LABORATORY STUDIES ON RESISTANCE IN *Plutella xylostella* (L.) TO *Bacillus thuringiensis*, SPINOSAD AND CARTAP HYDROCHLORIDE

By

RAJ KUMAR ARORA

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IN

ENTOMOLOGY

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CERTIFICATE - I

This is to certify that this dissertation entitled **"Laboratory studies on resistance in *Plutella xylostella* (L.) to *Bacillus thuringiensis*, spinosad and cartap hydrochloride"** submitted for the degree of **Doctor of Philosophy,** in the subject of **Entomology,** of the Chaudhary Charan Singh Haryana Agricultural University, Hisar is a bonafide research work carried out by **Shri Raj Kumar Arora** under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation has been fully acknowledged.

3/4/99

(V.K. Kalra)
Major Advisor
Senior Entomologist,
Department of Entomology,
CCS Haryana Agricultural University,
Hisar

CERTIFICATE - II

This is to certify that the dissertation entitled **"Laboratory studies on resistance in *Plutella xylostella* (L.) to *Bacillus thuringiensis*, spinosad and cartap hydrochloride"** submitted by **Shri Raj Kumar Arora** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar in partial fulfilment of the requirements for the degree of **Doctor of Philosophy**, in the subject of **Entomology**, has been approved by the Student's Advisory Committee after an oral examination on the same in collaboration with an External Examiner.

Vinod Kumar 11/6/99
MAJOR ADVISOR

11/6/99
EXTERNAL EXAMINER

Chauhan 11/6/99
HEAD OF THE DEPARTMENT

11/6/99
DEAN, POST-GRADUATE STUDIES

WITH AFFECTION,
RESPECT AND GRATITUDE
TO
MY FATHER
SHRI MADAN LAL ARORA

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CHAPTER-I

INTRODUCTION

Diamondback moth, *Plutella xylostella* (L.) (Plutellidae : Lepidoptera) is an important oligophagous pest of both wild and cultivated crucifers including oilseed and vegetable crops (Talekar and Griggs, 1986). It is cosmopolitan in distribution (CIE, 1967), particularly in south-east Asia (Robert and Wright, 1996).

In India, *P. xylostella* was first recorded by Fletcher (1914) on crucifer vegetables. The perusal of reports from across the country advocates it as one of the major constraints in the profitable cultivation of cole crops (Abraham and Padmanaban, 1968; Singh and Singh, 1982).

The pest occurs in an endemic form with high population densities on early (August to October) and late (February to April) sown cauliflower (Butani *et al.*, 1977). In case of severe infestation, the growing hearts are also damaged affecting the production of marketable curds (Panwar, 1995).

The control of *P. xylostella* has depended primarily and extensively on the use of insecticides recommended from time to time (Beri, 1958; Verma and Sandhu, 1968; Sudderudin and Kok, 1978; Liu *et al.*, 1982). However, the indiscriminate use of a number of commercial insecticides has led to the development of resistance in this insect in most countries of south-east Asia (Georghiou, 1981). In India, the preponderance of the reported resistance in this insect has increased due to the pronounced cultivation of early varieties of cauliflower with intensive use of conventional insecticides in perspective of higher value of the crop during off-season (Bhatia, 1988; Mehrotra, 1989). By and large, one of the counter measures suggested to combat the problem of resistance is the introduction and change over to the newer eco-friendly and more potent insecticides.

Recently, *Bacillus thuringiensis* and cartap hydrochloride have been found effective against *P. xylostella* (Kulkarni *et al.*, 1995; Kumar, 1996; Nagesh and Verma, 1997) and are, thus, in the process of being recommended to control it. Spinosad is newly introduced, environment friendly biopesticide which is effective against many borers and leaf eaters (Sparks *et al.*, 1996). The use of these pesticides against *P. xylostella* on cole crops is expected to increase many fold due to their different chemical nature, mode of action and low or practically no mammalian toxicity.

Bacillus thuringiensis (*B.t.*) is an aerobic, gram positive, spore-forming soil bacterium found commonly in the environment. It produces a number of insect toxins, the most distinctive of which are protein crystals formed during sporulation (Entwistle *et al.*, 1993). After proteolytic activation in the insect midgut, *B.t.* toxins bind to the brush border membrane of the midgut epithelium and produce pores that cause cells to swell and lyse (Gill *et al.*, 1992).

Spinosad is a mixture of two most active naturally derived factors spinosyn A and spinosyn D from a new species of soil bacterium, *Saccharopolyspora spinosa*, which acts both as contact and stomach poison (Anonymous, 1998). Intoxicated insects exhibit tremors characterised by continuous movement of mandibles and constant flexing of crochets on the pseudopodia.

Cartap hydrochloride is a derivative of nereistoxin, a naturally occuring insecticidal substance isolated from the marine segmented annelids, *Lumbrineris* (*Lumbriconereis*) *heteropoda* and *L. breviccira* (Sakai, 1969).

It kills insects by blocking ganglionic transmission through competitive binding to receptors of neurotransmitters in the central nervous system.

Though commercial use of these insecticides, except spinsosad, for the past many years across the world has resulted in the development of resistance in *P. xylostella* (Tabashnik *et al.*, 1990; Ferre *et al.*, 1991; Hama, 1992; Shelton *et al.*, 1993), yet the information on this aspect is not at hand from our country. Therefore, a need to quantify the development of resistance by *P. xylostella* to these chemicals was realised to generate base-line data with the aim of formulating strategies to manage this pest. A project was, thus, proposed with the following objectives:

1. To study the potential of development of resistance in diamondback moth to *Bacillus thuringiensis* var. *kurstaki*, spinosad and cartap hydrochloride.
2. To study the pattern of pesticide resistance and development of cross-resistance to other commonly used pesticides.
3. To study the biology of pesticide resistant *vis-a-vis* susceptible strain of this pest.

CHAPTER-II

REVIEW OF LITERATURE

Housefly, *Musca domestica* was the first insect to show fairly good degree of resistance to DDT in 1947 (Wilson and Gahan, 1948). So far, 504 species of insect-pests of public health, household and agricultural importance have shown resistance to almost all the groups of insecticides developed so far (Pimentel, 1993). Diamondback moth (DBM), *Plutella xylostella* (L.) was reported to be the first crop pest in the world to have developed resistance to DDT (Ankersmit, 1953; Johnson, 1953). The selection pressure caused by intensive chemical use against this pest resulted in the development of resistance to almost all the commonly used synthetic insecticides in different parts of the world (Verma and Sandhu, 1967; Chawla and Kalra, 1976; Sudderudin and Kok, 1978; Liu *et al.*,1982; Ho *et al.*, 1983; Tabashnik *et al.*, 1987; Hama, 1986). Hence, the farmers had to turn to the use of biopesticides where the chances of development of resistance were thought to be remote, but,of late,reports have appeared in the literature regarding the development of resistance to some of the biopesticides too. It is hypothesised that resistance develops at different rates between species and even between populations of the same species due to genetic, reproductive, behavioural/ecological and operational factors (Georghiou and Taylor, 1977a and b; Georghiou, 1980, 1983; Wood and Bishop, 1981). Since insecticides are only a part of resistance development process, if a laboratory selection is negative and no resistance develops, there is no guarantee that resistance will not develop in the field later (Pal and Brown, 1971). The related literature pertaining to the development of resistance, cross-resistance and biology

of *P. xylostella* due to selection pressure of *B.t.*, spinosad and cartap hydrochloride is reviewed hereunder :

2.1 Resistance to *B.t.*, spinosad and cartap hydrochloride

2.1.1 *B.t.*

The first commercial formulation of *B.t.* var. *kurstaki* was made available in 1938 in France (Weiser, 1986). However, the world war II impeded the expansion of work on its further development. Later, Thuricide was launched in the United States following the studies made by Steinhaus (1956).

In 1962, a subspecies *kurstaki* of *B.t.* was isolated in France (Kurstak, 1962) which contained δ-endotoxins (Hofte and Whiteley, 1989). These toxins offered safe and effective alternatives to conventional insecticides (Entwistle *et al.*, 1993) and were related harmless to beneficial arthropods including natural enemies (MacIntosh *et al.*, 1990). For the past more than two decades, the commercial use of *B.t.* var. *kurstaki* was considered reliable and chances of development of resistance to it were presumed to be remote because of its unique mode of action by various workers (Kreig and Langenbruch, 1981; Brunner and Stevens, 1986; de Barjac, 1987). However, a number of reports based on field trials have indicated reduced efficacy of *B.t.* to *P. xylostella* and apprehended the development of resistance (Kirsch and Schmutterer, 1988). Later, Tabashnik *et al.* (1990) while working in Hawaii, USA confirmed, presumably for the first time, that diamondback moth had developed substantial levels of resistance to *B.t.*

Resistance to *B.t.* was also reported in field populations of *P. xylostella* from Phillipines, Thailand, Malaysia and Japan and some other parts of USA (Jansson and Lecrone, 1990; Sinchaisri *et al.*, 1990; Gill *et al.*, 1992; Shelton and Wyman, 1992; Syed, 1992). During a survey conducted in USA, one of *P. xylostella* populations with *B.t.* selected upto 9 generations showed reduced efficacy, while another population collected from Hawaii, on the contrary, showed a reduction in the LC_{50} value (24 to 11 mg a.i./l) after selection upto five generations (Tabashnik *et al.*, 1992). Similarly, no resistance was detected by Yu and Nguyen (1992) while working in Florida, USA.

Shelton and Wyman (1992) screened eleven populations of *P. xylostella* from different states of USA (including one from Indonesia). As per their results, only 2 populations i.e. from New York and Florida showed high levels of resistance to *B.t.* Similarly, other workers also observed many-fold resistance in this pest to different formulations of *B.t.* (Jansson, 1992; Shelton *et al.*,1993). However, in general, the reports of many studies have shown significantly higher efficacy of *B.t.* in comparison to other synthetic insecticides particularly pyrethroids (Adams,1992; Chalfant,1992; Wyman, 1992; Leibee and Savage, 1993).

B.t. had been recommended as an essential component of IPM for *P. xylostella* in central America, atleast till early 1990s' (Andrews *et al.*, 1992), although the farmers did prefer the use of synthetic pyrethroids. However, Perez and Shelton (1997), while monitoring populations of *P. xylostella* for resistance/susceptibility to *B.t.* subspecies, disclosed that out of 18 populations collected from different states of central America,

14 had evolved resistance and LC_{50} values of Guatemala, Honduras and Costa Rica ranged between 4.3 to 18.3, 9.3 to 77.2 and 13.3 to 19.5 - fold higher, respectively, than the susceptible population from New York.

B.t. was introduced in Japan during 1981 and was used as an alternative insecticide for the control of *P. xylostella* (Hama, 1992). Resistance to *B.t.* was observed in some field populations of DBM. However, in the surveys conducted around Wakayama prefecture (Japan), certain populations of this pest were found to have developed resistance to *B.t.* by late 1980s' (Morishita and Azuma, 1987). Few other similar reports are also available in the literature from other parts of Japan (Tanaka and Kimura, 1991; Hama *et al.*, 1992; Tanaka, 1992). Hama (1992) was of the opinion that although a high level of resistance to *B.t.* was detected in Japan, yet the development of resistance was not fast, hence attributable to an incompletely recessive, autosomal allele.

In Malaysia, insecticide resistance in DBM was first reported by Ho (1965). Ho and Ng (1970) further observed that microbial insecticide, *Bacillus thuringiensis* was very effective in controlling this pest. In the low lands, Mohamad *et al.* (1979) obtained double yield of cabbage by using *B.t.* than control plots. The serious outbreak of DBM in late eighties was attributable to its resistance to *B.t.* in the areas of its extensive use (Syed, 1992). The LC_{50} (4.5 µg a.i./ml) and resistance ratio (112.5) were particularly higher in Cameron highlands as compared to those observed in lowlands by Iqbal *et al.* (1996).

Field evaluation of *B.t.* formulations in Thailand, where it was introduced in 1972, showed very promising results particularly in northern parts in the beginning (Rushtapakornchai *et al.*, 1980). However, in late 1980s' and early 1990s', some populations of this pest showed decreased susceptibility (Kobayashi *et al.*, 1992). Kuwahara *et al.* (1995) visualised that the insect had recovered its sensitivity to the conventional insecticides mainly due to the use of *B.t.* as an alternative insecticide.

Sun (1992) reviewed the insecticide resistance in DBM and anticipated that resistance to *B.t.* in *P. xylostella* could also occur in China, where this pesticide was introduced in 1984 (Sun *et al.*, 1986). However, Zhou and Ma (1993) while working in laboratory found DBM to be sensitive to *B.t.*, although two populations did show resistance. The resistance in *P. xylostella* to *B.t.* was endorsed by Shuai *et al.* (1994) in Guangdong Valley population and it was observed to be 5.7-fold in Shangai areas (Zhao *et al.*, 1996). Resistance in *P. xylostella* to *B.t.* was also recorded to the extent of 24-fold in Korea (Chou and Lee, 1994).

Inspite of the enormous reports of resistance to *B.t.*, there are few other reports which show that *P. xylostella* has not evolved resistance to this pesticide, as yet. In New Zealand, Cameron *et al.* (1997) observed two populations of this pest to be highly susceptible as compared to a resistant population from North America. Cameron and Walker (1998) also pointed out that tolerance level of *P. xylostella* to *B.t.* was relatively very low as compared to conventional insecticides. Likewise, four populations of this pest collected from central Chile did not show resistance to *B.t.* and the resistance factor was 0.56 to 1.0 only (Garrido and Arraya, 1997).

Similar results were obtained by Campos *et al.* (1997) in Brazil. Kranjajic *et al.* (1997) was of the view that the use of *B.t.* could be safely integrated with biocontrol agents to control *P.xylostella* and some other cabbage pests in Yugoslavia.

In India, a number of studies on *P. xylostella* have indicated that its susceptibility has decreased considerably to conventional synthetic insecticides (Verma *et al.*, 1972; Deshmukh and Saramma, 1973; Saxena *et al.*, 1989; Chawla and Joia, 1992; Raju and Singh, 1995; Kalra *et al.*, 1997). However, there are no reports on the development of resistance to *B.t.* Field experiments with *B.t.* formulation (Thuricide EC) initiated by Atwal and Singh (1969) in India afforded 83.2 per cent larval mortality of DBM after one week but Narayanan *et al.* (1970) reported 100 per cent larval mortality in laboratory, green house and field experiments within 36 hours. Singh *et al.* (1976) observed *B.t.* (Dipel WP, $16x10^3$ IU/mg) as the second best insecticide (96% mortality) after quinalphos to control *P. xylostella.* More than 96% mortality of this pest was also observed within 7 days after *B.t.* spray in pot culture experiments (Varma and Gill, 1977). Similarly, effective control of this pest insect was obtained with *B.t.* formulations under field conditions (Rajamohan and Jayraj, 1978; Kulkarni *et al.*, 1995; Nagamani *et al.*, 1995).

In laboratory experiments, *B.t.* was found highly toxic to two different populations of *P. xylostella* with LC_{50} values of 350.8 and 378.5 ppm in Tamil Nadu (Rabindra *et al.*, 1995). Kumar (1996) also observed that *B.t.* was highly effective against *P. xylostella* even at much lower doses than those suggested by formulators. He generated an additional

information that besides stomach action, *B.t.* also showed mortality due to contact action among larval stages, reduction in fecundity, hatchability and pupation. The bioassay in the laboratory and field experiments by Dilawari *et al.* (1996) with crystal endotoxins from HD-1 and HD-73 strains of *B.t.* var. *kurstaki* exhibited that Cry IA (a), Cry IIA encoded protein toxins (HD-1) and Cry IA(c) (H-73) were all toxic to the larvae of *P. xylostella.* Chromatographic analysis and lead dip bioassays have also confirmed that *P. xylostella* was most sensitive to *B.t.* var. *kurstaki* in India (Meenakshisundaram and Gujar, 1998).

2.1.2 Spinosad

Spinosad, a secondary metabolite of *Saccharopolyspora spinosa*, a soil bacterium, is relatively a new suspension concentrate formulation, registered in the USA as Tracer®Naturlyte Insect Control and recommended mainly for the control of cotton pests (Sparks *et al.*, 1996a). So far, no information is available in the literature on the development of resistance in *P. xylostella* to this product. However, literature pertaining to its technical development and control of other insect- pests is reviewed.

Spinosad is a water based product and contains no volatile organic solvents which make it more environmental friendly. Its physical and technical properties have been reviewed by Burton *et al.* (1996) and Thompson *et al.* (1996), respectively. Borth *et al.* (1996) inferred that spinosad is safe to the environment, farm workers and human diets. It was also found safe to coccinellids (Murray and Lloyd, 1997) but selective to hemipteran predators (Boyd and Boethel, 1998). It is also fairly

compatible with other insecticides (Johnson *et al.*, 1997b). According to Sparks *et al.* (1996a), out of more than 20 spinosyns (A-Y) identified so far on the basis of their chemical structure, spinosyn A was the most active principal in spinosad. Toxicological investigations showed that it was very active by injection but slow acting when applied topically to *Heliothis virescens* larvae (Sparks *et al.*, 1997; 1998).

A number of reports revealed excellent control of cotton pests *viz.*, *Heliothis armigera, H. virescens, H. zea, Spodoptera exigua* and *Lygus lineolaris* with spinosad (Mascarenhas *et al.*, 1996; Nead *et al.*, 1996; Porteous *et al.*, 1996; Sparks *et al.*, 1996b; Allen *et al.*, 1997; Johnson *et al.*, 1997a; Leonard *et al.*, 1997; Teague and Tugwell, 1997). Application of spinosad (0.02%) kept the population of citrus thrips, *Chaetanaphothrips orchidii* under check for ten weeks in a grape-fruit orchard (Tzur *et al.*, 1998). In laboratory studies, the LC_{50} values of spinosad ranged between 2 to over 5 ppm against *S. exigua, H. virescens, H. zea, Scirtothrips citri, Ceratitis capitata* (Adan *et al.*, 1996; Leonard *et al.*, 1996; Hasty *et al.*, 1997; Khan and Morse, 1997a and b; Lopez *et al.*, 1997) and 4.19 to 13.46 ppm against 10 different strains of *Pseudoplusia includens* (Mascarenhas and Boethel, 1997).

2.1.3 Cartap hydrochloride

It was first isolated from the marine segmented worms *Lumberineris heteropoda* and *L. breviccira* in 1934 and possesses a peculiar mode of action i.e. a post-synaptic blocker of the central nervous system (Sakai, 1969). In Japan, it is one of the chemicals recommended for the control of *P. xylostella* and is being used in over 30 per cent of total

crucifer growing areas (Sakai, 1986). After its intensive use in field for 15 years, Hama (1983) observed that *P. xylostella* was 7.5 times harder to be killed by cartap hydrochloride as compared to the susceptible strain. On the contrary, it was observed that inspite of 20 years of its use, high levels of resistance to this tertiary amine had rarely occured in Japan (Sakai, 1986; Kimura, 1989). However, in Kohno areas (Osaka prefecture) of Japan, a substantial increase in LC_{50} values (22 to 313 ppm) of cartap hydrochloride was reported by Sakai (1986). Similarly, higher LC_{50} values (500-1000 ppm and upto 1100 ppm) have also been worked out (Horikiri, 1989; Ozawa *et al.*, 1989, Hama *et al.*, 1990). Leaf dip bioassays with cartap hydrochloride against *P. xylostella* population from 10 locations in southwest Japan revealed LC_{50} values of 23.9 to 102 ppm (in 8 cases) and 272 to 403 ppm (in 2 cases) (Morishita *et al.*, 1992). Likewise, Murai *et al.* (1992) and Adachi and Futai (1992) recorded moderate resistance to this chemical compound in some of the population of this pest insect. Moreover, a 46-fold resistance in *P. xylostella* population has also been recorded with this pesticide in Japan (Hama *et al.*, 1992).

Reports from Taiwan also suggest that *P. xylostella* had acquired varying degrees of resistance to cartap hydrochloride, though in some cases, it was observed to be quite mild (Cheng, 1981a and b; Liu *et al.*, 1982; Cheng, 1986; Cheng *et al.*, 1992).

In China, selection with cartap hydrochloride for 35 generations resulted in the development of 25-fold resistance in *P. xylostella* (Chen *et al.*, 1993) and upto 87-fold resistance when selected for 80 successive generations (Chen *et al.*, 1995). A Korean population of DBM exhibited

9.1-fold resistance after 8th generation of selection with cartap hydrochloride (Cho and Lee, 1994). Branco and Gatehouse (1997), however, did not detect any resistance in this insect to cartap hydrochloride in Brazil during laboratory bioassays.

In India, cartap hydrochloride was introduced in 1987 and an effective dosage of 200 g a.i./ha was recommended for the control of *P. xylostella* (Peter *et al.*, 1989). Rai *et al.* (1992) recorded 87 percent mortality of pyrethroid-resistant *P. xylostella* with the application of cartap hydrochloride at 700 g a.i./ha whereas Kumar (1996) observed 95 percent mortality at 500 g a.i./ha. In a recent study, cartap hydrochloride at 0.05% afforded 85.47 per cent larval mortality of *P. xylostella* in cabbage fields (Nagesh and Verma, 1997). It has also been found to be highly toxic to multi-resistant population of *P. xylostella* in Punjab with LC_{50} values of 0.015 to 0.02 per cent (Joia and Udeaan, 1997).

2.2 Cross-resistance to commonly used insecticides in *Bacillus thuringiensis* var. *kurstaki*, spinosad and cartap hydrochloride-selected strains of *P.xylostella*

Cross-resistance enables resistant species to survive exposure to chemically related insecticides and generally results from a common detoxification pathway or a change in susceptibility to a common biochemical/physiological lesion (Metcalf, 1980).

2.2.1 *B.t.*-selected strain

As per the available literature, in general, there is a lack of cross-resistance between *B.t.* and conventional insecticides resistant strains of *P. xylostella*. This has been explained on the modes of action of different

groups of insecticides (Stone *et al.*, 1991). Various studies conducted on this subject with this pest insect have shown almost similar results. *P. xylostella* exposed to *B.t.* for 8 generations exhibited no cross-resistance to triflunuron, Lambda-cyhalothrin, prothiophos and cartap hydrochloride (Cho and Lee, 1994). Similar observations were made by other workers too, while working with fenthoate, benfuracarb, fenvalerate, chlorfluazuron, cartap hydrochloride and abamectin (Tabashnik, 1994; Sarnthoy *et al.*, 1997).

2.2.2 Spinosad-selected strain

Since spinosad is a recently discovered insecticide, there is no information available in the literature on its cross-resistance in *P. xylostella* to commonly used insecticides. The mode of action of spinsosyn A is distinct from that of organochlorine, pyrethroids, carbamates, organophosphates, cyclodienes, fiproles, pyrroles, avermectins, formamidines, neonicotinoids, acylureas and any other known insect control agent (Sparks *et al.*, 1998). Spinosyn A acts by altering the function of nicotinic acetylcholine and possibly γ-aminobutyric acid (GABA) gated chloride channels (Salgado *et al.*, 1997) which is different from all other known insecticides. Thus, even resistant strains possessing enhanced levels of mono-oxygenases or glutathion-S-transferases have not shown any cross-resistance to spinosyn A or spinosad (Sparks *et al.*, 1995).

2.2.3 Cartap hydrochloride-selected strain

The cartap hydrochloride-selected strain for 12 generations of DBM, when exposed to 20 commonly used insecticides of different groups, exhibited low cross-resistance to fenvalerate and deltamethrin having 6.47

and 4.89 resistance ratios, respectively (Cheng, 1986). It was concluded that cartap hydrochloride resistance was independent and had only a minor effect on both fenvalerate and deltamethrin, and this effect became evident only when cartap hydrochloride resistance reached a high profile. The insect did not develop such resistance to malathion and carbaryl (resistance ratios being 0.31 and 1.05, respectively), besides a number of other organophosphates.

While testing the sensitivity of cartap hydrochloride-selected strain (selected for 35 generations), Chen *et al.* (1993) observed slight cross-resistance to dichlorvos, malathion and fenitrothion and no cross-resistance to deltamethrin, cypermethrin, permethrin, methomyl and thiofenox.

As much as 19.9-fold cross-resistance to Lambda-cyhalothrin was observed in cartap hydrochloride-selected strain of *P. xylostella* (selected upto 8 generations) by Cho and Lee (1994). However, these values ranged between 2.2 to 3.4 with reference to prothiofos and triflumuron.

2.3 Biology of *P. xylostella* (selected *vis-a-vis* susceptible strain)

2.3.1 Mating duration

Variations exist in the mating duration of normal field populations of *P. xylostella.* Under laboratory conditions, mating of this insect has been observed to last for 1 to 2 hours (Jayarathnam, 1977; Yamada, 1978; Salinas, 1986), though it has been reported to be less than an hour also by many workers (Pivnick *et al.*, 1990a; Abro *et al.*, 1992; Kandoria *et al.*, 1994).

According to Campanhola *et al.* (1991), changes in mating behaviour could reduce the mating success of insects that were resistant to insecticides. Rowland (1991) was of the opinion that such changes could occur in

conjunction with the evolution of insecticide resistance either by chance or as a pleiotropic consequence of the genetic changes. However, the studies conducted by Groeters *et al.* (1993) revealed no difference in the mating duration of *B.t.*-resistant and susceptible *P. xylostella.*

2.3.2 Pre-oviposition period

The pre-oviposition period of *P. xylostella,* in general, has been observed to vary from 0.7 to 4.2 days by various workers (Bhalla and Dubey, 1986; Salinas, 1986; Kandoria *et al.*, 1994). As far as this insect is concerned, no report on the effect of insecticide pressure on pre-oviposition period is available in the literature. However, studies conducted on *Musca domestica* revealed no significant difference in the duration of this parameter both of DDT-resistant and susceptible strains (Pimentel *et al.*, 1951).

2.3.3 Oviposition period

As observed by various workers, there is considerable variation in the oviposition period of *P. xylostella.* The females of this insect have been reported to lay eggs for 2 to 18.6 days (Harcourt, 1957; Ho, 1965; Patil and Pokharkar, 1971; Kandoria *et al.*, 1994) in laboratory. Yadav *et al.* (1974) observed it to range from 1 to 9 days and pointed out that the variations could be due to the differences in rearing techniques and environmental conditions. Yamada (1978) found that most of the eggs of *P. xylostella* were laid in first few days and completed within 5 days at 22°C. According to some other reports, the egg laying started soon after mating and more than 58 percent eggs were laid on the first night of

egg laying and lasted upto 4 days (Abro *et al.*, 1992; Talekar and Shelton, 1993).

The oviposition period of *P. xylostella*, emerging from the pupae exposed to methomyl at 10, 50 and 100 ppm, did not vary from that of control, but it got prolonged significantly when exposed to 500 ppm (Nemoto, 1986). However, Motoyama *et al.* (1992) did not find any difference in oviposition periods of fenvalerate-selected and unexposed strain of DBM.

2.3.4 Post-oviposition period

There is not much information available on this parameter as far as *P. xylostella* is concerned. However, it has been reported to range between 2.5 to 7.4 days (Bhalla and Dubey, 1986; Kandoria *et al.*, 1994).

The difference in the post-oviposition periods of fenvalerate-selected, cypermethrin-selected and the parental strains of *Earias vittella* have been reported to be non-significant by Saini and Chopra (1988).

2.3.5 Fecundity

A high variation exists in the reported fecundity of normal (untreated) *P. xylostella* populations. The earliest recorded fecundity of this pest insect was reported to be 140±93.5 eggs per female when reared at 20°C temperature (Miner, 1947). Various later studies show it to range from 7 to 414 eggs per female in Malaysia (Ho, 1965; Ooi and Kelderman, 1979); 45.6 to 77.4 eggs per female in Japan (Yamada and Kawasaki, 1983); 107 to 300 eggs per female at different temperatures (Liu *et al.*, 1985). However, Talekar and Shelton (1993) while reviewing the fecundity of *P. xylostella* observed it to range from 11 to 188.

Reports from India also show high level of variations in the egg laying capacity of this pest. While Patil and Pokharkar (1971) reported it to range from 71 to 203 eggs/female at 26.3°C, Yadav *et al.* (1974) observed it to be 28 to 66 eggs at 25±3°C. On the other hand, Ram *et al.* (1987) observed 257.2 eggs and Kandoria *et al.* (1994) observed 99.7±27.7 eggs to be laid by a female, of this pest, while working under similar temperature conditions.

The vast variations have been attributed to several factors *viz.*, genetic composition of the insect, nutritional intake, nature of host plant, climatic conditions, the mating and the availability of the substrate (Tabashnik, 1985; Koshihara, 1986; Salinas, 1986; Lu *et al.*, 1988; Uematsu and Sakanoshita, 1989; Pivnick *et al.*, 1990b).

Reports are available in literature where fecundity of insects has been observed to be affected by the selection pressure of insecticides. In the earliest report, codling moths developing from the larvae exposed to sub-lethal concentrations of 4,6-dinitro-o-cresol showed markedly lower fecundity as compared to unexposed ones (Yothers and Carlson, 1946). Later, exposure to higher concentrations of DDT and lindane was reported to result in complete inhibition of oviposition in *Pectinophora gossypiella* and exposure to lower concentrations led to reduced fecundity of this pest (Haque and Venkatraman, 1970). Similar results have been obtained from the studies conducted on this pest by using sub-lethal concentrations of synthetic pyrethroids (Ross and Brown, 1982; Radwan *et al.*, 1984; Bariola, 1984).

Salama *et al.*(1981), while studying the effect of sub-lethal doses of *B.t.* var. *kurstaki*, observed retardation in fecundity of *Spodoptera littoralis*, *S. exigua* and *Heliothis armigera*. Hafez *et al.* (1993) observed that *Agrotis ipsilon* moths emerging from *B.t.* treated pupae had a low egg production. *Pericallia ricini* moths emerging from the 3rd instar larvae treated with sub-lethal concentrations of *B.t.* (Dipel 0.1) were less fecund (Mathur *et al.*, 1994).

Studies conducted on *Plutella xylostella* revealed that sub-lethal concentrations of fenvalerate resulted in complete inhibition of egg laying (Kumar and Chapman, 1984), though Motoyama *et al.* (1992) did not find any difference in the number of eggs laid by fenvalerate-selected and unselected strains of this pest insect. A reduction of 10 percent in the fecundity of *B.t.* resistant population of *P. xylostella* was observed by Groeters *et al.* (1994).

Scientific opinion, however, suggests that reproductive disadvantages of insects were not always associated with resistance and that the laboratory-selected strains suffered more reproductive disadvantages than resistant strains colonized from the field due to the differences in their relative fitness (Roush and Croft, 1986).

2.3.6 Incubation period

The incubation period of *P. xylostella* eggs, obtained from normal strain, has been recorded to range between 2 to 6 days (Miner, 1947; Ho, 1965; Abraham and Padmanaban, 1968; Patil and Pokharkar, 1971; Yadav *et al.*, 1974; Jayarathnam, 1977; Yamada and Kawasaki, 1983; Bhalla and Dubey, 1986; Chen and Su, 1986; Talekar and Shelton, 1993;

Kandoria *et al.*, 1994; Devi and Raj, 1995) and the variation is mainly attributed to temperature. Motoyama *et al.* (1992) did not observe any significant difference in the egg duration of fenvalerate-selected and unselected strains of *P. xylostella.*

2.3.7 Hatchability

As per the available literature, the hatchability of *P. xylostella* eggs has been observed to range between 80 to 100 per cent (Patil and Pokharkar, 1971; Yadav *et al.*, 1974; Bhalla and Dubey, 1986; Kandoria *et al.*, 1994) depending upon the temperature.

While working with *Earias vittella*, Saini and Chopra (1988) did not observe any significant difference in the per cent egg hatchability of fenvalerate-selected, cypermethrin-selected and parental strains.

2.3.8 Adult longevity

There are significant differences in the longevity of males and females of *P. xylostella* mainly influenced by variable temperature conditions in the laboratory. A wide range of adult longevity of 2 to 67 days for female was recorded at temperature ranging between 23-26°C (Miner, 1947; Harcourt, 1957). On the other hand, the average life span of 9-16 days was also reported by various workers (Patil and Pokharkar, 1971; Yadav *et al.*, 1974; Ooi, 1986; Ram *et al.*, 1987; Kandoria *et al.*, 1994) at temperature ranging around 25±1°C. However, when the temperature ranged between 16.1 to 34.1°C, the life span of female *P. xylostella* was observed to be 12.3 to 15.4 days (Bhalla and Dubey, 1986).

The longest male longevity of *P. xylostella* was reported to be 3-58 days (Harcourt, 1957), yet a number of reports cite the male longevity,

when fed with 10% sucrose solution, varying between 2-13 days (Patil and Pokharkar, 1971; Yadav *et al.*, 1974; Yamada and Kawasaki, 1983; Bhalla and Dubey, 1986; Ooi, 1986; Ram *et al.*, 1987; Kandoria *et al.*, 1994).

On an average, the reported adult longevity varied between 3 to 23 days at different temperatures (Abraham and Padmanaban, 1968; Hsu and Wang, 1971; Jayarathnam, 1977; Chen and Su, 1978; Yamada *et al.*, 1980; Yamada and Kawasaki, 1983; Leu and Lee, 1984; Liu *et al.*, 1985; Ooi, 1986; Devi and Raj, 1995).

The available reports on the effect of selection pressure of insecticides on the adult longevity of insects of either sex reveal that there is no change in the total life span of insecticide-selected and susceptible strains of insects (Saini and Chopra, 1988; Motoyama *et al.*, 1992).

2.3.9 Larval durations

In general, larvae of *P. xylostella* are reported to pass through four instars, though certain reports regarding the existence of 5th instar are also available in the literature (Harcourt, 1957; Patil and Pokharkar, 1971). The earliest studies by Miner (1947) show the four larval instars to be completed in 2.4, 1.6, 1.8 and 2.1 days, respectively (temperature 20-27°C). The results of various other studies are quiet similar, though the duration has been reported from 6 to 21 days (Miner, 1947; Harcourt, 1957; Metcalf *et al.*, 1962; Ho, 1965; Abraham and Padmanaban, 1968; Patil and Pokharkar, 1971; Yadav *et al.*, 1974; Dubey and Chand, 1977; Singh and Singh, 1982; Chen and Su, 1986; Ram *et al.*, 1987; Kandoria *et al.*, 1994). The large variations in the larval durations have

been explained by the variations in temperature and host crop on/under which food/conditions, the studies were conducted (Talekar and Shelton, 1993; Devi and Raj, 1995).

The reports are available in the literature regarding the slowing down of larval development of different insects when these were released/reared on insecticide treated food (Ross and Brown, 1982; Gist and Pless, 1982) and only a few studies have been conducted on the effect of sub-lethal doses of insecticides on the larval development of insects. The retardation in the larval development of *Spodoptera littoralis*, *S. exigua* and *Heliothis armigera vis-a-vis Bacillus thuringiensis* var. *kurstaki* has been observed by Salama *et al.* (1981). Mathur *et al.* (1994) also reported similar results while working with *Pericallia ricini* (Fab.) and Li *et al.* (1995) with *Choristoneura rosaceana* (Harris).

However, few reports pertaining to the effect of insecticide pressure on the larval duration reveal contradictory results in different insects. On one side, no difference in the total larval durations of fenvalerate-selected (9.9±1.8 days) and unselected (9.9±1.2 days) *P. xylostella* was found in the studies conducted by Motoyama *et al.* (1992). On the other hand, Saini and Chopra (1988) observed prolonged larval period of *E. vittella* in the fenvalerate-selected strain, though they could not produce such results in the cypermethrin-selected strain of this pest.

2.3.10 Pre-pupal duration

The pre-pupal duration of *P. xylostella* lasted for less than a day (Yadav *et al.*, 1974) and few reports also suggest that this stage may get extended from 1.5 to 2 days (Talekar and Shelton, 1993; Kandoria *et al.*, 1994). However, as per available literature, no studies have been conducted

on the effect of insecticidal selection on the duration of this stage of any insect.

2.3.11 Pupal duration

The pupal duration of *P. xylostella* varied between 4 to 15 days depending upon the temperature (Miner, 1947; Abraham and Padmanaban, 1968; Yadav *et al.*, 1974; Dubey and Chand, 1977; Singh and Singh, 1982; Yamada and Kawasaki, 1983; Talekar and Shelton, 1993; Devi and Raj, 1995).

Saini and Chopra (1988) did not observe any difference in the pupal period of fenvalerate-selected and cypermethrin-selected *Earias vittella* as compared to unselected strain. Similarly, the pupal periods of fenvalerate-selected and unselected strains of *P. xylostella* were also not significantly different from each other (Motoyama *et al.*, 1992).

2.3.12 Weights of different life stages

The available literature suggests that no studies have been conducted regarding the effect of insecticide-selection on the weights of different life stages of *P. xylostella.* However, studies conducted on *Earias vittella* reveal that the larvae and pupae of fenvalerate resistant strain of this pest were lighter than the cypermethrin-selected and parental strains (Saini and Chopra, 1988).

2.3.13 Measurements of various life stages

The 4th instar larvae and pupae of *P. xylostella* were reported to be 9.5 to 12 mm and 6.0 to 7.25 mm long, respectively (Abraham and Padmanaban, 1968; Patil and Pokharkar, 1971; Yadav *et al.*, 1974). Bhalla and Dubey (1986) reported that 1st, 2nd, 3rd, 4th instar larvae and pupae

were 1.30, 3.10, 4.67, 8.62 mm, 5.15 mm long and 0.18, 0.24, 1.03, 1.13 and 1.17 mm wide, respectively.

On an average, the adult measured 14 mm across wing expanse (Abraham and Padmanaban, 1968; Yadav *et al.*, 1974). The body of the adult male and female *P. xylostella* has been reported to be 4.97 and 4.98 mm long and 12.97 and 13.06 mm across wings (Bhalla and Dubey, 1986).

The perusal of available literature indicates that studies on the effect of selection pressure with insecticides to any insect species on this parameter have not been conducted.

CHAPTER-III

MATERIALS AND METHODS

The present studies were carried out in the laboratory of the Department of Entomology, Chaudhary Charan Singh Haryana Agricultural University, Hisar. The materials and methods used are described here under:

3.1 The insect and its rearing

Diamondback moth, *Plutella xylostella* (Plutellidae : Lepidoptera) was used as the test insect. The culture of the test insect was initiated by collecting about 200 larvae from the farmers' cauliflower fields around Hisar during the first week of August, 1997 in glass battery jars (20x15 cm) in the laboratory at 24±2°C temperature maintained by using desert coolers and electric heater. The freshly emerged moths were released in oven dried glass battery jars having cauliflower leaves to serve as substrate for oviposition. The cauliflower crop variety Snowball-16 was raised at the research farm of the Department of Entomology for getting insecticide free fresh leaves to serve as food for the larvae. Cotton swabs soaked in 10 per cent honey solution, placed on the muslin cloth, used for covering the top of the jar, were provided to serve as food for the moths. The swabs were recharged daily with honey solution and replaced after three days. Moths were shifted daily to new jars provided with fresh cauliflower leaves for oviposition. The jars with leaves carrying eggs were retained separately. The larvae after emerging from the leaf epidermis were provided with fresh cauliflower leaves to feed upon. At every successive instar, the larvae were gently transferred with the help of a camel hair brush, to other clean jars containing fresh leaves. After multiplying the culture in the laboratory for two successive generations, the whole stock was divided into four

lots. One lot was called the parental stock (to be referred herein after as PS). One each of the remaining three lots was used to study the development of resistance to three biopesticides *viz.*, *Bacillus thuringiensis* var. *kurstaki*, spinosad and cartap hydrochloride.

3.2 Preparation of insecticidal concentrations

The proprietary products of the nine insecticides belonging to five groups i.e. biopesticides (3), organochlorine (1), organophosphates (3), carbamate (1) and synthetic pyrethroid (1) were used in the present studies (Table 1). Sufficient volume of one per cent stock solution of each insecticide in acetone (except for *Bacillus thuringiensis* var. *kurstaki*) was prepared and stored in a deep freezer. These stock solutions were prepared afresh after every three months. From these stock solutions, further required concentrations of insecticides were prepared according to a flow chart devised for the purpose by using acetone as diluent. However, for preparing one per cent stock solution of *B.t.*, 10 ml of Delfin OF was diluted in 990 ml of distilled water. Further concentrations too, were prepared in distilled water.

3.3 Exposure of the insect to the insecticides

FAO method No.21 (Busvine, 1980) of topical application of insecticides to the larvae with slight modifications was followed for these studies.Instead of directly treating the larvae without any substrate, the larvae released on cut discs of cauliflower leaves of the size slightly smaller than the bottom of the petridish (2.5 cm diameter) were treated to simulate the application of insecticides in the field. To facilitate the movement of the larvae on both the sides of the leaf disc, the leaves bearing

Table 1. Details of the insecticides used

S.No.	Common name	Proprietory Products	Chemical Name	Manufacturer/ Formulator
1	2	3	4	5
(A)	**Biopesticides**			
1.	*Bacillus thuringiensis*	Delfin OF	*Bacillus thuringiensis* Beliner Var. *Kurstaki.* Spores & Crystalline delta-endotoxins (Potency: 23 million SU/ml *Spodoptera exigua*)	M/s Sandoz India Ltd.
2.	Spinosad (Spinosyn A + Spinosyn D)	*Spinosad 48SC	**Spinosyn A** 2-[(6-Deoxy-2,3,4-tri-O-methyl-alpha-L-mannopyranosyl)oxy]-13[(5-(dimethylamino) tetrahydro-6-methyl-1-2H-pyran-2-yl)oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-14-methyl-IH-as-idaceno(3,2-d)oxacyclododecin-7,15-dione **Spinosyn D** 2-[(6-Deoxy-2,3,4-tri-O-methyl-alpha-L-mannopyranosyl)oxy]-13[(5-(dimethylamino) tetrahydro-6-methyl-1-2H-pyran-2-yl)oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-4,14-dimethyl-IH-as-idaceno(3,2-d)oxacyclododecin-7,15-dione	M/s De-Nocil Crop Protection Ltd.
3.	Cartap hydrochloride	Padan 50SP	1,3-Bis(Carbamoylthio)-2-(N,N-dimethylamino) Propane hydrochloride	M/s Coromandel Indag Products Ltd.

* commercially not yet launched

Contd.....

1	2	3	4	5
(B)	**Organochlorine**			
4.	Endosulfan	Thiodon 35EC	6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide	M/s Hoechest Sheiring-AgroEvo Ltd.
(C)	**Organophosphates**			
5.	Malathion	Cythion 50EC	0,0-dimethyl phosphorodithioate of diethyl meracaptosuccinate	M/s Cyanamid India Ltd.
6.	Monocrotophos	Bilphos 36SL	Dimethyl (E)-1-methyl-2(methyl carbamoyl) Vinyl Phosphate	M/s Bayer India Ltd.
7.	Dichlorvos	Nuvan 76EC	2,2-Dichlorovinyl dimethyl phosphate	M/s Hindustan Ciba-Geigy Ltd.
(D)	**Carbamate**			
8.	Carbaryl	Sevin FLO-44AF	1-Naphthyl-N-methyl carbamate	M/s Rhone-Poulenc Agrochemical (India) Ltd.
(E)	**Synthetic Pyrethroid**			
9.	Cypermethrin	Cyperkill 25EC	(RS)-α-Cyano-3-Phenoxybenzy (1RS)-Cis,trans-3-(2,2-dichloroviny)-2,2-dimethyl cyclopropane Carboxylate	M/s Hexamar(E.I.D. Parry India)

thick midribs and veins were selected for cutting the leaf discs. One ml each of the insecticidal solutions were sprayed on each side of leaf discs with Potter's tower operating at 5 lb/inch^2 pressure. The larvae were released on the upper side before spraying. Keeping into account the larval habit of feeding on the underside of the leaf, the sprayed leaf discs were finally placed with underside down in the petridish. The sprayed leaf discs placed in the petridishes were dried at room temperature before covering with lid. A well fitted filter paper was placed in the cover of each petridish to prevent the escape of the larvae and to absorb the excess moisture released from the leaf discs. Thereafter, the petridishes were transferred to a BOD incubator maintained at 24±1°C. The surviving individuals, after exposure, were shifted to a clean glass battery jar provided with fresh untreated cauliflower leaf. These larvae were reared to the next generation.

The leaf residue bioassay method as described by Tabashnik and Cushing (1987) was followed to expose *Plutella xylostella* larvae to *B.t.* var. *kurstaki*. The leaf discs were dipped into the insecticidal solution for 5 seconds (Tabashnik *et al.*, 1990) and dried at room temperature. Such discs were placed in petridishes and larvae of the test insect were released on the leaf disc. The mortality data were recorded 72 hours after treatment (Tang *et al.*, 1996).

3.4 Development of resistance through selection

Third instar larvae from the pre-determined three lots of *P. xylostella* culture were used for exposure to the three bioinsecticides *viz.*, *B.t.* var. *kurstaki*, spinosad and cartap hydrochloride.

To begin with, 6 to 9 concentrations each of *B.t.* var. *kurstaki*, spinosad and cartap hydrochloride (based on the preliminary experiments conducted before taking up the present studies) were utilised. A set of control (spray of acetone alone) was also maintained with each exposure to work out the corrected mortalities. There were 50 larvae in each replication and in all 10 replications for each concentration were maintained. Mortality data were recorded 48 hours after treatment in case of spinosad and cartap hydrochloride and 72 hours in case of *B.t.* The surviving larvae from the concentrations affording around 85 percent mortality were selected and shifted to glass jar provided with fresh untreated leaves for further development. The survivors in the remaining concentrations were discarded. The progeny of the first surviving lot was termed as F_1 generation corresponding/pertaining to the insecticide to which it was exposed. In the same way, the exposures and selections upto 7 generations were conducted. The parental strain (PS) was also maintained all through without exposure to insecticides.

3.5 Quantification of insecticidal resistance

The degree of development of resistance to the three insecticides through different generations was determined by working out LC_{50} values in each generation and then by computing the resistance ratios. The resistance ratio for any generation was worked out by dividing LC_{50} value for that generation with the LC_{50} value of the parental strain. The observations on larval mortality were recorded by ensuring the larvae as dead when they did not resume activity after repeated proddings with a fine camel hair brush.

3.6 Cross-resistance studies

The studies on cross-resistance of *B.t.*-selected strain (BS), spinosad-selected strain (SS) and cartap hydrochloride-selected strain (CS) of *P. xylostella* to commonly used insecticides *viz.*, monocrotophos, malathion, endosulfan, dichlorvos, cypermethrin and carbaryl were made as per FAO method No.21 described earlier. The experiment was replicated five times and each replicate comprised of 10 third instar larvae. The LC_{50} values of these insecticides against the selected strains and that of the parental strain were computed and their comparisons were made for the studies on cross-resistance.

3.7 Biological studies

Sufficient fourth instar larvae from the three insecticide-selected and the parental strains were desexed visually (Liu and Tabashnik, 1997) so as to get atleast 10 adults of both the sexes. These larvae were reared separately in glass vials (7.8x2.5 cm) to initiate the studies on the biology of insecticide-selected and parental strains. These studies were carried out in the laboratory at 24±1°C in BOD incubator. Following biological parameters were studied:

1. Mating duration
2. Pre-oviposition period
3. Oviposition period
4. Post-oviposition period
5. Adult longevity
6. Fecundity

7. Incubation period
8. Hatchability
9. Number of larval instars
10. Larval durations
11. Pre-pupal duration
12. Pupal duration
13. Weights of various life stages
14. Dimensions of various life stages

The detailed methodology for studying these parameters is described here under:

3.7.1 Mating duration

Newly emerged one male and one female adult moth, comprising one replicate, were released in a glass jar (15x10 cm) provided with a piece of cauliflower leaf to serve as a cue and a cotton swab soaked in 10% honey solution to serve as food. In all, there were ten replicates for each strain. Mating pairs were observed after every fifteen minutes upto one hour initially. Since most of the pairs separated after one hour of mating, thereafter, the observations were recorded after every five minutes for the remaining mating pairs. The total mating duration (minutes) was determined for each individual pair.

3.7.2 Pre-oviposition period

The cauliflower leaf provided in the glass jar, containing the mated pair of moths kept under the flourescent tube light was observed with the help of a hand lens (20 x) through glass, without disturbing the moths, for the presence of the eggs at an hourly interval. The pre-oviposition

period was worked out from the time pairing elapsed and the beginning of egg laying in each case.

3.7.3 Oviposition period

The egg laying by the mated moths of all the four strains was observed at 12 hours' interval and the duration in days between initiation of egg laying and its completion was, thus, determined.

3.7.4 Post-oviposition period

The duration between the time the female stopped egg laying and its death was recorded as post-oviposition period (days). The observations were recorded at 12 hour intervals.

3.7.5 Adult longevity

The total duration (days) of adult male and female from emergence till death were computed. The individuals used for recording these observations were the same as those used in above discussed biological parameters (3.7.1 to 3.7.4).

3.7.6 Fecundity

The number of eggs laid by a female moth, on the cauliflower leaf and those on the glass jar and muslin cloth (used to cover the glass jar), were counted with the help of a hand lens (20 x). The pairs of moths were shifted daily to a new jar provided with a fresh cauliflower leaf for oviposition. The number of eggs laid by a single female in its life time was pooled to work out fecundity. Ten females of each strain were utilised for these studies.

3.7.7 Incubation period

Thirty eggs laid on the cauliflower leaf per replicate, kept in glass vials, were observed at 12 hours interval to determine the incubation period

(days) marked by hatching. In all, there were 10 replicates for each strain. The ruptured egg chorion observed with the help of a hand lens (20 x), was taken as a mark for egg hatching.

3.7.8 Hatchability

The hatchability (per cent hatching) of eggs was determined on the basis of number of eggs hatched successfully out of the total eggs kept under observation (30 eggs/replicate). Ten replicates of each of the four strains were maintained. The eggs which did not hatch were treated as infertile.

3.7.9 Number of larval instars

The number of larval instars this insect undergoes was determined by the moulting. The neonate larvae feed as miners on spongy mesophyll tissues of the leaf soon after their emergence, however, it comes out to moult (Talekar and Shelton, 1993). The exuviae of first instar larvae was detected with the help of a hand lens (20 x). The later instars being external feeders were marked by the presence of exuviae which were visible with naked eyes. The larvae were transferred to new glass vials, provided with fresh leaf, with the help of a fine camel hair brush. The leaf was changed as and when it started withering. The observations were made after 24 hours.

3.7.10 Durations of the larval instars and total larval duration

To record the durations of each larval instar and total larval duration, observations were made after every 12 hours. The presence of exuviae marked the transformation to the next instar. For each strain, 10 replications were run.

3.7.11 Pre-pupal period

The fourth instar larvae in the later part of its life became sluggish, suspended feeding and stopped movements, got reduced in size and initiated spinning of silken cocoon to cover its body before transforming to pupa. The time interval between inactivation and pupal formation was recorded as pre-pupal duration in days. The observations were recorded after every 12 hours in each of the four strains, replicated 10 times.

3.7.12 Pupal duration

Record of pupal duration in days was made from the time of formation of pupa till the emergence of the moths. Observations were recorded after every 12 hours in each of the 10 replicates of all the four strains.

3.7.13 Weights of various life stages

The newly formed (one day old) individuals of 2nd, 3rd and 4th instar larvae, pre pupae and pupae were weighed singly with the help of electronic digital balance (Model : FX-400 Afcoset). In all ten individuals were weighed in each case.

3.7.14 Dimensions of various life stages

Length and breadth of 10 randomly selected, one day old 2nd, 3rd and 4th instar larvae, pre-pupae, pupae and adult (male and female), in each of four strains, were measured. The observations were taken under a stereoscopic binocular microscope fitted with an ocular micrometer. The lengths were measured along the mid-dorsal line from head to the last abdominal segment. The width of the individuals was measured across wing expanse. A single individual comprised one replicate, and there were ten replicates for each strain.

3.8 Statistical analysis of the data

The mortality data was subjected to Abbott's formula for correction wherever required (Abbott, 1925). LC_{50} values of different insecticides were determined by Probit analysis (Finney, 1971). The data on the various parameters of the biology of the insecticide-selected and the parental strains was subjected to the analysis of variance (SAS Institute, 1989).

CHAPTER-IV

RESULTS

4.1 Toxicity of different insecticides against the parental strain of *P. xylostella*

The per cent LC_{50} values, in ascending order, for different insecticides for the third instar larvae of *P. xylostella* were spinosad 0.00033, *Bacillus thuringiensis* var. *kurstaki* 0.01493, cartap hydrochloride 0.01758, cypermethrin 0.02502, dichlorvos 0.04350, malathion 0.22310, carbaryl 0.32265, endosulfan 0.50192 and monocrotophos 1.36997 (Table 2). It suggests that spinosad was the most toxic and monocrotophos the least toxic insecticide to this insect. The relative toxicity of the different insecticides when compared, taking monocrotophos as standard, revealed that endosulfan, carbaryl, malathion, dichlorvos, cypermethrin, cartap hydrochloride, *B.t.* var. *kurstaki* and spinosad were 2.73, 4.42, 6.14, 31.49, 54.75, 77.93, 91.76 and 4151.42 times, respectively, more toxic than monocrotophos. Calculated regression lines for different insecticides are given in Fig.1 which indicate a homogeneous response of *P. xylostella* population to monocrotophos, spinosad, cartap hydrochloride and dichlorvos and comparatively heterogeneous response to rest of the insecticides.

4.2 Development of resistance in *P. xylostella* to *B.t.* var. *kurstaki*, spinosad and cartap hydrochloride

4.2.1 *B.t.* var. *kurstaki*

The third instar larvae of *P. xylostella* were selected for 7 generations with *B.t.* var. *kurstaki* at concentrations giving around 85 per cent mortality in each generation. The results of larval mortality at different concentrations

Table 2. Toxicity of different insecticides to the parental strain of *Plutella xylostella*

Insecticide	Heterogeneity $X^2_{(n-2)}$	Regression equation	LC_{50} (%)	Fiducial Limits	Relative Toxicity	Slope ± S.E.
Monocrotophos	$X^2_{(5)} = 0.54422$	Y = 3.1770 + 0.4407x	1.36997	0.579329 6.580847	1.00	0.4407 ± 0.34
Malathion	$X^2_{(5)} = 0.55654$	Y = 3.1255 + 0.5597x	0.22310	0.079376 0.627087	6.14	0.5597 ± 0.22
Endosulfan	$X^2_{(6)} = 0.38460$	Y = 3.1708 + 0.4942x	0.50192	0.128010 1.326596	2.72	0.4942 ± 0.30
Dichlorvos	$X^2_{(6)} = 0.29341$	Y = 2.0909 + 0.7995x	0.04350	0.019931 0.094951	31.49	0.7995 ± 0.17
Cypermethrin	$X^2_{(7)} = 0.49592$	Y = 2.8439 + 0.6344x	0.02502	0.010129 0.061847	54.75	0.6344 ± 0.20
Carbaryl	$X^2_{(7)} = 1.07955$	Y = 2.5692 + 0.5391x	0.32265	0.118326 0.879813	4.42	0.5391 ± 0.22
Bacillus thuringiensis var. *kurstaki*	$X^2_{(6)} = 0.67113$	Y = 2.4132 + 1.1897x	0.01493	0.009539 0.023396	91.75	1.1897 ± 0.09
Spinosad	$X^2_{(7)} = 0.25266$	Y = 1.3955 + 1.4313x	0.00033	0.000214 0.000506	4151.42	1.4313 ± 0.09
Cartap hydrochloride	$X^2_{(6)} = 0.27907$	Y = 2.6466 + 1.0482x	0.01758	0.010252 0.030159	77.92	1.0482 ± 0.11

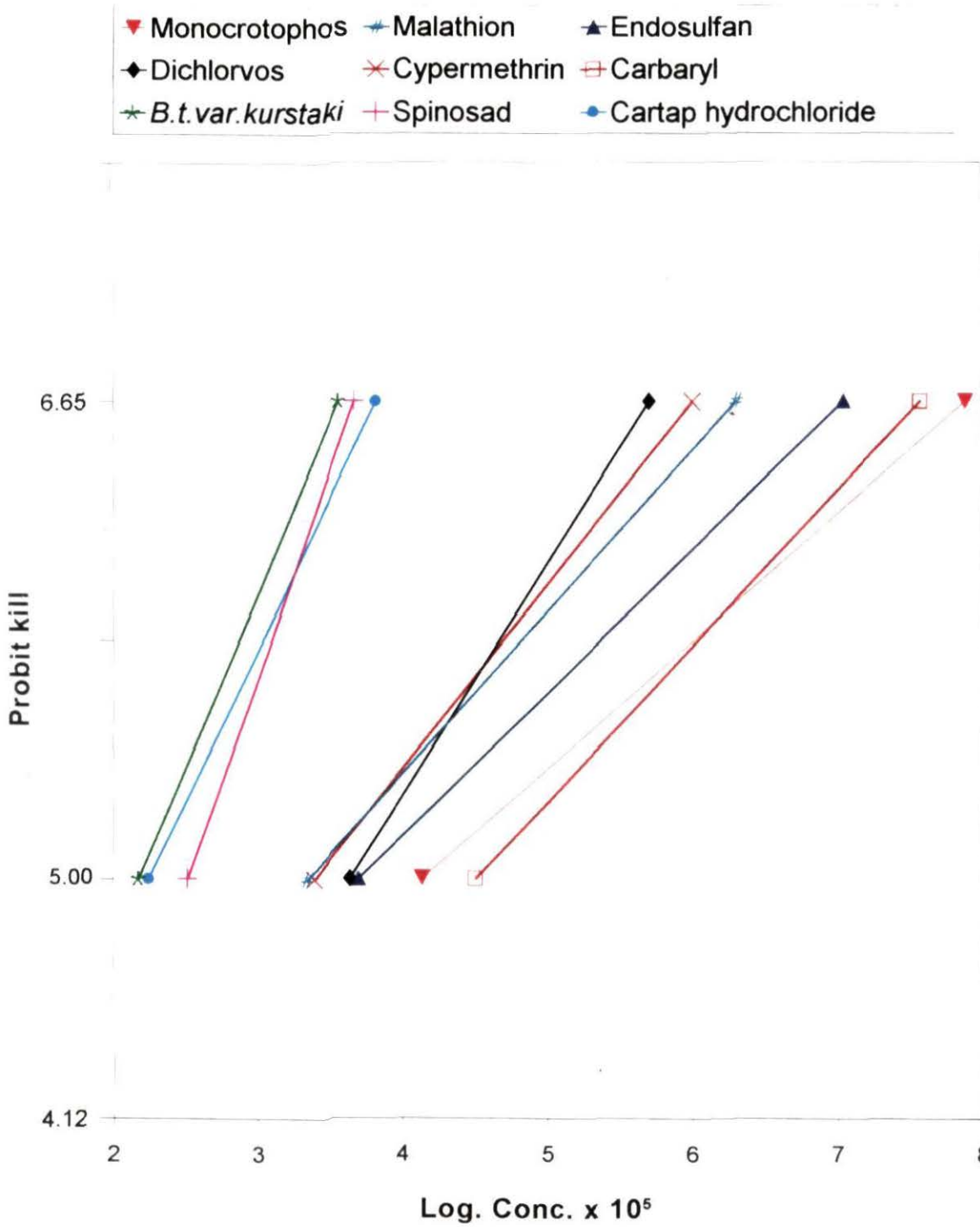

Fig. 1. Regression lines for various insecticides against parental strain of *Plutella xylostella*

of this insecticide during 7 generations are given in Table 3. Regression equations, LC_{50} values, slope values (of regression lines) and resistance ratios for different generations are presented in Table 4. The resistance ratio for the 7th generation as compared to 1st generation was 0.76 which suggests that the resistance did not develop in *P. xylostella* to *B.t.* var. *kurstaki* even after regular selection pressure upto 7th generation. Infact, the susceptibility of the *P. xylostella* population in the successive generations slightly increased to the bioinsecticide and the 7th generation became a bit more susceptible than the 1st generation (Fig. 2). The course of susceptibility through different generations is illustrated in Fig. 3.

4.2.2 Spinosad

Selection pressure of spinosad and responses produced as larval mortality in different generations has been presented in Table 5. The data indicate that there was not much difference in the insect mortality (77.5 to 81.25%) with 0.001 per cent spinosad in the 1st and 7th generation. The resistance ratio for 7th generation as compared to the 1st generation was 0.90 (Table 6) which indicates that resistance to spinosad did not develop in *P. xylostella* after continuous selection upto 7 generations. The LC_{50} values decreased (0.000299 to 0.000270%) with exposure to spinosad in the successive generations. The population of *P. xylostella* showed slightly increased susceptibility. The regression lines for the successive generations are drawn in Fig. 4.

Table 3. Details about selection pressure of *Bacillus thuringiensis* var. *kurstaki* to third instar larvae of *Plutella xylostella* during different generations

Generation	Concentrations of *B.t.* var. *kurstaki* used		Per cent mortality	Residual population rejected (x) selected (✓)
1	2		3	4
F_1	(i)	0.01	43.06	x
	(ii)	0.02	52.38	x
	(iii)	0.04	66.45	✓
F_2	(i)	0.02	54.26	x
	(ii)	0.04	69.02	x
	(iii)	0.06	77.50	✓
F_3	(i)	0.04	69.99	x
	(ii)	0.08	85.50	✓
F_4	(i)	0.04	67.4	x
	(ii)	0.08	84.2	✓
F_5	(i)	0.05	73.2	x
	(ii)	0.075	83.0	x
	(iii)	0.1	89.8	✓
F_6	(i)	0.04	65.5	x
	(ii)	0.08	82.8	x
	(iii)	0.1	88.5	✓
F_7	(i)	0.075	79.5	x
	(ii)	0.1	89.5	✓

Table 4. Toxicity of *Bacillus thuringiensis* var. *kurstaki* to third instar larvae of *Plutella xylostella* in different generations

Generation	Heterogeneity $X^2_{(n-2)}$	Regression equation	LC_{50} (%)	Fiducial Limits	Resistance Ratio	Slope±S.E.
I	$X^2_{(4)} = 2.64674$	Y = 2.3065 + 1.2315x	0.015387	0.012929 0.018312	1.00	1.2315 ± 0.03
II	$X^2_{(5)} = 6.7955$	Y = 2.8980 + 0.9911x	0.013205	0.010975 0.015889	0.85	0.9911 ± 0.04
III	$X^2_{(5)} = 2.5178$	Y = 2.2242 + 1.2748x	0.015041	0.012446 0.018178	0.97	1.2748 ± 0.04
IV	$X^2_{(4)} = 5.9506$	Y = 2.3447 + 1.2202x	0.014997	0.012570 0.017894	0.97	1.2202 ± 0.03
V	$X^2_{(4)} = 1.6023$	Y = 2.5982 + 1.1528x	0.012163	0.009978 0.014725	0.79	1.1528 ± 0.04
VI	$X^2_{(4)} = 2.3475$	Y = 2.9019 + 0.9627x	0.015105	0.010464 0.021427	0.98	0.9627 ± 0.07
VII	$X^2_{(4)} = 0.2262$	Y = 2.6659 + 1.1279x	0.011732	0.007415 0.018563	0.76	1.1279 ± 0.10

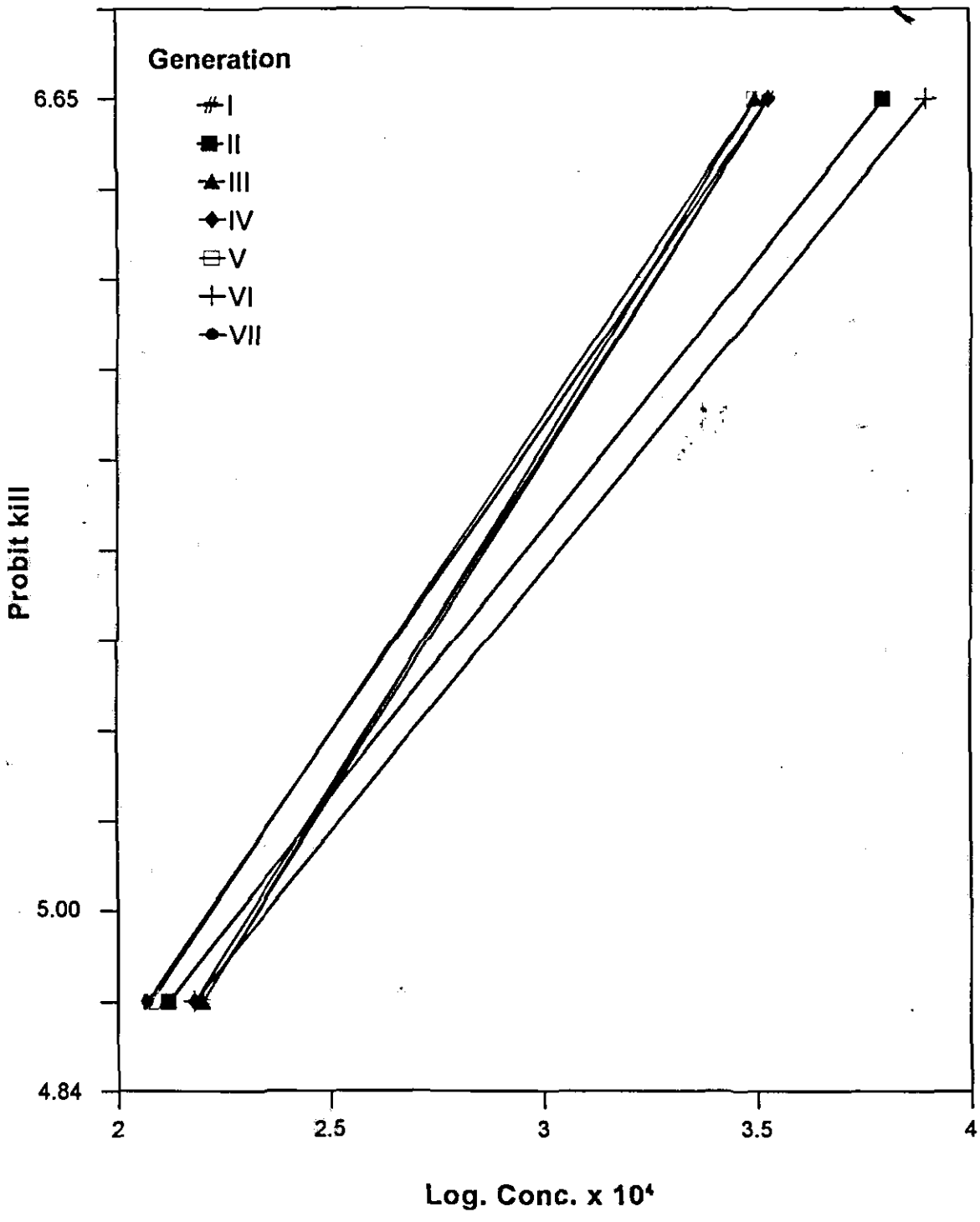

Fig. 2. Regression lines for *Bacillus thuringiensis* var. *kurstaki* against *Plutella xylostella* for 7 generations

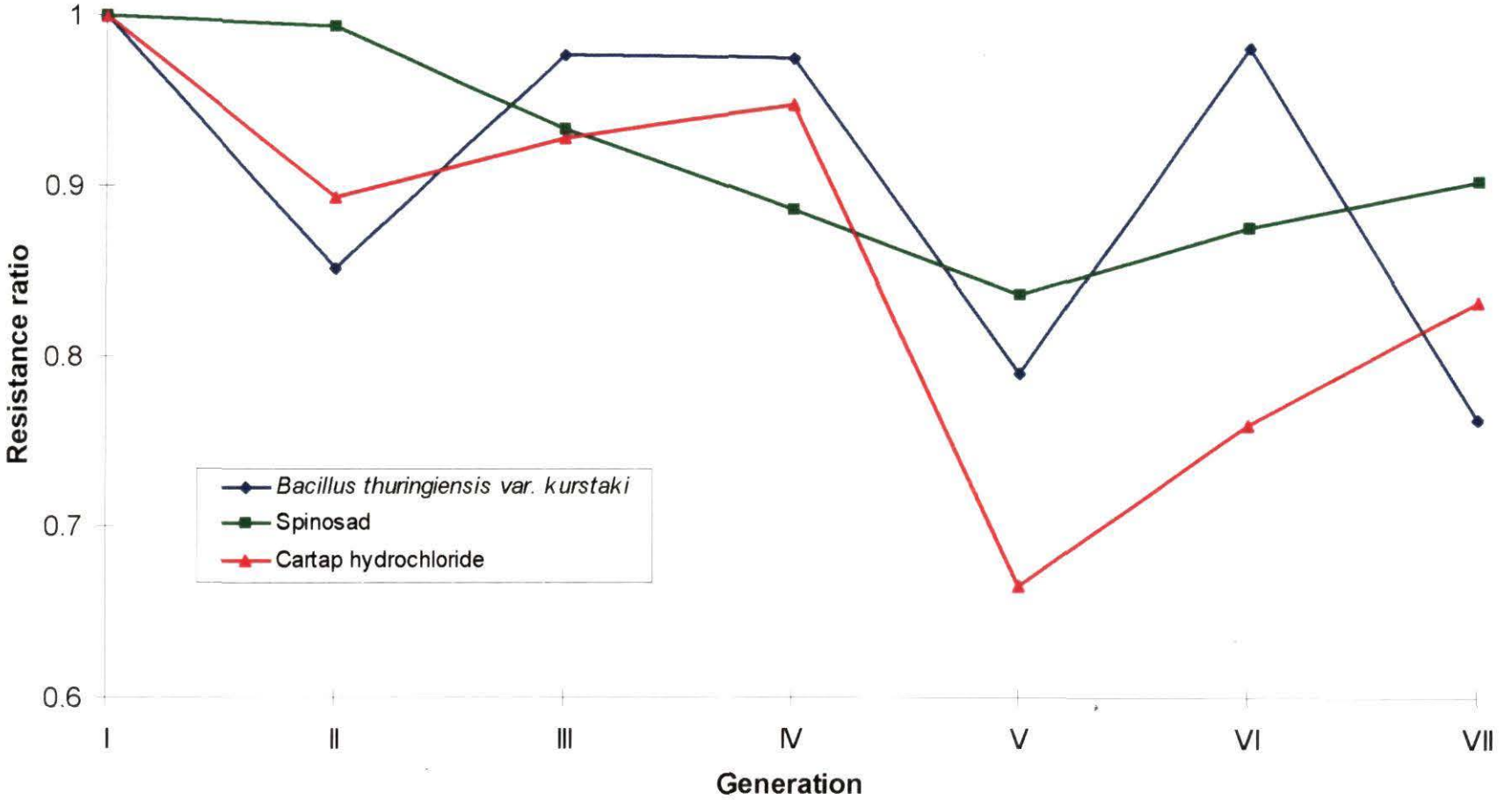

Fig. 3 . Pattern of development of resistance to *Bacillus thuringiensis* var. *kurstaki*, spinosad and cartap hydrochloride in *Plutella xylostella* upto 7th generation of selection

Table 5. Details about selection pressure of spinosad to third instar larvae of *Plutella xylostella* during different generations

Generation	Concentrations of spinosad used		Per cent mortality	Residual population rejected (x) selected (✓)
1	2		3	4
F_1	(i)	0.0004	54.75	x
	(ii)	0.0005	59.1	x
	(iii)	0.001	77.5	✓
F_2	(i)	0.0008	72.9	x
	(ii)	0.001	79.1	x
	(iii)	0.002	85.5	✓
F_3	(i)	0.001	78.0	x
	(ii)	0.002	82.95	✓
F_4	(i)	0.001	79.46	x
	(ii)	0.002	84.6	x
	(iii)	0.004	88.5	✓
F_5	(i)	0.001	80.8	x
	(ii)	0.002	85.8	x
	(iii)	0.004	90.0	✓
F_6	(i)	0.001	81.6	x
	(ii)	0.002	84.3	✓
F_7	(i)	0.0006	70.8	x
	(ii)	0.0008	74.0	x
	(iii)	0.001	81.25	✓

Table 6. Toxicity of spinosad to third instar larvae of *Plutella xylostella* in different generations

Generation	Heterogeneity $X^2_{(n-2)}$	Regression equation	LC_{50} (%)	Fiducial Limits	Resistance Ratio	Slope±S.E.
I	$X^2_{(4)} = 3.0405$	Y = 2.1292 + 1.1594x	0.000299	0.000251 0.000355	1.00	1.1594 ± 0.03
II	$X^2_{(5)} = 0.6643$	Y = 1.5867 + 1.3798x	0.000297	0.000243 0.000365	0.99	1.3798 ± 0.04
III	$X^2_{(5)} = 0.3746$	Y = 2.0608 + 1.2016x	0.000279	0.000237 0.000328	0.93	1.2016 ± 0.03
IV	$X^2_{(6)} = 5.7964$	Y = 2.1288 + 1.1846x	0.000265	0.000223 0.000314	0.88	1.1846 ± 0.03
V	$X^2_{(5)} = 0.4656$	Y = 2.1062 + 1.2062x	0.000250	0.000211 0.000297	0.83	1.2062 ± 0.03
VI	$X^2_{(5)} = 2.2319$	Y = 2.0303 + 1.2281x	0.000267	0.000201 0.000339	0.87	1.2281 ± 0.05
VII	$X^2_{(4)} = 0.1659$	Y = 1.6782 + 1.3657x	0.000270	0.000191 0.000382	0.90	1.3657 ± 0.07

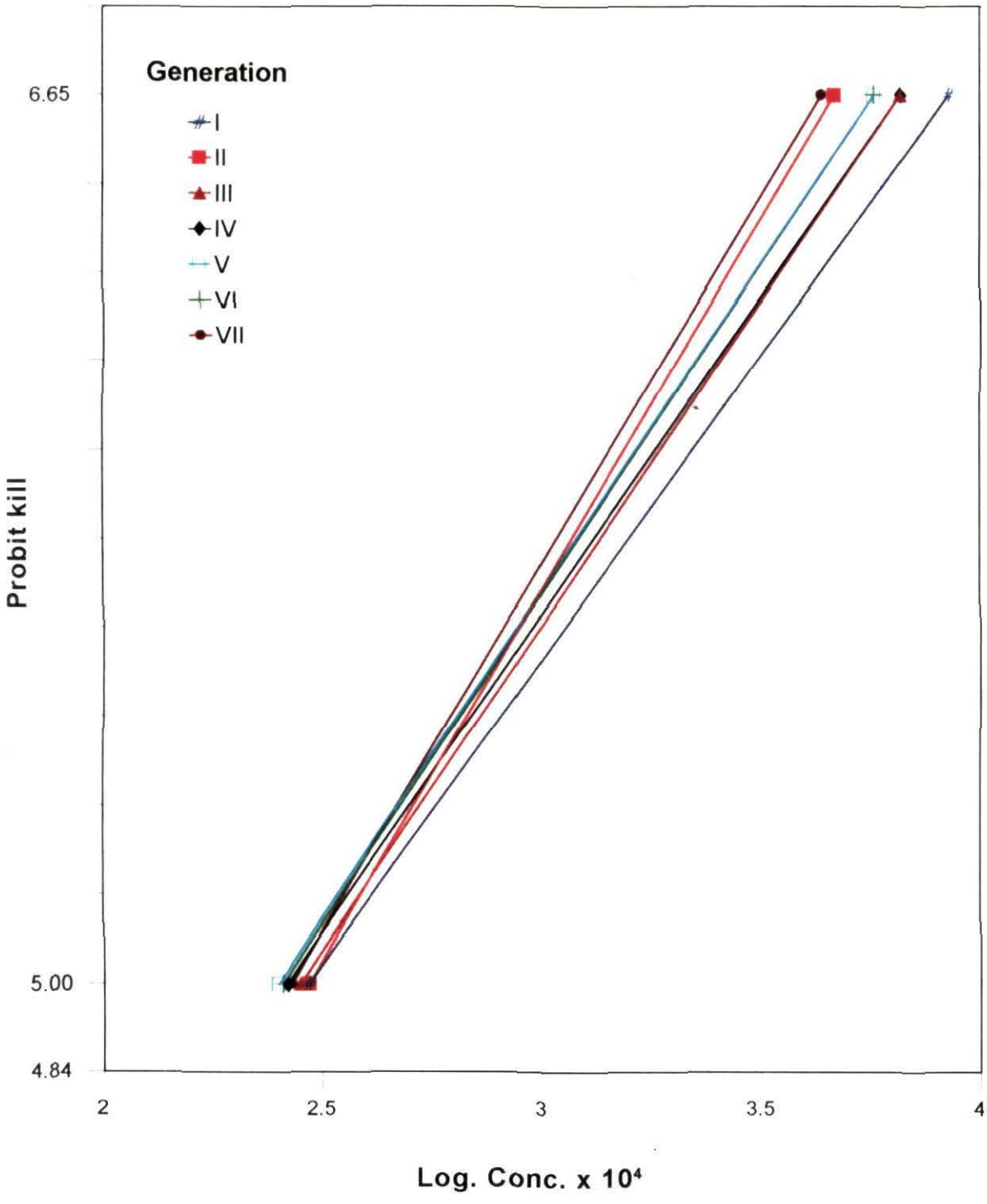

Fig. 4. Regression lines for spinosad against *Plutella xylostella* for 7 generations

4.2.3 Cartap hydrochloride

The selection pressure of cartap hydrochloride upto 7 generations of *P. xylostella* was given in every successive generation. The mortality data and the insecticidal concentrations used to obtain insect kill around 85 per cent is presented in Table 7. The regression equations, LC_{50} values slope values and resistance ratios for different generations find mention in Table 8. The resistance ratio (0.83) in the 7th generation as compared to the 1st generation remained low, indicating no resistance development in the insect. The regression lines for 7 generations (Fig. 5) are overlapping, thus indicating no change in the response to the toxicant in different generations.

4.3 Toxicity of different insecticides to *B.t.*, spinosad and cartap hydrochloride-selected strains of *P. xylostella*

4.3.1 *B.t.* var. *kurstaki*-selected strain

The *B.t.*-selected strain of *P. xylostella* exhibited only small variations in the LC_{50} values of different insecticides as compared to the parental strain (Table 2, 9). Malathion and carbaryl were slightly more toxic to the selected strain as compared to the parental strain. The LC_{50} values of malathion and carbaryl for *B.t.*-selected strain of this insect were 0.1908 and 0.2909 per cent as compared to 0.2231 and 0.32265 per cent for the parental strain, respectively. The *B.t.*-selected strain required slightly higher dose of the rest of the insecticides to achieve 50 per cent kill response as compared to the parental strain. The corresponding values of the other insecticides for the selected strain, in ascending order, were cypermethrin 0.0271 per cent, dichlorvos 0.05121 per cent, endosulfan

Table 7. Details about selection pressure of cartap hydrochloride to third instar larvae of *Plutella xylostella* during different generations

Generation	Concentrations of cartap hydrochloride used	Per cent mortality	Residual population rejected (x) selected (✓)
1	2	3	4
F_1	(i) 0.01	54.4	x
	(ii) 0.05	62.7	x
	(iii) 0.10	85.6	✓
F_2	(i) 0.075	73.55	x
	(ii) 0.09	78.10	x
	(iii) 0.10	83.0	✓
F_3	(i) 0.075	74.3	x
	(ii) 0.10	84.1	✓
F_4	(i) 0.05	63.86	x
	(ii) 0.075	74.53	x
	(iii) 0.1	83.36	✓
F_5	(i) 0.025	62.10	x
	(ii) 0.05	71.15	x
	(iii) 0.10	83.0	✓
F_6	(i) 0.1	80.9	x
	(ii) 0.15	82.9	✓
F_7	(i) 0.1	78.22	x
	(ii) 0.2	81.38	✓

Table 8. Toxicity of cartap hydrochloride to third instar larvae of *Plutella xylostella* in different generations

Generation	Heterogeneity $X^2_{(n-2)}$	Regression equation	LC_{50} (%)	Fiducial Limits	Resistance Ratio	Slope±S.E.
I	$X^2_{(5)} = 4.3584$	Y = 2.6095 + 1.0625x	0.017828	0.012762 0.024772	1.00	1.0625 ± 0.07
II	$X^2_{(5)} = 1.7126$	Y = 2.7894 + 1.0037x	0.015936	0.012553 0.020231	0.89	1.0037 ± 0.05
III	$X^2_{(4)} = 0.7119$	Y = 3.0561 + 0.8762x	0.016536	0.005777 0.047331	0.92	0.8762 ± 0.23
IV	$X^2_{(4)} = 1.8746$	Y = 3.0067 + 0.8946x	0.016899	0.013024 0.021926	0.94	0.8946 ± 0.05
V	$X^2_{(4)} = 2.8304$	Y = 2.7379 + 1.0905x	0.011864	0.010039 0.014022	0.66	1.0905 ± 0.03
VI	$X^2_{(4)} = 0.3867$	Y = 3.0507 + 0.9143x	0.013547	0.001577 0.019136	0.75	0.9143 ± 0.07
VII	$X^2_{(4)} = 0.7252$	Y = 3.2250 + 0.8176x	0.014823	0.013057 0.016827	0.83	0.8176 ± 0.02

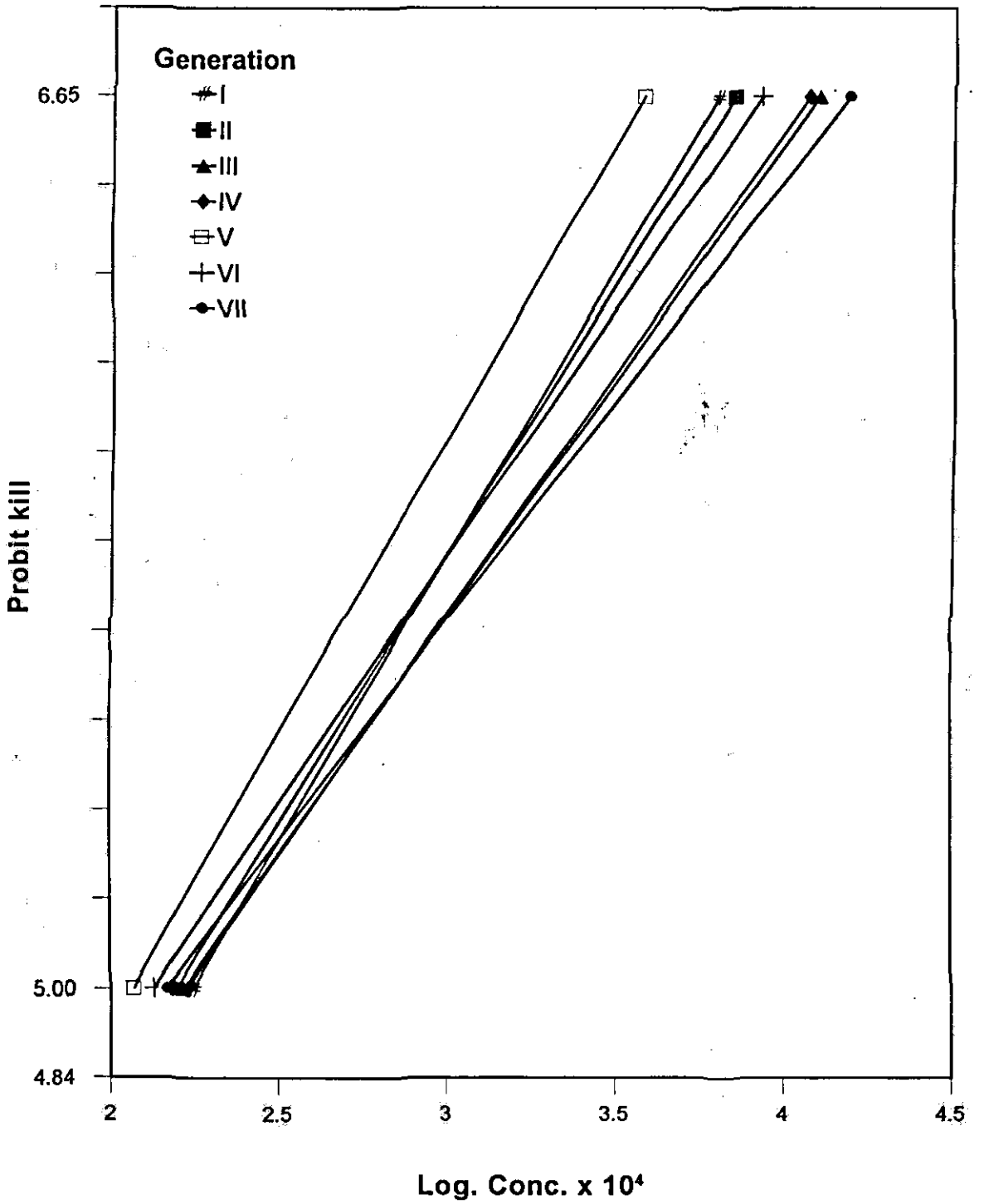

Fig. 5. Regression lines for cartap hydrochloride against *Plutella xylostella* for 7 generations

Table 9. Toxicity of different insecticides to *Bacillus thuringiensis* var. *kurstaki* - selected strain of *Plutella xylostella* larvae

Insecticide	Heterogeneity $X^2_{(n-2)}$	Regression equation	LC_{50} (%)	Fiducial Limits	Relative Toxicity	Slope ± S.E.
Monocrotophos	$X^2_{(5)} = 5.14051$	Y = 3.0071 + 0.3864x	1.43750	0.285614 7.234464	1.00	0.3864 ± 0.35
Malathion	$X^2_{(5)} = 1.03837$	Y = 2.6965 + 0.5381x	0.19080	0.020934 1.739620	07.53	0.5381 ± 0.49
Endosulfan	$X^2_{(6)} = 2.04376$	Y = 2.8034 + 0.4649x	0.53087	0.135051 2.086794	02.70	0.4649 ± 0.30
Dichlorvos	$X^2_{(7)} = 0.42654$	Y = 1.9497 + 0.8223x	0.05121	0.024288 0.107983	28.06	0.8223 ± 0.16
Cypermethrin	$X^2_{(6)} = 0.52515$	Y = 2.5802 + 0.7046x	0.02710	0.012325 0.059856	53.04	0.7046 ± 0.17
Carbaryl	$X^2_{(7)} = 1.06598$	Y = 2.4999 + 0.5600x	0.29090	0.111586 0.758647	04.94	0.5600 ± 0.21

0.53087 per cent and monocrotophos 1.4375 per cent. Cypermethrin came out to be the most toxic and monocrotophos the least, to both the strains of this insect. The relative toxicity of the different insecticides when compared among themselves, showed that cypermethrin, dichlorvos, malathion, carbaryl and endosulfan were 53.04, 28.06, 7.53, 4.94 and 2.70 times, respectively, more toxic than monocrotophos. The regression lines for different insecticides have been presented in Fig. 6.

4.3.2 Spinosad-selected strain

The toxicity of commonly used insecticides was calculated against spinosad-selected *P. xylostella* population also (Table 10). The comparisons of the LC_{50} values indicated that monocrotophos was the least toxic of all the insecticides used against this strain, whereas cypermethrin was found the most toxic. The per cent LC_{50} values were 0.0276, 0.0583, 0.20721, 0.2858, 0.29092 and 1.21278 for cypermethrin, dichlorvos, malathion, endosulfan, carbaryl and monocrotophos, respectively. Data pertaining to the relative toxicity of these insecticides reveal that cypermethrin, dichlorvos, malathion, endosulfan and carbaryl were 43.94, 20.80, 5.85, 4.24 and 4.17 times, respectively, more toxic than monocrotophos. The regression lines drawn for the six insecticides are given in Fig. 7 which indicate the similar trend.

4.3.3 Cartap hydrochloride-selected strain

The susceptibility of the cartap hydrochloride-selected strain of *P. xylostella* exhibited a similar trend as that of *B.t.* and spinosad-selected strains to the commonly used insecticides. The LC_{50} values were 0.02671

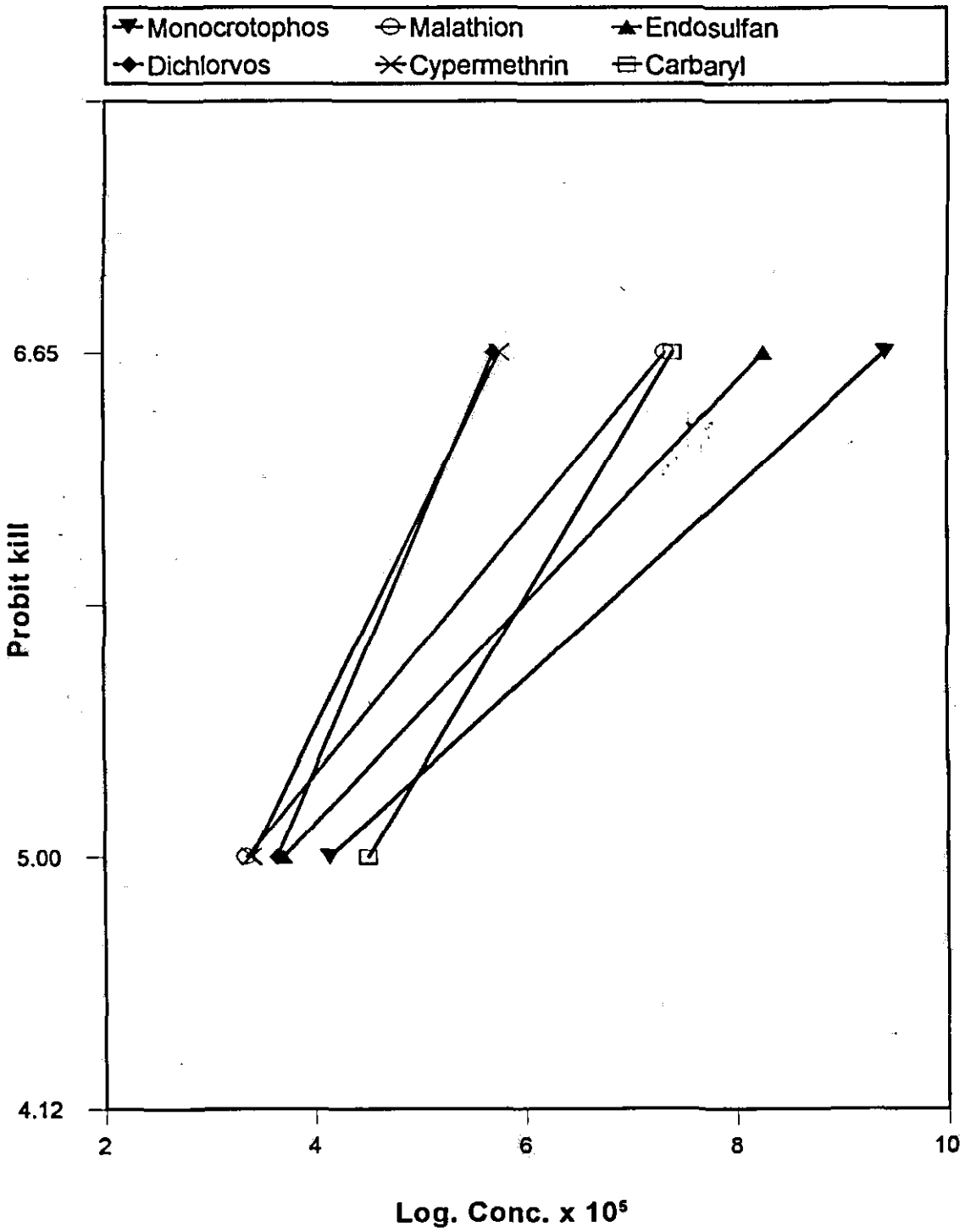

Fig. 6. Regression lines for various insecticides against *Bacillus thuringiensis* var. *kurstaki* - selected strain of *Plutella xylostella*

Table 10. Toxicity of different insecticides to spinosad - selected strain of *Plutella xylostella* larvae

Insecticide	Heterogeneity $X^2_{(n-2)}$	Regression equation	LC_{50} (%)	Fiducial Limits	Relative Toxicity	Slope ± S.E.
Monocrotophos	$X^2_{(6)} = 1.38082$	$Y = 2.5538 + 0.4812x$	1.21278	0.277315 5.38149	01.00	0.4812 ± 0.21
Malathion	$X^2_{(6)} = 0.80534$	$Y = 2.5120 + 0.5764x$	0.20721	0.077586 0.553415	05.85	0.5764 ± 0.26
Endosulfan	$X^2_{(6)} = 0.73631$	$Y = 3.3884 + 0.4663x$	0.28580	0.008536 2.095544	04.24	0.4663 ± 0.17
Dichlorvos	$X^2_{(6)} = 0.39455$	$Y = 2.0892 + 0.7729x$	0.05830	0.026083 0.130467	20.80	0.7729 ± 0.20
Cypermethrin	$X^2_{(7)} = 4.14762$	$Y = 3.0697 + 0.5608x$	0.02760	0.010852 0.070539	43.94	0.5608 ± 0.60
Carbaryl	$X^2_{(7)} = 1.10291$	$Y = 2.5245 + 0.5547x$	0.29092	0.018536 4.545754	04.17	0.5547 ± 0.21

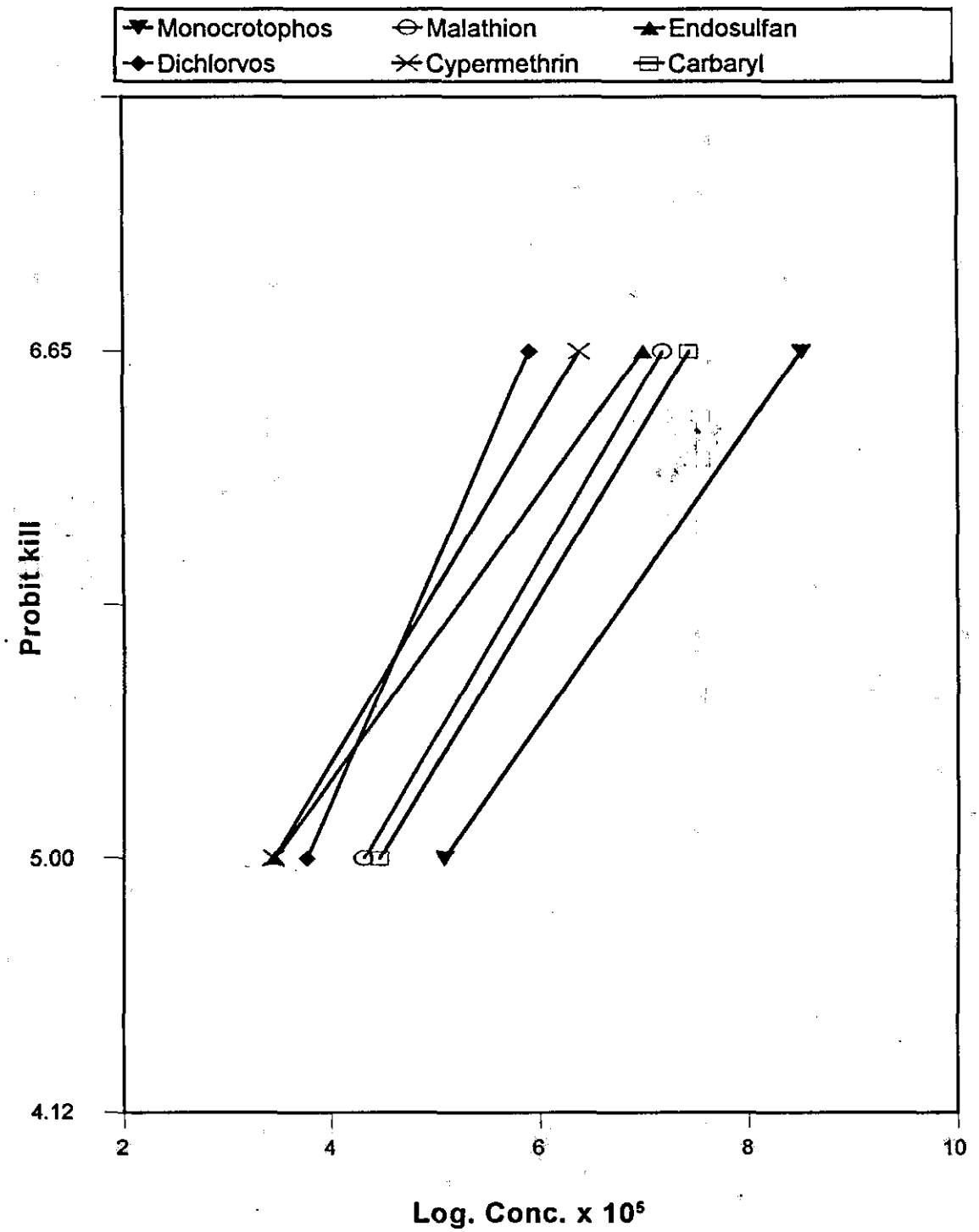

Fig. 7. Regression lines for various insecticides against spinosad - selected strain of *Plutella xylostella*

per cent for cypermethrin, 0.04134 per cent for dichlorvos, 0.24327 per cent for carbaryl, 0.26131 per cent for malathion, 0.58367 per cent for endosulfan and 1.35472 per cent for monocrotophos (Table 11). However, there was a modest decrease in the LC_{50} values of cypermethrin when compared to the LC_{50} value obtained for *B.t.* or spinosad-selected strains. The LC_{50} values calculated, in this case, also showed least toxicity of monocrotophos and the highest of cypermethrin to both the strains of this pest. The comparison of the relative toxicity values among the different insecticides to the cartap hydrochloride-selected strains revealed that cypermethrin, dichlorvos, carbaryl, malathion and endosulfan were 50.71, 32.77, 5.56, 5.18 and 2.32 times, respectively, more toxic than monocrotophos. Regression lines for different insecticides are drawn in Fig. 8.

4.4 Cross-resistance of *B.t.*, spinosad and cartap hydrochloride-selected strains of *P. xylostella* to commonly used insecticides

4.4.1 *B.t.*-selected strain (BS)

The *B.t.*-selected strain of *P. xylostella* did not exhibit any cross-resistance to the other insecticides used in these studies. However, as indicated by the toxicity data given in Table 12, this strain exhibited slightly higher resistance ratios to dichlorvos (1.18), cypermethrin (1.09), endosulfan (1.06) and monocrotophos (1.05) as compared to the parental strain (PS). This indicated that there was a mild decrease in the susceptibility of the *B.t.*-selected strain to these insecticides. On the other hand, this strain required slightly lower doses of malathion and carbaryl to achieve 50 per cent mortality as compared to that of the parental strain.

Table 11. Toxicity of different insecticides to cartap hydrochloride - selected strain of *Plutella xylostella* larvae

Insecticide	Heterogeneity $X^2_{(n-2)}$	Regression equation	LC_{50} (%)	Fiducial Limits	Relative Toxicity	Slope ± S.E.
Monocrotophos	$X^2_{(7)}$ = 1.42483	Y = 2.8101 + 0.4267x	1.35472	0.324409 5.657265	01.00	0.4267 ± 0.31
Malathion	$X^2_{(5)}$ = 0.56775	Y = 2.0940 + 0.6578x	0.26131	0.105128 0.649553	05.18	0.6578 ± 0.20
Endosulfan	$X^2_{(7)}$ = 2.64639	Y = 2.7930 + 0.4630x	0.58367	0.140840 2.418893	02.32	0.4630 ± 0.31
Dichlorvos	$X^2_{(7)}$ = 0.12642	Y = 2.0486 + 0.8161x	0.04134	0.016078 0.106322	32.77	0.8161 ± 0.21
Cypermethrin	$X^2_{(7)}$ = 0.14848	Y = 2.5539 + 0.7138x	0.02671	0.012697 0.056200	50.71	0.7138 ± 0.16
Carbaryl	$X^2_{(7)}$ = 0.22262	Y = 2.7932 + 0.5031x	0.24327	0.068202 0.867710	05.56	0.5031 ± 0.28

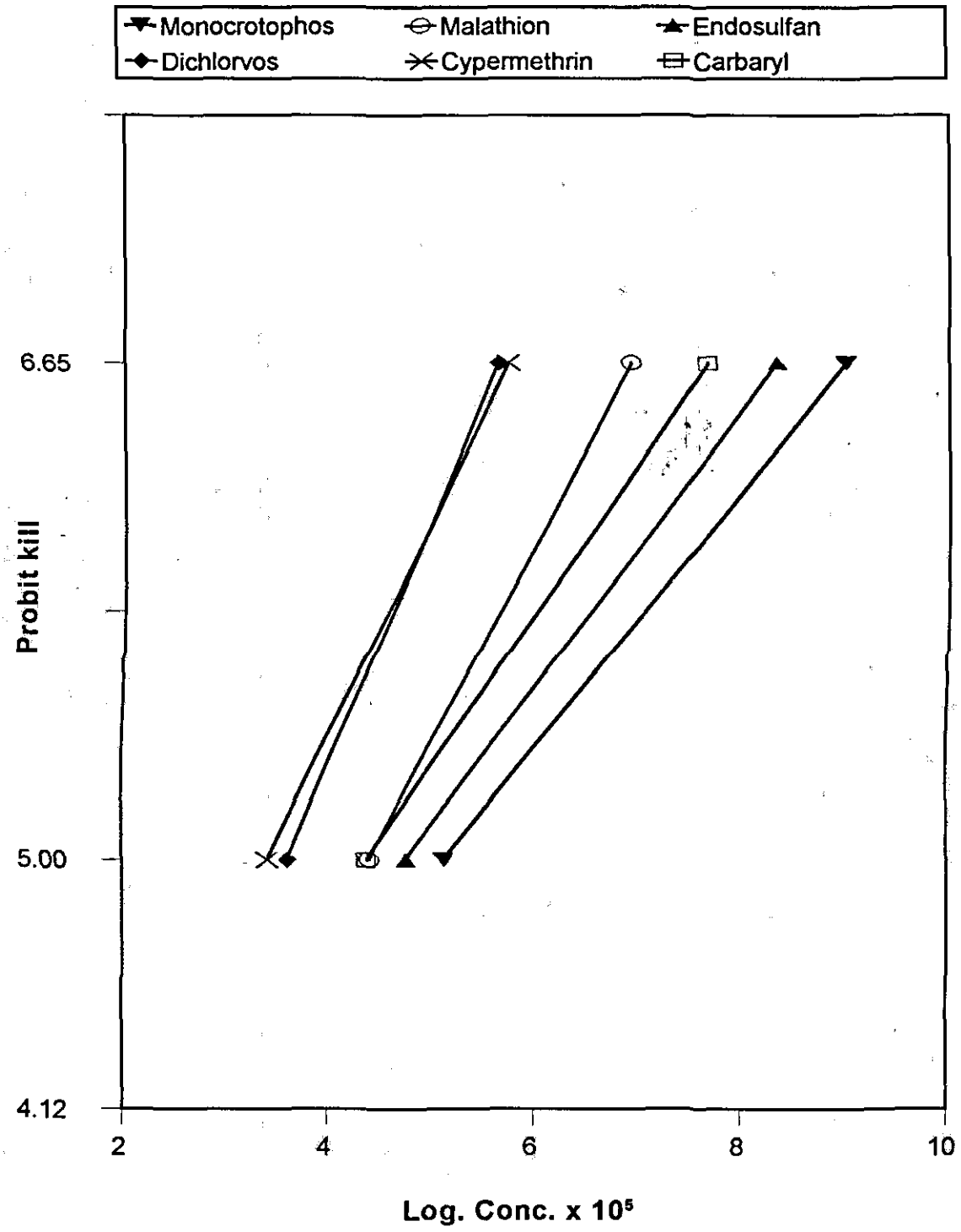

Fig. 8. Regression lines for various insecticides against cartap hydrochloride - selected strain of *Plutella xylostella*

Table 12. Cross resistance of *Bacillus thuringiensis* var. *kurstaki* - selected strain of *Plutella xylostella* to different insecticides

Insecticide	Heterogeneity $X^2_{(n-2)}$	Regression equation	LC_{50} (%)	Fiducial Limits	Resistance of BS over PS	Slope ± S.E.
Monocrotophos(BS)	$X^2_{(5)} = 5.14051$	Y = 3.0071 + 0.3864x	1.43745	0.285614 - 7.234464	1.05	0.3864 ± 0.35
Monocrotophos(PS)	$X^2_{(5)} = 0.54422$	Y = 3.1770 + 0.4407x	1.36997	0.579329 - 6.580847		0.4407 ± 0.34
Malathion (BS)	$X^2_{(5)} = 1.03837$	Y = 2.6965 + 0.5381x	0.19083	0.020934 - 1.739620	0.86	0.5381 ± 0.49
Malathion (PS)	$X^2_{(5)} = 0.55654$	Y = 3.1255 + 0.5597x	0.22310	0.079376 - 0.627087		0.5597 ± 0.22
Endosulfan (BS)	$X^2_{(6)} = 2.04376$	Y = 2.8034 + 0.4649x	0.53087	0.135051 - 2.086794	1.06	0.4649 ± 0.30
Endosulfan (PS)	$X^2_{(6)} = 0.38460$	Y = 3.1708 + 0.4942x	0.50192	0.128010 - 1.326596		0.4942 ± 0.30
Dichlorvos (BS)	$X^2_{(7)} = 0.42654$	Y = 1.9497 + 0.8223x	0.05121	0.024288 - 0.107983	1.18	0.8223 ± 0.16
Dichlorvos (PS)	$X^2_{(6)} = 0.29341$	Y = 2.0909 + 0.7995x	0.04350	0.019931 - 0.094951		0.7995 ± 0.17
Cypermethrin (BS)	$X^2_{(6)} = 0.52515$	Y = 2.5802 + 0.7046x	0.02716	0.012325 - 0.059856	1.09	0.7046 ± 0.17
Cypermethrin (PS)	$X^2_{(6)} = 0.49592$	Y = 2.8439 + 0.6344x	0.02502	0.010129 - 0.061847		0.6344 ± 0.20
Carbaryl (BS)	$X^2_{(7)} = 1.06598$	Y = 2.4999 + 0.5600x	0.29095	0.111586 - 0.758647	0.90	0.5600 ± 0.21
Carbaryl (PS)	$X^2_{(7)} = 1.07955$	Y = 2.5692 + 0.5391x	0.32265	0.118326 - 0.879813		0.5391 ± 0.22

BS = *Bacillus thuringiensis*-selected strain
PS = Parental strain

4.4.2 Spinosad-selected strain (SS)

The spinosad-selected strain of *P. xylostella* did not show cross-resistance to any of the insecticides tested (Table 13). Susceptibility of *this strain when compared to PS rather increased* when resistance ratios of 0.57, 0.89, 0.90 and 0.93 were worked out after the test insect was exposed to endosulfan, monocrotophos, carbaryl and malathion, respectively. The data also indicate that there was a mild decrease in the toxicity of dichlorvos and cypermethrin which offered resistance ratios of 1.34 and 1.105, respectively.

4.4.3 Cartap hydrochloride-selected strain (CS)

The cartap hydrochloride-selected strain of *P. xylostella* also did not exhibit cross-resistance to any of the six insecticides tested in the present studies (Table 14). The LC_{50} values of monocrotophos (1.35472), dichlorvos (0.04134) and carbaryl (0.24327) for CS were marginally lower than those of the parental strain (1.36997; 0.0435; 0.32265, respectively). The cross-resistance ratios of CS over PS for carbaryl, dichlorvos, monocrotophos, cypermethrin, endosulfan and malathion were 0.75, 0.95, 0.99, 1.07, 1.16 and 1.17, respectively. Slightly higher resistance ratios of cypermethrin, endosulfan and malathion indicate a decrease in susceptibility of this strain to these insecticides as compared to the parental strain.

4.5 Comparative biological studies on *B.t.*, spinosad and cartap hydrochloride-selected and parental strains of *P. xylostella*

The biological studies on four strains of *P. xylostella* i.e. three selected under insecticidal pressure and one parental strain were carried

Table 13. Cross resistance of spinsosad - selected strains of *Plutella xylostella* to different insecticides

Insecticide	Heterogeneity $X^2_{(n-2)}$	Regression equation	LC_{50} (%)	Fiducial Limits	Resistance of BS over PS	Slope ± S.E.
Monocrotophos(SS)	$X^2_{(6)} = 1.38082$	Y = 2.5538 + 0.4812x	1.21278	0.277315 - 5.381490	0.89	0.4812 ± 0.33
Monocrotophos(PS)	$X^2_{(5)} = 0.54422$	Y = 3.1770 + 0.4407x	1.36997	0.579329 - 6.580847		0.4407 ± 0.34
Malathion (SS)	$X^2_{(6)} = 0.80534$	Y = 2.5120 + 0.5764x	0.20721	0.077586 - 0.553415	0.93	0.5764 ± 0.21
Malathion (PS)	$X^2_{(5)} = 0.55654$	Y = 3.1255 + 0.5597x	0.22310	0.079376 - 0.627087		0.5597 ± 0.22
Endosulfan (SS)	$X^2_{(6)} = 0.73631$	Y = 3.3884 + 0.4663x	0.28559	0.008536 - 0.095544	0.57	0.4663 ± 0.26
Endosulfan (PS)	$X^2_{(6)} = 0.38460$	Y = 3.1708 + 0.4942x	0.50192	0.128010 - 1.326596		0.4942 ± 0.30
Dichlorvos (SS)	$X^2_{(6)} = 0.39455$	Y = 2.0892 + 0.7729x	0.05833	0.026083 - 0.130467	1.34	0.7729 ± 0.17
Dichlorvos (PS)	$X^2_{(6)} = 0.29341$	Y = 2.0909 + 0.7995x	0.04350	0.019931 - 0.094951		0.7995 ± 0.17
Cypermethrin (SS)	$X^2_{(7)} = 4.14762$	Y = 3.0697 + 0.5608x	0.02766	0.010852 - 0.070539	1.11	0.5608 ± 0.20
Cypermethrin (PS)	$X^2_{(7)} = 0.49592$	Y = 2.8439 + 0.6344x	0.02502	0.010129 - 0.061847		0.6344 ± 0.20
Carbaryl (SS)	$X^2_{(7)} = 1.10291$	Y = 2.5245 + 0.5547x	0.29027	0.018536 - 0.545754	0.90	0.5547 ± 0.60
Carbaryl (PS)	$X^2_{(7)} = 1.07955$	Y = 2.5692 + 0.5391x	0.32265	0.118326 - 0.879813		0.5391 ± 0.22

SS = Spinosad-selected strain
PS = Parental strain

Table 14. Cross resistance of cartap hydrochloride - selected strain of *Plutella xylostella* to different insecticides

Insecticide	Heterogeneity $X^2_{(n-2)}$	Regression equation	LC_{50} (%)	Fiducial Limits	Resistance of BS over PS	Slope ± S.E.
Monocrotophos(CS)	$X^2_{(7)} = 1.42483$	Y = 2.8101 + 0.4267x	1.35472	0.324409 - 5.657265	0.98	0.4267 ± 0.31
Monocrotophos(PS)	$X^2_{(5)} = 0.54422$	Y = 3.1770 + 0.4407x	1.36997	0.579329 - 6.580847		0.4407 ± 0.34
Malathion (CS)	$X^2_{(5)} = 0.56775$	Y = 2.0940 + 0.6578x	0.26131	0.105128 - 0.649553	1.17	0.6578 ± 0.20
Malathion (PS)	$X^2_{(5)} = 0.55654$	Y = 3.1255 + 0.5597x	0.22310	0.079376 - 0.627087		0.5597 ± 0.22
Endosulfan (CS)	$X^2_{(7)} = 2.64639$	Y = 2.7930 + 0.4630x	0.58367	0.140840 - 2.418893	1.16	0.4630 ± 0.31
Endosulfan (PS)	$X^2_{(6)} = 0.38460$	Y = 3.1708 + 0.4942x	0.50192	0.128010 - 1.326596		0.4942 ± 0.30
Dichlorvos (CS)	$X^2_{(7)} = 0.12642$	Y = 2.0486 + 0.8161x	0.04134	0.016078 - 0.106322	0.95	0.8161 ± 0.21
Dichlorvos (PS)	$X^2_{(6)} = 0.29341$	Y = 2.0909 + 0.7995x	0.04350	0.019931 - 0.094951		0.7995 ± 0.17
Cypermethrin (CS)	$X^2_{(7)} = 0.14848$	Y = 2.5539 + 0.7138x	0.02671	0.012697 - 0.56200	1.07	0.7138 ± 0.16
Cypermethrin (PS)	$X^2_{(7)} = 0.49592$	Y = 2.8439 + 0.6344x	0.02502	0.010129 - 0.061847		0.6344 ± 0.20
Carbaryl (CS)	$X^2_{(7)} = 0.22262$	Y = 2.7932 + 0.5031x	0.24327	0.068202 - 0.867710	0.75	0.5031 ± 0.28
Carbaryl (PS)	$X^2_{(7)} = 1.07955$	Y = 2.5692 + 0.5391x	0.32265	0.118326 - 0.879813		0.5391 ± 0.22

CS = Cartap hydrochloride-selected strain
PS = Parental strain

out with a view to find out, if the selection pressure of insecticides was in any way affecting the life processes of the insect. Findings of various biological parameters of these strains are presented below:

4.5.1 Mating duration

The average mating duration of *B.t.*, spinosad and cartap hydrochloride-selected strains of *P. xylostella* was 64.0 (50-70), 64.5 (55-70) and 64.0 (55-70) minutes, respectively (Table 15). On the other hand, the corresponding figures with respect to the parental strain were 65.5 (55-75) minutes. The variations in the mating durations among the insecticide-selected strains were non-significant. However, the mating period of 65.5 minutes in case of the parental strain was significantly higher than in case of *B.t.* and cartap hydrochloride-selected strains but it was on par with spinosad-selected strain.

4.5.2 Pre-oviposition period

The mean pre-oviposition period for the *B.t.*, spinosad and cartap hydrochloride-selected strains of *P. xylostella* was 0.31 (0.29-0.33), 0.32 (0.29-0.33) and 0.31 (0.29-0.33) days, respectively (Table 15), whereas the corresponding value for the parental strain was 0.31 (0.29-0.33) days. The differences among the pre-oviposition period of all the four strains were statistically non-significant and hence it could be concluded that the pre-oviposition period of this insect was not influenced due to the selection pressures of *B.t.*, spinosad or cartap hydrochloride in the larval stage.

4.5.3 Oviposition period

The mean oviposition periods for *B.t.*, spinosad, cartap hydrochloride-selected and the parental strains of *P. xylostella* were 6.85

Table 15. Various biological parameters of insecticide-selected and parental strains of *Plutella xylostella*

Parameter	*B.t.*-selected strain	Spinosad-selected strain	Cartap hydrochloride -selected strain	Parental strain	C.D. (P = 0.05)
1	2	3	4	5	6
Mating duration (min.)	64.0* ± 1.97** (50 - 70)***	64.50 ± 1.57 (55.00 - 70.00)	64.00 ± 1.52 (55.00 - 70.00)	65.50 ± 1.74 (55.00 - 75.00)	1.25
Pre-oviposition period (days)	0.31 ± 0.007 (0.29 - 0.33)	0.32 ± 0.008 (0.29 - 0.33)	0.31 ± 0.01 (0.29 - 0.33)	0.31 ± 0.005 (0.29 - 0.33)	N.S.
Oviposition period (days)	6.85 ± 0.13 (6.50 - 7.50)	6.85 ± 0.16 (6.00 - 7.50)	6.80 ± 0.15 (6.00 - 7.50)	6.80 ± 0.13 (6.12 - 7.50)	N.S.
Post-oviposition period (days)	7.75 ± 0.21 (7.00 - 8.50)	7.75 ± 0.20 (7.00 - 8.50)	7.80 ± 0.20 (7.80 - 8.50)	7.80 ± 0.21 (7.00 - 8.50)	N.S.
Fecundity (No. of eggs laid/ female)	93.20 ± 1.14 (87.00 - 98.00)	94.00 ± 1.22 (89.00 - 101.00)	90.90 ± 0.73 (87.00 - 93.00)	115.10 ± 1.75 (107.00 - 122.00)	5.4
Incubation period (days)	3.50 ± 0.01 (3.00 - 4.00)	3.40 ± 0.067 (3.00 - 3.50)	3.46 ± 0.069 (3.00 - 3.50)	3.50 ± 0.049 (3.00 - 4.00)	N.S.
Hatchability (% eggs hatched)	86.33 ± 0.41 (83.33 - 93.33)	86.33 ± 0.90 (83.33 - 93.33)	87.33 ± 0.93 (80.00 - 93.33)	84.10 ± 0.80 (83.33 - 90.00)	N.S.
Adult longevity (days) :					
Female	14.80 ± 0.15 (14.50 - 15.50)	14.70 ± 0.13 (14.50 - 15.50)	14.80 ± 0.16 (14.50 - 15.50)	14.80 ± 0.16 (14.50 - 15.50)	N.S.
Male	10.10 ± 0.15 (9.50 - 10.50)	10.00 ± 0.16 (9.50 - 10.50)	10.00 ± 0.16 (9.50 -10.50)	10.10 ± 0.15 (9.50 - 10.50)	N.S.

Contd.....

1	2	3	4	5	6
Larval duration (days) :					
1st instar	2.70 ± 0.13 (2.50 - 4.00)	2.80 ± 0.10 (2.50 - 4.00)	2.78 ± 0.16 (2.50 - 4.00)	2.80 ± 0.10 (2.60 - 4.00)	N.S.
2nd instar	2.90 ± 0.14 (2.50 - 3.50)	3.00 ± 0.16 (2.50 - 4.00)	2.90 ± 0.16 (2.50 - 3.50)	2.80 ± 0.18 (2.50 - 3.50)	N.S.
3rd instar	2.95 ± 0.12 (2.50 - 3.50)	3.00 ± 0.13 (2.50 - 3.50)	2.95 ± 0.12 (2.50 - 3.50)	3.00 ± 0.13 (2.50 - 3.50)	N.S.
4th instar	2.70 ± 0.15 (2.50 - 3.50)	2.80 ± 0.07 (2.50 - 3.00)	2.80 ± 0.07 (2.50 - 3.00)	2.70 ± 0.15 (2.50 - 3.50)	N.S.
Total larval duration (days)	11.25 ± 0.54 (10.00 - 14.50)	11.60 ± 0.47 (10.00 - 14.50)	11.35 ± 0.51 (10.00 - 14.00)	11.20 ± 0.56 (10.00 - 14.50)	N.S.
Pre-pupal duration (days)	1.70 ± 0.17 (1.00 - 2.00)	1.90 ± 0.16 (1.50 - 2.00)	1.80 ± 0.16 (1.00 - 2.00)	1.70 ± 0.16 (1.00 - 2.00)	N.S.
Pupal duration (days)	3.90 ± 0.19 (3.00 - 4.00)	3.90 ± 0.19 (3.00 - 4.00)	3.80 ± 0.20 (3.00 - 5.00)	3.90 ± 0.18 (3.00 - 5.00)	N.S.

* Average of 10 pairs/individuals
** S.E.(m)
***Range

(6.50-7.50), 6.85 (6.00-7.50), 6.80 (6.00-7.50) and 6.80 (6.50-7.50) days, respectively (Table 15). The insignificant differences in the above figures revealed that the selection with *B.t.*, spinosad and cartap hydrochloride for 7 generations did not affect the oviposition period of this insect pest.

4.5.4 Post-oviposition period

The mean post-oviposition periods for *B.t.*, spinosad and cartap hydrochloride-selected and parental strains of *P. xylostella* were 7.75 (7.00-8.50), 7.75 (7.00-8.50), 7.80 (7.00-8.50) and 7.80 (7.00-8.50) days, respectively (Table 15). It is clear from the data that the post-oviposition period of the three insecticide-selected and parental strains were statistically at par. It could, thus be, inferred that the selection pressure of *B.t.*, spinosad and cartap hydrochloride did not affect the post-oviposition period of this pest insect.

4.5.5 Fecundity

The fecundity of *B.t.*, spinosad and cartap hydrochloride-selected and parental strains of *P. xylostella* was 93.2 (87-98), 94.0 (89-101), 90.9 (87-93) and 115.1 (107-122) eggs/female, respectively (Table 15). It is evident from the data that the selection pressure of the three insecticides resulted in significant reduction of egg laying capacity of this insect as compared to the parental strain. However, the fecundity of insecticide-selected strains was statistically at par among each other.

Maximum number of eggs were laid within 24 hours of mating (Fig.9) when on an average 35.5, 38.4, 37.8 and 38.0 eggs were laid by the *B.t.*, spinosad and cartap hydrochloride-selected and parental strains, respectively. This number reduced drastically with the advancement in the age of the female in all the strains. The egg laying terminated by 7th day

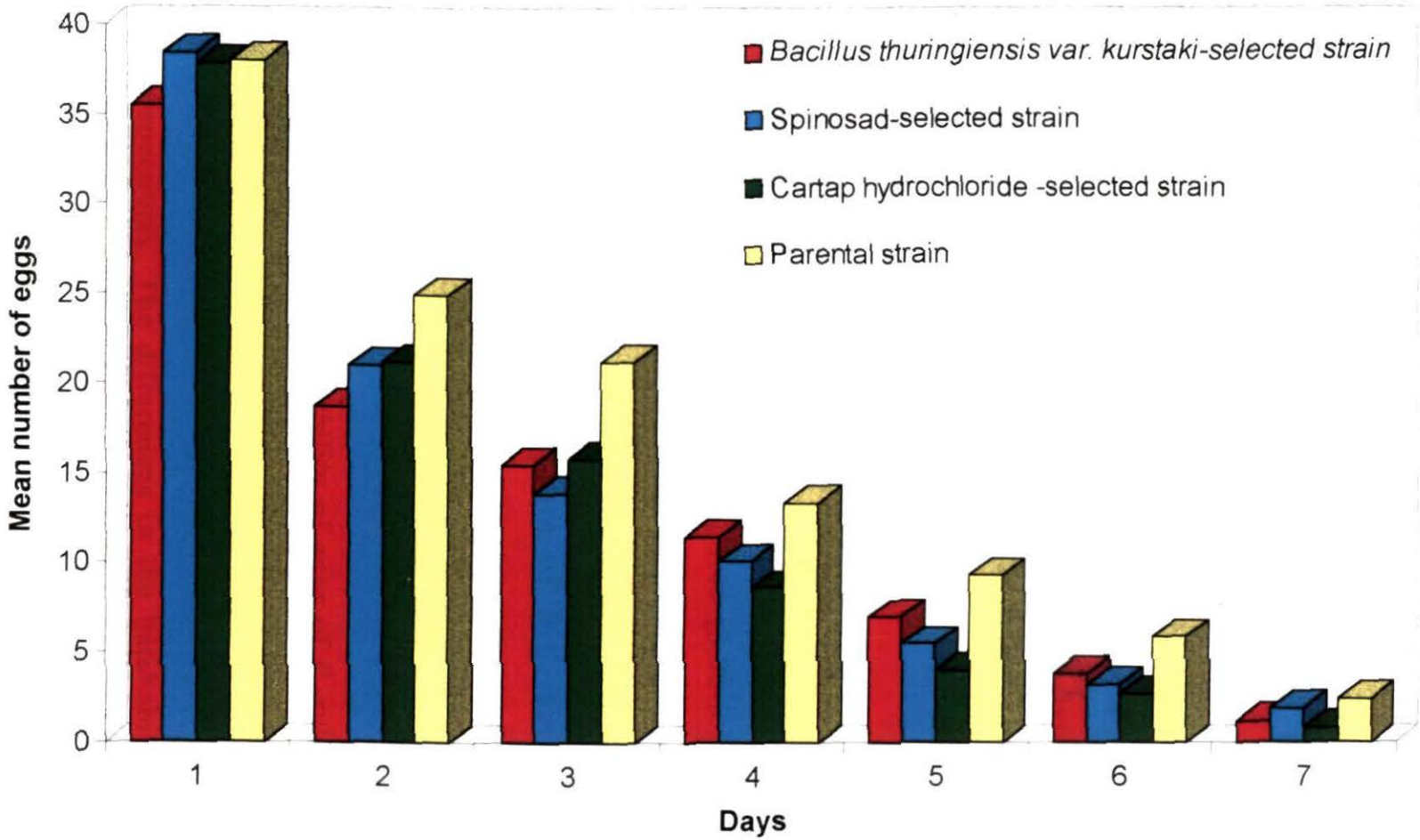

Fig. 9. Daily mean fecundity of biopesticides - selected and parental strains of *Plutella xylostella*

after mating when on an average 1.2, 1.9, 0.8 and 2.4 eggs were observed to be laid by the female of parental, *B.t.*, spinosad and cartap hydrochloride-selected strains, respectively. There were marginal differences in the oviposition rate of the four strains on the first day, but subsequently it was conspicuously higher in case of parental strain as compared to the three selected strains in the following days of oviposition. The detailed information on daily fecundity of the four strains is presented in Tables 16 to 19. On an average, *B.t.*, spinosad and cartap hydrochloride-selected and parental strain mothslaid 93.2, 94.0, 90.9 and 115.1 eggs in her life time.

4.5.6 Incubation period

The mean incubation period of the eggs laid by the females of *B.t.*, spinosad, cartap hydrochloride-selected and parental strains of *P. xylostella* was 3.50 (3.00-3.60), 3.40 (3.00-3.50), 3.46 (3.00-3.50) and 3.50 (3.00-4.00) days, respectively (Table 15). The variations in the incubation periods among all the strains were statistically non-significant and it could be implied that the selection pressure of *B.t.*, spinosad and cartap hydrochloride did not affect this parameter of the biological process of *P. xylostella.*

4.5.7 Hatchability

The mean percent hatchability of eggs of *P. xylostella* laid by *B.t.*, spinosad and cartap hydrochloride-selected and parental strains was 86.33 (83.33-93.33), 86.33 (83.33-93.33), 87.33 (80.0-93.33) and 84.10 (83.33-90.00), respectively. The differences in the hatchability were, however, statistically non-significant and hence it could be concluded that selection of *B.t.*, spinosad and cartap hydrochloride did not impair hatchability of the eggs laid by this insect.

Table 16. Daily fecundity of *Bacillus thuringiensis* var. *kurstaki* - selected strain of *Plutella xylostella*

Pair	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
1	32	19	15	9	12	5	2	0	0	0	0	0	0	0	94
2	38	18	15	11	9	7	0	0	0	0	0	0	0	0	98
3	35	10	12	18	9	5	3	0	0	0	0	0	0	0	92
4	45	16	11	14	10	0	0	0	0	0	0	0	0	0	96
5	30	28	12	8	6	5	5	0	0	0	0	0	0	0	94
6	32	24	20	7	3	3	0	0	0	0	0	0	0	0	89
7	41	15	22	14	2	1	0	0	0	0	0	0	0	0	95
8	38	17	19	11	8	4	0	0	0	0	0	0	0	0	97
9	34	21	12	10	7	5	0	0	0	0	0	0	0	0	90
10	30	19	16	13	4	3	2	0	0	0	0	0	0	0	87
Total	355	187	155	115	70	38	12	0	0	0	0	0	0	0	932
Mean	35.5	18.7	15.5	11.5	7.0	3.8	1.2	0	0	0	0	0	0	0	93.2
♀ laying egg (%)	100	100	100	100	100	90.0	40.0	0	0	0	0	0	0	0	

Table 17. Daily fecundity of spinsosad - selected strain of *Plutella xylostella*

Pair	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
1	32	21	12	9	8	6	2	0	0	0	0	0	0	0	90
2	51	16	14	10	0	0	0	0	0	0	0	0	0	0	91
3	36	24	13	11	6	5	0	0	0	0	0	0	0	0	95
4	47	24	12	9	9	0	0	0	0	0	0	0	0	0	101
5	33	17	10	10	9	8	7	0	0	0	0	0	0	0	94
6	31	19	18	7	8	4	5	0	0	0	0	0	0	0	92
7	44	23	17	15	0	0	0	0	0	0	0	0	0	0	99
8	32	18	16	10	6	4	3	0	0	0	0	0	0	0	89
9	42	21	18	9	4	2	0	0	0	0	0	0	0	0	96
10	36	27	9	11	5	3	2	0	0	0	0	0	0	0	93
Total	384	210	139	101	55	32	19	0	0	0	0	0	0	0	940
Mean	38.4	21.0	13.9	10.1	5.5	3.2	1.9	0	0	0	0	0	0	0	94.0
♀ laying egg (%)	100	100	100	100	80.0	70.0	50.0	0	0	0	0	0	0	0	

Table 18. Daily fecundity of cartap hydrochloride - selected strain of *Plutella xylostella*

Pair	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
1	36	21	16	10	5	3	2	0	0	0	0	0	0	0	93
2	30	17	21	11	6	2	2	0	0	0	0	0	0	0	89
3	41	25	19	7	0	0	0	0	0	0	0	0	0	0	92
4	36	19	17	10	3	2	0	0	0	0	0	0	0	0	87
5	56	16	12	6	2	1	1	0	0	0	0	0	0	0	94
6	33	23	23	0	6	4	2	0	0	0	0	0	0	0	91
7	40	24	12	8	5	3	0	0	0	0	0	0	0	0	92
8	34	18	15	11	8	7	0	0	0	0	0	0	0	0	93
9	30	23	9	17	5	3	1	0	0	0	0	0	0	0	88
10	42	26	14	6	0	2	0	0	0	0	0	0	0	0	90
Total	378	212	158	86	40	27	8	0	0	0	0	0	0	0	909
Mean	37.8	21.2	15.8	8.6	4.0	2.7	0.8	0	0	0	0	0	0	0	90.9
♀ laying egg (%)	100	100	100	90	80	90	50	0	0	0	0	0	0	0	

Table 19. Daily fecundity of parental strain of *Plutella xylostella*

Pair	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
1	36	20	18	18	10	8	2	0	0	0	0	0	0	0	112
2	41	13	24	12	10	7	0	0	0	0	0	0	0	0	107
3	33	29	14	10	14	9	2	0	0	0	0	0	0	0	111
4	31	19	20	25	0	9	8	0	0	0	0	0	0	0	112
5	43	24	20	12	8	2	0	0	0	0	0	0	0	0	109
6	48	33	25	0	13	0	1	0	0	0	0	0	0	0	120
7	39	32	28	12	9	2	0	0	0	0	0	0	0	0	122
8	40	26	19	9	12	10	3	0	0	0	0	0	0	0	119
9	32	29	24	17	8	5	2	0	0	0	0	0	0	0	117
10	37	24	20	19	9	7	6	0	0	0	0	0	0	0	122
Total	380	249	212	134	93	59	24	0	0	0	0	0	0	0	1151
Mean	38.0	24.9	21.2	13.4	9.3	5.9	2.4	0	0	0	0	0	0	0	115.1
♀ laying egg (%)	100	100	100	90.0	90.0	90.0	70.0	0	0	0	0	0	0	0	

4.5.8 Adult longevity

The perusal of the data pertaining to adult longevity of both male and female *P. xylostella* show that it was not affected by the insecticidal pressure (Table 15). The mean female longevity of *B.t.*, spinosad and cartap hydrochloride-selected and parental strains was 14.80, 14.70, 14.80 and 14.80 days, respectively, with a range of 14.50 to 15.50 days. The corresponding figures for the male *P. xylostella* were 10.10, 10.00, 10.00 and 10.10 days, respectively, (range 9.50 to 10.50 days). In general, longevity of males was observed to be shorter as compared to the females.

4.5.9 Larval durations

The 1st instar larva of *B.t.*, spinosad, cartap hydrochloride-selected and parental strains of *P. xylostella* took 2.70 (2.50-4.00), 2.80 (2.50-4.00), 2.70 (2.50-4.00) and 2.80 (2.60-4.00) days, respectively, to pass on to the next stage. The corresponding figures for the 2nd instar were 2.90 (2.50-3.50), 3.00 (2.50-4.00), 2.90 (2.50-3.50) and 2.80 (2.50-3.50) days, respectively. Similarly, the mean larval durations of 3rd instar larvae of the above said strains were recorded to be 2.95, 3.00, 2.95 and 3.00 days, respectively, (range 2.50 to 3.50 days). The corresponding figures, with respect to the 4th instar larvae were 2.70 (2.50-3.50), 2.80 (2.50-3.00), 2.80 (2.50-3.00) days, respectively, as compared to 2.70 (2.50 to 3.50) days of parental strain.

The differences in the larval durations of all the four instars of the insecticide-selected and normal strains of this insect, were found to be statistically non-significant. Thus, it could be inferred that the selection pressure of insecticides tried in the present studies had no marked affect on the larval durations of this pest.

The mean total larval period of the three insecticide-selected strains and that of the parental strain of this insect was 11.25 (10.00-14.50), 11.60 (10.00-14.5), 11.35 (10.00-14.00) and 11.30 (10.00-14.50) days, respectively (Table 15). The differences in the total larval durations were also statistically non- significant among all the strains.

4.5.10 Pre-pupal duration

The data generated on the pre-pupal period of *B.t.*, spinosad and cartap hydrochloride-selected strains of *P. xylostella* (Table 15) revealed no significant variations [1.70 (1.00-2.00), 1.90 (1.50-2.00), 1.80 (1.00-2.00) days, respectively] as compared to that of parental strain [1.70 (1.00-2.00) days].

4.5.11 Pupal duration

The pupal period of *B.t.*, spinosad and cartap hydrochloride-selected strains was recorded to be 3.90 (3.00-4.00), 3.90 (3.00-4.00) and 3.80 (3.00-5.00) days, respectively (Table 15). The differences in the durations of this parameter were, however, statistically non-significant as compared to parental strain [3.90 (3.00-5.00) days].

4.5.12 Larval weight

The 2nd instar larva of *B.t.*, spinosad and cartap hydrochloride-selected and parental strains of *P. xylostella* weighed 18.0, 19.0, 18.0 and 19.0 mg (range 15.0-20.0 mg), respectively, (Table 20) whereas the average larval weight of the 3rd instar was 35.0, 34.0, 35.0 and 35.0 mg (range 30.0 to 40.0 mg), respectively. The corresponding figures for 4th instar larvae of the three insecticide-selected and parental strain were 72.0, 71.0, 72.0 and 72.0 mg, respectively, with a range of 65.0 to 80.0 mg. The numerical differences in the mean larval weights of 2nd, 3rd and 4th

Table 20. Weight (mg) of different life stages of insecticide - selected and parental strains of *Plutella xylostella*

Stage	*B.t.*- selected strain	Spinosad-selected strain	Cartap hydrochloride -selected strain	Parental strain	C.D. (P = 0.05)
Larva					
2nd instar	18.0* ± 1.2** (15.0 - 20.0)***	19.0 ± 1.0 (15.0 - 20.0)	18.0 ± 1.2 (15.0 - 20.0)	19.0 ± 1.0 (15.0 - 20.0)	N.S.
3rd instar	35.0 ± 2.0 (30.0 - 40.0)	34.0 ± 2.1 (30.0 - 40.0)	35.0 ± 2.2 (30.0 - 40.0)	35.0 ± 2.0 (30.0 - 40.0)	N.S.
4th instar	72.0 ± 2.4 (65.0 - 80.0)	71.0 ± 2.8 (65.0 - 80.0)	72.0 ± 2.7 (65.0 - 80.0)	72.0 ± 2.7 (65.0 - 80.0)	N.S.
Pre-pupa	93.0 ± 2.4 (80.0 - 100.0)	92.0 ± 2.6 (80.0 - 100.0)	93.0 ± 2.4 (80.0 - 100.0)	92. 0 ± 2.3 (80.0 - 100.0)	N.S.
Pupa	101.6 ± 0.8 (98.0 - 105.0)	100.9 ± 0.8 (98.0 - 105.0)	101.5 ± 0.8 (98.0 - 105.0)	101.5 ± 0.8 (90.0 - 105.0)	N.S.

* Average of 10 individuals
** S.E.(m)
***Range

instar larva of the three insecticide-selected and the parental strains, individually, were statistically non-significant.

4.5.13 Pre-pupal weight

On an average, the pre-pupal weights of the *B.t.*, spinosad and cartap hydrochloride-selected strains were 93.0, 92.0 and 93.0 mg in a range of 80.0 to 100.0 mg, respectively (Table 20). These were observed to be statistically similar to that of the average pre-pupal weight of the parental strain (92.0 mg).

4.5.14 Pupal weight

The average pupal weight of 101.6 (98.0-105.0), 100.9 (98.0-105.0), 101.5 (98.0-105.0) and 101.5 (90.0-105.0) mg was worked out with respect to *B.t.*, spinosad, cartap hydrochloride-selected and parental strains, respectively (Table 20). The differences in the mean pupal weight of the three-selected strains as compared to that of the parental strain were found statistically non-significant.

4.5.15 Larval measurements

Since the 1st instar larvae feed by making mines in the leaves, the data pertaining to this parameter was started to be recorded from the 2nd instar. The average length of the 2nd instar larvae of *B.t.*, spinosad cartap hydrochloride-selected and parental strains was 1.84, 1.84, 1.82 and 1.86 mm, respectively, with a range of 1.40 to 2.00 mm (Table 21). The corresponding figures for the 3rd instar were 3.34 (3.00-3.60), 3.38 (3.20-3.60), 3.38 (3.20-3.60) and 3.40 (3.00-4.00) mm and those for the 4th instar were 6.62 (5.60-7.20), 6.60 (6.40-7.20), 6.60 (6.40-7.20) and 6.64 (6.00-7.20) mm, respectively.

Table 21. Measurements (mm) of different life stages of insecticides - selected and parental strains of *Plutella xylostella*

Stage	Parameter	*B.t.*- selected strain	Spinosad-selected strain	Cartap hydrochloride -selected strain	Parental strain	C.D. (P = 0.05)
1	2	3	4	5	6	7
Larva						
2nd instar						
	Length	1.84* ± 0.05** (1.40 - 2.00)***	1.84 ± 0.03 (1.40 - 2.00)	1.82 ± 0.05 (1.40 - 2.00)	1.86 ± 0.06 (1.40 - 2.00)	N.S.
	Breadth	0.18 ± 0.006 (0.14 - 0.20)	0.18 ± 0.003 (0.14 - 0.20)	0.18 ± 0.006 (0.14 - 0.20)	0.18 ± 0.004 (0.14 - 0.20)	N.S.
3rd instar						
	Length	3.34 ± 0.08 (3.00 - 3.60)	3.38 ± 0.09 (3.20 - 3.60)	3.38 ± 0.09 (3.20 - 3.60)	3.40 ± 0.10 (3.00 - 4.00)	N.S.
	Breadth	0.34 ± 0.006 (0.28 - 0.36)	0.33 ± 0.005 (0.30 - 0.36)	0.34 ± 0.006 (0.30 - 0.36)	0.34 ± 0.006 (0.30 - 0.36)	N.S.
4th instar						
	Length	6.62 ± 0.18 (5.60 - 7.20)	6.60 ± 0.20 (6.40 - 7.20)	6.60 ± 0.20 (6.40 - 7.20)	6.64 ± 0.20 (6.00 - 7.20)	N.S.
	Breadth	0.67 ± 0.01 (0.56 - 0.72)	0.67 ±0.02 (0.60 - 0.72)	0.67 ± 0.02 (0.60 - 0.72)	0.67 ±0.02 (0.60 - 0.72)	N.S.

Contd.....

1	2	3	4	5	6	7
Pupa						
	Length	5.67 ± 0.07 (5.30 - 6.00)	5.54 ± 0.04 (5.30 - 5.70)	5.52 ± 0.04 (5.40 - 5.80)	5.54 ± 0.04 (5.30 - 5.80)	N.S.
	Breadth	1.11 ± 0.02 (1.00 - 1.20)	1.11 ± 0.01 (1.00 - 1.20)	1.11 ± 0.01 (1.00 -1.20)	1.14 ± 0.01 (1.00 - 1.20)	N.S.
Adult						
Male						
	Length	4.82 ± 0.02 (4.60 - 5.10)	4.80 ± 0.018 (4.60 - 5.00)	4.80 ± 0.02 (4.60 - 5.10)	4.87 ± 0.05 (4.60 - 5.10)	N.S.
	Breadth	12.68 ± 0.02 (12.50 - 12.75)	12.67 ± 0.02 (12.50 -12.75)	12.70 ± 0.02 (12.50 - 12.80)	12.68 ± 0.02 (12.50 -12.80)	N.S.
Female						
	Length	4.92 ± 0.03 (4.70 - 5.00)	4.94 ± 0.04 (4.70 - 5.00)	4.92 ± 0.03 (4.70 - 5.00)	4.94 ± 0.04 (4.70 - 5.00)	N.S.
	Breadth	12.92 ± 0.06 (12.00 - 13.00)	12.94 ± 0.04 (12.00 -13.00)	12.92 ± 0.06 (12.00 -13.00)	12.92 ± 0.06 (12.00 - 13.00)	N.S.

* Average of 10 individuals
** S.E.(m)
*** Range

The mean breadth of the 2nd instar larva of the three insecticide-selected and parental strains was 0.18 mm (0.14 to 0.20 mm), and that of the 3rd instar, 0.34 (0.28-0.36), 0.33 (0.30-0.36), 0.34 (0.30-0.36) and 0.34 (0.30-0.36) mm, respectively. The corresponding figures for the 4th instar were 0.67 (0.56-0.72), 0.67 (0.60-0.72), 0.67 (0.60-0.72) and 0.67 (0.60-0.72), respectively.

The differences in the mean dimensions (length and breadth) of the 2nd, 3rd and 4th instar larvae of insecticides-selected/parental strains of this insect were found to be statistically similar (Table 21). It could, thus, be inferred from the above observations that the selection of *P. xylostella* with *B.t.* var. *kurstaki*, spinosad and cartap hydrochloride did not induce any change in the larval size which remained comparable to the parental strain.

4.5.16 Pupal dimensions

The mean length of the pupae of the *B.t.*, spinosad and cartap hydrochloride-selected strains of *P. xylostella* was 5.67 (5.30-6.00), 5.54 (5.30-5.70) and 5.52 (5.40-5.80) mm as compared to 5.54 (5.30-5.80) mm of the parental strain. The data with respect to breadth of *B.t.*, spinosad, cartap hydrochloride and parental strains were 1.11, 1.11, 1.11 and 1.14 mm with a range of 1.00 to 1.20 mm. The above values were statistically at par with each other (Table 21) and there was no evidence of any effect on the pupal measurements due to the selection pressure of the insecticides tried here.

4.5.17 Adult dimensions

The mean length of males of the *B.t.*, spinosad and cartap hydrochloride - selected and parental strains of *P. xylostella* were 4.82

(4.60-5.10), 4.80 (4.60-5.00), 4.80 (4.60-5.10) and 4.87 (4.60-5.10) mm, respectively, as compared to 4.92, 4.94, 4.92 and 4.94 mm (4.70 to 5.00 mm), respectively, of the females (Table 21). The corresponding figures with respect to the breadth of individuals of the above said strains of this insect were 12.68 (12.50- 12.75), 12.67 (12.50-12.75), 12.70 (12.50-12.80) and 12.68 (12.50-12.80) mm, respectively, in males and 12.92, 12.94, 12.92 and 12.92 mm, respectively, in females with a range of 12.00 to 13.00 mm. The variations in the values mentioned above were statistically non-significant, implying thereby no impact of selection pressure on this biological parameters of this insect.

CHAPTER-V

DISCUSSION

5.1 Toxicity of different insecticides against parental strain of *P.xylostella*

The comparison of the toxicity (LC_{50} values) of nine insecticides (Table 1) in the laboratory to the third instar larvae of *P. xylostella* indicated that spinosad was the most toxic (LC_{50} = 0.00033 per cent) and monocrotophos the least (LC_{50} = 1.36997 per cent). The toxicity of other insecticides, in descending order, was *Bacillus thuringiensis* (*B.t.*) var. *kurstaki* > cartap hydrochloride > cypermethrin > dichlorvos > malathion > carbaryl > endosulfan.

According to Verma *et al.* (1972), the LC_{50} values of malathion, dichlorvos, monocrotophos and endosulfan were 0.007023, 0.01122, 0.01222 and 0.1271 per cent, respectively, to the diamondback moth larvae collected from cauliflower fields around Hisar (Haryana). Later, Saxena *et al.* (1989) observed resistance to cypermethrin in population of this pest collected from Panipat (Haryana) (Resistance Ratio = 144.9). A considerable decrease in the relative toxicity of cypermethrin, dichlorvos, malathion, endosulfan and monocrotophos was observed in the Panipat (Haryana) population of *P. xylostella* by Kalra *et al.* (1997) (The respective LC_{50} values were 0.0420, 0.0565, 0.200, 0.775, 1.557 per cent). These results are in close conformity to those obtained in the present studies.

The LC_{50} value of spinosad, in the present investigations, against *P. xylostella* was 0.00033 per cent (3.3 ppm) being 4151.42 times higher than monocrotophos (the least toxic one). There is no information on the toxicity data of spinosad to *P. xylostella* in the available literature.

However, its LC_{50} values ranged between 2 to over 5 ppm to *Spodoptera exigua*, *H. virescens*, *H. zea*, *Scirtothrips citri* and *Ceratitis capitata* (Adan *et al.*, 1996; Leonard *et al.*, 1996; Hasty *et al.*, 1997; Khan and Morse, 1997 a and b; Lopez *et al.*, 1997).

In the present studies, *B.t.* var. *kurstaki* (potency : 23 million SU/ ml) proved to be 91.76 times more toxic (LC_{50} = 0.01493 per cent; 149.3 ppm) to *P. xylostella* as compared to monocrotophos (LC_{50} = 1.36997 per cent). However, Rabindra *et al.* (1995) worked out the LC_{50} values of *B.t.* var. *kurstaki* (potency : 16000 IU a.i./mg) to be 350.8 and 378.5 ppm with two populations of *P. xylostella* in Tamil Nadu. The variation in the toxicity can be explained on the basis of lower potency of the *B.t.* var. *kurstaki* formulation used in the latter studies. Later, Kumar (1996) observed high toxicity of *B.t.* var. *kurstaki* to *P. xylostella* at still lower doses. Further, *B.t.* var. *kurstaki* formulation having δ-endotoxins alone showed LC_{50} of 148 ppm against this pest (Meenakshisundaram and Gujar, 1998).

Cartap hydrochloride was 77.93 times more toxic (LC_{50} = 0.01758 per cent; 175.8 ppm) to *P. xylostella* as compared to monocrotophos. During 1980-82, the LC_{50} values of cartap hydrochloride in Japan ranged between 22 to 313 ppm against different populations of this pest (Sakai, 1986). However, LC_{50} value of 187 ppm comparable to that obtained in the present studies, were worked out by Adachi and Futai (1992) also from Japan. Similarly, Joia and Udeaan (1997) also reported the high toxicity (LC_{50} = 150 to 200 ppm) of this insecticide to *P. xylostella* in India.

5.2 Development of *B.t.* var. *kurstaki*, spinosad and cartap hydrochloride resistance in *P. xylostella*

5.2.1 *B.t.* var. *kurstaki*

No resistance in *P. xylostella* was observed to have developed when the pest was exposed to *B.t.* var. *kurstaki* for 7 successive generations in the present studies. Instead, a reduction in the resistance (Resistance ratio = 0.76) of this pest was observed in comparison to the response obtained from the parental population. Similar results were reported by Tabashnik *et al.*(1992) with Hawaiin population of this pest insect, where the LC_{50} values got reduced from 24 mg a.i./l to 11 mg a.i./l after exposure of the pest to *B.t.* var. *kurstaki* for 5 successive generations. However, the development of resistance to *B.t.* in insects has been reported to be associated with reduced binding of toxins to midgut membrane (Ferre *et al.*, 1991; Tabashnik *et al.*, 1992). The studies conducted in India have suggested that the affinity of δ-endotoxins to midgut proteases is very high and that is why the pest insect shows high sensitivity to this biopesticide (Dilawari *et al.*, 1996; Meenakshisundaram and Gujar, 1998).

Number of reports have indicated a substantial level of resistance to *B.t.* in *P. xylostella* (Tabashnik *et al.*, 1990; Sinchaisri *et al.*, 1990; Hama *et al.*, 1992; Shelton *et al.*, 1993; Syed, 1992). In most of these reports, the development of resistance has been attributed to its frequent use against this pest in the field. From India, till date, there is no indication of control failure of *P. xylostella* with *B.t.* and as high as 96 per cent control of this insect under field conditions have been achieved (Atwal and Singh, 1969; Narayanan *et al.*, 1970; Singh *et al.*,1976; Varma and

Gill, 1977; Rajamohan and Jayraj, 1978; Nagamani *et al.*, 1995; Kulkarni *et al.*,1995). Further, Kumar (1996) observed that *B.t.*(Potency : 23 million SU/ml) was highly effective against *P. xylostella* even at much lower doses (60 g/ha) than those suggested by formulators (625 g to 1.0 kg/ha). The reports from New Zealand (Cameron *et al.*, 1997; Cameron and Walker, 1998), Central Chile (Garrido and Arraya, 1997), Brazil (Campos *et al.*, 1997) and Yugoslavia (Kranjajic *et al.*,1997) further substantiate the findings of the present studies, where resistance to *B.t.* has not developed so far.

5.2.2 Spinosad

Exposure of *P. xylostella* to spinosad for 7 successive generations did not result in the development of resistance to this insecticide. On the other hand, the LC_{50} values of this insecticide decreased from 0.000299 in the first generation to 0.000270 in the 7th generation. The resistance ratios too, decreased proportionately.

As per the available literature, no such studies have been conducted on the development of resistance to spinosad in *P. xylostella.* However, recently the bioefficacy of spinosad to 10 different strains of *Pseudoplusia includens* (Walker) revealed that the LC_{50} values varied from 4.19 to 13.46 ppm (Mascarenhas and Boethel, 1997). Likewise, in topical bioassays of spinosyn A and D against *H. virescens* larvae, the LC_{50} values ranged between 1.28 to 2.56 µg/g (Sparks *et al.*, 1998).

5.2.3 Cartap hydrochloride

The selection pressure of cartap hydrochloride to third instar larvae of *P. xylostella* revealed no resistance development upto 7 generations.

Instead, a reduction in the resistance (Resistance ratio = 0.83) was observed in comparison to that of the parental population. However, the resistance to cartap hydrochloride in *P. xylostella* has been reported from Japan (Horikiri, 1989; Ozawa *et al.*, 1989; Hama *et al.*, 1990; Hama, 1992); Taiwan (Cheng *et al.*, 1992); China (Chen *et al.*, 1993; Chen *et al.*, 1995) and Korea (Cho and Lee, 1994), where it has been in use for many years against this pest.

The results of the present studies are in conformity with those obtained by Branco and Gatehouse (1997) in laboratory bioassays in Brazil who also did not encounter any resistance in *P. xylostella* to this insecticide. Cartap hydrochloride, since its introduction in India during 1987, is being reported to control *P. xylostella* effectively (Peter *et al.*, 1989; Rai *et al.*, 1992; Nagesh and Verma, 1997). Recently, Joia and Udeaan (1997) observed this insecticide to be highly toxic to the multi-resistant *P. xylostella* population in Punjab with LC_{50} values ranging from 0.015 to 0.020 per cent.

5.3 Cross-resistance of *B.t.* var. *kurstaki*, spinosad and cartap hydrochloride-selected strains of *P. xylostella* to different insecticides

5.3.1 *B.t.* var. *kurstaki*-selected strain

The *B.t.* var. *kurstaki*-selected strain of *P. xylostella* did not show cross-resistance to monocrotophos, malathion, endosulfan, dichlorvos, cypermethrin and carbaryl in the present studies, rather malathion and carbaryl exhibited slightly increased toxicity as compared to the parental strain.

No cross-resistance was observed by Cho and Lee (1994) while working with triflumuron, Lambda-cyhalothrin, prothiophos and cartap hydrochloride against *B.t.*-selected *P. xylostella.* Tabashnik (1994) and Sarnthoy *et al.* (1997) also did not detect any cross-resistance in *B.t.*-selected *P. xylostella* to fenthoate, benfuracarb, fenvalerate, chlorfluazuron, cartap hydrochloride and abamectin. The absence of any cross-resistance to the *B.t.*-selected insects has been attributed to its entirely different mode of action (Stone *et al.*, 1991).

5.3.2 Spinosad-selected strain

Spinosad-selected strain of *P. xylostella* also did not develop cross-resistance to the commonly used insecticides tested against it. As per available literature, studies have yet not been conducted on the development of cross-resistance to the spinosad-selected strain of this insect. Since the mode of action of spinosyn A is altogether different from other insecticides, the chances of development of cross-resistance in this case also seem to be quite dim (Salgado *et al.*, 1997; Sparks *et al.*, 1998). The results obtained in the present studies are in line of the hypothesis as given above.

5.3.3 Cartap hydrochloride-selected strain

There was no cross-resistance in the cartap hydrochloride-selected strain of *P. xylostella* to the six insecticides, i.e. monocrotophos, malathion, endosulfan, dichlorvos, cypermethrin and carbaryl tested in the present studies. Rather the LC_{50} values of monocrotophos, dichlorvos and carbaryl for the selected strain were marginally lower than those against the parental strain. The available literature gives contradictory picture. In one study, no cross-resistance to malathion and carbaryl was observed in this insect

which had been selected for 12 generations to cartap hydrochloride (Cheng, 1986). In another study, a slight cross-resistance in *P. xylostella*, selected for 35 generations to selection pressure of cartap hydrochloride, was observed to dichlorvos and malathion and no cross-resistance to cypermethrin (Chen *et al.*, 1993). The results obtained in the present studies, by and large, fall in line with those obtained in earlier studies. The variations may be explained by the facts that the exposure of the insect to cartap hydrochloride had been given for much greater number of generations in other studies.

5.4 Biology of *P. xylostella* (selected *vis-a-vis* parental strain)

The present studies indicate that out of the 14 biological parameters studied, only two *viz.*, mating duration and fecundity of *P .xylostella* were adversely affected in the *B.t.* var. *kurstaki*, spinosad and cartap hydrochloride-selected strains. There were no differences in the other parameters *viz.* Pre-oviposition, oviposition, post-oviposition and incubation periods, hatchability, adult longevity (male and female), larval (1st to 4th instar), pre-pupal and pupal durations, among the three insecticide-selected and parental strains. Further, weights and measurements of various life stages of this insect also did not differ in any of the four strains.

5.4.1 Mating duration

The mating durations of *B.t.*, spinosad and cartap hydrochloride-selected strains of *P. xylostella* were statistically at par among each other, though significant differences were observed when the mating durations of *B.t.* and cartap hydrochloride-selected strains were compared with that of the parental strain. However, studies conducted by Groeters *et al.* (1993)

did not show any difference between the mating durations of *B.t.*-resistant and susceptible strains of this insect. The variations in the present studies can be attributed to the physiological changes brought about by the insecticidal exposure either by chance or as a consequence of genetic changes as explained by Rowland (1991).

5.4.2 Fecundity

As per the present investigations, the average fecundity of *B.t.*, spinosad and cartap hydrochloride-selected strains of *P. xylostella* was significantly lower than that of the parental strain. According to Roush and Croft (1986), laboratory-selected strains of insects suffer more reproductive disadvantages than resistant strains colonized from the field (Roush and Croft, 1986). The variations in the present studies might be because of the phenomenon as explained above.Groeters *et al.* (1994) further observed lesser fecundity in *B.t.*-resistant strain of *P. xylostella* as compared to susceptible strain.

Reports are available on the reduction in fecundity of *P. xylostella* following its exposure to sub-lethal doses of fenvalerate (Kumar and Chapman, 1984), thereby, further corroborating the present findings. In few other studies also, a reduction in fecundity was observed when larvae or pupae of *Spodoptera littoralis, S. exigua, Heliothis armigera, Agrotis ipsilon* and *Pericallia ricini* were exposed to sub-lethal concentrations of *B.t.* var. *kurstaki* (Salama *et al.*, 1981; Hafez *et al.*, 1993; Mathur *et al.*, 1994).

No differences were observed in the pre-oviposition, oviposition, post-oviposition and incubation periods of all the 4 strains of *P. xylostella*

studied in the present studies. Likewise, egg hatchability, adult longevity (male and female), duration of each larval instar and total larval duration, pre-pupal and pupal durations also did not show any variation among the four strains. The weights and measurements of various life stages also did not differ in the four strains. The present findings are in line with the observations of the earlier workers with different insects including *P. xylostella* as far as pre-oviposition, oviposition, post-oviposition and incubation periods, hatchability, adult longevity and pupal duration are concerned (Pimentel *et al.*, 1951; Nemoto, 1986; Saini and Chopra, 1988; Motoyama *et al.*, 1992).

However, Saini and Chopra (1988) observed significant differences in the larval duration of fenvalerate-selected (resistant) *E. vittella* as compared to unexposed ones, but Motoyama *et al.* (1992) did not visualise any difference in the larval duration of the fenvalerate-selected and the parental population of *P. xylostella.* Also, no variations in the larval duration were observed in case of cypermethrin-selected strain of *E. vittella* compared to susceptible strain (Saini and Chopra, 1988). Further, the retardation in the larval development of some insects as a result of their exposure to the sub-lethal concentrations of insecticides including *B.t.* have also been encountered (Salama *et al.*, 1981; Hafez *et al.*, 1993; Mathur *et al.*, 1994).

Saini and Chopra (1988) observed significant variations also in the larval and pupal weights of fenvalerate-selected and unselected strains of *E. vittella* but such differences were not observed in cypermethrin-selected strain.

CHAPTER-VI

SUMMARY

The investigations entitled "Laboratory studies on resistance in *Plutella xylostella* (L.) to *Bacillus thuringiensis*, spinosad and cartap hydrochloride" were carried out in the laboratory of the Department of Entomology, Chaudhary Charan Singh Haryana Agricultural University, Hisar during 1997-98. The test insect, *P. xylostella* was collected from the farmers' cauliflower fields around Hisar, and multiplied in the laboratory at 24±2°C. This culture was divided into four lots, one of them being termed as parental strain and maintained without exposure to any insecticide through generations. The rest three were given selection pressures, one each with *B.t.* var. *kurstaki*, spinosad and cartap hydrochloride to its 3rd instar larvae in every generation. Selection pressure of spinosad and cartap hydrochloride was given as topical application with Potter's tower to the larvae released on cut leaf disc of cauliflower placed in the petridish. Both the sides of the leaf discs were treated. For selection in each generation, the test insect was exposed to many concentrations and the survivors of the concentration resulting into around 85 per cent mortality were retained for next generation and the rest were rejected. For *B.t.* var. *kurstaki*, leaf dip method was used and larvae were released on the treated leaf disc after getting it air dried under electric fan. Mortality counts were taken 48 hours after exposure in case of spinosad and cartap hydrochloride and after 72 hours for *B.t.* var. *kurstaki*. The selections were made for 7 successive generations for each of the bioinsecticides. Cross-resistance studies in the three biopesticide-selected strains to the commonly used insecticides *viz.*, monocrotophos, malathion, endosulfan, dichlorvos, cypermethrin and

carbaryl were conducted by working out the LC_{50} values. Studies on the comparative biology of the selected *vis-a-vis* the parental strains of *P. xylostella* were undertaken at 24±2°C.

A comparison of the toxicity as LC_{50} values of different insecticides to the parental strain indicated that spinsosad was the most toxic followed by *B.t.* var. *kurstaki*, cartap hydrochloride, cypermethrin, dichlorvos, malathion, carbaryl, endosulfan and monocrotophos (in descending order). Selection pressure of *B.t.* var. *kurstaki*, spinosad and cartap hydrochloride to *P. xylostella* manifested no resistance development to these biopesticides. The LC_{50} values and resistance ratios in the 7th generation were observed to be lower as compared to those of the 1st generation, indicating an increase in the susceptibility of this pest insect.

None of the three biopesticide-selected strains of *P. xylostella* exhibited cross-resistance to the commonly used insecticides, i.e. monocrotophos, malathion, endosulfan, dichlorvos, cypermethrin and carbaryl; rather, the *B.t.*-selected strain became more susceptible to malathion and carbaryl as compared to the parental strain. Similarly, the spinosad-selected strain showed higher susceptibility to endosulfan, monocrotophos, carbaryl and malathion and cartap hydrochloride-selected strain to carbaryl, dichlorvos and malathion.

Comparative biological studies on the parental and three biopesticide-selected strains revealed that out of the 14 biological parameters, i.e. mating duration, pre-oviposition and post-oviposition periods, fecundity, incubation period, hatchability, adult longevity, larval durations and total larval duration, pre-pupal and pupal periods, weights

and measurements of the various life stages, only two namely mating duration and fecundity were observed to be affected due to the biopesticide selection pressure.

Mating duration of the three biopesticide-selected strains of *P. xylostella* were at par among each other, but of the *B.t.* and cartap hydrochloride-selected strains, it was slightly lower than that of the parental strain. The fecundity of the three biopesticide-selected strains was affected adversely as compared to the parental strain.

Non-development of any resistance in *P. xylostella* to *B.t.* var. *kurstaki*, spinosad and cartap hydrochloride after the selection pressure for 7 successive generations and exhibition of no sign of cross-resistance to monocrotophos, malathion, endosulfan, dichlorvos, cypermethrin and carbaryl suggest that these insecticides can be used safely in alternate fashion for quite a long period of time. However, there is a need to undertake such studies further for atleast 20 to 25 generations to draw the final conclusion. The results may also serve as base line toxicity data to determine the relative development of resistance in the field population of this insect at a later date.

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*Original not seen