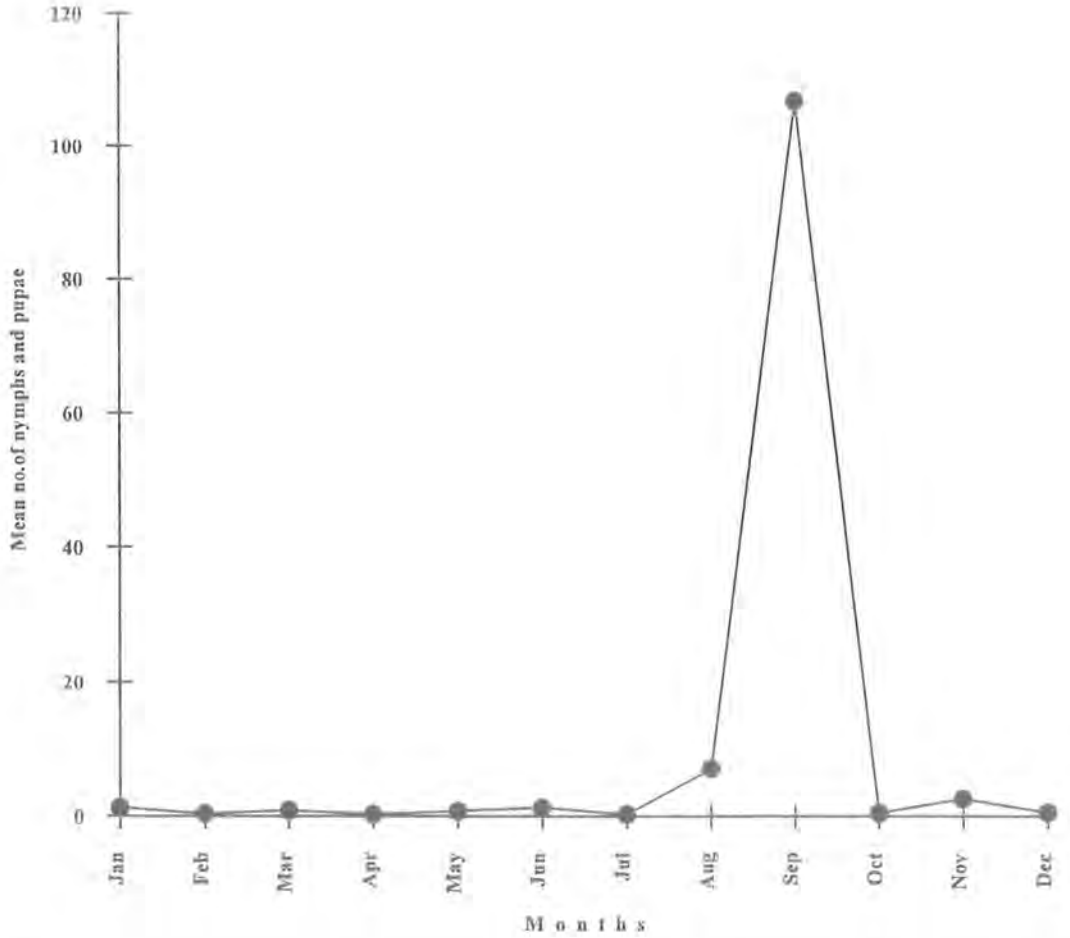


Fig.28 Population trends of *Bemisia tabaci* on *Chrysanthemum morifolium* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96

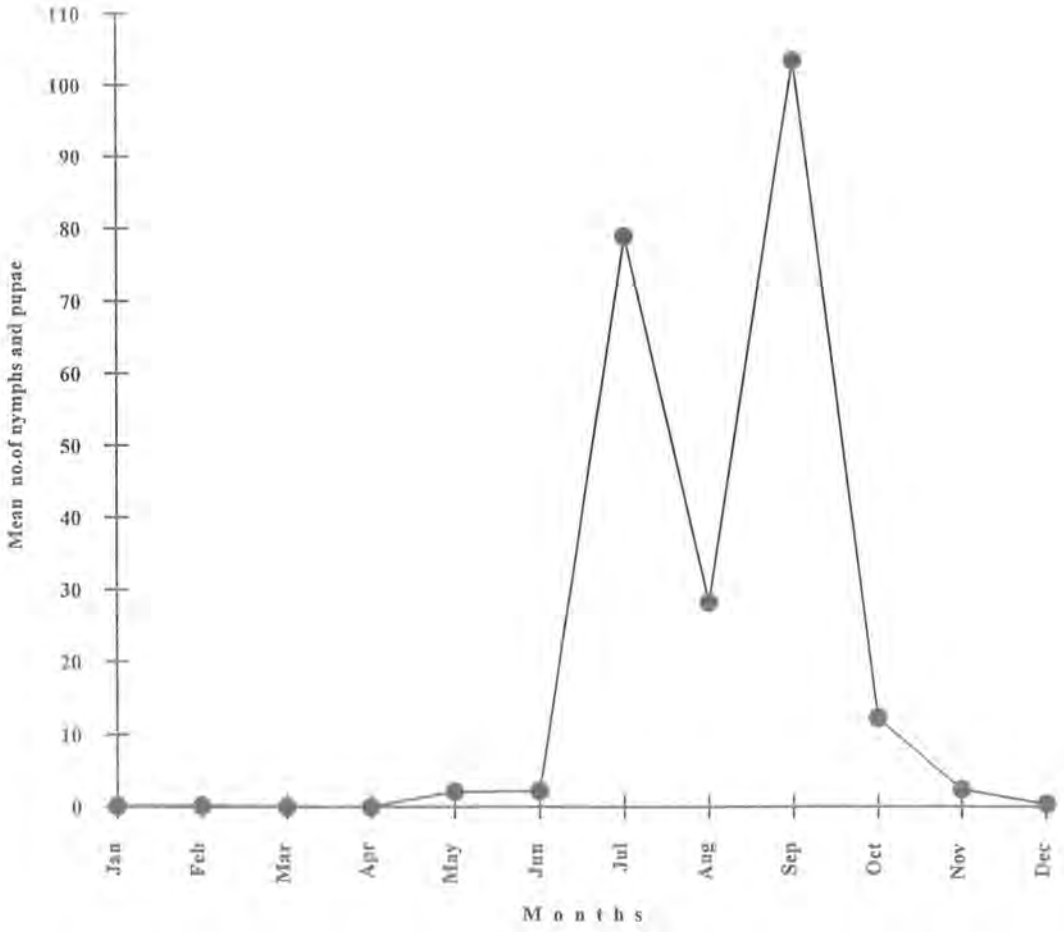


Fig.29 Population trends of *Bemisia tabaci* on *Ipomoea cairiea* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96

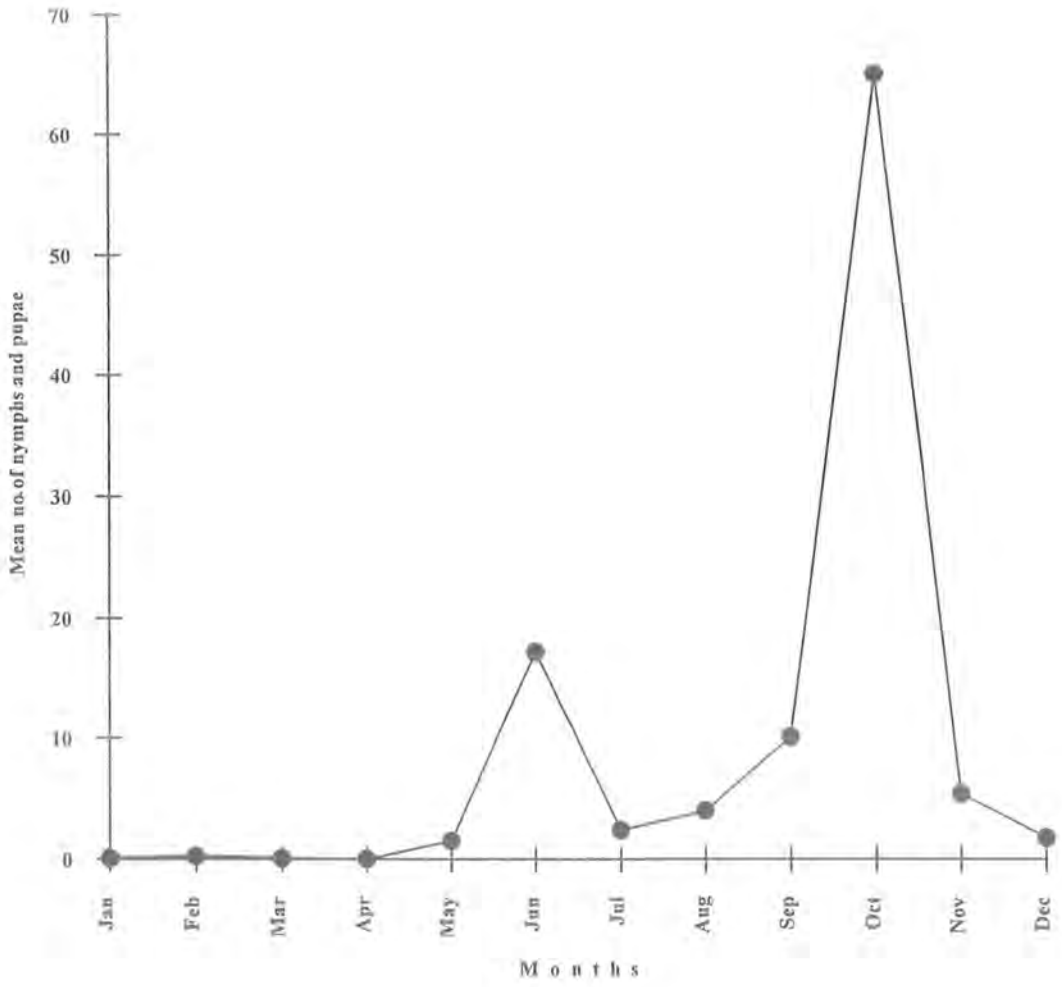


Fig.30 Population trends of *Bemisia tabaci* on *Tagetes erecta* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96

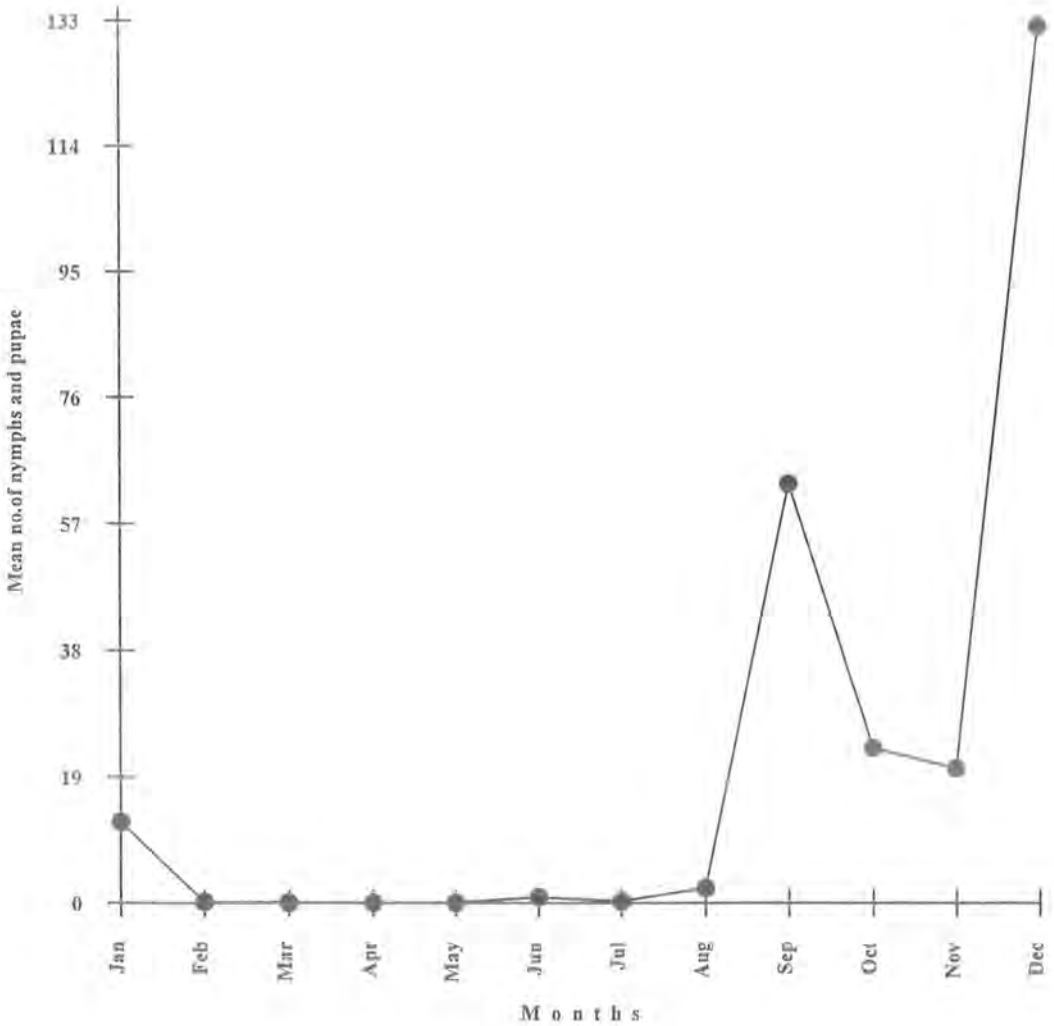


Fig.31 Population trends of *Bemisia tabaci* on *Ageratum conyzoides* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96

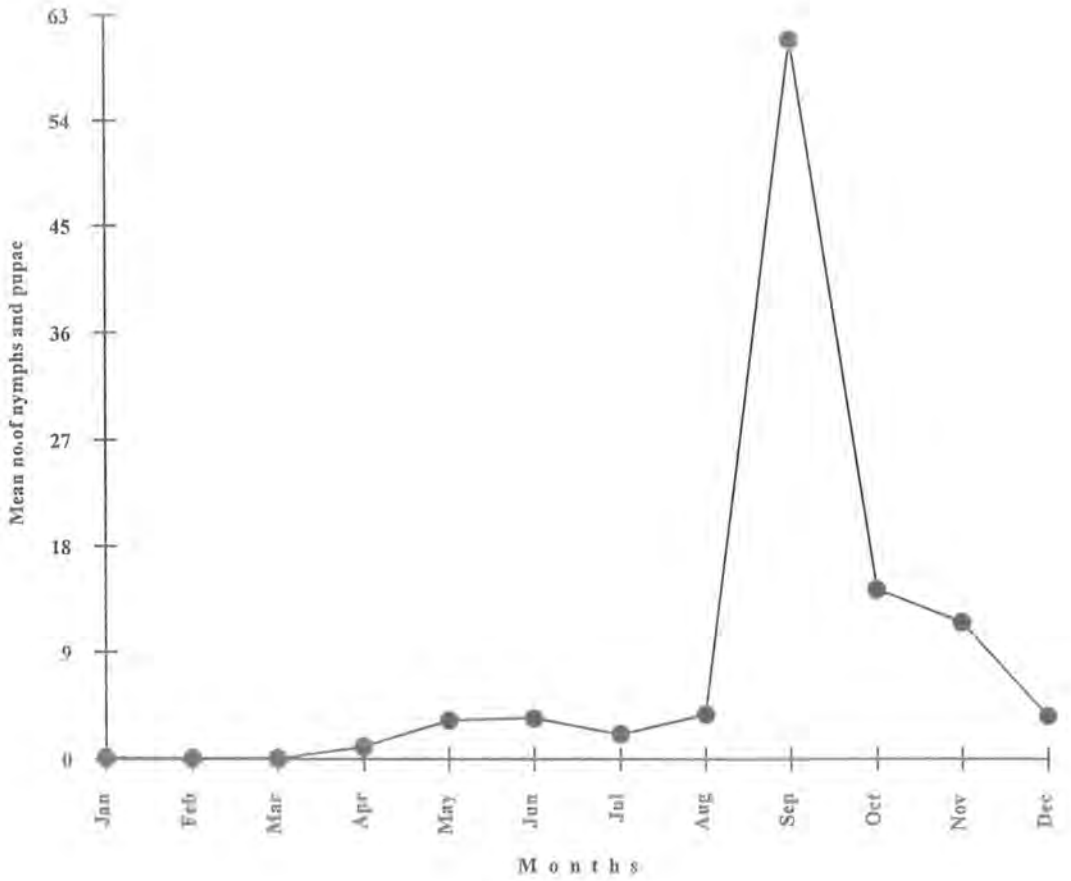


Fig.32 Population trends of *Bemisia tabaci* on *Helianthus annuus* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96

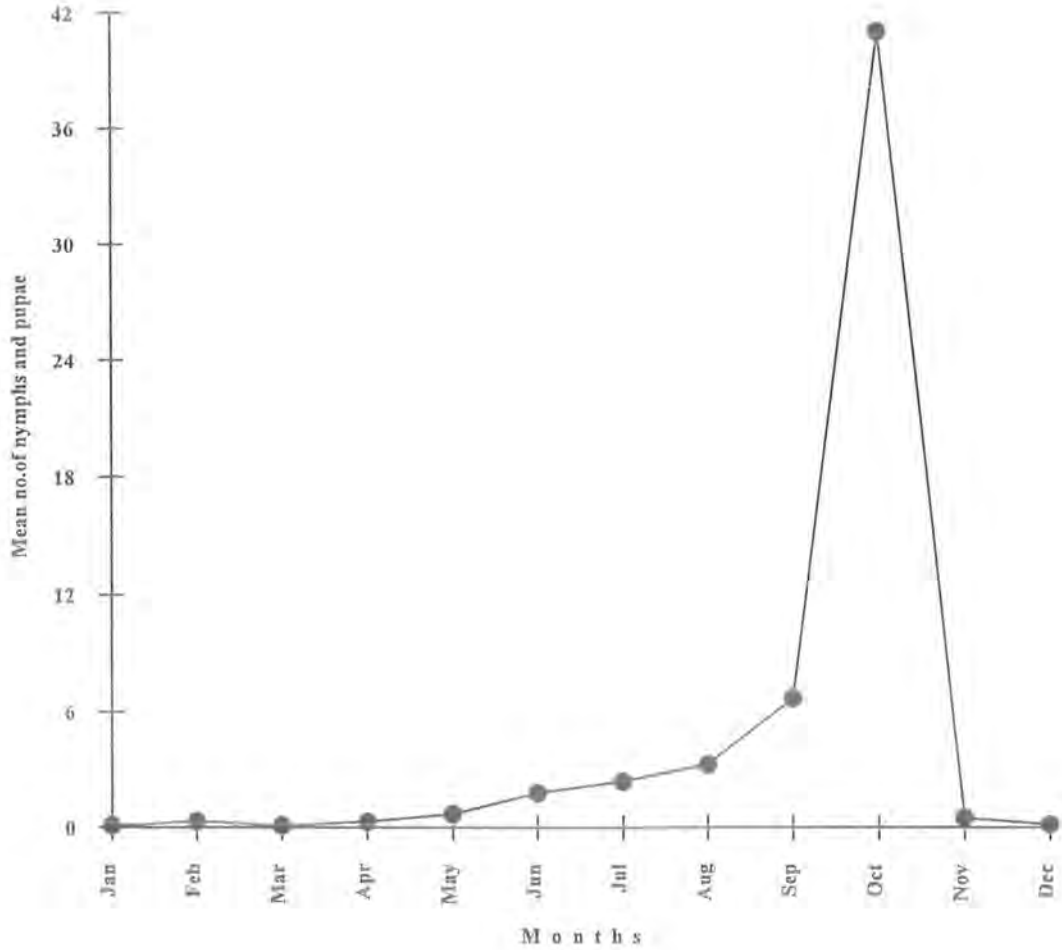


Fig.33 Population trends of *Bemisia tabaci* on *Hibiscus rosa-sinensis* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96

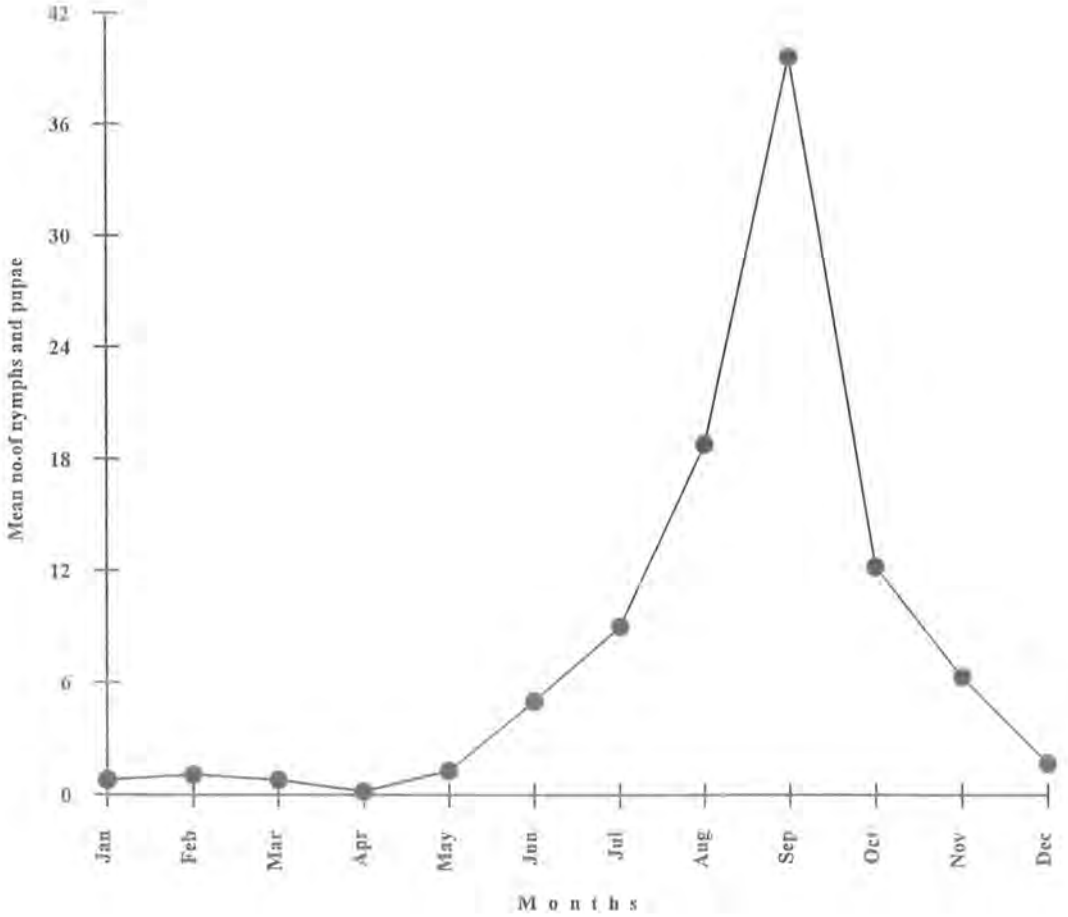


Fig.34 Population trends of *Bemisia tabaci* on *Lantana camara* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96

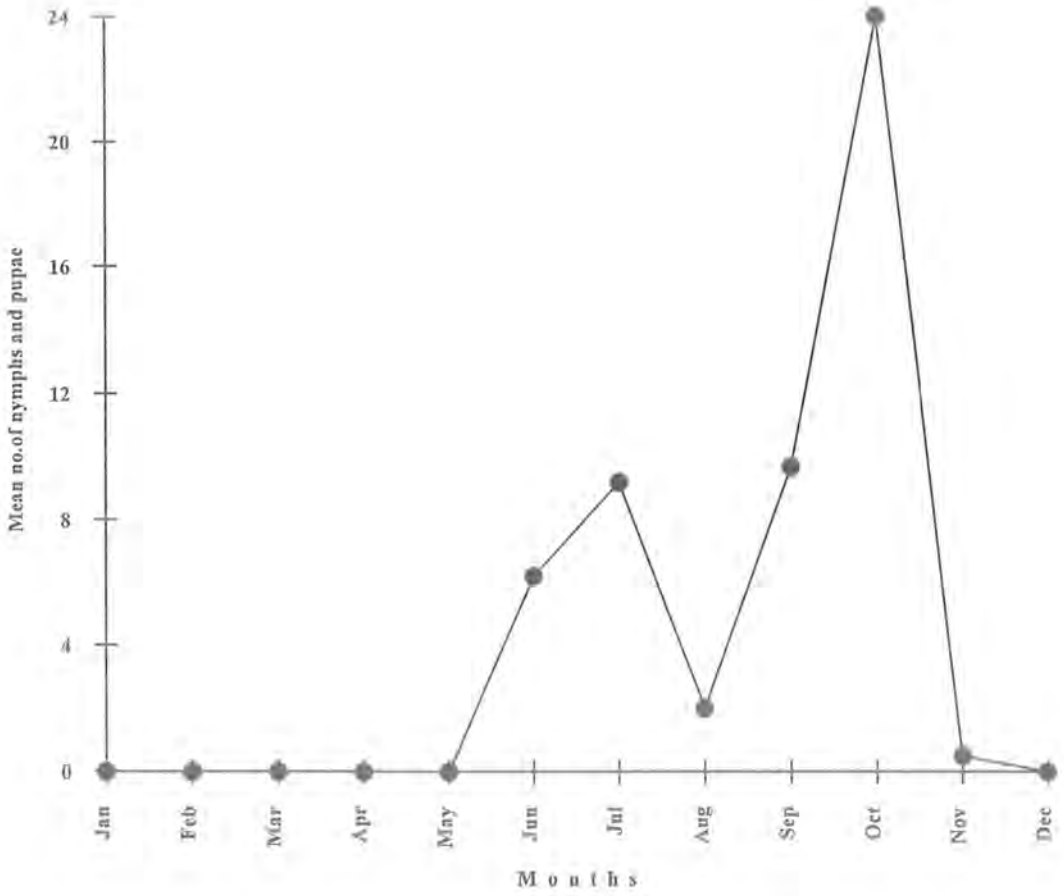


Fig.35 Population trends of *Bemisia tabaci* on *Gomephrena globosa* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96

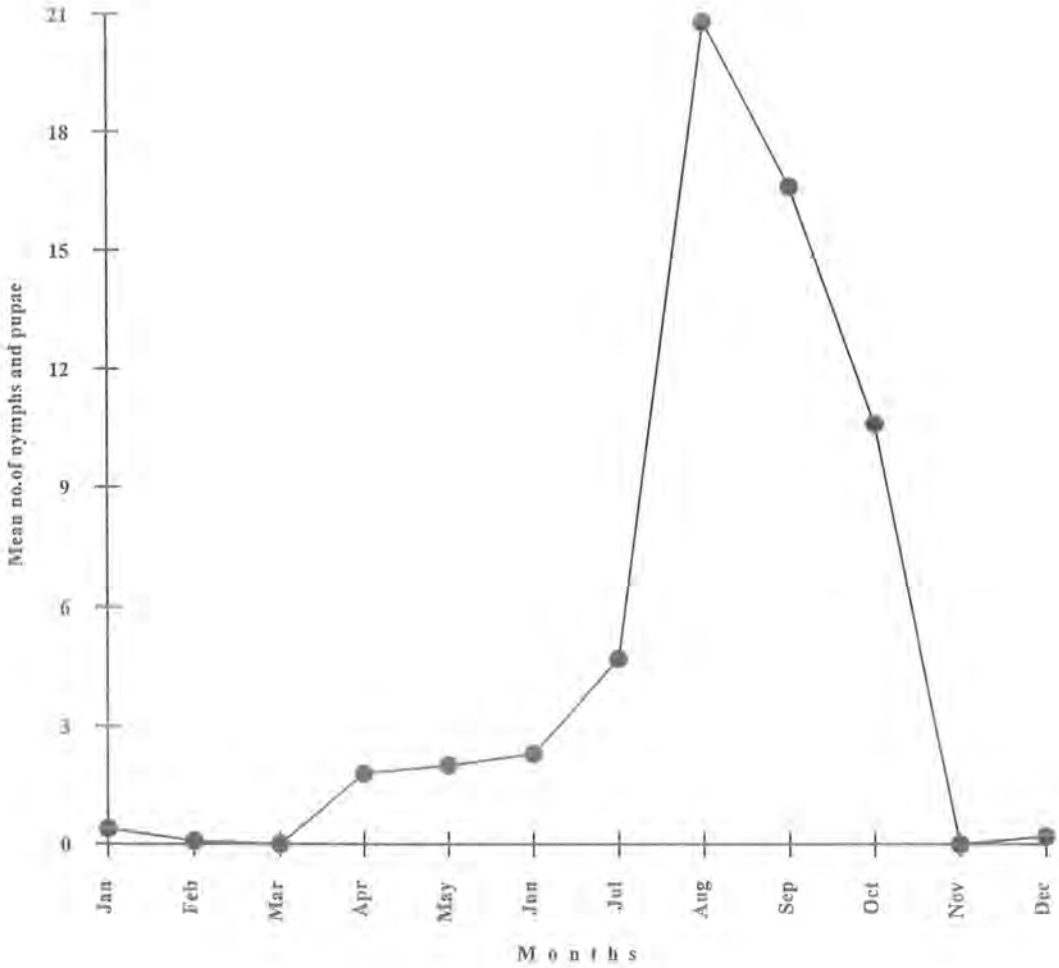


Fig.36 Population trends of *Bemisia tabaci* on *Solanum melongena* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96

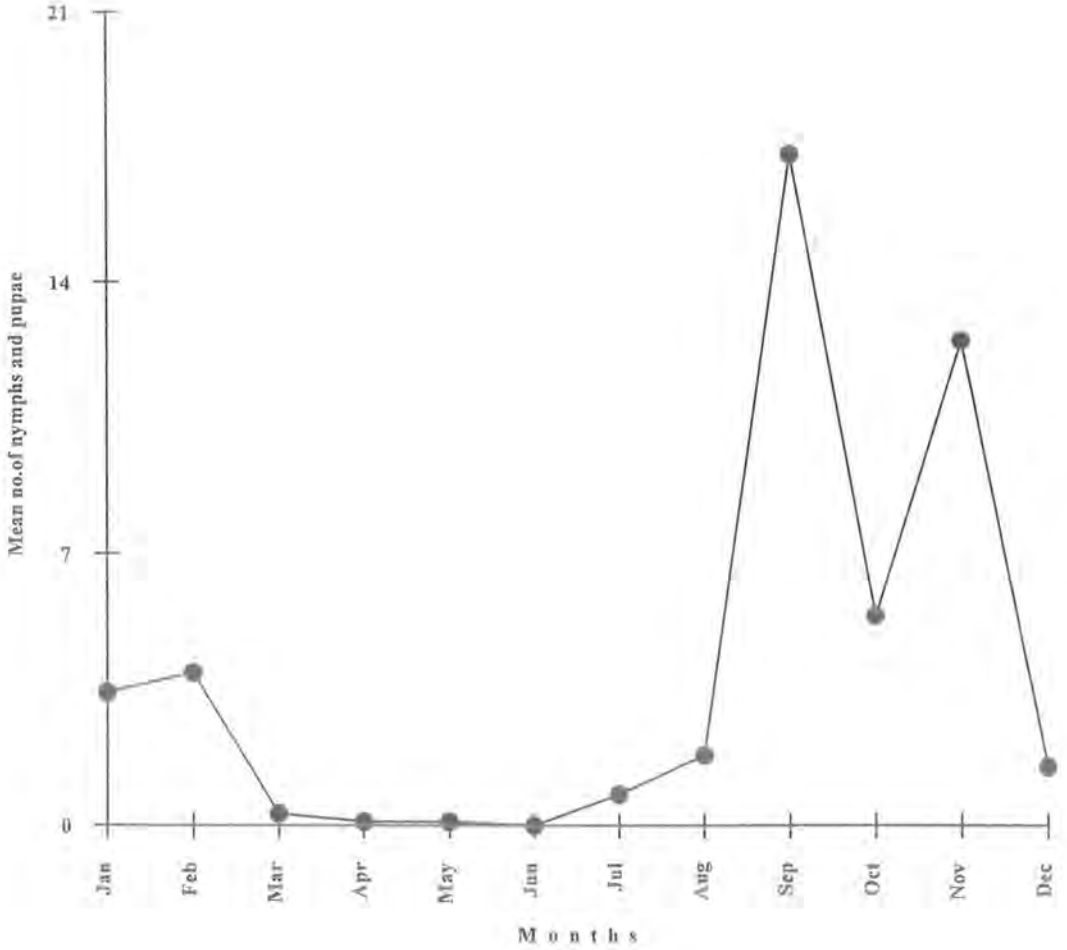


Fig.37 Population trends of *Bemisia tabaci* on *Euphorbia pulcherrima* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96

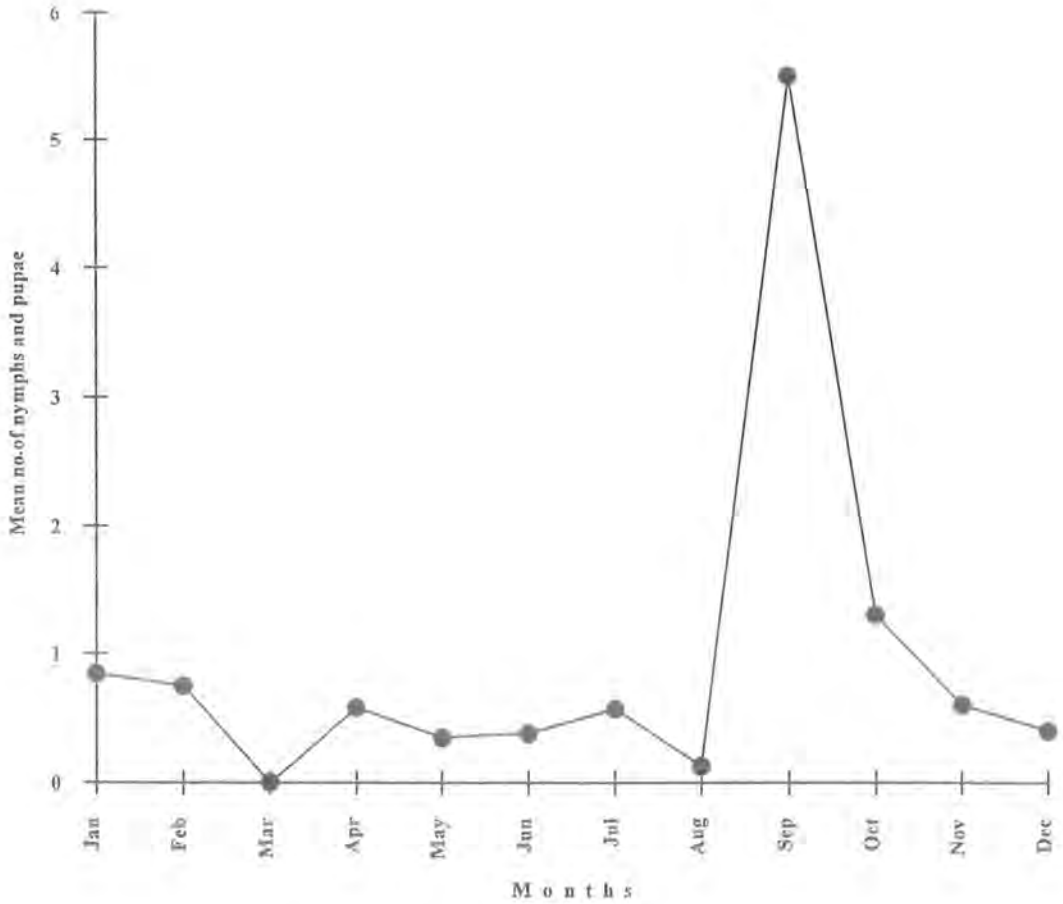


Fig.38 Population trends of *Bemisia tabaci* on *Ipomoea tricolor* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96

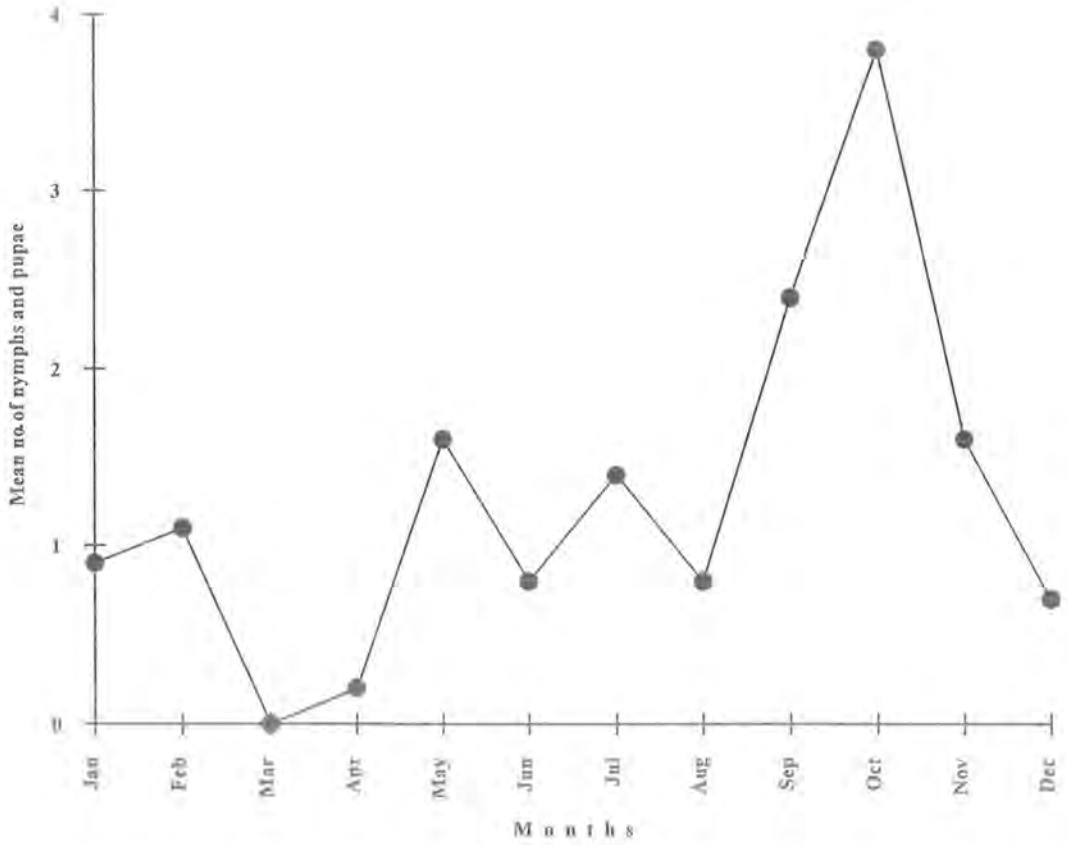


Fig.39 Population trends of *Bemisia tabaci* on *Hibiscus mutabilis* based on mean number of nymphs and pupae/100 cm^2 leaf area during 1993-96

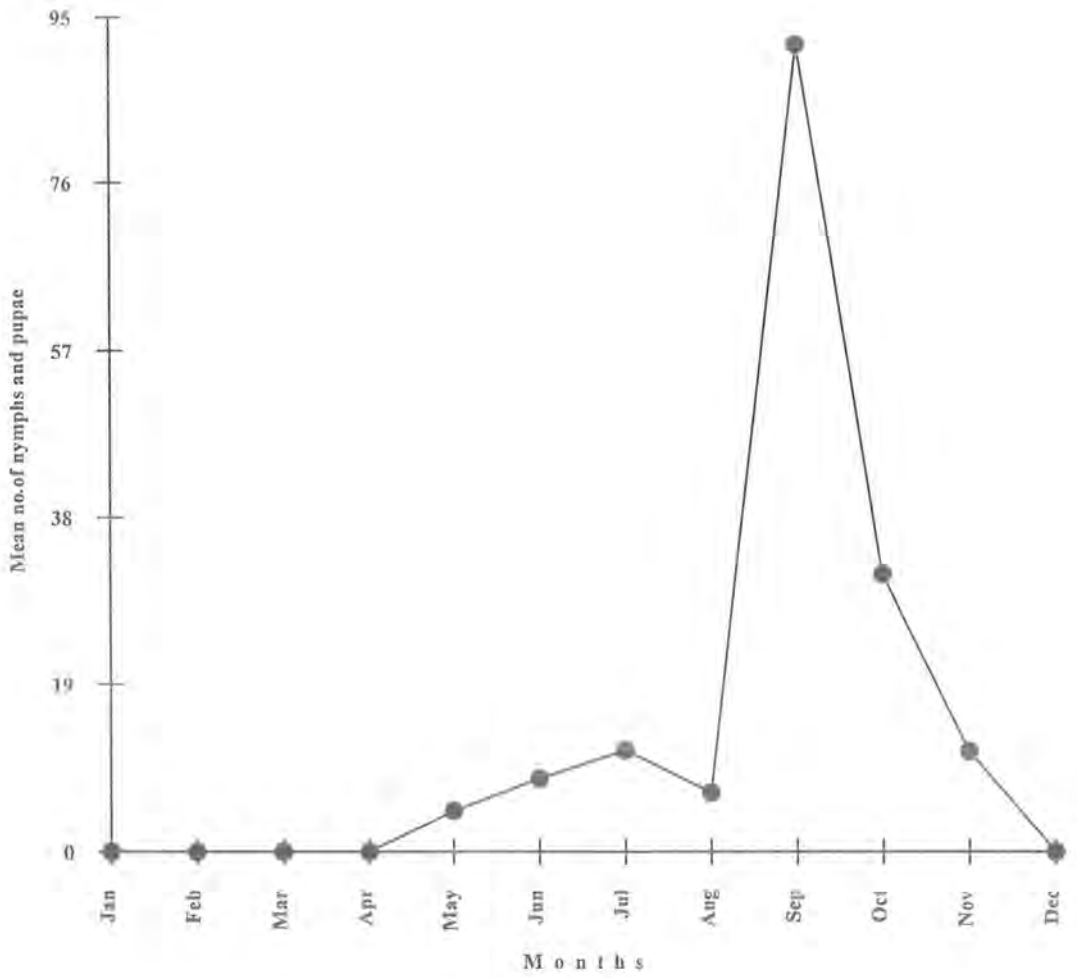


Fig.40 Population trends of *Bemisia tabaci* on *Corchorus triloailanis* based on nymphs and pupae/100 cm^2 leaf area during 1993-96

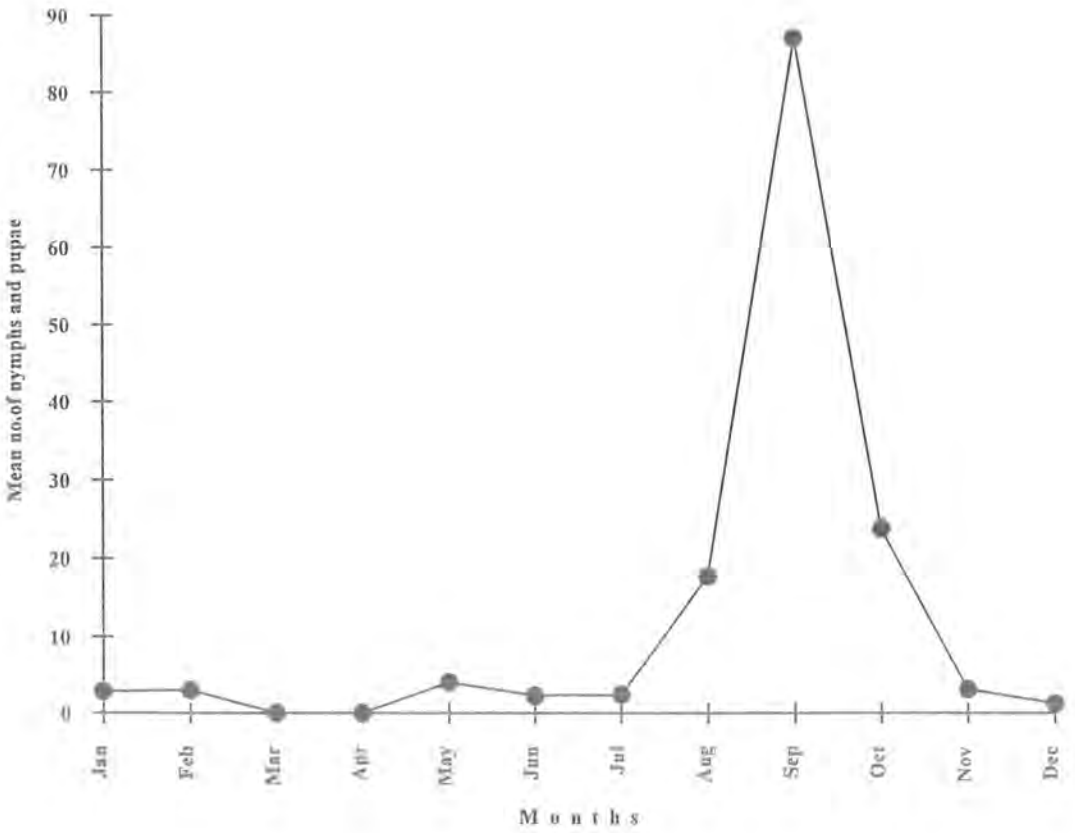


Fig.41 Population trends of *Bemisia tabaci* on *Euphorbia hirta* based on nymphs and pupae/100 cm^2 leaf area during 1993-96

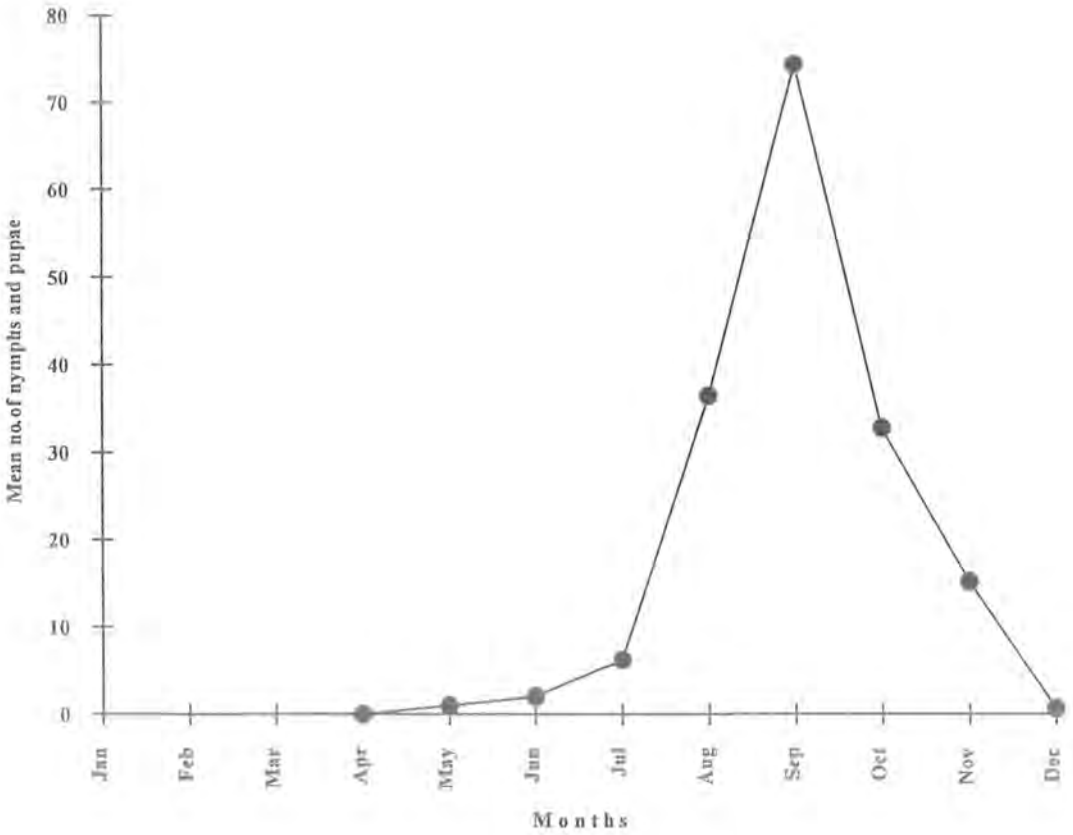


Fig.42 Population trends of *Bemisia tabaci* on *Phyllanthus pilulifera* based on nymphs and pupae/100 cm^2 leaf area during 1993-96

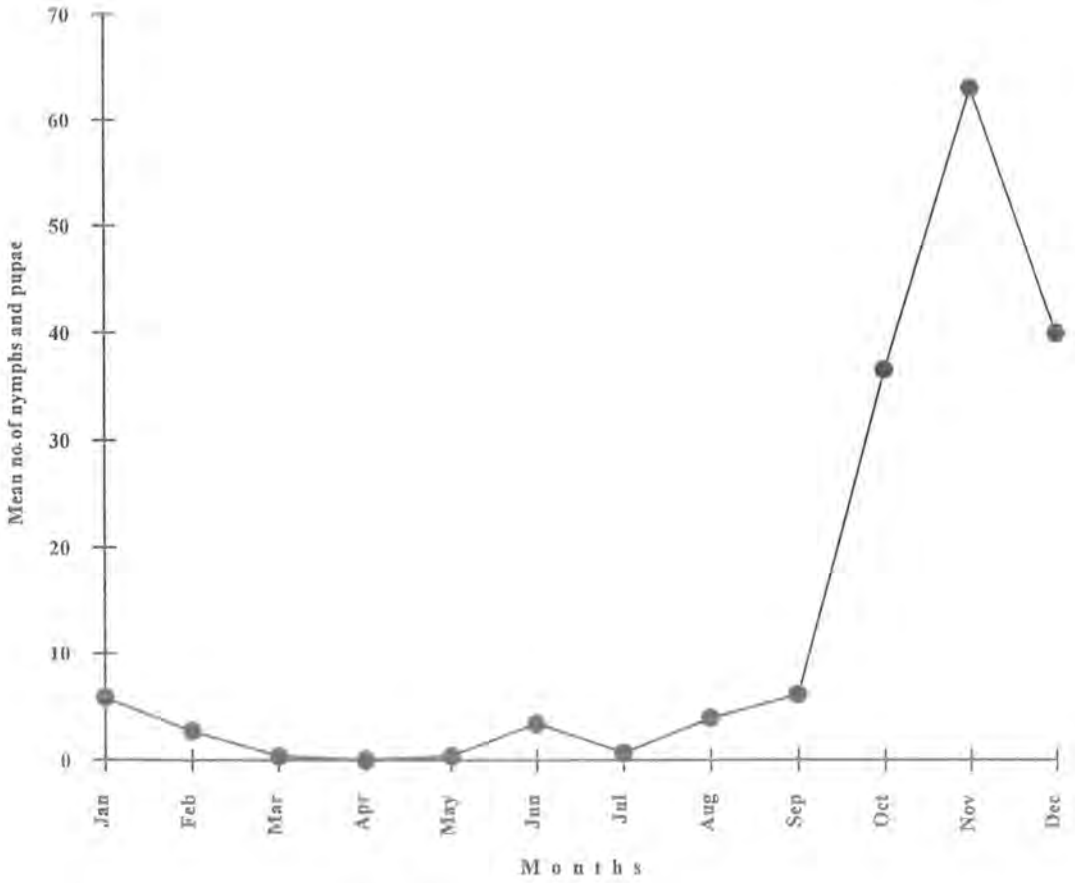


Fig.43 Population trends of *Bemisia tabaci* on *Conyza bonariensis* based on nymphs and pupae/100 cm^2 leaf area during 1993-96

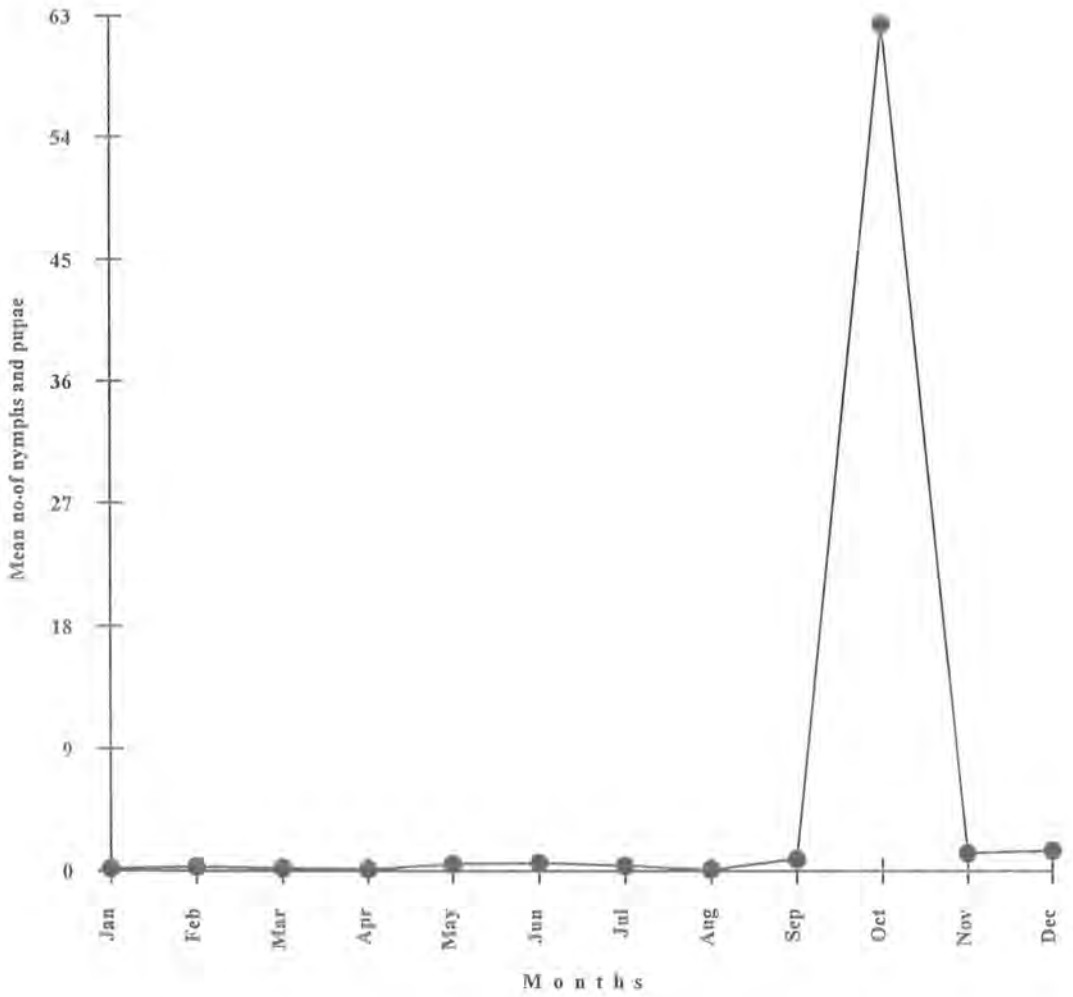


Fig.44 Population trends of *Bemisia tabaci* on *Convolulus arvensis* based on nymphs and pupae/100 cm^2 leaf area during 1993-96

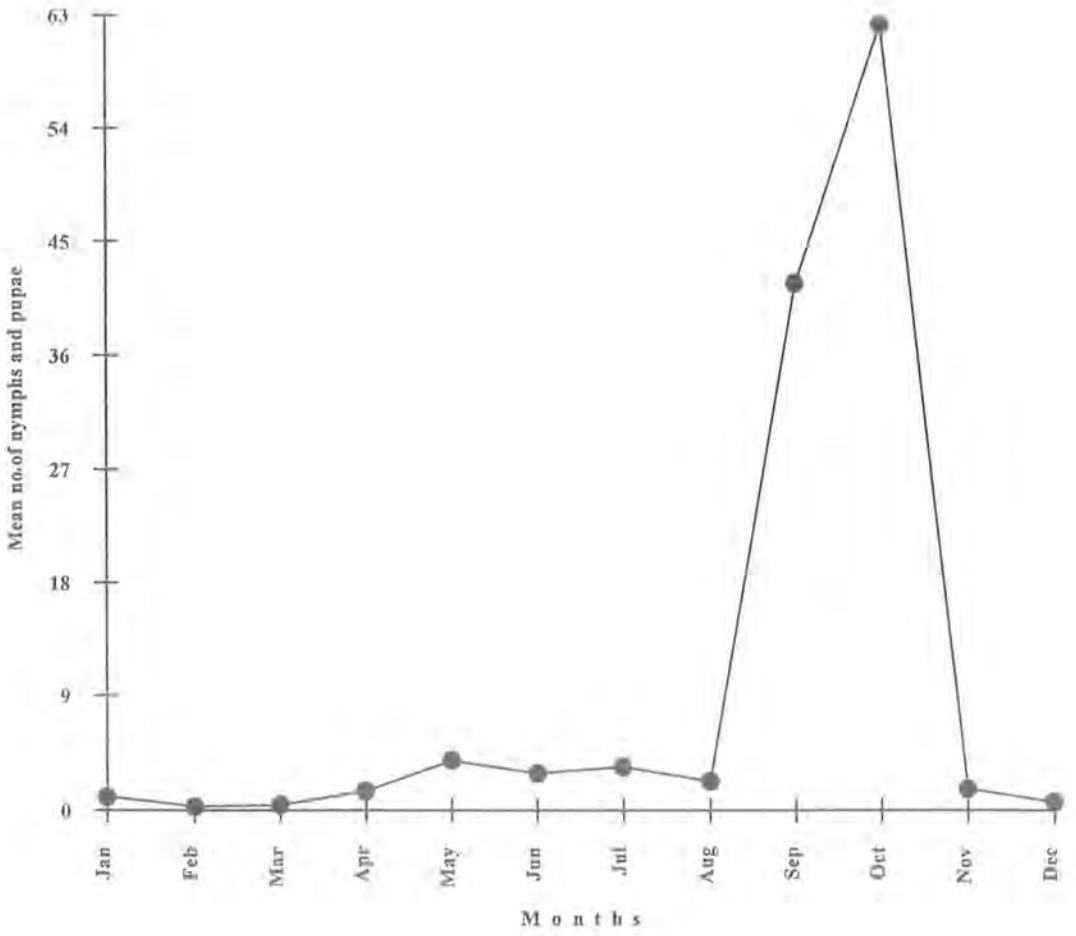


Fig.45 Population trends of *Bemisia tabaci* on *Eclipta alba* based on nymphs and pupae/100 cm^2 leaf area during 1993-96

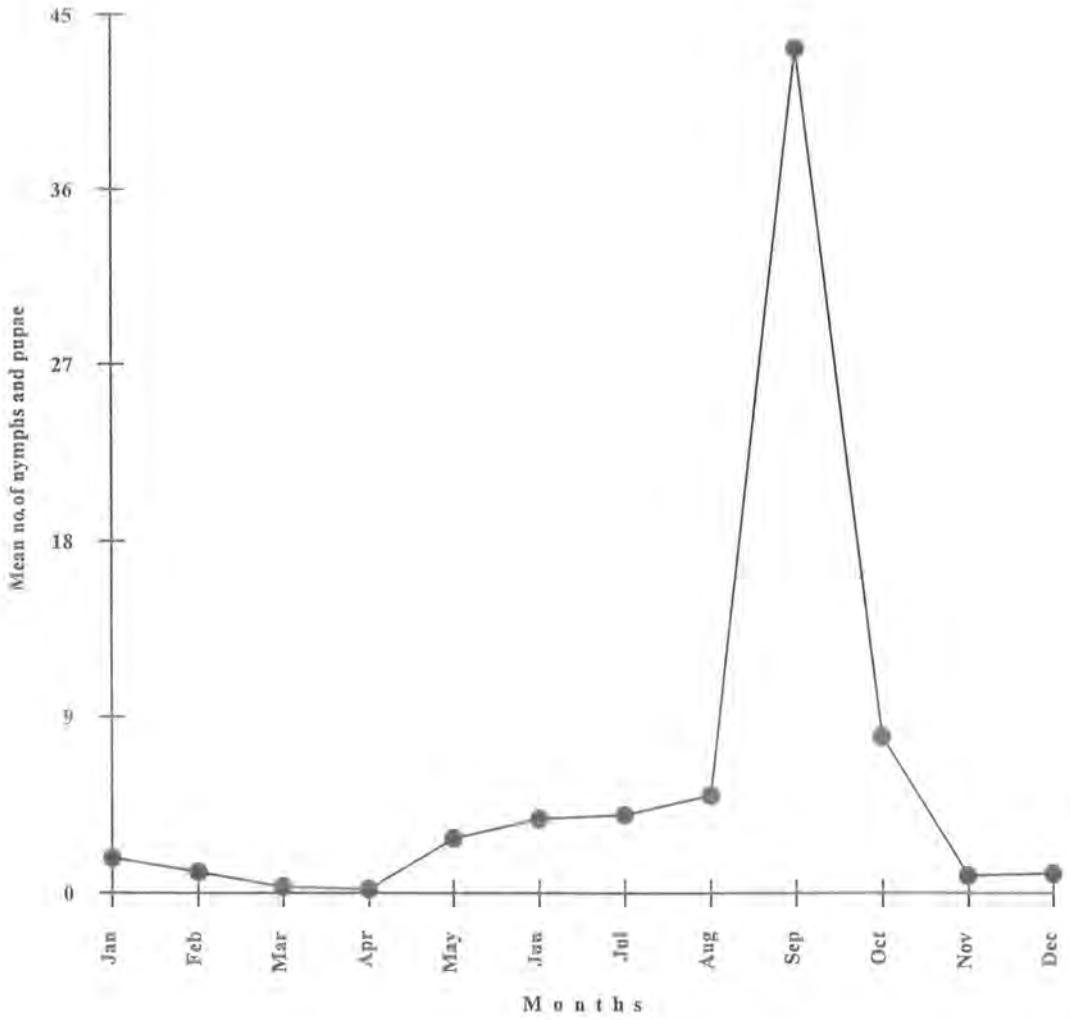


Fig.46 Population trends of *Bemisia tabaci* on *Malvastrum tricuspidatum* based on nymphs and pupae/100 cm^2 leaf area during 1993-96

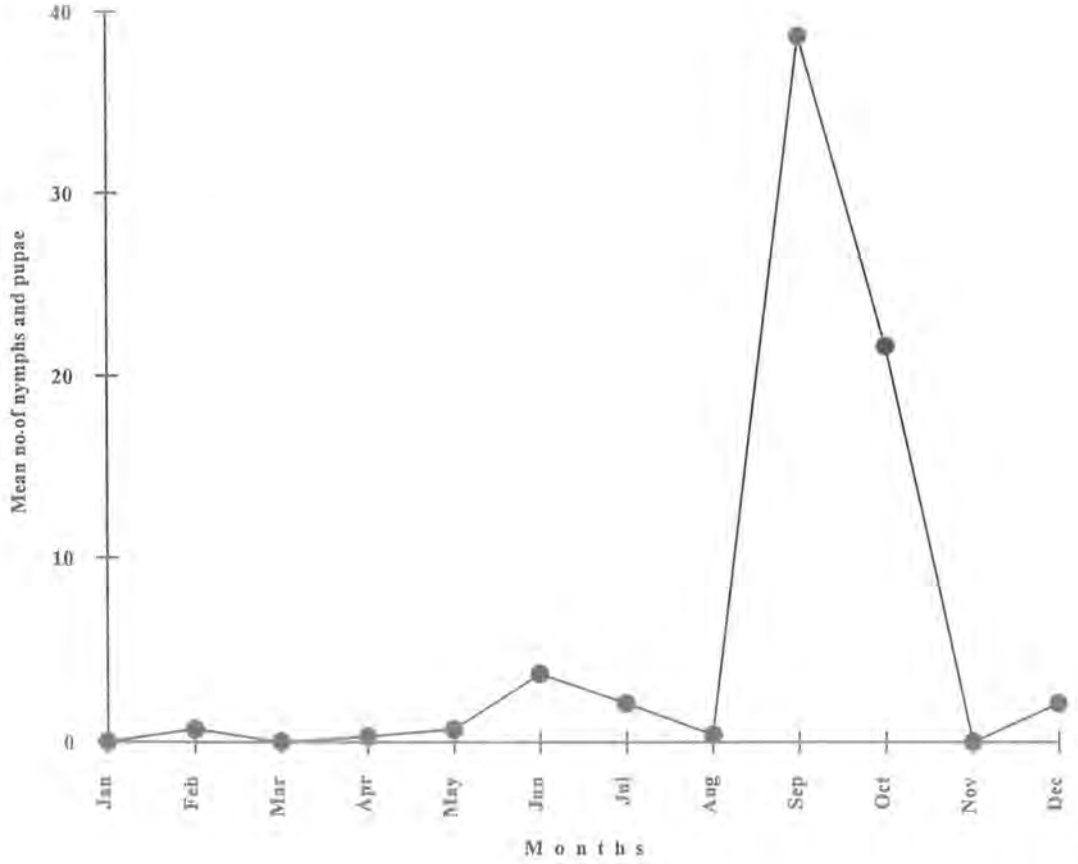


Fig.47 Population trends of *Bemisia tabaci* on *Solanum xanthocarpum* based on nymphs and pupae/100 cm^2 leaf area during 1993-96

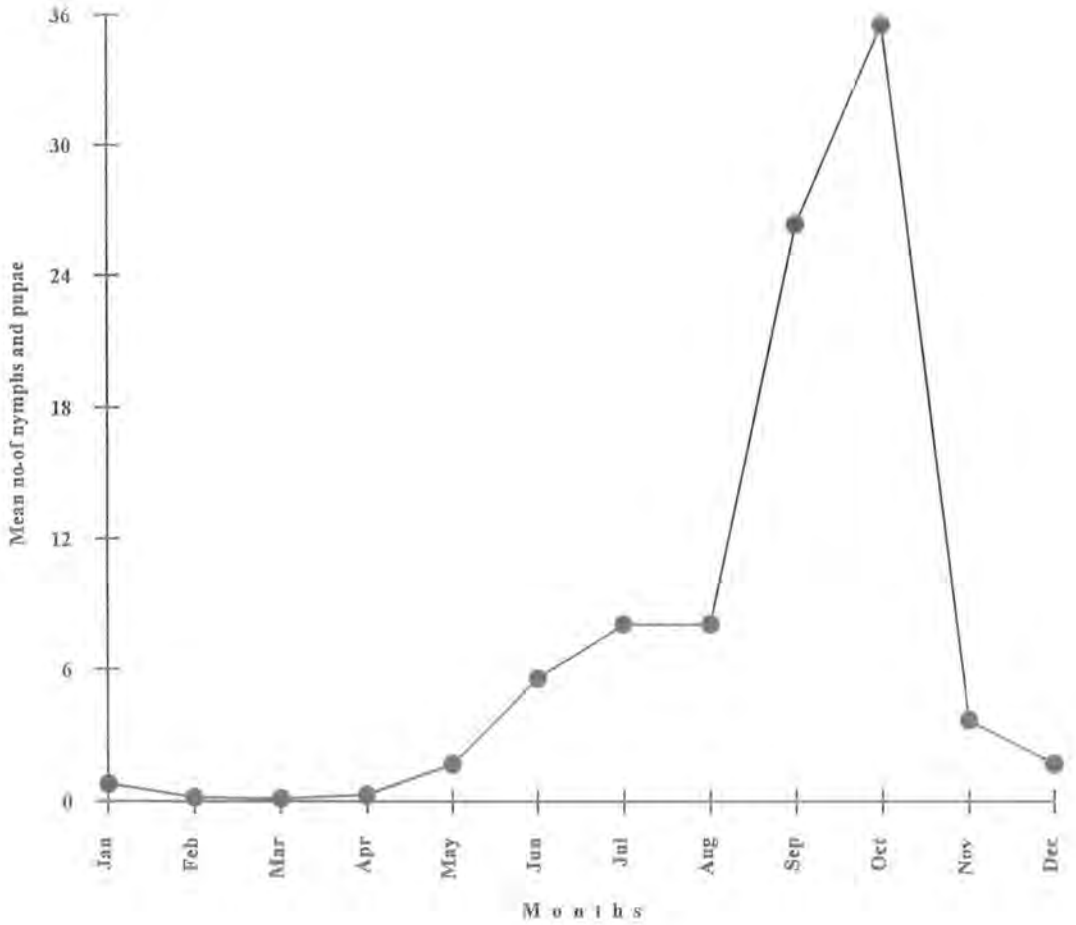


Fig.48 Population trends of *Bemisia tabaci* on *Achyranthes aspera* based on nymphs and pupae/100 cm^2 leaf area during 1993-96

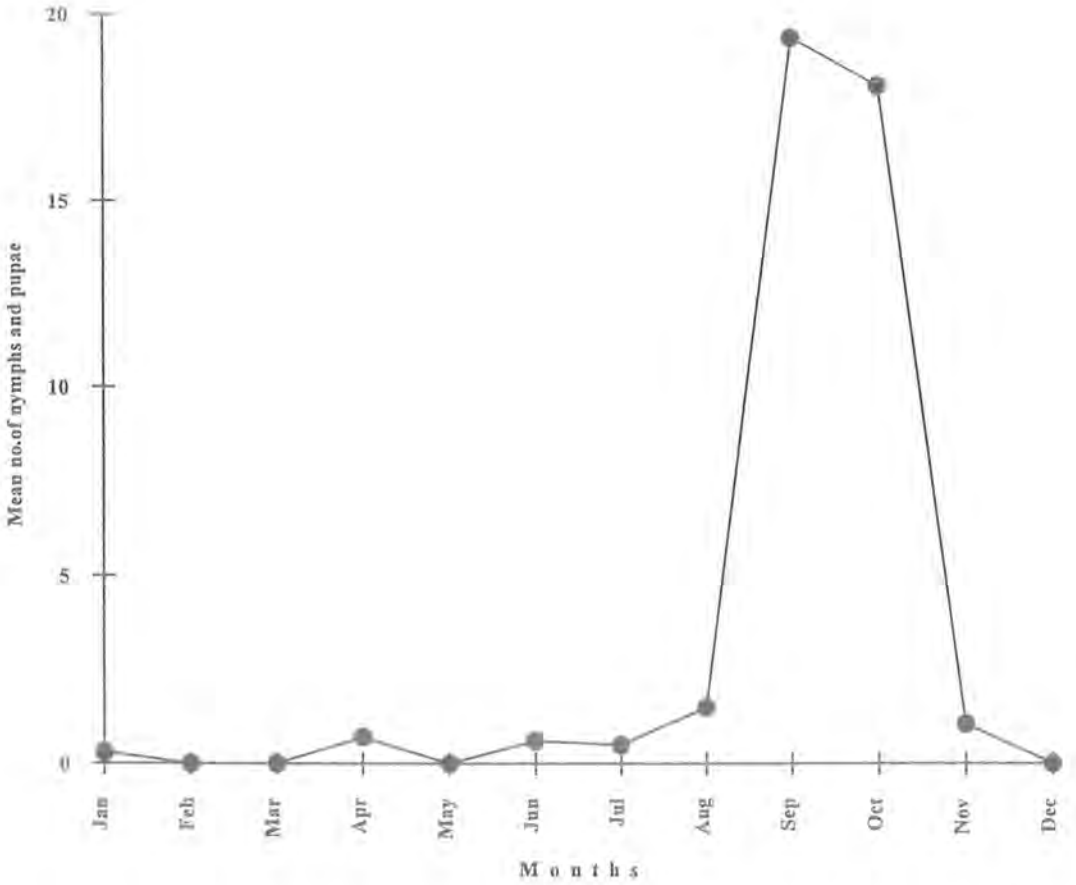


Fig.49 Population trends of *Bemisia tabaci* on *Polygonum* sp. based on nymphs and pupae/100 cm^2 leaf area during 1993-96

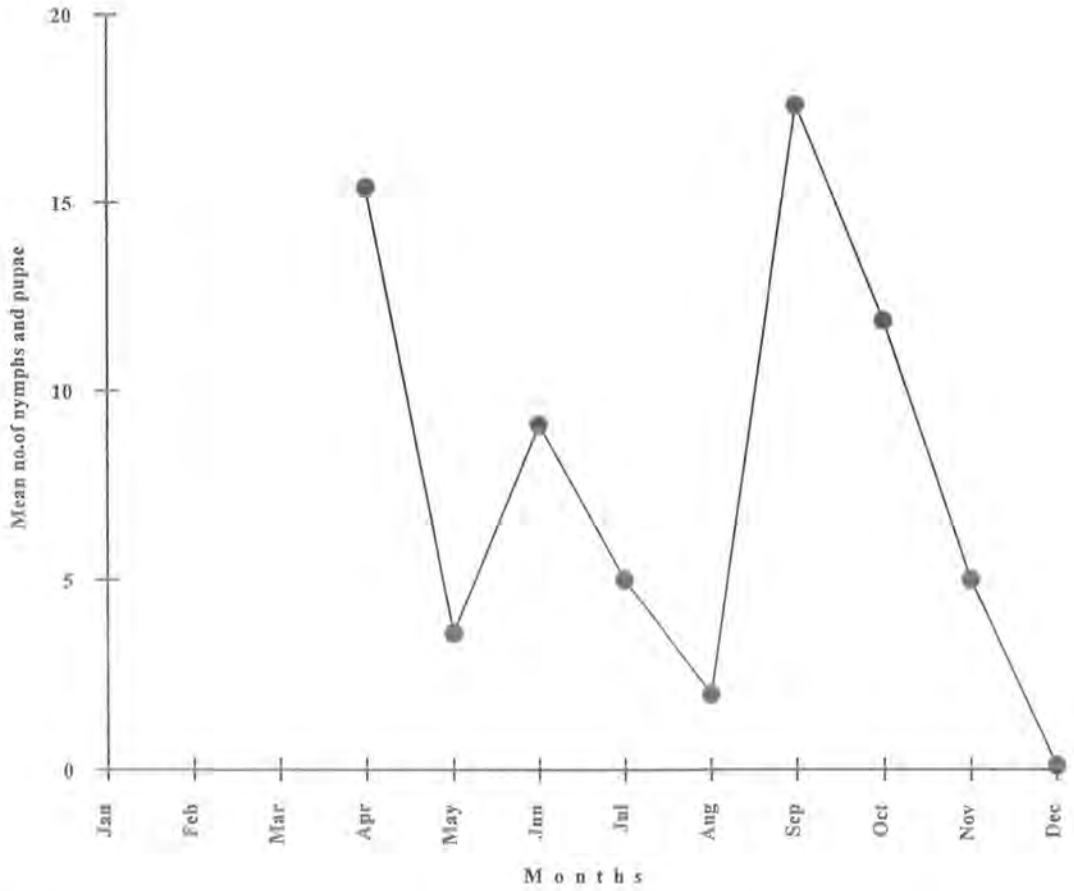


Fig.50 Population trends of *Bemisia tabaci* on *Citrullus tetragona* based on nymphs and pupae/100 cm^2 leaf area during 1993-96

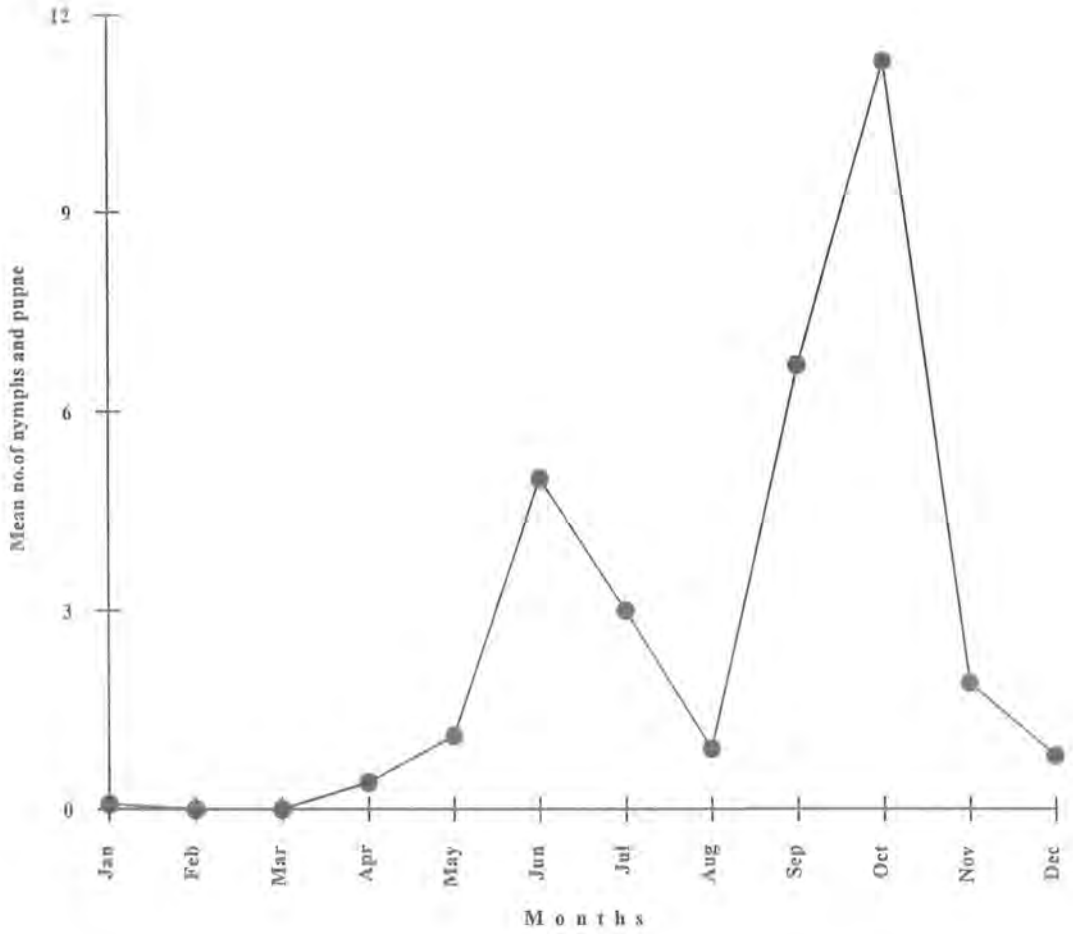


Fig.51 Population trends of *Bemisia tabaci* on *Xanthium stramarium* based on nymphs and pupae/100 cm^2 leaf area during 1993-96

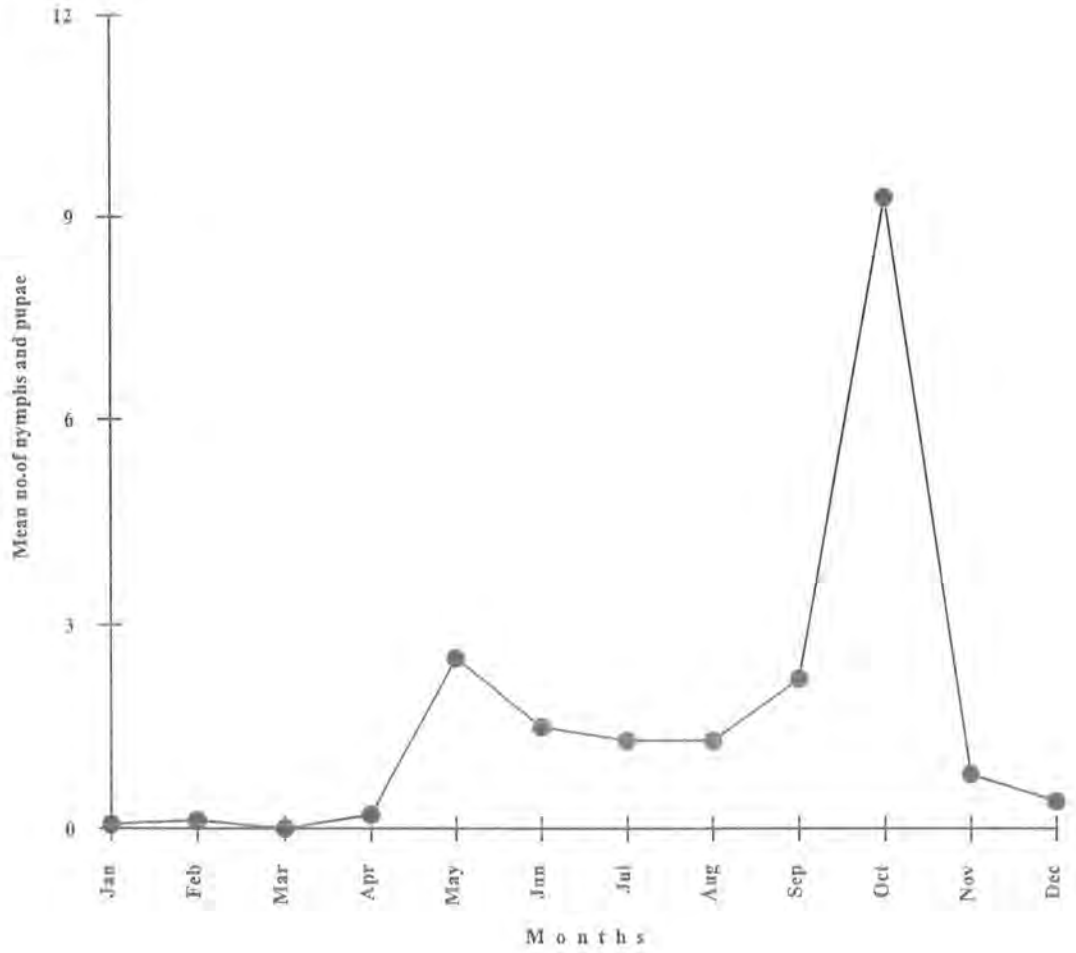


Fig.52 Population trends of *Bemisia tabaci* on *Physalis alkakengi* based on nymphs and pupae/100 cm^2 leaf area during 1993-96

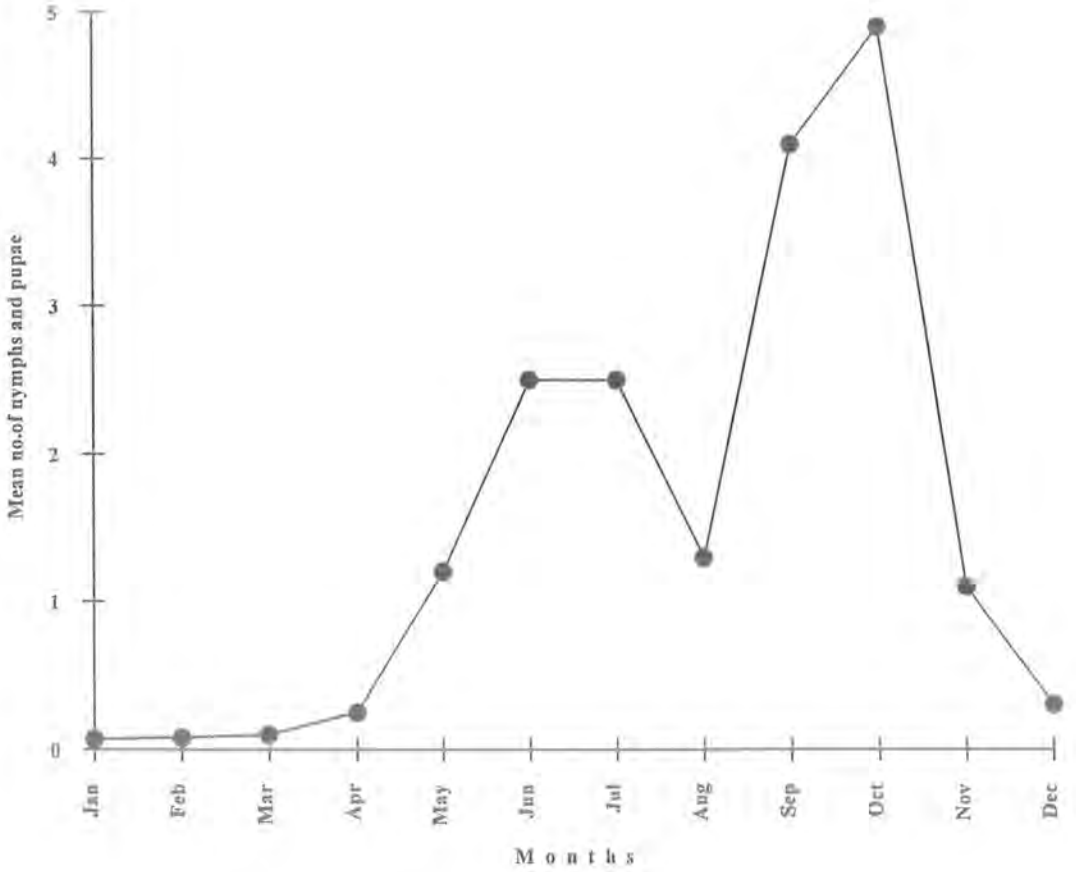


Fig.53 Population trends of *Bemisia tabaci* on *Solanum nigrum* based on nymphs and pupae/100 cm^2 leaf area during 1993-96

Table 1 Plant species on which *B. tabaci* was not recorded

Sr. No.	Botanical name	Vernacular Name	Family
1.	*Cordia obliqua*	Lasura	Boraginaceae
2.	*Dalbergia sissoo*	Shisham	Leguminoceae
3.	*Zizyphus mauritiana*	Beri	Rhamnaceae
4.	*Jasminum officinale*	Chambeli	Oleaceae
5.	*J. sambac*	Motia (small)	Oleaceae
6.	*Nyctanthes arbor-tristis*	Harsinghar	Oleaceae
7.	*Ficus religiosa*	Peepal	Moraceae
8.	*F. infectoria*	Pilkan	Moraceae
9.	*Parkinsonia aculeata*	-	Caesalpinaceae
10.	*Grewia asiatica*	Falsa	Tilliaceae
11.	*Azadirachta indica*	Neem	Meliaceae
12.	*Cestrum diurnum*	Den Ka Raja	Solanaceae
13.	*Ricinus communis*	Arend	Meliaceae
14.	*Vicia sativa*	Rewari	Legumiuosae
15.	*Carthamus oxyacantha*	Pohli	Compositae
16.	*Medicago denticulata*	Maina	Leguminosae
17.	*Echinochloa colonum*	Swanki	Gramineae
18.	*E. crus-galli*	Dhidhan	Gramineae
19.	*Cynodon dactylon*	Khabbal gass	Gramineae
20.	*Eruca sativa*	Taramera	Cuciferaceae
21.	*Cicer arietinum*	Chana	Leguminosae
22.	*Cajanus cajan*	Arhar	Leguminosae
23.	*Linum usitatissimum*	Alsi	Linaceae
24.	*Nicotiana tabacum*	Tambaku	Solanaceae
25.	*Medicago sativa*	Lucern	Leguminosae
26.	*Portulaca oleracea*	Kulfa	Portulaceae
27.	*Mangifera indica*	Aam	Anacardiaceae
28.	*Avena fatua*	Jangli Jai	Poaceae
29.	*Lathyrus aphaca*	Jangli Matar	Leguminosae
30.	*Rosa indica*	Gulab	Rosaceae
31.	*R. damascena*	Gulab	Rosaceae
32.	*Melia azedarach*	Dharaikh	Meliaceae
33.	*Brassica oleracea var. capitata*	Band Gophi	Brassicaceae
34.	*Bambusa vulgaris*	Bans	Poaceae
35.	*Musa paradisiaca*	Kaila	Cannaceae
36.	*Amygdalus communis*	Badam	Rosaceae
37.	*Callistemon lanceolatum*	-	Myotaceae

38.	*Citrus aurantifolia*	Lemon	Rutaceae
39.	*Citrus acida*	Khattai	Rutaceae
40.	*Citrus reticulata*	Kennu	Rutaceae
41.	*Citrus sinensis*	Musammi	Rutaceae
42.	*Eugenia jambolana*	Jaman	Myrtaceae
43.	*Populus euphratica*	Popular	Salicaceae
44.	*Avena sativa*	Jai	Poaceae
45.	*Hordeum vulgare*	Jau	Poaceae
46.	*Oryza sativa*	Chawal	Poaceae
47.	*Pennisetum glaucum*	Bajra	Poaceae
48.	*Saccharum officinarum*	Ganna	Poaceae
49.	*Sorghum bicolor*	Jawar	Poaceae
50.	*Triticum aestivum*	Gandum	Poaceae
51.	*Zea mays*	Makai	Poaceae
52.	*Phoenix dactylifera*	Khajoor	Arecaceae
53.	*P. sylvestris*	Jangli khajoor	Arecaceae
54.	*Pterospermum acerifolium*	Beeri patta	Sterculaceae
55.	*Ficus benjamina*	-	Moraceae
56.	*Prosopis juliflora*	Kekar	Mimosaceae
57.	*Bambusa vulgaris var. striata*	Bans	Poaceae
58.	*Corianderum sativum**	Dhanian	Umbelliferae
59.	*Euphorbia pilulifera**	Dhodhak	Euphorbiaceae
60.	*Foeniculum vulgore**	Sonf	Umbelliferae
61.	*Papaver rhoeas**	Garden poppy	Papaveraceae

* = Only adults were found

Table 2 **Crops on which *Bemisia tabaci* was recorded with priod of activity during 1993-96**

Host	Period	Status
Sesbania sesban	January-October	**
Helianthus annuus	February-May	**
Vigna radiata	March-December	*
Vigna cylindrica	March-October	**
Glycine max	March-May	*
Lens culinaris	March-April	***
Corchorus capsularis	May-November	*
Hibiscus canabinus	May-October	**
Cyamopsis tetragonoloba	June-November	**
Sesame indicum	June-October	*
Brassica campestris var.*sarson*	September-May	**
Trifolium alexandrinum	October-December	***

* = Major ** = Minor ***= Incidental

Table 3 **Vegetables on which *Bemisia tabaci* was recorded with period of activity during 1993-96**

Host	Period	Status
Mentha viridis	January-December	*
Lufa cylindrica	January-December	**
Capsicum frutescens	January-December	*
Lycopersicum esculentum	January-December	**
Solanum melongena	January-December	*
Spinacea oleracea	January-December	**
Ipomoea batatas	January-December	*
Cucumis sativus	February-May	*
Benincasa hispida.	March-December	**
Cucurbita pepo	March-December	**
Cucumis melo	March-December	*
Abelmoschus esculentus	March-December	*
Citrullus lanatus	March-November	*
Cucumis melo var. utilissma	March-May	**
Momordica charantia	April-December	**
Citrullus lanatus var. fistulosus	April-November	*
Cucumis melo var. phutt	May-October	*
Lagenaria siceraria	May-October	**
Colocasia esculenta	May-January	**
Brassica oleracea var. botrytis.	June-March	**
Brassica rapa	August-February	**
Raphanus sativus	August-February	**
Beta vulgaris	September-January	***
Daucus carota	September-November	***
Cucurbita pepo var. melopepo	October-May	*
Solanum turberosum	October-May	**
Raphanus sp. (red radish)	October-May	***
Pisum sativum	October-January	***
Trigonella foenum-graecum	October-February	***

*** = Major** **** = Minor** ***** = Incidental**

Table 4 Fruit and forest plants on which *Bemisia tabaci* was recorded with period of activity during 1993-96

Host	Period	Status
Salmalia malabarica	January-December	**
Acacia nilotica	January-December	**
Albizzia lebbek.	January-December	*
Bauhinia purpurea	January-December	*
Ficus carica	January-December	**
Morus alba	January-December	*
Morus laevigata	January-December	**
Eucalyptus citriodora	January-December	**
Psidium guajava	January-December	*
Leucaena leucophylle	January-November	**
Cassia fistula	March-January	**
Pongamia pinnata	April-January	**
Broussonetia papyrifera	April-January	*
Vitis vinifera	Apri-February	**
Punica granatum	May-January)	*
Moringa oleifera	June-December	**
Unidentified sp. A	August-March	**
Ehretia laevis	October-December	**

* = Major **= Minor

Table 5 Ornamental plants on which *B.tabaci* was recorded with period of activity during 1993-96

Host	Period	Status
Tecoma stans	January-December	**
Chrysanthemum morifolium	January-December	*
Ageratum conyzoides	January-Deceember	*
Ipomoea tricolor	January-December	*
Helianthus annuus	January-December	*
Erythrina suberosa	January-December	**
Althaea rosea	January-December	**
Lawsonia inermis	January-December	**
Hibiscus mutabilis	January-December	*
Hibiscus rosa-sinensis	January-December	*
Lantana camara	January-December	*
Gardenia jasminoides	January-December	*
Euphorbia pulcherrima	January-December	*
Jasminum multiflorum	January-December	**
Unidentified species (ver.Gur Mukhi)	February-March	***
Verbena hybrida	February-May	**
Hamelia patens.	March-December	**
Ipomoea carnea	April-January	**
Ocimum basilicum	April-February	**
Bougainvillea glabra	April-February	**
Solanum melongena	April-February	*
Gerbera jamesonii	May-December	**
Tagetes erecta	May-March	*
Antigonon leptopus	May-November	**
Elettaria cardamum	May-November	**
Ipomoea cairiea	May-February	*
Lathyrus odoratus	May-February	**
Ruellia tuberosa	May-January	**
Gomphrena globosa	June-November	*
Celosia argentia var.cristata	June-March	**
Campsis undulata	July-December	**
Ipomoea purpurea	July-November	**
Jasminum rigidum	July-October	***
Argyrela speciosa	July-February	**
Vitex agnus-costus	August-November	**
Buddieja paniculata	August-November	***
Impatiens balsamina	August-December	**
Mathiola incaria	September-November	***
Cestrum nocturnum	September-October	**
Tropaeolum majus	October-December	***
Cineraria hybrida	December-April	***

*** = Major ** = Minor *** = Occasional**

Table 6 **Weeds on which *Bemisia tabaci* was recorded with period of activity during 1993-96**

Host	Period	Status
Achyranthes aspera.	January-December	*
Chenopodium album	January-December	**
Chenopodium murale	January-December	**
Conyza bonariensis	January-December	*
Eclipta alba	January-December	*
Convolvulus arvensis	January-December	*
Euphorbia prostrata	January-December	**
Rhynchosia sp.	January-December	**
Abutilon indicum	January-December	**
Malvastrum tricuspidatum	January-December	*
Rumex dentatus	January-December	**
Datura metel	January-December	**
Nicotiana plumbaginifolia	January-December	**
Solanum nigrum	January-December	*
Solanum xanthocarpum	January-December	*
Withania somnifera	January-December	**
Cassia absus	January-December	**
Verbena officianalis.	March-January	**
Citrullus tetragona	April-December	*
Trianthema portulacastrum	April-December	**
Phyllanthus pilulifera	April-December	*
Tribulus terrestris	April-November	**
Amarantus viridis	April-February	**
Heliotropium euphorium	April & July	***
Xanthium stramarium	April-January	*
Polygonum sp.	April-February	*
Physalis alkakengi	April-February	*
Euphorbia hirta	May-February	*
Digera arvensis	May-December	**
Ipomoea aquatica	May-December	**
Unidentified weed-4 (Wild Jute type)	May-December	**
Corchorus triloailanis	May-November	*
Cleome viscosa	May-October	**
Unidentified weed-2(Abutilon type)	May-October	**
Unidentified weed-3 (Jantar type)	June-December	**
Unidentified weed-5 (Chappa)	July-December	***
Unidentified weed-1 (Chibbar type)	July-November	**

Corchorus olitorius	July-October	***
Leuces cephalotes	August-November	***
Cnicus arvensis	September-May	**
Euphorbia helioscopia	October-February	**
Melilotus indica	October-February	**
Malva parviflora	October-May	**
Mentha longifolia	November-June	**
Anagallis arvensis	December-February	***

* = Major ** = Minor *** = Incidental

Table 7 MEAN NUMBER OF NYMPHS AND PUPAE OF *BEMISIA TABACI* PER 100 CM^2 ON VARIOUS MINOR CROPS DURING 1993-96

Host	J	F	M	A	M	J	J	A	S	O	N	D
Cyamopsis tetragonoloba	0.0	0.0	0.0	0.0	0.0	0.0	0.6	2.4	1.2	0.5	0.3	0.0
Sesbania sesban	0.2	0.0	0.0	0.0	8.8	0.0	1.2	0.0	12.0	0.7	0.9	0.0
Vigna cylindrica	0.0	0.0	0.0	0.1	0.4	1.5	0.4	0.2	0.3	0.3	0.0	0.0
Brassica campestris var.sarson	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	15.0	1.3	0.4	0.2
Helianthus annuus	0.0	0.0	1.0	3.2	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hibiscus canabinus	0.0	0.0	0.0	0.0	0.8	1.3	0.6	0.8	1.1	0.3	0.0	0.0
Colocasia esculenta	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.3	0.1	0.0	0.0
Brassica oleracea var. botrytis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	3.8	0.6	0.0	0.0
Cucurbita pepo	0.0	0.0	0.2	0.6	0.1	0.1	0.0	0.0	0.2	0.5	0.0	0.3
Momordica charantia	0.0	0.0	0.0	0.1	0.2	0.2	0.2	0.1	0.7	1.1	0.2	0.4
Cucumis melo var. flencuosus	0.0	0.0	0.2	4.5	7.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Benincasa hispida	0.0	0.0	0.0	0.4	0.7	0.5	0.1	0.0	11.5	1.0	1.4	0.8
Salanum turberosum	0.3	0.0	0.8	0.2	0.3	0.0	0.0	0.0	0.0	5.5	0.6	0.3
Raphanus sativus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	17.0	0.7	0.6	0.1
Spinacea oleracea	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.8	0.5	0.4	0.0
Lycopersicum esculentum	0.4	0.3	0.0	0.2	0.2	0.3	0.4	0.0	0.7	2.0	0.7	0.4
Lufa cylindrica	0.1	0.0	0.1	0.6	0.5	0.9	0.4	0.1	1.9	1.6	0.1	0.0
Brassica rapa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.1	0.2
Lagenaria siceraria	0.0	0.01	0.2	0.6	0.1	0.1	0.04	0.01	0.2	0.5	0.1	0.3

Table 8 MEAN NUMBER OF NYMPHS AND PUPAE OF *BEMISIA TABACI* PER 100 CM^2 ON MINOR FRUIT AND FOREST TREES DURING 1993-1996

Host	J	F	M	A	M	J	J	A	S	O	N	D
Cassia fistula	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.6	0.3	0.2	0.0
Ficus carica	0.8	0.3	0.6	0.1	0.4	0.8	0.7	0.5	0.3	0.3	0.8	1.0
Vitis vinifera	0.1	0.4	0.0	0.0	0.4	0.1	0.5	0.5	0.3	0.1	0.4	1.0
Acacia nilotica	0.2	0.6	0.2	0.2	1.0	0.4	0.4	0.1	1.1	1.4	0.3	0.0
Leucaena leucophylle	0.1	0.1	0.0	0.0	0.2	0.1	0.1	0.3	0.1	1.1	0.0	0.0
Eucalyptus citriodora	0.0	0.0	0.0	0.1	0.4	0.5	0.1	0.5	5.2	1.3	1.7	0.9
Salmalia malabarica	0.0	0.2	0.3	0.0	0.1	0.4	0.2	0.2	0.5	0.0	0.1	0.8
Morus laevigata	0.1	0.01	0.1	0.02	0.1	0.6	0.7	1.3	1.0	0.5	0.2	0.3
Moringa oleifera	-	-	-	-	-	-	-	-	3.9	0.6	0.4	0.4
Pongamia pinnata	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.4	0.2	0.1	0.0	0.0
Ehretia laevis	-	-	-	-	-	-	-	-	-	6.4	0.6	0.2
Unidentified sp. A	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.3	0.1	0.0	0.0

Table 9 MEAN NUMBER OF NYMPHS AND PUPAE OF *BEMISIA TABACI* PER 100 CM^2 ON MINOR ORNAMENTAL PLANTS DURING 1993-96

Host	J	F	M	A	M	J	J	A	S	O	N	D
Impatiens balsamina	-	-	-	-	-	-	-	-	5.4	3.9	0.4	0.03
Verbena hybrida	0.0	0.1	0.0	0.2	0.6	-	-	-	-	-	-	0.5
Bougainvillea glabra	0.1	0.1	0.0	0.04	0.04	0.05	0.2	0.09	0.4	0.2	0.2	0.07
Celosia argentia var. cristata	0.1	0.05	0.06	0.0	0.0	1.1	0.6	0.5	1.8	4.6	0.3	0.07
Elettaria cardamum	0.4	0.0	0.0	0.0	0.0	0.0	1.8	0.2	0.0	0.0	0.0	0.0
Lathyrus odoratus	1.9	0.5	0.0	0.0	0.2	0.3	0.0	0.3	9.9	0.7	0.09	2.5
Gerbera Jamesonii	0.0	-	-	0.0	0.06	1.3	0.4	0.3	0.7	1.9	1.8	0.5
Ipomoea carnea	0.02	0.0	0.0	0.0	0.2	0.3	0.2	0.4	3.9	2.0	0.2	0.2
Althaea rosea	0.07	0.3	0.2	0.4	0.6	0.9	3.9	0.4	8.3	39.3	0.4	0.3
Erythrina suberosa	0.3	0.3	0.1	1.2	8.0	2.7	9.1	0.5	1.0	0.3	0.08	0.07
Hamelia patens	0.0	0.0	0.0	0.0	0.2	2.5	0.2	0.3	1.3	1.3	0.3	0.7
Lawsonia inermis	0.2	0.5	0.9	0.5	0.3	0.6	1.2	0.0	2.9	1.4	1.8	3.1
Ocimum basilicum	0.2	0.2	0.0	0.09	0.3	0.3	0.6	0.7	1.7	1.9	1.2	0.3
Ruellia tuberosa	0.1	-	-	0.0	0.2	0.5	2.8	0.4	4.0	11.4	0.7	0.5
Argyrela speciosa	0.02	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tecoma stans	0.4	0.4	0.2	0.0	0.0	0.7	0.4	0.2	9.9	2.9	0.7	0.1
Antigonon leptopus	0.0	0.0	0.0	0.0	0.5	2.3	0.6	1.5	6.9	2.0	1.3	0.0
Compsis undulata	-	-	-	-	-	-	0.5	30.1	167.3	64.4	3.1	4.2
Ipomoea.purpurea	-	-	-	-	-	-	0.0	0.0	1.1	0.3	0.3	0.0
Vitex agnus-costus	-	-	-	-	-	-	-	1.3	3.2	0.5	0.6	0.0
Jasminum multiflorum	0.1	0.1	0.2	0.2	0.4	0.5	0.4	0.4	0.5	0.2	0.1	0.2
Cestrum nocturnum	0.1	0.0	0.0	0.0	0.0	0.3	0.1	0.5	0.0	8.8	0.5	1.1

Table 10 MEAN NUMBER OF NYMPHS AND PUPAE OF BEMISIA TABACI PER 100 CM^2 ON MINOR WEED PLANTS DURING 1993-96

Host	J	F	M	A	M	J	J	A	S	O	N	D
Abutilon indicum	0.18	0.05	0.04	0.1	0.3	0.05	0.1	0.3	0.2	0.3	0.9	0.05
Withania somnifera	0.04	0.13	0.03	0.05	0.03	0.5	0.3	0.6	8.0	7.0	0.4	0.1
Amarantus viridis	0.1	0.7	0.0	0.4	0.4	0.5	0.3	0.2	1.6	4.4	0.8	0.5
Chenopodium album	0.14	0.28	0.0	0.1	0.3	0.3	0.09	0.0	0.0	0.6	0.3	0.3
Tribulus terrestris	0.0	0.0	0.0	0.0	1.3	0.3	0.1	0.9	8.0	0.7	0.8	0.0
Cassia absus	0.0	0.0	0.0	0.0	0.3	11.0	0.5	0.5	25.0	6.0	0.0	0.8
Cleome viscosa	-	-	-	-	0.1	0.09	1.0	1.0	2.1	6.1	-	-
Dhatura metel	0.06	0.2	0.02	0.05	0.5	0.0	0.7	0.1	28.0	7.0	0.1	0.0
Euphorbia prostrata	0.8	1.2	0.0	0.0	0.0	-	1.2	-	-	30.4	0.0	0.0
Euphorbia helisesocopia	0.8	1.2	0.0	0.0	0.0	-	1.2	-	-	30.4	0.0	
Ipomoea aquatica	0	-	-	0	0.2	0.0	0.4	0.8	0.2	0.2	0.05	0.2
Trianthema portulacastrum	0	0	0	0.8	1.2	0.3	0.3	0.3	1.4	8.3	0.3	9.1
Chenopodium. murale	0.3	0.2	0.2	0.3	0.1	1.3	0.7	0.3	0.2	1.6	1.9	0.6
Cnicus arvensis	0.1	0.3	0.1	0.04	0.04	0	-	-	0.4	1.2	0.8	0.14
Rhynchosia sp.	0.05	0.08	0.07	0.02	0.03	1.1	1.4	4.4	9.0	8.4	0.4	0.0
Rumex dentatus	0.07	0.06	0.03	0.05	0.03	0.6	0.1	0.2	0.0	0.5	0.3	0.02
Malilotus indica	0.2	0.0	0.0	0.0	0.0	-	-	-	0.0	-	1.2	0.3
Malva parviflora	0.2	0.3	0.3	0.2	1.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2
Digera arvensis	0.0	0.0	0.0	0.0	0.6	0.5	0.7	0.3	2.8	29.01	1.3	0.9
Nicotiana plumbaginifolia	0.07	0.09	0.03	0.2	0.2	0.2	0.04	0.2	0.9	5.2	0.8	0.2
Mentha longifolia	0.6	0.2	0.0	0.4	0.0	3.5	0.0	0.0	0.0	0.0	0.0	1.4
Verbena officianalis.	0.0	0.00	0.0	0.7	0.6	0.4	0.2	0.0	14.3	3.0	1.1	0.8
Unidentified sp.1 (Chibbar type)	-	-	-	-	-	-	-	5.0	30.0	8.0	-	-
Unidentified sp.2 (Abutilon type)	-	-	-	-	-	5.5	1.3	0.5	-	-	-	-
Unidentified sp.3 (Jantar type)	-	-	-	-	-	0.0	6.0	3.9	5.9	2.2	1.9	0.0
Unidentified sp.4 (wild jute type)	-	-	-	-	0.0	4.8	0.7	2.2	148.0	9.1	0.0	2.7

Carry over

Cotton whitefly does not undergo diapause during winter and continues to breed on a large number of plants at a slow rate. During winter, in cotton areas it was recorded from 114 hosts including 32 crops, 31 ornamental and 35 weed plants and 16 fruit and forest trees. Maximum population was on *M. viridis* (mint), *S. melongena* (brinjal), *C. pepo var. melopepo* (squash), *L. esculentum* (tomato) and *S. turberosum* (potato) among the crops and vegetables, *A. conyzoides* (Jaretum), *H. annuus* (ornamental sunflower), *I. tricolor* (morning glory), *L. camara* (lantana) and *C. morifolium* (gul-e-daudi) among ornamental plants; *X. strumarium* (cocklebus), *E. hirta*, *A. aspera* (puth kanda), *E. prostrata* (dodhal booti), *M. tricuspidatum* and *C. arvensis* (bindweed) among weeds and *B. papyrifera* (wild mulbery), *M. alba* (mulbery), and *F. carica*. These hosts carried almost 90% of the total population during this period.

After winter temperature starts rising, the whitefly starts multiplying rapidly on *V. radiata*, *L. culinaris*, *V. cylindrica*, *G. max* and *H. annuus* from crops; *S. melongena*, *C. pepo var. melopepo*, *C. melo*, *C. sativus*, *C. lanatus var. listulosus* and *C. melo var. utilissma* among vegetables; *A. rasea*, *C. morifolium*, *L. camara*, *H. annuus* and *H. mutabilis* among ornamental plants, *E. prostrata*, *C. tetragona*, *X. strumarium*, *M. tricuspidatum*, *S. nigrum* and *A. aspera* among weeds; *M. alba* and *A. lebbek* among fruit and forest trees are important and play major role for shifting of *B. tabaci* from these hosts to cotton.

Population trends on cotton

During cotton season it continues to develop on cotton as well as on other alternate hosts at much faster rate with a peak in September-October. Population

dynamics of *B. tabaci* was studied on cotton at three sites at Khanewal during 1994-1996. Weekly data are presented in **Fig. 54-56**. During 1994 whitefly population was low in June and was at economic threshold level and remained so at the end of July. From August to October population was very high with a peak at the end of September **(Fig.54).** Population trend during 1995 and 1996 was almost similar with a peak in the second week of September in 1995 **(Fig.55)** and in the third week in 1996 **(Fig.56)** and declined afterwards.

Host preference for oviposition and survival

Whitefly is highly polyphagous species and as reported above was recorded from 145 plants species but some of these were more preferred than others. Suitability of a plant depends on a number of factors such as preference for oviposition, survival and development period etc. Suitability of the recorded plants, based on preference for oviposition, development period and survival was studied.

To determine oviposition preference of whitefly, six recorded host plants including *G. hirsutum* as control were offered in multiple choice tests in cages under field conditions when day temperature was 30-35°C and night temperature 15-22°C. Six potted plants included *G. hirsutum, S. melongena, L. camara, X. strumarium, A. aspra* and *C. tetragona* were placed in a circle. It was replicated five times. Total leaves of each host were counted. Two-day-old ten pairs of whitefly per leaf were released in the centre of the cage to give equal chance for oviposition. After 24 hours adults were removed from the cages and number of eggs laid on each host plant was counted. After six days, number of eggs hatched was recorded. Nymphal development and survival was recorded at weekly intervals.

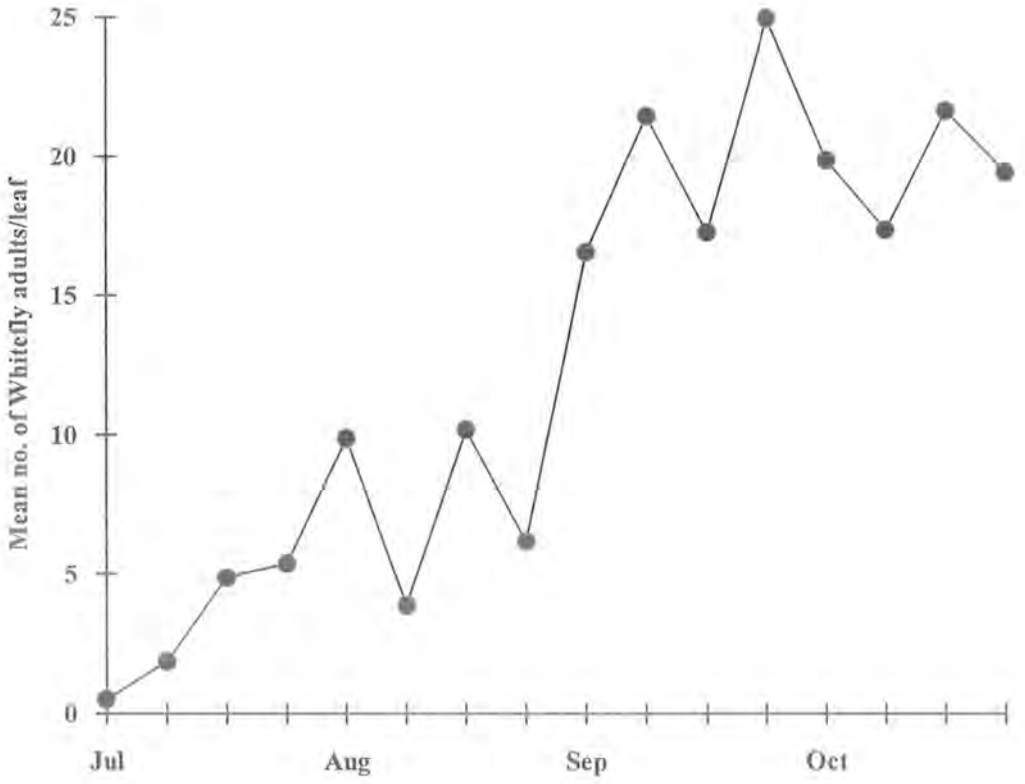


Fig.54 Mean number of *Bemisia tabaci* adults on cotton during 1994

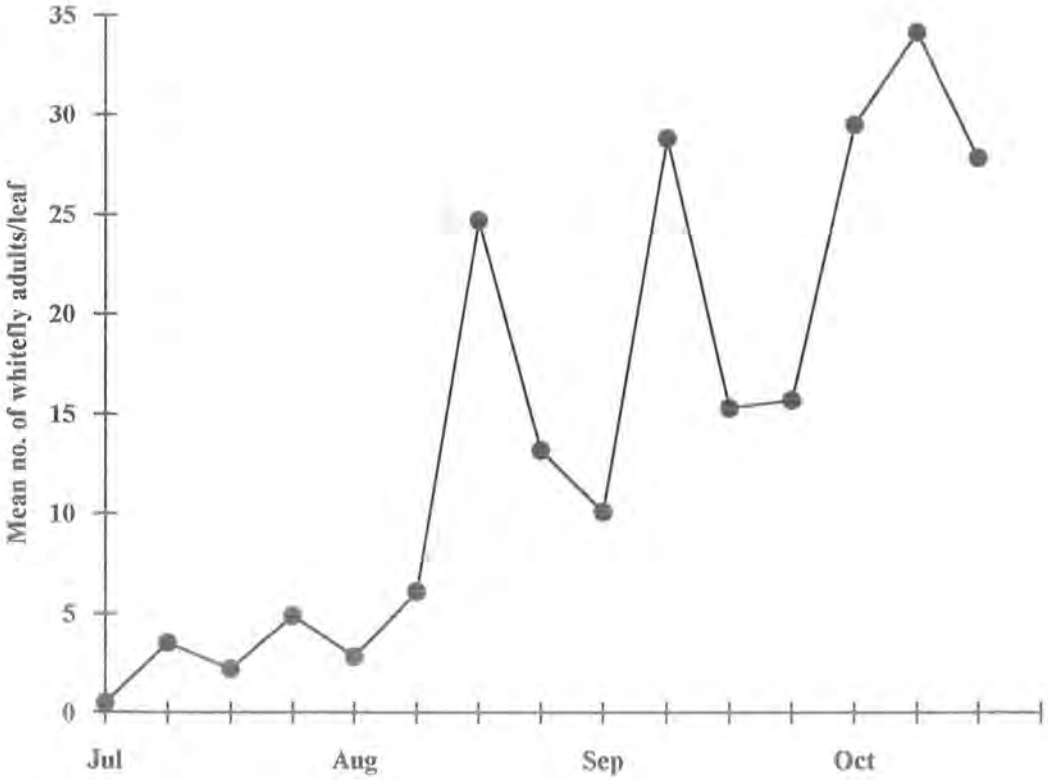


Fig.55 Mean number of *Bemisia tabaci* adults on cotton during 1995.

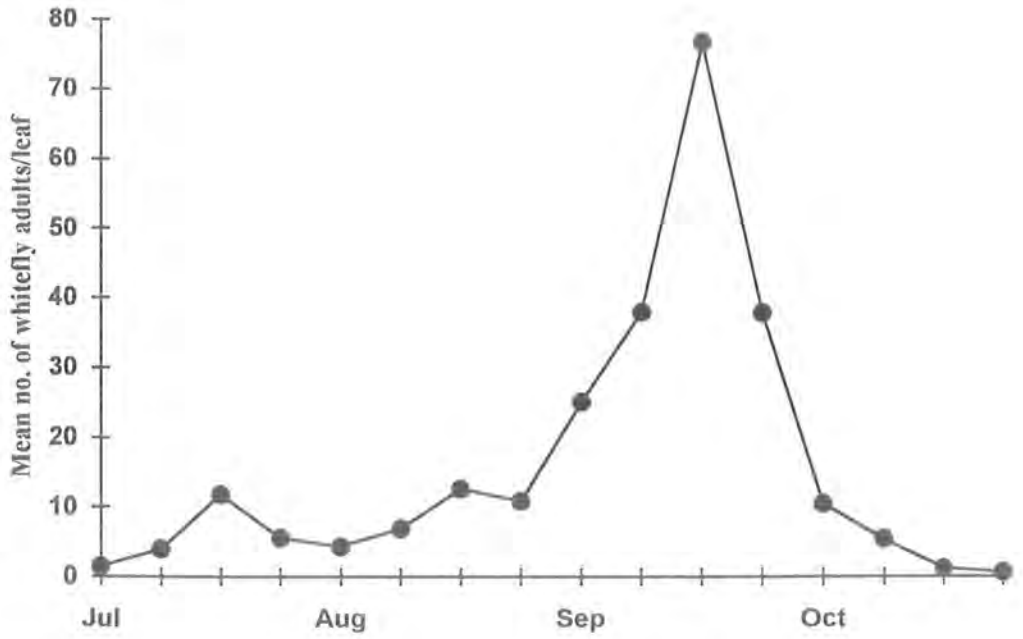


Fig.56 Mean number of *Bemisia tabaci* adults on cotton during 1996.

S. melongena, G. hirsutum from crops and *X. strumarium, A. aspera* and *C. tetragona* from weeds, *L. camara* from ornamental were most preferred for oviposition followed by others (**Table 11**). Maximum egg mortality was on *G.hirsutum* followed by *X. strumarium* and others. Development of nymphs was lowest on *A. aspera* followed by *G. hirsutum* (**Table 11**) *S. melongena* was the most suitable host followed by *L. camara, C. tetragona, G. hirsutum, A. aspera* and *X. strumarium* in descending order for whitefly development (**Table 11**).

Table 11 Rate of oviposition and survival of whitefly immatures on different hosts in the field when maximum temperature was 30 to 36°C ($\bar{X}$=33.3) and minimum temperature ranged 15 to 22°C($\bar{X}$= 18.9)

Host plants	No.of eggs		Number of nymphs and pupae survived		Survival %
	Laid	Hatched	Nymphs	Pupae	
Gossypium hirsutum	1751	1345	879	796	45.5
Solanum melongena	2517	2086	1679	1473	58.5
Lantana camara	1547	1335	966	839	54.2
Xanthium strumarium	1733	1310	841	694	40.0
Achyranthes aspera	637	574	358	279	43.8
Citrullus tetragona	266	222	164	138	51.9

On some of the annual plants whitefly breeds only for a few months although these were present for longer periods. In order to determine their suitability for development 17 annual and five perennial plants were compared in microcages in the laboratory. Cotton was kept as control. This experiment was carried out on two vegetables, eight ornamentals, ten weeds and two fruit and forest trees.

Oviposition and survival data are presented in **Table 12**. Most preferred hosts for oviposition were *H. patens* followed by *S. melongena, L. camara, M. alba, C. melo* and *P. alkakengi* and others (**Table 12**). Suitable hosts for development and survival were *C. triloailanis* followed by *C. melo, P. alkakengi, L. camara, C. argentia, S. melongena. H. patens and M. alba* were preferred for oviposition but were not suitable for development and survival. Although on *C. triloailanis, H. annuus* and *C. argentia*

less number of eggs were laid but survival rate was much higher compared to other hosts. *C. triloailanis* was the most suitable for development and survival among the tested hosts followed by others (**Table 12**).

Table 12 Comparative development of *Bemisia tabaci* on different annual and perennial hosts under caged conditions.

Host plant		Eggs		No.developed		%
Botanical Name	**English Name**	**Laid**	**Hatched**	**Pupae**	**Adults**	**Survival**
Annual						
Cucumis melo	Melon	116	116	103	96	82.8
Solanum melongena	Brinjal	172	144	116	108	62.8
Celosia argentia	Cock's Comb	52	46	42	34	65.4
Hamelia patens	Hamelia	185	160	42	35	19.0
Helianthus annuus	Sunflower(orn.)	88	63	63	54	62.5
Lantana camara	Lantana	164	162	145	109	66.5
Ruellia tuberosa	Ruellia	74	36	21	20	27.0
Achyranthes aspera	Achyranthes	86	81	63	47	54.7
Amarantus viridis	Amaranthes	47	43	23	20	42.6
Cassia absus	Casia	67	31	15	14	20.9
Chenopodium murale	Chenopodium	31	25	18	8	25.8
Citrullus tetragona	Citrullus	93	77	52	52	55.9
Convolvulus arvensis	Bind weed	57	53	27	23	40.4
Corchorus triloailanis	Corchorus	68	63	63	63	92.6
Physalis alkakengi	Physalis	103	92	76	69	67.0
Solanum nigrum	Night Shade	73	53	45	39	53.4
Withania somnifera	Withania	27	22	15	13	48.1
Perennial						
Eucalyptus citrodora	Eucalyptus	4	2	0	0	0.0
Morus alba	Mulberry	129	51	24	19	14.8
Lawsonia inermis	Lowsonia	82	30	10	9	11.0
Abutilon indicum	Abutilon	6	2	0	0	0.0
Malvestrum tricuspidatum	Malvestrum	67	67	52	50	44.8
Gossypium hirsutum	Cotton	126	107	87	82	65.1

Development period

Development period of *B. tabaci* was studied on 16 plant species at 27±3°C (**Table 13**). Eggs hatched from 4.5 to 5.5 ($\bar{x}$ = 4.6) days. The nymphs completed development in 9.5 to 11.5 ($\bar{x}$ = 10.3) days. Adults from pupae emerged in 4 to 5.5 ($\bar{x}$ =4.9) days. The total development period was 18.5 to 22.5 ($\bar{x}$ = 19.7) days (**Table 13**).

Table 13 Comparative development of *Bemisia tabaci* on different host plants at 27±3°C.

Host Plants	Mean developmental period in days			
	Eggs	Nymph	Pupae	Total
Solanum melongena	4.5	9.5	4.5	18.5
Cucumis melo	4.5	9.5	5.0	19.0
Abelmoschus esculentus	4.5	10.5	5.0	20.0
Lantana camara	4.0	9.5	4.5	18.0
Solanum nigrum	4.0	9.5	5.0	18.5
Physalis alkakengi	4.5	9.5	5.0	19.0
Helianthus annuus	4.5	10.0	5.0	19.5
Citrullus tetragona	4.5	11.0	5.5	21.0
Achyranthes aspera	4.5	10.5	5.0	20.0
Corchorus triloailanis	4.5	10.5	4.5	19.5
Withania somnifera	5.5	11.5	5.5	22.5
Celocia argentia	5.0	11.0	5.5	21.5
Convolvulus arvensis	4.5	11.0	5.0	20.5
Chenopodium murale	5.5	11.0	4.5	21.0
Amarantus viridis	4.5	11.0	4.0	19.5
Gossypium hirsutum	4.5	10.5	5.0	20.0

Vertical distribution

Studies on vertical distribution of the whitefly are useful for efficacious application of pesticides. Therefore, distribution of immature stages was studied on cotton plant. Second and third instar nymphs and pupae were counted on all the parts on main stem and side branches of five plants of cotton variety CIM-70 daily during peak period of whitefly activity in July and August. First expanded leaf was considered as node one.

Distribution of nymphs and pupae on leaves of the main stem is given in **Table 14.** In July 1992 when plants were about 45 cm high, whitefly population was minimum on leaves up to third node and then gradually increased and was maximum on 9th node. Thereafter, it declined reaching zero on sixteenth node. In August population trend was almost similar. Up to second node population was low. From third node onwards it increased being maximum on tenth node and then declined and was

minimum on seventeenth node. In 1993 population trends were almost similar to that during 1992. It was maximum on node tenth in July and on eighth in August.

Table 14 **Number of nymphs and pupae of *Bemisia tabaci* on leaves on main stem on cotton variety CIM-70 from top to base**

No.of expanded leaf	Mean number of nymphs/ pupae per leaf			
	1992		1993	
	July	August	July	August
1	0.0	0.0	0.0	0.1
2	0.4	0.5	0.0	0.3
3	0.1	2.2	0.0	0.9
4	5.0	5.0	0.0	1.8
5	7.6	5.6	0.2	2.9
6	11.4	10.1	1.6	4.8
7	18.5	10.9	3.0	9.3
8	29.7	12.0	5.3	10.2
9	37.5	12.3	7.8	8.2
10	30.0	16.2	8.5	6.2
11	23.1	12.3	8.2	5.0
12	18.7	8.5	5.2	3.8
13	9.5	4.3	3.1	3.1
14	1.6	4.9	2.9	1.7
15	0.4	4.6	1.3	1.1
16	0.0	2.9	0.7	1.3
17	0.0	0.6	0.1	0.1

To determine the population level on side branches nymphs and pupae of all branches were counted from top to base. Distribution trend of nymphs and pupae is presented in **Table 15**. Overall population was higher from fourth to tenth branches as compared with other branches. In July major proportion of the population found was from fourth to ninth branches. As the plant grew whitefly shifted upwards and in August highest population was from node second to ninth in both the years **(Table 15)**.

Table 15 **Number of nymphs and pupae on leaves on side branches from top to base**

Side branch number	Means number of whitefly nymphs per branch			
	1992		1993	
	July	August	July	August
1	1.0	3.0	0.0	0.6
2	2.2	4.2	2.3	4.9
3	0.8	8.1	2.9	9.3
4	1.2	5.6	6.0	14.7
5	1.8	4.8	9.8	13.0
6	2.6	7.0	11.7	16.5
7	4.6	10.0	20.5	14.1
8	4.0	9.6	9.4	9.0
9	1.6	7.2	8.0	4.5
10	0.6	2.6	5.0	1.2
11	0.6	1.6	0.0	0.0
12	0.4	1.4	0.0	0.0

To determine how the whitefly infestation starts on branch leaves, the number of nymphs and pupae were counted from leaves of side branches starting from top to base. Nymphs and pupae on each leaf from top to base is presented in **Table 16.** Within the branch close to main stem leaves were more susceptible in carrying nymphal population than the young leaves (**Table 16**).

Table 16 **Number of nymphs and pupae per leaf within the branch from top to base**

Leaf number	Mean number of whitefly nymphs and pupae per leaf			
	1992		1993	
	July	August	July	August
1	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0
3	0.1	0.0	0.1	0.0
4	0.2	0.1	6.0	0.1
5	0.5	0.2	1.7	0.2
6	0.6	0.3	4.7	0.8
7	0	0.6	0	1.8
8	0	0.7	0	5.1

Control measures

In Integrated Pest Management (IPM) a number of methods such as chemical, biological etc. are used in such a way that effect of one is complementary to other. For whitefly control feasibility of chemical and biological controls was studied.

Chemical control

a) Efficacy of two formulations and three volumes of application

At present, mainly insecticides are used for the control of *B. tabaci* on cotton. Efficacy of two insecticides from different chemical groups including karate (pyrethroid); and nuvacron (organophosphate) in ultra low volume formulation and two volumes of application of E.C. formulation was studied. Untreated plot was kept as control for comparison. To study the morality due to insecticides whitefly adults and immatures were counted on 10 leaves, 48 hours, one week and two weeks after each spray in the field

b) Effect of insecticides on whitefly

The whitefly population was significantly lower in the plot where nuvacron was used as high and low volume as compared with control 48 hours after spray. However, these difference were non significant as compared with control when nuvacron was used as ultra low volume **(Table 17).** The population was significantly higher where nuvacron was used as compared with untreated check one and two weeks after spray.

The whitefly number was significantly higher in the plot where ultra low volume formulation of nuvacron was used as compared with high and low volumes of application 48 hours and one week after spray. The differences between low and high volumes one week after spray were non significant. In the ultra low volume population

was also significantly higher than low volume but their difference was non significant where nuvacron was used as high volume two weeks after spray.

In the plots where karate was sprayed whitefly population was almost the same or higher both in the treated and control 48 hours after application. One and two weeks after it was higher where karate was used compared with the control. In low volume and ultra low volume it was always higher where karate was used as compared with control (**Table 17**).

Table 17 Mean number of whitefly adults and immatures per leaf from the plots treated with nuvacron and karate at high, low and ultra volumes of application

Treatments	Mean number of nymphs and adults								
	High volume			Low volume			Ultra low volume		
	48 hours	One week	Two weeks	48 hours	One week	Two weeks	48 hours	One week	Two weeks
Nuvacron	3.6a	7.2b	8.7b	4.3b	7.4b	7.4a	6.4cd	8.7c	8.8b
Control *	7.0d	6.8a	7.3a	7.0d	6.8a	7.3a	7.0d	6.8a	7.3a
Karate	5.9c	9.7d	12.1c	6.9d	11.1e	14.5d	10.2e	12.1f	19.8e
Control *	5.0c	6.8a	7.6a	5.0c	6.8a	7.6a	5.0c	6.8a	7.6a

*Same control was kept for all the three volumes of each insecticide, separately
Means followed by the same letter are not significantly different at 5% leve of significance

To determine the effect of insecticides on whitefly in addition to field, observations on mortality were also made in the laboratory. Three leaves with maximum nymphs and pupae were brought to the laboratory 48 hours, one week and two weeks after each spray. The number of nymphs and pupae were counted and the leaves were dipped in the bottle with water in it and kept under hurricane lamp glass for emergence of whitefly adults.

Whitefly adults emergence was significantly lower in the plot where nuvacron was used as high volume 48 hours after spray as compared with control but these differences were non significant when this chemical was used as low and ultra low volumes 48 hours after spray. Adult emergence was also significantly lower where nuvacron was used one week after spray as compared with control in all the volumes of application. Significantly more number of adults emerged where nuvacron was used as low and ultra low volume as compared with control two weeks after spray but these difference were non significant when nuvarcon was used as high volume. Adults emergence was significantly lower 48 hours after spray where karate was used as high volume but these difference with low volume were non significant. In low and ultra low volumes emergence was higher one and two weeks after spray where karate was used compared with control (**Table 18**).

Table 18 **Percentage of whitefly adults that emerged from the fields collected nymphs and pupae where different formulations of nuvacron and karate were used and the control plots**

Treatments	Adult emergence (%)								
	High volume			Low volume			Ultra low volume		
	48 hours	One week	Two weeks	48 hours	One week	Two weeks	48 hours	One week	Two weeks
Nuvacron	20.9a	25.2a	51.6abc	30.4b	34.7b	53.8de	30.1b	34.3b	54.9e
Control*	30.6b	40.1c	50.4ab	30.6b	40.1c	50.4ab	30.6b	40.1c	50.4ab
Karate	32.5c	48.2c	51.0ab	34.5d	43.3d	80.0a	38.6e	46.3e	53.2cd
Control*	35.8d	41.3c	52.1bcd	35.8d	41.3c	52.1bcd	35.8d	41.3c	52.1bcd

*Same control was kept for all the three volumes of each insecticide, separately.
Means followed by the same letter are not significantly different at 5% level of significance.

c) **Effect of insecticides on parasitoids**

To determine the effect of insecticides on parasitoids, three leaves with maximum infestation of whitefly from each plot at all intervals were kept in the laboratory as mentioned above.

Parasite emergence was significantly higher from insecticides free plots compared with different application volumes of nuvacron and karate, 48 hours, one and two weeks after spray. Higher number of parasites emerged from plots where ultra low volume was used as compared with low and high volumes at all intervals. The differences in parasites emergence were significant in most of the case **(Table 19)**.

Both nuvacron and karate in high application volume proved significantly more toxic to whitefly parasitoids as compared with low and ultra low volumes 48 hours, one and two weeks after spray **(Table. 19)**.

Nuvacron proved more toxic to whitefly parasitoids and significantly lower number of adults emerged as compared with plots treated with karate at all intervals **(Table. 19)**. Overall adverse effect of insecticides use was much greater on parasitoids than on whitefly.

Table 19 Percentage of parasitoid adults that emerged from the fields collected nymphs and pupae where different formulations of nuvacron and karate were used and the control plots

Treatments	Adults emergence (%)								
	High volume			Low volume			Ultra low volume		
	48 hours	One week	Two weeks	48 hours	One week	Two weeks	48hours	One week	Two weeks
Nuvacron	15.0f	9.9e	7.7a	16.0e	11.6d	10.0b	22.4b	15.7c	9.9b
Control *	26.4a	18.8b	23.5e	26.4a	18.8b	23.5e	26.4a	18.8b	23.5e
Karate	20.5c	14.9c	12.9c	19.0d	16.2c	11.2bc	25.7a	19.2b	10.2b
Control *	27.0a	23.1a	20.6d	27.0a	23.1a	20.6d	26.0a	23.1a	20.6d

*Same control was kept for all the three volumes of each insecticide, separately.
Means followed by the same letter are not significantly different at P = 0.5 level

d) Evaluation of some non-conventional and conventional chemicals against whitefly

Efficacy of some non-conventional and botanical insecticides such as *Eruca sativa* (vegetable oil) oil alone and in combination with detergent (zip) and neem soap, biosal 0.32% EC (neem extract) was studied. Two conventional insecticides, acetamiprid 20 SP (mospilan) and diafenthiuron 500 SC (polo) were kept as control for comparison. An untreated plot was kept as control. The details are given in **Table 20**. The cotton cultivar CIM 240 was sprayed four times at weekly interval during peak activity period of pest, during August and September. Number of nymphs and adults of whitefly were recorded 24 hours, 72 hours and one week after each spray.**(Fig.57-58).**

From the conventional insecticides both acetamiprid and diafenthriuron gave almost 80% mortality of adults one, three and seven days after application. From the non-conventional insecticide *Eruca sativa* oil + detergent and biosal gave highest mortality during this period. Mortality of adults with *E. sativa* oil + detergent was slightly better than *E. sativa* oil or detergent alone **(Fig.57)**.

Nymphal mortality was highest with acetamiprid followed by *E. sativa* oil + detergent, diafenthriuron, biosal and others **(Fig.58).** Mortality with *E. sativa* oil or detergent was better up to three days and declined sharply afterwards.

Table 20 Insecticides with the treatment and their dose rate

Treatment	Dose/ha
Eruca sativa oil	2500ml
Detergent	2500ml
Eruca sativa oil + detergent	2500ml + 2500ml
Eruca sativa oil + neem soap	2500ml + 1kg
Biosal 0.32 EC	2500ml
Acetamiprid 20 SP (mospilan)	375gm
Diafenthiuron 500 SC (polo)	500ml
Untreated check	-

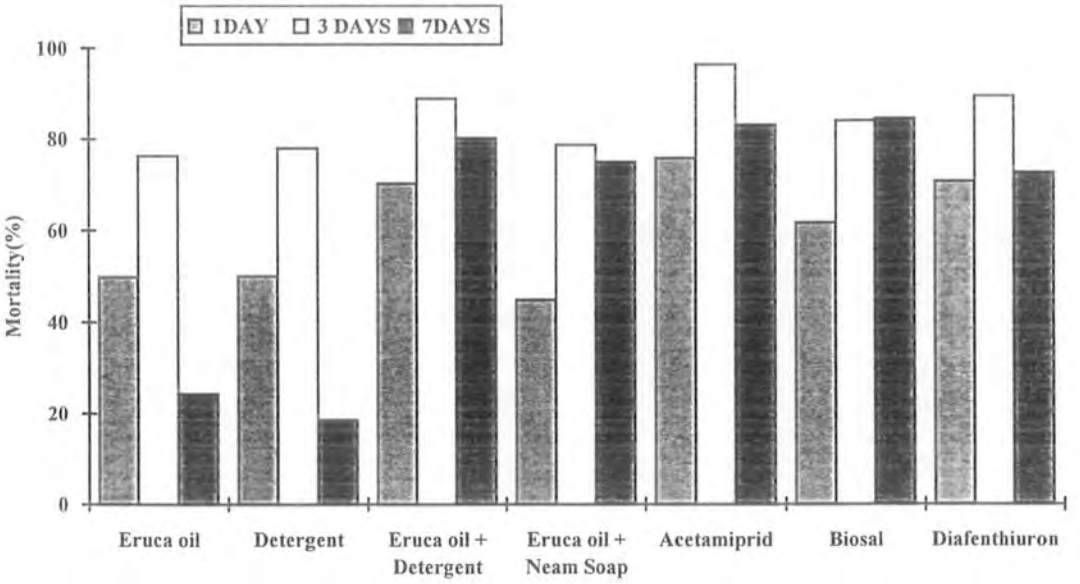


Fig.57 Mortality of *Bemisa tabaaci* nymphs with some conventional and non conventional chemicals at different intervals.

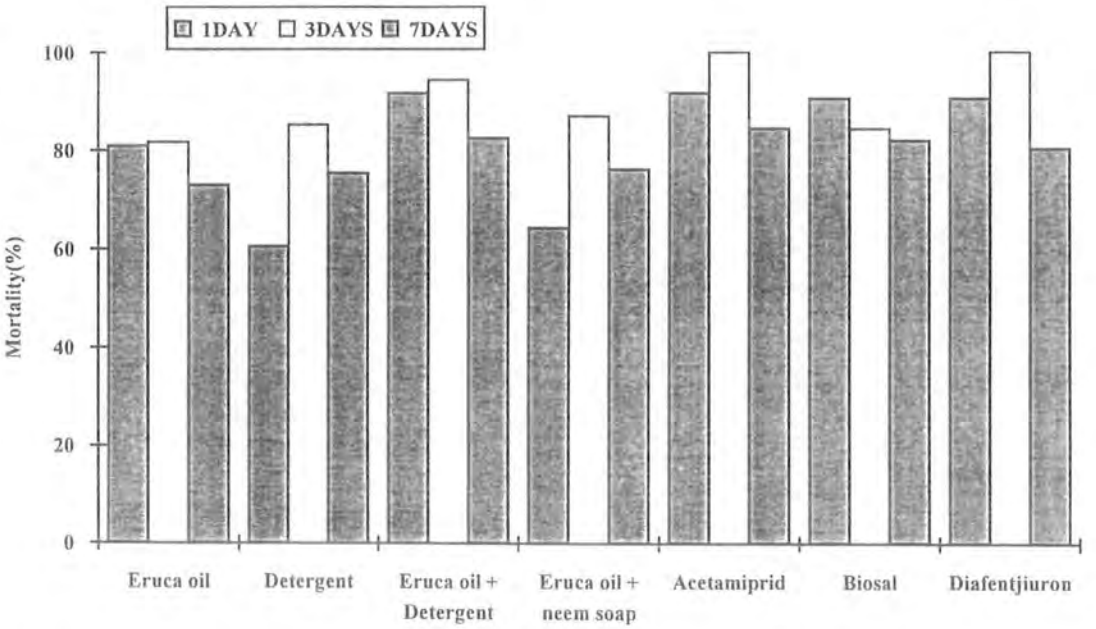


Fig.58 **Mortality of *Bemisa tabaaci* adults with some conventional and non conventional chemicals at different intervals**

Biological control

a) Parasitism

Biological control is major component of integrated pest management. In Pakistan natural enemies of *B. tabaci* have been reported by Mohyuddin *et al.* (1989). To determine the effect of natural enemies and their role in regulating whitefly population on cotton was studied. Incidence of various natural enemies on host plants other than cotton was also studied to determine the possibility of such other hosts as reservoir for their shifting to cotton at the appropriate time. As identification of various parasitoids was not possible, therefore total parasitism was recorded.

To study the parasitism in *B. tabaci,* leaves of different host plants infested with whitefly nymphs and pupae were brought to the laboratory throughout the year. Leaf pieces with nymphs and pupae were cut and kept in gelatin capsules for the emergence of parasite.

Encarsia species and *Eretmocerus species* were common parasitoids of whitefly nymphs and pupae. From January to March, maximum parasitism was on *Lantana camara* (4.0 to 18.9%), on *S. melongena* (2.3 to 4.3%) and on *E. pulcherrima* (2.5 to 5.0%). From April to June highest parasitism was on *S. melongena*, (4.0%) on *C. melo* (9.1%) and on *V. radiata,* (7.0%), on *A. lebbek* (8.9%) on *M. alba* . (14.3) on *L. camara* (5.6%) and on *A. rosea.* (8.3%), on *A. aspera* (28.0%) and on *X. strumarium* (19.5%) during this period. From July to December parasites remained active mainly on *S. melongena* (13.3%), *L. camara* (8.9%), *A. aspera* (15.0%), *X. strumarium* (14.3%), *A. rasea* (37.5%), *Ipomoea batatas* (25.0%) and on *Gossypium hirsutum* it ranged between 1.2 to 8.9%. It shows that parasitism remained high on those plants which received no or very little spray of insecticides against whitefly.

b) Effect of conservation of natural enemies on whitefly infestation

In a separate experiment effect of conservation of natural enemies on infestation of *B. tabaci* was studied. Two plots each measuring 0.4 ha. were selected, in one plot farmer used insecticides and was compared where natural enemies were conserved and insecticides were not used against bollworms. Botanical insecticide such as biosal was used for the control of *Amrasca devastans* (Dist.). Pest and natural enemies were recorded at 15 days interval. For parasitoids 10 leaves with whitefly nymphs and pupae from each plot was brought in the laboratory. Leaves pieces with nymphs and pupae were kept in the gelatin capsules.

Whitefly activity started during July in both the plots. Population remained almost at the same level till the end of August. Thereafter, whitefly increased and number per leaf was seven at the end of October where farmer use insecticides. The parasitism in this plot was very low. Applications of insecticide was responsible for low parasitism in this plot (**Fig. 59**). In other plot where farmer did not use insecticide whitefly numbers fluctuated at low level and was 0.5 per leaf at the end of October. The parasitism remained high from July to October with a peak (40%) at the end of October (**Fig. 60**).

c) Predators

The number of predators in both these plots was also estimated. The relative abundance of predators is given in **Fig.61**. The main predators include *Brumoides suturalis* (F.), *Coccinella septempunctata* L., *Scymunus* sp., and *Paederus fuscips* Curtis, *Chrysoperla carnea* Steph., *Orius sp.*, *Geocoris spp.* From July to September number of predators was more abundant where insecticides were not sprayed compared with where farmer used insecticides (**Fig.61**).

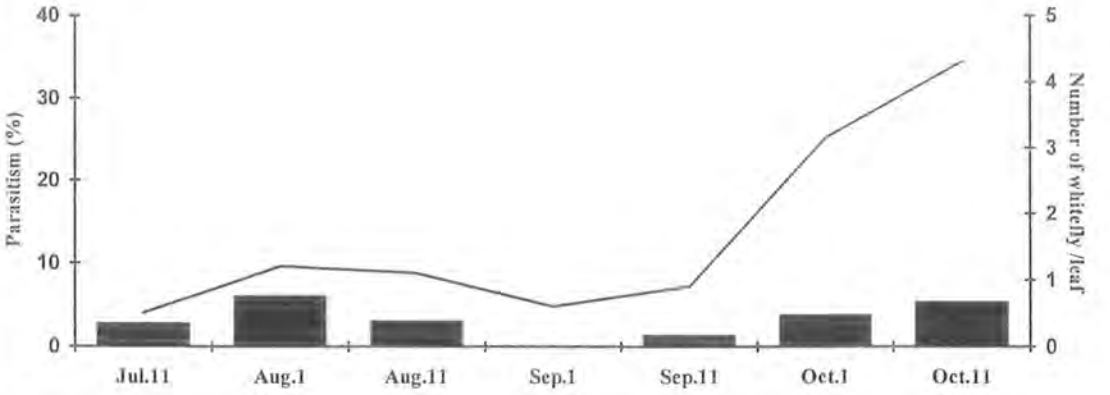


Fig.59 Whitefly population (line) and total parasitism (bar) on it on cotton where farmer used pesticides at Khokhran

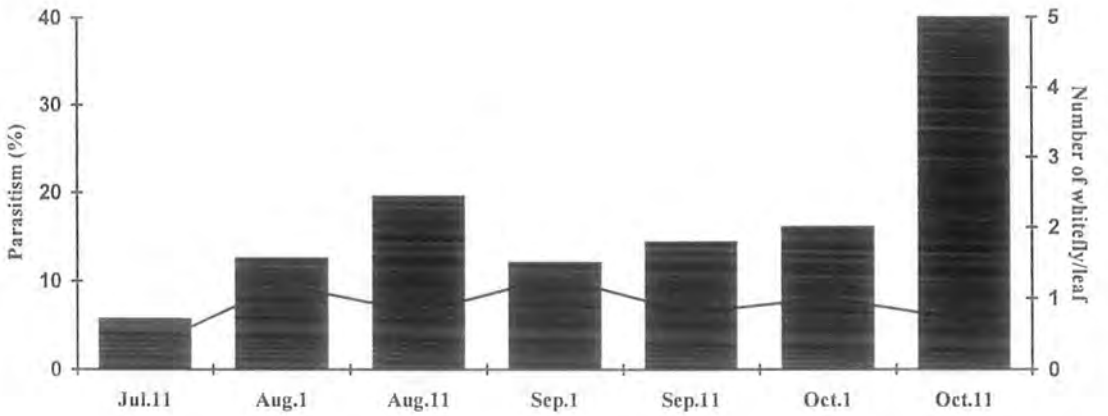


Fig.60 Whitefly population (line) and total parasitism (bar) on it on cotton where no pesticide was used at Khokhran

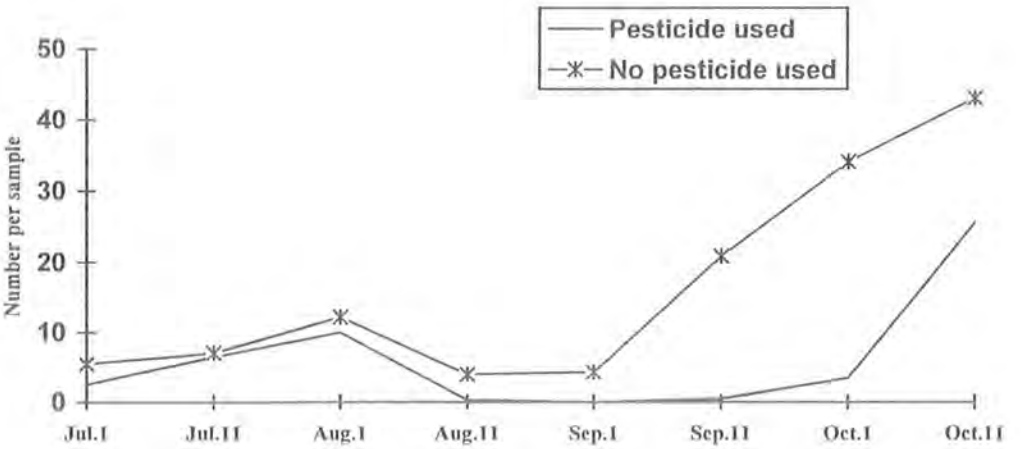


Fig.61 Relative abundance of predators in cotton at Khokhran

d) **Efficiency of *Chrysoperla carnea* in the field**

In 1996 *A. devastans, Thrips tabaci* (Lind.) and *B. tabaci* remained active on cotton throughout the season. *Orius* sp., *Geocoris* sp., spiders and *C. carnea* were the main predator associated with these pests. Due to severe attack of *A. devastans* insecticidal application started in July. *Orius* sp. *Geocoris* sp. and spiders were eliminated but *C. carnea* fluctuated and survived at low level. Whitefly population was not controlled with commonly used insecticides and became more serious. Gradually *C. carnea* also developed and its population was 120 thousand/ha soon after when whitefly reached to its maximum. Activity of this predator coincided with that of whitefly and played key role in bringing whitefly population to very low level at the end of September **(Fig.62).** Further studies are needed to find out whether it has developed resistance to insecticides or not.

e) **Predators population on alternate host plants**

Predators associated with whitefly infestation on different plant species were *B. suturalis*, Coccinellids, *Scymunus* sp., and *Paederus fuscipes*, *C. carnea., Orius sp., Geocoris spp.*, and unidentified species of spiders. Population levels varied on different plant groups depending on the whitefly infestation and climatic conditions.

From January to March weeds carried the highest number of predators followed by fruit and forest plants (**Table 21**). From April-June maximum predators population was recorded from crops followed by ornamental plants, fruit and forest trees and weed plants. From July to September highest population was recorded on fruit and forest plants followed by weeds and others. In October, November and December maximum population was on weeds followed by fruit and forest plants and others (**Table 21**).

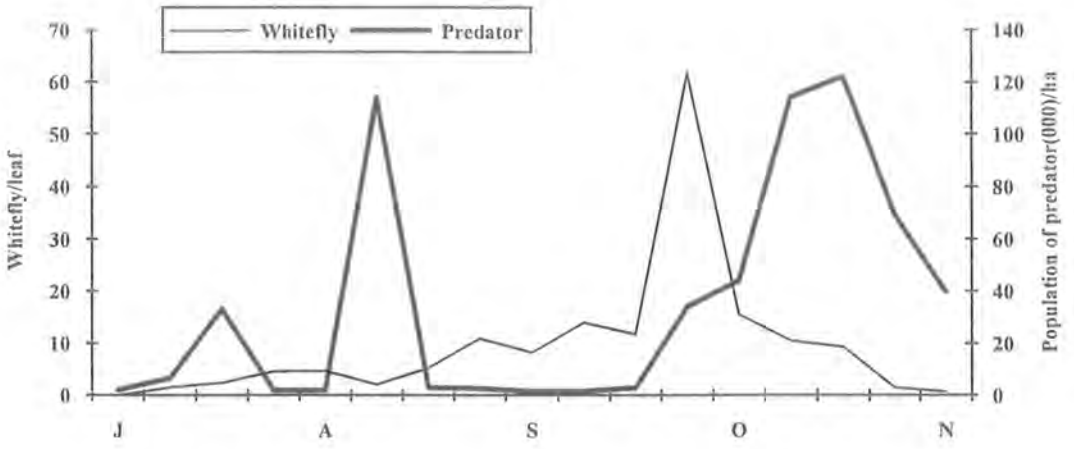


Fig.62 **Population level of *Bemisia tabaci* and *Chrysoperla carnea in the filed during 1996.***

Table 21 Abundance of various predators on different vegetation

Plant Groups	Predators population %			
	Jan.-Mar.	Apr.-Jun.	Jul.-Sep.	Oct.-Dec.
Crops	11.9	29.7	5.2	5.2
Ornamental plants	24.4	29.4	14.4	13.0
Fruit and forest plants	28.1	23.4	54.7	37.7
Weeds	35.6	17.6	25.7	47.2

Among different predator species, highest percent of *C. carnea, Orius sp.*, and *Geocoris sp.* were found on crops followed by others. The *Coccinellids* were more common on ornamental plants followed by fruit and forest trees, weeds and cropes however, spiders were maximum on weeds followed by fruit and forest trees, ornamental plants and crops **(Table 22)**.

Table 22 Population of different predator species on different plants

Predator Species	Population (%)			
	Crops	Ornamental plants	Fruit and forest plants	Weeds
Chrysoperla carnea	31.3	6.7	4.2	7.5
Orius spp.	18.2	1.2	1.0	2.3
Geocoris spp.	7.0	2.1	4.1	0.0
Coccinellids	14.0	31.4	28.8	21.3
Spiders	29.5	58.6	63.0	69.2

DISCUSSION AND CONCLUSION

Mohyuddin *et al.* (1989) recorded eleven species of parasitoids from whitefly nymphs and pupae. They stated that parasitoids should keep the whitefly under control, if insecticides are not used. Indiscriminate and excessive use of insecticides eliminates the natural enemies, resulting in increase of whitefly population. The studies carried out on the effect of insecticides on the pest and its natural enemies showed that the population of predators and parasitoids was much lower where insecticides were used compared with where farmers did not use insecticides .

In the USA whitefly developed resistance to organophosphate and pyrethroids as reported by Horowitz *et al.* (1988) and Probakher (1985-88 and 89), to these two groups and carbamate was reported by Bryan *et al.* (1992). In Pakistan, Ahmad (1996) reported that whitefly has become resistant to organophosphate such as dimethoate, methamidophos and monocrotophos and pyrethroids like cypermethrin and deltamethrin. In 1991, when virus became more severe whitefly assumed the status of the most destructive pest of cotton and plant losses were more than what would have been with whitefly feeding only. As a result of combination of development of resistance and elimination of natural enemies whitefly caused tremendous loss to the cotton crop in 1992 and 1993 crop seasons.

To develop IPM on cotton use of biological control through conservation of natural enemies and use of chemical (selective) insecticides as well as their integration as control measures were tried. Four conventional

insecticides, such as nuvacron and karate which have been used for a long time and two new insecticides diafenthiuron and acetamiprid which have recently been introduced for whitefly control and some non-conventional insecticides such as *Eruca sativa* oil, detergent and botanical insecticides were used against whitefly. monocrotophos and lambda-cyhalothrin did not work and the whitefly population was always above the threshold level and were more toxic to natural enemies after spray. Diafenthiuron and acetamitrid were effective against whitefly and provided excellent control of this pest. However, non-conventional insecticides provided better control of whitefly and they were also safe for the natural enemies. The population of predators in the plots treated with these chemicals was 40% higher as compared with conventional insecticides. Vertically whitefly distribution showed that about 10% of the population occurred on top part of the plant and maximum population was in the middle part, from 4th to 9th node followed by lower part. The diurnal activity of whitefly indicated that adults are more active from five to eight O' clock in the morning and then decreased gradually. Late in the evening the adults become active again and ceased their activity after sunset. In view of the pest occurrence on different parts of the plant and its activity during day hours, the spray application needs to modify. The drop nozzles needs to be fixed on the tractor mounted sprayer and the angles of the nozzles should be directed to those areas where maximum population is present for better penetration of insecticides and desirable control of this pest. It is also

recommended that spray should be done early in the morning before the adults become active or late in the evening when maximum adults are inactive.

The whitefly has been recorded from 145 plant species, of these 48 were major and 97 minor. Most of these hosts are present in summer alongwith cotton. However, in winter it was recorded from 114 hosts including 32 crops, 31 ornamental plants, 35 weed plants and 16 fruit and forest trees. All of them were not suitable for development but some of them such as *Solanum. melongena, Cucurbita pepo* var. *melopepo, Helianthus annuus, Lantana camara, Achyranthes aspera and Convolvulus arvensis* were most important and these hosts carried almost 90% of the total population during winter. After winter temperature become high and it multiply more rapidly on such as *Lens culinaris, Vigna radiata, V. cylindyca, Glycine max, H. annuus, S. melongena, C. pepo* var. *melopepo, Cucumis melo, C. sativus, Citrullus lanatus var. listulosus, C. melo* var. *phut, Althea rosea, Chrysanthemum morifolium, L. camara, Hibiscus mutabilis, Euphorbia prostrata, C. tetragona, Xanthium strumarium, Malvestrum tricuspidatum, S. nigrum, A. aspara, Morus alba* and *Albizzia lebbek* play major role during pre-cotton season and it shifts from these hosts to cotton.

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CHAPTER-II

PINK BOLLWORM
PECTINOPHORA GOSSYPIELLA (SAUNDERS) (LEPIDOPTERA : GELECHIIDAE)

INTRODUCTION

Pectinophora gossypiella (Saunders) (*Lepidoptera : Gelechiidae*) is the most serious pest of cotton in Pakistan. In India the pest was first recorded in 1842 and thought to be a native of this country (Marlatt, 1918), Pearson, 1958; Metcalf *et al.*, 1962; Noble, 1969; Pruthi, 1969 and Watts, 1969). The first authentic record of its occurrence in the Punjab was in 1894 when it was known as *Platyedra gossypiella*. It was reported to be serious in 1917 and 1922 in the eastern Punjab where climate was relatively moderate, however, its infestation was comparatively low due to severe climate in western Punjab including Multan and D.G. Khan areas (Bindra, 1928). Hussain and Bindra (1931) reported that with the increase in the availability of irrigation water and use of fertilizers delayed crop maturity and helped in the multiplication of this pest. Rate of infestation varied greatly from year to year. The attack was higher in years of high rainfall compared with hot and dry years. At high population level repeated insecticidal applications after every 5-7 days were required for its control.

It starts in going diapause as larvae however, a part of its population remains active and adults continue to emerge throughout the year with first peak in April when suitable hosts for oviposition are not available. Emergence during this period is regarded as suicidal. However, moths emerging during July and August oviposit on cotton.

Chemical control of the pink bollworm is difficult as the larvae remain protected in the bolls for most of the time. Therefore, there is a need to develop its integrated pest management (IPM). To develop this oviposition behaviour, larval

feeding, preference for squares and boll age, role of possible carryover sources, control measures with insecticides, pheromone and the cultural practices were investigated. These findingsare presented here.

REVIEW OF LITERATURE

Pectinophora gossypiella (Saunders) is the most important pest of cotton, not only in Pakistan but also in other cotton growing countries of the world.

It was first recorded in India in 1842, and became serious after the introduction of upland cotton (Saunders, 1843). It spread through Sri Lanka, Burma, Malaysia (Lefroy 1906) and China (Hunter, 1918). First record in Australia was in 1911 (Wilson, 1972). In Africa it was recorded for the first time in Tanzania (Vosseler, 1904), in Egypt about 1906-1907 (Willcocks, 1916), Sudan in 1914-1915 (Ripper and George, 1965), Malawi in 1939 (Smee,1940) and Zimbabwe in 1959 (Wheelpan,1960). Its spread in the United States has been summarised by Noble (1969).

Studies on its biology reported from various parts of the world have been reviewed by Pearson (1958). Its biology has been studied in Australia by Willcocks (1916), in Hawaii by Busck (1917), in Uganda by Taylor (1936) in Sudan by Ripper and George (1965) in the United States by Owen and Calhoun (1932) and Noble (1969), in Pakistan by Haq (1973) and Ahmad (1977).

Pearson (1958) reported that mating takes place soon after emergence. However, El-Sayed and El-Rahman (1960) reported that in Egypt it takes place 3-4 nights after emergence. According to Lukefahr and Griffin (1957) initiation of mating requires light intensity below 3 foot candle for at least seven hours, maximum mating occurs an hour or two later and continues for an hour. However, Haq, (1973) reported that in Pakistan mating takes place during day time between 11 am-5 pm at 15.6-32.2°C and lasts for 48-81 minutes. In cages males mated about four times and females

twice. Trapped females mated up to six times with a mean of 1.1 times (Noble, 1969). Kaae and Shorey (1973) reported that on calm nights mating always occurs on the upper surface of cotton leaves near top parts of the plant, on windy nights on lower leaves and in the shade in the moonlit nights.

Oviposition is influenced by temperature and humidity (Haq, 1973). Ingram (1994) stated that preoviposition period is two days but Haq (1973) reported that it extend from 2 to 14 days. El-Sayed and El-Rahman (1960) reported that oviposition started on third night after mating and females laid eggs throughout their lives. It was maximum on the second night. They further reported that maximum eggs were laid at 25°C and minimum at 18°C. The females seem to be sterile at 35°C. The adult life prolonged with high humidity. Lukefahr and Griffin (1957) reported that oviposition started on the second night and was maximum on the third night and about 80% eggs were laid before mid night.

Eggs are usually laid singly, sometimes they are laid in batches of 2-10 on leaves, squares and bolls, (Pearson, 1958; Haq, 1973 and Noble, 1969). In the West Indies fecundity of females varied from 100 to 200 eggs (Ingram, 1981). In a recent review the number of eggs has been reported to be from 100 to 500, depending on the size and longevity of the female (Ingram,1994).

Pink bollworm larvae damage buds and flowers before bolls formation (Ingram, 1994). Bolls formed from the rosetted flowers were 40% fewer than that of healthy ones. (Butler and Henneberry, 1976). Tascano *et al.* (1974) reported 12 to 24-day-old as susceptible bolls and Watson and Fullerton (1969) reported half-grown, Lukefahr and

Griffin (1962) less than 20-day-old bolls and Steenwyk (1976) considered 14 to 20-day-old bolls as susceptible to the pink bollworm attack. High moisture contents and rapid accumulation of lipids in seeds during this stage were considered to be responsible for this preference. (Gaskey and Gallup, 1931; Ali and Rana, 1963; Memon and Malik, 1967).

The effect of climate on the pink bollworm infestation was studied in India. Bindra (1928) reported that pink bollworm was more serious in humid and cool areas. He stated that temperature in western Punjab (Multan) were too high for the larvae to survive, therefore infestation was low. Khan (1938) reported that pupal exposure above 27.8°C or below 24.4°C and moths to 30°C caused reduction in oviposition potential. He further proved that relative humidity of 60-80% was most favourable for the development of gonads in pupae and consequently oviposition. Haq (1973) reported that infestation was higher in areas with more than 38 cm rainfall and less in areas where rainfall was less than 20 cm.

The pink bollworm passes winter in diapause as larvae. Because of this habit timely and effective management of cultural practices such as shredding of sticks, soil tillage and irrigation could greatly reduce overwintering population as reported by various authors. The importance of post harvest irrigation and tillage practices in reducing diapausing pink bollworm population in the United States was reported by Fye (1979) and Watson *et al.* (1973). Ingram (1981) reported that 100% pink bollworm diapausing larvae were killed with slashing and different tillage practices across the rows. Rice and Reynolds (1971) reported that depth is critical because most of the overwintering larvae occur in the top 50 mm soil layer. However, Ingram (1994)

reported that shredding and tillage do not cause any mortality in areas where diapausing larvae occur in or on soil in hibernaculae.

Simwat *et al.* (1982 and 1985), Nandal *et al.* (1984) and Nandal and Singh (1985) reported post harvest larval mortality by stocking sticks vertically and horizontally, in the shade and sun with boll bearing portion facing in all directions and concluded that vertical stocking of sticks about 2 m high directed towards West and South gave highest larval mortality.

Matthews *et al.* (1965) reported outbreaks of the pink bollworm in Zimbabwe in 1959 and Malawi in 1962 due to close ginning season to next cotton crop. El-Tigani and Khagali (1978) referred out-breaks in Sudan where ginning season had been extended close to the next cotton season..

A large number of plant species, mostly of family Malvaceae, have been reported as hosts of the pink bollworm in various parts of the world by Noble (1969). Pearson (1958) stated that the pink bollworm attacks all types of cottons (*Gossypium* spp.) including cultivated and wild and many species of genus *Abelmoschus* particularly *A. esculentus* (okra), *A cannabinus* (kenaf) and *A. sabadariffa* (roselle) and *Corchorus alitorius* (jute). In addition to these Matthews *et al.* (1965), Sohi (1984) and Gutierrez *et al.* (1986) have reported some additional plant species as its hosts. Noble (1969) in the USA and Ingram (1981) in the West Indies reported that okra is favoured host and it shifts from okra to cotton (Ingram, 1981). Therefore, as reported by Ripper and George (1965) its cultivation was prohibited during summer. In Pakistan Haq (1973) and Cheema *et al.* (1976) also reported okra as its host. Hussain and Bhindra

(1931) from India and Ahmad (1976) from Pakistan reported that alternate host plants are not important for build up of pink bollworm population on cotton.

Bishara (1930) reported that ratooning of cotton increased the pink bollworm attack during cotton season in Egypt. Taylor (1936) mentioned severe attack of the pink bollworm in Uganda when ratoon cotton was not uprooted. Noble (1969) stated that ratoon or standover cotton was common practice in the U.S.A. It was abandoned when pink arrived.

Pheromone of *P.gossypiella* has been isolated and identified by Hummel *et al.* (1973) and Bierl *et al.* (1974). It has been named as gossyplure. It is a 1:1 mixture of the (Z.Z)-and (Z.E)-7,11- hexadecadienyl acetate. Its various formulations have been tested and used for monitoring, mass trapping and mating disruption in various parts of the world. Monitoring of the pink bollworm moth with sex pheromone traps in Pakistan was studied by Ahmad (1979). Application of insecticides based on moth catches by using gossyplure baited trap have been recommended by a number of workers. In Israel Melamed and Shoham (1975) recommended spraying of insecticide when trap catches reached 8 moths/night, Teich *et al.* (1977) stated five moths/night as threshold in India. Taneja and Jayaswal (1981) reported need for spray on cotton when 8 moths/trap/night are caught but Dhawan and Sidhu (1984) reported best control with 4 moths/trap/night.

The use of insecticides against cotton pests have been reviewed by Ingram (1994) and according to him these are being used since insecticidal properties of DDT were discovered in 1946.

In Mexico 3 to 11 applications of insecticides were given for the control of this pest whereas in Egypt two applications gave good control (Pearson, 1958). As reported by Lowry and Burger (1965) it developed resistance against DDT by 1958-60 in Mexico and in 1962 in Texas in the USA and control with this insecticide was not possible. Therefore, other insecticides such as organophasphate and carbamate and mixture of DDT and organophasphate or DDT and BHC were used (Noble, 1969). Reynolds (1980) reported that use of insecticides for cotton production in California was very limited before the invasion of the pink bollworm in Arizona and California. After the establishment of this pest application went up to 10-15 times. At present organophosphates, carbamates and synthetic pyrethroids are being used for the control of pink bollworm in the USA. Haynes *et al.* (1986, 1987) devised pyrethroid resistance monitoring method through insecticide and pheromone baited sticky traps.

In India, Sidhu and Dhawan (1978) recommended 4 to 5 applications of carbamate, DDT, endosulfan and organophasphate for the pink bollworm control at 10 to 14-days interval at flowering. Sidhu *et al.* (1979) and Sidhu and Dhawan (1981) tested many insecticides of different groups and found that some organophosphate insecticides were as good as carbamate for the control of pink bollworm. Jayaswal and Saini (1981) mentioned synthetic pyrethroids as effective as carbamate but Bhamburkar and Kathane (1984) and Gupta and Katiyar (1985, 1987) reported pyrethroids as the most effective insecticides against the pink bollworm.

In Egypt, Saleh and El-Din (1982), Khalil *et al.* (1983) Watson and Guirguis (1983) reported good control of this pest with organophosphates and pyrethroids. However, Watson *et al.* (1988) mentioned that successive sprays of chlorpyrifos,

fenpropathrin and cyanophos provided 90% reduction in larval population of the pink bollworm. In China Wan and Wan (1982) recommended deltamethrin but Luo *et al* (1986) reported mixture of fenvalerate and BT as effective against the pink bollworm.

In Pakistan Waheed *et al.* (1979) studied comparative efficacy of pyrethroids and endosulfan on cotton pests. They concluded that pyrethroids were better for the control of bollworms including pink than endosulfan. Attique and Khaliq (1983), Attique and Rashid (1983), Ahmad *et al.* (1983) and Attique *et al.* (1984) tested various formulations of pyrethroids, organophosphates and carbamates and concluded that pyrethroids were more effective than other groups against the bollworms including pink.

Some 147 parasitoids and predators of the pink bollworms were listed by Thompson (1946) and Hertig (1975). Greathead (1966) reported about 25 species of natural enemies from the pink bollworm from Africa. Cheema *et al.* (1980a) recorded 28 species of parasitoids from pink bollworm in Pakistan. They also studied biology, ecology, host range and status of *Apanteles anagaleti* Mues., *Bracon gelechiae* Ashm., *Bracon greeni* Ashm., *Bracon hebetor* Say., *Rogas aligharensi* Qadri., *Goniozus sp.*, *Elasmus johnstoni* Ferriers., *Habrocytus chrysos* (Wlk.), and *Brachymeria tachardiae* (Cam.). Singh and Sidhu (1982) and Singh *et al.* (1988) reported that *A. angalati* overwinters in *Sathbrota simplex* Wlsm in India. Sekhon and Varma (1983) reported that *Chelonus* sp. and *Camptothlipsis* sp. parasitizes diapausing larvae of the pink bollworm in India. Hakel (1986) reported that *Exeristes roborator* (F.), *Perisierola* sp., *Elasmus platyedrae* Ferr. *Habrocytus* sp. and *Bracon brevicornis* Wesm. were found parasitizing diapausing larvae of the pink bollworm in Egypt.

A number of attempts on biological control of the pink bollworm have been made in various parts of the world. Oatman (1978) reported that three *Bracon* species were introduced into Egypt, of these *B. mellitor* Say and *Bracon kirkpatricki* Wlkn. did not get establish and *B. lefroy* (D. & G.) reported to be established. He further recorded that they were imported and released in Texas, Mexico and Puerto Rico between 1933 and 1955, but none of them became established. Fye and Jackson (1973) and Legner (1979) reported that although some species could overwinter but were not established in the USA. The possibilities of introducing natural enemies against this pest in Pakistan have been discussed by Habib and Mohyuddin (1981). Two species of parasites, *Chelonus blackburni* Cam. (native to Hawaii) and *B. kirkpatricki* (native to East Africa) were imported from India. These were multiplied and released. *Chelonus blackburni* was recovered soon after releases from Multan but whether or not it became established is not known.

Mass rearing and augmentation of *Bracon nigrorufum* (Cushm.) was done by Gong *et al.* (1984) and of *Dibrachys cavus* (wlk.) by Chu (1978) and Cock (1985) in China. *Trichogramma brasiliensis* (Ashm.). *B. kirkpatricki* and *C. blachburni* by Parsad *et al.* (1985) in India.

In the U.S.A. Noble (1969) reported predacious mite, *Melichares* (*Blottisocius) tarsal* (Berlease) feeding on caged pink bollworm eggs, while *Glycyphagus destructor* (Schrank), *Parasitus* sp., and *Tyrophagus putrescentiae* (Schrank) are known to feed on larvae in stored seeds, whereas *Chrysopa rufilabris,* a beetle *Enoclerus quandrisignatus* (Say) and an unidentified species of spiders on larvae in flowers. Orphanides *et al.* (1971) reported that 12 insect species feed on eggs. In addition to

these nine spider species feed on its larvae. Irwin *et al.* (1974) tested seven predator species against pink bollworm eggs in California. Among these *Chrysoperla carnea* Stephens, *Geocoris pallens* Stal. and *Nabis americoferus* Carayon were most effective. Henneberry and Clayton (1985) reported that *Collops marginellus* Leconte, *Hippodamia convergens* (Guer.), *Sinea confusa* Caudell and *Orius tristicolor* (White) proved effective as egg predators in the laboratory.

Pearson (1958) recorded mite predator, *Pyemotes ventricosus* (Newprint) in Africa which attacked the pink bollworm larvae in seed stores and fields. This species and *P. berfsi* (Oudeman) are giving good control of diapausing larvae in Barbados (Ingram, 1981), in Egypt (Abdul Nasr *et al.* 1978) and in India (Singh and Singh, 1984 and Naresh and Balan, 1985). Cheema, *et al.* (1980b) recorded 63 species of predators of the pink bollworm in Pakistan. Among these 23 were insects, 26 spiders, one mite, one mammal, and 12 birds.

Design of the pheromone traps can affect their catching capacity. Therefore, efficacy of different traps designs have been investigated by a number of workers. Sharma *et al.* (1971, 1973a and b) developed series of trap designs one after the other. Bierl *et al.* (1974) modified his trap design making it from a 14 litre ice cream container to form a closed cylinder 6.0 mm high and 200 mm in diameter with six equally spaced 22 mm holes and all these used stickum as trapping medium. In Israel Neumark *et al.* (1972) used polystyrene cups filled with water and a drop of detergent in it.

In India Reed *et al.* (1975) and in Malawi Marks (1976) used modified different Sharma trap designs. Coudriet and Henneberry (1976) used five trap designs and found

no significance difference in trap catches of the pink bollworm. Foster *et al.* (1977) compared four trap designs and found that Delta trap was significantly better than the other three.

Flint *et al.* (1976) reported mass trapping of the pink bollworm as effective control measure. Huber *et al.* (1979) reported that infestation was 1.9% where mass trapping was done as compared with 4.2% in the immediate three preceding years when mass trapping was not done.

Control of the pink bollworm with Nomate PBW a sex pheromone by using mating disruption was reported for the first time in the U.S.A. by Brooks *et al.* (1979). Since then synthetic gossyplure has been used as microencapsulated, chips, flakes and twist tie formulations successfully for the control of pink bollworm in the U.S.A. (Shorey *et al.*, 1984 and Staten *et al.*, 1987) and in Egypt (Critichely *et al.*, 1983 and 1985; Campion *et al.*, 1989; El-Adle *et al.*, 1988; McVeigh *et al.*, 1983 and Moawad *et al.*, 1991). In Pakistan successful control of the pink bollworm by using mating disruption with these formulations was reported by Attique (1985) and Critchely, *et al.* (1991). Single application of a twist tie formulation containing combined pheromones of *P.gossypiella*, *Earias vittella* (F.) and *E. insulana* (Boisd.) have been used by (Critchely *et al.*, 1987; Qureshi and Ahmed, 1989; Chamberlain *et al.*, 1992) and slow release PVC resin formulation by Chamberlain (1993).

MATERIAL AND METHOD

Adult activity

To monitor the activity of pink bollworm moths, pheromone vials baited with 1mg of gossyplure of Z : Z and Z : E isomer were used in the traps throughout the year from 1994 to 1996, at two localities including Central Cotton Research Institute, Multan (CCRI) and 65 km North East of Multan at Punjab Seed Corporation Farm, Khanewal. Pheromone was replaced at two-week-interval and the traps were changed when necessary (3-4 weeks). Moth catches data were recorded daily at Multan and at fortnightly intervals at Khanewal.

Oviposition

Egg laying sites of the pink bollworm were studied on variety NIAB-78 during 1992 and 1993 crop seasons. The crop was sown in the first week of June. Sucking pests were controlled with dimethoate. No insecticide application was given against bollworms. Five randomly selected plants of variety NIAB-78 were cut from the base three days a week from August to October. Stem, leaves, squares, flowers and bolls were examined under binocular and number of eggs laid on each part were noted.

To determine when the pink bollworm attack starts, five cotton varieties, *Gossypium hirsutum* CIM-70 (short stature and early maturing), NIAB-78 and CIM-109 (medium statured and late than CIM-70), CIM-135 and Alseemi-515 (long stature and late maturing) were planted each on 0.4 ha in the second week of June during 1992 and 1993 at the farmer field. All fruiting bodies of twenty plants from each variety were removed at weekly interval from July to November. Squares, flowers and bolls were examined individually for pink bollworm oviposition. Insecticides were not used for

the control of the bollworms. Cultural practices such as application of fertilizer, irrigation and control of sucking pests etc., were the responsibility of the farmer.

To determine the oviposition preference for squares of different ages, all squares from twenty plants of each above mentioned variety were removed. The squares were separated in to small (1-10-days) and large (11-21-days) and were examined for oviposition.

Effect of pink bollworm attack on squares and bolls

To investigate whether or not the squares attacked by the pink bollworm develop in to bolls, rosette flowers of CIM-70, NIAB-78, CIM-109, CIM-135 and Alseemi-515, varieties were tagged from August to October during 1992 and 1993. Same number of unattacked flowers on the same plant were kept as control. Shedding in rosette and healthy flowers was recorded daily. The observations continued till both categories of bolls reached maturity.

To determine the effect of pink bollworm attack on number of seeds and weight of lint on bolls developed from rosette flowers of NIAB-78, CIM-109, CIM-135 and Alseemi-515 were kept up to opening. On opening equal number of bolls developed from rosette and healthy flowers were plucked in November and December. Number and weight of seeds and lint per boll from rosette and normal flowers were recorded.

Boll age preference

The susceptibility of bolls to attack of the pink bollworm based on boll age and moisture content was studied at Multan. A 0.4 ha field of cotton was selected every year from 1992 to 1994. The local commercial cultivars S-12 in 1992 and 1993 and CIM-240 in 1994 were used for these studies. One hundred white flowers were tagged

weekly from September to November, the peak period of the pink bollworm activity at Multan. Ten bolls from the age groups of 7, 14, 21, 28 and 35 days were harvested at weekly interval. Bolls of individual category were weighed immediately after collection and dissected to determine whether the larvae were present or not. The infestation of bolls was based on the presence of larvae, larval mines or exit holes. These bolls were then placed in an oven at 110°C for 24 hours and re-weighed to determine the moisture contents.

Pupation

Mature larvae of pink bollworm come out of the bolls and drop on ground for pupation. To determine the time when larvae come out from the bolls, in September three hundred 14-28-day-old bolls were plucked from the unsprayed field and placed in three conical funnels each having 0.6m diameter. This was repeated three times during the cotton season in 1992 and 1993. A glass jar containing detergent was kept below the funnel to collect larvae dropping from the bolls. This was carried out in the cotton field under normal conditions. During the day larvae were counted hourly from 8.00 to 7.0. The night collection was counted in the morning at 7.00.

Carry over sources

Pink bollworm larval mortality was determined in the ginning waste. The samples of cotton waste were taken from ten ginneries, three kgs from each factory at weekly interval from December to May. These were brought to the laboratory and examined for double seeds and alive and dead larvae. Total population and mortality from 120 kgs of waste were noted every month. Pink bollworm activity was also determined by placing one pheromone baited trap in each factory to catch the adults from December to August.

Survival of the pink bollworm larvae in heaps of different sizes during 1993-94 in the field was studied. Ginning waste was kept in three wiregauze cages measuring 1.2 x 1.2 x 2.4m in three replicates. The heap size was 0.6, 1.2 and 1.8 m high. Emergence of males was recorded with one pheromone baited delta trap per cage from January to October. Females were recorded by visual observation. Moth emergence based on 2.83 cu. m of ginning waste was noted.

To investigate if ginning waste could be used as organic matter in the soil without being source of infestation of the bollworm during next cotton season. The waste was spread in cotton field at the rate of 19.8 tons/ha. In one treatment irrigation was applied as such. In the other treatment field irrigation was done after mixing the waste with the soil with disc plough. Nine 1.2 x 1.2 x 1.2m conical screen cages with a glass jar on the top, in three replicates, were placed in each treatment. Emergence of the pink bollworm moths in these cages was recorded daily from March to October. Percentage of organic matter and nitrogen, phosphorus and potassium ppm available in the soil were determined at the start of the experiment and 90-days after application of the waste. Contents of these nutrients were also quantified before the start of the experiment.

After cotton has been harvested a part of pink bollworm population is known to survive in the soil, double seeds and left over bolls. To determine survival of the larvae under different practices the following experiments were conducted at CCRI, Multan.

To study effect of irrigation on the mortality of pink bollworm larvae in the soil, two fields were selected where sticks were removed manually. One was irrigated during March and other was not. Emergence of moths was recorded by placing total of

27 conical wireguaze cages measuring 1.2 x 1.2 x 1.2m with a glass jar on the top in three replicates. Number of moths emerged in the two treatments was recorded.

Effect of ploughing on survival of the pink bollworm larvae was studied. After the cotton had been harvested, one field was selected where sticks were removed manually. This was divided into three plots. One plot was ploughed with furrow turning plough, the second with munnah plough and third was kept as control where ploughing was not done. This was replicated twice. Moth emergence was recorded by placing total of 27 conical wiregauze cages in three replicates for moth catches as mentioned above

To study the effect of destroying of sticks in the field with disc plough and rotavator on pink bollworm mortality, one block of cotton measuring 90 x 90 m was divided into three equal plots. In one plot sticks were slashed with rotavator, in the second with disc plough and in the third sticks were removed manually and no ploughing was done and kept as control. Moth emergence was recorded in 18 wiregauze cages in three replicates.

After picking cotton, farmers cut the sticks alongwith leftover bolls and pile them up at their farm houses. To study effect of this practice on carry-over of pink bollworm, cotton sticks were piled in two ways. One hundred fifty small bundles were kept with tops upwards (vertically). The same number of bundles was stored according to conventional practice followed by the farmers as large heap and sticks were placed horizantally. Samples of 200 bolls from bundles kept vertically and 200 each from upper and lower sticks of large heap were collected from January to July in the fourth week of each month and dissected. Number of alive and dead larvae were recorded.

Rate of the pink bollworm infestation on alternate hosts was studied in Multan and Khanewal areas. *Abelmoschus esculentus* (okra), *Althaea rosea* (hollyhock), *Hibiscus cannabinus* (kenaf), *H. rose-sinensis* (china-rose), *H. mutabilis* (cotton-rose), *Malvaviscus arboreus* (turks cap), *Abutilon* spp. and *Malva parviflora* (small mallow) were examined for pink bollworm infestation during 1992 and 1993 throughout the year. Fruits, shoots and leaves were examined for the presence of eggs and larvae.

Trap comparison

To compare the efficacy of four types of traps including Delta red, Delta white, Funnel white and Universal traps were placed randomly in a single row at the Punjab Seed Corporation Farm, Khanewal. This was replicated three times. Position of traps was not changed during study period of 25 days. Nomate PBW fibre dispenser was used in all the traps. In another trial in addition to these four, yellow funnel trap was also included and they were baited with the above mention sex pheromone. They were installed in a single row in cotton fields. This was replicated five times. The trap position was changed every fifth day so that each trap had an equal chance for attracting moths at all sites. The moth catches were recorded daily for each trap separately from January to May.

Control measures

One dose of Naturalyte 105, 20SC and four dosage of Naturalyte 105, NAF-85 EC were tested against pink bollworm. Deltamethrin and bifenthrin were kept as standard for comparison. There were eight treatments including untreated check. The area for each plot was 150 sq.m with four replicates. CP-15 knap sack hand held sprayer was used with a capacity of 20 lit. fitted with hallow cone nozzle and delivering

250 lit. of water per ha, operated at a pressure of 40 P.S.I. (= 276 Kpa). Two application were made at 12 days interval treating one row at a time during both applications. Randomly selected 25 susceptible bolls from each treatment were collected and kept in the laboratory for 4-5 days and then dissected. Pink bollworm damage was recorded on the basis of alive larvae or presence of mine. Boll damage percentage and larvae were recorded at 7 and 12 days after each application.

Pink bollworm was controlled through mass trapping, using pheromone baited traps at two locations. First trial was conducted at Khokhran on a 2.4 ha block of S-12 and replicated three times. Thirty traps were placed on 13th September because of late planting. These traps were replaced after 2-3 weeks. Another adjoining 2.4 ha plot was kept as control. The distance between the traps was 25m. No plant protection measures were taken during the season in both the blocks where mass trapping was done and the control. Infestation was determined by sampling 400 bolls (14-21-day old) per block from both treatments at weekly intervals. Number of pink bollworm moths in the trapped area was also recorded.

Another trial was conducted 65 km North East of Multan at the Punjab Seed Corporation, Khanewal on a 12-ha. block in three replicates. At squaring stage 144 traps were placed. These traps were replaced after 2 to 3 weeks. Another 12-ha., 200 meters away from the plot where pheromone was used was kept as control. The distance between the traps was 25m. In pheromone treated area one application of disyston, 12 kgs/ha against sucking pests and one application of each endosulfan and guzathion for *Earias* species was given. One granular and five foliar applications of endosulfan, guzathion carbryl, endosulfan and guzathion were given to the area kept as

control. The pink bollworm infestation was determined by sampling 400 bolls (14 to 21-day-old) from both plots at weekly intervals.

The pink bollworm control through mating disruption with 20 cm PB-Rope was investigated about 40 km North East of Multan at farmers' field. Application of PB-Rope, a sex pheromone was given only once at the pin square stage on 11-ha. during 1991. The ropes were tied 10 to 12 cm on the top of the plant at the rate of 625 pieces/ha. The distance between the pieces was 4.5 m. Similar block of cotton was kept as control where insecticides were used for the control of the bollworms. Pink bollworm infestation was determined by dissecting 100 bolls at weekly intervals.

During 1992 efficacy of two type of Tea Bags (pheromone impregnated plastic bags) was compared with 20 cm PB-Rope on 12-ha. at farmer field. The crop was sown on 29th June. Pheromone application was made once on 30th July at the pin square stage. A total of 500 pieces of PB-Ropes, 500 small Tea Bags and 250 of large Tea Bags per ha. were tied manually 16-20 cms above the ground with the main stem. Distance within and between the rows was 4.5 m for PB-Rope and small Tea Bags and 6.5 m for large Tea Bags. One block of 2.4 ha. was kept without Tea Bags where farmer used his own pesticide regime. In the center of pheromone treated plot, 0.2 ha. plot was kept as untreated check where one foliar application was made for the control of sucking pests and no insecticide was used to control *Earias* spp. To assess the communication disruption in the pink bollworm, three pheromone baited traps were placed each in pheromone and insecticide treated area. The pink bollworm infestation was determined by dissecting 100 bolls from each treatment at weekly intervals.

RESULTS

Adults activity

Adult activity was monitored with delta traps having rubber septa impregnated with 1 mg of *Pectinophora gossypiella* pheromone, gossyplure (1:1 mixture of Z.Z and Z.E-7, 11 hexadecadienyl acetate) at Central Cotton Research Institute, Multan and Punjab Seed Corporation Farm, Khanewal from 1994 to 1996.

Moth catches at both localities showed that the moth continue to emerge throughout the year **(Fig.1-6).** The minimum number was caught during January, February. From March onwards catches increased and the first peak was in the middle of April and then it declined gradually and was minimum in June. This is "suicidal" emergence because suitable host plants were not available for the oviposition. The moth catches again increased gradually from July onwards with a peak in September-November. This is the time when susceptible stages of cotton are available. Although the overall moth population was lower at Multan **(Fig.1-3)** than at Khanewal **(Fig.4-6)** but the emergence patterns were similar at both the places.

Oviposition

Females of *P. gossypiella* laid eggs singly or in groups of 2-4 eggs on all parts of the cotton plant including terminal buds, lower side of small and large leaves, leaf petioles, main stem, squares and immature bolls. During August, when bolls were not present, most of the eggs were laid on squares **(Table 1)**. During September and October when both squares and bolls were present, maximum oviposition take place on the bolls. The eggs were laid on top and base of the bolls near the sutures.

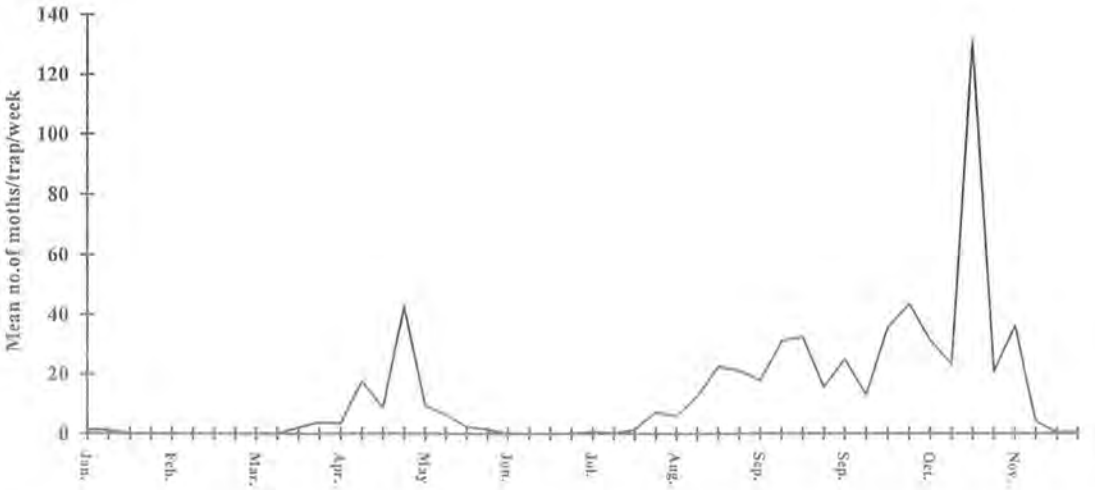

Fig.1 Mean number of *Pectinophora gossypiella* male moths caught/trap/week at Multan during 1994

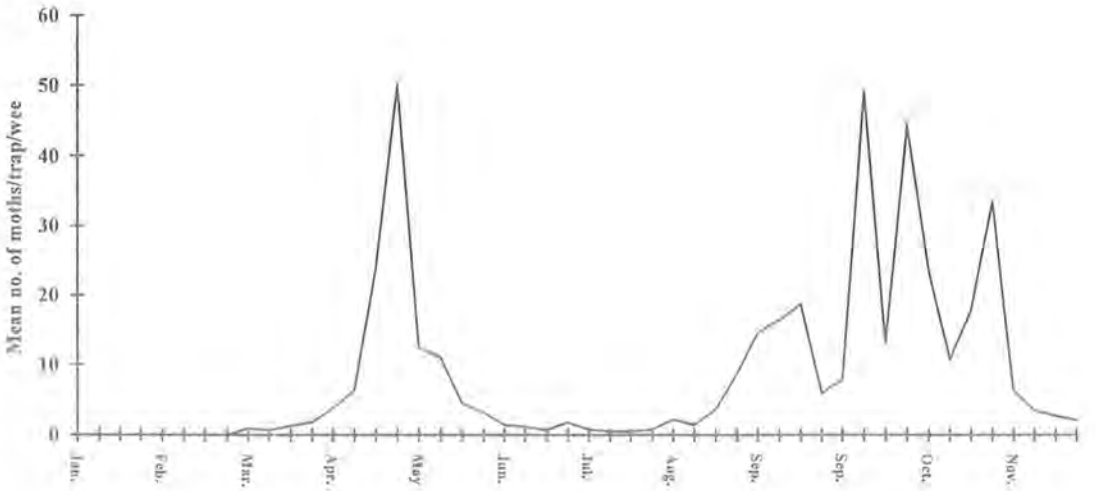

Fig.2 Mean number of *Pectinophora gossypiella* male moths caught/trap/week at Multan during 1995

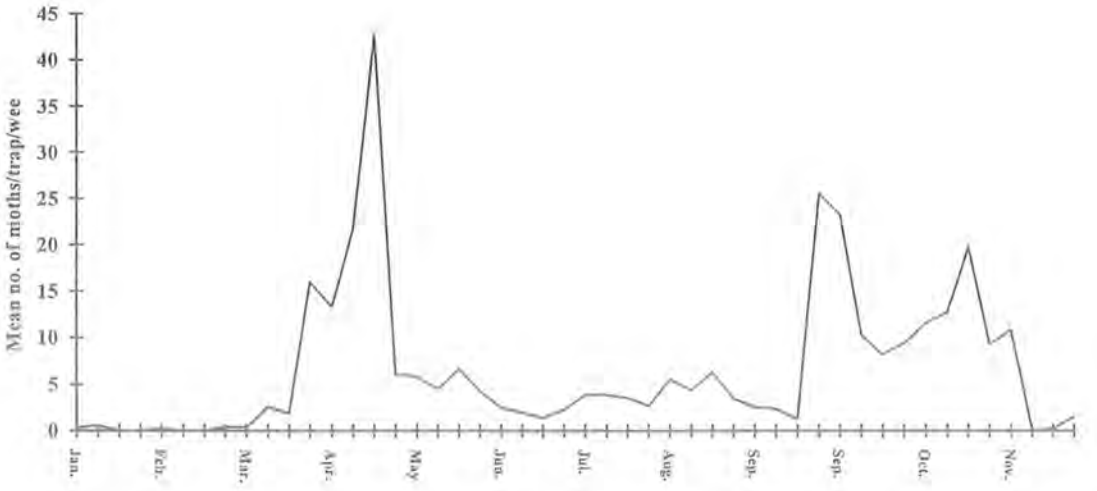

Fig.3 Mean number of *Pectinophora gossypiella* male moths caught/trap/week at Multan during 1996

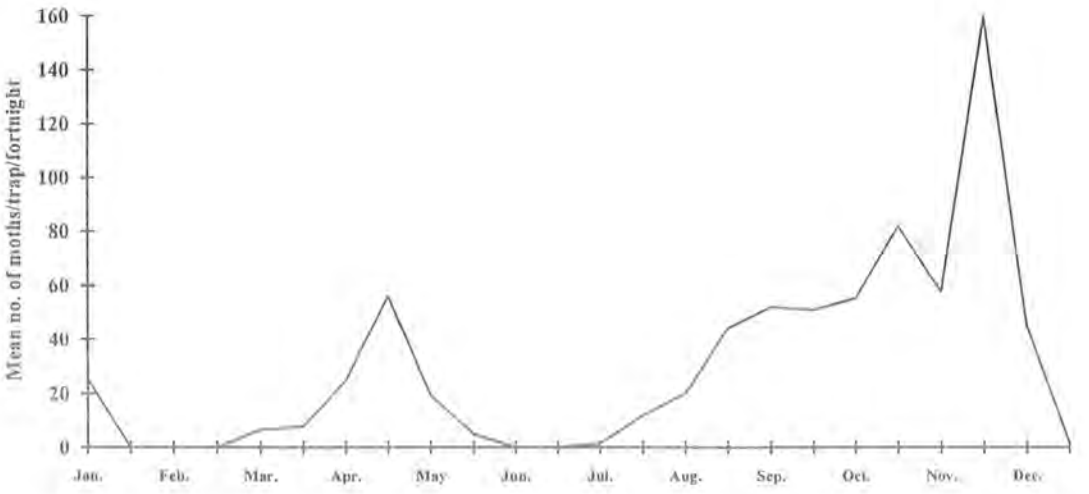

Fig.4. Mean number of *Pectinophora gossypiella* male mothscaught/trap/fortnight at Khanewal during 1994

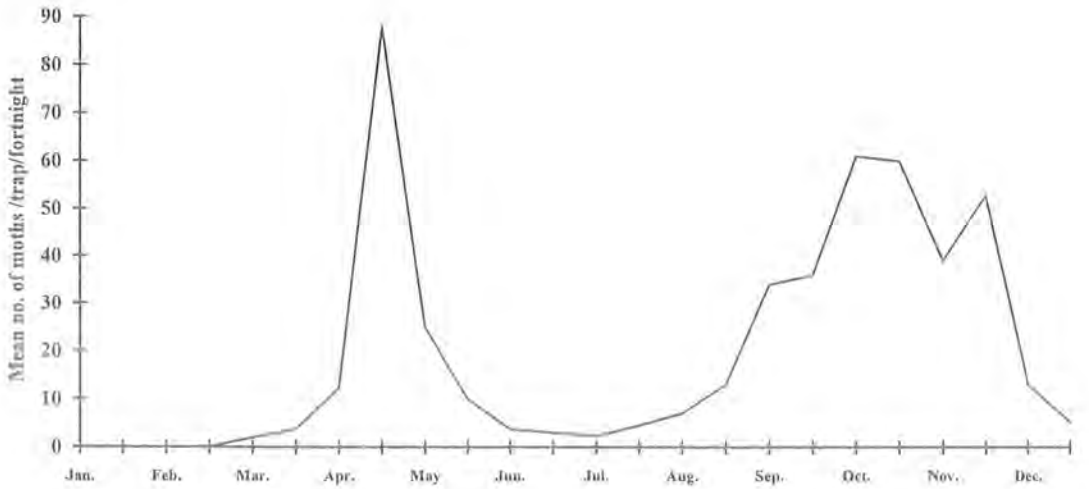

Fig.5. Mean number of *Pectinophora gossypiella* male mothscaught/trap/fortnight at Khanewal during 1995

Fig.6. Mean number of *Pectinophora gossypiella* male mothscaught/trap/fortnight at Khanewal during 1996

Fecundity of females developed from diapausing larvae is low, being 30 to 90 ($\bar{X}$=75) eggs per female in the month of August. During September and October number of eggs per female is 100-200 ($\bar{X}$=125). Oviposition, duration of egg and larval stages are influenced by temperature and relative humidity. In the Punjab, temperatures range from 38-45°C during July-August. Out of 500 eggs 200 died (40%) during July-August. During September-October when temperature range was 33-35°C egg mortality was low. From 400 eggs 20 (5%) died during this period. Eggs laid in early August hatch within three days. The larvae of first generation complete development in 10-12 ($\bar{X}$=10.8) days. Egg to adult stage took 16-18 ($\bar{X}$=16.5) days. Females of first generation oviposit in the third week of August on squares and bolls. On hatching, larvae feed for 12-15 ($\bar{X}$=13.5) days. Total development period from egg to adult is about 15-20 days. Next generation females begin oviposition in the first week of September. Incubation period during September is 3-5 ($\bar{X}$=4.2) days. The eggs and larvae of third generation completed development in 20-25 ($\bar{X}$=23.0) days. The eggs of fourth generation hatch in 5-7 ($\bar{X}$=6.5) days in October. The life cycle is completed in 25-30 ($\bar{X}$=28.0) days. Eggs of fifth generation are laid at the end of October. They hatch in October to early November. Full-grown larvae of the fifth generation do not pupate and enter diapause. There are five overlapping generations from July/August to November. Thus with the fall of temperature from July onwards the development period was prolonged.

Table 1 Distribution of *Pectinophora.gossypiella* eggs on cotton plant

Months	Eggs laid on different plant parts (%)						
	Terminal Bud	Small Leaf	Large Leaf	Leaf petiol	Main stem	Squares	Bolls
August	10.5	2.4	12.2	1.9	0.5	72.5	-
September	5.3	4.6	9.3	6.3	3.5	0.3	70.7
October	3.5	4.0	2.6	1.9	3.0	0.0	85.0

- = Bolls not available

Period of infestation to squares

To determine when the pink bollworm attack starts, sqaures of randomly selected 20 plants of each of five varieties were removed at weekly interval and examined for oviposition. The results showed that pink bollworm attack on floral buds depends upon time of square formation. In CIM-70, an early maturing variety, fruiting started 30-35 days after germination and the bollworm attack started in early July followed by NIAB-78 and CIM-109 which pick-up fruit few days later. In CIM-135 and Alseemi-515 floral buds appeared 45 days after germination, in the first week of August, therefore, infestation appeared late as compared with other varieties. In September when squares, flowers and bolls were available in all varieties, infestation decreased on squares and increased on the bolls **(Table 2)**. Overall infestation during different years was variable. It was low on bolls in early season and on squares late in the season. As the season progressed, infestation on squares further decreased and maximum attack was on bolls in September and October. Infestation pattern in bolls was also similar to those of floral buds.

Table 2 ***Pectinophora gossypiella* attack (%) on floral buds and bolls in five varieties of cotton**

Varieties	July		August		September		October	
	Square	Boll	Square	Boll	Square	Boll	Square	Boll
CIM-70	0.36	1.75	3.70	1.50	-	0.46	0	0.93
NIAB-78	0.22	0.81	0.34	0.26	0.93	0.91	0	0.82
CIM-109	0.20	1.35	0.70	0.68	0.84	1.26	0	1.35
CIM-135	0.00	0.00	0.32	0.00	0.00	1.25	0	1.20
Alseemi-515	0.00	0.00	0.36	0.53	0.22	1.06	0	2.41

Ovipositon preference for squares of different ages

In the Multan area squares turns into flower in 21 days. Oviposition preference for square of different ages was studied. Squares of 20 randomly selected plants of each

variety including CIM-70, NIAB-78, CIM-109, CIM-135 and Alseemi-515 were removed at weekly intervals during 1992 and 1993. One to ten day-old and 11 to 21 day-old-squares were separated and examined in the laboratory for oviposition.

Females preferred nearly half-grown (7-10-day-old) buds for oviposition **(Table 3)**. In 1992 on an average 87 to 93% ($\bar{X}$= 90.4) eggs were laid on young squares of all the varieties and on older squares 7 to 13% ($\bar{X}$= 9.6) eggs were laid. In 1993 the number of eggs on young squares was 82 to 94% ($\bar{X}$= 88.4) whereas they were 6-18% ($\bar{X}$= 13.6) on older squares **(Table 3)**.

Table 3 Percentage of *Pectinophora gossypiella* eggs on small and large squares during 1992-93

Varieties	1992		1993	
	1-10 day-old-square	11-21 day-old-square	1-10 day-old-square	11-21 day-old-square
CIM-70	93	7	82	18
NIAB-78	92	8	90	10
CIM-109	87	13	87	13
CIM-135	89	11	94	6
Alseemi-515	91	9	89	11

Larval feeding

The newly hatched larvae from the eggs laid on leaves and petioles enter the nearest squares or bolls. They take 60-90 ($\bar{X}$= 65.0) minutes to enter these. During this period, larvae are subjected to predation and mortality due to abiotic factors and pesticides. Those hatched from the eggs laid under the calyx, squares or bolls take 30-60 ($\bar{X}$ = 55.0) minutes to enter these parts and are less exposed to pesticides and natural mortality factors.

Most of the larvae enter through the base of about 10-day-old-squares. Larvae do not survive if infested squares are shed at an early stage. There is no mark of entry on squares. The larvae go on feeding on their internal parts. In the infested squares third or fourth instar larvae stitch the petals together. This prevents the flower from opening. These are known as rosette flowers. Larvae feed on anthers and styles of flowers. On an average 34-58 % larvae complete their development before the petals are shed. If the larvae are not fully-grown at the time of bolls formation, these enter to young bolls from the top and consume entire contents of the bolls to complete development. They pupate in 2-3 days later. The infested bolls are shed. On an average 42 to 60% ($\bar{X}$= 48.0) larvae complete their development on squares, flowers and ovaries of young bolls and 40 to 58% ($\bar{X}$= 44.0) on squares and flowers.

Newly hatched larvae enter through any part of the bolls. The entry point can be noticed because a yellow spot develops on the lint at the entry point. After entering in 14 to 28-day-old bolls larvae feed on the boll contents including the lint near the entry part at least for 24 hours. They feed on lint, seed coat and kernal completely before attacking the second seed. Two seeds are sufficient to complete larval development in the same loculus but a small proportion of papulation also makes a neat circular tunnel by cutting through the interlocular septa to feed on more seeds in adjoining loculi. However, in young bolls all contents are consumed resulting in their shedding. Bolls older than a week remain attached to the plant and dry up. Older bolls do not shed but exhibit partial damage and early opening.

To estimate loss in yield in open bolls where one or two loculi were damaged, all the mature bolls were removed from twenty randomly selected, plants of five

varieties including CIM-70, CIM-109, CIM-135, NIAB-78 and Alseemi-515. Both with one and two damaged loculi of each variety were weighed separately and yield loss was compared with healthy bolls.

In bolls in which one loculus was damaged yield loss per boll varied from 23.3 to 34.2% and where two loculi were damaged from 37.0 to 52.4% depending on the boll weight. Maximum yield loss was in Alseemi followed by CIM-135, CIM-109, CIM-70 and NIAB-78 in descending order **(Table 4)**.

Table 4 Loss of seed cotton due to partial damage by *Pectinophora gossypiella* in different cotton varieties

Varieties	Weight of healthy boll (gm)	Weight of boll with one or two damaged loculi (gm)		Loss in yield with one or two damaged loculi		Percentage loss with one or two damaged loculi	
		1	2	1	2	1	2
NIAB-78	3.7	2.8	2.33	0.90	1.37	24.3	37.0
CIM-109	3.6	2.64	1.92	0.96	1.68	26.7	46.7
CIM-70	4.0	3.07	2.28	0.93	1.72	23.3	43.0
CIM-135	4.3	2.90	2.35	1.40	1.95	32.6	45.3
Alseemi-515	5.0	3.29	2.38	1.71	2.62	34.2	52.4

Effect of rainfall on infestation

Rate of infestation varies considerably from year to year depending on a number of factors such as size of population entering diapause at the end of the season, suicidal emergence during spring and summer before squares are available and climatic factors such as temperature and rainfall etc. Haq (1973) reported that low rainfall adversely affect the pink bollworm infestation. During 1994 and 1995 rainfall and infestation of the pest were compared. In 1994 rainfall was less than 40cm once in August and then remained dry during the active period of crop and pest. Overall infestation was very

low with a peak (10%) damage at the end of October. In 1995 there was more rains in August-September. The infestation remained above the economic threshold level throughout the season. Maximum attack was 35% at the end of the season. Thus it reveals that frequent rains during August and September provide favourable conditions and it builds up high population **(Fig. 7)**.

Effect of pink bollworm attack in squares on development of bolls

The squares attacked by the pink bollworm develop into rosette flowers. To investigate whether these flowers develop into bolls or not, both healthy as well as rosette flowers of CIM-70, NIAB-78, CIM-109, CIM-135 and Alseemi-515 were tagged in equal numbers from August to the first week of October during 1992 and 1993.

Shedding of rosette flowers was significantly lower in CIM-70 as compared with NIAB-78, CIM-109 AND CIM-135 but these differences between CIM-70 and Alseemi-515 were not significant when effect of months was pooled. Pattern of shedding of healthy flowers was similar to that of rosette flowers but the differences between the varieties were non significant. Although shedding of rosette flowers in all the varieties was much lower than healthy ones but statistically differences were non significant (Table 5).

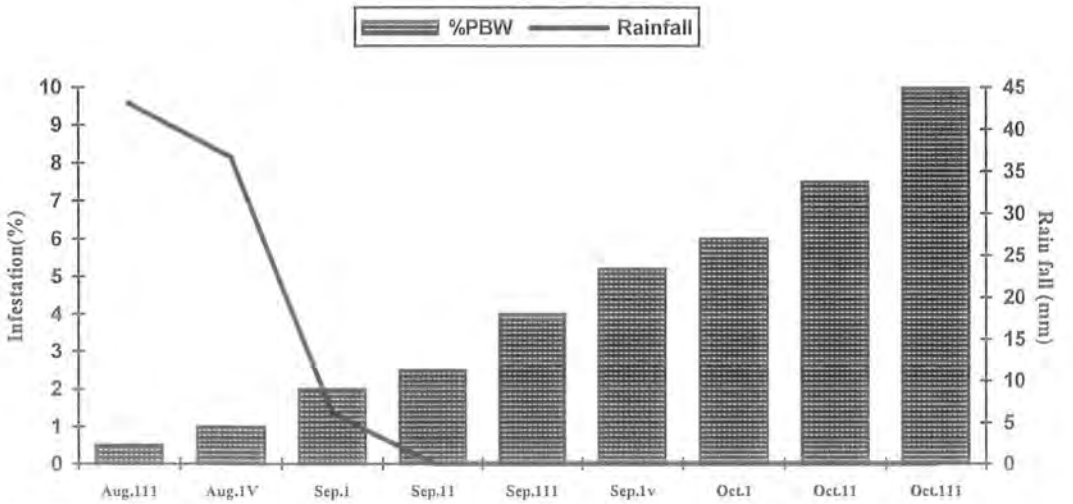

Fig.7(a) **Infestation of *pectinophora gossypiella* and rainfall during 1994**

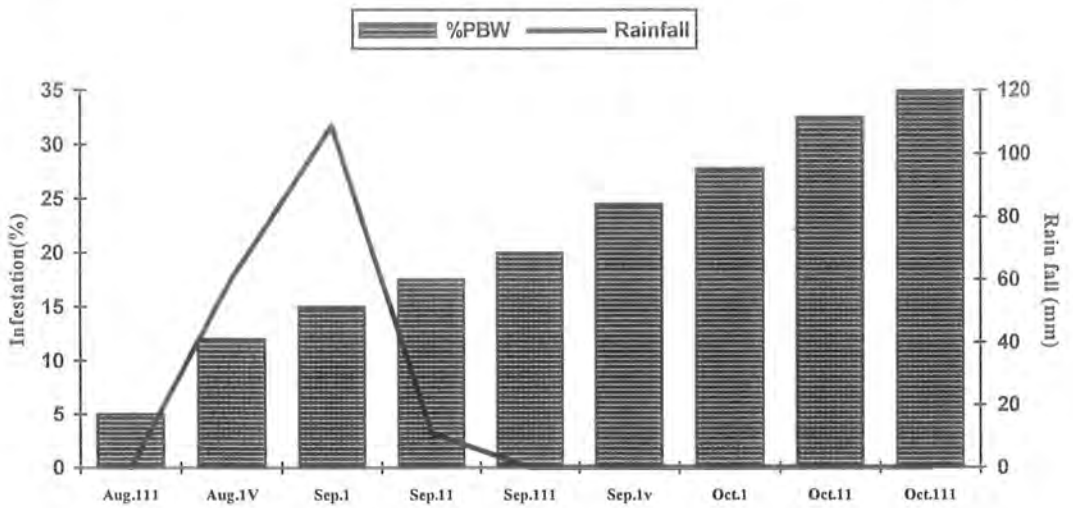

Fig.7(b) **Infestation of *pectinophora gossypiella* and rainfall during 1995**

Table 5 **Total number of rosette and healthy flowers tagged during 1992-93 and shedding percentage in different varieties**

Varieties	No.of flowers tagged *	Shedding (%)	
		Rosette	Healthy
CIM-70	1025	43.5a	23.2a
NIAB-78	487	73.6b	39.6ab
CIM-109	425	74.7b	44.7ab
CIM-135	72	79.6b	49.5b
Alseemi-515	27	51.9ab	35.8ab

* = Same number were tagged for both categories separately
Effect of months pooled
Means followed by the same letters are not significantly different at 5% level of significance.

All the varieties suffered heavy shedding of rosette and healthy flowers during August as compared with September and October and the differences during these months were significant at 5% level in all the cases (**Table 6**).

Table 6 **Shedding percent of rosette and healthy flowers in different periods of crop development**

Month	Rosette	Healthy
August	87.2c	68.4c
September	58.7b	33.1b
October	47.9a	13.1a

Effect of variety pooled
Means followed by the same letters are not significantly different at 5% level of significance.

Young bolls formed from rosette and healthy flowers shed in early stages of development. Shedding started two days after boll formation and continued up to ninth day. Maximum shedding occurred from fourth to sixth day after tagging of flowers. Majority of the bolls did not shed during this period and reached maturity. There was

no difference in the pattern of boll shedding between the varieties but shedding was higher in bolls developed from rosette flower as compared with healthy one.

Effect of pink bollworm attack on number of seeds and lint/boll

To investigate the effect of pink bollworm attack on bolls, healthy and rosette flowers were tagged on the same plant of NIAB-78, CIM-109, CIM-135 and Alseemi-515. The bolls formed from these flowers were picked up on opening. Weight of boll, number and weight of seeds and lint per boll were recorded for both the categories.

All bolls formed from rosette flowers were not normal. Eight to ten percent were shriveled on one side, resulting in deformed shape. Overall number of seeds and weight of lint produced from rosette flowers were less compared with bolls developed from healthy flowers (**Table 7**). It seems that fertilization may have been affected causing these differences.

Table 7 **Mean boll weight, number and weight of seeds and lint per boll from rosette and healthy flowers**

Varieties	Rosette flower				Healthy flower			
	Boll weight (gm)	No. of seeds/ boll	Seed weight/ boll (gm)	Weight of lint/boll (gm)	Boll weight (gms)	No.of seeds/ boll	Seed weight boll (gm)	Weight of lint/boll (gm)
NIAB-78	2.60	22.49	1.69	0.89	2.66	22.99	1.71.	0.91
CIM-109	2.50	21.13	1.59	0.88	2.98	25.17	1.87	1.07
CIM-135	2.35	18.12	1.48	0.85	2.69	21.17	1.67	0.99
Alseemi-515	3.75	20.50	2.55	1.20	-	-	-	-

- = Rosette flowers not available

Boll age preference to pink bollworm attack

The studies were made to determine the susceptibility of bolls of different ages to pink bollworm attack. White flowers of S-12 and CIM-240 were tagged. Bolls of

different ages developed from these flowers were removed and dissected to determine the pink bollworm attack.

The results revealed that attack was minimum up to 7-day-old bolls. As the boll age increased, damage also increased and was maximum up to 21-day-old bolls **(Table 8)**. The infestation level decreased afterwards but this decline was sharp when the bolls became 28-day-old. Therefore, 14-28-day-old bolls were most susceptible to pink bollworm attack.. Bolls older than 28 days are less susceptible, most probably due to the hard boll skin which restrict the penetration of first instar larvae. At Mulltan similar trends were found in both S-12 and CIM-240 varieties. It appears that water contents does not affect the susceptibility of bolls **(Table 8).**

Table 8 Relationship of boll age and moisture content to *Pectinophora gossypiella* attack

Varieties	Boll age (days)	Moisture content of bolls (%)	Infestation (%)
S-12	07	83.3	9.2
	14	84.4	28.4
	21	70.6	37.5
	28	76.9	27.7
	35	74.6	19.2
CIM-240	07	81.0	4.3
	14	84.3	24.3
	21	87.9	32.5
	28	78.4	22.5
	35	72.8	17.8

Pupation

During active period of the pest (August to October) when weather is hot, full-grown larvae make a small circular hole in the green boll and drop on the ground for pupation. To determine the time when these larvae come out, 14-28 day-old-bolls were placed in conical funnels. A glass jar containing detergent was kept below the funnel

for larval collection. During the day larvae were counted at hourly intervals from 8.00 to 1900 hours and at night collection was counted in the morning at 7.00.

The maximum number of larvae left the bolls during day and the peak was from 11.00 to 15.00 hours when the temperatures are highest. Dropping rate decreased gradually after 15.00 hours and was minimum during night **(Table 9)**.

Table 9 **Number of *Pectinophora gossypiella* larvae collected in the jars after they left bolls for pupation during different time of the day, based on 900 bolls in three replicates in each season.**

Hours	1992		1993		Mean	
	No.	%	No.	%	No.	%
07-0800	3.6	2.2	1.3	0.4	2.5	0.4
08-0900	3.6	2.2	5.0	1.3	4.3	1.6
09-0100	4.6	2.8	12.7	2.3	8.6	3.1
1000-1100	4.6	2.8	22.0	5.7	13.3	4.8
1100-1200	11.0	6.3	56.0	14.6	33.5	12.2
1200-1300	43.0	25.9	88.4	23.0	65..7	21.9
1300-1400	29.0	17.5	67.7	17.6	48.3	17.6
1400-1500	15.0	9.1	49.3	12.9	32.2	11.7
1500-1600	17.6	10.6	25.7	6.7	21.6	7.6
1600-1700	9.7	5.9	21.7	5.6	15.7	5.7
1700-1800	10.6	6.4	18.3	4.8	14.5	5.3
1800-1900	7.6	4.6	7.3	1.9	7.6	2.8
1900-0700	5.9	3.6	8.7	2.3	7.3	2.7

During active season pupation within bolls was never found. Ingram (1994) mentioned that in the West Indies larvae may tunnel to the cuticle, leaving this as a transparent window, and pupate inside. In Pakistan pupation occurs either on the soil debris, in the top five cm soil or in the soil crevices, mostly in the plant periphery. For pupation larvae spin an elongated loose white silken cocoons. Pupal period was 4-10 days during summer (from August to October).

In the end of October a part of the larval population start entering diapause in the bolls or in the soil. Cocoons of these larvae are quite different from non-diapausing larvae. These are thick, closely woven, spherical in shape with no exit hole. These are known as hibernaculae. The larvae remain curled up in the cocoons, until conditions become suitable for pupation. Some of these have short diapause and adults continue to emerge during winter, others have long diapause and adults from these continue to emerge up to August next year.

Carry over sources

Different workers have reported different sources of carry over such as ginning waste, leftover bolls, alternate host plants, ratoon cotton and survival of larvae in the soil, double seeds etc. The role of each of this source was determined separately.

Ginning waste

a) Pink bollworm larval mortality in the ginning waste

To study role of ginning waste as source of infestation, samples of 30 kg. were taken from the heap of ten ginneries during December to May at weekly intervals. These were examined to determine number of alive and dead larvae of the pink bollworm. Emergence of moths in the ginneries was also recorded from December to August by placing one pheromone baited trap in each factory. Number of dead and alive larvae recorded per 120 kg waste are given in **Table 10**.

Larval mortality in the ginning waste was different in different years. Larval population gradually decreased from December to May with a peak in January. The mortality increased from December to May (**Table 10**). It was higher during March in 1991, 1992 and 1993. All larvae were found dead during May in 1991, in April during

1992 and 1993 (**Table 10**) when temperature were more than 40°C. However, moths were continuously caught in the pheromone baited traps placed in these factories even afterwards. Average number of moths per trap was 27 in April 18 in May, 11 in June, 15 in July and 22 in August when factories were closed. It was not certain whether these moths were coming from outside or emerging from the hidden larval population in the cracks and crevices at the premises of the factories.

Table 10 **Number of *Pectinophora gossypiella* larvae collected from sample weighing 120 kg. of ginning waste and larval mortality during 1991, 1992 and 1993**

Months	1991-92			1992-93			1993-94		
	No.of larvae		Mortality (%)	No.of larvae		Mortality (%)	No.of larvae		Mortality (%)
	Total	**Dead**		**Total**	**Dead**		**Total**	**Dead**	
Dec.	-	-	-	-	-	-	1265	383	30.3d
Jan.	5948	2396	40.3c	4971	964	19.4d	2495	888	35.6d
Feb.	5415	2570	47.5c	4005	1434	35.8c	1101	565	51.3c
Mar.	2872	1713	59.6b	304	157	51.6b	296	212	71.4b
April	875	856	97.8a	54	54	100a	140	140	100a
May	675	675	100a	0	0	0	0	0	0

- = Sample not taken 0 = No waste available
Means followed by the same letters are not significantly different at 5% level of significance.

b) Emergence of moths in ginning waste in heaps of different sizes

To determine the survival of larvae in the ginning waste, it was kept in heaps of 0.6, 1.2 and 1.8 m high in open filled in cages measuring 1.2 x 1.2 x 2.4 m. In February when experiment was started 0.12 larvae per kg. were recorded. The number of moths emerged from 2.83 cu. m of ginning waste are presented in **Table 11.**

Moth emergence was completed by the end of April from the small heap of the waste (0.6 m). From other two cages moth emergence continued till the first week of

May. However, in the biggest heap, one moth emerged in October during 1993. Overall population level was higher in 1993 than 1992. Emergence was rapid in small than in the large heaps.

Waste of these heaps was examined at the end of October for live larvae. From the waste kept in 1.8 m high heap five dead larvae were found in double seeds but no larva was found from the waste of other two cages. Almost all the adults from the double seeds emerged before cotton was planted.

Table 11 **Number of *Pectinophora gossypiella* moths emerged based on 2.83 cu. m ginning waste kept in heaps of different sizes at Multan.**

	Mean number of moths emerged					
Months	**0.6m**		**1.2m**		**1.8m**	
	1992	**1993**	**1992**	**1993**	**1992**	**1993**
March	0.7	40.6	0.3	26.6	0.25	19.79
April	0.7	46.9	0.6	32.8	0.88	21.88
May	0.0	0.0	0.3	4.6	0.15	18.75
October	0.0	0.0	0.0	0	0	1.04

c) Emergence of pink bollworm moths from the ginning waste spread in the field.

Survival of the pink bollworm larvae in the ginning waste when it is spread in the field for use as a source of organic matter was studied. The waste containing on an average one larva per kg was applied in the soil. There were two treatments, (i) ginning waste was spread on the soil surface @ 19 ton /ha and was irrigated and (ii) ginning waste applied at the same rate but mixed in the soil with disc plough and then irrigated. Nine wiregauze cages in each treatment were placed with glass jars on the top to catch the moths till October. Organic matter contents of the field before and after the application of waste were determined.

Moth emerged till May in both the treatments with peak emergence in April (**Table 12**). No emergence took place after this month. More moths emerged from the waste spread on the soil than where it was mixed with soil. However, the differences between the treatments were non-significant. Overall 33.3% less moths emerged from plot where ginning waste was mixed in soil compared with surface spreading indicating mortality in larval population when mixed with soil.

Pre-treatment organic matter was 0.41% and post-treatment 0.84% after three months. Hence almost 100% organic matter increased in the soil. This experiment shows that ginning waste can be used as organic matter to improve the soil texture without acting as source of pink bollworm infestation to the next year's crop.

Table 12 **Number of *Pectinophora gossypiella* moths recorded from ginning waste spread on the soil and mixed in it at Multan.**

Months	Number of moths emerged from	
	Ginning waste spread on soil + irrigation	Ginning waste mixed with disc plough + irrigation
March	3c	0c
April	47a	40a
May	13b	2b

Means followed by the same letters are not significantly different at 5% level of significance.

Crop residue

a) Effect of irrigation on survival of pink bollworm larvae

After cotton has been harvested, a part of the pink bollworm population overwinters in the soil. To study the effect of irrigation on the survival of the pink bollworm larvae, two fields were selected where sticks were removed manually. One was irrigated during February and the other was kept as control without irrigation.

Emergence of moths was recorded by placing 27 conical wireguaze cages measuring 1.2m x 1.2m x 1.2m with a glass jar on the top for collection of moths.

The number of moths emerged from the field where irrigation was not given was significantly higher than where irrigation was done **(Table 13)** indicating that about 56% larvae died because of irrigation.

Table 13 Effect of irrigation on survival of *Pectinophora gossypiella* diapausing larvae in soil based on emergence of moths in irrigated and non irrigated fields

Treatment	No. of moths emrged/ha	Reduction over control %
Irrigation	5222a	55.73
No irrigation	11797b	-

Means followed by the same letters are not significantly different at 5% level of significance.

b) Effect of ploughing on survival of the pink bollworm larvae

To determine the effect of ploughing on survival of the pink bollworm larvae, two fields were selected where sticks were removed manually. Each field was divided into three plots. One plot from each field was ploughed with furrow turning plough and the second with munnah plough, and the third was kept as control where ploughing was not done. Moth emergence was recorded by placing 27 conical wiregauze cages as described above.

Maximum number of moths emerged from the plots where no ploughing was given followed by munnah plough and furrow turning plough in decending order **(Table 14)**. Furrow turning plough gave highest mortality of pink bollworm larvae and the differences were significant as compared with munnah plough as well as where ploughing was not done.

Table 14 **Effect on survival of *Pectinophora gossypiella* larvae where ploughing was done with munnah and furrow turning plough and control at Multan**

Treatment	No.of moths emerged/ha.	Reduction over control (%)
Furrow turning plough	7600a	21.96
Munnah plough	8191b	15.89
No ploughing	9739c	-

Pre-treatment population = 0.093 larvae/sq.m.
Means followed by the same letters are not significantly different at 5% level of significance.

c) Effect of destruction of sticks on pink bollworm larvae

To study the effect of destruction of sticks in the field on carry over of the pink bollworm larvae, one block of cotton measuring 90 x 90 m was divided into three equal plots. In one plot sticks were slashed with rotavator, in the second with disc plough and from the third sticks were removed manually. Moth emergence was recorded in 18 wiregauze cages in three replicates.

Slashing of sticks caused significant larval mortality compared with where sticks were removed manually. Minimum moths emerged from the plot where sticks were shreded with rotavator followed by where stick were destroyed with disc plough and where sticks were not ploughed in the soil (**Table 15**). Mortality of the pink bollworm with rotavator was 53% and with disc plough 23% and the differences among these two treatments were non-significant. However, differences between shredding and control were significant at 0.05 level. The mortality in fields where shredding was done must be higher than given above because some larvae from the bolls must have been added to the soil than where sticks were completely removed. Thus shredding of sticks has addative effect over simple ploughing.

Table 15 Effect of mechanical destruction of cotton sticks on survival of *Pectinophor gossypiella* larvae in the field at Multan.

Practice	Number of moths emerged	Reduction (%)
Rotavator	10846a	53.83
Disc plough	12845a	23.01
Control	16684b	-

Means followed by the same letters are not significantly different at 5% level of significance.

To determine the effect of ploughing of sticks at the rate of 10 tones/ha in the field on organic matter, nitrogen, phosphorus and potassium contents were analysed and quantified before ploughing and 90 days after crushing.

Organic matter increased 49% and nitrogen 22%, phosphorus by 6.3 ppm potassium by 15.4 ppm where shredding was done compared with where sticks were removed manually **(Table 16)**. Slashing of sticks not only cause mortality in pink bollworm but also increased fertility of the soil.

Table 16 Effect of addition of plant material on soil on the nutrient index

Nutrient	Pre-treatment level	90 days after treatment	Control
Organic matter %	0.37	0.73	0.38
Nitrogen (%)	0.35	0.45	0.34
Phosphorus (ppm)	8.2	8.7	8.2
Potash (ppm)	204	241	205

Effect of ginning on mortality in the pink bollworm larvae

To study effect of ginning on larval mortality, one kg of cotton seed and 3 kgs of seed cotton from the same lot were collected from one ginnery from October to December every month. Double seeds from the seed cotton were separated by hand and compared with ginned seeds for larval mortality.

Number of double seeds was different in cotton picked at different times. They were rare in the bolls opened during or before October. Maximum number of double seeds was found in the bolls that matured in December (**Table 17**).

In the seed cotton collected from ginneries, number of double seeds was much higher than in the seeds collected after ginning. This indicates that considerable mortality occurs from ginning process.

Table 17 Percentage of double seeds before and after ginning in seed cotton picked at different times of the year at Multan

Months	1993		1994		1995	
	Seed cotton	**Seed**	**Seed cotton**	**Seed**	**Seed cotton**	**Seed**
October (Ist pick)	1.0	0.0	2.0	0.01	2.4	0.2
November (2nd pick)	3.0	0.2	3.5	0.5	5.0	0.4
December (3rd pick)	7.0	0.3	5.0	0.7	7.6	0.7

Left over bolls on cotton sticks

To compare larval mortality in left over bolls on sticks, sticks were piled in two ways. One hundred fifty small bundles each containing about 300 sticks were stored with tops upwards (vertically). The same number of bundles was stored in heap in the conventional practice followed by the farmer i.e., horizontally. Two hundred bolls were sampled from the upper half, 200 from the lower half of the heap and 200 bolls from upright bundles every month.

In March larval mortality was low both in the bundles and heap. It increased with the passage of time. In small bundles and upper half of the heap 72% larvae died by May when 24% had died at lower half of the heap. In June and July alive larvae were found only from the bolls in lower half of heaps (**Table 18**). Survival of pink

bollworm larvae up to July in the heap lying partly in shady places indicated that left over bolls on the sticks serve as source of carryover if not properly managed.

Table 18 **Number of dead and alive larvae of *Pectinophora gossypiella* and percentage mortality in sticks kept in small bundle and heaps at Multan**

Months	In bundles			In heaps					
				Top			Bottom		
	Alive	Dead	Mortality %	Alive	Dead	Mortality %	Alive	Dead	Mortality %
March	61	3	4.7	64	5	7.2	67	9	11.8
April	30	21	41.2	26	19	42.2	62	10	13.9
May	12.0	32	71.9	11	27	71.1	45	14	23.7
June	0	13	100	0	12	100	11	9	45
July	0	7	100	0	8	100	7.0	4	34.8

a) Pattern of emergence of *Pectinophora gossypiella* in bolls and seeds

Bolls

To verify emergence of the pink bollworm moths from left over bolls, at different temperatures, 1400 bolls in 1995 and 700 in 1996, containing pink bollworm diapausing larvae, were collected at harvest. Half of these were kept in the open where winter and summer day and night temperature varied greatly and the same number was kept at room temperature (27±3°C). Moth emergence was recorded daily.

During 1995 the moths emerged from bolls kept in the open from January to April with a peak in the second week of March. From the boll kept at room temperature, adults continue to emerged from October to August with a peak in July **(Table 19).** During 1996 when bolls were kept in the open moth emergence continued from November to the second week of May with a peak in third week of April **(Table 19)**. After middle of May no moth emerged from these bolls. When these were

dissected larvae were found dead. From the bolls kept at room temperature adults emergence was low from December to April. However, 50.0% moths emerged in July but no moth emerged in August (**Table 19**).

Table 19 **Emergence of *Pectinophora gossypiella* moths from bolls kept at 27±3°C and in the open during 1995 and 1996 at Multan**

Month	1995				1996			
	Laboratory		Open		Laboratory		Open	
	Number	%	Number	%	Number	%	Number	%
October	6	0.9	0	0.0	0	0.0	0	0.0
November	53	7.6	0	0.0	0	0.0	4	1.1
December	30	4.3	0	0.0	6	1.7	1	0.3
January	83	11.9	2	0.3	40	11.4	1	0.3
February	14	2.0	62	8.9	94	26.9	25	7.1
March	18	2.6	222	31.7	13	3.7	25	7.1
April	21	3.0	72	10.3	9	2.6	63	18.0
May	20	2.8	0	0.0	0	0.0	36	10.3
June	88	12.6	0	0.0	0	0.0	0	0.0
July	278	39.7	0	0.0	?	50.0	0	0.0
August	13	1.8	0	0.0	0	0.0	0	0.0

Seeds

Moth emergence pattern was studied by keeping 1,500 double seeds each at room temperature (27±3°C) and in the open where temperatures varied considerably. Details given in table.

Moth emergence from seeds kept at room temperature started in March and was completed by August. There were two peaks first in April and second in July. In August remaining larvae were found dead within the seeds due to unknown factors. Moth emergence from the seeds kept in open was low. It started in February and completed in the first week of May with a peak in April (**Table 20**). Moth did not

emerge from 47.4% larvae and died within the seeds, possibly because of high temperature of May and June. At room temperature moth continue to emerge up to August whereas in the open maximum moths emerged in April when maximum temperature was above 40°C for few days. Larval mortality occurred in May when temperature was 44°C and in June 46°C. It is evident that at room temperature and in the open maximum adults emerged during April when average temperature was 27°C. It indicates that emergence of moths from diapausing larvae depends on the micro-environments of the places where they hibernate.

Table 20 Emergence of moths from the double seed kept at different temperature at Multan

Months	Room Temperature (27±3°C)		Open (24±45°C)		Temperature°C		
	Adult emergence				Mean		Ave.
	Number	Percentage	Number	Percentage	Maximum	Minimum	
February	-	-	58	3.9	30.5	2.5	17.5
March	225	15.0	317	21.1	30.5	9.0	19.2
April	375	25.0	358	23.9	42.0	14.0	27.0
May	60	4.0	56	3.7	44.5	20.0	33.3
June	79	5.3	0	0.0	46.0	23.0	34.4
July	525	35.0	0	0.0	43.5	230	32.0
August	8	0.5	0	0.0	39.0	26.5	32.2

Ratoon cotton

Ratoon cotton is also considered a source of carry over of the pink bollworm. To determine the level of infestation, squares, flowers and green bolls of ratoon cotton were examined during 1992 and 1993. During both the years after winter, first generation larvae of the pink bollworm were available on newly formed squares and flowers at the end of March. Infestation was 0.12% in March, 1.9% in April, 2.8% in

May and 4.1% in June indicating that low levels of infestation occurs on ratoon cotton in the Multan district.

Alternate host plants

Role of alternate host plants as source of carry over of the pink bollworm was studied. Two cultivated crops *Abelmoschus esculentus*. (okra) and *Hibiscus cannabinus* (kenaf), five ornamental plants *Althea rosea* (hollyhock), *Hibiscus rosa-sinensis* (china-rose), *Hibisucus mutabilis* (cotton-rose) and *Malvaviscus arboreus* (turks cap), *Malva parviflora* (small mallow) and two weeds, *Abutilon* spp. were examined during 1992 and 1993.

A total of 2760 fruiting parts of *Althaea rosea* were examined from April to September. No fruiting part and larva were found from January to March. In April 2.5% (16 out of 650), in May 2.5% (20 out of 800), in June 1.3% (10 out of 800) and in July 0.3% (1 out of 300) damage fruits were found. Low level of infestation was also found on *Hibiscus mutabilis*. In January 0.8% (1 out of 150) and in February 2.0% (2 out of 100) during both the years. From 11,280 fruits of *Abutilon* spp. two pink bollworm larvae were reared in November in both the years.

No pink bollworm larva was found on 2,750 fruits of *H. cannabinus*, 1,180 fruits of *H. rosea-sinensis*, 885 of *M. arboreus*, 550 of *M. parviflora* and 20,000 of *A. esculentus*.

Control Measures

Chemical control

At present insecticides are extensively used in Pakistan. Pesticides worth rupees more than seven billions are imported every year. Of these 80% are sprayed on cotton. Behaviour of the pink bollworm is different from *Helicoverpa armigera* (Hub.) and *Earias* spp. Its larvae remain protected in the bolls for most of the development period, thus out of the reach of insecticides. Therefore, for good control pesticide should be sprayed when moths, eggs and newly hatched larvae are present. Thus time of application of insecticides is critical. More than 35% bolls may get infested if insecticide applications are not made at the proper time.

Efficacy of three insecticides for control of pink bollworm was studied. These included two formulations of naturalyte 105, 48EC and naturalyte 105, 20SC, deltamethrin 2.5EC and bifenthrin 10EC. Four dosage of naturalyte 105, 48EC and one dose of each of naturalyte 105, 20SC, deltamethrin and bifenthrin. Untreated plot was kept as control. Each insecticide was applied twice at an interval of 12 days.

The first application of all the insecticides reduced infestation of the pink bollworm compared with untreated check. Among the pyrethroids, bifenthrin was better than deltamethrin **(Table 21)**. Overall larval survival was lower with higher doses of naturalyte 48EC than its lower dosages. Effectiveness of naturalyte 20SC was comparable to that of naturalyte 48EC. It seems that insecticides were effective only for 3-4 days after application. After this newly hatched larvae were not killed and entered the bolls thus increasing infestation **(Table 21)**. Overall larval mortality was higher with the first application than with second. Interval between the two sprays seems

responsible for this increase in damage and larval survival. Thus repeated applications within 5-7 days at high population level are required to control the pest.

Table 21 Percentage of bolls damage and number of larvae recovered after the application of three insecticides at Multan during 1992

Insecticides	Dose g.a.i./ h	Ist spray				2nd spray			
		7 days		12 days		7 days		12 days	
		Boll damaged (%)	*Alive larvae	Boll damaged (%)	*Alive larvae	Boll damaged (%)	*Alive Larvae	Boll damaged (%)	*Alive larvae
Naturalyte 105, NAF-85 48EC.	12.5	6	3	7	5	11	5	14	8
	25.0	4	2	5	5	8	4	16	7
	75.0	3	1	5	2	8	2	15	7
	100.0	3	0	4	2	10	4	12	1
Naturalyte 105, 20SC	50.0	2	1	5	2	8	3	9	4
Deltamethrin 2.5EC	12.5	5	3	4	0	5	1	10	4
Bifenthrin 10EC	50.0	3	1	2	1	4	0	11	2
Control		8	6	9	7	13	8	12	9

* = No. of larvae per 50 bolls

Biological control

In Pakistan possibilities of biological control of various cotton pests have been discussed by Habib and Mohyuddin (1981). Of the 28 species of parasitoids recorded from the pink bollworm, *Apanteles angaleti* Mues. *Bracon gelechiae* Ashm.(Braconidae), *Goniozus* sp. (Bethylidae) and *Elasmus johnstoni* Ferriere (Elasmidae) were most common as reported by Cheema *et. al;* (1980a).

The predators recorded feeding on the pink bollworm eggs and larvae include 23 insect species, 26 spiders, one mite, one mammal and 12 birds. Most of the predators are abundant before the applications of insecticides are started. Their populations decreased as a result of indiscreminate and excessive use of insecticides.

The granular insecticides or seed dressing may do less damage to natural enemies. Mohyuddin *et al.* (1997) conserved the predators by growing lucern before harvesting wheat so that predators shift from wheat to lucern and from lucern to cotton when it is cultivated in June.

Cultural control

Cotton is the only host plant of the pink bollworm in Pakistan. After the crop has been harvested the diapausing larvae survive in the soil or in left-over bolls on the cotton sticks. Cultural control measures can be used effectively for keeping the pink bollworm diapausing population down between the seasons. After the sticks have been cut manually simple irrigation had pronounced effect on larval mortality and caused almost 56% reduction in larval population where irrigation was given. Reducation of larval population with munnah plough was 15% and furrow turning plough 21%. Destruction of cotton sticks with rotavator gave 54% mortality and disc plough gave 23% larval mortality. Sticks cut manually and stored in small bunddles with boll bearing portions upright position caused 100% larval mortality before the cotton is planted. This indicated that destruction of cotton sticks alongwith bolls as early as possible or proper management of sticks if they are to be used for fuel purposes are very important. These practices can reduce the pink bollworm population to a minimum level. Large scale benefits of these management practices can only be possible if adopted in all the cotton growing areas.

Use of sex pheromones

Insect pheromones are extremely powerful species specific tools for pest control. Different pheromone formulations of *P. gossypiellia* viz; Pectone, Nomate

PBW, PB Rope and Chips have been used for monitoring and control by mass trapping and mating distruption techniques.

a) Efficacy of different type of traps

For monitoring efficacy of five types of traps including Delta white and red, white and yellow funnel and universal traps was determined. Out of these, Universal trap was locally manufactured whereas others were imported and are expensive. Their import involves foreign exchange. To eliminate error because of trap position in catches four traps in an experiment, Delta red, Delta white, Funnel white and Universal traps were put in the field in one line and replicated three times. Position of the traps was kept the same for 25 days and moth were collected every day. In the other experiment after 25 days five traps in addition to above mention four traps Yellow funnel was used in one line and replicated five times. Trap positions were changed after every five days.

The average number of moths caught/trap for five days where positions were not changed are given in **Table 22**. More moths were caught in Delta white followed by Delta red, Universal and Funnel white in decending order. Overall catches were significantly higher in these traps when placed on the corners compared with when these were placed away from the corners between the other traps. Maximum moths were caught in Delta white, Delta red and Yellow funnel, when they were placed between the traps and position was changed. Overall Universal trap proved inefficient and Yellow funnel trap superior than the other traps.

Table 22 *Pectinophora gossypiella* males caught in different type of traps at Multan

Trap Design	Number of males caught/trap/5 days		
	Trap position unchanged	Trap placed on the corners	Trap placed in between the other trap
Funnel White	11.1	22.5	12.1
Funnel Yellow	-	114.0	26.2
Universal	12.5	4.0	6.3
Delta white	20.9	26.0	30.6
Delta red	16.9	53.0	28.1

b) Mass trapping

To determine the effect of mass trapping on pink bollworm infestation, in September 2.4 ha cotton field was selected at Khokhran. A total of 30 traps, 12/ha were used in the trial area. Cotton block of the same size 200 m away was kept as control where no plant protection was done.

Moth catches were low in pheromone treated block in September with a peak in October **(Table 23)**. Pink bollworm infestation in both the areas was almost equal when trial was started. Infestation was reduced one week after the traps were installed as compared with the control. It remained at the same level till the end of season but in the control infestation gradually increased and was 26% at the end of October when moth catches were also highest **(Table 23)**.

Table 23 Rate of infestation of *Pectinophora gossypiella* in control and where traps were placed and number of moths of traped area at Khokhran

Observation period		Boll infestation (%)		No.of males trapped
Months	Weeks	Mass trapped	Control	
September*	II	7.3	8.0	-
September	III	6.0	15.0	120
September	IV	5.7	16.0	390
October*	I	6.0	16.0	413
October	II	5.3	20.0	1015
October	III	7.3	21.0	1036
October	IV	8.3	26.0	1088

* = Dates when pheromone and new traps were placed or replaced.

The suitability of pink bollworm control with mass trapping of males was compared with the area where farmer used insecticide at Khanewal. Two blocks of cotton each of 12 ha was selected. In one block Pheromone baited traps at the rate of 12 traps/ha were used. Same size of cotton block treated with insecticide was kept as control for comparison. To monitor males activity four traps were placed in the insecticide treated area. One application of disyston was given at the end of July with first irrigation and two foliar insecticides were applied against *Earias* species during the season. Control received one granular application of disyston and was sprayed five times for the control of pink and spotted bollworm during the season.

In August pink bollworm males population trapped in the pheromone treated area was low. Maximum moths were caught during September and October when pest was most active (**Table 24**). Infestation in pheromone and insecticide treated areas was zero when trial was started. In the middle of August infestation started and was below economic injury level in both the treatments. However, it was much lower in mass trapped block as compared with insecticide treated area. During September and October infestation remained above the threshold level where farmer used his own pesticide regime. Fifty percent reduction in insecticide use was achieved in mass trapping area. It indicated that mass trapping reduced the pink bollworm infestation considerably but the growers have to use insecticides for other bollworms.

Table 24 **Rate of infestation of *Pectniphora gossypiella* where mass trapping was done and control and number of males caught in mass trapped area at Khanewal**

Observation period		Boll infestation (%)		No. of males trapped
Months	Weeks	Pheromone**	No pheromone***	
August*	I	0.0	0.0	0
August	II	0.4	1.9	2476
August	IV	2.3	6.4	2317
September*	II	3.5	12.9	1886
September	IV	2.7	15.3	10684
October	II	5.6	7.5	2735
October	IV	6.0	9.3	10195

* = Dates when new traps were placed or replaced.
** = Sex pheromone treated = 1 granular + 2 foliar applications.
***= Insecticide treated (control) =1 granular +5 foliar applications.

c) Feasibility of pink bollworm control with communication disruption

To determine the efficacy of gossyplure containing *P. gossypiella* pheromone, two blocks of cotton each of 11-ha were selected during 1991. In one block 20cm PB Ropes were applied at the rate of 500 pieces/ha and compared where farmer used insecticides.

In the pheromone treated area, moth catches remained zero throughout the season. Male moths were active throughout the season in the insecticide treated area. Gradually moth catches increased with a peak 1004/trap/week in the middle of September **(Fig.8)**. In the pheromone treated area catches were almost zero resulting 100 percent mating disruption **(Fig.9)**, however, gravid females entered from the nearby fields and caused low level of infestation. In the pheromone treated area two insecticidal application of dimethoate against *Amrasca devastans* (Dist.) in whole block and two of Polytrin-C, (mixture of profenofos + cypermethrin) against *Earias* species, each on 1.6 hectares were given. In the plot where farmer used insecticide, two

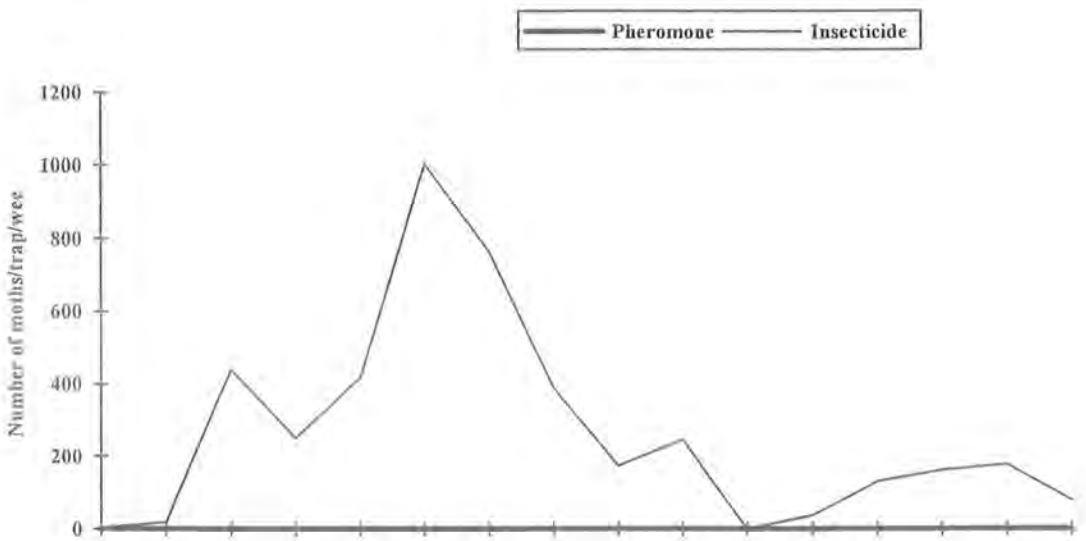

Fig.8 Number of *pectinophora gossypiella* male moths caught/trap/week in the pheromone and insecticide treated plots during 1991

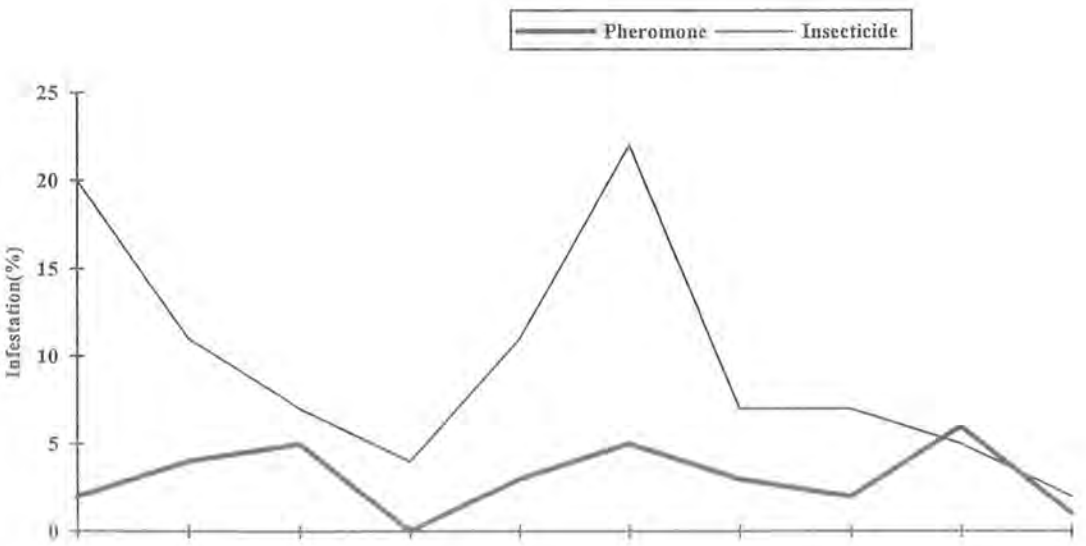

Fig.9 Infestation of *Pectinophora gossypiella* in the PBW rope and insecticide treated plots during1991

applications of insecticide were given against *A. devastans* and three for bollworms. Inspite of five applications of insecticide, pink bollworm infestation in the block where farmer used insecticide went up to 22% as compared with 6% in the pheromone treated block. In addition to good quality, higher yield of seed cotton was obtained. The yield of seed cotton was 2408 kgs/ha in the pheromone treated block as compared with 2156 kg/ha in the insecticide treated area.

To reduce the cost of pheromone Shin-Etsu Chemical Co., Ltd., Japan impregnated gossyplure in two sizes of plastic bags described as small and large Tea bags. To compare the efficacy of this formulation with PB Rope, a trial was conducted during 1992. BP Rope 20cm and small Tea bags both at the rate of 500 pieces/ha and large bags 250 pieces/ha were applied on 10 ha. A block of 2.4 ha 200 m. away was selected where farmer used insecticide.

Male moth catches were low during early August in the pheromone and insecticide treated areas. Population gradually increased with a peak at the end of September when maximum catches were 360/trap/week. Mating suppression was 99% in the pheromone treated plots **(Fig.10)**. Infestation in flowers and bolls was also very low as compared with insecticide regime**(Fig.11)**. Sucking pests were controlled with dimethoate two times in both the blocks. In pheromone treated area *Earias* spp. were controlled with profenofos+cypermethrin two times at recommended dose rate. In insecticide treated plot, bollworms were controlled with dimethoate+cypermethrin, profenofos+cypermethrin and bifenthrin. Thus reduction in pesticide use was almost 33%. Moth suppression was also 99% in the centre of pheromone treated plot where only one foliar application was given against sucking pests and no insecticide was used against *Earias* species.

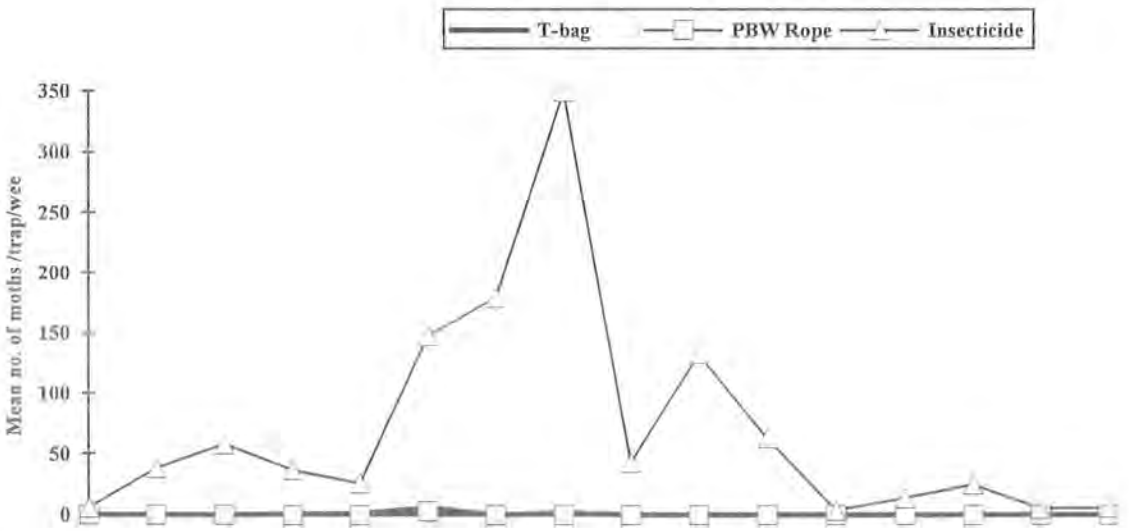

Fig.10 Mean number of male moths of *Pectinophora. gossypiella* caught in plots withT-bag,(average of small and large) PBW rope and insecticide during 1992

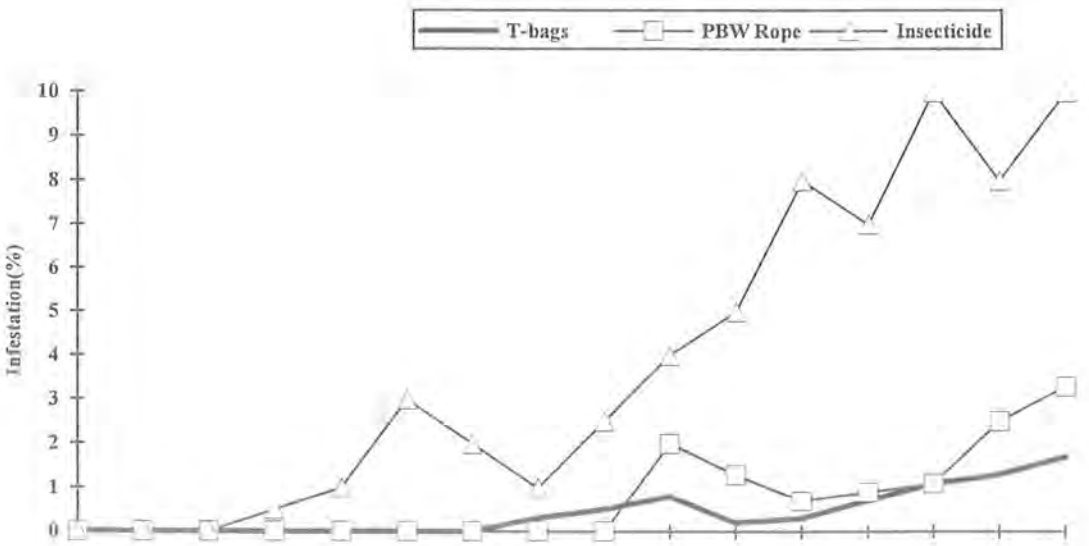

Fig.11 Infestation of *Pectinophora gossypiella* in the plots treated with T-bag,(average of small and large) PBW rope and insecticide s during 1992

Small and large Tea bags returned from the field to Japan were analysed at different intervals after placement. Hundred days after placement left over of the originally loaded pheromone was 21% in the small Tea bag and 27% in the large Tea bags (personal comm.). The yield of seed cotton was 1866 kgs/ha in large, 1737 kgs/ha in small Tea bags and 2080 kgs/ha in PB-Rope as compared with 1507 kgs/ha where insecticides were used. In the centre of the pheromone treated area where only sucking pests were controlled and no application of insecticide against bollworms was made, the yield of seed cotton was 1388 kgs/ha.

DISCUSSION AND CONCLUSION

The pink bollworm *Pectinophora gossypiella* (Saunders) has been reported to attack 70 host plants worldwide (Noble, 1969). In Pakistan it has been reported feeding on *Altheae rosea, Abelmoschus esculentus, Hibiscus cannabinus,* and *Euphorbia dracunculoides* by Haq (1973) and from *Abutilon* spp. by Cheema *et al.* (1976). As a result of a survey carried out in the Punjab, to determine the effect of alternate hosts as source of infestation on cotton, it was recorded in extremely small numbers from *A. rosea, Hibiscus mutabilis* and *Abutilon* spp. indicating that these are incidental hosts and probably are not a major source of infestation on cotton. It was not recorded on *H. cannabinus, H. rosa-sinensis, Malvaviscus arboreus, Malva parviflora* and *A. esculentus*. Pink bollworm record from *H. cannabinus*, *E. dracunculoides* and *A. esculentus* reported by Haq (1973) seem erroneous most probably because of misidentification of the host plants or pest species. Although Pearson (1957) in Africa and Noble (1969) in the USA reported *A. esculentus* as most favoured host of the pink bollworm, it was not found on this host indicating that it may be either because the strain of the pink bollworm is different or the variety of okra is not the same that is found in Pakistan.

Cheema *et al.* (1980a) reported 28 species of parasitoids. Maximum incidence of parasitoids attacking the pink bollworm larvae and pupae varied from 0.5 to 6% which is extremely low. They seem to play very little role in controlling the pest because the larvae are concealed in the bolls and pupae in the soil. Thus they are most of time out of reach of the natural enemies therefore, incidence of larval and pupal parasitoids is difficult to improve. However, egg stage is exposed to parasitoids and predators for three to seven days depending upon the

temperature and thus is more vulnerable to the natural enemies. Mohyuddin *et al.* (1997) reported that augmentative releases of *Trichogramma chilonis* Ishii gives good control of all the cotton bollworms including pink.

A number of cultural practices such as destruction of leftover bolls on the sticks, irrigation and ploughing to kill the diapausing larvae in the soil have been recommended by various workers. In developing Integrated Pest Management (IPM) of the pink bollworm feasibility of various cultural practices in addition to given above such as mortality of diapausing larvae in ginning waste and mixing in the soil, pattern of moth emergence from diapausing larvae at different temperatures, use of sex pheromone and chemical control were studied.

For control of the pink bollworm destruction of sticks and bolls on cotton sticks before 31 January was recommended as early as 1930s. This practice was made mandatory under West Pakistan Agriculture Pest Ordinance of 1959. This recommendation seems to have been made without considering the economic implication of this control measure. Therfore, this has not been adoped by the farmers because sticks are source of energy for them. They cannot afford to buy expsnsive fuel for household when ready fuel is available from the sticks. Destruction of sticks can destroy the pink bollworm in the bolls but large number can survive if not managed properly. The present study indicated that 100% pink bollworm larvae could be destroyed without destroying the sticks by putting them in small bundles and keeping them in upright position. This practice seems to be economically feasible because stocking the sticks in small bundles in upright position should not involve much cost.

After the cotton has been harvested the pink bollworm larvae are present in the field. Irrigation during winter killed 54-57% larvae as indicated by number of moths that emerged as compared with control. One irrigation during winter if adopted by the farmers can considerably reduce the infestation of the pink bollworm next year.

Munnah and furrow turning ploughs caused 15 and 20 percent mortality respectively, compared with where sticks were removed manually. However, slashing of sticks with rotavator or disc plough gave 23 and 54 percent larval mortality compared with the control. The proper and timely tillage practices and irrigation can reduce more than 70% of the population.

Double seeds have been reported as source of infestation in the next season. In the present study it was found that as result of ginning most of the larvae are killed because double seeds are crushed and the number of larvae in the double seeds were extremely low (0.01-0.2%) where ginning was done at 750-825 rpm. The population that may survive is killed when temperature is 45± 3 °C during May. However, if waste is mixed with soil and irrigated, it not only completely destroy the pink bollworm larvae but also add organic matter and improve soil fertility. If mixing is done where temperatures are moderate the mortality in the residual population in double seeds may not be completed.

Control of the pink bollworm with insecticides is difficult because the larvae after hatching are expoed for 30 to 90 minutes before they enter the squares or bolls and then they are out of reach of insecticides. The most vulnerable stages are eggs and adults. To achieve its control well timed spray of insecticide

synchronizing with emergence of adults is required. For this purpose pheromone traps may be used for adults monitoring. The insecticides gave control of larvae upto 7 days after spray. The moths continue to merge during most of the active period of the pest. Therefore, most frequent sprays will be required if monitoring of the adult population is not done with sex pheromone traps. In Israel, Melamed and Shoham (1975) and in India, Taneja and Jayaswal (1981) recommended that spraying of insecticide should be done when trap catches are eight moths/trap/night. However Teich *et al.* (1977) reported five moths as threshold in India. Studies on the economic threshold of adults catches to correlation with infestation have not been made in Pakistan, till such studies are made, threshold developed in India may be used as cotton in India and Pakistan grow in similar ecological condition.

Pink bollworm could be controlled with mass trapping in a limited area as its application is economically not feasible because the pheromone is expensive and laborious to apply therefore, difficult to undertake on large areas. Communication disruption with pheromone has been affective but at present farmers have not adopted because of high cost. Moreover, farmers have to spray for bollworms such as *Earias* spp. and *Helicoverpa* even if pheromones are used.

Cultural control is the most important component for the management of overwintering population of the pink bollworm. Irrigation and ploughing of soil after the cotton has been harvested gave about 80% mortality of the diapausing population in the soil. Diapausing larvae in the left over bolls on the sticks can be destroyed by keeping them vertically in small bundles. Thus pink bollworm could be reduced effectively if all cotton growers follow cultural practices properly.

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CHAPTER-III

AMERICAN BOLLWORM, *HELICOVERPA ARMIGERA* (HUBNER) (LEPIDOPTERA : NOCTUIDAE)

INTRODUCTION

The African bollworm, earlier called as American bollworm, *Helicoverpa (Heliothis) armigera* (Hubner) (Noctuidae : Lepidoptera) is widely distributed in Africa, India, Central and South Eastern Asia to Japan, the Philippines, the Middle East, Southern Europe, Eastern and Northern Australia, New Zealand and the Pacific Islands (Pearson, 1958 and CAB, 1968).

It is highly polyphagous (Pearson, 1958; Bilapate, 1984; Zaluchi *et al,* 1986) attacking a great variety of plants including crops, vegetables, fruit and forest trees, ornamental and weed plants. It has been recorded feeding on 127 plants in Africa, Asia and Australia (Reed and Pawar, 1982). Bhatnager and Davis (1978) reported 105 host plants in India whereas Saleem and Yunus (1982) recorded it from 41 plant species, belonging to 14 families in the Punjab and Mohyuddin (1989) reported 65 host plants including 35 crops and vegetables, 24 weeds and 6 ornamentals from Pakistan.

It is known by a number of common names depending on the crop it attacks. For example on gram it is known as gram pod borer or gram caterpillar, on cotton as cotton bollworm, on corn as corn earworm, on tomato as tomato fruitworm and on tobacco as false tobacco budworm (Metcalf *et al.* 19951; Metcalf and Flint, 1962; Lea, 1982; Kumar and Sangapapa, 1984; Hsu, 1985, Pawar, *et al;* 1985).

In 1967 its severe infestation was recorded in some areas of Punjab, Pakistan on Deltapine varieties, causing failure of cotton crops in certain areas. After 70's *H. armigera* became serious pest on gram, maize, tobacco and cucurbits in certain areas. From 1989 onwards, it became the major pest of cotton and caused considerable

damage both to quantity and quality of cotton. Its infestation was more serious on varieties with succulent leaves, more leaf area and in fields where nitrogen fertilizers were used at higher dose. Cotton yield was reduced considerably during 1994 and 1997 due to severe infestation of this pest.

It is a direct pest and feeds on buds, flowers and bolls of cotton. Its economic threshold levels are low and thus control measures become necessary at very low population densities. Matthews and Tunstal, (1968) reported a loss of 112 kgs/ha in seed cotton at population level of 0.23 egg per plant and 820 kgs/ha with 1.0 egg per plant. They concluded that control measures become necessary when egg count reaches 0.5 per plant in Malawi. However, recent work of Van-dan Berg and Cock (1993) from East Africa indicated great variations (50-80%) in the viability of eggs. They suggested that egg based threshold level should be used with caution and proposed a threshold level of two larvae per plant for control purposes.

In the absence of basic knowledge of key mortality factors, in Pakistan control measures are mainly based on chemicals. A study in Pakistan indicated that because of resistance against major groups of insecticides older larvae are less vulnerable to insecticides (Ahmad *et al.* 1995).

Keeping in view importance of the pest in the production of cotton and other high value crops, population dynamics on cotton and alternate host plants, oviposition and larval distribution on cotton, pupation and diapausing behaviour and key mortality factors regulating its population were undertaken to develop better control strategies for this pest. Results of these studies are reported here.

REVIEW OF LITERATURE

It is highly polyphagous (Pearson, 1958; Bilapate, 1984; Zaluchi *et al.* 1986) and its larvae have been found feeding on a wide and diverse range of cultivated and wild plants. It has been reported from 63 plant species belonging to 27 families in Australia (Matthews, 1987), 127 plants in Africa, Asia and Australia (Reed and Pawar, 1982). 105 plants in India (Bhatnagar and Davis, 1978) and 65 host plants in Pakisan (Mohyuddin, 1980).

Matthews (1987) revised the genus *Heliothis* and split it into two independent genera, *Heliothis* and *Helicoverpa* as advocated by Hardwick (1965). He placed *Heliothis armigera* found in the Indo-Pakistan subcontinent in genus *Helicoverpa*. It is known by different common names depending on the crop it attacks. (Hsu *et al.* 1985; Metcalf *et al.* 1951; Lea, 1982; Pearson, 1958).

Inspite of its large host range plant species preference is present (Johnson *et al.*, 1975, Roome, 1975; Zaluchi *et al.*, 1986). Cotton supports the highest population, however, is not considered to be the most preferred host (Johnson *et al.*, 1975; Firempong and Zaluchi, 1990).

Its biology and ecology have been studied by a number of workers on different hosts in different parts of the world. Moth emergence has a circadian rhythm, starting at dusk and continues until mid night after which it virtually ceases (Tripathi and Sharma, 1984; Riley *et al.*, 1992). Peak emergence takes place between 2000 to 2200 hours (Roome, 1975 and Singh and Singh, 1975) and the moths pass through wing drying, crumpled wing and butterfly stages. These phases are completed in an hour or so but

time increases when moths emergence occurs in late hours of the night (Riley *et al.*,1992).

Host selection in *Heliothinae* has been comprehensively reviewed by Fitt (1990) and for moths in general by Ramaswamy (1988) and oviposition stimuli have been identified by various workers (Jackson *et al.*, 1984; Tingle and Mitchell, 1984, Fitt, 1990).

H. armigera, females release pheromone in the early hours of the morning. (Ramaswamy, 1990) mating call begins two to five hours after emergence, peak mating period is on fourth day, and preoviposition period ranges one hour to four days (Jayaraj, 1982).

Although females may lay infertile eggs before mating, mainly ovipositon is stimulated by mating. Periods of mating, preoviposition, oviposition, incubation and rate of infertility depend on temperature (Singh and Singh, 1975; Jayaraj, 1982). Females do not oviposit at 10°C and more eggs are laid at 21-27°C than at 15 and 30°C (Henneberry and Clayton, 1991). The females are highly selective for oviposition. The host has to be in suitable condition for development (Hardwick, 1965). Geographical variations in the preference for oviposition sites have sometimes been noted and host preference has been needed to change over the time. Pattern for oviposition is not consistent on cotton (Quaintance and Bruce, 1905; Parsons, 1940; Mistric, 1964; Tunstall *et al.*, 1966; Beeden, 1974, Patel *et al.*, 1974; Hillhouse and Pitre, 1976; Joyce, 1977; Wilson *et al.* 1980; Bernhardt and Phillips, 1982; Fitt, 1990 and Hassan *et al.*, 1990) but females prefer upper surfaces of leaves, young growing points and squares. Gonzales *et al.*, 1967; Fye, 1972; Beeden, 1974; Hillhouse and Pitre, 1976; Joyce,

1977; Wilson *et al.*, 1980; Bernhardt and Phillips; 1982; Mabbatt *et al.*, 1979; Mabbett and Nachapong, 1984; Farrer and Bradley, 1985; Maramawati, 1988 and Uthamasamy, 1992). Dillon *et al* (1994) reported higher mortality of eggs during early season as compared with late in the season. Egg distribution differs significantly between the cotton varieties depending on their leaf characteristics (Fitt, 1987, 1988 and 1990). Pearson (1958) reported that one third eggs are laid on the floral buds and rest are scattered all over the plant. However, it has been reported that egg location on the plant had little effect on subsequent establishment of the larvae (Ferrer and Bradley, 1985). Females preferred hairy over glabrous leaf surfaces for oviposition (Cullen, 1969; Lukefahr *et al.* 1971).

It has five to seven larval instars depending on the food and climatic conditions but temperature plays a significant role. Moulting often takes place on the upper surface of leaves in sunlight, possibly to hasten drying of the new cuticle (Reed, 1965; Hardwick, 1965). The duration of larval period varies considerably depending on the temperature and diet. Reed (1965) recorded on an average larval period of 21.1 ± 3.2 days including a pre-pupal period of 2.72 days when larvae were reared on cotton flowers at temperatures ranging from 21 to 27°C. Little information is available on the larval distribution on the plant and available information shows that small larvae are generally found on squares and terminals and large larvae on the bolls (Quaintance and Bruce, 1905; Wilson *et al.*, 1980; Ramatho *et al.*, 1984; Farrar and Bradley, 1985). Larvae pupate in the soil and the depth depends on texture and moisture contents of the soil (Reed, 1995).

H. armigera undergoes facultative diapause which is induced by short photoperiod, 11.5 to 12.5 hours day length and low temperature between 19-23°C. (Komarova, 1959; Hardwick, 1965; Phillips and Newson, 1966; Roach and Adkinson, 1970; Cullen and Browning, 1978; Roome, 1979; Hackett and Gatehouse, 1982a and b and Fitt and Daly, 1990). Under natural condition there is no (Coaker, 1959; Nel 1961) or very little (Reed, 1965; Hackett and Gatehouse 1982a and b) diapause in *H. armigera* in tropical region. However, Wilson (1979) reported that in Namoi Valley, Australia more than 80% pupae formed during late April to May undergo diapause and do not resume activity until September. Pyke (1995) reported that 21-74.5% pupae undergo diapause depending on temperature. Summer aestivation was observed in this species where diapausing populations are exposed to high temperature (35°C) for longer periods and it may last for more than 80 days in Sudan. It is broken by a fall in temperature and rains following dry conditions (Hackett and Gatehouse, 1982a and b).

The pest has variable number of generations per year, depending on climatic conditions and availability of food. Meng *et al.* (1962) reported three generations of *H. armigera* in China per year at a latitude of 40°N, four between 30°N and 40°N five between 25°N and 30°N and up to six or even more in south. In Pakistan where extreme variations in temperature and rainfall occur from coastal areas to high mountains tracts, it may complete two to six generations depending on latitude, temperature, rainfall and availability of food (Hardwick, 1965).

The role of biotic and abiotic mortality factors in different developmental stages has been summarized in life-table form in India (Nanthagopal and Uthamsamy, 1989), in South African by Van dan Berg and Cork 1993), and in China by Shijun and Yanquin (1994). They reported higher generation increase rate late in the season. Patel

and Mittal (1986) reported that the pest can multiply 19.1 times per generation with daily finite increase rate of 1.63 females/day. High mortality of eggs and early larval instars mainly due to abiotic factors, predators, parasites, cannibalism and food competition has been reported by Twine (1971 and 1974) and Twine *et al.* (1983), Evans (1985), Shijum and Yanquin (1989), Kyi *et al.* (1991), Dillon (1993) however, pearson (1958) regarded these factors unimportant in regulating *H. armigera* populations.

Importance of cultural control measures was reported in Australia by Pyke (1995). Information on the major parasitoids of *H. armigera* in Africa has been summarized by Henrard (1937), Pearson (1958), Risbec (1960), Greathead (1966) and Greathead and Gerling (1988). Natural enemy complex in the USA has been reviewed by Johnson *et al.* (1986), King and Coloman (1989) and King and Jackson (1989). In Pakistan 16 species of parasitoids attacking *H. armigera* have been reported by Mohyuddin (1989). Van dan Berg and Cork (1993) reported 300 species of parasitoids and predators in East Africa.

Pyrethroid resistance in *H. armigera* has been reported in Australia (Gunning *et al.* 1984, Forrester *et al.* 1993), Thailand (Ahmad and McCaffery, 1988), China (Xia, 1993) and India (McCaffery *et al.*, 1989; Armes *et al.*, 1992). In Pakistan Ahmad *et al.* (1997) tested seven pyrethoids and found different resistance levels in different populations. Highest level of resistance was found to cypermethrin and cyfluthrin followed by alpha-cypermethrin and deltamethrin. Resistance to zeta-cypermethrin, bifenthrin and lambda-cyhalothrin was generally low in most of the strains. They further reported that isomeric content may have a marked effect on the development of resistance to pyrethroids. Factors involved in the mechanism of resistance were

reported as decreased nerve sensivity (Ahmad *et. al.*, 1989; Gunning *et al.*, 1991), enhanced detoxification by monooxygenases (Ahmad and McCaffery, 1991; Gunning *et al.*, 1991; Forrester *et al.* 1993) and esterase's. (Gunning *et al.*,1996) and reduced cuticular penetration (Ahmad, 1988; Gunning *et al.*, 1991).

METHODS AND MATERIALS

Alternate host plants and population dynamics

Population dynamics of *Helicoverpa armigera* (Hubner) on alternate host plants were investigated from 1994 to 1996 in the cotton belt of Multan, Khanewal, Sahiwal, Bhakar and Layyah. Sampling was done twice a month from Multan and Khanewal and once a month depending on the availability of the host from other localities. Flowers/fruits and leaves of 100 plants were examined from crops, vegetables, fruit and forests, trees ornamentals and weed plants for its eggs and larvae. For *Triticum aestivum* (wheat), *Cicer arietinum* (gram) and *Trifolium alexandrinum* (berseem) etc., 100 tillers were examined as sampling units. The data are presented as per 100 fruiting bodies leaves or tillers depending on the size and structure and species of plant.

Eggs and larval distribution

Distribution of *H. armigera* eggs and larvae within plant was studies on cotton commercial cultivar CIM-240, sown in the first week of June at Mouza Khanpur Qazian about 60 km South-West of Multan and experimental area of Central Cotton Research Institute, Multan during 1994 and 1995. In 1994 randomly selected 180 plants at Location-I and 120 at Location-II and 150 during 1995 in Location-I were examined for eggs and larval distribution during peak period of pest's activity in September-October. On each observation date, twenty plants, five each from four sites from an area of 0.4 ha, were examined. Position of eggs and larvae of different instars were recorded on plant map starting from top to base, keeping terminal as zero node. Egg and larval population on individual structure is presented as percentage.

Host suitability

Host preference of *H. armigera* was studied by offering vegetative and reproductive parts of different hosts to newly hatched larvae in batches of 15 larvae per host and replicted three times. Each larva was kept in plastic tubes (cap. 20ml) covered with screwed cap with small holes in it for aeration. These tubes were then kept at 27 ± 2°C in an incubator. Food of each host was changed daily. Larval mortality during the developmental stages was also noted up to pupation. Pupal period was also recorded.

Parasitism

In order to study parasitism, eggs, larvae and pupae were collected from different hosts throughout the year. Eggs were kept in glass tubes (cap. 50ml). Moist tissue paper was provided in glass tubes to avoid desiccation. Observations were recorded daily till eggs hatched or parasitoids emerged. For larval parasitoids field collected larvae of different instars were reared on their respective host plants, individually in glass tubes (cap. 20 ml) or petri dishes (dia. 9 cm) till either pupated or died or parasitoids emerged. Food was changed daily or as needed. Dead larvae were dissected to see the cause of death. Field collected pupae or those formed from full-grown field collected larvae were kept in plastic cups (cap. 300 ml) containing moist soil, and less than 20 pupae in each cup. The cups were covered with muslin cloth and tightened with rubber rings to check the escape of emerging moths or parasitoids. Observations were recorded daily till the emergence of moths or parasitoids were completed.

Pupation

For pupation and diapause behaviour, full-grown larvae were collected from cotton fields from October to December at 15 days interval. Larvae collected on each

date were kept in plastic trays. Each tray measuring 38 x 28 x 14 cm consisted of separate chambers, 'a' for larval rearing, 'b' for pupation. A lot of 20 larvae was kept in upper chamber 'A' alongwith green food consisting of squares, flowers and small bolls. Chamber 'B' was filled with 8-10 cm layer of clay loam soil, covered with a thin layer of plant trash for the pupation of larvae. Chamber 'A' was covered with a wooden lid made up of wire guaze. Sides were plugged with cotton to prevent the escape of larvae. Larvae ready for pupation crawled down into the chamber 'B' through the holes in chamber 'A'; and pupated on or in the soil. Pupae formed from each date were kept separately in 250 ml plastic cups filled with loose moist soil, covered with muslin cloth. Trays containing larvae or pupae were kept in the cotton field and emergence of moths was recorded every day and is given as percentage.

Life tables

Last generation larvae pupated under field condition were collected at 15 days intervals during November 1995 to January 1996. Pupae collected on different dates were kept separately in plastic cups (cap. 250 ml) containing loose and moist soil and covered with muslin cloth. The cups were placed in the field as mentioned above and moths emergence was recorded daily.

The key mortality factors on all developmental stages were carried out at the experimental station of Central Cotton Research Institute, Multan during 1994 and 1995 crop seasons on unsprayed cotton variety, CIM-240, planted on third and fifth June on 0.2 ha during both the seasons. Plant to plant distance was 0.3 m and row to row 0.8 m with two rows of cotton and five plants in each row. Each plot was separated from other plots by an empty area of 1.5 m to prevent larval migration. Eggs used in these studies were obtained by releasing moths on cotton plants covered with muslin

cloth sleeve cages measuring 0.9 x 1.8 x 1.8 m, open at lower end. Laboratory reared four-day-old five females and five males were released in each cage at 1900 hours. It was replicated three times. The whole procedure was repeated five times in early (June-July), mid (August-September) and late season (October-November). Next morning cages and moths were removed. Eggs laid on each plant were counted and marked with black ink. The eggs were examined for hatching, dislodging and predation daily. Eggs that did not hatch and shrivelled were brought to the laboratory, examined under binocular microscope for parasitism and other possible mortality reasons. Natural infestation was eliminated by crushing the newly laid eggs every morning. After hatching larvae entered into the floral buds. Early instar larvae could only be counted by examining the floral buds. To minimize removal of fruiting parts, first three observations were made on every third day. Alive larvae recovered from fruiting parts were again released in the same plot. Dead larvae were examined under microscope to determine possible mortality reasons. Third instar to full-grown larvae mostly fed on flowers and bolls and could be seen easily. These were counted daily till the larvae were ready for pupation.

Diapause

Larvae ready to pupate were collected from all plots and released in one plot covered with field cage. Moth emergence was recorded daily. The pupae from which moths did not emerge up to one month were searched in the soil and examined for mortality factors. Life tables were constructed as described by Harcourt (1960).

RESULTS

Adults activity

Moth activity of *Helicoverpa armigera* (Hubner) was monitored with mercury bulb light trap throughout the year from 1994-96 at Central Cotton Research Institute, Multan. Data were recorded daily and weekly averages are presented in **Fig.1-3**.

Adults were active throughout the year. Moth catches were generally low during January and February and then June to August. From March onwards population gradually increased with a peak in the third week of April in 1994, **(Fig. 1),** fourth week in 1995 **(Fig.2)** and second week in **(Fig.3)**1996. Catches were more from September to December with a peak in the middle of October during cotton season in all the year **(Fig.1-3)**.

Oviposition behaviour of *Helicoverpa armigera* on cotton

H. armigera eggs are distributed all over the plant. To reduce the use of insecticides for its control by treating that part of the plant where maximum eggs are laid, distribution of eggs was studied at different rates of infestation during 1994 and 1995 on cotton cultivar CIM-240.

The female laid eggs on all parts of the plant including terminal buds, leaves, fruiting parts and occasionally on the internodes of main stem and side branches at different pest densities. The eggs were mostly laid on the upper surface of leaves and a few eggs were found on underside. Generally females laid eggs singly but sometimes 2-4 eggs were found at one place. It is not known whether these were laid by a single or more females.

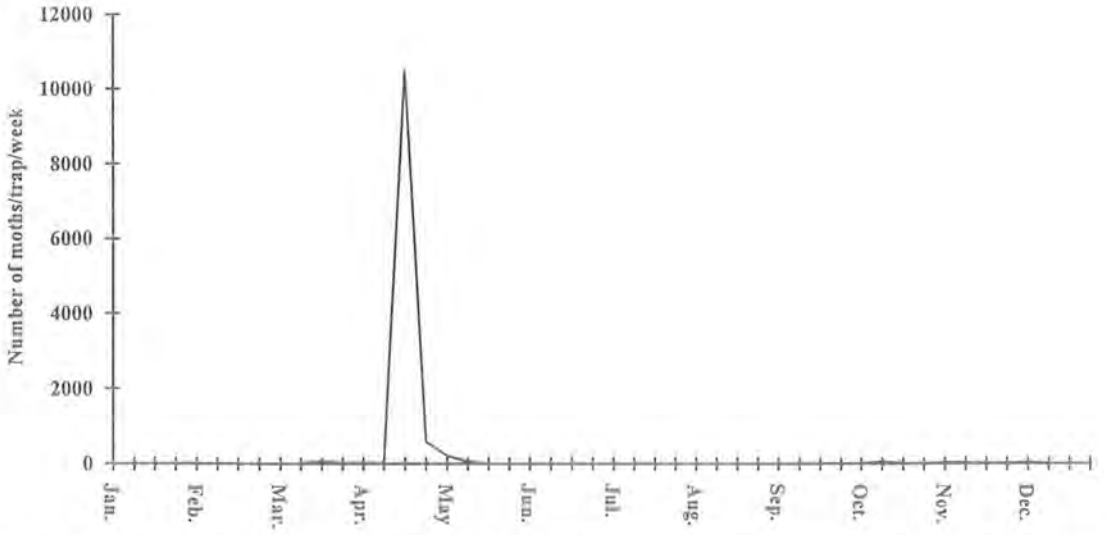


Fig.1 Number of moth catches of *Helicoverpa armigera* /week/light trap at Multan during 1994

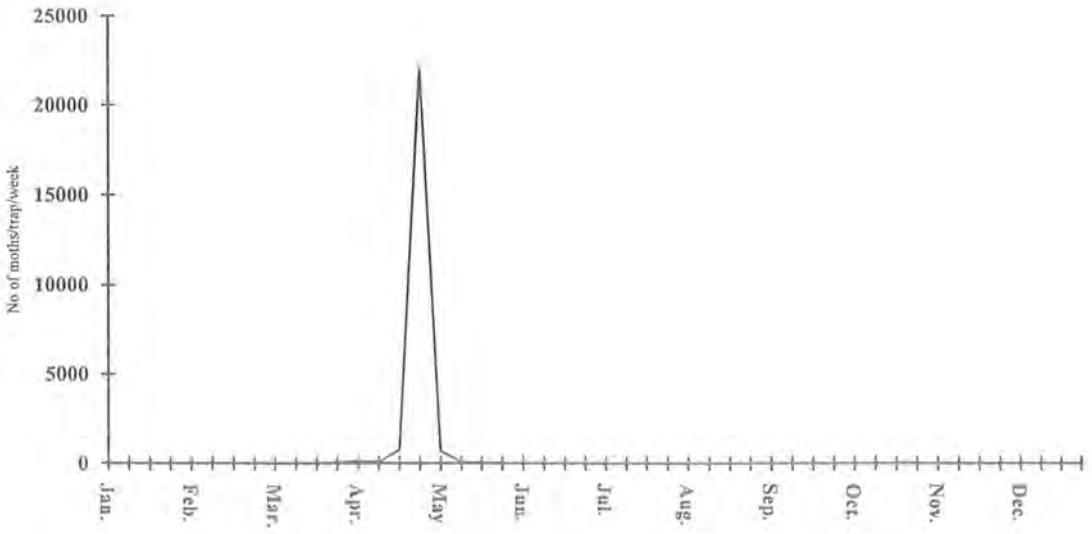


Fig.2 Number of moth catches of *Helicoverpa armigera* /week/light trap at Multan during 1995

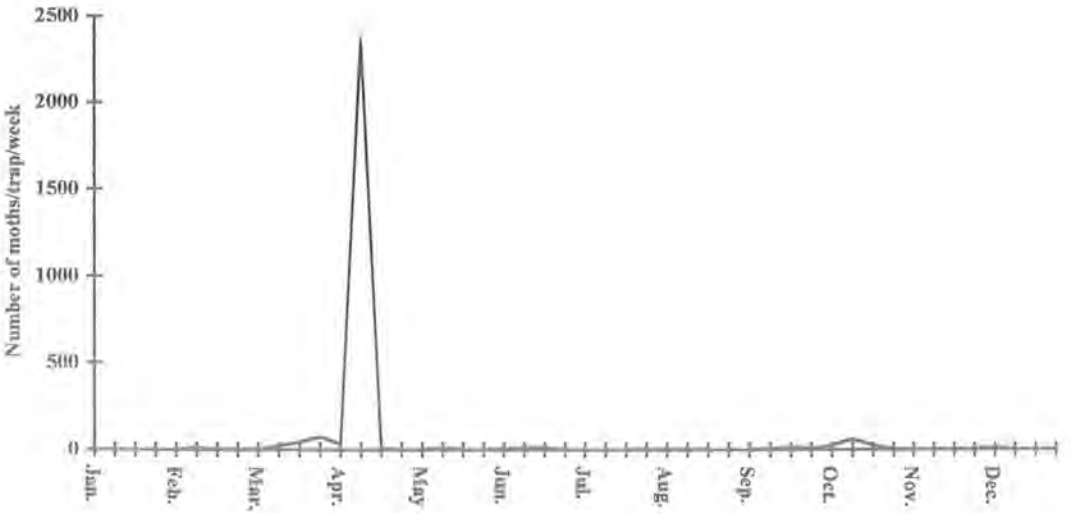


Fig.3 Number of moth catches of *Helicoverpa armigera* /week/light trap at Multan during 1996

Distribution of *H. armigera* eggs on cotton plant

The studies on distribution of *H. armigera* eggs on cotton plant were made. Eggs on different parts of the plant at three levels of infestation were recorded from the top, middle and bottom portions of the plant. About 70% eggs were laid on top (1 to 10 nodes on main stem) followed by middle (11-20) and bottom (21 to ground level) portion of plant at all levels of infestation **(Table-1)**.

Oviposition preference for main stem, side branches and fruiting parts was also determined. At low infestation level maximum eggs were found on the main stem but at medium and high levels of infestation maximum eggs were laid on side branches. Females laid minimum eggs on fruiting parts at all the levels of pest infestation **(Table 2)**.

On main stem, eggs were recorded on terminal bud, unexpanded and expanded leaves and internodes. The highest number of eggs (26.5%) were found on the first expanded leaf at low pest pressure while on the second expanded leaf both at medium (24.7%) and high (14.0%) levels of infestation. At all levels of infestation eggs were found consistently upto seven expanded leaves, thereafter egg deposition was irregular. Minimum eggs were laid towards the bottom part of the plant at all the pest pressures **(Table 3).**

From the side branch leaves, maximum eggs were laid on first expanded leaf both at low (46.6.%) and medium (41.3%) pest infestation levels. However, at high level of infestation 34.4% eggs were found on unexpanded leaf. Egg deposition decreased gradually towards main stem at all pest pressures indicating that females prefer young leaves as compared with old **(Table 4).** Among the fruiting structures at

different positions of side branches, maximum eggs were laid on the first fruiting point immediately after terminal and it decreased towards the main stem (**Table 5**).

Egg laying preference of *H. armigera* when all fruiting phases; squares, flowers and bolls were present was determined. Egg deposition on various fruiting structures varied greatly and maximum eggs were laid on squares (78-88%) followed by flowers(8-17%) and bolls(3-4%) at all levels of pest infestation (**Table 6**).

Table 1 Distribution of *Helicoverpa armigera* eggs on different portions of cotton plant at different levels of pest infestation

Plant Portion	% Eggs found at different pest pressure		
	Low	Medium	High
Top	74.5	70.4	81.3
Middle	15.3	19.4	11.5
Bottom	10.2	10.2	7.2

Table 2 Distribution of *Helicoverpa armigera* eggs on different parts of cotton plant at different levels of pest infestation

Plant Parts	% Eggs found at different pest pressure		
	Low	Medium	High
Main Stem	71.2	39.1	39.5
Branches	25.6	51.5	54.4
Fruiting Parts	3.2	9.4	6.1

Table 3 Distribution of *Helicoverpa armigera* eggs on main stem of cotton plant under different levels of pest infestation

Main Stem	% Eggs*at different levels of infestation		
	Low	Medium	High
Terminal Bud	10.3	4.2	9.2
Unexpand. Leaf	15.6	7.9	12.7
Expanded.Leaf 1	26.5	19.3	14.0
Expanded.Leaf 2	24.1	24.7	14.3
Expanded.Leaf 3	14.7	18.0	14.7
Expanded.Leaf 4	5.8	12.2	11.3
Expanded.Leaf 5	1.8	8.3	7.4
Expanded.Leaf 6	0.5	4.2	7.5
Expanded.Leaf 7	0.2	0.4	2.9
Expanded.Leaf 8-16	0.5	0.8	2.6
Internodes	0.0	0.0	3.4

*= Precentages based on total eggs deposited on main stem.

Table 4 Distribution of *Helicoverpa armigera* eggs on leaves of side branches of cotton plant at different levels of pest infestation

Side Branch	Eggs on side branch leaves (%)		
	Low	Medium	High
Terminal Bud	11.1	4.6	13.8
Unexpanded Leaf	34.3	23.3	34.4
Expanded.Leaf 1	46.6	41.3	32.7
Expanded.Leaf 2	6.1	25.4	17.2
Expanded.Leaf 3	1.2	5.4	1.8
Expanded Leaf 4-7	0.7	0.0	0.1

Table 5 **Distribution of *Helicoverpa armigera* eggs on fruiting bodies at different levels of pest infestation**

Fruiting Point	% Eggs based on total eggs on Fruiting Points		
	Low	Medium	High
Fruiting Point 1	55.0	58.3	41.7
Fruiting Point 2	44.0	30.4	39.1
Fruiting Point 3	1.0	11.3	18.2
Fruiting Point 4-6	0.00	0.00	1.0

Table 6 **Distribution of *Helicoverpa armigera* eggs on squares, fowers and bolls different fruiting parts of cotton plant at different levels of pest infestation**

Fruiting Parts	% Eggs found		
	Low	Medium	High
Squares	86.7	88.3	78.8
Flowers	8.9	8.2	17.7
Bolls	4.4	3.5	3.5

Biology

The biology of *H. armigera* was studied on cotton at 28±2°C . Moths started mating 3.5±1.5 days after emergence, during 10.00-02.00 hours and continued for 2±1.5 hours. Oviposition started 2±1 days after mating with peak on 4th or 5th days. Eggs laid on the first and second days were 10-15% unfertilized. Generally eggs were laid singly but occasionally 3-5 eggs were also found on the terminal parts of the plant. A single female laid on average 350±200 eggs in oviposition period of 6.5±1.5 days. Newly hatched larvae fed on egg shells and nearby soft plant tissues and took more time to moult as compared with later instars. First instar completed development in 3±1 days. Each of the later instar lasted about two to three days. Time taken to complete six instars was 14±2 days after hatching. Prepupal and pupal periods lasted for 11±2 days. Males fed on 10% solution of sucrose lived for 8±3 days and females 13±4 days

Under field conditions *H. armigera* eggs hatched within two days during June-July when generally temperatures were more than 40°C and took 4-6 days during September-October when temperature were 35±2°C. Larvae completed their development within 14 days in June-July and in 24 days during September-October depending upon temperature. Prepupal and pupal period was 8±2 days during this period.

Number of generations

H.armigera remained active throughout the year where summer temperatures were 45±3°C and winter was mild. After cotton, from December to April the non-diapausing population completed two overlapping generations on *Cicer arietinum* (chickpea), *Cucurbita pepo melopepo* (squash), *Solanum turberosum* (potato),

Lycopersicon esculentum (tomato), *Trifolium alexandrinum* (bersim), *Pisum sativum* (peas), *Triticum aestivum* (wheat), *Abelmoschus esculentus* (okra), *Nicotiana tabacum* (tobacco), *Lagenaria ciceraria* (gourd), *Solanum melongena* (brinjal) and *Helianthus annuus* (sunflower) etc. From May to July third and fourth generations were completed on *Zea mays* (maize), *Sorghum vulgare* (sorghum), *A. esculentus*, *N. tabacum*, *L. esculentum*, *H. annuus* and *S. melongena* etc. From August to November it completed 3-4 generations on *A. esculentus*, *N. tabacum*, *S. melongena*, *S. tuberosum* and *L. esculentum* but *Gossypium hirsutum* (cotton) was the major host. Thus it seems to complete 7-8 generations in a year..

Host suitability

African bollworm attacks a number of plant species in the field throughout the year. To compare development and suitability of *H. armigera,* some winter and summer hosts were tested separately at 27±3°C. These include cultivated crops, weeds, ornamental plants and fruit trees. Cotton was kept as control with both the sets. Fifteen newly hatched larvae were offered to respective hosts and replicated three times. Food was changed daily.

Data on development period and survival are presented in **Table** 7. Development was fast on *Triticum aestivum* followed by *Pisum sativum, Malva parviflora, Trifolium alexandrinum, Hibiscus rosa-sinensis , Bauhinia purpurea, Cicer arietinum, Rosa indica, Abutilon sp., Mangifera indica, Helianthus annuus* and *Glycine max* among the winter hosts. The most suitable host for development was *M. parviflora* and it was as good as *G. hirsutum* followed by *T. aestivum, H. rosa-sinensis, C. arietinum, M. indica, R. indica, B. purpurea, H. annuus, Abutilon sp., P. sativum,*

T. alexandrinum and *G. max*. Larvae on summer hosts took more time to complete development, maximum on *Glycine max* followed by *H. annuus*, *M. indica*, and *Abutilon* sp. but none of them was as good as *G. hirsutum*. Under forced feeding it could develop even on *M. indica* which is not a recorded host (**Table 7**).

Table 7 Development period and survival percentage of *Helicoverpa armigera* on different hosts at 27± 3°C in the Laboratory

Plant species	Development period (days)		Larvae completed development (%)
	Larvae	Pupae	
Cicer arietinum	16.1	11.7	46.1
Pisum sativum	13.8	11.0	17.0
Trifolium alexandrinum	14.0	12.0	13.3
Trifolium aestivum	14.6	9.9	53.3
Helianthus annuus	17.3	15.5	20.0
Glycine max	22.0	12.0	6.7
Malva parviflora	15.0	10.8	66.7
Hibiscus rosa-sinensis	15.43	11.14	46.7
Rosa indica	18.25	11.0	26.7
Abutilon	20.3	10.7	20.0
Mangifera indica	19.4	12.0	30.0
Bauhinia purpurea	15.0	12.3	20.0
Gossypium hirsutum	15.0	11.0	66.8

Alternate hosts and population dynamics

During the present survey *H. armigera* was recorded feeding on leaves and fruiting parts of 61 plant species including crops, ornamental and weed plants belonging to 56 genera of 20 families. These hosts have been classified as major and minor. On the basis of duration of feeding and level of infestation major hosts are given in **Table 8** and minor in **Table.9**. On the basis of pest presence major and minor host are given **Fig.4** and **5.**

Host Plant	J	F	M	A	M	J	J	A	S	O	N	D
Cicer arietinum												
Cucurbita pepo melopepo												
Pisum sativum												
Solanum tuberosum												
Trifolium alexandrinum												
Rosa indica												
Convolvulus arvensis												
Cucumis sativus												
Solanum melongena												
Lycopersicon esculentum												
Helianthus annuus												
Glycine max												
Lagenaria ciceraria												
Carthamus oxyacantha												
Nicotiana tabacum												
Zea mays												
Abelmoschus esculentus												
Sorghum vulgare												
Gossypium hirsutum												

Host & Pest Present

Host Present, Pest Not Present

Host & Pest Not Present

Fig. 4 Incidence of *Helicoverpa armigera* on Major Host Plants

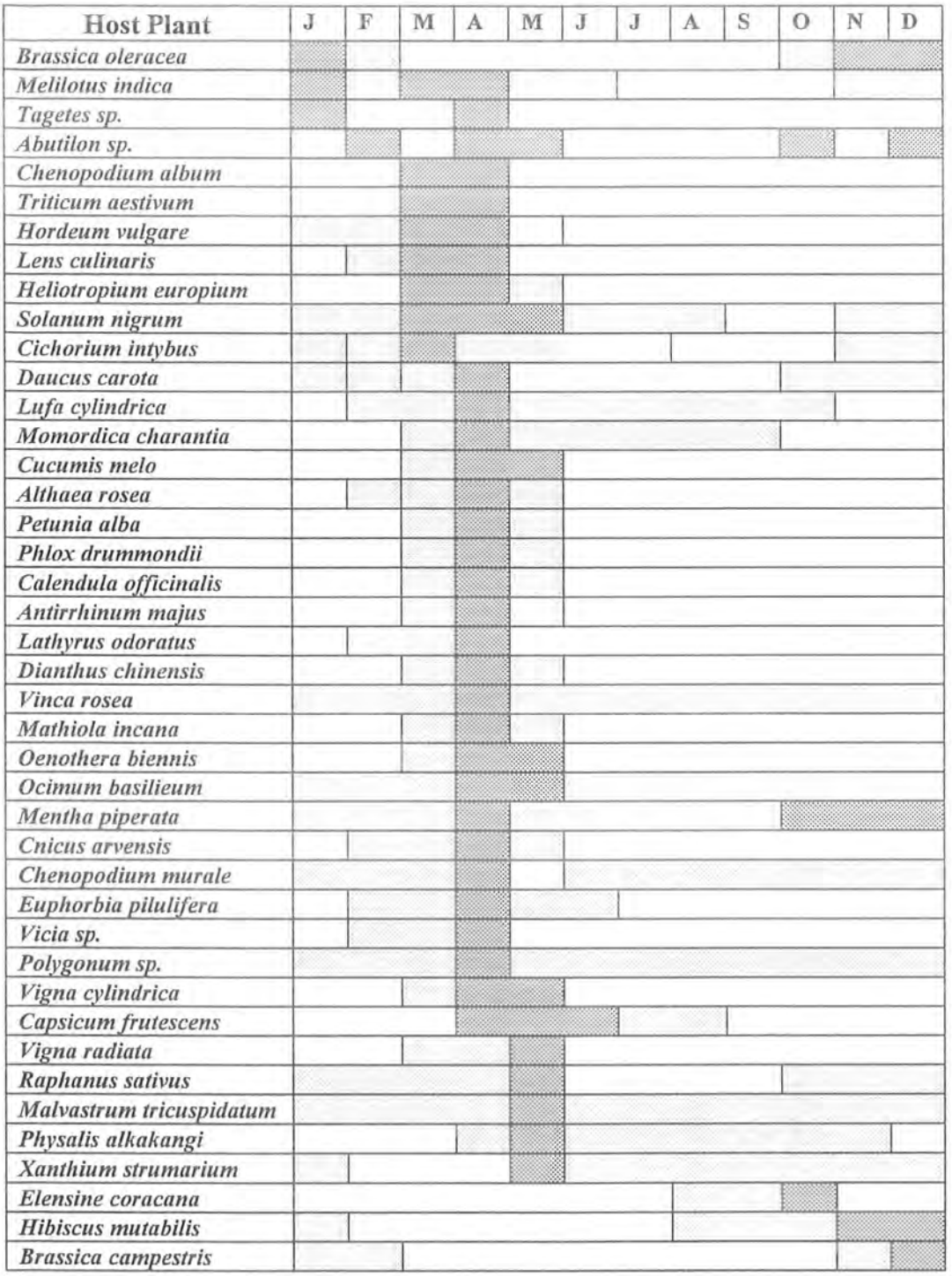


Fig. 5 Incidence of *H. armigera* on Minor Host Plants

During January and February population of *H. armigera* fluctuated at low levels on all the 19 major hosts. It was highest per 100 fruiting parts on *C. arietinum* (1.1) and *Cu curbita pepo melopepo* in January (1.1), on *C. pepo melopepo* in February (2.2), in March (17.0) and in April (35.2). It was maximum in May on *Abelmoschus esculentus* (21.1), on *Nicotiana tabacum* in June (7.0), on *A. esculentus* in July (3.1) and in August (1.9), on *G. hirsutum* in September (4.0), on *A. esculentus* in October (13.5), on *R. indica* in November (5.8) and in December (4.2)

On minor hosts highest number of larvae per 100 fruiting parts were on *Tagetes sp.* (0.5) and *Melilotus indica* (0.5) in January. It was found only on *Abutilon* sp. in February (0.2), highest on *Hordeum vulgare* in March (2.4), on *Tagets sp.* in April (7.0), on *Ocimum basilieum* in May (2.7), on *Capsicum frutescens* in June (0.2).No larva was found on these hosts from July to September. Highest population was on *Elensine coracana* in October (1.6), on *Hibiscus mutabilis* in November (0.6) and *Brassica compestris* in December (2.0) **(Table 9).**

Table 8 Mean number of larvae of *Helicoverpa armigera* per 100 fruiting bodies/leaves on major host plants in cotton growing area from January (J) to December (D).

Host	J	F	M	A	M	J	J	A	S	O	N	D
Cicer arietinum	1.1	0.6	5.3	7.1	-	-	-	-	-	-	0.0	0.0
Cuecurbita pepo melopepo	1.1	2.2	17.0	35.2	-	-	-	-	-	-	-	0.0
Pisum sativum	0.5	0.8	1.8	1.9	-	-	-	-	-	-	-	0.0
Convolvulus arvensis	0.8	0.2	4.0	4.4	03	0.6	0.0	0.0	0.0	-	0.0	0.0
Solanum tuberosum	0.1	0.0	3.0	4.8	-	-	-	-	-	0.0	0.5	0.5
Trifolium alexandrinum	0.2	0.1	0.6	1.3	0.9	-	-	-	-	-	0.2	0.8
Rosa indica	0.7	0.6	3.1	14.1	8.0	1.5	0.5	0.0	0.0	5.9	5.8	1.8
Solanum melongena	0.1	0.0	0.3	3.2	2.4	0.0	0.0	0.0	0.0	1.2	0.2	0.1
Cucumis sativus	-	-	2.7	10.9	-	-	-	-	-	-	-	-
Lycopersicum esculentum	0.0	0.0	1.9	5.6	1.9	0.0	-	-	0.0	0.0	0.5	0.0
Helianthus annuus	-	-	0.5	4.5	3.6	0.0	0.0	0.0	-	-	-	-
Glycine max	-	-	1.0	3.3	0.3	-	-	-	-	-	-	-
Lagenaria ciceraria	-	-	2.0	15.0	9.7	0.6	0.7	0.0	0.0	-	-	-
Carthamus oxyacantha	-	-	-	19.6	6.0	-	-	-	-	-	-	-
Nicotiana tobacum	-	-	0.0	1.0	16.2	7.0	0.0	0.0	-	-	-	-
Zea mays	-	-	0.0	0.3	4.0	6.1	0.0	-	-	0.0	0.0	0.0
Abelmoschus esculentus	-	-	-	6.8	21.1	5.3	3.1	1.9	0.2	13.5	2.0	0.3
Sorghum vulgare	-	-	0.0	0.0	7.2	5.8	1.7	0.0	0.0	0.0	0.0	
Gossypium hirsutum	-	-	-	-	10.1	4.0	0.1	0.4	4.0	4.1	4.2	0.3

- = Pest not available

Table 9 Mean number of larvae of *H. armigera* per 100 fruiting parts on minor hosts in cotton growing areas from January(J) to December(D).

Host plants	J	F	M	A	M	J	J	A	S	O	N	D
Brassica oleracea	0.3	0	0	0	-	-	-	-	-	-	0.3	0.5
Melilotus indica	0.5	0.0	0.3	0.3	-	-	-	-	-	-	-	-
Tagetes sp.	0.5	0.0	0.0	7.0	-	-	-	-	-	-	-	-
Abutilon sp.	0.0	0.2	0.0	0.9	0.08	0	0	0	0	0.7	0.0	0.4
Chenopodium album		0.0	0.5	2.4	-	-	-	-	-	-	-	-
Triticum aestivum		0.0	0.3	1.3	-	-	-	-	-	-	-	-
Hordeum vulgare		0.0	2.4	3.0	-	-	-	-	-	-	-	-
Lens culinaris		0.0	0.5	0.3	-	-	-	-	-	-	-	-
Heliotropium europium			1.0	1.2	-	-	-	-	-	-	-	-
Solanum nigrum		0.0	1.5	0.6	0.3	0	0	0	0	0	0	0
Cichorum intybus		00	1.0	-	-	-	-	-	-	-	-	-
Daucus carota	-	-	0.0	1.5	0	0	0	0	0	0	0	0
Lufa cylindrica	-	-	0.0	1.65	-	-	-	-	-	-	-	-
Momordica charantia	-	-	0.0	2.5	-	-	-	-	-	-	-	-
Cucumis melo	-	-	0.0	2.3	0.1	-	-	-	-	-	-	-
Althaea rosea	-	-	0.0	0.3	0	0	-	-	-	-	-	-
Petunia alba	-	-	0.0	0.5	-	-	-	-	-	-	-	-
Phlox drummondii	-	-	0.0	2.3	-	-	-	-	-	-	-	-
Calendula officinalis	-	-	0.0	0.7	-	-	-	-	-	-	-	-
Antirrhinum majus	-	-	0.0	3.6	-	-	-	-	-	-	-	-
Lathyrus odovatus	-	-	0.0	0.4	-	-	-	-	-	-	-	-
Un idetified plant var.pink	-	-	0.0	2.5	-	-	-	-	-	-	-	-
Vinca rosea	-	-	0.0	2.0	-	-	-	-	-	-	-	-
Stock	-	-	0.0	1.0	-	-	-	-	-	-	-	-
Oenothera biennis	-	-	0.0	2.0	0.6	-	-	-	-	-	-	-
Ocimum basilieum	-	-	0.0	4.4	2.7	-	-	-	-	-	-	-
Mentha piperata	-	--	0.0	1.3	-	-	-	-	-	0.8	0.2	0.8
Cnicus arvensis	-	-	0.0	2.7	-	-	-	-	-	-	-	-
Chenopodium murale	-	-	0.0	1.0	-	-	-	-	-	-	-	-
Euphorbia pilulifera	-	-	0.0	4.0	-	-	-	-	-	-	-	-
Vicia sp.	-	-	0.0	1.0	-	-	-	-	-	-	-	-
Polygonum sp	-	-	0.0	1.0	-	-	-	-	-	-	-	-
Vigna cylindrica	-	-	0.0	3.7	0.3	-	-	-	-	-	-	-
Capsicum frutescens	-	-	0.0	1.1	0.5	0.2	-	-	-	-	-	-
Vigna radiata	-	-		0.0	1.5	-	-	-	-	-	-	-
Raphanus sativus	-	-		0.0	1.0	-	-	-	-	-	-	-
Malvastrum tricuspidatum	-	-		0.0	1.0	-	-	-	-	-	-	-
Physalis alkakangi	-	-		0.0	0.4	-	-	-	-	-	-	1.0
Xanthium stramarium	-	-			1.0	-	-	-	-	-	-	-
Elensine coracana	-	-	-	-	-	-	-	-	-	1.6	-	-
Hibiscus mutabilis	-	-	-	-	-	-	-	-	-	-	0.6	0.3
Brassica campestris	-	-	-	-	-	-	-	-	-	-	-	2.0

- = Host not available
0 = Pest not available

Infestation of *H. armigera* on various crops before cotton is cultivated

Infestation of *H. armigera* on different crops was recorded at three locations. At Layyah and Bhakkar where *C. arietinum* was available, at Sahiwal vegetables, maize and *H. annuus* and at Multan, vegetables were major hosts before cotton is cultivated. Shifting of *H. armigera* from one to another host round the year varied in different areas depending on the pattern of cultivated crops and natural vegetation **(Fig. 6)**.

In arid/semi arid areas of Layyah and Bhakkar, *H. armigera* was found in small number on *C. arietinum* during February and highest infestation was found during March and April. After the harvest of *C. arietinum* in April, the pest was found on *Carthamus oxyacantha* and then on *G. hirsutum* and other hosts.**(Fig.6)**

In the Multan areas, during winter it feeds on *S. turberosum, T.alexandrinum, R. indica, P. sativum, C. arvensis* and *S. melongena*. From February onwards it was generally found on *C. sativus, H. annuus, G. max, L. esculentum* and *L. siceraria*. Most of these hosts terminated in April-May. Thereafter, pest shifts to *A. esculentus, S. vulgare* and then on *G. hirsutum*.**(Fig.6)**

In the Sahiwal areas, *H. armigera* feds on *Lycopersicon esculantum* and *S. tuberosum* during winter and early spring. From March to June maximum population was found on *Solanum tabacum, H. annuus and Zea mays*. From these crops it shifts to cotton.

Distribution of *H. armigera* larvae on cotton plant

To determine distribution of *H. armigera* larvae top, middle and bottom portions of cotton cultivar CIM-240 were examined at different levels low, medium and high rate of infestations during September and October.

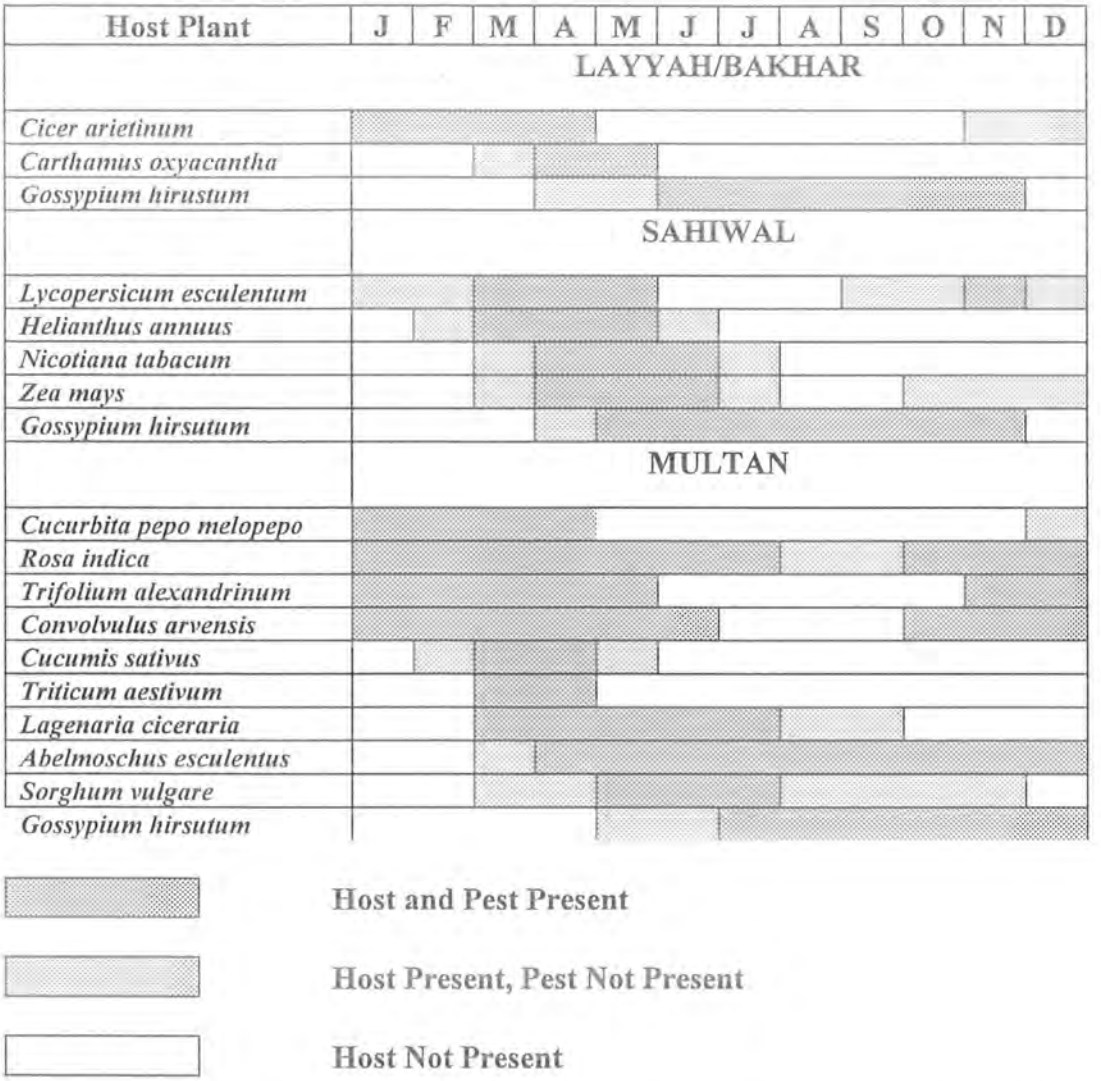


Fig.6 Infestation of *Helicoverpa armigera* on different host plants at different locations

Maximum number of small larvae were found on top portion of plant followed by middle. These were not found on the bottom portion of plant during September and October **(Table 10).** Maximum number of half-grown larvae was found on the middle portion of plant during both these months followed by top portion during September and lower part in October **(Table 10).**

Larval feeding preference for vegetative and reproductive parts was also studied. During September and October maximum number of young larvae was found infesting squares followed by terminal bud, flowers and leaves in decending order. Maximum number of half-grown larvae was found in flowers followed by squares and bolls during both these months. However, very small number of larvae was also recorded on leaves and terminal buds in September. No full-grown larvae was found on vegetative structures. Highest number of larvae were found in bolls followed by flowers and squares during this period. Thus 27.9% of small larvae were found on vegetative parts in September and 28.6% in October compared with 71.1 and 71.4 on reproductive parts during these month. Similarly 90-95% of half-grown larvae were found on reproductive structures during this period all full grown larvae wee found on reproductive parts and no larva was found on vegetative parts **(Table 11)**.

Table 10 **Distribution of *Helicoverpa armigera* larvae on different portions of cotton plant**

Month	Plant Portion	Larval stage %		
		Small	Medium	Large
September	Top	67.8	27.1	5.1
	Middle	16.7	72.2	11.1
	Bottom	0.0	20.0	80.0
October	Top	46.2	30.8	23.0
	Middle	25.0	66.7	8.3
	Bottom	0.0	50.0	50.0

Top = Up to 10th Main Stem nodes
Middle= 11th to 20th Main Stem Nodes
Bottom= 20th Main Stem Node to the ground level

Table 11 **Distribution of *Helicoverpa armigera* larvae on different parts of cotton plant**

Month	Plant Part	Larval stage %		
		Small	Medium	Large
September	Terminal buds	20.9	3.3	0.0
	Leaves	7.0	6.6	0.0
	Squares	53.5	36.7	22.2
	Flowers	18.6	46.7	33.4
	Bolls	0.0	6.7	44.4
October	Terminal buds	23.8	0.0	0.0
	Leaves	4.8	4.8	0.0
	Squares	42.9	33.3	9.1
	Flowers	28.5	38.1	18.2
	Bolls	0.0	23.8	72.7

Pupation

Full-grown last instar larvae when ready to pupate fell or crawled to the ground and mostly enter in to the crackes and crevices of the soil for pupation. Majority of the larvae pupated away from the plants on the slope of the bed. Pupation took place at different depths in the soil. Most of it occurred in 2.5 to 5.0 cm depth. A small proportion of the larvae pupated at 7.5 to 10.0 cm depth and on soil surface in irrigated loamy soil. Larvae make earthen cell before entering the prepupal stage. Larvae that pupated on the soil surface spun loose web during prepual stage. In non-irrigated sandy soil mostly pupation take place at 12.5 to 17.5 cm (**Table 12**). In both the situation upper layer of the soil gets dry quickly, therefore, soil temperature and moisture contents seems to be limiting factor for pupation depth .

Table 12 Percentage of *H. armigera* pupae found per m²at different depth

Period	Percent Larvae found					
	On surface	Upto 2.5	Upto 5.0	Upto 7.5	Upto 10.0	Upto 12.5
November	2.6	41.0	37.6	16.6	2.2	0.0
December	0.8	36.1	46.4	12.8	2.8	1.1
January	1.4	49.1	40.9	7.9	0.6	0.0

Diapause

H. armigera has obligatory diapause. Pattern of adult emergence in full-grown larvae collected from cotton field was studied during October, November, December and January. The larvae were kept in the plastic trays in field until pupation **(Fig.7a)** and for adult emergence **(Fig.7b)** as described in materials and methods. Adult emergence from these pupae is presented in **(Fig. 8)**. There was great variation in emergence of moths. Adults from non-diapausing population began to emerge 10 days after pupation in October, 15 days in November, 10-25 days in December and 25 days in January. When mean maximum and minimum day and night temperatures were 31°C and 17°C in October ($\overline{X}$ = 24°C), 27°C and 14°C in November ($\overline{X}$ = 20°C), 21°C and 8.5°C in December ($\overline{X}$ = 16°C) and 19°C and 6.6°C in January ($\overline{X}$ = 9°C). Mean sunshine hours ranged from 9.25 in October and 6.6 in December.

The length of the pupal period was up to 65 days when larvae pupated during October-November and it increased as the winter progressed being up to 70 days in larvae that pupated in December and 70-90 days that pupated in January. Maximum adults from all the pupae formed from field collected larvae in October to December emerged within 40 days, thereafter, the emergence rate became slow due to low temperatures and shorter day length. Adults from the remaining pupae emerged within 90 days. Emergence of moths within 40-50 days after pupation seem to be of non-diapausing population.

Diapause in pupae formed from November to January in cotton field and collected during this period was also studied. Moths began to emerge 10-days after the collection of pupae in November. There was no moth

Fig. 7a Plastic tray used for rearing and pupation of *Helicoverpa armigera* larvae

Fig. 7b Plastic tray and cups used for the emergence of adults of *Helicoverpa armigera*.

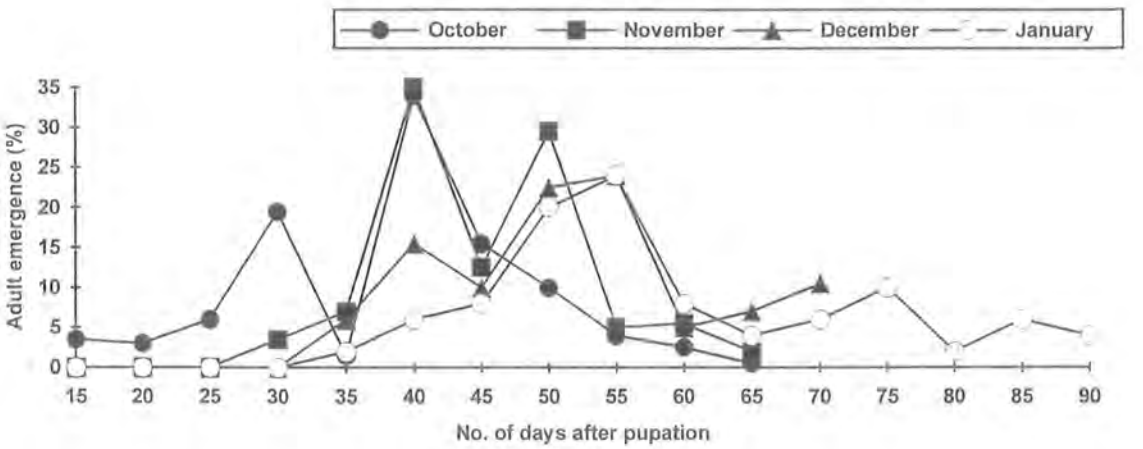


Fig.8 Emergence of *Helicoverpa armigera* adults from pupae that formed in trays from field collected larvae from October to January at Multan

emergence up to 25-days from the pupae that collected in December and 30-days collected in January (Fig.9). Emergence pattern from the pupae collected in the first and second half of November was almost similar to that mentioned above and about 60% of the adults completed their emergence within 50-days after the pupae were collected. A part of the population emerged in 50 to 90-days. Long diapausing individuals emerged 145 (2.5%) and 150 (1.3%) days after the collection (**Fig. 9**). Emergence pattern from the pupae collected in December and January was almost similar to those pupae collected in November. Pupal period was 90 days in December and 75 days in January. Pupae collected in December and January from cotton filed were probably from the last generation and were exposed to low night temperature 10-7°C ($\overline{X}$=9.0) and 6-8 sunshine hours. Thus it seems that there are three types of populations i.e., non-diapausing, short diapausing and long diapausing.

Life tables of *H. armigera* on cotton.

Primary mortality factors responsible for the reduction in *H. agmigera* eggs, larvae and pupae of first generationin during June-July, of second generation during August and September and of third generation during October-November were investigated under field situation in 1994 and 1995 crop seasons. Key mortality factors are given in **Table 13-15.**

Life table of the first generation

a) Egg mortality

First generation life table was prepared during early stage of crop growth when plants were 30-55 days old with no crop canopy and temperatures were around 45±3°C in June and 40±3°C in July, relative humidity varied from 12-18% in June and 45-87%

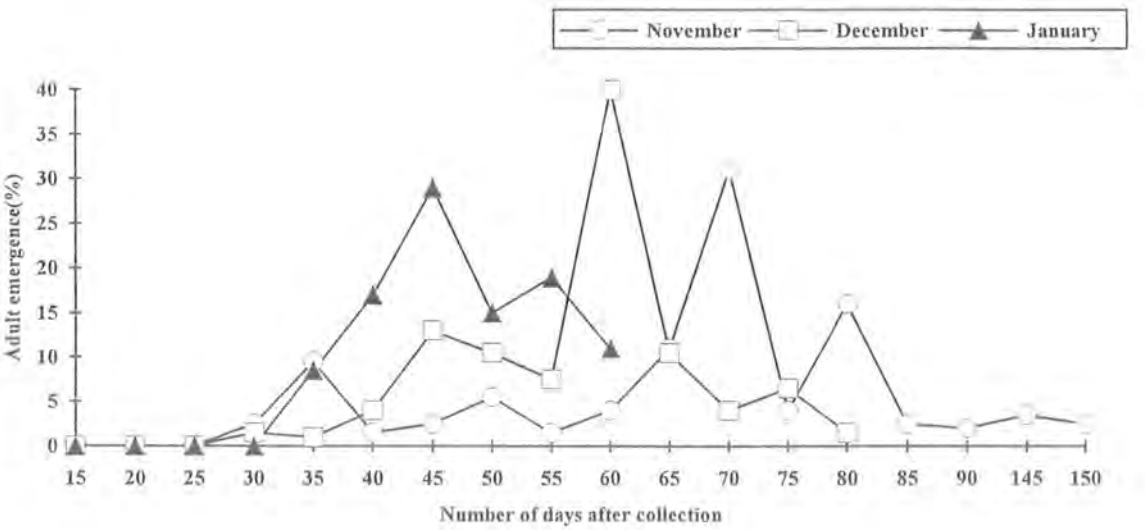


Fig.9 Emergence of *Helicoverpa armigera* adults from field collected pupae during different months and kept inplastic trays in the field

in July. The females laid eggs on the upper surface of the top leaves which were exposed to all type of biotic and abiotic mortality factors. The eggs suffered heavy mortality (42.1%) due to desiccation in the month of June while 31.5% dislodged and dropped due to the action of strong winds (wind speed 2.3-16.9, $\overline{X}$ = 6.9 km/hr.) and rains. Unfertile eggs were 1.9%. Sucking predators caused 12.5% eggs reduction during this period. Parasitism was extremely low. Only 1.9% eggs were parasitized by *Trichogramma chilonis* Ishii during this period. Overall 89.9% of the eggs laid on early crop were eliminated due to various factors and survival rate was 10.1% **(Table 13).**

b) Larval mortality

In the absence of crop canopy and fruiting structures larvae were subjected to various mortality factors from hatching to pupation. Key population reduction factors during first and second instars were dropping and predation. Highest reduction due to dropping and predation by chewing insects in first instar larvae was 41.0% and in second instar 26.3% followed by sucking predators 8.0% in the first instar and 13.9% in the second instar. Larval movement in search of feeding site is quick at this stage, initially larvae dislodged and may be predated on the ground later on. Rate of dropping decreased gradually as they become older. Missing larvae and their predation due to chewing predators including coccinellid beetles, *P. fuscipes* and spiders was still highest (24.1%) in the third instar followed by predation due to sucking predators like *Chrysoperla carnea, Orius* sp., *Geocoris* sp. etc., and parasitism **(Table 13).** In the fourth, fifth and sixth instars missing/predation due to spiders, crabid beetle and birds followed by parasisim play significant role in reducing the population of *H. armigera* larvae. Parasitism was 1.4% in the fourth, 4.0% in the fifth and 6.1% in the sixth instar larvae of the pest. However, it never exceeded on cotton because of excessive use of

pesticides in the wide spread adjoining areas. It is interesting to note that parasitism on insecticide free *C. arietinum* crop may go up to 60% or more and also high in the larvae feeding on *T. alexandrium, C. pepo var. melopepo* and *Capsicum frutescens* growing as pre-cotton crops in the cotton growing belt.

c) Pupal mortality

Full-grown larvae pupate on the soil under the plant debris or in the cracks and crevices. During pre-pupal and pupal stages, they are exposed to ground predators, climatic factors like dryness or excessive moisture. Depending upon the type of soil and climate mortality is different. Decaying was an important factor causing 15.4% reduction of pupae during early season of crop growth in irrigated conditions. Number of termites were found in the predated pupae but is not certain wether termites were primary or secondary predators. However, ants like *Pheidola* sp. and *Cataglyplus* sp. were major pupal predators. Predation, and interculturing caused major mortality of the pupae. No parasite was recorded from the pupae. Overall pupae suffered 30.7% mortalities during early season.

Life table of the second generation

a) Egg mortaliy

In August-September, temperatures become mild ($\bar{X}$=31.5±5°C) and favourable for crop and pest growth. Eggs suffered comparatively less mortality (70.8%) as compared with early season (89.9%). Major contributors of egg reduction were dropping (33.9%), predation (18.1%), desiccation (15.1%), infertility (1.2%) and parasitoids (2.5%) (**Table 14**).

b) Larval mortality

In the first and second instars dropping/missing and predation of larvae were important. From third instar onwards, diseases, chewing insect, spiders and bird predators were the major contributors. Among bird predators *Acridotheres tristis* (L). *Strunus valgaris* L, *Passar domesticus* L., *P. hispaniolensis, Transcapicus tshusi,* Wegtails, Indian Robin, Green bee eaters etc., visited cotton field frequently and were seen feeding on *Helicoverpa* larvae. Due to low temperature and higher humidity diseases caused by bacteria, virus and fungi were also important during this period. Parasitism was low from third to sixth instars and considered as minor contributor **(Table 14).**

c) Pupal mortality

Pupal mortality factors were almost similar to that of first generation. Due to dense plant canopy and flood irrigation, maximum pupal reduction was due to decaying followed by interculturing, insect predators and desiccation **(Table 14).** No pupal parasite was reared.

Life table of the third generation

a) Egg mortality

In October-November, at late stages of crop growth average day (23.5°C) and night (11.0°C) temperatures were lowest of the season. Highest reduction was due to egg dropping followed by insect predators and infertility. Desiccation and parasitism were minor contributors. Overall survival of eggs during late stages of crop growth was almost 40% **(Table 15).**

b) Larval mortality

Larval mortality factors in the third generation were almost similar to that of first and second generations. Maximum reduction of first and second instar larvae was due to dropping followed by insect predation. In addition to insects and bird predators, diseases also played significant role in the reduction of fourth, fifth and sixth instar larvae of the pest. Role of parasites remained very low during this period.

c) Pupal mortality

Highest pupal mortality was due to the rottening of pupae with moisture followed by adults that failed to come out from the soil and predation. No pupal parasite was recorded (**Table 15**).

Table 13 Life table of the first generation of *Helicoverpa armigera* on cotton

X	lx	dxF	dx	100qx
Egg	3840	Desiccation	1617	42.1
		Dropping	1210	31.5
		Infertility	74	1.9
		Trichogramma chilonis	71	1.8
		Chrysoperla carnea, Orius sp., *Geocoris* sp. *Coccinella* spp., *Paederus fuscip.*	481	12.5
		Sub-total	**3453**	**89.9**
Larvae	387	*C. carnea, Orius* sp.,*Geocoris* sp.*Coccinella* sp., *Paederus* sp	31	8.0
1st instar		Missing/Predation by chewing insects	162	41.0
		Sub-total	**193**	**49.9**
2nd instar	194	*C. carnea, Orius* sp.,*Geocoris* sp.	27	13.9
		Missing/Predation by chewing insects	51	26.3
		Sub-total	**78**	**40.2**
3rd instar	116	*Microplitis* sp.,*Campoletis chlorideae*	2	1.7
		C. carnea, Geocoris sp.	15	12.9
		Missing/Predation by chewing insects	28	24.1
		Sub-total	**45**	**38.8**
4th instar	71	*Microplitis* sp.,*Campoletis chlorideae*	1	1.4
		C. carnea,.,Geocoris sp.	4	5.6
		Missing/Predation by chewing insects and birds	17	23.9
		Sub-total	**22**	**31.0**
5th instar	49	*Microplitis* sp.,*Campoletis chlorideae*	2	4.0
		Missing/Predation by chewing insects and birds	14	28.6
		Sub-total	**16**	**32.6**
6th instar	33	*Microplitis* sp.,*Campoletis chlorideae*	3	6.1
		Missing/Predation by chewing insects and birds	5	12.1
		Sub-total	**7**	**21.2**
Pupae	26	Decay	4	15.4
		Interculture	2	7.7
		Desiccation	1	3.8
		Ants, Termites*	1	3.8
		Sub-total	**8**	**30.7**

Survival rate = 0.47%

X= The age interval
lX= The number of individuals surviving at the beginning of age interval X
dxF= The factor responsible for the death of individuals of age interval X
dx X= The number of individuals dying within age interval X due to dxF
100qX= Percent mortality

Table 14 Life table of the second generation of *Helicoverpa armigera* on cotton

X	lx	dxF	dx	100qx
Egg	2145	Desiccation	324	15.1
		Dropping	728	33.9
		Infertility	26	1.2
		Trichogramma chilonis	53	2.5
		Chrysoperla carnea, Orius sp., *Geocoris* sp. *Coccinellids, Paederus sp.*	388	18.1
		Sub-total	**1519**	**70.8**
Larvae Ist instar	626	*C. carnea, Orius* sp., *Geocoris* sp.	100	16.0
		Missing/Predation by chewing insects	110	17.6
		Sub-total	**210**	**33.6**
2nd instar	416	*C. carnea, Orius* sp.,*Geocoris* sp	88	21.2
		Missing/Predation by chewing insects	37	8.9
		Sub-total	**125**	**30.1**
3rd instar	291	*Microplitis* sp.,*Campoletis chlorideae*	3	1.0
		C. carnea,. Geocoris sp.	28	9.6
		Bacteria, Viruses,Fungi	9	3.1
		Missing/Predation by chewing insects	44	15.1
		Sub-total	**84**	**28.9**
4th instar	207	*Microplitis* sp.,*Campoletis chlorideae*	4	1.9
		C. carnea,. Geocoris sp.	17	8.2
		Bacteria, Viruses, Fungi	12	5.8
		Missing/predation by chewing insects, spiders and birds	21	10.1
		Sub-total	**54**	**26.1**
5th instar	143	*Microplitis* sp.,*Campoletis chlorideae*	3	2.0
		Bacteria, Viruses,Fungi	17	11.1
		Missing/predation by chewing insects, spiders and birds	39	25.5
		Sub-total	**59**	**38.6**
6th instar	94	*Microplitis* sp.,*Campoletis chlorideae*	1	1.1
		Missing/predation by chewing insects, spiders and birds	20	21.3
		Bacteria, Viruses,Fungi	7	7.5
		Sub-total	**28**	**29.8**
Pupae	66	Decay	24	36.4
		Interculture	7	13.6
		Pheidola sp, *Pcataglypius* sp.	5	7.6
		Sub-total	**38**	**57.6**

Survival rate = 1.07%

X= The age interval
lX= The number of individuals surviving at the beginning of age interval X
dxF= The factor responsible for the death of individuals of age interval X
dx X= The number of individuals dying within age interval X due to dxF
100qX= Percent mortality

Table 15 Life table of the third generation of *Helicoverpa armigera* on cotton

X	lx	dxF	dx	100qx
Egg	5770	Dropping	2250	39.0
		Infertility	260	4.5
		Trichogramma chilonis.	143	2.5
		*Chrysoperla carnea, Orius*sp.,*Geocoris* sp.*Coccinella spp., Paederus sp.*	789	13.7
		Sub-total	**3442**	**59.7**
Larvae Ist instar	2328	*C. carnea,Geocoris* sp.	370	15.9
		Missing/Predation by chewing insects	384	15.9
		Sub-total	**754**	**32.4**
2nd instar	1574	*C. carnea,Geocoris* sp.	297	18.9
		Missing/Predation by chewing insects	120	7.6
		Sub-total	**417**	**26.5**
3rd instar	1157	*Microplitis* sp.,*Campoletis chlorideae*	26	2.2
		C. carnea,.,Geocoris sp.	81	7.0
		Bacteria, Viruses, Fungi	62	5.4
		Missing/Predation by chewing insects and birds	110	9.5
		Sub-total	**279**	**24.1**
4th instar	878	*Microplitis* sp., *Campoletis chlorideae*	17	1.9
		Bacteria, Viruses, Fungi	46	5.2
		Missing/Predation by chewing insects, spiders and birds	118	13.5
		Sub-total	**181**	**20.6**
5th instar	697	*Microplitis* sp.,*Campoletis chlorideae*	19	2.7
		Bacteria, Viruses, Fungi	57	8.2
		Missing/Predation by chewing insects, spiders and birds	138	19.8
		Sub-total	**214**	**30.7**
6th instar	483	*Microplitis* sp.,*Campoletis chlorideae*	11	2.3
		Bacteria, Viruses, Fungi	78	16.1
		Missing/Predation by chewing insects, spiders and birds	128	26.3
		Sub-total	**217**	**44.9**
Pupae	266	Decay	94	35.3
		Adults	32	12.0
		Pheidola sp, *Cataglyplus* sp.	23	8.6
		Sub-total	**149**	**56.0**

Survival rate = 2.03%

X= The age interval
lX= The number of individuals surviving at the beginning of age interval X
dxF= The factor responsible for the death of individuals of age interval X
dx X= The number of individuals dying within age interval X due to dxF
100qX= Percent mortality

Generation increase rate

On the whole 99.53%, 98.93% and 97.97% individuals including eggs, larvae pre-pupae and pupae died due to biotic and abiotic factors in early, mid and late stages of crop growth. Adults emerged in the sex ratio of 1:1. Fecundity of the females varied from generation to generation depending upon the climatic conditions. The pest survived at the rate of 0.47%, 1.07% and 2.03% whereas generation increased rate was 1.06, 2.03 and 2.30 folds during early, mid and late stages of crop growth.

Population growth rate increased gradually with maximum at the end of the season but actual carryover of the pest from cotton to next hosts may be much lower as compared with survival rate because no irrigation or ploughing was done in the field where these studies were carried out. However, after cotton from November to January the farmer provide irrigation and ploughing for the prepation of seed bed for wheat, maize and sunflower and this may cause considerable reduction in pupal stage.

Table 16 Population and generation growth of *Helicoverpa armigera* on cotton

Parameter	Ist generation	2nd generation	3rd generation
Adults emerged	18	23	117
Males	8	11	56
Females	10	12	61
Fecundity/Female	410	367	218
No. at the start of generation	3840	2145	5770
No. at the end of generation	4100	4404	13298
Survival rate	0.47	1.07	2.03
Population growth rate	1.06	2.03	2.03

Control Measures

African bollworm, *Helicoverpa armigera* (Hub.) is a polyphagous. It feeds on 61 host plants which include 31 cultivated crops, 16 weeds and 14 ornamentals plants. It is distributed in almost all the cotton growing areas of this region. The amount of damage depends upon climatic conditions. From 1989; it became key pest of cotton, causing considerable damage to both quality and quantity of the produce in 1994 and 1997. High reproductive potential, favourable climatic conditions, wide host range, introduction of more susceptible varieties, resistance of commonly used insecticides and elimination of natural enemies due to indiscriminate use of insecticides played major role in its establishment as key pest of cotton in the near past.

Among the control measures, cultural practices, biological control, and use of chemicals are important. In Pakistan, it is controlled generally with chemicals and the success of control measure depends primarily on the complete understanding of its biology, ecology, host range and population dynamics. The contribution of each control measure in keeping this pest below economic threshold level was investigated during the course of this study.

Chemical control

a) Efficacy of insecticides on eggs of *H. armigera*

Eggs and small larvae are most vulnerable stages for the control of *H. armigera*. To determine the efficacy on eggs, seven insecticides were tested. Eggs deposited on tissue paper in the laboratory were treated with two methods namely leaf dip method and aerosal spray method. In the dip method tissue paper with fresh (white) and two days old (brown) eggs were dipped once in the insecticides as given in **Table 16**. In the

aerosal method three strokes were given for complete coverage of insecticides. The white and brown eggs were kept separately in the petri dishes till they hatched or died. When eggs were treated by aerosal method, methomyl and thiodicarb gave 100% mortality of white eggs and the differences with the other insecticides were significant. The differences among these two treatments were non-significant. All other insecticides gave significantly lower mortality as compared with these two chemicals.

Mortality of brown eggs was less than white eggs. Overall methomyl and thiodicarb were most effective and gave significantly higher mortality as compared with other insecticiedes. The differences between these two compounds as well as other insecticides were significant **(Table 16).**

Table 16 Mortality of white and brown eggs of *Helicoverpa armigera* After 24 hours of aerosol spray in the laboratory

Treatments	Dose (a.i.g/ha)	*Egg mortality (%)*					
		White		Brown		Total	
		No. Treated	% mortality	No. Treated	% mortality	No. Treated	% mortality
Beta cyfluthrin 25EC	77.2	136	11.8 ^{c}	152	0.0 ^{e}	288	4.73 ^{c}
Bifenthrin 10EC	247.1	156	5.1 ^{e}	139	0.0 ^{e}	295	2.71 ^{f}
Cyhalothrin 2.5 EC	102.0	156	8.3 ^{d}	168	7.7 ^{d}	324	8.02 ^{d}
Methomyl 40SP	247.1	174	100.0 ^{a}	156	96.8 ^{a}	330	97.88^{a}
Profenofos 50EC	494.2	166	78.3 ^{b}	163	31.3 ^{c}	329	55.01 ^{c}
Thiodicarb 80DF	395.4	158	100.0 ^{a}	179	85.0 ^{b}	337	91.99 ^{b}
Triazophos 40EC	494.2	190	11.1 ^{c}	178	0.0 ^{e}	368	5.71 ^{e}
Untreated check	xxxx	166	0.0 ^{f}	145	0.0 ^{e}	311	0.00 ^{g}

*= Values based on transformed data using Arsenic transformation
Mean followed by the same letters are not signficantly different at 5% level of significance

When eggs deposited on tissue papers were treated in pesticide solution as leaf dip method, methomyl and thiodicarb gave 100% mortality followed by profenofos, triazophos, cyhalothrin, bifenthrin and betacyfluthrin of white eggs and the differences of methomyl and thiodicarb were significant as compared with all other insecticides. However, the differences between these two chemicals were non-significant.

Mortality of brown eggs was highest with methamyl (92.8%) followed by thiodicarb(89.2%) and profenofos (75.0%) and others. The differences between all the treatments were significant. Mortality with other insecticides was not much different as compared with untreated check (**Table 17**).

Table 17 Mortality of white and brown eggs of *Helicoverpa armigera* when treated with leaf dip-method under laboratory conditions

Treatments	Dose (a.i.g/ha)	Egg mortality (%)					
		White		Brown		Total	
		No. Treated	% mortality	No. Treated	% mortality	No. Treated	% mortality
Beta-cyfluthrin 25EC	77.2	171	2.9 ^{f}	73	0.0 ^{e}	244	2.1 ^{b}
Bifenthrin 10EC	247.1	140	4.3 ^{e}	91	0.0 ^{e}	231	2.6 ^{f}
Cyhalothrin 2.5 EC	102.0	170	11.8 ^{d}	112	3.6 ^{d}	282	8.5 ^{c}
Mehtomyl 40SP	247.1	162	100.0 ^{a}	74	77.0 ^{a}	236	92.8 ^{a}
Profenofos 50EC	494.2	219	97.2 ^{b}	97	24.7 c	316	75.0 ^{c}
Thiodicarb 80DF	395.4	173	100.0 ^{a}	95	69.5 ^{b}	268	89.2 ^{b}
Triazophos 40EC	494.2	124	63.7 ^{c}	87	0.0 ^{e}	211	37.4 ^{d}
Untreated check	xxxx	135	3.7 ^{ef}	103	0.0 ^{e}	238	2.1 ^{f}

*= Values based on transformed data using Arsenic transformation
Mean followed by the same letters are not signficantly different at 5% level of significance

Efficacy of insecticides on white and brown eggs in the field was also tested. Two concentration of profenofos and one concentration of eight insecticides as given in **Table 18** were tested. Crops with mix population of white and brown eggs was sprayed with hand held knap sack sprayer by the farmers using 250 lit. of water/ha. Three rows of 30m length were sprayed with each insecticide. One row was sprayed at a time. Three hours after spray leaves with white and brown eggs from each treatment were plucked and brought to the laboratory. Leaves with white and brown eggs of each treatment were kept separately in the petri dishes. Observations on eggs was taken daily up to one week. From the eggs hatched mortality was calculated.

Maximum mortality of white eggs was with methomyl (92.1%) followed by thiodicarb (85.3%), profenofos with higher dose (80.6%), amitraz (74.5%) and 60.1% with low dose of profenofos and differences among the treatments were significant. Other insecticides gave no significant mortality of white eggs **(Table 18)**.

Mortality percentage of brown eggs was almost similar to that of white eggs. Highest with methomyl 92.5% followed by thiodicarb 85.3% and higher dose of profenofos 79.4%, the differences between these three as well as other treatments were significant.**(Table 18)**.

Table 18 **Mortality of white and brown eggs of *Helicoverpa armigera* with insecticide when treated with Knap Sack sprayer under field conditions**

Treatments	Dose (a.i.g/ha)	Egg mortality (%)					
		White		Brown		Total	
		No. Treated	% mortality	No. Treated	% mortality	No. Treated	% mortality
Amitraz 40EC	494.2	270	74.4 ^{d}	147	42.2 ^{e}	417	56.0
Beta-cyfluthrin 25EC	77.2	257	8.9 ^{g}	109	5.5 ^{gh}	366	8.6 ^{g}
Bifenthrin 10EC	247.1	296	7.8 ^{g}	223	4.0 ^{h}	519	6.2 ^{h}
Cyhalothrin 2.5 EC	102.0	335	5.6 ^{h}	138	8.0 ^{g}	474	6.3 ^{h}
Deltamethrin+ triazophos 36EC	533.7	236	3.4 ^{I}	225	8.0 ^{g}	451	5.6 ^{h}
Methomyl 40SP	247.1	315	92.1 ^{a}	124	93.5^{a}	439	92.5^{a}
Profenofos 50EC	308.9	248	60.1 ^{e}	122	61.5 d	370	60.5 ^{d}
Profenofos 50EC	494.2	206	80.6 ^{c}	134	77.6 ^{c}	340	79.4 ^{c}
Thiodicarb 80DF	395.4	292	85.3 ^{b}	115	85.2 ^{b}	407	85.3 ^{b}
Triazophos 40EC	494.2	246	15.8 ^{f}	98	14.3 ^{f}	344	15.4 ^{f}
Untreated check	xxxx	225	5.3 ^{h}	151	4.6 g^{h}	376	5.0 ^{h}

*= Values based on transformed data using Arsenic transformation
Mean followed by the same letters are not signficantly different at 5% level of significance

b) **Efficacy of four insecticides on larvae of *H. armigera***

Efficacy of four insecticides on small and large larvae of *H. armigera* was studied on cotton cultivar CIM-448 during 1997. The insecticides included naturlyte, deltamethrin, thiodicarb and profenofos. The plot size with each insecticide was 12 x 32 m and replicated four times. Untreated check was kept as control for comparison. Two insecticidal applications were given at eight days intervals with Knap Sack sparyer. Single row was sprayed at a time using 250 lits. of water per hectare. Larvae were categorised as small (first to third instar) and large (fourth to sixth instar) larvae and counts were taken five and eight days after application.

Five and eight days after first application, mortality of small larvae was almost equal in naturalyte and thiodicarb. Mortality percentage after second application during these interval was higher than the first application. Mortality percentage of large larvae with these insecticides was less than the small larvae after first application. However, after second application mortality percentage was much higher than the first application at both the intervals **(Table-19).** Mortality trends of small and large larvae with profenofos after first and second appllication was almost similar to that of naturalyte and thiodicarb. Deltamethrin gave lower mortality of small and large larvae at both intervals as compared with other three insecticides **(Table 19).**

Table 19 Mortality percentage of small and large larvae of *Helicoverpa armigera* with naturalyte, deltamethrin, thiodicarb and profenofos five and eight days after application at Multan.

No. of application	Stages of larvae	naturalyte		deltamethrin		thiodicarb		profenofos	
		5days	8days	5days	8days	5days	8days	5days	8 days
First	Small	81.6	83.9	51.1	62.9	83.1	80.6	77.5	66.1
Second	Small	86.1	100.0	77.8	65.2	100.0	87.9	91.3	82.6
First	Large	79.5	53.2	53.1	47.5	85.5	65.6	66.3	63.9
Second	Large	97.3	92.3	83.8	52.2	100.0	100.0	94.6	61.5

c) Evaluation of some insecticides against *Helicoverpa armigera*

Two insect growth regulators Match and Gimstar and two combinations of Agree (B.T. formulation) and Polytrin-C (mixture of cypermethrin+profenofos) were compared for their efficacy against *H. armigera*. Larvin (thiodicarb) and fury (zetacypermethrin) were used as standard for comparison. There were seven treatments including untreated check with 20 x 10m plot size in three replicates. Crop was treated

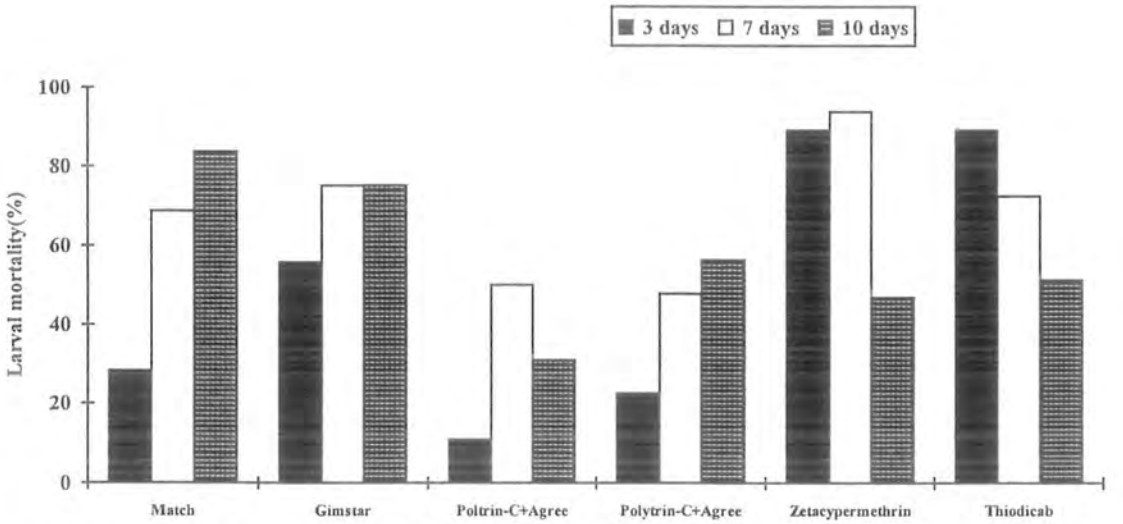
in September when number of larvae were more than 20,000/h. Mortality data were recorded 3, 7 and 10 days after treatment.

All the treatments were effective as compared with untreated check. Among the treatments Fury proved better than other treatments 3 and 7 days after application. Among the insect growth regulators, Match and Gimstar gave significantly higher mortality of the pest as compared with mixture of B.T. and Polytrin-C. Mortality with these two treatments was less 3 days after application as compared with 7 and 10 days. Dose rate of 200ml of Agree with Polytrin-C was better than 100ml and was effective for longer time **(Fig.10)**.

Biological control

A large number of parasitoids, predators and pathogens have been reported from Pakistan by various workers. However, egg parasite, *Trichogramma chilonis,* larval parasites, *Campoletis chlorideae Microplitis* sp., *Apantales* sp., *Bracon hebetor* and an unidentified species of *Ichneumonid* were reared with low level of incidence from Multan and Sahiwal areas. *T. chilonis* was reared from chillies up to 1.2%. From Layyah area, incidence of larval parasitoids on *Cicer arietinum* was 26-30% in March and up to 60% in April. On *Carthamus oxyacantha* 14.1% in May, on *Convolvulus. arvensis* (12.6%), on *Chenopodium album* (20.0%) where insecticides are not used as compared with *Solanum tuberosum* (7.7%), *Abelmoschus esculentus* (2.7-4.4%), *Cucurbita pepo melopepo* (4.6%) and *Gossypium hirsutum* (4.6%) which are sprayed extensively.

Insect predators like *Adonia varigata*, *Coccinella septumpunctata, Chilomenus sexmaculata, Orius* sp., *Geocoris* sp., *Chrysoperla carnea, Paederus fuscupes, Polistes*



Pre-treatment Population=20(000) Larvae/ha

Fig.11 Mortality of *Helicoverpa armigera* larvae with insect growth regulators and insecticides

spp., *Vespa* spp., unidentified species of spiders and bird predators were present and fed on eggs and larvae of this pest. *Pheidola* sp. and *Cataglyphus* were major predators of pupae. From September onwards predators like *Orius* sp., *Geocoris* sp., and *C. carnea* started shifting to cotton and their population was abundent at the end of season. On an average insect predators caused 10% egg mortality. Insects, spiders and birds predation was much higher but had not been properly estimated. Ants and crabid beetle consumed 18.9% of the pupal population.

a) Feeding efficiency of *Chrysoperla carnea* on *Helicoverpa armigera*

Feeding efficiency of *Chrysoperla carnea* larvae in different instars was determined by offering 10-30 eggs/larva of *H. armigera* daily at 27±3°C. First instar larvae of *C. carnea* on average consumed 21.6 eggs or 18.2 first or 13.3 second or 4.0 third instar larvae of the pest during its mean developmental period of 3.5 days. First instar larvae of the predator failed to consume larger larvae of the pest and were mostly damaged by the pest as a result of the defensive mechanism. Second and third instars of the predator were completed on average in 3.5 and 7.5 days, respectively while mean duration of total larval period was 14.5 days. Feeding efficiency of predator increased as its larvae become older **(Table 20).** It consumed 216.6 eggs or 191.2 first instar or 147.3 second instar or 92.0 third instar larvae of *H. armigera* in its life span.

Table 20 Feeding efficiency of *Chrysoperla carnea* larvae on *Helicovera armigera*

Predator Larval instar	Mean Duration (days)	Number consumed			
		Eggs	Ist Instar larvae	Second Instar larvae	Third instar larvae
Ist Instar	3.5	21.6(6.2)*	18.2(5.2)*	13.3(3.8)*	4.0(1.2)*
2nd Instar	3.5	39.2(11.2)*	30.0(8.6)*	22.5(6.4)*	9.0(2.6)
3rd Instar	7.5	180.8(24.1)*	143.0(19.1)*	11.5(14.9)*	79.0(10.5)*
Total	14.5	241.6(16.7)*	191.2(13.2)*	147.3(10.2)*	92.0(6.3)*

Cultrual control

Pre-cotton season plants like *C. pepo melopepo, T. alexandrinum, A. esculentus, Lagenaria siceraria, T. aestivum, L. esculentum, S. tuberosum, Sorghum valgare*, in the areas of Multan, *Z. mays, N. tabacum, H. annuus and C. arvensis* in Sahiwal and *C. arietinum and C. oxyacantha* in non-irrigated area (Layyah) play significant role in the survival and population build-up of *H. armigera*. Proper management of this pest on cultivated crops and destruction of weeds can reduce its pre-cotton carryover to cotton significantly.

Although population growth rate increases gradually with maximum at the end of the season but actual carryover of the pest from cotton to next host may be much lower as compared with survival rate. This reduction may be caused by irrigations and ploughings given for the preparation of the seed bed for *T. aestivum, Z. mays* and *H. annuus* after *G. hirsutum*. One irrigation and three to five ploughings resulted in 43.9% mortality of the pupae. Depending upon the depth of pupation mortality was variable, maximum being 50% at 10cm depth.

DISCUSSION AND CONCLUSION

Recently *Helicoverpa armigera* has become serious pest of cotton most probably because of elimination of natural enemies and development of resistance to insecticides. Mohyuddin *et al.* (1989) recorded eight species of parasitoids from this pest and indicated that these should keep it under control on cotton. It seems that excessive and indiscriminate use of insecticides has created this problem because of elemination of natural enemies. During these studies incidence of the parasitoids was higher on *Cicer arietinum* and other hosts where insecticides were not used compared with cotton probably because of this reason. Moreover, Mohyuddin *et al.* (1997) demonstrated that augmentative releases of egg parasitoid *Trichogramma chilonis* Ishii increased parasitism and gave excellent control of bollworms including *H. armigera*.

In Pakistan Ahmad *et al.* (1997) reported that this pest has developed resistance to both organophosphate and pyrethroid insecticides. They have reported various degree of resistance to various insecticides including cypermethrin (13-205 times), cyfluthrin (3.6-177), a low to moderate resistance to deltamethrin (2.2-81), alpha-cypermethrin (10-133), bifenthrin (1.4-9.7), lambda-cyhalothrin (2.1-7.0) and zeta-cypermethrin (2.1-32). Pyrethroids were considered most effective insecticides for a long time, for control of lepidopterous pests including *H. armigera* but this pest has developed resistance against this group. Thus development of resistance is also a major factor in high infestation of this pest on cotton and other crops.

Of the eleven insecticides tested methomyl, thiodicarb and profenofos were most effective against eggs as well as young larvae of *H. armigera*. These insecticides and chlorpyrifos are being extensively used in all the cotton growing areas. At present the farmer have very limited choice for the control of this pest. However, if their use is continued, the pest is sure to develop resistance against these insecticides also. These insecticides should be used in rotation with others rather than continuously. Therefore, it is imperative that resistance management strategy should be developed through rotation so that the efficacy of the various insecticides is maintained for longer periods.

H. armigera is a polyphagous species. Its alternate hosts seem to play a significant role in its population build up on cotton. For example in arid areas *Triticum aestivum, Cicer arietinum* and *Carthamus oxyacantha* are its major hosts. After *C. arietinum* and *T. aestivum* have been harvested in April, its population shifts to *C. oxyacantha*. The farmers leave this weed growing till the susceptible stages of cotton are available. If the farmers destroyed this weed in time, infestation of *H. armigera* can be reduced.

In irrigated areas such as Multan and Sahiwal its populations seem to shift from *Helianthus annuus, Nicotiana tabacum, Zea mays, Sorghum vulgare* and *Abelmoschus esculentus* and other vegetables. These being economics crops cannot be harvested before cultivation of cotton. Therefore, these should be grown in areas where cotton is

not extensively cultivated. The Government may have to declare different zones for their cultivation.

Desiccation, dropping of eggs and early instar larvae and predation were key mortality factors on eggs, larvae and pupae in first, second and third generations of *H. armigera*. More than 30% mortality is caused by predators in different stages of the pest. Their role especially of *Chrysoperla carnea* can be increased if they are encouraged by growing alfalfa as recommended by Mohyuddin *et al.* (1997).

Cultural practices such as irrigation, ploughing and planking cause considerable pupal mortality. The pest can be controlled by adopting above mentioned cultural practices, the judicious use of insecticides (The insecticides against which it has developed resistance should not be used for some times) and their rotation, conservation and augmentation of natural enemies.

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Pyrethroid resistance of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan

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Abstract

The status of pyrethroid resistance in some field populations of *Helicoverpa armigera* (Hübner) from Pakistan was determined using an IRAC leaf-dip method. Resistance factors varied between populations, and the general trend was for a moderate to high resistance to cypermethrin and cyfluthrin; a low to moderate resistance to deltamethrin and alpha-cypermethrin; and a comparatively low resistance to bifenthrin, lambda-cyhalothrin and zeta-cypermethrin. Depending on which physiological mechanisms are shown to be present in Pakistani field strains, the latter group of pyrethroids may serve as useful tools in the management of insecticide resistance.

Introduction

Cotton is attacked by a number of sucking and chewing s in Pakistan; amongst the latter, bollworms are currently most important in economic terms. Like most other on producing countries, pest control has largely relied on nical insecticides. The indiscriminate use of insecticides, icularly during the 1980s and 1990s, contributed to the rgence of cotton bollworm, *Helicoverpa armigera* (Hübner) idoptera: Noctuidae) as a primary pest of cotton in nt years. In 1994, *H. armigera* became the major cause of stantial yield reduction of cotton in Pakistan. Control of pest was not always adequate probably due in part to the elopment of insecticide resistance. Moderate to high ls of resistance to cypermethrin and monocrotophos and derate resistance to endosulfan were recorded in field ulations of *H. armigera* in Pakistan (Ahmad *et al.*, 1995). eighbouring India, Jadhav & Armes (1996) also recorded resistance to cypermethrin, fenvalerate, endosulfan and alphos in this pest. Here we report the results of stance monitoring of *H. armigera* to a range of pyrethroids ch are currently considered the most potent insecticides bollworm control and are widely used on cotton. Any rences between pyrethroid compounds might be utable in resistance management programmes aimed at erving the usefulness of this important chemical group.

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Materials and methods

Insects

Fifth or sixth instar larvae of *H. armigera* were collected from various locations in Pakistan during 1991 to 1995. Each collection was made from a 5 acre block of a particular host crop. The larvae were fed in the laboratory on a semi-synthetic diet (modified from Ahmad & McCaffery, 1991), which consisted of chickpea flour (300 g), ascorbic acid (4.7 g), methyl-4-hydroxybenzoate (3 g), sorbic acid (1.5 g), streptomycin (1.5 g), corn oil (12 ml), yeast (48 g), agar (17.3 g) and distilled water (1300 ml) with a vitamin mixture. Adults were fed on a sucrose solution with the addition of vitamins and methyl-4-hydroxybenzoate.

Insecticides

Commercial formulations of cypermethrin (Arrivo, 100 g/l EC, FMC), alpha-cypermethrin (Bestox, 50 g/l EC, FMC), zeta-cypermethrin (Fury, 181 g/l EC, FMC), deltamethrin (Decis, 25 g/l EC, AgrEvo), cyfluthrin (Baythroid, 50 g/l EC, Bayer), bifenthrin (Talstar, 100 g/l EC, FMC) and lambda-cyhalothrin (Karate, 25 g/l EC, Zeneca) were obtained from the respective manufacturers.

Bioassays

Newly moulted second instar larvae from the F_1 laboratory generations were exposed to different insecticides using the leaf dip technique as recommended by the

Table 1. Toxicity of pyrethroids in leaf dip bioassays to second instars of field strains of *Helicoverpa armigera* from Pakistan.

Insecticide	Strain/location	Slope ± s.e.	LC_{50} (ppm)	95% fiducial limits	RR[a]
Cypermethrin	Vehari[1]	1.15 ± 0.09	0.74	0.56–0.99	–
(Arrivo 100 g/l EC)	Shershah[2]	1.39 ± 0.12	9.4	7.27–12.2	13
	Sahiwal[3]	2.31 ± 0.22	20	16.5–24.3	27
	Lodhran[4]	1.52 ± 0.13	41	32.2–52.5	56
	Khanewal[5]	2.61 ± 0.26	18	14.8–21.2	24
	Okara[6]	2.29 ± 0.22	20	16.0–23.7	27
	Shujabad[7]	1.61 ± 0.14	39	30.6–49.1	53
	Hyderabad[8]	1.81 ± 0.16	13	10.6–16.6	18
	Kaghan[9]	1.65 ± 0.14	13	10.6–16.8	18
	Khanpur[10]	2.50 ± 0.24	36	30.1–43.6	49
	Multan[11]	2.04 ± 0.18	12	9.97–15.1	16
	Kabirwala[12]	1.88 ± 0.17	111	89.1–138	150
	Dadu[13]	2.36 ± 0.23	80	65.9–96.8	108
	Lar[14]	2.32 ± 0.23	152	125–184	205
Alpha-cypermethrin	Vehari	1.67 ± 0.15	0.24	0.19–0.31	–
(Bestox 50 g/l EC)	Sahiwal	2.15 ± 0.19	2.5	2.05–3.06	10
	Lodhran	1.45 ± 0.12	4.8	3.73–6.12	20
	Shujabad	2.28 ± 0.22	4.1	3.36–4.96	17
	Hyderabad	2.53 ± 0.25	7.0	5.78–8.36	29
	Khanpur	1.66 ± 0.15	2.9	2.27–3.63	12
	Multan	2.06 ± 0.19	5.8	4.69–7.07	24
	Nawabshah[15]	1.76 ± 0.15	19	15.4–24.1	79
	Kabirwala	1.37 ± 0.12	8.6	6.62–11.2	36
	Dadu	2.25 ± 0.22	20	16.1–23.9	83
	Lar	2.41 ± 0.23	32	26.2–38.2	133
Zeta-cypermethrin	Islamabad[16]	2.41 ± 0.24	0.19	0.16–0.23	–
(Fury 181 g/l EC)	Shershah	1.92 ± 0.17	0.39	0.31–0.48	2.1
	Lodhran	1.72 ± 0.15	3.5	2.79–4.39	18
	Khanpur	2.06 ± 0.20	1.9	1.55–2.35	10
	Multan	1.87 ± 0.17	2.9	2.32–3.59	15
	Kabirwala	2.14 ± 0.21	3.6	2.94–4.41	19
	Dadu	1.61 ± 0.15	6.1	4.80–7.73	32
	Lar	1.95 ± 0.18	2.7	2.17–3.31	14
Deltamethrin	Vehari	1.16 ± 0.10	0.37	0.28–0.49	–
(Decis 25 g/l EC)	Sahiwal	1.90 ± 0.17	3.3	2.66–4.08	8.9
	Lodhran	1.69 ± 0.14	7.6	6.01–9.49	21
	Okara	2.60 ± 0.26	6.9	5.72–8.22	19
	Hyderabad	1.58 ± 0.14	0.81	0.64–1.03	2.2
	Multan	1.79 ± 0.16	3.1	2.48–3.87	8.4
	Kabirwala	1.28 ± 0.11	14	10.9–18.8	38
	Dadu	2.11 ± 0.20	17	13.7–20.6	46
	Lar	1.85 ± 0.17	30	24.4–37.9	81
Cyfluthrin	Islamabad	2.17 ± 0.21	0.22	0.18–0.27	–
(Baythroid 50 g/l EC)	Shershah	1.44 ± 0.12	0.80	0.62–1.02	3.6
	Lodhran	1.53 ± 0.13	20	15.8–25.8	91
	Hyderabad	1.04 ± 0.09	2.1	1.55–2.84	9.5
	Multan	1.69 ± 0.16	6.7	5.27–8.38	30
	Kabirwala	1.45 ± 0.12	31	23.9–39.2	141
	Dadu	2.07 ± 0.20	33	26.7–40.4	150
	Lar	1.99 ± 0.18	39	31.9–48.4	177
Bifenthrin	Vehari	1.44 ± 0.13	0.67	0.52–0.86	–
(Talstar 100 g/l EC)	Shershah	1.74 ± 0.15	0.91	0.73–1.14	1.4
	Sahiwal	1.19 ± 0.09	2.6	2.00–3.48	3.9
	Lodhran	1.74 ± 0.15	6.5	5.16–8.09	9.7
	Khanewal	2.35 ± 0.23	3.6	3.00–4.40	5.4
	Shujabad	2.12 ± 0.19	6.4	5.21–7.81	9.6
	Hyderabad	3.33 ± 0.36	3.2	2.71–3.74	4.8
	Kaghan	2.30 ± 0.22	8.3	6.83–10.1	12
	Khanpur	2.75 ± 0.27	6.6	5.56–7.91	9.9
	Multan	2.26 ± 0.22	2.0	1.66–2.46	3.0
	Nawabshah	1.93 ± 0.18	17	13.4–20.6	25
	Kabirwala	1.46 ± 0.13	14	10.9–18.0	21
	Dadu	1.97 ± 0.18	12	9.73–14.8	18
	Lar	1.81 ± 0.17	8.9	7.09–11.1	13

Table 1. Continued overleaf.

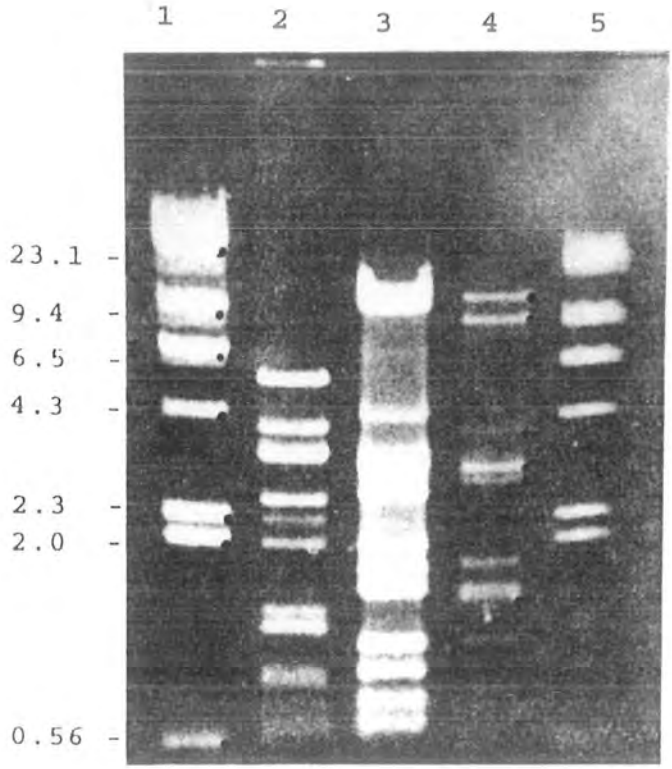

Fig 14 Agarose gel (0.8%) electrophoretic patterns of Sau 3A restriction digest of phage DNA's are as follows:

Lane 1: Phage Marker
Lane 2: Phage Sung.
Lane 3: Phage P008.
Lane 4: Phage TK1.
Lane 5: Phage Marker.

Chapter 5:

Discussion

DISCUSSION

In this study the total lactic count, titratable acidity and pH of indigenous dahi samples collected from twin cities of Pakistan are presented in (Table 1). The minimum and maximum total lactic count, titratable acidity and pH ranges from log 6.13 to log 8.97, 1.67 to 0.92% and 3.69 to 4.65 respectively. The values recorded in the present investigation are partially agreed with Naeem and Rizvi (1983). They reported the minimum and maximum range of viable bacterial count and pH values from $1.1x10^6$ to $9.5x10^7$ and 3.4 to 4.9. The variation may be due to different environmental conditions and quantity of raw milk used for manufacture of dahi. Dave *et al* (1991) on the other hand have recorded the total lactic count ranged from $49x10^7$ to $37x10^8$/g. The titratable acidity of a good finished product is around 0.85 to 0.90% had been reported by Davis (1975). He observed that to get this amount of acidity the fermenting product should be removed from 45°C when the titratable acidity is around 0.65 - 0.70%, while in the present study the acidity varies from 1.67 to 0.92%.

Table (2) showed the population range of total lactic count, titratable acidity and pH with numbers of indigenous dahi samples. It was observed that maximum number of lactic count of dahi samples are within the range of log 7.55-8.25, while their corresponding titratable acidity and pH values vary from 0.92 - 1.10% and 3.85 - 4.22 respectively. The difference may be due to procedure of dahi making, environmental temperature, starter culture composition, quality and quantity of inoculum, incubation time and temperature. However among these factors suggested, starter is a crucial component and any change may cause a lot of economic loss, as this product is being prepared from a heterogenous mixture of undefined lactic acid bacteria along with contaminants (Masud *et al.*, 1988) Therefore, the quality of dahi varies with the type of starter culture used. In order to provide a good quality fermented milk product to the consumer there is a need to identify and characterize the wild strains of lactic acid bacteria present in this cultured milk product.

The organisms identified in this study are listed in Table 3 . The presence of such bacteria have been reported in earlier studies (Naeem and Rizvi, 1983; Isani *et al.*, 1986). Moreover, it is again observed that all the isolated bacteria from indigenous dahi were thermophilic and mesophilic in nature (Jay, 1978).

In this study *L. delbrueckii ssp. bulgaricus* and *S. thermophilus* constituted the dominant microflora of dahi. Thus these species may play an important role for the preparation of this fermented milk product, as also noted by Warsey (1983) and Mohanan *et al.*,(1983). Similarly the successful manufacture of yoghurt depends upon the correct lactic fermentation being carried out by a mixture of *S. thermophilus* and *L. delbrueckii ssp. bulgaricus* (Accolas *et al.*, 1980) rather than single strain culture due to the symbiotic relationship between species of bacteria (Rasic and Kurmann, 1978; Tamine and Deeth 1980). Moreover the presence of *L. helveticus* in our dahi samples is consistent with the finding of Thomas (1985) who reported that *L. helveticus* may be used in yoghurt starter culture with *S. thermophilus* and *L. delbrueckii ssp. bulgaricus*. These two species of *lactobacilli* are mainly used for acid and flavor production in yoghurt making (Kosikowski, 1982).

The high number *L. delbrueckii ssp. bulgaricus* recorded in the present investigation may be due to the long incubation time. Mohanan *et al.*,(1983) reported that when milk was inoculated with the mixed culture of *S. thermophilus* and *L. delbrueckii ssp. bulgaricus* for preparation of dahi, the *streptococci* initially grow at much faster rate than the *lactobacilli* but after sixteen hours of incubation the *lactobacilli* became dominant. Furthermore, they are of the view that the inoculum added to milk has a predominance of *lactobacilli* and as in the case of old or sour curd the relative growth rates of the two organisms would be different.

The presence of *S. lactis* and *S. cremoris* in our study is of great interest. Kosikowski (1982) reported that these organisms are mostly used for the preparation of different types of cheese. However, they may be used for the preparation of yoghurt where multistrains starter culture is used to produce the desired acidity. Kosikowski (1982) also suggested that these strains have a different optimum temperature about 72°F for multiplication than the optimum temperature of yoghurt starter. The role of these strains in dahi has not yet been clarified, however, they may play a role in the preparation of this product in winter (Laxminarayana *et al.*, 1977). Furthermore, Kosikowski (1982) reported that *S. cremoris* and *S. lactis* are slime producers, releasing complex carbohydrate capsular materials which increase viscosity. In the Netherlands, 90% of the yoghurt is made with slime producing bacteria. Therefore, it may be assumed that these bacteria contribute to the formation of dahi by their unique biological properties.

The presence of *L. casei* in dahi is in line with the finding of Naeem and Rizvi (1983), but their role is again unknown. Roginski (1988) reported that *L. casei* is used in the production of a popular Japanese fermented milk product called yakult.

Although only nine of our samples of dahi contained *L. acidophilus*, their presence was also reported by Naeem and Rizvi (1983) and Isani *et al.* (1986). Kosikowski (1982) was of the opinion that this microorganism is mostly used for the preparation of acidophilus milk, where it produces acid ranging from 1.2 to 2.0%. However their presence in dahi may be objectionable due to their high acid production capability.

On the basis of the results obtained in this study, it is concluded that in high quality fermented milk products a small change in the starter may be acceptable. The indigenous dahi contains a mixture of lactic acid bacteria, and the quality of dahi varies with the type of predominant species in the starter used for inoculation. Therefore, it can be suggested that efforts should be made to select suitable indigenous strains of bacteria to local environmental conditions in order to produced a uniform high quality fermented milk product.

The starter culture used for the preparation of a fermented product have been selected on the basis of their acidification rates and organoleptic quality of final product (Batt, 1986). In manufacturing yoghurt with the defined starter the *S. thermophilus* is used in conjunction with *L. bulgaricus* as starter, the cocci forming the greater proportion of the population at the time of inoculating the milk. The production of acid in the early stages of yoghurt making depends substantially on the activity of cocci while *lactobacilli* are responsible for continuing the acidification process. These properties depend upon the strains used for inoculation. It could be seen that *L. delbrueckii ssp. bulgaricus* strains produced a high rate of acid production as compared to *S. thermophilus*. This may be due to their genetic variation. Similar views are expressed by Jeffrey (1985). It was further observed that 8 strains of *S. thermophilus* and 7 strains of *L. delbrueckii ssp. bulgaricus* were considered to be slow starter strains which may be due to that as the lactic acid is not produced by a starter culture from lactose at the desired rate of speed therefore, the culture is called "slow". Almost any condition leading to an impairment in the growth of starter culture bacteria results in a "slow" starter (Kosikowski, 1982).

Later on these bacterial strains were compared individually at different temperatures i.e. 37°C, 40°C and 45°C by using their developed acidity (final T.A. minus initial T.A.) for further selection of starter culture for yoghurt making. The results of Table (6) revealed that temperature had a considerable effect on the growth of *S. thermophilus* strains however, there was no difference between 37°C and 40°C respectively. It was further observed that there was also a pronounced differences among the strains for their developed acidity on their respective temperature. It was also noted that their rate of acid production increases with the increase of temperature. More acid production was recorded at higher temperature.

On the basis of these observations these strains were further divided into two groups for

their developed acidity. The strain No 3, 5, 7, produced higher amount of developed acidity and are ranked as high (H) while 4, 1, 2 and 6 produced a relatively less developed acidity are ranked as weak (w). Similar results were also recorded for the examined strains of *L. delbrueckii ssp. bulgaricus*. It was observed from Table (7) that temperature also has a marked effect on the growth of *L. delbrueckii ssp. bulgaricus* strains are in descending order i.e. 45°C < 40°C < 37°C respectively. Furthermore, there was also a marked difference among the strains for their developed acidity. The strains No.1,7,2,3,4 produced higher amount of developed acidity and are ranked as (H) while 5,8,6 produced low developed acidity are ranked as (w).

Finally, two strains each ranked as high and weak lactic acid producers of *S. thermophilus* and *L. delbrueckii ssp. bulgaricus* were randomly selected for comparative studies for their optimum growth temperature, generation time and titratable acidity in order to make a suitable pairs for yoghurt making at a particular temperature. The result of these three experiments are summarized in Table (8).

The results obtained from the present study related with optimum growth temperature indicated that there is a correlation between generation time and acidity and both these factors depend upon the optimum growth temperature. The strains that have less generation time produce more acid at its respective optimum temperature. It is further observed that there was a difference in generation time between the species of *streptococci* and *lactobacilli*. The generation time of *L. delbrueckii ssp. bulgaricus* strains are less than *S. thermophilus* therefore *L. delbrueckii ssp. bulgaricus* produced more acid than *S. thermophilus*. It was further observed for *S. thermophilus* that coagulation of milk occurred sooner at temperatures much higher than those temperatures at which the greatest amount of cell growth occurred. This indicates that the optimum temperature for growth did not coincide with the temperature at which the rate of acid production is the greatest. These results support the view of Radke - Mitchell and Sandine (1986) and Masud *et al.*, (1992).

It was earlier reported by Sampcłinski *et al.*, (1978) that optimum growth temperature is

one of the unique characteristics of bacteria. This trait would be expected to influence the growth compatibility of strains, especially those with different optimum growth temperature. It was also observed that all examined strains of *S. thermophilus* and *L. delbrueckii ssp. bulgaricus* have maximum growth at 45°C as compared to other tested temperatures. Moreover, their was also a difference in their generation time among the examined strains of *S. thermophilus* and *L. delbrueckii ssp. bulgaricus*. This may be due to the differences in their genetic make up. Jeffery, (1985) reported that some of the properties essential for successful milk fermentation are encoded by genes located on plasmid DNA. Furthermore, it was reported by Yu *et al.*, (1983) that if the plasmid profile of the two organism is similar or even identical, there may be difference in their nucleotide composition, nucleotide sequence or even both. *L. delbrueckii ssp. bulgaricus* have 43°C optimum temperature and are thermophilic in nature and have more generation time and acidity at 43°C. Similar observations are also reported by Breed *et al.*, (1957); Martly, (1983); and Radke - Mitchell and Sandine (1986).

The present study also showed that there was an inverse relationship between titratable acidity and pH values of each strain at different time intervals (Fig 1 to 4). Growth temperature is correlated with the pH and acidity. It was observed that there was variation for their rate of acid production among the examined strains of *S. thermophilus* and *L. delbrueckii ssp. bulgaricus*. Maximum acidity recorded after 8 hour of incubation at 45°C for the strains of *S. thermophilus* varies from 0.72 to 1.28% while for *L. delbrueckii ssp. bulgaricus* strains it varies from 1.23 to 1.35%. The difference was again presumably due to the generation time and genetic make up. The acidity for *L. delbrueckii ssp. bulgaricus* at 45°C varies from 0.22 to 1.97 % . These results are also in line with the findings of Masud *et al.*, (1992). The present study further revealed that there was a gradual increase in acidity with the passage of time by the individual strains at their optimum temperatures. It also appeared that there was no marked difference among the examined strains for rate of acid production and there was a considerable diversity between the individual strains of *L.*

delbrueckii ssp. bulgaricus and *S. thermophilus* with respect to their rates of acid production confirming the observations of Accolas *et al.*, (1980) and Masud *et al.*, (1992). This diversity is probably more significant than the generalization that the *L. delbrueckii ssp. bulgaricus* exhibit maximum rate of acid production as compared to the other strains. These results also support the findings of Mohanan *et al.*, (1983). Later on, two strains each of *L. delbrueckii ssp. bulgaricus* (H and w) and *S. thermophilus* (H and w) were randomly selected for mixed culture studies to establish the best combination and ratio that would produce the maximum acidity within 3 and 1/2 hours at 42°C. The results of these experiments are summarized in Table (9). It is seen that increased acid production as measured by TA (% lactic acid) was recorded when both strains were used together as compared to individual. This may be attributed to the mutual stimulatory action of the two organisms (Moon and Reinbold, 1976; Accolas *et al*, 1980). Some of the amino acids released as a result of proteolytic activity of *L. delbrueckii ssp. bulgaricus* have been reported to stimulate the growth of *S. thermophilus* (Bautista *et al.*, 1966; Higashio *et al*, 1977 a; Shankar and Davis, 1976) while the stimulatory action of *S. thermophilus* on the growth of *L. delbrueckii ssp. bulgaricus* has been ascribed to formic acid and pyruvic produced by *S. thermophilus* (Higestiho *et al*, 1977b; Shankar and Davis, 1976). It was further observed that higher rate of acid production was recorded in 1:2 combination of *S. thermophilus* and *L. delbrueckii ssp. bulgaricus* as compared to other tested combinations. This may be attributed to the symbiotic relationship between two organism while all other combinations possible for pairing strains of *S. thermophilus* and *L. delbrueckii ssp. bulgaricus* did not produced a high titratable acidty at this particular temperature. Similar views are expressed by Lal *et al* (1978). Rao *et al* (1982) also observed that 1:2 ratio of *S. thermophilus* and *L. delbrueckii ssp. bulgaricus* produced higher titratable acidity as compared to other tested combination of these two strains.

Since 1:2 combination produced maximum acidity in all the examined strains of *S. thermophilus* and *L. delbrueckii ssp. bulgaricus* therefore, this combination was further examined both for chemical and organoleptic evaluation.

The mean values recorded for different parameters for making yoghurt from buffalo milk by using different selective combinations is shown in Table (10). It was observed that there is a

marked difference among the combination strains of *S. thermophilus* and *L. delbrueckii ssp. bulgaricus* for titratable acidity acetaldehyde and diacetyl contents this may be due to their associative growth properties (Rake-Mitchell and Sandine, 1986). It was further observed that titratable acidity recorded for these different combinations were within the permissible range as described by Dehaast *et al.*,(1979) and Morley, (1979).

Reports of earlier studies suggest that the presence of acetaldehyde is the primary importance for good yoghurt flavor (Keenan and Bill, 1968 and Sandine and Elliker, 1970). In yoghurt its maximum production is reached about 3hrs at 42°C. Similarly in our study all the tested combinations of *S. thermophilus* and *L. delbrueckii ssp. bulgaricus* strains also produced appreciable level of acetaldehyde. The amount recorded are well comparable to 23 to 33 ppm of acetaldehyde produced by mixed yoghurt culture as reported by Hamdan *et al.*,(1971b). However, the difference recorded among these combinations may be attributed to difference in volatile aroma compounds producing ability of these strains during incubation at this particular temperature. Similarly different studies have reported that the diacetyl content of yoghurt may range between trace to greater than 0.90 ppm the diacetyl content recorded in these combinations are within the range suggested in the literature that will contribute to the pleasant and delicate flavour and aroma in the final product (Warsey, 1983 and Lal *et al.*,1978).

The yoghurt prepared by all the selected combination was subjected to organoleptic evaluation by a pannel of judges. The results of these studies are presented in Table (10). The values shown are the mean score given by the pannel of five judges on Hedonic scale. The pannel results revealed that the culture combination of *S. thermophilus* (H) and *L. delbrueckii ssp. bulgaricus* (w) got higher score (12). This might be due to the chemical properties that reflect the organoleptic properties. This supports the findings of Robinson *et al.*,(1977).

On the basis of these observations it is concluded that there was a variation among *S. thermophiles* and *L. delbrueckii ssp. bulgaricus* strains for their biochemical properties for different tested combinations. Moreover, chemical properties on yoghurt are likely to effect organoleptic properties of the final product.

Extensive studies have been conducted for many decades on the dairy starter culture preservation, specially with reference to the lactic acid bacteria. The end product prepared with a starter depends upon the type and the quality of the starter used. All starter cultures for industrial use in Pakistan are imported from outside sources which are not only uneconomical but also hard to maintain locally. Although traditionally prepared yoghurt (dahi) is consumed by a large population of Pakistan this is never 100% wholesome. It is almost always prepared with the help of some unidentified and undesired mixture of lactic acid bacteria which usually contains a large number of contaminants. This kind of yogurt has a very short shelf life and is by no means safe for health.

Thus there has always been a need to conduct an extensive study in the field of dairy industry for manufacturing good quality yogurt with the help of indigenous strains of yogurt starter cultures, viz., *S. thermophilus* and *L. delbrueckii ssp. bulgaricus*. This was only possible by selecting stable and resistant strains from local raw milk and preserving them in such media that could provide cryoprotection during their storage. Therefore, the present study was conducted on this aspect utilizing the selected strains of yoghurt culture characterized by Masud *et al.*, (1997).

Spectrophotometric study of presently studied strains by Masud *et al.*, (1992) showed that their log phase ended after 4 to 5 hr. of incubation at 370C. Peebles *et al.*, (1969) had reported earlier that the cells harvested at the end of the log phase show a higher survival rate as compared to those harvested during the stationery phase. Therefore, in the present study cells were harvested ruing the log phase before resuspension in the five cryoprotectives, viz., 10% Skim milk as the control, ß-glycerophosphate, D-mannitol, Sorbitol and Na-glutamate.

Although presently used cryoprotectives had been shown earlier to provide a high survival, but they had never been used for the preservation of indigenous strains. The present study therefore

presented a comparison between indigenous strains of *S. thermophilus* and *L. delbrueckii ssp. bulgaricus* with respect to how these cryoprotective effect the survival of indigenous strains after freeze-drying.

Indigenous strains of *S. thermophilus* and *L. delbrueckii ssp. bulgaricus* individual as well as in a 1:1 mixture were lyophilized in 5 different cryoprotectives. Table 12 a and 12 b shows mean log viable count before and after freeze-drying along with their log cycle reduction and percent log survival after freeze-drying.

It was observed that there was no significant difference between log viable count of all indigenous strains before and after freeze drying in all the cryoprotectives except sorbitol which significantly lowered the survival rate after freeze-drying. In all other cases a high survival rate was perhaps due to optimum initial bacterial load for lyophilization which plays a vital role after freeze-drying. These results are consistent with the findings of Kilara *et al.*,(1976) and Bozoglu *et al.*, (1987).

Although similar techniques were employed for propagation of all strains yet low viable count was recorded in the case of sorbitol. This might be due to some kind of a toxic effect produced by sorbitol soon after suspension before freeze-drying. Similar low counts in sorbitol have also been observed in some earlier studies (De Valdex *et al.*, 1983b; Champagne *et al.*, 1991b).

High viable counts of indigenous strains in ß-glycerophosphate are similar to the observations of De Valdez *et al* (1983b) who suggested ß-glycerophosphate as a better cryoprotective for LAB. On the other hand Kilara *et al.*, (1976) proposed that Na-glutamate also offers good cryoprotection to *S. thermophilus,* ranging from 57-94%, and present findings are comparable to those results. There are some differences between present findings and those of Champagne *et al.*, (1991b) regarding survival rate which might be due to varied storage conditions as well as intergeneric difference (Kilara *et al.*, 1976).

Tables 13a and 13b show a comparison of log mean viability and percent log survival during 12 months of storage of lyophilized cultures using different cryoprotective agents at 0°C. Present observations for 12 months storage at 0°C are comparable with the findings of Obayashi *et al.*, (1961) whereby a very low log cycle reduction was observed during storage at 0°C compared to storage at 37°C. It was observed that there is a marked difference between strains for the viable count among all the tested cryoprotectives. High survival rate was recorded for *S. thermophilus* as compared to *L. delbrueckii ssp. bulgaricus* strains. This may be due to differences in their size as well as their chemical composition of the cell walls (Bozoglu *et al.*, 1987). It was further observed that viable count of *S. thermophilus* and *L. delbrueckii ssp. bulgaricus* continuously decreased with the passage of time at 0°C.

Later on the 10 filtrates were spotted neat and at serial dilution on lawns of *L. lactis ssp. diacetylactis* (F 7/2) and *L. lactis ssp. lactis* respectively. Two of the filtrates showed zones of inhibition in spot test using the soft agar overlay technique. A variable size of plaques formation occurred with one filtrate (Fig 8:1). This variation for plaques morphology may be caused by different types of phages. Similar views are expressed by Luria *et al.* (1978). In this study a large size (3mm) plaque was selected for further characterization of phage. This particular phage give uniform clear plaque morphology against both the tested host strains and designated as phage Tk1. The lytic activity of phage Tk1 was determined by spectrophotometry at 37°C (Fig. 2). The phage appeared to be virulent in nature to both of the host strains. These observations are in line with the findings of Henning *et al.* (1986) who studied interactions between 60 phage isolates and 100 strains of *S. lactis*, *S. lactis ssp. diacetylactis* and *S. cremoris*. Most phages were specific for one group but some phages attacked strains from all the three groups. Lactic phages with multiple host range have been isolated by several workers (Jarvis and Meyer 1986). Four out of 14 phages isolated by Saxelin *et al* (1986) were found to lyse more than one host. Prevalence of the phages capable of attacking more than one host is due to the commonly adopted practice of using multiple strain starters. Moreover, it is know that phages may adopt to the original non-permissible host by a host range mutation, which can alter it's absorption specificity or by host-controlled modification (Moeller *et al.*, 1986). It was further observed that lytic activity started approximately after one hour of infection in both host strains and OD decreased dramatically during the examined period indicating total lysis of the culture. However, a lower value of OD 600 was recorded in L. *lactis ssp. lactis* as compared to *L. lactis ssp. diacetylactis* (F 7/2). This lytic activity may have resulted due to the repression of host protein synthesis by phage Tk1. The similar pattern of lytic activity was recorded by Sanders and Klaenhammer (1980).

The study further revealed that the rate of absorption to the phages to the both tested strains may be the same. As it has been reported that the rate at which a given phage absorbs to a host depends on the number of suitable receptors (Luria *et al.*, 1978). It may be assumed that phage replication in both the tested strains is the same.

The infection cycle of bacteriophage Tk1 in M17 media was characterized by its one step growth kinetics. Fig (7) graphically represents the increase in PFU per milliliter as a function of time during infection at 37°C. Latent period reported for lactic streptococcal phages range from 9 to 139 min with a majority occurring within 40 to 50 min at 30°C. Moreover, their burst size are highly variable and ranges from 9 to 105 particles per infected cells (Keogh 1973). Phage Tk1 exhibited identical latent period of about 15 min. the rise period 25 min and the burst size was 80 to 85 phages in both the tested strains. Latent period of 80 to 100 min. rise period of 30 to 60 min and a average burst size of 40 to 60 phages particles were reported for *S. lactis* phage by Kozak *et al.* (1973). The latent period and rise period of our phage was much shorter whereas the burst size was much larger than those reported. This may be due to the use of M17 medium for propagation of lactic streptococci and their phage at this particular temperature. The M17 medium enhances the growth of lactic streptococci due to the high buffering capacity of the medium and has proved to be superior to other media in demonstrating and distinguishing between lactic streptococcal phages (Terzaghi and Sandine, 1975). Shorter latent period of more liberation on burst or both are characteristic of phage races that develop quickly and achieve high population in cheese whey. These phages can cause complete failure of starter culture even when present in low concentration at the start of cheese making (Lawrence *et al.*, 1976).

The ultrastructure of indigenous phage Tk1 was examined by electron microscopy after negative staining with phosphotungstic acid. This revealed that phage belonged to group B1 in the phage taxonomic classification of Bradley (1967), a small isometric type with an average head size of 53 nm and 130 nm noncontractile, flexible and regularly striated tail (Fig. 8). The morphology

of this phage is similar to the findings of Huggins and Sandine (1977) who have reported the head size in the range of 56 to 75 nm, tail length of 128 to 305 nm, and tail width of 8 to 14 nm for 38 temperate phages induced from lysogenic strains of *S. cremoris, S. Lactis* and *S. lactis ssp. diacetylactis.*

The starter culture for production of dahi is a heterogenous mixture of lactic acid bacteria. However, there is a large variation in the quality of the final product because crucial parameters such as milk quality, starter composition and the quality and quantity of inoculum and incubation temperature during the preparation are not appropriately monitored. This may add to the chances of starter culture failure due to phage contamination. However, the effects of phage contamination may not necessarily result in complete batch failure or an inferior product quality, because of to the practice of using multiple strain starters.

Bacteriophages TK1, RK5, KS1 and KS2 were isolated from the indigenous whey samples of dahi against *L. lactis subsp. diacetylactis.*

Plaque sizes are presented in Table 15. All these phages formed clear plaques on their indicator strain. The observed variation for plaque sizes could be attributed to different types of phages (Luria *et al.*, 1978).

The infection cycle of indigenous isolated phage KS1 in M17 broth was characterized by its one-step growth kinetics are shown in Fig.9:1. Similar curves were obtained with the other phages. The latent period, rise period and burst size of these phages are listed in Table 9:2. It was observed that though all these phages had the same host but exhibited slightly different latent period, rise period and burst size. Latent period ranging from 32 to 56 min and burst sizes from 2 to 125 have been reported to lactococcal phages (Neve and Teuber, 1991; Powell and Davidson, 1985). The phage KS1 had a shorter latent period and a large burst size followed by Tk1, KS2 and RK5 respectively. The difference may be that it is very difficult to obtain an exact correlation between

colony forming units and the number of cells in chain forming micro-organisms like lactic *streptococci* (Loof *et al.*, 1983).

It was further noted that the latent period of our phages were much shorter whereas their burst size was much larger than those reported. This can be due to the use of M17 medium for the growth and propagation of lactic *streptococci* and their phages. Similar views are expressed by Masud *et al.*, (1996). Moreover, shorter latent period or more liberation on burst or both are the characterization of virulent phages which can completely destroy the starter culture even present in low concentration (Lawrence *et al.*, 1976). The differences observed in their growth curve among the examined phages may be due to the disparity in their individual mode of replication.

All these bacteriophages were stable at 4°C no loss of titer was observed during 6 months storage. These phages were partially inactivated by $CHCl_3$ in 24h (Table 16). The effects of temperature and pH on these phages are shown in table 3. None of these phages was inactivated by temperature upto 40°C in one hour and all the phages except RK5 were completely inactivated by 60°C in one hour. Different studies reported that temperature effect the multiplication of bacteriophages. Sozzi *et al.*, (1978) determined the minimum, maximum and optimum growth temperatures for a variety of lactic acid bacteria and their phages, came to the general conclusion that bacteriophages multiplied at temperature where the bacteria actively grew. As the bacterial strain used in this experiment is a mesophilic in nature therefore the phages may also be mesophilic in nature.

The Table (16) further revealed that none of the phages were effected by pH 6, 7 and 8 in 24h. However, these phages were partially inactivated by pH3 in 24h. The phage TK1 and SK5 were remarkably stable at low pH, they were not affected by pH4 in 24h and were only partially inactivated by pH3 in 24h. The possible reason for this difference may be due to starter being used

for the preparation of indigenous dahi is a heterogeneous mixture, therefore, continuous use of such culture may made them resistant to survive at low pH.

For clear morphological distinction of these phages, the following criteria were considered, head diameter, tail length diameter, flexibility and contractility of the tail. All these phages investigated had isometric heads and exhibited non-contractile and flexible or stiff rods (Fig. 10).

It was observed that these phages morphologically are similar to each other and therefore could be distinguished only on the basis of their different head and tail length. The phage KS1 was substantially different from TK1, RK5 and KS2 with respect of their structure and particle size. Phage TK1, RK5 and KS2 were morphological practically identical; head diameter and tail length were 51 ± 2nm and 151 ± 3nm for TK1, 49 ± 2nm and 133 ± 3nm for RK5 and 49 ± 2nm and 135nm ± 3nm for KS2 respectively while for KS1 the value recorded for head and tail is 18.13 ± 2nm and 77.5 nm ± 3nm only.

Capsomeres comprising the head may be seen in all these phages, generally in the form of more or less prominent dark central zones. All these phages have a noncontractile tails 2 to 2.5 times larger than the head diameter and 1 to 10 nm thick, either straight and curved. The overall appearance of these phages particles suggests that these phages could be classified by the system of Bradely (1967) into group B. Other studies (Terzaghi, 1976; Lembke *et al.*, 1980; Relano *et al.*, 1987; Prevots *et al.*, 1990) had also shown that small isometric headed phages (morphotype B1) were predominant among lactococcal phages, ranging from 60 to 80% in France, Germany and New Zealand.

On the basis of these studies it is concluded that the phages present in this region are quite different from those reported elsewhere. Therefore, further study is needed to compare these indigenous phages to the well characterized phages at molecular level in order to generate data which can be used to construct resistant starter culture which can be used throughout the world.

The protein profile of indigenous phages isolated from Pakistan and three standard phages which belong to different groups revealed differences in protein profiles (Figs. 10). Phage P008 showed prominent bands of molecular weight 115Kd, 89Kd, 56Kd, 36Kd, 27.5Kd and 20Kd nearly similar to the protein profile as reported by Raddy (1974). Phage P107 (lane-3) showed peptide bands of 140Kd, 80Kd, 61Kd, 36Kd, 29Kd and 20Kd as weakly stained and two bands of 100Kd and 56Kd that were highly stained. Phage P335 (lane-4) showed strong bands of molecular weight 180Kd, 165Kd, 162Kd, 139Kd, 95Kd, 74Kd, 61Kd, 50Kd, 42.7Kd, 22.7Kd and 14.5Kd. Phage RK5 (lane-5) showed strong bands of 180Kd, 115Kd, 95Kd, 74Kd, 61Kd, 35Kd and 22Kd. Phage Tk1 (lane-6) has strong bands of molecular weight 225Kd, 139Kd, 95Kd, 74Kd, 61Kd, 42.7Kd and 14.5Kd. Phage KS2 (lane-7) has major protein bands of molecular weight 280Kd, 162Kd, 88Kd, 74Kd, 50Kd, 32Kd and 22Kd. Phages KS1, KS2, RK5 and TK1 show some homology to phage P335 but differ from phage P008. Reference phages P008, P107 and P335 which belong to different phage groups show clearly different protein profiles with exception of few similar bands(Braun *et al.*, 1989; Conveny *et al.*, 1987; Jarvis, 1984; Jarvis and Mayer, 1986; Loof *et al.*, 1983; Powell and Davidson, 1986; Powell *et al.*, 1989; Prevots *et al.*, 1990; Raddy, 1974). Lactococcal phages classified within same DNA homology group had similar protein profile (Braun *et al.*, 1989; Daly and Fitzgerald, 1982; Prevots *et al.*, 1990; Relano *et al.*, 1987). Different phage groups revealed different protein profiles as confirmed by our results whereas within the groups all members showed a similar or even identical protein composition (Braun *et al.*, 1989). A protein of molecular weight 27Kd was found in all the tested isolates of Pakistan supposed to be a protein of LC3 which is 26 Kd in molecular weight (Lillehang *et al.*, (1991). Phage P335 is a newly emerging phage first isolated in Germany (Lembke *et al.*, 1980). Thus, from the data obtained so far, it is safe to say that our phage isolates form a distinct group which do not share total homology with any of the three standard phages (P008, P107 and P335) which were

representative of three different groups. The difference may be attributed due to their genetic make up. Similar views are expressed by Conveney *et al.*, (1987).

The western blot analysis of four local isolates RK5, KS1, KS2 and TK1 and three reference phages P008, P107 and P335 has been performed using polyclonal antisera raised against phage P008 (Fig. 12). All phages RK5, KS1, KS2, TK1, P107 and P335 cross reacted at variously. Phage RK5, KS1 and KS2 showed strongest level of antigenicity with three prominent bands of 98Kd, 56Kd and 41Kd, however, phage KS1 is a variant, it has a band of 120Kd strongly stained absent in other phages. Local isolate RK5, KS1 and KS2 share homology with phage P335 with three of its prominent bands 98Kd, 56Kd and 41Kd while phage RK5 and KS2 share one band of 115Kd with P008 and one very faint band of 98Kd. Two bands of molecular weight 56Kd and 41Kd of RK5, KS1 and KS2 were found similar with phage P107. Blot analysis showed that local phages RK5, KS1 and KS2 were more similar to phage P335 than phages P008 and P107, or in other words local phage isolate were not absolutely similar to any of the reference phages which represent three different phage groups. These results correlate well with the amount of proteins visible at these position on SDS polyacrylamide gel after Coomassie blue staining. A different Phage 197 reported by Schouler *et al.*,(1992) showed peptide bands of 25Kd, 35Kd, 45Kd and 46Kd to be immunogenic against *Lactococcus lactis*. The number and intensity of the bands appearing in the immuno blot depend on the phage analyzed. Phage P001 and c6A preparation showed only a very strong band at the position of the 45Kd protein (Schouler *et al.*, 1992). Similarly Kim and Batt (1991) also observed that peptide band of 35Kd, 38Kd, 40Kd and 43Kd of actively reactive bands of bacteriophage F4-1 against *Lactococcus lactis* where 35Kd and 43Kd being the major reactive bands as compared to 38Kd and 40Kd. Correlation between DNA homology group and overall protein composition of the phage has been confirmed by western blotting and ELISA test (Kim and Batt, 1991; Schouler *et al.*, 1992).

Restriction analysis using enzyme EcoR1 of the DNA samples prepared from three local phages TK1, SK2, RK5 and two standard phages P008 and P335 has been presented in (Fig. 1.3). Restriction endonuclease EcoR1 was used because it usually makes more cuts in the genomes of bacteriophages reported by Loof *et al.*, 1983). In our case, EcoR1 cuts TK1 DNA at two sites which generated three fragments of sizes: >21.2Kb, 7.5Kb, 2.55 Kb. The approximate genome size of TK1 calculated from the fragment sizes is thus 31.25 Kb. Genome size of small isometric headed noncontractile bacteriophages has 30Kb (Conveny *et al.*, 1987). DNA of phage KS2 when digested with EcoR1 resulted in four fragments of sizes: >21.2Kb, 9.5Kb, 7.5Kb and 2.4Kb. The fragment of 9.5Kb was visible as a low intensity band may be the result of partial digestion of phage DNA. Genome size of KS2 is approximately >31.1Kb. The total length of genome of phage P008 is 29.7Kb and P335 is 36.4Kb (Loof *et al.*, 1983; Braun *et al.*, 1989). The restriction pattern showed that our local isolates were genetically different from both P008 and P335, as the high mobility bands of all phages differed in size.

Later on phage TK1 was further digested with another restriction endonuclease SAU 3A along with P008 and Sung (Fig. 1.4). It was observed that SAU 3A cleared phage P008 DNA into at least 9 fragments as compared to TK-I. These observations suggest that both the phages are different in their DNA sequences. Moreover, earlier studies reported that DNA's of same Lactococcal bacteriophages are highly resistant to cleavage by many restriction endonuclease (Coveney *et al.*, 1987; Jarvis and Meyer, 1986; Powell and Davidsen, 1986). This effect was also recorded in our experiments. Different authors showed that lactococcal phage classified in the same DNA homology group had similar protein composition (Jarvis, 1984; Loof *et al.*, 1983; Raddy, 1974; Relano *et al.*, 1987).

The restriction endonuclease profiles of bacteriophage TK-I are different from corresponding patterns of all lactococcal bacteriophages previously described(Loof *et al.*, 1983;

Powell *et al.*, 1986; Raddy, 1974). However, the taxonomic significance of such comparison may be limited, since phages that show different restriction enzyme profiles may reveal strong DNA homologies (Jarvis and Meyer, 1986). As found by Daly and Fitzgevold (1982), such lack of correlation between phage restriction profiles and phage DNA homologies may be caused by host-induced modifications of phage-DNA.

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Appendix-A

PROTOCOLS FOR VARIOUS TESTS

1. Measurement of bacterial population

This was carried out by using a modified procedure of Radke-Mitchell and Sandine(1986).

1. Growth was monitored turbidimetrically (Bausch and Lamb spectronic 21) for zero through 8-h.
2. 0.5 ml. sample was added to 4.5 ml. of 0.2% EDTA.
3. pH was adjusted to 12.5 with 10N NaOH.
4. This solution dissolved the casein particals in the milk, leaving turbidity only due to bacterial cells present in the sample.
5. This mixture was read immediately at 410 nm.
6. Generation time (G) at each temperature of incubation was determined using the equation G=0.693/K.
7. Growth rate (K) was determined by equation K=2.303 (log b - log a)/t, where log a and log b are the logarithmic absorbance readings taken during exponential growth and (t) the time minutes.

2. Total titratable acidity (Robinson and Tamime; 1976)

1. 9 ml. of the sample was taken at one hour interval and was titrated against 0.1 N NaOH by using Phenolpthalene as an indicator.
2. The acidity was calculated by the volume of 0.1 N NaOH used for neutralization.

3. Determination of volatile aroma compounds

This was carried out by using a modified procedure of Warsy, (1983).

1. 200 g of samples in each of the two 500 ml conical flask was weighed in, marking one as O_2 and the other as N_2.

2. The pH of O_2 marked flask was adjusted to 3.5 by addition of lactic acid and was fixed for distillation under O_2 gas flow. N_2 marked flask was adjusted to a pH 6.5 by addition of NaOH 33.3% and it was fixed for distillation under N_2 gas flow.
3. Both flasks were heated on a moderately heated electric hot plate.
4. In separate graduated tubes having 5 ml of distilled water, 15 ml distillate was collected. Each tube was kept erect in a beaker full of crushed ice during collection of distillate.
5. The contents of VAC were analysed on gas chromatograph using internal standard methods under following conditions.

Internal standard

1 ml of ethyl methyl ketone (1 mg/ml) solution was added in each tube and after mixing, 1 µ l of the sample was injected in the gas chromotograph.

Gas chromatograph

Perkin-Elmer fractometer model 900, of Perkin Elmer Corporation, Norwalk, Connecticut, USA, was used.

Column

Stainless steel column 2 M long having carbopak 60/80 supporting material, 0.2% carbowax 1600 stationary phase.

Temperature

The temperature of the column was 85°C, of ijector and manifold was 190°C.

Carrier gas

Nitrogen was carrier gas flowing at the rate of 20 ml/minute and detection was by flame ionization.

Peaks of retention time and quantities of VAC were directly recorded by 3380 A, integrator unit of Hewlett Packard of USA.

4. Purification of phages

1. 500 ml of bacteriophage was prepared by adding 10 ml of Phage (10^{11} PFU) with 2.5 ml 1 M $CaCl_2$ in 3 hours grown bacterial broth culture (2% v/v) at 37°C. The multiplicity of infection was approximately 0.01 bacteriophages per cell. With continuous incubation the lysis occurred in three hours.
2. After complete lysis cell debris was removed by centrifugation at 5000 rpm for 20 min.
3. 9% (w/v) solid polyethylene glycol (PEG 6000) and 0.5M NaCl (sodium chloride) were added. This was dissolved by slow stirring on a magnetic stirrer at room temperature.
4. It was left overnight at 4°C or at least for 2 hours to allow the bacteriophage particles to precipitate.
5. The precipitated bacteriophage particles were pelleted by centrifugation at 11,000 g for 10 minutes at 4°C. The supernatant was discarded and the centrifuge bottle were kept in titled position for five minutes to allow the remaining fluid to drain away from the pellet and then fluid was removed by a pipette.
6. Using a wide bore pipette equipped with a rubber bulb, the bacteriophage pellet was gently resuspended in buffer A (pH 7.5): 4 ml for each 500 ml of phage lysate. Wash the walls of the centrifuge bottles thoroughly because the bacteriophage precipitate sticks to them, especially when they were old.
7. Equal volume of chloroform was added to the bacteriophages suspension and vortexed for 30 seconds. The organic and aqueous phases were separated by centrifugation at 1600g for 15 minutes at 4°C. Recovered the aqueous phase containing the bacteriophage.

8. Step gradient with three densities 1.3, 1.5 & 1.7 was prepared in buffer A and then carefully layered the bacteriophage suspension over it.
9. Centrifuged it in SW 50 at 48,000 rpm for 2 hours at 4°C.
10. A bluish band of bacteriophage particles would visible at interphase between 1.3 & 1.5 gm/ml layer. If the yield of bacteriophage is low, placing the gradient against a black background and shining a light from above often helps to detect the band of bacteriophage particles.
11. The bacteriophage particles were collected by puncturing the side of the tube. First placed a piece of scotch tape on the outside of the tube, level with the bacteriophage band, using a 21 gauge needle, puncture the tube through the tube and the band of bacteriophage particles was collected. The bacteriophages were not mixed with material from other bands that were visible with gradient. These bands may consist of various types of bacterial debris and unassembled bacteriophage components or broken tails.
12. The band of bacteriophage particles was collected and stored at 4°C in CsCl in a tightly capped tube.
13. The dialyzing tubing was boiled in Buffer B (pH 7.5) for 15 min. The presence of EDTA diminished any chances of the presence of nucleases. The tubing was filled with bacteriophage band and clipped from both sides.
14. Removed the cesium chloride from the purified bacteriophage preparation by dialysis at 4°C for 1 hour against a 1000 fold volume of buffer C pH. 7.5.
15. The dialysis tube was then transferred to a fresh flask of buffer and dialyzed for an hour again.
16. Purified bacteriophages were stored at 4°C.

BUFFER A

100 mM Tris-Cl

10 mM $MgCl_2$

pH = 7.5, storage at 4°C

BUFFER B

10 mM Tris-Cll

1 mM EDTA

pH = 7.5, storage at 4°C.

BUFFER C

50mM Tris-Cl

10mM $MgCl_2$

10mM NaCl

pH = 7.5, storage at 4°C.

T.E

10 mM TrisCl

1 m MEDTA

pH = 7.5, storage at 4°C.

Cesium chloride solutions for step gradient, prepared in buffer A.

DENSITY	CsCl/100 ml	CsCl/10 ml
1.30	40.615	4 .615
1.50	68.110	6.811
1.70	95.606	9.560

5. Lytic pattern of bacteriophages

The methodology used to determine the lytic pattern of bacteriophages is one which was first adopted by Sander and Klaenhammer, 1980. Before starting the experiment one must know that m.o.i. must not be greater than 0.1. 2.5 ml broth was inoculated with 2% v/v bacterial culture. When O.D reached at 0.26 at 600 nm, add 100 ml phage and 50 ml of 1 M $CaCl_2$ and O.D was recorded immediately. Optical density increased up to 60 to 90 minutes then starts decreasing. The increase / decrease in O.D values were recorded until it become minimum and culture become clear. O.D values were recorded at 600 nm on SHIMADZU sp-UV-120-01 spectrophotometer.

The percentage drop in the O.D Value after host lysis by bacteriophages was calculated by using the given formula:

[O.D of bacterial culture before phage inoculation -]
[O.D of bacterial culture after phage inoculation] X 100
[O.D of bacterial culture before phage inoculation]

A graph between time and O.D Vales was plotted which shows the time required for complete lysis.

6. One step growth experiment

One-step growth curve experiment was conducted according to the procedure of Chow *et al.*,(1988).

1. Bacterial cultures were grown in M-17 broth. Until the optical density at 600 nm reach 0.085.
2. To 0.9 ml of this culture, 0.1 ml of 1 M $CaCl_2$ and 0.1 ml of diluted treated phage was added at a multiplicity of infection of 0.01.

3. The tube were held at room temperature for 10 min. to allow phage absorption.
4. Centrifuged for 3 mins. in a microcentrifuge.
5. Supernatant was discarded.
6. The pellet was suspended in 10 ml of prewarmed M-17 broth.
7. Serial dilutions were made.
8. The tubes were then incubated at 30°C.
9. Sample were titerated at 3 to 5 min. intervals on double layers techniques (Terzaghi and Sandine, 1975).
10. The burst size was determined by dividing the average count from the latent period into the average count from the rise period.

7. Electron microscopy

1. Carbon coated copper grid (400 mesh x 3.05 mm) was soaked in 20 ml of purified phage for 1 minute, removed the excess phage.
2. Transferred the grid in 1% Phosphotungstic acid (PTA) or 2% Uranyl acetate (UA) for 1 minute.
3. Excess strain was removed through filter paper.
4. The grids were then examined with JEOL TEM 100 CXII electron microscope at accelerating voltage of 80 KV.
5. The micrographs were taken at magnification of 53,000 and 80,000.

8. Sample preparation of bacteriophage proteins

1. The method of Braun *et al.*, (1989) was used for the preparation of protein samples.
2. Purified phage particles were dialyzed against double distilled water and were suspended in an equal volume of sample buffer and 5% β.mercaptoethanol.
3. The phage particles were disintegrated by boiling at 100°C for 5-7 minutes.
4. After 5 mins. of incubation on ice, the protein samples were centrifuged at 10,000 rpm for one mins. to pellet out the debris.

9. SODIUM DODYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

SDS-PAGE was carried performed as desrcibed by Laemmli (1970) in minigels and is outlined below:

1. Glass plates were cleaned thoroughly and wipped with 70% athenol.
2. Acrylamide, tris buffer, SDS and water were mixed and degassed for 10-15 mins, then ammonium persulphate and TEMED were added.
3. Minigel apparatus was set up and separation gel was poured avoiding any gas bubble.
4. It was overlaid with aqueous saturated butanol and allowed to polymerize for at least 3 hours.
5. Butanol was washed first with water and then 3 X with tris buffer.
6. Stracking gel was poured as the separation gel.
7. Combs were placed slowly avoiding any air bubbles under the combs.
8. It was layered with aqueous saturated butanol and allowed to polymerize.
9. After washing with chamber buffer, the samples were added and run at the desried voltage.
10. Gel was removed after electrophoresis and stained.

Coomassie blue stain

Isopropanol	250.0 ml
Acetic Acid	100.0 ml
Coomassie blue R-250	0.5 gm
Water ad.	1.0 liter

Destain

Isopropanol	250.0 ml
Acetic Acid	100.0 ml
Water ad.	1.0 liter

Stained the gels overnight destained to a clear background. The gels were stored in 5 to 10% acetic acid.

Reagents for SDS-PAGE

1. Acrylamide-Bisacrylamide (30.3%)

Acrylamide	30.0 g
Bisacrylamide	0.8 g
Water ad.	100.0 ml

Filtered and stored at 4°C in a brown bottle.

2. 1 M Tris HCl pH 6.8

Trizma bass	2.42 g
Water ad.	20.00 ml

pH was adjusted with 1 N HCl.

3. 1.5 M Tris HCl pH 8.7

Trizma bass	18.2 g
Water ad	100.0 ml

pH was adjusted with 1 N HCl.

4. Sample preparation buffer

1 M Tris pH 6.8	0.625 ml
B-mercaptoethanol	0.500 ml
Glycerol	1.00 ml
SDS	0.230 ml
0.1 % bromophenol blue	0.100 ml
Water ad	10.00 ml

5. Chamber buffer

Trizma bass	30.29 g
Glycine	144.20 g
SDS	10.00 g
Water ad	1.00 ml

Diluted 1:10 before use.

RESOLVING AND STACKING GEL MIXTURE

CONTENTS	STACKING GEL	RESOLVING GEL	
	5%	12%	15%
	3 ml	5 ml	5 ml
WATER	2.1	1.6	1.1
30% Bis-Acrylamide	0.5	2.0	2.5
1.5 M Tris.Cl (pH 8.8)	-	1.3	1.3
1.0 M Tris.Cl (pH 6.8)	0.38	-	-
10 % SDS	0.03	0.05	0.05
10 % APS	0.03	0.05	0.05
TEMED	0.003	0.03	0.03

10. Generation of polyclonal antisera

1. The method of Schouler *et al.*, (1992) was used to generate polyclonal antisera against bacteriophage P008 (10^{12} PFU/ml), after being emulsified in an equal volume of Freud's complete adjuvant.
2. The emulsion was intradermaly injected at 10 different sites on the lower back of the rabbit, using 100 ml inoculum per dose (Harlow and Lane, 1988).
3. After 2 weeks, a second injection (booster dose)of antigens in Freud's incomplete adjuvant, were given in a similar way.
4. Rabbits were bled after a week (8 days after last inoculation).
5. The blood (5 ml) was incubated at 37°C for 30 minutes and then centrifuged at 3,000 rpm for 15 minutes, at 4°C.
6. Serum was carefully taken using a pasteur pipette and stored at -20°C.

11. Western blotting

1. SDS-PAGE was performed (Laemmli, 1970) in a mini vertical slab gel apparatus (Bio-Rad Mini PROTEIN-II).
2. The gel was washed 3 times in transfer buffer (TB) for 20 minutes each.
3. Western blotting was carried out by direct electrophoretic transfer of proteins from the gel to a nitrocellulose filter by the method of Burnette, 1981.
4. Electrophoretic transfer of proteins from polyacrylamide gel to nitrocellulose paper (NCP) was accomplished in an Electrophoretic Transfer Cell (Mini Trans-Blot, BIO-RAD) by means of the transfer buffer.

The protocol steps are given below:

IST DAY

1. Soak gel 3 times in TB X 20 min.
2. Cut two whatman filters (WF) of the size of Fiber pad and one NCP of the size of the gel,(mark the orientation of these filters and NCP).Soak WF and NCP in TB for 30 min. Set up the apparatus with grey slide (cathode) of gel cassette holder down as follows (a)Fiber Pad (b) WF (c) SDS gel(d) NCP (e) WF (f) Fiber Pad. Close apparatus and run at 100 for 1 hour.
3. Remove NCP and incubate in TBSB at room temperature for 1 hour.
4. Wash 3 X TBST for 5 min.
5. NCP incubate in 1^{o} antibody + TBST overnight at $4^{o}C$.

2ND DAY

6. Wash 3 X TBST for 5 min. Incubate in TBSB + 2^{o} antibody, i.e. anti-rabbit IgG peroxidase-Conjugated in the ratio of 1:1000 at room temperature for 2 hours.
7. Wash 3 X TBS for 5 min.
8. Add developer and wait for the colour.
9. Wash with D.H_2O to stop the reaction.
10. Dry NCP and photographed.

Buffers for western blotting

TRANSFER BUFFER (TB) pH 8.3,

25 mM TrisCl	3.03 gm
Glycine	14.40 gm
0.192M Methanol	200 ml
D.H_2O Make up to	1000 ml

TRIS BUFFER SALINE (TBS)pH 7.5

20 mM Tris-Cl	2.42 gm
50 mM NaCl	29.22 gm
D.H_2O Make up to	1000 ml

TBST

TBS	99ml
Tween-20	1ml

TBSB

TBS	100 ml
BSA	0.4 gm

DEVELOPER

4-Chloro-1-Naphthol	0.015 gm/5ml CH_3OH
10 mM TrisCl	0.03 gm/25 ml D.H_2O
30% H_2O_2	15 ml(add just before use).

12. Phage DNA preparation

1. In 2 ml of phage suspension 80 µl 0.5 M EDTA, 52 µl proteinase -K (20 mg/ml) and 108 l 10% SDS were added and incubated at 55°C for 90 min.
2. Equal volume phenol (pH = 8.0) was added in above mixture. Mixed it gently for 5 minutes, kept on ice for 5 minute, and centrifuged at 10,000 rpm for 10 minutes. Extract aqueous layer in a separate eppendorff tube, without disturbing protein layer.
3. Equal volume of chloroform : isoamyl alcohol (24 : 1) was added in aqueous layer, mixed it gently for 5 minutes, then kept on ice for 5 minutes. Centrifuged it at 10,000 rpm for 10 minutes. Aqueous layer was extracted with blunt end tip, in separate eppendorff, without disturbing protein layer.
4. DNA was precipitated by adding 1/10 volume of 3M sodium acetate and 2 volumes of absolute ethanol. Mixed gently otherwise DNA will shear. Kept it at -20°C overnight.
5. Next day centrifuged it at 3500 rpm for 30 minutes at 4°C or 10,000 rpm for 10 minutes in microfuge to pellet DNA.
6. DNA pellet was washed with 1 ml 70% ethanol and removed any traces of sodium acetate. Mixed the content gently and centrifuged it at 3500 rpm for 20 minutes at 4°C. Discard the supernatant.
7. DNA pellet was vaccum dried and then resuspended in 500 µl of T.E (10mM Tris Cl, 1 mM EDTA pH 8.0).Mixed it gently.
8. 2 ml RNase (10 µg/ml) was added in DNA suspension and incubated it at 37°C for 90 minutes.
9. The aqueous phase was extracted with equal volume of phenol pH 8, as in step 2.
10. The aqueous phase was extracted with equal volume of chloroform : isoamyl alchol (24:1), as in step 3.

11. The DNA was precipitated by adding 1/10 volume of 3M. Sodium acetate and equal volume of isopropanol or 2 volume of absolute ethanol. Kept it -70°C for 10 minutes.
12. Centrifuged it at 10,000 rpm for 10 min to pellet the DNA.
13. Washed the pellet with 500 ml of 70% ethanol, mixed and then centrifuge it at 10000 rpm for 10 minutes.
14. DNA pellet was vaccum dried and then resuspend in 50 µl T.E (10 mM Tris-Cl mM EDTA) pH = 8.0. Kept it at -20°C overnight.
15. DNA concentration was calculated by using 2,3 wavelength programme for measuring concentration of nucleic acid by using formula :

O.D at 260 nm x correction factor x dilution factor = x µg/ml.

Correction factor = 50 and

Dilution factor = 50 (294 µl H_2O + 6 µl DNA suspension).

13. DNA digestion with restriction endonuclease (EcoR 1)

1. Three ml of each DNA sample was digested with EcoR1.

   Typical reaction was:

D.H_2O	x µl
Reaction buffer H	2 µl
DNA	x µl (equivalent to 3 µg)
Enzyme	1 µl
Total volume	20 µl

2. Incubated it at 37°C for two hours or overnight. Reaction was stopped by adding 5 µl gel loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol).

3. Heated it at 65°C for 10 min, briefly centrifuged (30 second) and loaded to 1% agarose gel.
4. Gel has run at 50 volts for 4 hours.
5. Then stained the gel for 30 minutes in 15 µl of ethidium bromide (10 mg/ml) in 300 ml of deionized water.
6. Observed the gel under UV light and photographed with Polaroid camera.
7. Fragment sizes of digested DNA was calculated against standard curve of marker BOEH. MANNHEIM III on log paper.