

**POTENTIAL FOR ACQUISITION OF RESISTANCE TO
PLANT CHEMICALS IN DIAMONDBACK MOTH
(*Plutella xylostella* L.): A CASE STUDY WITH NEEM**

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**DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE**

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(*Plutella xylostella* L.): A CASE STUDY WITH NEEM**

JAYADEVI, H.C.

**Thesis submitted to the
UNIVERSITY OF AGRICULTURAL SCIENCES, BANGALORE
in partial fulfillment of the requirements
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Doctor of Philosophy

**in
AGRICULTURAL ENTOMOLOGY**

BANGALORE

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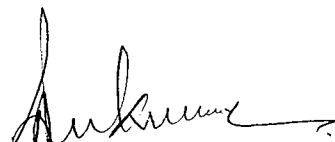
*Affectionately Dedicated to
Appaji, Amma, Doddappaji & Annaiah*

**DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
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CERTIFICATE

This is to certify that the thesis entitled, “**POTENTIAL FOR ACQUISITION OF RESISTANCE TO PLANT CHEMICALS IN DIAMONDBACK MOTH (*Plutella xylostella* L.): A CASE STUDY WITH NEEM**” submitted by Ms. **JAYADEVI, H. C.** in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **AGRICULTURAL ENTOMOLOGY** to the University of Agricultural Sciences, Bangalore, is a *bonafide* record of research work done by her during the period of her study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bangalore
February, 2006



(A. R. V. Kumar)
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Major Advisor

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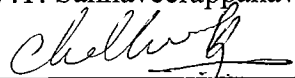


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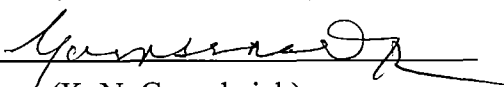
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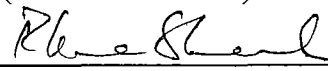
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I. INTRODUCTION

I. INTRODUCTION

Insecticidal resistance is the “development of an ability in a population of insects to tolerate doses of toxicants which would otherwise prove lethal to the majority of individuals in a normal population of the same species” (WHO, 1957). Considering difficulties in defining the various terms involved in the WHO definition, conventionally and for the practical needs, the resistance is measured more in terms of folds of change in the median lethal concentrations (or doses) compared to a reference strain.

Perhaps the first documented insecticidal resistance is that of the San Jose scale to lime sulphur identified during 1908 (Melander, 1914). Since, the discovery of the biological activity of DDT, the list of insects showing resistance to various insecticides has only increased world wide. By 2004, as many as 530 insect pests were reported to have developed resistance to one or more insecticides (Bills *et al.*, 2004) and it appears the number of such insects has reached a plateau (Denholm *et al.*, 2002).

However, the levels of resistance seem to vary greatly among the insect populations. Different populations of a single species from different geographical areas are known to vary in their response even to the same insecticide as a consequence of local variations in management practices (Tabashnik *et al.*, 1987; Mohan and Gujar, 2003). This variation is attributed to differential rates of development of resistance as determined by many local ecological and genetic factors (Roush and McKenzie, 1987). The rate of evolution of resistance is maximum when selection pressure is high (Poopathi and Baskara, 2002), as long as sufficient numbers of progeny are available for continuing the generations.

Apart from these, genetic nature of resistance is also expected to influence the development pattern of the resistance. Autosomal or sex-linked inheritance of resistance, dominance or otherwise of the resistance alleles, and the number of genes governing the resistance are also implicated to affect the rates of development of resistance in insects (McKenzie, 1996; Tabashnik *et al.*, 1992). Coupled with the above parameters, levels of homozygosity in resistance alleles achieved by a population are expected to influence further risk of resistance development. As a consequence, measures such as heritability and response to selection help understand the risk of resistance development. Therefore, a

thorough knowledge of the genetics of resistance helps in assessing the risk of resistance and provides opportunities to develop both 'insecticide resistance' and 'insect pest' management strategies like 'refuge' strategies (Gould and Tabashnik, 1998).

In conjunction with the genetic factors, the associated changes in fitness parameters of a population moving towards acquiring insecticide resistance is expected to influence the stability of the insecticide resistance already acquired (Georghiou, 1972; Tabashnik *et al.*, 1995). Because the development of resistance is invariably expected to incur some fitness costs, best expressed in terms of the reduced biotic potential of the population. However, once the insect develops a high level of insecticide resistance, the stability of the insecticidal resistance acquired may be determined more by the nature of the resistant alleles, number of loci, extent of homozygosity achieved by the selected population *etc.* of the resistant population (Tabashnik *et al.*, 1995). So that in completely homozygous populations, irrespective of the cost of resistance, the levels of resistance achieved, in principle, may remain stable for a long time.

Development of resistance to one insecticide may have repercussions beyond the limits of the chemical in question. Once the insect develops resistance to an insecticide, the use of alternative insecticides for the management will be limited due to the inherent capacity of insects to develop positive cross resistance to other insecticides of the same or a different group of chemicals for which the insect was never previously exposed (Yamamoto *et al.*, 1993; Khambay *et al.*, 2001). However, such insects may also become sensitive to other insecticides, termed as negative cross resistance (Pittenderigh *et al.*, 2000). Negative cross resistance is of great importance in promoting continued use of a set of insecticides or the management of a target pest. Although, the hypothesis is in place, there are no practical examples to meet this requirement, barring an exception (Yamamoto *et al.*, 1983; Yamamoto *et al.*, 1993). In this context, it is pertinent to note that in the recent past, indoxacarb, a representative of the oxadiazine group of insecticides is expected to show negative cross resistance in insects resistant to pyrethroids (Khambay *et al.*, 2001; Gunning and Devonshire, 2002). But opposing views do exist on this issue (Anonymous, 2006).

It is also contended that the rate of development of resistance varies as an inverse function of the number of selection agents (McKenzie and Byford, 1993; Prabhaker *et al.*, 1998). Most of these studies however used synthetic insecticides as the basis for

understanding the principle. Our earlier experience with neem suggests that neem is a very potent chemical of great practical utility in pest management. Intensive studies with this wonder plant, has suggested the plant to contain a large variety of terpenoids and non terpenoids, which are all potentially useful as insecticides (Kraus, 1995). However, azadiarchtin-A is the most abundant of these chemicals (Rembold, 1996) and many commercial neem products based on azadirachtin are now available in the market for insect pest management (Gahukar, 1996). Both crude extracts of neem and its products have been shown to be efficient insecticides. Azadirachtin is also unique in that, the molecule is expected to act on a wide variety of behavioural and physiological targets in the insect leading to many impacts on the biology of the insect that culminates in the reduced propagation of the target insect over time (Vollinger, 1987; Schmutterer, 1995). Thus azadirachtin is unlike synthetic insecticides in its modes of action on insects. Further, crude extracts of neem is expected to contain a large variety of potential insecticidal chemicals that ultimately improve the potency of the overall insecticidal property. As a result, the crude extract is expected to be far superior, in insect pest management, to even the azadirachtin, whose use efficiency has been shown to reduce with increasing purity in a product (Ashok, 2001; Jayadevi, 2002; Kumar *et al.*, 2003). Due to these varied factors, neem is expected to have strong Built-in Resistance Prevention mechanism, so that insects may not really be able to develop resistance against this insecticide (Rice, 1993). Two earlier studies point to this possibility as insects developed only marginal resistance to neem (Vollinger, 1995; Feng and Isman, 1995).

An approach to understand many of these possibilities as related to neem in particular and a novel chemical in a model insect system would be of immense use. Logically, a synthetic insecticide is expected to have a single mode of action on the target insect while a chemical such as azadirachtin is unique in that, being a single molecule is having large many biological impacts on the insect. Further, a crude extract of neem is a mixture of chemicals with multiple modes of action on the target insect. Thus the three exhibit a gradation of insecticidal intensity on insects. Thus, the present study was planned with the objective of understanding how a target insect develops resistance to these different gradations of selection intensities.

A logical question then is to identify a suitable model system. In principle, it is possible to test this aspect in any model insect. However, the model systems are generally

selected on the basis of the previous experience of the researcher. Based on previous experience (Kumar *et al.*, 2000; Jayadevi, 2002; Kumar *et al.*, 2003) with diamondback moth (DBM), *Plutella xylostella*, was selected for the present study.

Plutella xylostella (L.) (Lepidoptera: Plutellidae) is one of the major hurdles for the production of cabbage world wide. Insecticide application is the major mode of managing this pest in the country and elsewhere in the world. Farmers often increase the doses of insecticides, use mixtures of several chemicals and spray up to 25 times within a cropping season (Khan *et al.*, 1991, Kamala, 2006). The sole reliance on insecticides has thus resulted in the development of high levels of resistance to all major groups of insecticides (Talekar and Shelton, 1993) in DBM. As a result, a new insecticide is expected to have a utility life of just two to three years in cabbage ecosystem (Rushpakornchai and Vattanatangum, 1986; Pernag *et al.*, 1988; Zoebelein, 1990; Talekar and Shelton, 1993). DBM has developed resistance to as many as 69 insecticides, which is the maximum for any insect pest (Bills *et al.*, 2004). It has developed resistance to every group of insecticides introduced for its management (Zoebelein, 1990; Kim *et al.*, 1991; Mohan and Gujar, 2003 and Sayyed and Wright, 2004) and it was the first insect to have developed resistance to *Bt* sprays under field conditions (Tabashnik *et al.*, 1990; Ramirez *et al.*, 1995). DBM is potentially capable of developing resistance even to neem (Vollinger, 1995). Further, a large number of studies have been carried out at UAS, Bangalore on neem using DBM as the model system. Hence, the methods of rearing, culturing, assaying *etc.* have been standardized in the lab conditions (Jayappa, 2000; Kumar *et al.*, 2000; Ashok, 2001; Jayadevi, 2002; Kumar *et al.*, 2003). The insect completes over 15 generations under lab conditions, so that reasonable progress can be made quickly.

More importantly, during the early part of this decade, indoxacarb, then freshly introduced for the purpose of management of DBM in and around Bangalore, was being widely used to the extent of nearly 50 % of all sprays by cabbage farmers. However, of late, due to the attributed reasons of control failure, less than 4 per cent of the farmers around Bangalore are currently using the insecticide for the management of this pest (Kamala, 2006). Further, cypermethrin, believed to be completely useless in the early 90s with a high degree of insecticidal resistance in DBM, is expected to have negative cross resistance with indoxacarb (Khambay *et al.*, 2001; Wing *et al.*, 2000; Gunning and Devonshire, 2002). In addition, there are no studies under laboratory conditions to develop

resistant populations for either indoxacarb or cypermethrin. Therefore, keeping these factors in mind, and using DBM as a model system the present study was planned with the following specific objectives:

Objectives:

1. To evaluate the susceptibility of laboratory and field cultures of diamondback moth to neem seed kernel extract, a commercial neem formulation and a currently effective synthetic insecticide
2. To select DBM for resistance to the neem seed kernel extract, a commercial neem formulation and a currently effective synthetic insecticide
3. To study the rates of evolution of resistance among the populations selected for resistance
4. To assess the genetic changes and assess the fitness costs, if any, in selected cultures
5. To assess the biochemical changes in selected strains of DBM

II. REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Settled agriculture is believed to be the first step towards increasing the carrying capacity of the world for Human populations. During the last 8-10,000 years of settled agriculture, the last few decades are crucial in ushering in the era of Green Revolution, when the agricultural productivity increased by leaps and bounds. But with the modern practices came the associated pest and disease problems. Control of pests was thus attempted early on by using many methods. Plant chemicals among them were easily available and extensively used until the discovery of biological activity of such inorganic chemicals as sulphur and arsenates during the 1800s. Evolution of chemical insecticides (Matsumura, 1975; Appendix I- list of chemicals discovered or introduced for pest management) essentially began with readily available material such as arsenicals, petroleum oils, and botanical insecticides (nicotine, pyrethrin and rotenone). The first synthetic organic insecticides that appeared for public use were Dinitro compounds and thiocyanates (Matsumura, 1975). These early compounds were restricted in their use. However, indiscriminate use of lime sulphur resulted in survival of San Jose scale under the crust of chemicals in 1908 (Melander, 1914). Thus the first major hurdle in the use of chemicals for pest management – ‘resistance’ was identified.

The discovery of insecticidal property of DDT by Muller (1939) and its release as Gesarol® for the control of mosquitoes, ushered in a new era of synthetic pesticides. In 1941 DDT was used for the first time in the control of an agricultural insect pest, Colorado potato beetle. Later in 1943, DDT was introduced to USA by Knippling –Director of USDA (Perkin, 1982). 1940s onwards it was extensively used against flies, mosquitoes, bedbugs, louse, agricultural pests, pests of livestock, poultry, *etc.* (Brown, 1958). However, DDT was first introduced for the control of malaria and other vector borne diseases in India among armed forces in 1944 (Anonymous, 2002). At this moment DDT was viewed as a panacea for all kinds of insect problems – agriculture, veterinary and medical. The promise of better world by DDT brought in an array of new compounds for pest management in the 1940’s including more organochlorines, new organophosphates (1944) and carbamates (1947). Initially insecticides released were of broad spectrum activity, but as the time passed, they are now tending to be more species or group specific and ecofriendly (Casid and Quistad, 1998; Ishaaya and Degheele, 1998; Fig. 1).

Coming back to the early era of insecticide usage, the world soon realized the limitations of the promise of DDT, and the first report of resistance to this chemical came as early as 1947 from Italy in the ubiquitous housefly (Brown, 1958). Simultaneously, there were more reports of resistance pouring in but this time in two spotted spider mite (1949), spider mite (1952) and 1954 in boll weevil to Chlorinated hydrocarbons (Roussel and Clower, 1955). Resistance to DDT in DBM was recognised only by 1968 in India (Verma and Sandhu, 1968) around Ludhiana.

Further, many more followed the humble house fly to demonstrate the power of the biological world to withstand the man made weapons in an eternal war with insects- the Pest War (Georghiou, 1990; Denholm *et al.*, 2002). The tally now stands at a whopping 500 and odd species (Bills *et al.*, 2004), but probably, to our relief, that seems to be the limit (Fig. 2; Appendix-II).

2.1 Insecticidal resistance

The word “resistance” was used initially for the instances where insecticide dosages that were formerly effective, met with control failure. When the phenomenon first appeared it was checked for the quality of the insecticide used and also for the thoroughness of its application rather than to relate it to a change in the genetic susceptibility levels of the insect population (Brown, 1958). Soon the quantitative methods of testing insect’s resistance were formulated. Such quantitative tests, like, a) either applying the insecticide to the insect or b) by applying the insect to the insecticide deposit and measuring what was then called the “physiological resistance”, using normal or standard strains as points of reference, were developed. If the amount needed for equivalent mortality has increased by five to ten times, then it was considered as “resistance” (Decker and Bruce, 1952) in house flies. In 1950, Knipling pointed out that resistance levels of ten times the normal or even less would result in unsatisfactory control of houseflies and mosquitoes by chlorinated hydrocarbons. In 1956 Keiding (1956) reported that a ten-fold increase in LC_{50} levels was evidently sufficient to invalidate organophosphorus compounds as residual sprays for housefly control.

Resistance is basically an attribute that has developed and come to be recognised among insect populations consequent to the application of the insecticide. For pre-existing

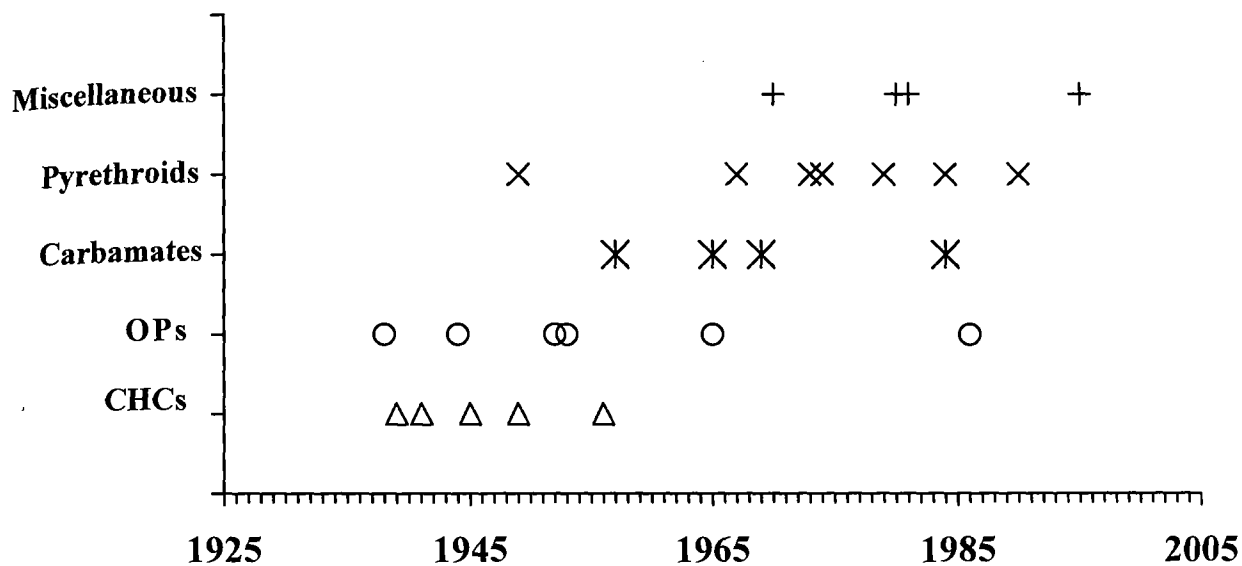


Figure 1: Insecticides belong to different chemical groups introduced at different time periods. (Appendix I)

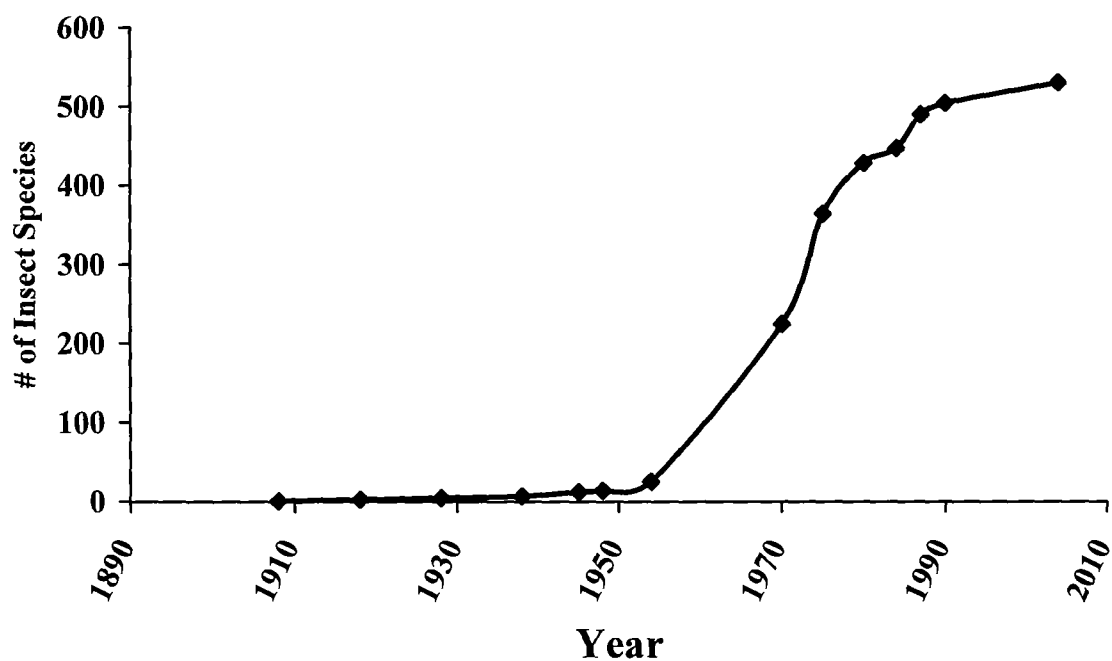


Figure 2: Growth pattern in the number of insecticide resistant insects reported at different times in the world. (Data: Appendix II)

resistance, *e.g.*, that of the boll-weevil or grasshoppers for DDT, a separate word such as “refractoriness” was also used. For developed resistance, unfortunately the phrase “acquired tolerance” has often been used, but in course of time, all these simply were termed “resistance” (Brown, 1958).

2.1.1 Resistance – the definitions

Until 1957 there were no clear cut definitions for insecticidal resistance. But many scientists had defined resistance as,

- 1) If the amount of pesticide required to get a median lethal mortality is 5-10 times more than that of the normal / susceptible population it is then called as the Resistance.
- 2) The added ability to withstand an insecticide acquired by breeding from those individuals which survive exposure to that particular toxicant insufficient to wipe out the whole colony (Brown, 1958).
- 3) Resistance is the developed ability of an organism to tolerate the doses of toxicants as a consequence of the application or frequently coming in contact with that toxicant which was lethal to the same population earlier or to the populations which are not exposed (Hoskin and Gordon, 1956).

WHO definition of Resistance The WHO Expert Committee on Insecticides (1957) has defined and clarified the term Resistance as “Resistance to insecticides is the development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species. The term ‘behaviouristic resistance’ describes the development of the ability to avoid a dose which would prove lethal”.

This is the most widely accepted and used definition of insecticide resistance. It is to be noted however, that the definition does not stipulate the number of folds of the median lethal values between the susceptible and the resistant populations. As a result, many ideas such as 5 to 10 times change in the dosage *etc.*, have apparently remained as conventional methods of ascribing meaning to the term resistance among the practicing entomologists.

2.1.2 Assessment of Resistance

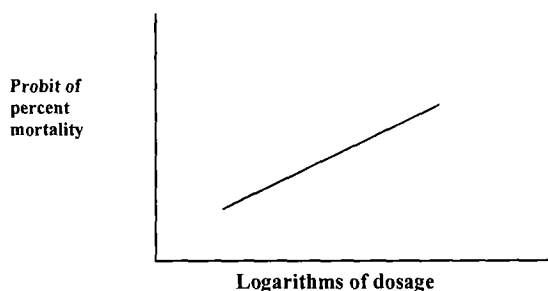
Recognition of resistance (measurement of development of resistance) is critical to further understanding of the utility of the chemical and other biological features of the insect associated with the resistance. In the early days, the measurement of resistance began with the expression of per cent mortality at a particular concentration. Based on quantitative test results relative difference in the per cent mortality was used to express the resistance. In 1925, Abbott came up with the formula for corrected per cent mortality, which became the most widely used formula in insect mortality assessments due to any treatment. It is given as:

$$\% \text{ Corrected Mortality} = \frac{(y - x)}{(100 - x)} \times 100$$

Where, x = % mortality in control

y = % mortality in treatment

Bliss (1935) was the first person to propose that the method of plotting the logarithms of dosage vs probit per cent mortality would yield a straight line (See fig.). Further, Finney - 1949, 1952, 1971 formulated the present method of calculation of Median lethal doses through regression equation.



Now Finney's (Finney, 1971) method of probit analysis is the most accepted and widely used method for assessing the efficacy of a chemical and consequently for the assessment of resistance in terms of median lethal mortality levels of a toxin. However, with any chosen method of bioassay, the time at which effects are measured must be kept constant. As the median lethal dose values decrease and slope increases as time after treatment increases (Hoskin and Gordon, 1956; Jayappa, 2000; Liu *et al.*, 2003).

2.1.2.1 Test of resistance : Discriminate dose

In 1962 resistance test methods were standardized and published in the "Bulletin of Entomological Society of America" as *Standardised Laboratory Detection Methods for Resistance Determination in Agricultural Arthropod Pests*. Further "First Conference on Test Methods for Resistance in Insects of Agricultural Importance" (Anonymous, 1967)

recommended and developed a method for determining resistance under field conditions. They called it as field level test or Discriminating dose – 99% and was based on susceptible population assay.

However, from then onwards, development of resistance in field populations was identified based on the response to discriminating doses. Wherein, dose that gives 99 per cent mortality of the susceptible lab population was used as the discriminating dose. For the actual test, the collected field populations were treated with the discriminating dose. Susceptible populations are expected to provide 100 per cent mortality, while reduction in the mortality levels suggests the development of resistance (Anonymous, 1967).

Discriminating dose technique would mask several biological attributes relevant to understanding the phenomenon of resistance. Hence, a ratio of the median lethal dose of field collected test population to that of an identified susceptible population (Vines *et al.*, 1984) is widely preferred.

$$\text{Resistance Ratio (RR)} = \frac{\text{LC}_{50} \text{ of Resistant population}}{\text{LC}_{50} \text{ of Susceptible population}}$$

Above equation provides a simple and unambiguous method of understanding the extent of variation among populations in their response to a selected insecticide or to know the variation in the susceptibility of selected populations to different insecticides and *vice versa*, in terms of resistance ratio (RR) keeping an identified susceptible population as the reference. Resistance ratios are also useful to know the rate of development of resistance in a population to an insecticide on selection (Crow, 1956; Brown and Payne, 1988).

2.1.3 Development of resistance to insecticides in Insects: A progression

Before 1947, there were 7 insects in which resistance was recorded. However, quickly many more insects were added to this list (Denholm *et al.*, 2002). In view of the steady increase in the use of synthetic organic insecticides following the introduction of DDT after World War II there was also a commensurate increase in the number of species becoming resistant to insecticides. From a single case of resistance to lime sulphur which was detected in a scale insect in 1908, the number of resistant arthropods grew to 14 by

the end of 1940s, 490 (Georghiou, 1987, 1990) species by the end of 1986 and it has proliferated to reach 530 species of insects and mites that had developed resistance to one or more types of insecticides (Bills *et al.*, 2004; Fig. 2).

The frequency of occurrence of resistance in various orders of arthropods (Perry *et al.*, 1998) reflects the extent to which members of each order have been subjected to chemical stress. The large number of resistant species in the order Diptera (36% of the total) is an indication of the widespread use of insecticides by international organizations to control flies and mosquitoes that are vectors of human diseases. Even larger numbers of resistant species are found among insects and mites that are pests of agricultural crops. There are at least seventeen agricultural insect pests which are resistant to more than twenty insecticides (Bills *et al.*, 2004; Appendix III).

Analysis of these seventeen species suggested the following. Fifteen of them are polyphagous indicating the potency of polyphagous insects to develop resistance to a greater degree compared to the specialist herbivores. Nine insect pests are sucking type suggesting the independence of mouth parts or the type of feeding habit in the development of insecticidal resistance ($\chi^2 = 0.029$; $p > 0.05$). Contrary to the popular belief (Forgash, 1984), considering the life span, although 12 insect pests have short life cycle, life span *per se* does not seem to have any special role in causing the development of resistance among insects. Four of these insects belong to a single family, Noctuidae, Lepidoptera, and are polyphagous. Among all Arthropoda, *Tetranychus urticae* is resistant to maximum number of insecticides (72) with *Plutella xylostella* being the next that is resistant to 69 insecticides (Bills *et al.*, 2004). Thus, among insects, *P. xylostella* appears to be the most versatile pest that can, in principle, develop resistance to any insecticide used for its management. Accumulation of resistance being a foregone conclusion in insect pest management in general, it is not surprising that the life of any newly introduced insecticide for the management of *P. xylostella* is only 2 -3 years (Talekar and Shelton, 1993).

When arranged by chemical groups of insecticides (Georghiou, 1990) 58 per cent of the species are resistant to the cyclodiene class, 52 per cent to DDT, 52 per cent are resistant to organophosphorus compounds, 17 per cent to carbamates, 9.5 per cent to

pyrethroids and about 10 per cent to miscellaneous compounds. However, according to 2004 arthropod resistance data base (www.cips.msu.edu/resistance/), higher per cent of species show resistance to organophosphate (43% of insects) insecticides (Fig. 3). Quite a few are resistant to more than one class of insecticides. These percentages reflect not only the extent of usage of each type of insecticide, but also possibly the relative persistence of the chemical in the environment, for example, the organochlorine-type insecticides are the most persistent and organophosphates are the most abundant (many) insecticides.

Studies on Insecticide resistance

The identification of the resistance in an insect species to an insecticide involves either testing the susceptibility of different populations of the insect from different geographical area to different insecticides or the same population to different insecticides. However, it is also done through selection studies, wherein an insect is selected for resistance to an insecticide to test its ability to develop resistance under laboratory or green house conditions. Such studies also provide an opportunity to better understand the genetic and biochemical mechanisms involved in the development of resistance to an insecticide in a selected population *vis-à-vis* unselected population.

Knowledge on different facets of resistance *viz.*, rate of development of resistance, genetics (Liu and Tabashnik, 1997) and ecological factors (McKenzie, 1996) have contributed for the evolution of different pest management techniques. Keeping these in mind, an attempt is made herein to understand the knowledge levels of these factors as related to insecticide resistance. But in order to develop a focus, DBM is used as the primary target subject for compiling the review. Further, indoxacarb, cypermethrin and neem and its formulations are the main insecticidal compounds around which the review is centred.

2.2 Population variation in response to insecticide

An excellent review on variation in susceptibility of DBM populations across the world for different insecticides was compiled by Sannaveerappanavar (1995). The present review adds to this knowledge with some considerations for other insects.

A number of studies have demonstrated the differential susceptibility of insect pests such as *Helicoverpa armigera* (Gunning *et al.*, 1984), *Blattella germanica* (Siegfried

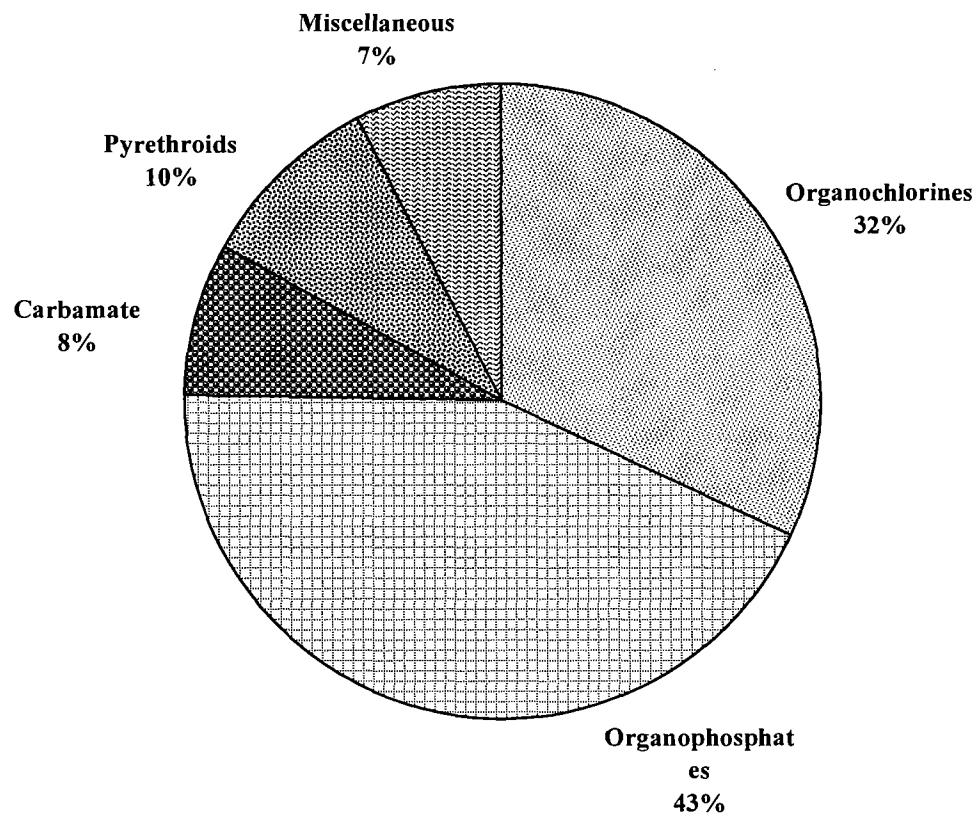


Figure 3: Current status of per cent of insect species resistant to different groups of insecticides (Source: Bills *et al.*, 2004)

et al., 1990), *Liriomyza trifolii* (Ferguson, 2004) and *P. xylostella* (Tabashnik *et al.*, 1987; Sannaveerappanavar, 1995; Mohan and Gujar, 2003; Table 1) from different locations and to different insecticides. *Plutella xylostella* populations, from different geographical areas, in specific, are known to vary in their susceptibility to different insecticides. The phenomenon appears to be quite commonly observed from all over the world and reports of variation in susceptibility levels of local populations are available for India (Mohan and Gujar, 2003; Sannaveerappanavar and Viraktamath, 2006), China (Li *et al.*, 1998a), Taiwan, Thailand (Zoebelein, 1990), Korea (Song, 1991) and Australia (Baker and Kovaliski, 1999; Table 2). The variation has been recorded irrespective of the chemical group to which an insecticide belongs and include synthetic insecticides (Branco and Gatehouse, 1997), microbial insecticides (*Bt*), chitin synthesis inhibitors and to other new groups of insecticides (Pereira *et al.*, 2006a) and even when they were tested for the first time (Liu *et al.*, 2003).

From these studies it can also be concluded that, very high levels of resistance have been recorded against *Bt* formulations (Sayyed and Wright, 2004) even though they were effective during early 1990's (Zoebelein, 1990; Song, 1991). Further, towards pyrethroid insecticides considerable variation in the levels of resistance ranging from eight to more than 25,000 fold has been observed in DBM (Ovalle and Cave, 1989; Sannaveerappanavar, 1995; Baker and Kovaliski, 1999). Pyrethroids perhaps are the group to which currently the next highest level of resistance is recorded compared to *Bt* formulations. In the recent past more specific and ecofriendly insecticides with novel modes of action have been introduced for the management of DBM. However, against these also high levels of resistance has been recorded. Resistance is documented for acyl-urea compounds (Furlong and Wright, 1994), phenyl pyrazole compound (Fipronil- 400 fold RR), spinosad (1170 fold) and indoxacarb (330-fold RR; Sayyed and Wright, 2004) from different parts of the world. Nonetheless, acyl-urea compounds, indoxacarb and spinosad remain effective against DBM in many parts of India and are currently the most widely used chemicals for the management of this pest in cabbage and cauliflower ecosystems both in India (Joia *et al.*, 2003; Jayadevi *et al.*, 2003a) and also in China (Liu *et al.*, 2003).

One of the most potent botanical insecticide commonly recommended in cabbage ecosystem is neem and neem based formulations (Sannaveerappanavar and Viraktamath,

Table 1: Some examples of studies on variation in susceptibility of different insect pests to insecticides across different geographical areas.

Sl. No.	Country	Type of study / Place	Insecticides tested	Resistance ratio (RR) (fold)	Remark	Reference
1	<i>H. armigera</i> (Lepidoptera: Noctuidae)	Field population- Australia	Permethrin Fenvalerate Cypermethrin Deltamethrin	15 20 30 20		Gunning <i>et al.</i> , 1984
2	<i>Bemisia tabaci</i> (Homoptera: Aleyrodidae)	3 different locations USA – 3 Strains, BL, BR and CV	Strains Chlorpyrifos Fenthion Malathion Methyl parathion Monocrotophos parathion Sulprofos Fenvalerate Permethrin DDT Propoxur	BL 1.6 3.7 7.1 20.2 2.3 6.0 42.5 3.5 12.0 6.5 2.2 RR 2.6 4.0 7.1 22.5 3.3 6.8 38.5 2.0 29.0 9.7 3.8	CV 3.9 3.1 5.8 21.4 5.0 1.5 54.0 1.0 1.3 7.1 1.2	Prabhaker <i>et al.</i> , 1985
3	<i>H. armigera</i> (Lepidoptera: Noctuidae)	Field collected - Thailand	Insecticides <i>Cis</i> -cypermethrin <i>Trans</i> -Cypermethrin Fenvalerate DDT Carbaryl Diazinon, Monocrotophos & Endosulfan	RR 102 82 51 125 28 2		Ahmad and McCaffery, 1988

Contd... Table1

Sl. No.	Country	Type of study / Place	Insecticides tested	Resistance ratio (RR) (fold)	Remark	Reference
4	Beet Armyworm, <i>Spodoptera exigua</i> , (Lepidoptera: Noctuidae)	Field populations	Insecticide - Strains Fenvalerate Permethrin Methomyl	Monterey 11 - 29	Guassave Kern 22 10 7 - 16 19	Brewer <i>et al.</i> , 1990
5	<i>Plutella xylostella</i> (Lepidoptera: Plutellidae)	Field collected- Korea <i>Bacillus thuringiensis</i>	Strains Pyungchang Oksan JMC	44 11 12		Song, 1991
6	Oblique leaf roller <i>Choristoneura</i> <i>rosaceana</i> (Lepidoptera: Tortricidae)	Field population, Organic and conventional managed fruit orchards, from valley of British Columbia	Azinphosmethyl (Azin) Tebufenozide (Teb) Methoxyfenozide (Meth) Indoxacarb	Organic site- more susceptible, Conventional site- more resistant	Positive cross resistance (CR) betn. Azin & Teb, Meth, Indoxacarb did not show CR Azin	Smirle <i>et al.</i> , 2002
7	Leaf miner- <i>Liriomyza</i> <i>trifolii</i> (Diptera: Agromyzidae)	Chrysanthemum, USA Strains - CA-1, CA-2 and GA-1	Cyromazine Abamectin, Spinosad	CA-1 18.1 22.0 >188	CA-2 GA-1 8.2 5.4 no resistance 30.6 1,192 1.9	Ferguson, 2004

Table 2: Studies on variations in susceptibility of *Plutella xylostella* to different insecticides in different parts of the world.

Sl. No.	Country (1)	Location / strain (2)	Insecticides tested / Strains (3)	Resistance ratio (RR) (fold) (4)	Remarks (5)	Reference (6)
1	Thailand	Field collected cabbage from Bangkok	DDT, Lindane, endrin, Methyl parathion, & some OP carbamate, pyrethroid, CSI, Bt & Methamidophos	>3000 >125 >25		Zoebelein, 1990
2a	Brazil	Field collected Strain I	Deltamethrin Methamidophos	4 2		Branco and Getchouse, 1997
2b		Strain II	Deltamethrin Methamidophos	47 9		Branco and Gatehouse, 1997
3	China	Field collected	Quinalphos Fenvalerate Cypermethrin	46.1 38.9 8.0		Luo <i>et al.</i> , 1995
4	Thailand	BS colony	Bt- Toarow CTR XenTariR	668 66.5		Imai and Mori, 1999
5	Australia	South Australia (many populations)	OP(Chlorpyrifos Methamidophos and Mevinphos), Pyrethroid(esfenvalerate & Permethrin), Cabamate (Methomyl)	OP- 5 to 200 Pyrethroids- 8 to 400 Methomyl -6		Baker and Kovaliski, 1999
6	Panipet, Haryana	Field collected	Monocrotophos Malathion Endosulfan Dichlorvos	138.74 28.47 6.09 5.03		Kaira <i>et al.</i> , 1997

Contd.... Table 2

	(1)	(2)	(3)	(4)	(5)	(6)
7	India	Tested for geographical area variation	Strain Panipet(Haryana) Ranchi (Bihar) Jaunpur (UP) Delhi Bangalore	High - Low Cyp, Fen - Delta Fen, Delta- Cyp Fen, Delta - Cyp Delta- Cyp, Fen Delta- Cyp, Fen (All- high R - Quinalphos (OP))	Cypermethrin (Cyp), Deltamethrin (Delta), Fenvalerate (Fen)	Saxena <i>et al.</i> , 1989
8	Honduras	Field collected	Strains Zamorano Tatumbra Honduras	Methomyl-Methamidophos-Cyber 45.29 - 224.15 - 411.75 fold 20.75 - 66.54 - 189.96 fold 14.37 - 70.84 - 158.13 fold		Ovalle and Cave, 1989
9a	China	2-Field collected strains -Zhangjiakou population	Avermectin Malathion Hexaflumuron Deltamethrin	1.8 2.9 0.5 1.7		Li <i>et al.</i> , 1998
9b	China	Wuhan population	Avermectin Malathion Hexaflumuron Deltamethrin	4.3 2.2 3.2 2.4		Li <i>et al.</i> , 1998
10	India	Field population from across India	Flufenoxuron & Fenvalerate Monocrotophos Cartap Fipronol Biobit, Cry1Ab and RH2485	highly tolerant, 3.7-4.5 10-24 120-505 Susceptible & effective		Mohan and Gujar, 2003
11	India	Populations around Bangalore,	Organochlorines, OPs, carbamates and pyrethroids	Varied susceptibility		Sannaveerapp anavar and Viraktamath, 2006

Contd.... Table 2

	(1)	(2)	(3)	(4)	(5)	(6)
12a	USA	Field – Indoxacarb	Minnesota Texas	0.0049 g ai/lt 0.0213 g ai/lt		Liu <i>et al.</i> , 2003
12b	USA	Field – λ -Cyhalothrin	Minnesota Texas	0.0050 g ai/lt 0.0147 g ai/lt		Liu <i>et al.</i> , 2003
13	Malaysia, Cameron islands	Field collected - Cameron islands	Fipronil Spinosad Indoxacarb Bt-Cry1Ac Cry1Ca	400 1,170 330 2,840 1,410		Sayed and Wright, 2004
14	India	Field population – Punjab Indoxacarb (LC ₅₀ values –III instar)	Indoxacarb Jagraon Samrala Phagwara Khanna	LC ₅₀ (% a.i.) 0.00006 0.00006 0.00007 0.00003		Joia <i>et al.</i> , 2003
15	Hawaii	Geographical area variation-Field variation	DDT, Diazinon, Permethrin, Fenvalerate	DDT- 130, Diazinon- 15, Permethrin-5, Fenvalerate-3	Inter-island variation< 10km distance	Tabashnik <i>et</i> <i>al.</i> , 1987
16	Field collected	Acylurea compounds,	Teflubenzuron, Chlorfluazuron	T-110folds, C- 8200folds		Furlong and Wright, 1994
17	India- Karnataka	Bt products & Acyl urea compounds to 3 populations – malur, Hassan and Belgaum	Insecticides Strains - Malur Biobit 43.95 Dipel 47.03 Delfin 24.91 Flufenuron 12.96 Lufenuron 6.63	Hassan- Belgaum 4.97 - 7.84 4.79 - 7.19 0.93 - 9.5 0.66 - 0.7 0.06 - 0.33		Pereira <i>et al.</i> , 2006a
18	India – Karnataka	Populations from around Bangalore	Carbaryl Methomyl Alphamethrin Cypermethrin	>180.01 36.31 >46138.07 26507.81	Deltamethrin- 2814.07 Fenvalerate - 27848.21 fold	Sannaveerapp anavar, 1995

1997; Saucke *et al.*, 2000). The crude extract of the neem seed kernels commonly called NSKE has been found very effective in the management of DBM (Schmutterer, 1992; Saucke *et al.*, 2000; Jayadevi, 2002) in cabbage ecosystem. However, nothing much is known about the geographical variation in susceptibility of insect populations in general and DBM in particular to neem and its formulations.

Thus the insects in general and *P. xylostella* in particular seem to have overwhelmingly demonstrated a capacity to show local variations in the susceptibility to different insecticides. The response to insecticides can vary greatly within small geographical areas. This variation in response has been largely attributed for the differences in the management practices across sampled locations (Tabashnik *et al.*, 1987; Mohan and Gujar, 2003). As a result, despite high migration capabilities, many insect populations may remain as 'genetic islands' and demonstrate ecological independence and evolutionary fate depending on the local management practices. Thus the local variations seem to be a universal phenomenon and perhaps the biological strength that predisposes an insect to acquire resistance to many xenobiotics.

2.3 Selection for insecticide resistance

Selection experiments are usually carried out under laboratory or green house conditions to know the possibility of development of resistance, rates of development of resistance and mechanisms of resistance to insecticides.

It has been observed that the rate of development of resistance varies across insecticides and insect pests (Table 3). Insect-insecticide combination is the ultimate governing factor in the development of resistance. The rate of development of resistance is affected by different factors, genetic, biological and operational factors (Forgash, 1984) and the resistance causing selection pressure (Roush and McKenzie, 1987). Insects with short life cycle are expected to develop resistance faster than insects with long life cycle, simply due to the greater turn over rate of the population. However, under laboratory selection studies, rate of development is mainly influenced by selection pressure (Brown and Payne, 1988) and reproductive potential of the genotype rather than the genetic basis of the character (McKenzie, 1985; Whitten and McKenzie, 1982; Roush and McKenzie, 1987).

Table 3: Some of the selection studies carried out under laboratory in different insect pests for resistance to insecticides.

Sl. No.	Insecticide (1)	Insecticide (2)	Selection / selection pressure (3)	No. of generation selected (4)	Resistance level (fold) (5)	Remark (6)	Reference (7)
1	<i>Pectinophora gossypiella</i> (Lepidoptera: Gelechiidae)	Bt-CryAc	Lab selected on field collected strain	36	3,100		Tabashnik <i>et al.</i> , 2002
2	<i>Heliothis virescens</i> (Lepidoptera: Noctuidae)	Bt- CryIAC	Lab	12 19 30	7-8 >500 >10,000		Gould <i>et al.</i> , 1995
3	<i>Bemisia tabaci</i> (Homoptera: Aleyrodidae)	Imidacloprid- Bifenthrin- Fenvalerate	Lab selection- @60-80%	8	21.90 7.12 4.13		Sethi <i>et al.</i> , 2002
4	<i>Heliothis virescens</i> (Lepidoptera: Noctuidae)	Permethrin (Pyrethroid)	Lab selection @ LD ₈₀ conc.(70-90% mortality)	12	37		Jensen <i>et al.</i> , 1984
5	<i>Diatraea saccharalis</i> , (Lepidoptera: Pyralidae)	Fenvalerate (Fen) Monocrotophos (Monoc)	Lab	12	Fen- 3.6 Monoc- 7.6		Vines <i>et al.</i> , 1984
6	<i>Heliothis virescens</i> (Lepidoptera: Noctuidae)	Methyl parathion	Lab	F ₂₁ @ 80% seln. Pressure	18.93		Wolfenbarger <i>et al.</i> , 1982
7	<i>H. virescens</i>	Spinosad	Lab	19	669		Wyss <i>et al.</i> , 2003

Contd... Table 3

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
8	<i>Heliothis armigera</i> (Lepidoptera: Noctuidae)	<i>Bt- Cry1Ac</i>	Lab	RES -Ac (F4) RES-Bt (F14)	93 205		Kranthi <i>et al.</i> , 2006
9	<i>Bemisia argentifolii</i> (Homoptera: Aleyrodidae)	Bifenthrin- Endosulfan- Chlorpyrifos - Binf-End-Chl- (rotation)1:2 (Binf+End)-	Green house selection - 27 generation	Bifenthrin- Endosulfan- Chlorpyrifos- Binf-End-Chl- (rotation) 1:2(Binf+End)-	752.0 36.0 47.0 16.0-5.0-7.0 17.0 (F23)	Use of mixture & rotation delay rate of development of resistance	Prabhaker <i>et al.</i> , 1998
10	Horn fly, <i>Haematobia irritans</i> (Diptera: Muscidae)	Treated with six insecticide combination	50 generation	Permethrin (F50)- Diazinon (F45)- Ivermectin (F50)- Perm-Diaz(1:2)- F50 Perm-Daiz(rotation)- Perm-Iver (rotation)-	628 5.65 6.18 8.54 (Per & Diaz); 4.21 (Per)-4.81(Diaz) 61.0-2.95 40.8-2.38	Use of mixture & rotation delay rate of development of resistance	McKenzie and Byford, 1993
11	<i>Culex quinquefasciatus</i> (Diptera: Culicidae)	<i>Bacillus sphaericus</i>	Lab selection for 5 year	Selected at 3 conc. I-330 mg toxin/lit II- 660 mg toxin/lit III- 990 mg toxin/lit	6013 8990.3 9984.6	Higher seln pressure, higher rate of development of resistance	Poopathi and Baskaran, 2002
12	Green peach aphid, <i>Myzus persicae</i> ,	Azadirachtin & Refined Neem seed extract (NSE) at equivalent conc. of Aza	Lab	40	Aza- 9 NSE- no, resistance	Could not select for resistance to crude neem	Feng and Isman, 1995

Contd.... Table 3

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
13	Sugar cane borer, <i>Diatraea saccharalis</i>	Azinphosmeth yl Carbofuran	Lab at LD ₉₀ and LD ₉₅ level	6 gen.- carbofuron 5 gen. – Azinphosmethyl Lab selection at LC ₇₀	Carbofuron -8 Azinphosmethyl- no resistance developed		Reagan <i>et al.</i> , 1974
14	Sugar cane borer, <i>Diatraea saccharalis</i> (Lepidoptera : Crambidae)	Tebufenzoide	Lab selection- 4 generation	Lab selection at LC ₇₀	Population lost	Ovicidal action	Rodriguez <i>et al.</i> , 2001
15	<i>Musca domestica</i>	Indoxacarb- Oxadiazine insecticide	Lab selection- NYINDR	3 - topical application	>118		Shono <i>et al.</i> , 2004
16	Predatory mite <i>Metaseiulus</i> <i>occidentalis</i> (Acari : Phytocidae)	Abamectin	Lab	20	3.8	Increased rate of survival of adult female, & no. of eggs/ female	Hoy and Ouyang, 1989
17	<i>Bemisia tabaci</i> (Homoptera: Aleyrodidae)	Thiamethoxa m, Acetamiprid	Lab selection- Adults @ LC ₁₀	12 15	589 110	TR- No CR to acetamiprid, AR-CR to Thiamethoxan >500fold	Horowitz <i>et al.</i> , 2004

Toxicological observations indicate that there exists a dynamic variation in tolerance to a poison among individuals of a given population. However, truly sublethal doses of insecticides do not induce resistance in susceptible populations, or increase the level of enzymes responsible for the metabolism of the chemical. On the contrary, the cumulative effect of daily sublethal doses of an insecticide may render the insect more susceptible to the poison (Brown, 1958). Therefore, selection for insecticide resistance should aim at discriminating among different genotypes based on response in terms of dose response regression lines. At very high or very low doses, there will not be any discrimination among genotypes in favour of resistance. Hence, selection can not take place and as a result, selection can only take place at those doses which discriminate between susceptible and resistant genotypes (Roush and McKenzie, 1987).

As a consequence, in most of the studies selection pressure at concentrations of slightly more than the median lethal concentrations, which cause up to 80 per cent mortality (Wolfenbarger *et al.*, 1982; Sethi *et al.*, 2002) have been used, as too low concentrations will not impose any selection for resistance in the population. However, too high selection pressure of > 90 per cent may not allow populations to develop (Brown, 1958; Roush and McKenzie, 1987). Thus the selection pressure is crucial for the development of insecticidal resistance whether in field or in the lab.

2.3.1 Rate of development of resistance

Many factors appear to influence the rate of development of resistance in insects. As the selection concentrations / pressure increased, there was increase in the rate of development of resistance (Poopathi and Baskara, 2002). In most of the studies, selections have been carried out at more than 70 per cent selection pressure (Gould *et al.*, 1992) and below 90 per cent (Kotze and Sales, 2001). However, in many of the studies reviewed here, selection pressures were not indicated (Table 3 and 4) and as a result generalized rule on this aspect is difficult to develop in an empirical way. Thus the current rules of selection pressure are largely based on a theoretical foundation, but are fairly well substantiated (See Roush and McKenzie, 1987 for example). Rate of development of resistance is also influenced by the number of selection agents, which is here designated as the 'selection intensity'. Resistance development is rapid when only one insecticide is applied continuously from generation to generation (Prabhaker *et al.*, 1998), but is slower

when selections were carried out with mixtures of two insecticides and/or by rotation of two or more insecticides (McKenzie and Byford, 1993).

Some of the studies on selection for insecticide resistance in DBM against different insecticides have been compiled, to understand more about the response of DBM to selection with different insecticides (Table 4). Published literature suggested that the level of resistance to an insecticide was independent of the number of generations for which an insect was selected ($r = 0.1153$; $n = 27$; $p < 0.05$; Fig. 4). However, the rate of development and level of resistance attained were high on selection with *B. thuringiensis* formulations (Cry toxins). Within ten generations of selection $> 10,000$ fold of resistance ratio (RR) was observed in DBM (Tabashnik *et al.*, 1991; Maruyama *et al.*, 1999). Even on selection with methyl parathion and parathion (Organophosphates) relatively high levels (1000- 2600 fold RR) of resistance was observed (Kao *et al.*, 1989). Medium levels of 20 to 500 fold of resistance ratios were recorded against chitin synthesis inhibitors (Ismail and Wright, 1991). Selections with pyrethroids for 24 generations developed only < 100 fold resistance (Kim *et al.*, 1990) and to deltamethrin in 65 generations, more than 1000 fold of RR was recorded (Liu *et al.*, 1995). But surprisingly, high levels of resistance ratios have been recorded against pyrethroids in field populations of *P. xylostella* (Table 2; Ovalle and Cave, 1989; Sannaveerappanavar, 1995). Thus in essence, the rate of development of resistance and the levels of resistance seen in DBM appear to be largely independent of the group of insecticide but the *Bt* toxins may be unique in bringing about very high levels of resistance.

The review, thus suggested *Bt* toxins, chlorinated hydrocarbons, OPs, carbamates, pyrethroids and acyl urea compounds have been explored among the synthetic insecticides for the development of resistance (Table 4). However, among the pyrethroids, one of the most widely used insecticide cypermethrin was not explored for the development of resistance in DBM. Similarly, it was observed that some of the recent novel insecticides such as indoxacarb, avermectins, spinosins *etc.*, that represent novel chemical groups are yet to be explored for understanding the rates of development of resistance in DBM. Among these, indoxacarb has been studied only in house fly for its ability to induce resistance. The house fly within three generations developed a resistance ratio of more than 118 fold (Shono *et al.*, 2004).

Table 4: Some of the selection studies carried out under laboratory in *Plutella xylostella* for resistance to different insecticides.

SLNo.	Insecticide (1)	Group of insecticide (2)	Selection (3)	No. of generation selected (4)	Resistance level (fold) (5)	Remark (6)	References (7)
1	Teflubenzuron	Acyl urea compounds	Lab and field	6 months	Field -31 Lab1 sel-18 Lab 2 sel-36		Lin <i>et al.</i> , 1989
2	<i>Bacillus thuringiensis</i>	<i>Bt</i> toxins	Lab selection- two populations	5 9	150-190 430-820	Resistance development is rapid in lab than field	Tabashnik <i>et al.</i> , 1991
3	Fenvalerate	Pyrethroid	Lab selection	24	66.2		Kim <i>et al.</i> , 1990
4	Parathion,	OP	Lab selection	30	>2600		Kao <i>et al.</i> , 1989
5	Methyl parathion	OP	Lab selection	30	>1000		Kao <i>et al.</i> , 1989
6a	Chlorfluazuron	Acyl urea compounds	Lab selection	6	109		Ismail and Wright, 1991
6b	Teflubenzuron	Acyl urea compounds	Lab selection	6	315		Ismail and Wright, 1991
7	Chlorfluazuron	Acyl urea compounds	Lab selection TL strain BK strain	14 15	318 303		Fahmy <i>et al.</i> , 1991
8a	Dimehypo	OP	Lab-on 4 th instar- topical application	35	51	R development followed 'S' shaped curve	Chen <i>et al.</i> , 1993
8b	Cartap	Tertiary amines	Lab-on 4 th instar- topical application	35	25	R development followed 'S' shaped curve	Chen <i>et al.</i> , 1993

Contd.... Table 4

Sl.No.	(1)	(2)	(3)	(4)	(5)	(6)	(7)
9a	Dimehypo	OP	Lab- Seln 4 th instar- topical application	85	178		Chen <i>et al.</i> , 1995
9b	Cartap	Tertiary amines	Lab- Seln 4 th instar- topical application	80	87		Chen <i>et al.</i> , 1995
10	Diafenthion	Thio-urea compounds	Field cage	25 (Malaysia) 55 (Thailand)	No resistance development		Uk <i>et al.</i> , 1995
11	Deltamethrin	Pyrethroid	Lab selection	65	1163	Slow-initially, Faster-mid and rapid-later stage of selection	Liu <i>et al.</i> , 1995
12a	ToarrowR-CT	<i>Bt</i> -Product	Lab – Diet incorporation	10	>30,000		Maruyama <i>et al.</i> , 1999
12b	<i>Bt</i> toxins-Cry1Ac	<i>Bt</i> -Product	Lab – Diet incorporation	10	>45,000		Maruyama <i>et al.</i> , 1999
13	Chlorfluazuron	Acyl urea compound	Lab	6	23.78		Wu <i>et al.</i> , 1998
14	Abamectin	Macrocyclic lactones	Lab	25	100		Zhang <i>et al.</i> , 2001
15	Fipronil	Phenyl pyrazole	Lab	5 (G3-G8)	From 440 fold to 770 fold increased		Sayed and Wright, 2004
16a	Biobit-Cry1Ac	<i>Bt</i> - product	Lab	4	1.65		Pereira <i>et al.</i> , 2006b
b	Flufenoxuron	Acyl urea compound	Lab	4	5.96		Pereira <i>et al.</i> , 2006b

Contd... Table 4

Sl.No.	(1)	(2)	(3)	(4)	(5)	(6)	(7)
17	Cry1C protoxin	<i>Bt</i> -toxin	Lab	26	I instar – 12,400 II- 63,100 RR		Zhao <i>et al.</i> , 2000
18	Chlorfluazuron	Acyl urea compound	Lab selection	6	112		Fauziah <i>et al.</i> , 1992
	Teflubenzuron	Acyl urea compound	Lab selection	6	312		Fauziah <i>et al.</i> , 1992
19	Chlorfluazuron	Acyl urea compound	Lab selection – 2 strains – TL & BK	9 10	TL strain- 276 fold BK strain- 216 fold RR		Fahmy and Miyata, 1992b
20	Fenvalerate	Pyrethroid	Sel. in lab – 3 rd instar @ 50% sel. Pressure, larva dip method	F25- Fenvalerate+PBO Fenvalerate	17 63	Higher – only Fenvalerate, Comparatively low- Fen + PBO	Takeda <i>et al.</i> , 1992

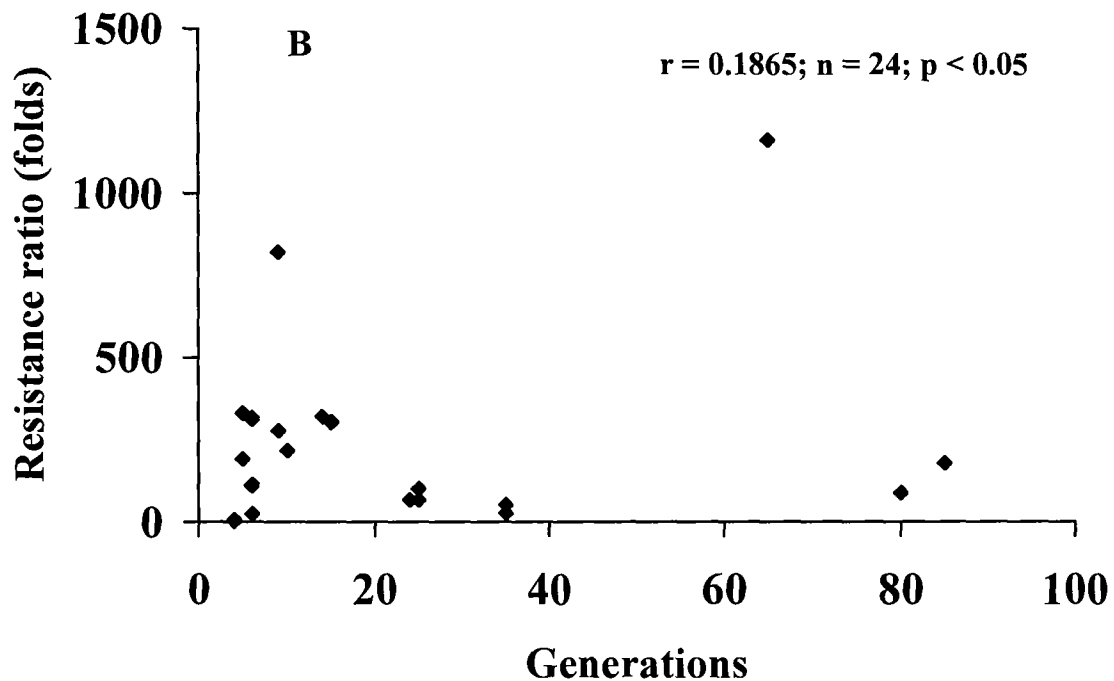
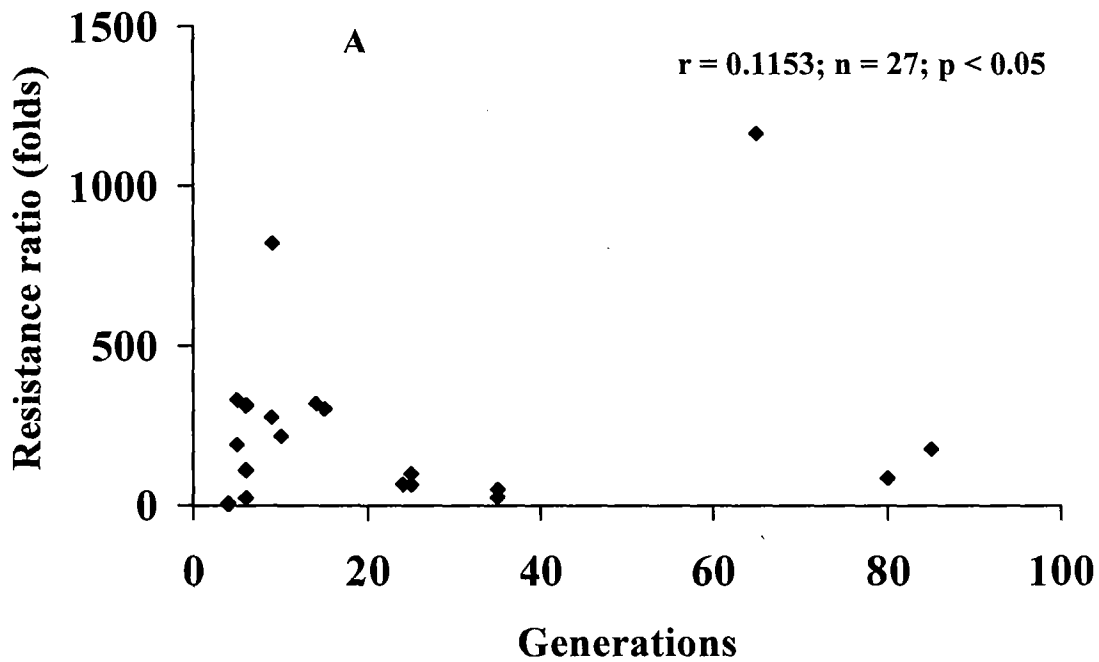


Figure 4: Relation between number of generations of selection and the acquisition of resistance ratio achieved in different insects to various insecticides. A. represents the whole set of data compiled for 27 studies. B. Represents the data for 24 sets after excluding three very abnormally high data points.

One other class of insecticides, botanicals have only been explored once from the point of view of DBM. The insect could only develop 1.7 fold resistance to neem seed kernel extract after 35 generations of selection (Vollinger, 1995), largely indicating lack of development of resistance to neem in general. Only other study was an attempt to select for pure azadirachtin and crude extract of neem seed kernel for resistance in Green Peach Aphid. The aphid developed 9 fold of resistance after 40 generations of selection against pure azadirachtin (Feng and Isman, 1995) but failed to develop resistance to crude extract of neem seeds at equivalent azadirachtin concentration.

2.3.2 Trends in rates of development of resistance: LC_{50} values

An interesting question to ponder would be the nature of the trend in the rates of development of resistance. Some laboratory selections carried out for longer durations have demonstrated the trends very clearly. The house flies selected with methoxychlor showed sigmoid response in just 10 generations, while, resistance to gamma- BHC, chlordane and Dieldrin took more than 45 generations to achieve a plateau in resistance levels (Decker and Bruce, 1952; Brown, 1958). Development of resistance to cartap and dimehypo in DBM populations under laboratory selection also followed sigmoid curve (Chen *et al.*, 1993). Thus the contention that the resistance develops slowly in the beginning and more rapidly later to reach a plateau at the end with rigorous continued selection appears to be quite meaningful (Crow, 1956). Crow (1956) provides many examples of how the rate of development of insecticidal resistance should proceed in a sigmoid manner ultimately reaching a plateau.

Like all other traits under selection, it is anticipated that the development of insecticidal resistance also picks up slowly in the beginning due to low levels of resistance alleles in the population. Corresponding to the high variance then onwards, the population responds genetically with quicker changes towards higher gene frequency to demonstrate a faster growth rate. Ultimately due to attainment of homozygous condition for the resistance factors (alleles?) the changes in response levels are anticipated to reduce resulting in a plateau at the end. The interesting factor here is the meaning of the lag phase. It is largely attributed to the fact that the resistance alleles are essentially detrimental and thus under homozygous conditions, the survival attributes will be greatly affected by natural selection leading to a plateau in the response to selection (Crow, 1956). Therefore, an inherent assumption is that the resistance alleles, irrespective of whether

dominant or otherwise, would essentially reach a maxima under continued and vigorous selection. But how many studies fit this theoretical expectation?

An attempt therefore, was made to ascertain the nature of the trends in development of insecticidal resistance in many insects using published data from many studies including those cited above (Table 5). Unfortunately however, large many studies did not provide detailed data on the median lethal doses at different generations of selection. Therefore, selection studies, in which information on the LC_{50} values at different generations of selection available were used for further analysis to understand the nature of growth of resistance. Data on 25 selection studies collected from eight scientific papers were used and the LC_{50} or RR values given were plotted across number of generations of selection. Then the best function was estimated in an MS-Excel spread sheet. The best fit function, as the analysis revealed, in all the studies was an exponential function (Table 5). In majority of the selection studies the increase in LC_{50} values over generation followed significant exponential function (Mckenzie and Byford, 1993; Sethi *et al.*, 2002), while a few only indicated fit to be relatively poor (Vines *et al.*, 1984). Interestingly, the studies where the fit was poor were also the studies where the resistance levels were quite low. Thus it appears that exponential pattern of growth is the rule by and large for the development of insecticidal resistance in insects. However, this raises the question of why Crow's explanation of sigmoid nature of the growth is not evident in these studies.

In principle, the exponential growth indicates a potential for limitless growth. Sigmoid growth is essentially an exponential growth with a biological limit – K. Thus it is possible that most or all the studies included for the analysis basically truncated the selection process even before the sigmoid pattern appeared. A careful examination of these studies indicated the values not to plateau off at the fag ends of the selection. Thus, the exponential growth seen in the development of resistance as is evident from the 25 studies substantially suggests the sigmoid growth to be the rule in the development of insecticidal resistance (Crow, 1956 and also see Brown, 1958). As a corollary, exponential growth of resistance ratios in a selection process is a necessary and sufficient condition for indicating the development of insecticidal resistance.

Table 5: Analysis of data on rate of development of resistance over generation on selection with insecticides in different insect pests.

SL.No.	Test Insect	Selection agent	Insecticide tested	N**	$y = a e^{bx}$	Correlation coefficient (r)	Reference
1	<i>Liriomyza trifolii</i> , Study on rate of	Unselected	Permethrin	10	$y = 331.12e^{-0.2168x}$	0.9066*	Parrella and Trumble, 1989
2	loss of resistance	Unselected	Chlorpyrifos	10	$y = 21.93e^{-0.1046x}$	0.7374*	
3	Horn fly	Permethrin alone	Permethrin	22	$y = 0.4573e^{0.1595x}$	0.9697*	McKenzie and Byford, 1993
4		Ivermectin	Ivermectin	17	$y = 129.23e^{0.0265x}$	0.6612*	
5		Permethrin-Diazinon mixture	Permethrin-Diazinon mixture	20	$y = 1.6757e^{0.0124x}$	0.3463 ^{NS}	
6		Permethrin:Diazinon	Permethrin	20	$y = 0.857e^{0.0394x}$	0.7819*	
7		Permethrin:Diazinon	Diazinon	19	$y = 1.0249e^{0.0142x}$	0.5803*	
8		Selected by Permethrin - Diazinon rotation	Permethrin	21	$y = 0.532e^{0.1049x}$	0.9010*	
9		Selected by Permethrin - Diazinon rotation	Diazinon	17	$y = 0.936e^{0.0075x}$	0.2492 ^{NS}	
10		Permethrin-Ivermectin altering	Permethrin	24	$y = 0.5128e^{0.0996x}$	0.9383*	
11		Permethrin-Ivermectin altering	Ivermectin	17	$y = 113.16e^{0.0195x}$	0.5865*	
12	<i>Heliothis virescens</i>	Methyl parathion	Methyl parathion	13	$y = 4.5881e^{0.1137x}$	0.7099*	Wolfenbarger <i>et al.</i> , 1982
13	<i>Bemisia tabaci</i>	Imidacloprid	Imidacloprid	6	$y = 69.409e^{0.3597x}$	0.9696*	Sethi <i>et al.</i> , 2002
14		Bifenthrin	Bifenthrin	6	$y = 131.24e^{0.2156x}$	0.9873*	
15		Fenvalerate	Fenvalerate	6	$y = 2367e^{0.1614x}$	0.9966*	
16	<i>Heliothis virescens</i>	Unselected	Cry1Ac	6	$y = 0.0556e^{-0.0835x}$	0.5987 ^{NS}	Gould <i>et al.</i> , 1995
17		Selected	Cry1Ac	6	$y = 5.2608e^{0.1417x}$	0.8001 ^{NS}	

Contd... Table 5

Sl.No.	Test Insect	Selection agent	Insecticide tested (LC ₅₀)	N**	$y = a e^{bx}$	'r'	Reference
18	<i>Heliothis virescens</i>	Permethrin	Permethrin	6	$y = 2.0951e^{0.3233x}$	0.8476*	Jensen <i>et al.</i> , 1984
19	<i>Diatraea saccharalis</i>	Monocrotophos	Monocrotophos	11	$y = 1.0239e^{0.1032x}$	0.4849 ^{NS}	Vines <i>et al.</i> , 1984
20		Fenvalerate	Fenvalerate	12	$y = 0.0309e^{0.1313x}$	0.8522*	
21	<i>Bemisia argentifolii</i>	Bifenthrin selected	Bifenthrin selected	15	$y = 0.2713e^{0.1567x}$	0.9214*	Prabhaker <i>et al.</i> , 1998
22		Endosulfan	Endosulfan	15	$y = 0.0134e^{0.126x}$	0.9825*	
23		Chlorpyrifos	Chlorpyrifos	15	$y = 0.0118e^{0.1347x}$	0.9702*	
24	Mixture of	Bifenthrin+Endosulfan	Bifenthrin+Endosulfan	13	$y = 0.0239e^{0.0586x}$	0.8056*	
25	Mixture of	Bifenthrin+Endosulfan	Bifenthrin+Endosulfan	12	$y = 0.0225e^{0.0724x}$	0.9937*	

** N- number of times bioassay carried out in selection experiment

* Correlation coefficient 'r' is significant at $p \leq 0.05$

^{NS} Correlation coefficient 'r' is not significant at $p \geq 0.05$

2.3.3 Trends in rate of development of resistance: The Slope

Crow's argument on the genetic variability contributing for the sigmoid growth of insecticidal resistance, can best be gauged by the phenotypic variability in the population at different times of selection. The slope of the dosage mortality regression lines therefore are ideal indicators of the extent of variability in the population under selection. Hoskin and Gordon (1956) have shown that when specific resistance is developed in a population, the dosage-mortality regression line becomes flatter as the LC_{50} increases, it becomes steeper again as the population becomes homogeneous for resistant individuals (Fig. 5A). A large number of studies on the genetics of insecticidal resistance confirm this possibility. As the F_1 progeny of the reciprocal crosses end up having flatter slopes. Further, in contrast to this sequence of events with those in which no specific resistance is involved, either the dosage-mortality line merely becomes steeper because of the absence of the most susceptible phenotypes (Fig. 5B) (as described above) or simply shifts without change in the slope if only cross resistance or vigour tolerance is involved (Fig. 5C). In essence, the slopes of the logdose-probit (ldp) mortality lines, during the course of development of resistance should tend to follow a 'U' shaped curve (Hoskin and Gordon, 1956).

On the contrary, Chilcut and Tabashnik (1995) provided evidence and argue that slope is not the good indicator of the change in resistance levels. As slope of the probit mortality line is due to phenotypic variation that is influenced by both genetic and environmental changes, it may not be a true representative of just the genetic variation. In principle, the slope, the indicator of the phenotypic variation, is indicative of only one third of the genetic variation in insecticide tolerance, as various other parameters of bioassay conditions may also contribute for much of this variation (Omer *et al.*, 1993, Tabashnik and McGaughey, 1994). Therefore, it is very unlikely that the slope may provide sufficient evidence of the progression of the resistance. However, it is possible that specific insect-insecticide combination may provide different patterns of changes in slope and need to be understood in the light of the Hoskin and Gordon's (1956) contentions. Thus it is worth examining the nature of change of slopes across generations for any insect-insecticide combination in a selection study.

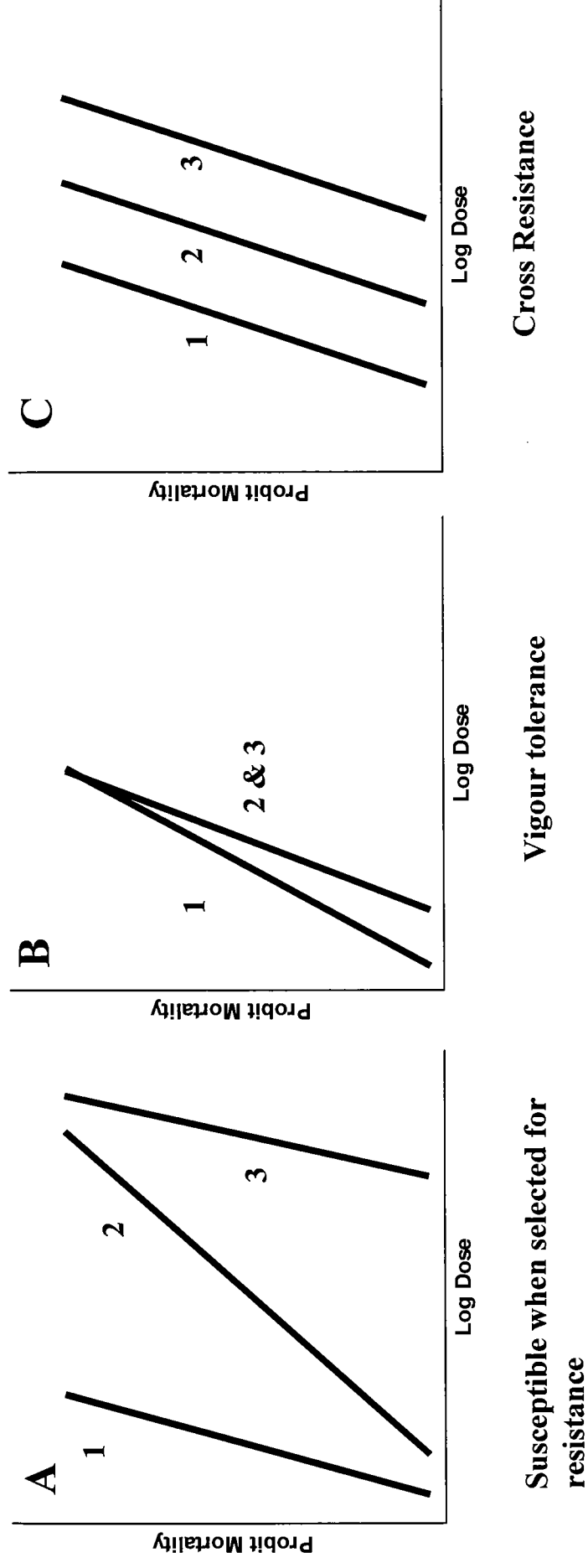


Figure 5 : Changes in dosage –mortality regression lines resulting from selection pressure depending on the nature of resistance acquired by the insect (Hoskin and Gordon, 1956). Numbers of lines 1-3 represent the different stages of selection. 1 = Susceptible, 2 = during the course of selection and 3= completely resistant.

2.4 Genetics of insecticidal resistance

It is widely accepted that the development of insecticide resistance by insects is due to the selection of variants in the population carrying pre-adaptive genes for resistance. Therefore, the frequency of these resistant alleles (or genes) presents in the population, and the nature of these genes (either single or multiple, dominant or recessive) are important in influencing the growth and stability of the insecticidal resistance in a population. Selection pressure, that is the proportion of individuals dying during the course of selection, can greatly influence the frequencies of these resistant alleles. The importance of selection pressure in influencing the rate of development of resistance has already been discussed.

Considering population to be selected, one can anticipate that the rate of development of insecticidal resistance might vary with the initial relative frequency of the resistant alleles (McKenzie, 1996). An indirect evidence to this effect would be that the populations that are previously exposed to the selection agent (insecticide) tend to show faster development of resistance, which could be simply be a situation of higher allelic frequency in the population that was selected later (Motoyama *et al.*, 1992; Tabashnik *et al.*, 1994; McKenzie, 1996; Zhao *et al.*, 2000). Considering that the resistant alleles are pre-adaptive and existing in the populations, it is quite likely that the sub populations of insects may vary greatly in the frequency of the alleles (Roush and McKenzie, 1987). It is known that the populations of a single species vary greatly in their ability to develop resistance in the first place and likely that some of the populations may completely fail to develop resistance. For example, two different populations of DBM selected with Chlorfluazuron resulted in development of 318 fold (F_{14}) in TL strain and 303 fold (F_{15}) of resistance in BK strain (Fahmy *et al.*, 1991). Such studies indirectly suggest the variation in the frequency of the resistant alleles.

The frequency of 'R' – the resistant alleles can be estimated using homozygous resistant strains and the homozygous susceptible strains. Frequency of resistant alleles for *Bt* in *H. virescens* was estimated to be 5×10^{-3} to 5×10^{-4} (Tabashnik, 1994) under laboratory selection conditions and in the field it was estimated to be 1.5×10^{-3} (Gould *et al.*, 1997). But, in *P. xylostella*, based on laboratory studies the allelic frequency was estimated to be 0.12 for resistance to Cry1Ac (Tabashnik *et al.*, 1997). Frequency of alleles has also been estimated based on the response of the F_2 progenies of sib-mating

within isofemale lines. Through this F_2 screening methodology the frequency of alleles conferring resistance to the Cry1Ab *Bt* toxin was estimated as 5.3×10^{-3} to 2.6×10^{-3} in *Ostrinia nubilalis* (Andow *et al.*, 1998 and 2000) and $<3.6 \times 10^{-3}$ in *Scirphophaga incertulas* (Bentur *et al.*, 2000). The studies thus show considerable variation in the frequency of resistant alleles to an insecticide in natural populations.

Number of genes that regulate a trait is basic to the spread of any trait in a population. It is suggested that all quantitative traits are essentially governed by a set of genes that provide strong additive effect. As a result, this neo-Darwinian thinking championed by many evolutionary geneticists insists that traits such as insecticidal resistance are basically quantitative in nature (Wright, 1952; Lande, 1981). So much so, that the mathematical calculations only aim at providing a non-monogenic answers (Lande, 1981). Thus it is pertinent to understand the importance of number of genes involved in providing, in the present case, the insecticidal resistance. Any polygenic trait is expected to spread at a greater rate in a population under selection relative to a monogenic condition (Lande, 1983; Orr and Coyne, 1992); monogenic traits on the other hand tend to get less quickly diluted even after relaxation of the selection (McKenzie, 1985). The caveat however, is the rate of development of resistance in a monogenic condition may still be rapid, provided the frequency of the resistant alleles is relatively higher than the mutation rate (Georghiou and Taylor, 1977; Hoy *et al.*, 1980; Whitten and McKenzie, 1982).

There are evidences to suggest that insecticidal resistance could be a monogenic or polygenic trait depending on the insect-insecticide combination (Table 6 and 7). Further to this, as a corollary to the quantitative nature of the trait, the selection can potentially be a response in polygenic mode to begin with, which later dissipates into a monogenic trait over increased intensity of selection (McKenzie, 1996). Nevertheless, a general belief is that the laboratory selections lead to polygenic resistant traits due to inherent genetic bottlenecks associated with the lab cultures that can only select for additive effects, while field populations that were not previously exposed to the novel chemistry tend to develop monogenic resistance traits (McKenzie, 1985. 1996; Roush and Hoy, 1981; Roush and McKenzie, 1987). As a result, polygenic resistance traits are rare under field conditions. Essentially the argument suggests that the laboratory selections should select for resistance with existing variation, with minimal scope for encountering the monogenic mutant alleles

Table 6: Studies on genetics of resistance, (mechanism of inheritance, degree of dominance, heritability and number of genes) involved in different insect pests against different insecticides tested.

Sl. No.	Insecticide (1)	Insecticide (2)	Selection- Field / Lab (3)	No. of gen. selected (4)	Resistance level (fold) (5)	Type of study (6)	Genetics (7)	References (8)
1	<i>Pectinophora gossypiella</i> (Lepidoptera: Gelechiidae)	Bt-Cry 1Ac	Lab	36 generation	3,100	Genetics- Reciprocal cross, Back cross- Monogenic model test	Autosomal, One with 3 or more locus, Recessive	Tabashnik <i>et al.</i> , 2002
2	<i>Bemisia tabaci</i>	Imidacloprid Bifenthrin- Fenvalerate	Lab	8	RR 21.90 7.12 4.13	Imidacloprid Bifenthrin Fenvalerate	$h^2=0.16$ $=0.07$ $=0.02$, Autosomal, recessive, more than one gene	Sethi <i>et al.</i> , 2002
3	<i>Heliothis virescens</i> (Lepidoptera: Noctuidae)	Methyl parathion	Lab	21 @ 80% seln. pressure	18.93	RS(2.8) > SR reciprocal cross- Male sex linked,	D=>1.0(RS) $=0.46$ (SR): Dominant, monogenic	Wolfenbarger <i>et al.</i> , 1982
4	<i>H. virescens</i> (Lepidoptera: Noctuidae)	Spinosad	Lab	19	669	Genetic studies-	Autosomal, recessive, single gene (χ^2 test)	Wyss <i>et al.</i> , 2003
5	<i>Heliothis armigera</i> (Lepidoptera: Noctuidae)	Bt- Cry1Ac	Lab	RES -Ac (F4) RES-Bt (F14)	93 205	D= -0.16 = - 0.14 D=0.14 $=0.09$	$n_E=0.53$, $n_E=1.11$; autosomal, semi-dominant, single gene	Kranthi <i>et al.</i> , 2006

Contd... Table 6

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
6	Colorado potato beetle, <i>Leptinotarsa decemlineata</i> (Coleoptera: Chrysomelidae)	Bt-CryIIIA		-		F ₁ cross & Back cross D=0.77 (RS) =0.76 (SR)	Autosomal, Incompletely dominant, more than one gene (χ^2 test)	Rahardja & Whalon, 1995
7	<i>Heliothis virescens</i> (Lepidoptera: Noctuidae)	Permethrin resisitant	Lab selected	800		F ₁ cross	Dominant, Monogenic inherited	Pimprale <i>et al.</i> , 1997
8	Tobacco bud worm, <i>Heliothis virescens</i> (Lepidoptera: Noctuidae)	cypermethrin	Field collected	-	-	F ₁ - assay - h^2 =0.85 $\pm$ 0.40; Response to selection predicted=4.6 2; actual=4.66	Estimate of h^2 capable of accurately measuring the response to selection.	Firko and Hayes, 1990
9	<i>Pectinophora gossypiella</i> (Lepidoptera :Gelechiidae)	Bt-CryIAC	Lab selection	3	> 100	F ₁ cross Low conc.- Intermediate- High conc.	Autosomal, Codominant, Partially recessive, Completely recessive	Liu <i>et al.</i> , 2001
10	Colorado potato beetle, <i>Leptinotarsa decemlineata</i> (Coleoptera: Chrysomelidae)	Imidacloprid	Field collected	Adult Larvae	100.8 13.2	D= -0.23 (3DAT), =-0.10 (7DAT); Back cross- (F ₁ x S)	Autosomal, Incompletely recessive, >one locus involved resistance	Zhao <i>et al.</i> , 2000

Contd... Table 6

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
11	Sweet potato white fly, <i>Bemisia tabaci</i> (Aleyrodidae: Homoptera)	Methidathion (OP)	Lab	8	8.6	$h^2=0.49@1^{st}$ gen., & 0.344 @ 8 th gen.	In this h^2 values over gen, calculated	Bloch and Wool, 1994
12	<i>Lucilia cuprina</i> , (Diptera: Calliphoridae)	Diflubenzuron		>6 years	8,990	Maternal influence on inheritance, Codominant (R x S); Incompletely recessive (SxR)	Maternal effect inheritance, Polygenic, MFOs – R mechanism (Aldrin epoxidase activity)	Kotze & Sales, 2001
13	<i>Musca domestica</i>	Indoxacarb	Lab	3	>118	F ₁ cross - Completely recessive	Factors located on autosome 4 & minor factor on autosome 3	Shono <i>et al.</i> , 2004
14	Two spotted spider mite <i>Tetranychus urticae</i>	Chlorfenapyr, Etoxazole	Lab		483 fold (Chlor), >1,00,000 fold (Etoxazole)	Autosomal, Completely dominant gene- both,	F ₂ -monogenic inheritance-both	Uesugi <i>et al.</i> , 2002
15	<i>Blattella germanica</i>	Chlorpyrifos (Dursban)	Field collected		20	MFO & hydrolytic enzymes involved in R	Incompletely dominant, Autosomal > one genetic factor (Chi sq test)	Siegfried <i>et al.</i> , 1990

Contd... Table 6

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
16	Western corn borer, <i>Diatrotica virgifera</i> (Coleoptera: Chrysomelidae)	Methyl parathion	Field collected NY and P strain assayed for genetics of resistance.	NY P	5.2 7.9	F1, reciprocal crosses	Incompletely dominant, Autosomal	Parimi <i>et al.</i> , 2003
17	<i>Pectinophora gossypiella</i> (Lepidoptera: Gelechiidae)	<i>Bt- Cry1Ac</i>	Lab selected two strain AZP, APHIS-98R		>100	Same resistance allele r_1 , r_2 , & r_3 but frequency is different		Tabashnik <i>et al.</i> , 2004
18	<i>Chrysomela tremulae</i> (Coleoptera: Chrysomelidae)	<i>Bt-Cry3Aa</i>	Lab selection, 60 generation		>6,400	D= 0.07, Completely recessive	D= 0.07, Completely recessive	Augustin <i>et al.</i> , 2004
19	German cockroach, (<i>Blatella germanica</i>)	Pyrethroid field population	Field, genetic studies		>140	Autosomal, Incompletely dominant		Cochran, 1994
20	<i>Scirpophaga incertulas</i> (Lepidoptera: Pyralidae)	<i>Bt-Cry1Ab</i> toxin	Field collected	Assessed for gene frequency		<3.6 x 10 ⁻³	490 iso-female screened-Based on F ₂ progeny response	Bentur <i>et al.</i> , 2000

Table 7 : Studies on genetics of resistance (inheritance, degree of dominance, heritability and number of genes) in different strains of *Plutella xylostella* against different insecticides tested.

Sl.No.	Insecticide (1)	Insecticide group (2)	Selection-Field /Lab (3)	No. of gen. selected / strain(4)	Resistance level (5)	Remark-type of study (6)	Genetics (7)	Reference (8)
1	Teflubenzuron	Acyl urea compound	Field		>1000	Crossing studies	Autosomal Monofactorial (χ^2), highly recessive	Cheng <i>et al.</i> , 1990
2	Fenvalerate	Pyrethroid	Cabbage field -Hawaii			F ₁ cross	Polygenic, $h^2=0.20$	Tabashnik & Cushing, 1989
3	Phenthoate	OP	Lab selection	Strains- OKR -OSS		Heritability studies	$h^2=0.42$ OKR =0.41 OSS	Bliss <i>et al.</i> , 1989
4	Fenvalerate	Pyrethroid	Lab selection	F ₁ cross		Inheritance	Autosomal, Incompletely recessive	Kim <i>et al.</i> , 1991
5	Bt- Commercial product	Bt-product	Lab selection	Resistant		F ₁ cross, BC-F ₁ x resistant	Autosomal, Recessive, Single locus (Chisq), Lande - <1	Tabashnik <i>et al.</i> , 1992
6	Toarrow CT	Bt-product	Lab selection	Strain- RO ROO-	78.5 704	F ₁ cross, F ₂ back cross	Incompletely recessive, autosomal, single gene	Hama <i>et al.</i> , 1992
7	Bt-Cry1A(b)	Bt-toxin	Field collected		50	F ₁ cross	Recessive, sex linked (Autosomal allele- binding)	Ramirez <i>et al.</i> , 1995

Contd... Table 7

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
8	Toarrow CTR-XenTariR-	<i>Bt</i> -product	Field collected	Toarrow CTR-XenTariR-	668 fold 66.5 fold	F1 cross with susceptible	Poly factorial	Imai and Mori, 1999
9	Cartap	Tertiary amines	Lab selected	Cartap resistant		F1 cross	Autosomal, Incompletely dominant, and by 2 or more gene(s)	Cheng <i>et al.</i> , 1999
10	Dimehypo	OP	Lab selected			F1 cross, $D=0.39$ (SxR), $D=0.28$ (RxS), $h^2=0.052$	Autosomal, Incompletely dominant, and by 2 or more gene(s)	Li <i>et al.</i> , 1998
11	Cry1Ac	<i>Bt</i> -toxin	Lab selected	F1 cross and back cross			Incompletely dominant, one allele on separate loci	Sayyed and Wright, 2001
12	Avermectin	Macro cyclic lactones		F1 cross and back cross			Autosomal, Incompletely recessive, and polygenic	Zhang <i>et al.</i> , 2001
13	BTK –(Cry1Aa, Cry1ab, Cry1Ac, & Cry2A)	BTK – (Cry1Aa, Cry1ab, Cry1Ac, & Cry2A)	Lab selected (BTK-SEL)	F5 – BTK	112 fold R to BTK	F1 cross and back cross $D=-0.35$ & 0.24-Partially recessive,	Autosomal, $n_E=0.20$ & 0.21; Single gene (χ test)	Sayyed <i>et al.</i> , 2000

Contd... Table 7

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
14	Fipronil	Phenyl pyrazole	Lab selected	5 (G3-G8)	770	$D_{LC}=0.37$; $h^2=0.18$ $G=17$;	Autosomal, Incompletely recessive, Monogenic (chisq test) / single locus	Sayed and Wright, 2004
15	Cry1C protoxin	<i>Bt</i>	Lab selection —	26	I instar – 12,400 fold II- 63,100 fold RR	II instar $D=-0.28$ (SxR); $= -0.22$ (RxS);	Autosomal Incompletely recessive/ Recessive, Monogenic (χ^2)	Zhao <i>et al.</i> , 2000
16	Chlorfluazuron	Acyl urea compound	Field collected- BBT strain		130 fold RR	$D=-1$;	Autosomal, Completely recessive, Monogenic major gene	Kobayashi <i>et al.</i> , 1992
11	Chlorfluazuron	Acyl urea compound	Lab selection – 2 strains – TL & BK	9- TL strain 10 - BK strain	276 216	$h^2=0.27$ (TL); $= 0.064$ (BK)	Heritability of TL more than BK strain	Fahmy and Miyata, 1992
12	Fenvalerate	Pyrethroid	Lab selected		8200 fold RR		Autosomal, recessive, >1 loci	Motoyama <i>et al.</i> , 1992

due to genetic bottlenecks leading to polygenic traits. As a result, any resistance leading to the selection of a rare mutant that can spread and remain viable as a resistant line can best represent the field situation. Consequently, the selection under field conditions is

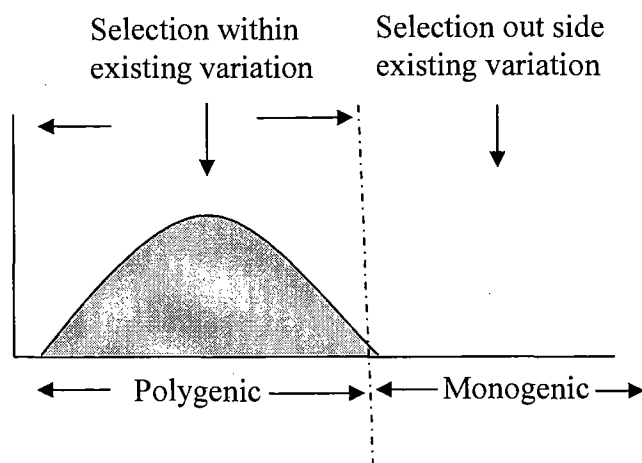


Figure 6: Impact of selection within existing variation and outside the phenotypic variation that leads to polygenic and monogenic nature of selection of insecticidal resistance (McKenzie, 1996)

expected to be taking place outside the limits of the existing dose-response variation in the population (McKenzie, 1996; Fig. 6). Yet, a population that is previously exposed to the intended insecticide on laboratory selection may lead to monogenic resistance trait. A compilation of 31 insect-insecticide resistance studies that evaluated the number of genes involved in the resistance suggested a lack of difference in the numbers of mono or polygenic traits ($\chi^2 = 2.13$; $p > 0.05$; Table 8). Therefore, it is interesting to see how many of these studies involved the selection for resistance among the populations that were previously exposed to the particular insecticide.

On similar lines, the nature of the resistant alleles as a potential factor in influencing the rate of development of insecticidal resistance has not been well tested. But yet, in *Lucilia cuprina*, dominant alleles conferring resistance to dieldrin demonstrated high resistance development at a much faster rate compared to diazinon, whose resistance was governed by a recessive allele (McKenzie and Whitten, 1984). Thus, the expectation is that the recessive alleles may reduce the rate of spread of the phenotype in the population so that the response patterns may be quite shallow. On a similar ground, the nature of inheritance, whether autosomal or sex linked may influence the rates of

Table 8: Summary of the analysis of the genetics of resistance to different insecticides in different insect pests (Table 6 and Table 7)

Inheritance	Degree of dominance	No. of genes	No of studies	Total
I. Sex linked inheritance				
Maternal	Incompletely recessive	Polygenic	1	
Paternal	Dominant	Monogenic	1	
II. Autosomal Inheritance				
Autosomal	Recessive/ Incompletely recessive		3	
Autosomal	Recessive/ Incompletely recessive	Monogenic	10	
Autosomal	Recessive/ Incompletely recessive	Polygenic	5	
				18
Autosomal	Dominant/ Incompletely dominant	Monogenic	5	
Autosomal	Dominant/ Incompletely dominant	Polygenic	3	
Autosomal	Dominant/ Incompletely dominant		3	
				11

development of resistance. Autosomal inheritance may result in ten fold fewer susceptible individuals among the progeny compared to a sex linked trait (our calculations).

2.4.1 Methods of studying the genetics of resistance

A knowledge of genetics of resistance is helpful in (a) detecting and monitoring resistance, (b) assessing the risk of resistance, (c) required for modelling the evolution of resistance, (d) developing strategies for delaying the resistance in pests and (e) to promote development of resistance in beneficial organisms as the various facets of insecticide resistance are linked to the genetics of resistance.

Genetic studies on insecticidal resistance aim at understanding, the number of genes involved, number of allele's involved, gene amplification, inheritance and the nature of gene/s involved. Nature of genetics of the trait, the insecticidal resistance, influence the rate of development, stability and loss of insecticide resistance in insect pests. Resistance can be governed by autosomal /sex linked chromosomes, dominant or recessive genes or by one or more number of genes (Roush and McKenzie, 1987) that influence the rate of development of resistance. Hence, to delay the development of resistance, it is necessary to have information on genetics of insecticidal resistance which in turn helps develop insecticide resistance management techniques.

As part of the genetic studies, inheritance, the number of genes involved, the numbers of alleles involved, degree of dominance, response to selection and heritability are generally studied in insecticidal resistance studies.

2.4.1.1 Inheritance

Inheritance of a trait may take place either through autosomal or through sex chromosomes. Depending on the type of chromosomes involved, they are called as autosomal or sex linked inheritance, respectively.

Inheritance of resistance whether by autosomal or sex linked, are assayed through response of F₁ progenies of the reciprocal crosses. A majority of the resistance traits against different insecticides in most insects are autosomally inherited (Table 6). Exceptionally, however, in *H. virescens* resistance against methyl parathion is inherited

through paternal chromosomes (Wolfenbarger *et al.*, 1982) and in *L. cuprina*, against diflubenzuron is maternally inherited (Kotze and Sales, 2001). In *P. xylostella* resistance to different insecticides, such as pyrethroids (Kim *et al.*, 1991), *Bt* toxins (Ramirez *et al.*, 1995), chitin synthesis inhibitors (Cheng *et al.*, 1990) and cartap (Cheng *et al.*, 1999) are all inherited autosomally. Out of 31 studies compiled from literature only in two the resistance was inherited as a sex linked trait and in DBM, all the resistance traits were inherited only through autosomal chromosomes (Table 7). Thus the insecticidal resistance in general is expected to be inherited autosomally (Table 8).

2.4.1.2 Nature of Resistance Alleles : Methods to study

Nature of the resistant alleles, *i.e.*, dominant or recessive, is also known to influence the rate of development of resistance. The allele that is expressed at the cost of its counterpart in heterozygous condition is a dominant allele. Three criteria can be used to identify the dominant genes. If the trait is regulated by a dominant allele then, a) it will be expressed in all generations, b) it is passed on from the affected parent to at least about 50% of his/her progeny, and c) a parent that does not express the trait does not transmit it to any of his/her progeny. On the contrary, if a trait is regulated by a recessive allele then, it is not expressed in heterozygous condition but expresses itself only in homozygous (aa) individuals.

However, in some systems the phenotype may not fit either of the two conditions mentioned above. Such a situation is generally termed as co-dominance or incompletely dominant situation.

Two different approaches are followed to understand the nature of resistance trait.

1. Based on logdose-probit (ldp) mortality lines and
2. Degree of dominance (D) - Stone (1968) method

2.4.1.2.1. Based on Probit mortality lines

Based on the relative position of the F_1 progeny ldp mortality line with respect to resistant (RR) homozygous population and the susceptible (SS) homozygous population, the nature of the gene can be predicted. Wherein, the F_1 is the progeny of homozygous resistant and susceptible populations (Brown, 1958). If the F_1 ldp mortality line falls towards the ldp mortality line of resistant population then it is expected to be controlled by

a dominant allele and recessive when it is towards the susceptible population. However, if it is midway between the resistant and susceptible population ldp mortality lines, then it is generally regarded as a co-dominant trait (Brown, 1958). This method is perhaps, the most widely used method in ascertaining the nature of the insecticide resistance alleles, largely because it is simple and easy to accomplish.

2.4.1.2.2. Degree of Dominance : Stone (1968) method

The nature of allele/s regulating the resistance can also be estimated in terms of degree of dominance, proposed by Stone (1968). The methodology involves the calculation of LC_{50} of the F_1 progeny and parental populations (*i.e.*, resistant and susceptible populations). Using this information, 'D' the degree of dominance is calculated following Stone (1968). The 'D' can take values from -1 to +1. If 'D' is -1 then it is completely recessive, a value of 0 indicates complete lack of dominance and if the trait is completely dominant 'D' takes a value of +1. The method can be conveniently adopted for calculation of the degree of dominance of resistance alleles using either lab selected or field resistant populations.

Information relating to 31 studies that dealt with the genetics of resistance was collected during this review which included studies on DBM as well as other insects (Table 6 & 7). In nineteen of these studies, resistance was found to be governed by incompletely or completely recessive genes and in 12 studies it was by dominant alleles (Table 8). Although, more often than not, the resistance in different insect pests appeared to be governed by recessive or incompletely recessive alleles (Tabashnik *et al.*, 2002; Wyss *et al.*, 2003; Table 6), the frequencies compiled suggest a lack of difference between the two ($\chi^2 = 1.69$; $p > 0.05$). Thus the probabilities of resistance being governed by either dominant or recessive alleles remain equal.

An over all consideration of all the studies compiled suggested the possibility that the nature of the resistant alleles is independent of both the insect and the insecticide. Thus specific combination of insect–insecticide combination appears to be the determining factor in deciding the nature of the resistant alleles. For example, in DBM both recessive and dominant alleles have been implicated to confer resistance to different insecticides (Tabashnik *et al.*, 1992; Cheng *et al.*, 1999; Zhang *et al.*, 2001). Similarly, for the same

insecticide, *Bt* toxin, Cry1Ac, both recessive and dominant alleles have been shown to be involved in *P. gossypiella* (Tabashnik *et al.*, 2002) and *P. xylostella* (Sayyed and Wright, 2001), respectively. However, there is also a need for clarity as some of the studies provide opposing ideas of the nature of the allele in a single insect. For example Hama *et al.*, (1992) considered the resistance to a *Bt* product based on Cry1Ac to be a recessive trait, while Sayyed and Wright (2001) indicated the incompletely dominant nature of the alleles involved in the resistance to the same toxin, Cry1Ac. As a result, it is important, and even necessary that even the established facts be cross verified.

An interesting question to pursue would be to understand the association between the nature of inheritance, whether autosomal or sex linked, and the nature of resistance whether dominant or recessive. Unfortunately, the literature reviewed did not provide a reasonably good sample size for understanding this relationship, primarily because only two of the 31 studies compiled indicated the involvement of sex linked inheritance for insecticidal resistance. Thus independent of these two studies, the recessive and the dominant nature of the alleles involved in autosomally inherited traits seem to be a random phenomenon (Table 8). Further considering the distribution of dominance or recessive nature of the alleles across mono- or polygenic resistance was also found to be random (2 x 2 contingency $\chi^2 = 0.043$; $p > 0.05$).

2.4.1.2.3 Degree of dominance and the resistance management

Whether, the resistance is governed by recessive or dominant gene will influence the rate of development of resistance and consequently the management practices. If the resistance is governed by a dominant allele even after several generations of selection either in the lab or in the field populations, they continue to have susceptible 'S' alleles in the selected population (Fig. 7), as phenotypic resistance continues to be represented by heterozygotes also. Consequently, on relaxing the selection pressure, the population can come back to a susceptible condition over generations due to the spread of susceptible alleles in the population. However, if resistance is governed by a recessive allele (r), then it is likely that the homozygous condition is attained at a quick pace depending on the extent of selection pressure imposed. Once the population attains a homozygous condition, a relaxation in selection pressure will not allow the population to revert to the susceptible condition due to lack of availability of susceptible alleles in the population

Resistance by dominant gene 'R'

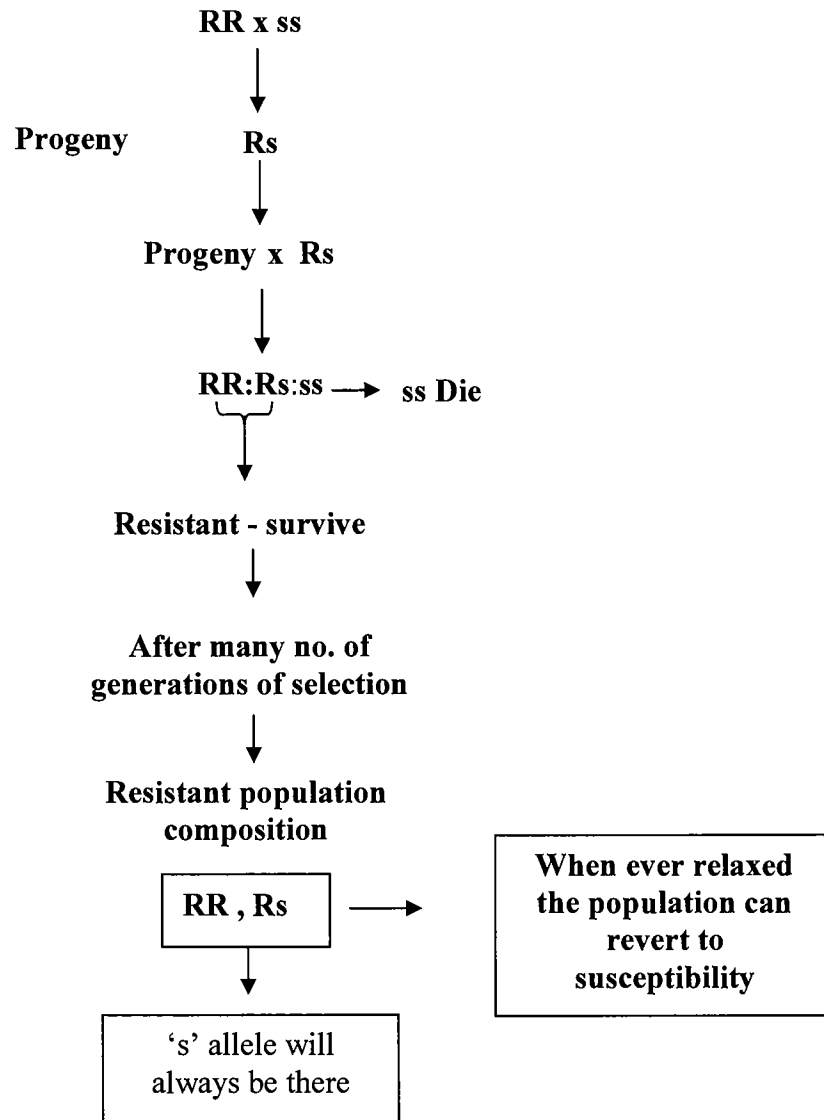


Figure 7: Nature of distribution of alleles in the populations selected for resistance and its consequences for the degree of susceptibility when the resistance is governed by a dominant allele.

(Fig. 8). Evidences to this effect of continued stable resistance level are available (Brown, 1958; Tabashnik *et al.*, 1995). In such situations, the stability of the resistance can best be broken with planned studies to integrate the dominant alleles (the susceptible trait) into the population under selection. Refugia concept therefore, becomes very relevant for such situations. A case in point is the potentially recessive nature of the resistance in cotton bollworms to *Bt* toxins (Gould and Tabashnik, 1998; Liu *et al.*, 2001). Refugia in such cases would serve as the source of susceptible alleles for delaying the development of resistance in the population under selection. However, pertinent to note here is the fact that refugia can no less be important in delaying the rate of development of resistance even when the resistance is a dominant trait, it is only the relative contributions that differ between the two.

2.4.1.3 Number of genes governing insecticidal resistance

Resistance may be governed by single gene (Monogenic) or more than one gene (Polygenic). The number of genes governing resistance can be worked out based on the responses of F_1 progeny of the homozygous resistant (RR) and susceptible (SS) populations and those of the F_2 progeny of the back crosses with suitable parents (Tabashnik, 1991; Zhao *et al.*, 2000; Uesugi *et al.*, 2002). These studies provide the data for calculation of both the nature and the number of alleles responsible for the resistance trait. Two basic methods are employed to study this. First of this method employs the potential frequency distribution of the various combinations of the expected allelic frequencies and the dose response-values generated based on these phenotypic frequencies will be accordingly re-calculated to provide the expected frequencies in back cross F_2 progeny. These values will be compared using chi square test with the observed values of the backcross F_2 progeny. Results of the chi-square test provide the basic idea of how many alleles are possibly involved in conferring the resistance, depending on the logic on which the expected frequencies are drawn. But most studies compare the values for the monogenic two-allele frequencies and depending on the test results decide whether the resistance trait is monogenic or not. If the Chi square test provides significant differences, then the trait is considered to be polygenic. On the contrary, expecting that all quantitative traits are essentially polygenic, insecticide resistance trait is also measured to evaluate the number of factors involved (Lande, 1981). These values are calculated primarily using the median lethal doses and the variance in the response pattern of the homozygous parent stocks, F_1 and back cross F_2 progeny.

Resistance governed by recessive 'r' gene/s

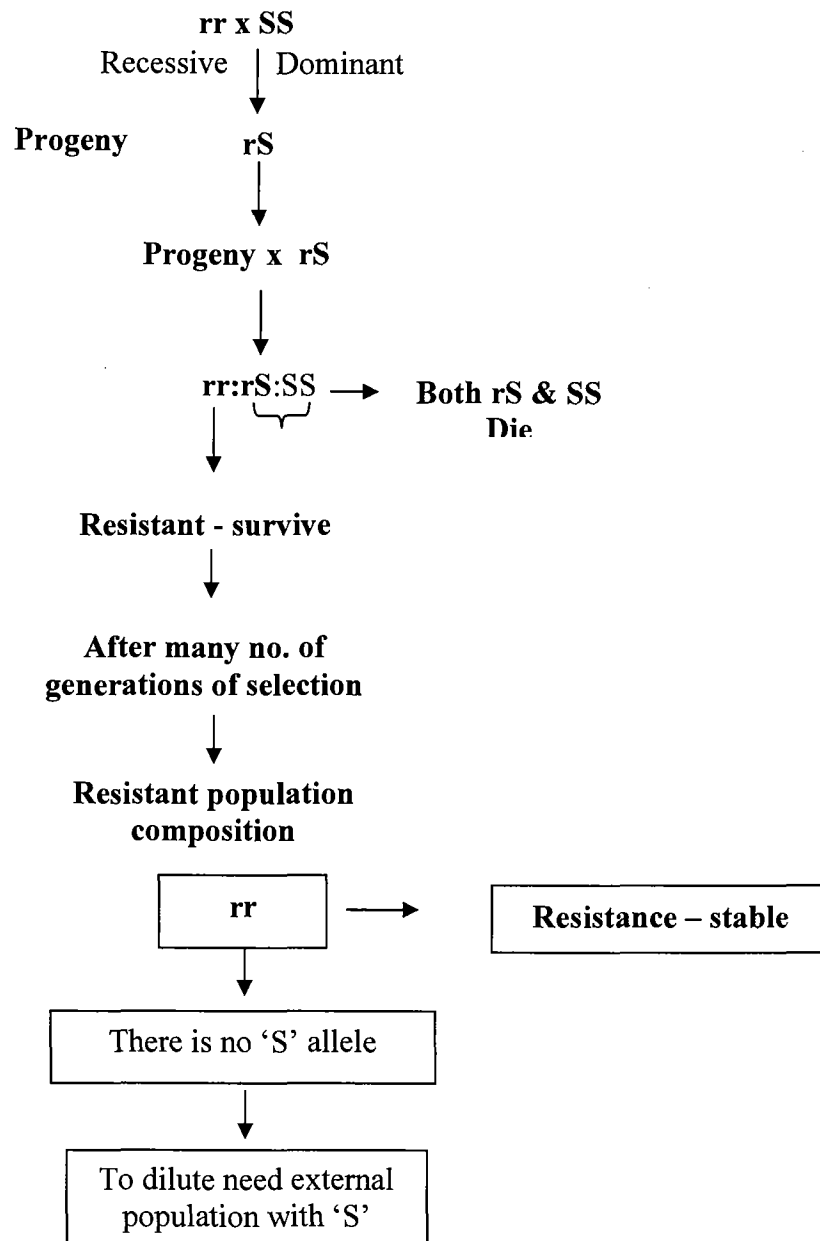


Figure 8: Nature of distribution of alleles in the populations selected for resistance and its consequences for the degree of susceptibility when the resistance is governed by a recessive allele.

2.4.2 Heritability (h^2)

Heritability is the proportion of phenotypic variance accounted by additive genetic variation (Falconer, 1989). It is also defined as the proportion of the genes or trait transmitted by parents to their offspring.

The rate at which the phenotypic frequencies change when a given amount of selection is applied is a direct function of the heritability. Greater the heritability of a given trait, that is the more its variation among individuals of the same population due to heredity, more rapidly it will evolve in the population under the influence of a given intensity of selection.

Heritability (h^2_B) in a broader sense is also defined as the proportion that genetic variance contributes to the total phenotypic variance ($h^2_B = V_G / V_P$). Heritability is a precise measure that compares the total amount of phenotypic variability with the amount due to variation in genetic constitution. If h^2_B score is one, indicates that all of the variation in the population is due to the difference among the genotypes and no variation is caused due to the influence of the environment. Similarly, a h^2_B value of 0 suggests that all of the variation seen in a population is caused by environment, genetic differences among individuals does not have any influence on the trait considered for measurement.

From this broad sense of heritability, narrower sense of heritability can be estimated from which direct estimation of the rate at which evolution can occur are predicted (Wilson and Bossert, 1971). Heritability in the narrower sense (h^2_N) is defined as the speed with which a trait is evolving in a population (R), increases as the product of its heritability (h^2_N) and the intensity of the selection process (S).

$$R = h^2_N S,$$

where, R- response of the population to selection

S-Selection differential (indicative of the proportion of the population included in the selection)

$$h^2_N = V_A / V_P$$

Where, V_A = Variance due to additive effect of genes = 'R'

V_P = Total phenotypic variance = 'S'

Hence, $h^2_N = R / S$

The rate of evolution is proportional to the genetic variance of the population. Response to selection will be very high during the initial generations of selection as the genetic variation in the population is high. However, 'R' being proportional to h^2 the population selected over generations tends to show reduced 'R' and consequently lower h^2 values. This is also because, the variation in population decreases as the population moves towards homozygosity (Falconer, 1989).

The realized heritability of resistance can be estimated by regression of median lethal dose (LD₅₀) on cumulative selection differential (Tanaka and Noppun, 1989) and by other techniques (Ferrari *et al.*, 1982, Wolfenbarger *et al.*, 1982). However, these methods are laborious and are not generally used in insecticide resistance studies to estimate the heritability. Falconer (1989) provides a method of estimation of heritability of liability (Firko and Haye, 1990). Following which, Tabashnik, (1992) described a method for estimating realized heritability of resistance to insecticides. This method uses data that are collected routinely during artificial selection studies.

Estimation of realized heritability of resistance based on the method illustrated here can be useful in several ways. Perhaps most important, is that it provides standardized means to quantify results of selection experiments and thus enables quantitative comparison among experiments. In laboratory selected populations if there is lack of response to selection ($h^2=0$) it indicates an inability of the field populations to evolve resistance.

Among the studies compiled (Table 7), h^2 values in *P. xylostella* varied from 0.42 against fenthioate (Bliss *et al.*, 1989) to a low of 0.052 against dimehypo (Li *et al.*, 1998). Other intermediary values are against fenvalerate ($h^2= 0.20$; Tabashnik and Cushing, 1989), and fipronil (0.18; Sayyed and Wright, 2004). An excellent compilation of heritability values by Tabashnik (1992) in different insect pests also suggested similar variation among different insects ranging from 0.42 to 0.047.

Heritability (h^2) values also influence the rate of development of resistance. One such measure is the calculation of the parameter 'G', the number of generations required

for ten fold increase in resistance to an insecticide. 'G' values have been calculated only in very few studies. In *P. xylostella* a ten fold increase in resistance to fipronil requires 17 generations (G) (Sayyed and Wright, 2004). However, higher the h^2 value, lower will be the number of generations required for ten fold increase in resistance level, as 'G' is inversely proportional to heritability (Tabashnik, 1992). As a consequence it can be anticipated that the ten fold increase in resistance occurs much faster in early generations of selection and while the same gets delayed as the selection progresses. Further, the two are also related to the selection pressure. Number of generations required for a 10 fold increase in LC_{50} decreases as the proportion killed by insecticide ($100 - p$) increases (Tabashnik, 1992).

However, this can be best gauged when the heritability is kept constant (Tabashnik, 1992). At constant heritability values, the rate of development of resistance (G) is inversely proportional to selection pressure. This is of particular importance and provides a platform for meaningful comparison of rates of development of resistance for different insecticides (Tabashnik, 1992).

2.5 Fitness and insecticidal resistance

Resistance to an insecticide occurs in an insect either due to increased metabolic detoxification of the insecticide or due to target site insensitivity. As a result of change in the normal biochemical function, there will be an expected diversion of the resources towards the mechanisms involved in resistance. Thus resistance is expected to incur certain fitness cost. Fitness cost is the reduced fitness of individuals bearing 'R' resistance alleles over the homozygous susceptible (SS) individuals that may express in many different ways such as, reduced or increased developmental time, life time fecundity or egg viability. All these factors together are expected to get reflected in the form of reduced biotic potential, the intrinsic rate of increase-'r'. Impact of fitness cost is expected to be particularly strong during the early stages of resistance evolution (Groeters *et al.* 1993; Tabashnik *et al.*, 1995).

A number of evidences exist for the fitness cost of insecticidal resistance. Larvae of *Pectinophora gossypiella* fed on *Bt* transgenic cotton plants developed slowly and laid less number of eggs than susceptible individuals (Carriere *et al.*, 2001). However, males of the *H. virescens* selected for spinosad resistance developed slowly and emerged later

resulting in their reduced frequency due to decreased reproductive competence (Wyss *et al.*, 2003). Resistance to pyrethroids incur biological cost in terms of increased larval period, reduced number of eggs and less hormone produced by the females in *H. virescens* (Campanhola *et al.*, 1991).

Considering DBM, resistance to *Bt* resulted in reduced fecundity and egg hatchability (Groeters *et al.*, 1994), longer duration of development and reduced adult longevity (Shirai *et al.*, 1998). However, increased fecundity on selection with methomyl than in control has been attributed to hormoligosis effect of insecticide in DBM (Sota *et al.*, 1998). But surprisingly, a DBM population selected for resistance to chlorfluazuron resulted in higher intrinsic rate of increase with increased fecundity and shorter generation time than the unselected control populations (Yamada *et al.*, 1993). While such a phenomenon is aberrant, there are also situations where detectable fitness cost was not evident. DBM selected against fenvalerate resistance did not incur any apparent fitness cost, but there was a decrease in resistance on relaxation from selection (Motoyama *et al.*, 1992). *A priori*, if there is no fitness cost, it is then anticipated that the allelic frequencies should remain stable under relaxation. On the contrary, if there is a fitness cost, then it is anticipated that the frequencies move in favour of susceptible allele under relaxation. Thus, irrespective of the mechanism involved and the nature of the cost, if there is decrease in resistance levels under relaxation from selection, then there should be some fitness cost involved in sustaining the resistance. Thus on the whole, many studies indicated here suggest some form of fitness cost associated with insecticidal resistance in insects in general and *P. xylostella* in particular.

2.6 Relaxation and stability of resistance

Stability of resistance in the absence of selection pressure determines many pest management tactics. Many factors *viz.*, ecological, genetic and fitness parameters can potentially influence the stability of resistance. Resistance management tactics like alternation of different pesticides and window method of application of pesticides assume that frequency of resistance alleles to each compound decline rapidly in the absence of selection pressure (Georghiou, 1972). The dilution of the resistance can be due to immigration of susceptible individuals or because of selection against carriers of 'R' alleles *i.e.* fitness cost involved or both. However, under laboratory conditions, stability of resistance may depend mainly on nature of gene/allele and fitness parameters.

2.6.1 The nature of allele

If the resistance is governed by dominant allele then the phenotypic resistance is not in full agreement with the genetic composition as considerable proportion of individuals could be heterozygous to resistance allele (Fig.7). As a consequence, relaxation would help the spread of these alleles in the system leading to increased susceptibility and a consequent breakdown of resistance. Thus, in principle, resistance governed by dominant alleles would bring back the market life of an insecticide that became useless due to accumulation of resistance in the past. However, if the resistance is governed by a recessive allele, phenotypic agreement is restricted to the homozygous recessive allele condition. As a result, high resistance levels essentially mean a high proportion of homozygous individuals in the population (Fig. 8). Consequently, the proportion of susceptible alleles remain extremely low or nil leading to high stability of the resistance to the target chemical in the population. Essentially, the chemical becomes eternally useless against the target pest population, with a caveat of recurrent mutation for susceptibility.

This hypothesis was tested in Diamondback moth, *P. xylostella* after prolonged selection with the microbial insecticide, *B. thuringiensis* (Tabashnik *et al.*, 1995). A laboratory strain of diamondback moth derived from a moderately resistant field population was selected with *Bt* for 21 generations. After an apparent plateau in resistance level, the population was relaxed from selection to know the stability of resistance. The resistance to *Bt* in DBM is governed by autosomally inherited, recessive gene. Hence, once the resistance achieved a plateau, it is expected to remain constant on relaxation. The level of resistance remained stable even after 20 generations of rearing without exposure to *B. thuriengensis* in extremely highly resistant, NO-YA isofemale line. The stability was because, the pair of adults that founded the NO-YA line was homozygous for alleles conferring a high level of resistance at the locus or loci controlling the resistance. Assuming that no susceptible alleles were introduced into this line by mutation or gene flow (Tabashnik *et al.*, 1995) the resistance would be expected to remain stable despite fitness costs, if any, associated with the resistance.

2.6.2 Fitness cost

Fitness cost is the main factor for instability of resistance during the initial developmental stage of resistance *i.e.*, in a population with heterozygous individuals (Tabashnik *et al.*, 1995). This gets nullified once all individuals in the population reach the homozygous condition. Thus, the hypothesis was tested in Diamondback moth, *P. xylostella* after prolonged selection with the microbial insecticide, *B. thuringiensis* (Tabashnik *et al.*, 1995). After 21 generations of selection when resistance level reached a plateau, different strains were relaxed from selection to know the stability of resistance. As resistance is governed by recessive gene, it was expected to remain stable. However, in most of the isofemale lines the resistance declined. The instability of resistance to *Bt* in DBM was due to decreased biotic potential of resistant individuals relative to susceptible individuals in the absence of *Bt* (Groeters *et al.*, 1993; Tabashnik *et al.*, 1991).

In *H. virescens* resistance to pyrethroids, cypermethrin and permethrin was due to metabolic and target site insensitivity mechanism of resistance (Campanhola and Plapp, 1989). Resistance decreased in the absence of selection pressure and was due to reduced biotic potential identified in the form of changes in developmental time, decreased fecundity, egg viability, mating success and pheromone production compared to susceptible populations (Campanhola *et al.*, 1991).

Thus the fitness costs would cause a decline in resistance only in heterogeneous populations that include individuals that were not homozygous for resistance (Groeters *et al.* 1993, 1994; Tabashnik *et al.* 1991, 1994). Recurrent mutation from resistant to susceptible genotypes is another major factor promoting instability of resistance. But, mutation rates are expected to be too low to cause substantial changes in genotypic frequency (Hartl, 1988). However, in some insects stability of insecticidal resistance is maintained even after removal of selection as the resistant 'R' individuals showed an increased biotic potential relative to susceptible ones (Beeman and Nanis, 1986).

But there are also views that decrease in resistance to an insecticide under relaxation may not always involve fitness cost (Motoyama *et al.*, 1992). Some times, resistance to insecticide decrease on relaxation of selection to reach a certain level at which it remains stable with out reaching the level of susceptible population (Chen *et al.*, 1995; Tang and Shelton, 1995) in DBM. Hence, as a rule, barring extremely few

exceptions, resistance to most of the insecticides declines on relaxation from selection (Fahmy and Miyata, 1992a; Zhao *et al.*, 2000; Table 9).

Further, considering the rate of decrease in resistance, the decline in resistance over generations followed an exponential decay function (Parrella and Trumble, 1989; our analysis; Table 5). When different strains of DBM resistant to *Bt* were relaxed from selection, average rates of decline in resistance per generation were highest in the strains with the highest levels of resistance compared to other strains which were moderately resistant to *Bt* (Tabashnik *et al.*, 1994).

2.6.3 Mechanism of resistance and stability

Apart from the genetics of resistance which influences the stability of resistance, the mechanism of resistance also influences the rate of reversion. Metabolic detoxification, target site insensitivity and decreased penetration are the common modes of resistance mechanisms against insecticides. When resistance is due to metabolic detoxification of an insecticide by enzymes, increased detoxification is due to the increased activity of the enzyme that may call for energetic investment towards sustaining resistance. As a consequence, many normal biological processes *viz.*, developmental time, fecundity *etc.*, may be affected leading to loss of fitness. Thus in the absence of insecticide (selection pressure), resistant populations show a tendency to increase their susceptibility.

On the contrary, resistance can also accrue due to target site insensitivity such as physical or structural changes of binding sites against *Bt* toxins, reduced number of target sites, reduced number of Na⁺ channels against DDT, *etc.* Such mechanisms, apparently, may not involve recurring energetic investments for sustaining the insecticidal resistance leading potentially to a possibility that the levels of resistance remain stable through generations of relaxation period (Campanhola and Plapp, 1989).

Considering these possibilities and going by the assertion that the fitness costs are of particular importance in the early phases of development of insecticidal resistance, one can argue that the increased levels of resistance under sustained selection are achieved due to a possibility of development of more than one mechanisms of resistance against a selection agent. Therefore, it is of prime importance to understand the mechanisms of

Table 9 : Some of the studies on level of resistance on relaxation from selection during selection for resistance to different insecticide in *Plutella xylostella*.

Sl.No.	Resistance to Insecticide	Type of study Field /Lab	Resistance after selection	No. of generation relaxed	Resistance level after relaxation	Remark	Reference
1	Chlorfluazuron	Lab selected		9	Reached level of susceptible strain		Fahmy and Miyata, 1992a
2	<i>Bt</i> -subsp <i>kurstaki</i>	Field collected	>1500 fold	3 7	300 150	Decreased and remained almost stable- single loci	Tang and Shelton, 1995
3	Bt toxins- in Taiwan	Field monitoring	Toarrow-CT -42 fold of RR (1990) Bacilex-27fold of RR (1989)	2 year	5 folds (1992)		Adachi and Grey, 1996
4A	Dimehypo	Lab selected	167	5	57 fold		Chen <i>et al.</i> , 1995
4B	Cartap	Lab selected	87	5	74 fold		Chen <i>et al.</i> , 1995
5	Fevalerate, Phosmet	Field collected		20	Equivalent to susceptible strain		Zhu <i>et al.</i> , 1998
6	<i>Bt</i> - Cry 1Ac	Lab selected	5800	6 isofemale lines tested 20 generation	5800	Remained stable in line at 5800fold	Tabashnik <i>et al.</i> , 1995
7	Teflubenzuron, Chlorfluazuron	Lab selected	110 8200	31	0.7 1.3	Stability studies, Relaxation after 31 generations	Furlong and Wright, 1994
8	Cry1C protoxin	Lab selected- 26 generation	63,100	10	235		Zhao <i>et al.</i> , 2000

insecticidal resistance through time *vis-à-vis*, fitness. There apparently do not seem to be any verification or substantiation of this possibility. Nevertheless, the argument if true, is expected to have much wider ramifications in terms of genetics, and ecology of insecticidal resistance.

In essence, the foregoing discussion throws up some interesting possibilities. Fitness cost, nature of the allele and the mechanisms of resistance, all put together, the loss of resistance to an insecticide through time, under relaxation, is a foregone conclusion but for a few exceptions. Thus, in insects such as DBM, where the rates of acquisition of resistance are enormous, insecticides that have become useless at times may have an opportunity to become viable options for reuse over time. Cross resistance studies are expected to provide some insights into these possibilities.

2.7 Cross resistance

2.7.1 Positive cross resistance

Essentially cross resistance means the ability of an insect or a population that is known to be resistant to a particular insecticide (in this case) demonstrating high levels of tolerance to another insecticide for which there was no previous exposure. Such a positive response to one leading to other can occur as a consequence of similar or identical mechanisms of resistance such as common metabolic enzymes, similar target sites *etc.* For example, DDT and pyrethroids share the same target site on the voltage-sensitive sodium channels, and the insensitivity in the insect to one results in the development of resistance to both (Plapp, 1976). Wide variation in the susceptibility of *P. xylostella* and in other insects to the pyrethroids, even before their widespread field applications started is thought to have resulted from cross-resistance associated with reduced sensitivity of this target site arising from the previous and extensive use of DDT (Davis *et al.*, 1975; Brown *et al.*, 1982; Zoebelein, 1990). Similarly, resistance due to reduced sensitivity of acetylcholinesterases confers cross-resistance between organophosphates (OPs) and carbamates (Brown and Bryson, 1992). Further, similarity in metabolic mechanisms of resistance to OPs, carbamates and pyrethroids in *P. xylostella* results in cross resistance among these three groups of insecticides (Kim *et al.*, 1990; Liu *et al.*, 1995). However, cross resistance between unrelated groups such as *Bt* selected strains being resistant to cartap and thiocyclam has also been observed (Hama *et al.*, 1992). Generally, metabolic resistance is believed to confer a broader cross-resistance than target site insensitivity

(Casida and Quistad, 1998). The spectrum of cross-resistance not only depends on the target sites and metabolic pathways of an insecticide, but also on the insect being selected and the insecticide used for selection (Table 10). Even in *P. xylostella*, cross resistance is documented between pyrethroid selected resistant strain to organophosphates and carbamates (Kim *et al.*, 1990; Liu *et al.*, 1995; Table 11)

Cross resistance to insecticides among insect pests has been a well documented phenomenon and is widely prevalent among many insects. The phenomenon is of particular importance within each chemical group of insecticides and development of resistance to one renders many other members of the group ineffective. As a result, the problem of cross resistance has put an eternal demand for novel pesticide molecules with different modes of action.

2.7.2 Negatively correlated resistance

While most documented cross resistance situations suggest a positive association between any two insecticides under considerations, in some exceptional situations, it has been observed that resistance to one insecticide makes it hyper-sensitive to another insecticide and on occasions it could be between any two counterparts. Such a mechanism is termed Negative Cross Resistance. Negative cross resistance is defined as a situation in which an insect population that is resistant to one insecticide is hyper sensitive to a second insecticide and insects that are hyper sensitive to the first compound are tolerant to the second (Pittenderigh *et al.*, 2000). The concept has been pursued vigorously for a long time with many evidences of potential one way or two-way negative cross resistance between insecticides. Largely because, the concept allows the use of any two counterpart insecticides or toxins eternally for the management of a crop pest, provided the two are negatively cross resistant. Unfortunately however, only one evidence exists in practice for the deployment of a pair of negatively cross resistant insecticides for pest management. The green rice leafhopper, *Nephotettix cincticeps*, has developed cross-resistance to whole aryl N-methylcarbamates due to alteration of the acetylcholinesterase (AChE). The altered AChE that was insensitive to the N-methylcarbamates, was hyper-sensitive to N-propylcarbamates. For the field resistant strain, the combination of N-methyl and N-propylcarbamates was effective, while N-propylcarbamate was powerful in controlling the strain highly resistant to N-methylcarbamates (Yamamoto *et al.*, 1983). This also served

Table 10: Level of cross resistance recorded against different insecticides in different species of insect pests selected for resistance to an insecticide.

Sl.No.	Resistant to Insecticide	Group of insecticide	Type of study Field /Lab	Generation & Resistance level	Cross resistance - Insecticides tested	Cross resistance level	Results	Reference
1	<i>Heliothis virescens</i> (Lepidoptera: Noctuidae)	Permethrin (Pyrethroid)	Lab selection @ LD80 conc.(70-90% mortality)	F8 -37 fold	Cypermethrin	7.9 fold @ LD ₅₀ ; 50 fold @ LD ₉₅ level		Jensen <i>et al.</i> , 1984
2	<i>Heliothis virescens</i> (Lepidoptera: Noctuidae)	Permethrin (Pyrethroid)	Lab selected	800 fold	Chlorfenapyr (Pyrethroid insecticide)	Negative cross resistance Betn. Cyp(x) vs Chlorf (y)	Y=-1.065x +3.513	Pimprale <i>et al.</i> , 19997
4	<i>Diatrea saccharalis</i>	Azinphos methyl	Lab selected for 6 generation	Resistance not developed	Carbofuron- Monocrotophos- Methomyl-	3.5 4 11 fold RR		Reagans <i>et al.</i> , 1974
4	<i>Diatrea saccharalis</i>	Carbofuron	5 gen. selected	8 fold RR	Methyl parathion- Methomyl-	2 16 fold		Reagans <i>et al.</i> , 1974
5	<i>Musca domestica</i>	Indoxacarb	3 generation	>118 fold RR	DCJW- Spinosad- Fipronil- Cyfluthrin- Chlorfenapyr- Dimethoate- Tetrachlorvinphos- Methomyl- Abamectin- Dieldrin- γ -chlodane- Lindane- Toxaphene-	3.2 (RR-fold) 4.1 0.45 4.4 0.73 1.0 10 1.0 2.1 2.5 1.3 9.4 2.3	DCJW (Bioactivated form of Ind) (-)ve cross resistant to Fipronil (0.45) & Chlorfenpyr (0.75)	Shono <i>et al.</i> , 2004

Table 11: Level of cross resistance in different selected strains to different insecticides in *Plutella xylostella*

Sl.No.	Resistant to Insecticide	Group of insecticide	Type of study Field /Lab	Generation & Resistance level	Cross resistance – Insecticides tested	Cross resistance level	Results	Reference
1	Fenvalerate	Pyrethroid	Lab selection	24 - 66.2 fold	Deltamethrin other pyrethroids OP Carbamates	145 fold 17.4 - 45 fold 2.5 4.3 folds		Kim <i>et al.</i> , 1990
2	Parathion,	OP	Lab selection		Methyl parathion	500		Kao <i>et al.</i> , 1989
3	Methyl parathion	OP	Lab selection		Parathion	>1000		Kao <i>et al.</i> , 1989
4	Dimehypo,	OP	Lab selection	35-51 fold	Dimehypo, Cartap, Thiocyclam, DDVP, Malathion, Fenitrothion	+ ve CR		Chen <i>et al.</i> , 1993
	Cartap	Tertiary amine	Lab selection	35 -25fold	Deltamethrin, cypermethrin, permethrin, Methomyl, Thiofanox	(-) ve Cross resistance		Chen <i>et al.</i> , 1993
5	Bt - Toarrow CT	Bt product	Lab selection	RO strain- 78.5	Cartap Thiocyclam	46 fold 52 foldRR		Hama <i>et al.</i> , 1992
6	Deltamethrin	Pyrethroid	Lab selection	F65-1163 fold RR	Cypermethrin, DDVP, Dimehypo and methomyl	(+) ve cross resistance		Liu <i>et al.</i> , 1995
7	Chlorfluazuron	Acyl urea compound	Lab selection	F6-23.78 fold	Neristoxin derivative –Cartap, Shachongdan	0.21 fold, 0.23 fold	(-) ve cross resistance	Wu <i>et al.</i> , 1998

as an example of negative cross resistance occurring within the same chemical group of insecticide.

Negative cross resistance can be due to enhanced activities of detoxifying enzymes accompanied with resistance to one insecticide that may increase the bioactivation of another. For example, increased activities of cytochrome P₄₅₀s (monooxygenases) were possibly responsible for observed negative cross-resistance to cypermethrin in a chlorpyrifos-selected strain of the German cockroach, *Blattella germanica* (Scharf *et al.*, 1997), to diazinon in the pyrethroid resistant horn fly, *Haematobia irritans* (Cilek *et al.*, 1995), to chlorpyrifos in dicofol selected two-spotted spider mite, *Tetranychus urticae* (Hatano *et al.*, 1992) and to chlorfenapyr in pyrethroid resistant *He. virescens* (Pimprale *et al.*, 1997) and *Ha. irritans* (Sheppard and Joyce, 1998). Similarly, enhanced activities of esterases in pyrethroid resistant *Hel. armigera* resulted in enhanced conversion of indoxacarb to more toxic metabolite DCJW (N-decarbomethoxylated JW 062) that resulted in the negative cross resistance (Wing *et al.*, 2000; Gunning and Devonshire, 2002). In addition to increased metabolic activation, structural changes in insecticide target sites accompanied with resistance to an insecticide may render the insect more sensitive to other insecticides that share the same target site in an opposing way. For example, the alteration in acetylcholinesterase resulting from selection with dimethoate conferred negative cross-resistance to propoxur in the olive fruit fly, *Bactrocera oleae* (Vontas *et al.*, 2001). Finally, allosteric effects at target sites may result in negative cross-resistance between two types of insecticides that share the same target, but different sites. For example, due to allosteric effects of sodium channels, negative cross-resistance to N-alkylamides (Elliott *et al.*, 1986) or dihydropyrazoles (Khambay *et al.*, 2001) is expressed in pyrethroid resistant *Musca domestica* (Table 12).

A more recent group of insecticides, oxadiazine compounds that contain indoxacarb for example, are also shown to be negatively correlated with the pyrethroid resistant insect pests (Yamamoto *et al.*, 1993). However, the phenomenon was not uniform across all insects resistant to pyrethroids and thus might be specific to insect and the mechanism of resistance. Pyrethroid resistant *M. domestica* was observed to be hyper sensitive to oxadiazine compound, indoxacarb (Shono *et al.*, 2004; Khambay *et al.*, 2001). *Helicoverpa armigera* populations from Australia resistant to pyrethroids (Gunning and Devonshire, 2002) and India (Ramasubramanian and Regupathy, 2004), were hyper

Table 12: Studies on the different level of negative cross resistance recorded in different resistant insect pests

Sl. No.	Resistant to Insecticide (1)	Group of insecticide (2)	Type of study Field /Lab (3)	Generation & Resistance level (4)	Mechanism of resistance (5)	Cross resistance - insecticides tested (6)	Cross resistance Level (fold) (7)	Reference (8)
1a	<i>Musca domestica</i>	Pyrethroids-Permethrin	Lab selected-LPR strain	5900 fold RR	Increased oxidative metabolism by Cyp P ₄₅₀ & <i>kdr</i>	Indoxacarb	1.3	Shono <i>et al.</i> , 2004
1b	<i>Musca domestica</i>	Multi resistant	YPER strain	>18,000 fold RR	<i>Super kdr</i> & monooxygenase	Indoxacarb	0.9	Shono <i>et al.</i> , 2004
1c	<i>Musca domestica</i>	Cyclodiene resistant	OCR strain	-	Altered GABA receptor	Indoxacarb	0.5	Shono <i>et al.</i> , 2004
2	<i>Musca domestica</i>	Resistant to pyrethroids	Homozygous <i>kdr</i> allele, <i>Super kdr</i> allele		<i>Kdr</i> resistant	Dihydropyrazole-Indoxacarb related compounds, Negative cross resistant to almost all insecticides	<i>Kdr super-kdr</i> 1. 0.6 2. 0.61 3. 1.4 4. 1.1 5. 0.88 6. 3.0 7. 0.66 8. 0.48 9. 0.86 0.04 – 0.5 fold of RR in different populations	Khambay <i>et al.</i> , 2001
3	<i>Haematobia irritans</i>	Pyrethroid, Permethrin	Field population	95.0 fold RR		Diazinon (OP)		Cilek and Knapp, 1993
4	<i>Helicoverpa armigera</i>	Pyrethroid	Field population - Australia	Resistant to pyrethroid	Esterase mediated resistance to pyrethroids	Indoxacarb	Negative cross resistant	Gunning and Devonshire, 2002

Contd... Table 12

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
5	Codling moth, <i>Cydia pomonella</i> , (Lepidoptera: Tortricidae)	OP compound-Azinphosmet hyl	2- lab selected colonies	R-LO strain - 3.56 fold; R-CL strain - 2.61 fold RR	AChE inhibitor	Insecticide - strain Chlorpyrifos Methyl parathion	R-LO ; R-CL (RR- fold) 0.10 0.42 0.26 0.18	Dunley and Welter, 2000
6	<i>Musca domestica</i>	OC & OP compound resistant	Bioresmethrin-Deltamethrin-DDT-	38 390 33	<i>Super kdr</i> -resistance	<i>N-alkylamides</i> - 7 compounds(a-g)	RR range 0.24 -0.64 fold	Elliott <i>et al.</i> , 1986
7	<i>Helicoverpa armigera</i> ,	Pyrethroid	Field collected pyrethroid resistant		Resistant due to increased esterase	Indoxacarb	RR range 0.42 - 0.63 fold to Ind	Ramasubramanian and Regupathy, 2004
8	<i>Helicoverpa armigera</i> ,	Pyrethroids - (Tamil nadu-India)- F14 gen. selection	Fenvalerate-Cypermethrin-Deltamethrin-	5 5 4	Increased MFOs and Carboxyl activity	Indoxacarb-Spinosad-	Fen : Cyp: Delt 0.5 : 0.5 : 0.5 0.5 : 0.5 : 1.0	Ramasubramanian and Regupathy, 2004
9	<i>Heliothis virescens</i> (Lepidoptera: Noctuidae)	OP & Pyrethroid selected	OP-R- Profenfos seld. F5 gen; PYR-R- Cypermethrin F4 gen.	18.1 19.6		Profenfos-Cypermethrin-Indoxacarb-Spinosyn A-	OP-R : PYR-R 18.1 : 4.53 12.9 : 19.6 15.8 : 5.57 2.19 : 2.99	Anonymous, 2006... Absence of -ve resistance Pyr seld.

Contd.... Table 12

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
10	<i>Haematobia irritans</i>	Pyrethroid, Permethrin	Field population	Resistant to pyrethroids	Enhance monooxygenase activity – Diazinon – converted to Diazoxon (More toxic metabolite)	Diazinon	Range RR- (0.1-0.7)	Cilek <i>et al.</i> , 1995
11	Olive fruit fly, <i>Bactocera oleae</i>	OP- Dimethoate	Lab selected & Field collected		AChE mechanism	Carbamate	(-) ve cross resistance	Vontas <i>et al.</i> , 2001
12	<i>Tetranychus urticae</i> (Acari: Tetranychidae)	Dicofol -OC	Lab selected		MFO's	Chlorpyrifos	Due to conversion of Chlorpyrifos to more toxic chlorpyrifos oxon	Hatano <i>et al.</i> , 1992
13	<i>Musca domestica</i>	Resistance to pyrethroids			Mechanism – (Super-kdr resistance)	RR<1fold, n-alkylamides	Negative Cross resistance to n-alkylamides	Khamby <i>et al.</i> , 2001
14	<i>Nephotettix cincticeps</i>	N- methylcarbamates	Field population		alteration of the acetylcholinesterase	N-propylcarbamates and Oxadiazone compounds		Yamamoto <i>et al.</i> , 1983; Yamamoto <i>et al.</i> , 1993

sensitive to indoxacarb, while *Heliothis virescens* resistant to pyrethroid did not show negative cross resistance to indoxacarb (Anonymous, 2006). Even in *Spodoptera frugiperda* and *P. xylostella* resistance to permethrin did not indicate a possibility of negative cross resistance to indoxacarb (Yu and McCord, 2007).

Pittenderigh *et al.* (2000) define negative cross resistance as potentially a bidirectional phenomenon, but all the studies compiled in the present study (Table 14) indicate all the examples, except one, to suggest a unidirectional phenomenon. The concept being of great value in the management of insect pests, it is important that the meaning of the negative cross resistance be correctly understood. A pertinent point to be understood therefore, is the concept of hyper sensitivity. It is of utmost importance to understand the meaning of this concept when negative cross resistance has to be characterised. Apparently, different examples cited here ascribe different meaning to the term ‘hyper-sensitivity’. A comparison on a common platform would facilitate better identification of the meaningfully negatively cross resistant pairs of insecticides.

2.8 Mechanisms of resistance and methods of detection

The most common types of resistance found in insects are increased enzymatic detoxification and target site insensitivity (Agosin, 1985; Oppenoorth, 1985; Scott, 1991). According to several authors (Wilkinson, 1983; Oppenoorth, 1985; Scott, 1991) increased enzymatic detoxification is the most commonly occurring resistance mechanism although altered target sites are also quite common. Monooxygenases-mediated resistance is probably the most frequent type of metabolism based insecticide resistance (Hodgson and Kulkarni, 1983; Oppenoorth, 1985; Brattsten *et al.*, 1986; Scott, 1991), although esterases are also important (Oppenoorth, 1985). Monooxygenases are also referred to as cytochrome P450s, or Mixed Function Oxidases (MFOs) and detoxification mediated by them has the potential to confer cross-resistance to toxins independent of their target sites (Wilkinson, 1983; Oppenoorth, 1985; Scott, 1991). Glutathione S-transferases are of high significance for detoxification of organophosphates (Yu, 1996). However, cytochrome P₄₅₀s are also responsible for the activation of many organophosphates (Hodgson *et al.*, 1991), which are currently the most widely used class of insecticides.

A point to note is that each of these terms referred to enzymes represent independent groups and in each class, different enzymes could potentially be responsible for the detoxification of different toxins (Table 13). Further, it is also likely that more than one mode of resistance might be operating in an insecticide resistant population. As a result, it is not uncommon to see higher activity of multiple groups of enzymes.

2.8.1 Based on synergists through Bioassay

The term "synergism" is used for cases where two compounds together show a higher activity than that predicted from the sum of their individual activities. Often one component is not toxic or far less active than the counterpart component at the dosage employed, but when combined with the latter markedly increases the activity and is called a "synergist" and hence can be defined as an agent that increases the effectiveness of another agent when combined with it (Crow, 1956). As related to insecticides, a synergist is used at higher doses (for example, 5-10 times more than the insecticide) in many cases and the costs justify their use in limited cases like natural pyrethrum. Synergists are used with the purpose of suppression of a defensive mechanism that would reduce the heterogeneity of a resistant population possessing this factor, thus making them similar to susceptible strain (Crow, 1956).

Synergists are specific in their action towards specific groups of enzymes and hence, their use along with the designated insecticides against resistant populations helps identify the group of enzyme involved in the detoxification of the insecticide. Some of the synergists which are specific against different enzymes are,

Esterase inhibitors: Triphenyl phosphate (TPP), S,S,S-tributyl phosphorotrithioate (TBPT it is also called as DEF) and 1,1-di-(4-chlorophenyl) ethanol (DMC).

MFOs : Piperonyl butoxide (PBO), Butacid 8EC and MGK-264 [2-(2-ethylexyl)- 3 α , 4, 7, 7 α -tetrahydro-4,7-methano-(1*H*)-dione]

Glutathione-S-transferases: Diethylmaleate (DEM)

However, these can also be used in recognizing the type of resistance mechanism involved through the bioassays. It is expressed commonly by taking the ratio of the LD₅₀ of an insecticide alone to the LD₅₀ of the insecticide with the synergist (Yamamoto, 1973; Hodgson and Tate, 1976; Casida and Quistad, 1995). If there is an increase in the

Table 13: Summary of different enzymes expected to be involved in the detoxification of different groups of insecticides
(Oppennoorth, 1985; Scott, 1991).

Mechanism	Organochlorine	Organophosphates	Carbmates	Pyrethroids	Miscellaneous
Mixed function oxidases					
(MFOs)	**	**	**	**	**
Hydrolases (Esterases)		**	**	**	**
Glutathion-S-transferases	**				
DDT-ase	**				
Reduced penetration	**	**		**	**

effectiveness of an insecticide when combined with the synergist, it indicates involvement of that particular enzyme in resistance mechanism. In *P. xylostella*, PBO is the commonly used synergist to test involvement of mixed function oxidases in detoxification (Chen *et al.*, 1993; Ismail and Wright, 1992) against different insecticide resistance. However, TPP, TBPT and DEF are also used in identifying increased activity of esterases and DEM for glutathione-S- transferase enzymes (Noppun *et al.*, 1987; Kao and Sun, 1991; Ismail and Wright, 1992; Table 14).

2.8.2 Biochemical method

Activity of enzymes can be measured through the differential rate of metabolism of the substrates. This method is useful in making more specific assertion of the role of monooxygenases, esterases and glutathione-S-transferases in the detoxification of the insecticide. The activities of the enzyme preparations from final instar larval midguts are verified with specific model substrates.

The important model substrates used in knowing monooxygenase (MFOs) activity are, *O*-demethylation substrates like, *p*-nitroanisole, 7-methoxyresorufin and 7-ethoxyresorufin, then one *O*-deethylation substrate, ethoxycoumarin and aldrin epoxidase activity through aldrin. Wherein, the *O*-demethoxylation or *O*-deethoxylation of the substrate is measured with the help of UV-spectrophotometer or fluoro-spectrophotometer to quantify the MFOs in resistant populations (Yang *et al.*, 2004). In DBM resistance to pyrethroids (Hung and Sun, 1989) and chitin synthesis inhibitors (Lin *et al.*, 1989) are mainly through the activity of MFOs.

Esterases are a family of enzymes that catalyze the hydrolysis of a variety of esters, peptides, amides and halides. In insects, esterases are the major enzymes involved in the detoxification of organophosphate and carbamate groups of insecticides (Oppenoorth, 1965; Plapp, 1976). Activity of the esterases in insects is measured in terms of the amount of conversion of the two substrates alpha-naphthyl acetate or beta-naphthyl acetate to respective alpha or beta- naphthol (Scharf *et al.*, 1997). *Plutella xylostella* selected to OP resistance have shown higher activity of esterases (Maa and Chaung, 1983; Doichuanngam and Thornhill, 1989; Table 14).

Table 14 : Studies on biochemical mechanisms involved in resistance to different insecticides in *Plutella xylostella*

Sl.No.	Resistant to insecticide (1)	Insecticide group (2)	Strain - Selected/ Field (3)	Resistance level (folds) (4)	Model substrate/ synergist (5)	Level of expression (fold) (6)	Remark (7)	Reference (8)
1	Fenvalerate	Pyrethroid	Field strains	300 to >11,000	Aldrin epoxidase, 7-Methoxyresorufin 7-Ethoxyresorufin 7-Ethoxycoumarin	<2 16 5 18	PBO good synergist and MFO are major detoxifying enzymes of fenvalerate	Hung and Sun, 1989
2	Teflubenzuron	Acyl urea compound	Lab and field	Field -31 Lab 1 sel-18 Lab 2 sel-36	Aldrin epoxidase, 7-Methoxyresorufin 7-Ethoxyresorufin 7-Ethoxycoumarin	3 7-28		Lin <i>et al.</i> , 1989
3	Malathion	OP	Lab selected - 8 generation	Resistant		Increased esterase activity		Doichuanngam and Thornhill, 1989
4	Parathion,	OP	Lab selection	>2600	7-Methoxyresorufin DCNB	Increased 7-Methoxyresorufin activity	Increase of microsomal oxidase activity	Kao <i>et al.</i> , 1989
5	Methyl parathion	OP	Lab selection	>1000	7-Methoxyresorufin DCNB	Increased DCNB activity	Increased glutathione-S-transferase activity	Kao <i>et al.</i> , 1989

Contd....Table 14

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
6	Chlorfluazuron	Acyl urea compound	Lab selection	109	Synergist, PBO, DEF before treatment	34 fold increased toxicity	MFO & esterase involved in resistance	Ismail and Wright, 1992
7	Teflubenzuron	Acyl urea compound	Lab selection	315	Synergist, PBO, DEF before treatment	28 fold increased toxicity		Ismail and Wright, 1992
8	Dimehypo, Cartap	OP Tertiary amine	Lab selection - 35 generation	51 25	Synergist, PBO	6.28 4.85fold synergism	MFOs major mech. R to Dimehypo & cartap	Chen <i>et al.</i> , 1993
Other insects								
Sl.No.	Insect	Resistant to insecticide	Strain - Selected/ Field	Resistance level (folds)	Model substrate	Level of expression	Remark	Reference
1	<i>Musca domestica</i>	Indoxacarb	Oxidiazine-3 gen.	>118	PBO-Diehy maleate-TEP	7.7 fold of synergism with PBO	P450 MFOs - mech. Of resistance to Ind in H.fly	Shono <i>et al.</i> , 2004
2	Sweet potato white fly, <i>Bemisia tabaci</i> (Aleyrodidae: Homoptera)	Methidathion (OP)	Lab selection-8 generation	8.6	β -NA	Increased- β -NA activity	Mechanism of resistance, Fitness	Bloch and Wool, 1994

PBO- Piperonyl butoxide; β -NA- β -Naphthyl acetate; TEP- S,S,S- tributyl phosphorothioate; DCNB-Dichloro nitrobenzene

Esterases either act due to change in the form or by increased production due to gene duplication, which can be analysed through isozyme assays. Isozymes are the multiple molecular forms of the given enzyme occurring in membranes of the same species catalyzing the same physiological reaction. These are identified through electrophoresis on polyacrylamide gels wherein isozymes are resolved to get a zymogram (Biochem book). Resistance to malathion in ST strain of DBM was associated with the presence of Est8b and Est 9b esterases while the presence of Est 4b band was unique to susceptible strain (Maa *et al.*, 1992; Maa and Liao, 2000).

Glutathione S-transferases (GST) are the enzymes involved in the transfer of a chemical group from one molecule to another. In insects glutathione-S-transferases are mainly involved in metabolic detoxification of the OP insecticides (Plapp, 1976) and also to some extent indoxacarb (Ahmad and Hollingworth, 2004). Glutathione S-transferase activity is quantified in terms of the amount of conjugation of the substrate to glutathione. The most commonly used substrates are 1-chloro-2,4-dinitrobenzene (CDNB) and 3,4-dichloronitrobenzene (DCNB) (Kao *et al.*, 1989). However, *p*-nitrophenyl acetate (PNPA) can potentially be used as a substrate to know the activity of both esterases and glutathione-S-transferases (Scharf *et al.*, 1997). Increased activity of GST towards DCNB was observed in *P. xylostella* resistant to methyl parathion and parathion (Kao *et al.*, 1989).

2.9 Review of literature specific to the insect and the insecticides used in the study

2.9.1 Diamondback Moth, *Plutella xylostella* L.

Cabbage is an important vegetable cash crop for many farmers around cities in south India and occupies four per cent of the area of all vegetables grown in the country. Due to introduction of heat tolerant varieties, it is now grown throughout the year without any clear cut sowing season. India is one among the leading producers of cabbage standing next only to China in the over all production of head cabbages (Chadha, 2001).

Among the lepidopteran insect pests, *Crociodolomia binotalis*, *Hellula undalis* and *Spodoptera litura*, and diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera : Plutellidae) are the major insect pests. DBM is believed to have originated in Mediterranean area (Harcourt, 1954), and is now found all over the world where ever crucifers are grown (Tsunoda, 1980). The larvae feed on all stages of the crop and feeding results in development of holes on the leaves, which enlarge as the plant grows. Larvae

might attack and nibble at the growing primordia. Severe damage to primordia causes complete loss of the crop with an expected threshold levels of 4 to 7 larvae per plant (Srinivasan and Veeresh, 1986). The larval incidence has been shown to be of importance in greatly reducing the crop yield parameters such as number of harvestable heads, head diameter, head weight and ultimately the yield (Chellaiah and Srinivasan, 1986, Jayadevi, 2002).

DBM is of particular concern as a serious pest of crucifers as the insect is extremely difficult to manage. Its prolific breeding abilities, capacity to adopt to variable environmental conditions, short life cycle (Chellaiah and Srinivasan, 1986) and absence of effective natural enemies (Lim, 1986) are believed to be the factors responsible for the insect to become a number one enemy of cabbage farmers. In India, farmers completely depend on insecticides to manage DBM (Kamala, 2006). Tropical countries where crucifers are grown through out the year, DBM completes nearly 20 generations per year, and the sole reliance on insecticides for control facilitates the rapid build up of resistance (Talekar and Shelton, 1993). To overcome the resistance, farmers often increase doses of insecticides, use mixtures of several chemicals and spray up to 25 times within a cropping season of less than three months (Khan *et al.*, 1991, Kamala, 2006). These high levels of use has caused it to become resistant to almost all insecticides in use and on an average, a new insecticide is expected to have a utility life of just two to three years in cabbage ecosystem (Rushpakornchai and Vattanatangum, 1986; Pernag *et al.*, 1988; Zoebelein, 1990; Talekar and Shelton, 1993). DBM has developed resistance to as many as 69 insecticides which is the maximum for any insect pest (Bills *et al.*, 2004). It has developed resistance to every group of insecticides, organochlorines (Zoebelein, 1990), organophosphates (Baker and Kovaliski, 1999), Carbamates (Zoebelein, 1990), pyrethroids (Kim *et al.*, 1991), cartap (Mohan and Gujar, 2003), phenyl pyrazole (Sayyed and Wright, 2004), spinosad, indoxacarb (Sayyed and Wright, 2004) and IGRs (Cheng *et al.*, 1990). It is the only insect which has developed resistance to *Bt* sprays under field condition (Tabashnik *et al.*, 1990; Ramirez *et al.*, 1995). DBM is potentially capable of developing resistance even to neem (Vollinger, 1995).

2.9.2 Cypermethrin

The present day pyrethroids are compounds based on natural pyrethrums from *Chrysanthemum roseum* Adam, *C. carneum* Steud. and *Pyrethrum cinerariefolium* Vis.

However, synthetic pyrethroids were mainly developed to improve photo-stability and increased insecticidal activity while their mammalian toxicity is reduced (Davis, 1985). Synthesis of synthetic pyrethroids started as early as 1924, immediately after the discovery of active chemical in *C. cinerariifolium*, the pyrethrum (Staudinger and Ruzicka, 1924). However, only by 1949 allethrin, the first synthetic pyrethroid was introduced (Schechter *et al.*, 1949) which was stable and of longer residual activity. Allethrin, prothrin and natural pyrethrin-I are used in mosquito coils because of their high volatile nature (Elliott, 1977). These constitute the first generation of pyrethroids. Next was the second generation comprising of dimethrin (1961), tetramethrin (1965), resmethrin, bioresmethrin (1969) and bio-allethrin (1965). However, they were not stable under sunlight, hence are not used in agricultural pest management in open fields but were used in the management of insect pests in glasshouse conditions. Then most photo-stable, third generation pyrethroids were synthesized and fenopropathrin (1971) was commercialized as an acaricide by 1980. During this period many more *viz.*, permethrin, cypermethrin, deltamethrin and fenvalerate were introduced. These were highly photo-stable and are currently widely used in the management of agricultural insect pests under open field conditions. From 1975- 1983 fourth generation pyrethroids, cypothrin (Tick control), flucythrinate (broad spectrum, phytophagous mites), cyfluthrin (Cotton pests) flumethrin, cyhalothrin *etc.* were introduced and many are in their developmental stages (Perry *et al.*, 1998).

These pyrethroids are more volatile and act as contact insecticides, and act on voltage gated Na^+ channel in the nerve membrane. The molecules bind to these Na^+ channels and keep them open for long periods than required resulting in hyper-excitation leading to convulsions, paralysis and death of the treated insect (Tatebayashi and Narahashi, 1994). Insects have developed resistance to pyrethroids either due to *kdr* mechanism of resistance or metabolic detoxification of insecticide by MFOs (Oppernoorth and Welling, 1976) or esterases (Ishaaya and Casida, 1980; Ishaaya *et al.*, 1983).

In India, DBM showed tolerance to the pyrethroids as early as 1982 (Awate *et al.*, 1982), within about 2-3 years of their introduction to the market. The resistance level of 2.4 (Li *et al.*, 1998) to 26507.81 fold (Sannaveerappanavar, 1995) have been recorded against cypermethrin. There is very high variation between the strains of DBM in their susceptibility even to deltamethrin (Branco and Gatehouse, 1997). By 1990, 48 species

(9.5%) of insect pests were resistant to pyrethroids (Georghiou, 1990) and the number rose to 53 (10%) species by 2004 (Bills *et al.*, 2004).

Resistance to DDT confers positive cross resistance to pyrethroid insecticides, in general (Perry *et al.*, 1998). Resistance to pyrethroids with *kdr* mechanism of resistance were known to confer negative cross resistance to N-alkylamides (Elliott *et al.*, 1986) or dihydropyrazoles (Khambay *et al.*, 2001) in *M. domestica*. Negative cross resistance is also due to enhanced activities of esterases in pyrethroid resistant *Hel. armigera* that resulted in the enhanced conversion of indoxacarb to more toxic metabolite DCJW (N-decarbomethoxylated JW 062) and resulted in negative cross resistance (Wing *et al.*, 2000; Gunning and Devonshire, 2002). However, *He. virescens* resistant to pyrethroids did not show negative cross resistance to indoxacarb (Anonymous, 2006). Even in *Spodoptera frugiperda* and *P. xylostella* resistance to permethrin did not show negative cross resistance to indoxacarb (Yu and McCord, 2007).

2.9.3 Indoxacarb

Indoxacarb (DPX-MP062) is a recently introduced foliar insecticide belonging to oxadiazine group. The compound was discovered in 1992 by the E.I. DuPont Co. (McCann *et al.*, 1992; Lohm *et al.*, 1999). The compound is mainly effective against lepidopteran insect pests, *Helicoverpa sp.*, *Spodoptera sp.*, *P. xylostella*, *Trichoplusia sp.*, *etc.*, to some sucking pests *Lygus lineolaris* and *Empoasca sp.* and beetle *Leptinotarsa decemlineata* (Harder *et al.*, 1996; Wing *et al.*, 2000; Jayadevi, 2002; Liu *et al.*, 2003). In insects, indoxacarb appears to be decarbomethoxylated to DCJW by an esterase/amidase (Wing *et al.*, 1998). DCJW is more effective in blocking sodium channels (Tsurubuchi and Kono, 2003; Tsurubuchi *et al.*, 2001) than indoxacarb (Wing *et al.*, 2000; Lapied *et al.*, 2001; Tsurubuchi *et al.*, 2001). However, indoxacarb and DCJW have also been shown to affect mammalian nicotinic acetylcholine receptors (Zhao *et al.*, 1999) and have a weak effect on mammalian GABA receptors (Narahashi, 2002).

Indoxacarb was the most effective insecticide in the management of DBM in cabbage ecosystem (Jayadevi, 2002; Liu *et al.*, 2003) during early part of this decade. It was also recommended in place of other conventional insecticides (Mohan and Gujar, 2003). DBM populations from different locations also varied in their susceptibility to indoxacarb (Joia *et al.*, 2003). However, field populations of DBM from Malaysia,

Cameroon islands, quickly recorded very high levels of resistance to indoxacarb, 330 fold (Sayyed and Wright, 2004).

Selection with indoxacarb in *M. domestica* developed resistance of >118 fold in three generations. Thus, it indicates the possibility of insects developing resistance to indoxacarb at an alarming rate. However, the oxadiazines are expected to give good control of the insect pests, as insect pests which are resistant to pyrethroids are expected to exhibit negative cross resistance to indoxacarb (Khambay *et al.*, 2001; Wing *et al.*, 2000; Gunning and Devonshire, 2002). But, further strong evidences to this effect are lacking and are believed to be due to the higher rates of detoxification of the insecticides relative to their rate of activation by esterases (Huang, 2002).

2.9.4 Neem Seed Kernel Extract (NSKE)

Neem (*Azadirachta indica* A. Juss.; Meliaceae) is one of the most important botanical insecticide used in insect pest management from time immemorial. Neem has a wide variety of actions on the biology of insects. The biological effects of neem on insects are multitudinal and include repellence (Singh, 1985), feeding deterrence, ovipositional deterrence (Saxena *et al.*, 1981), mating disruption (Saxena *et al.*, 1993), insect growth regulatory effect on metamorphosis (Jagannadh and Nair, 1992), reduced fecundity and viability of eggs (Vollinger, 1987). It is expected that a combined impact of all these effects (Singh and Singh, 1987) would greatly reduce the population in subsequent generations on crop plants.

Neem is a rich collection of biologically active chemicals, consisting of tri-, di-, and monoterpenoids (amoorastatins, vipinins, meliacins, salannins, *etc.*), meliacarpins, flavonoids, dihydrochalcones, tannins, *etc.* Some of the most active compounds against insects found in neem are, azadirachtin A, azadirachtin B, nimbolinin, salannin, meliontriol, *etc.* (Kraus, 1995). Azadirachtin A itself is expected to act on insects in a wide variety of ways causing both behavioural and physiological effects on the insect. Biological activity of at least 50 of these compounds on insects has been documented. Hence, neem with its multiple compounds and multiple modes of physiological and behavioural actions is expected to have a strong impact on the biology of the target insects and as a result may have a 'built- in resistance prevention (BIRP)' mechanism (Rice, 1993). This was also supported by the fact that *P. xylostella* and *Aphis fabae* (Green

peach aphid) failed to develop resistance over 35 and 40 generations, respectively, of selection for resistance against neem (Vollinger, 1995; Feng and Isman, 1995). Further, studies by Verkerk and Wright (1993) suggested crude extract of neem to be superior to pure azadirachtin as an insecticide. This was also substantiated by the fact that the specific activity of the azadirachtin decreased with increasing concentration of azadirachtin in the commercial neem formulations (Kumar *et al.*, 2003). As a corollary, crude extracts of neem provided excellent control of insect pests in field (Schmutterer, 1992; Srinivasan and Krishnamoorthy, 1993; Sannaveerappanavar, 1995; Jayadevi, 2002). Hence, neem seed kernel extract (NSKE) at 4 % by w/v is widely recommended as the best management strategy for the lepidopteran pests of vegetable cabbage in India (Srinivasan and Krishnamoorthy, 1993; Jayadevi *et al.*, 2003b). Methodology for the preparation of high quality NSKE was also standardized and recommended by UAS, Bangalore (Jayappa, 2000; Anonymous, 2000). In India, however, there have been no attempts to evaluate the possibility of any insect developing resistance to crude extracts of neem.

2.9.5 Neemazal-F

Azadirachtin (=azadirachtin-A) is the major chemical component responsible for insecticidal property (Schmutterer, 1995; Kraus, 1995) of neem seeds, both by its activity against insects and also by its relative content in the seeds (Rembold, 1996). Consequently, many commercial neem formulations (CNFs) based on neem are currently available in the market in India and can serve as potential replacements for difficult to prepare NSKE (Ermel and Kleeberg, 1995; Gahukar, 1996; Gopinathan, 2002). However, the concentration of the active ingredient, azadirachtin, varies greatly in these products ranging from 300 to 50,000 ppm. Neemazal- F is a highly concentrated formulation of azadirachtin available in India and is also one of the CNFs readily available as an alternative to crude extract of neem (Gopinathan, 2002; Kleeberg, 2002). The product is now widely recommended for pest management in a wide variety of crops and insect pests. It was also found quite effective for the management of DBM and other Lepidoptera in cabbage (Jayadevi, 2002).

Summary of Review

Insecticidal resistance is a vast subject that has held the attraction of all economic entomologists since the first report of its occurrence in 1908. As a result, the present

review is not an exhaustive review of the subject. Nevertheless, an attempt has been made to compile the salient factors such as tracing the historical development of the subject, theoretical bases of resistance including genetics, mechanisms of resistance, rates of development and loss of resistance besides considering the methodologies of study.

In summary, the review suggested the following :

1. It appears that the number of insects becoming resistant to insecticides is reaching a plateau.
2. More number of insect species are resistant to OPs, which has the highest number and amount of insecticides among all chemical groups.
3. Populations from different geographical area vary in their response to different insecticides and each population acts as a potential "Genetic Island".
4. There is no relation between number of generations selected and the level of resistance achieved in different insecticides.
5. Insecticidal resistance increases as an exponential function under laboratory conditions. As a corollary, exponential change in LC_{50} values should indicate the response to selection.
6. The shape of movement of the slope of probit regression lines for populations under selection need not be 'U' shaped.
7. Mode of inheritance, autosomal or sex linked inheritance influences the rate of resistance development. Sex linked inheritance grows ten times slower compared to autosomal inheritance.
8. Degree of dominance of resistance influences the rate and stability of resistance. If resistance is recessive and once the population attains homozygosity, the resistance remains stable. Hence refuges become extremely important for IRM strategies.
9. Polygenic trait is expected to spread at a greater rate in a population under selection relative to a monogenic trait.
10. From 31 studies compiled, it was found that resistance alleles could be either recessive or dominant and both are equally probable. Similarly, it is equally probable that the resistance is either monogenic or polygenic.
11. Heritability (h^2) and response to selection (R) are high at initial generations of selection when the variation in the population is high. However, these decrease as populations move towards homozygosity on selection.

12. As number of generations required for ten fold increase in resistance or LC_{50} (G) is inversely proportional to heritability (h^2), 'G' increases as h^2 decreases
13. Resistance to insecticide in insects is almost always associated with fitness costs.
14. Stability of insecticidal resistance is determined by nature of the resistant allele, number of loci, extent of homozygosity achieved by the selected population and fitness parameters of resistant populations.
15. Although negative cross resistance is envisaged as a bidirectional phenomenon by some authors, most studies tend to show only unidirectional situations.
16. There was lack of clarity in the description of oversensitivity as related to negative cross resistance.

Keeping these points in mind, it will be of interest to know how these various factors influence an insect being selected for resistance to neem. Neem is unique in having an array of active ingredients affecting insect biology, behavior and physiology. However, the most active component of neem, azadirachtin alone also demonstrates almost similar impacts on insects. Hence, it would be pertinent to reexamine the rates of evolution of resistance to neem and formulations based on azadirachtin in comparison to a synthetic insecticide. The three it is expected would impose entirely different selection intensities on the insect leading to differing ramifications. Therefore, the present study is planned to examine this issue using DBM as the model system. DBM is one of the cosmopolitan insect pests known to occur all over the world where ever crucifers are grown. To its credit, it is resistant to maximum number of insecticides (69) among all insect pests in the world. Ease of culturing adds to these advantages.

Further, to the best of our knowledge, there are no studies on development of resistance to DBM using indoxacarb and cypermethrin. Therefore, indoxacarb and cypermethrin representing synthetic insecticides having single modes of action, Neeamazal-F as proxy to azadirachtin, having a single chemical with multiple modes of action and crude extract of neem, NSKE, representing multiple compounds having multiple modes of action were used to see the response to selection in the model insect, DBM.

III. MATERIAL AND METHODS

III. MATERIAL AND METHODS

Material used and methods employed during the selection for resistance to indoxacarb, cypermethrin, Neemazal-F and NSKE in *Plutella xylostella* are described briefly. Further, procedures involved in biochemical estimation of enzymes, studies on genetic mechanism of insecticidal resistance and also life table parameters have been described. All the laboratory experiments were carried out at the Department of Entomology and in collaboration with other departments at UAS, GKVK, Bangalore that include, School of Ecology and Conservation, Crop Physiology. Some of the facilities available at the Project Directorate -Biological Control, Hebbal, Bangalore were also used.

3.1 Test Insect

Diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Plutellidae) was used as the test insect to understand the evolution of insecticidal resistance to selected insecticides. Mechanism of resistance, genetics and effect of selection on life history parameters were also studied to understand the possible reasons for the development of resistance.

Mass rearing of the test insect

3.1.1 Laboratory strain (JJ-Lab) of DBM

A lab strain, JJ-Lab that had completed more than 100 generations in the laboratory was used as the susceptible strain for almost all comparative studies. This strain, initiated by Jayappa (2000) was collected from cabbage fields in and around Bangalore during September, 1997, and is being maintained in the laboratory on mustard seedlings adopting the method of Liu and Sun (1984) with suitable modifications.

Bold seeded mustard (*Brassica juncea*) seedlings were raised in plastic petri dishes (10 x 1.5 cm). The petri dishes were filled with vermiculite to a depth of one cm. Bold type mustard seeds were then sown evenly over the entire surface. Watering was done slowly over the surface as and when required. The seeds germinated in about two to three days at room temperature. The petri plates were maintained in sufficient light condition to facilitate good growth of seedlings. Seedlings of 3 to 4 cm length were then used for rearing DBM.

The larvae were reared on the mustard seedlings raised in petri dishes in the laboratory to pupal stage. After pupation, the pupae were placed in the ovipositional cage (35 x 10 x 35 cm) that had a glass top with wire mesh on the three vertical sides. After the emergence of moths, one day old moths were provided with three to four day old seedlings for oviposition. After every 24 h seedlings with eggs were taken out and labeled with date of oviposition and transferred to rearing trays. Fresh seedlings were given daily for oviposition up to five days from the day of emergence of the adults. Ten per cent honey or sugar solution on cotton wads served as the food for adult moths.

In the rearing trays, eggs hatched in about three to four days and the young larvae feed on the leaves, first by mining and then on the entire leaves. When all the eggs hatched, population would be high and the seedlings had been entirely consumed, fresh seedlings were placed in the rearing tray. The larvae migrated to the fresh seedlings by themselves or were transferred by gently tapping the consumed seedlings on to a sheet of paper and then on to the fresh seedlings using a camel hair brush. Fresh seedlings were provided as and when required. When the larvae are in the third instar well grown mustard leaves wrapped with moist cotton to maintain the turgidity of leaves were provided in the plastic tray (45 x 25x 10 cm). The leaves are grown in the green house on raised seedbeds. Whenever the problem of white rust, *Albugo candida*, was noticed, a fungicide spray of DM-45 at the rate of 2 g per liter of water was given (Ashoka, 2001). Spraying of fungicide was deliberately avoided one week prior to laboratory experiments *i.e.* bioassays. Fresh leaves were carefully examined before providing to the larvae to ascertain freedom from other insect stages. When the larvae were in the fourth instar, rough folded papers were placed in the rearing tray all around the petri dishes, or all around the plastic tray, to facilitate pupation. The larvae pupated in the paper folds, on the rim of the plastic trays, on the seedlings or on the outer surface of the petri dishes. The pupae were collected daily with the help of forceps and were placed in the oviposition cage for the emergence of adults. The pupae were also stored in the refrigerator for few days to synchronise the moth emergence, whenever large number of larvae of uniform age group were required for bioassays. These pupae could be stored at least up to seventeen days with out significantly affecting the fecundity and the emergence pattern (Jayappa, 2000).

The rearing was done at room temperature with periodical disinfection of rearing material using formaldehyde to prevent fungal and bacterial infection to the seedlings and the larvae. All necessary precautions were taken to keep away the ants, spiders and lizards, which would feed on the larvae and pupae.

3.1.2 Field strains

Field strains were started by collecting fourth instar larvae and pupae from the cabbage and cauliflower (Table 15) fields of five different taluks around Bangalore. The five field strains were collected from Devanahalli, Doddaballapura, Hoskote, Malur and Shidlaghatta taluks from around Bangalore. In all these five taluks, cabbage and cauliflower are grown intensively and DBM is the major insect pest. Therefore, most of the cost involved in the production goes for the management of this insect pest. All the five taluks (Plate 1) are located within a radius of 50 km around Bangalore and are separated by at least 20 km from one another. At the time of insect collection, information on stage of the crop and insecticides used for crop protection were also collected (January, 2005; Table 15). Pupae were sorted and stored in refrigerator until all the final instar larvae also pupated. Later all the pupae were removed and placed in room condition ($25 \pm 2^{\circ}\text{C}$), so that the adult emergence is synchronized and sufficient number of larvae were available for bio-assays in F_1 generation. Strains from different taluks were maintained separately. The second instar larvae of F_1 progeny were used for all bio-assays. Five selected insecticides (Table 16) were assayed in F_1 generation for ascertaining the susceptibility of the five field populations. The susceptibility to different insecticides of the five field strains were compared with that of the laboratory strain, JJ-Lab.

3.1.3 Mustard and Radish leaves

Bold seeded mustard variety seeds were sown evenly with appropriate spacing on raised seedbeds (180 x 120 x 10 cm) in green house condition. Need based watering and manuring were done. By strict vigilance, leaf webber, mustard sawfly and other pests were removed by hand picking and destroyed. Fungal diseases on mustard leaves were managed by using DM-45. Suitable mature leaves from approximately 30 – 35 day old plants were used for all the bioassays.

However, after 10 generations of rearing and selection of the field collected strains, mustard could not be maintained in sufficient quantity in the green house condition.

Table 15 : Details of cultures of Diamondback moth, *Plutella xylostella* collected from around Bangalore during January 2005 and used for the study.

Locality/ Strain	Source Crop	Stage of the crop	Date of collection	No. of sprays given	Chemical
LAB (susceptible)	Cabbage		August, 1997	Maintained on mustard seedlings without exposure to any chemical	>110 generation old
Hoskote	Cauliflower	Harvesting	January, 2005	No data	
Doddaballapura	Cauliflower	Harvesting	January, 2005	7	Monocrotophos, Doom, Spinosad (Success), Rocket – (Cypermethrin+ Profenophos), Acephate (Staphane)
Malur	Cauliflower	Curd formation stage	January, 2005	3	Chop-(Chlorpyrifos+ Cypermethrin), Phenthoate (Krin), Kiran
Devanahalli	Cabbage	Harvesting	January, 2005	8	Spinosad, Rocket – (Cypermethrin+ Profenophos)
Shidlaghatta	Cauliflower	Harvesting	January, 2005	5	Spinosad (Success)-4 times, Methomyl(Lannate)

Table 16: Insecticides used for testing the initial susceptibility for cross resistance studies on *Plutella xylostella* (2nd instar) collected from different locations around Bangalore during 2005.

Sl. No.	Insecticide	Trade Name	Conc. of a.i	Chemical group	Manufacturer
1	Indoxacarb	Avaunt TM	14.5SC	Oxadiazine	E.I. DuPont India Pvt. Ltd 8 th Floor, DLF Plaza Tower, DLF Qutab Enclave, Phase-I, Gurgaon-122 002, Haryana
2	Cypermethrin	Cymbush TM	25EC	Pyrethroid	Syngenta crop protection, Private Ltd. 14, J.Tata road, Mumbai-400 020
3	Spinosad	Success TM	2.5SC	Microbial insecticide	DE-NOCIL crop protection Pvt. Ltd, Unit No.1, First Floor, Corporate park, V.N. Purav Marg, Chembur, Mumbai-400 071
4	Azadirachtin	Neeamazal-F TM	50000 ppm	Commercial neem formulation	EID Parry India Ltd, Neem division, Thiyyagavalli factory, Coimatore, Tamil Nadu
5	Neem Seed Kernel Extract (NSKE)	-	-	-	Prepared afresh every time using the seeds collected from Botanical garden of UAS, GKVK, Bangalore
6	Endosulfan	Endoin [®]	35EC	Organochlorine	Insecticides (India) Ltd. E-443-444, RIICO industrial area, Chopanki (Bhiwadi) – 301 707, Rajasthan
7	Chlorpyrifos	Lethal TC TM	20EC	Organophosphate	Insecticides (India) Ltd. E-443-444, RIICO industrial area, Chopanki (Bhiwadi) – 301 707, Rajasthan
8	Carbaryl	Sevin [®]	50WDP	Carbamate	Bayer crop science India Ltd, Regd. Office, Aventis house, 54/A, M.V. road, Chakala, Andheri (E), Mumbai- 400 093
9	Imidacloprid	Atom [®]	17.8SL	Neonicotinoid	Indofil chemicals company, Nirlon house, DR. ANNIE Besant Rd, Worli, Mumbai-400 030
10	Novaluron	Rimon [®]	10EC	Chitin synthesis inhibitor	Indofil chemicals company, Nirlon house, DR. ANNIE Besant Rd, Worli, Mumbai-400 030

Therefore, mustard leaves were replaced by radish (*Raphanus sativus*) leaves for all selection treatments. Radish was also grown following the same procedure as that of mustard plants. Leaves from 30-35 day old plants were used for selection and further rearing of the resistant strains from III instar onwards.

3.2 Insecticides used

3.2.1 Spinosad

Spinosad is a mixture of the two most active naturally occurring metabolites (spinosyns A and D) produced by fermentation of the soil actinomycetes, *Saccharopolyspora spinosa* (Kirst *et al.*, 1992; Thompson *et al.*, 1997). The mode of action of spinosad is characterized by excitation of the insect nervous system, leading to involuntary muscle contractions, prostration with tremors, and paralysis due to activation of nicotinic acetylcholine receptors. Additionally it also acts on GABA mediated nervous system (Salgado *et al.*, 1997; Thompson *et al.*, 1997). It is commercially available as Success[™] for the management of DBM in cabbage and cauliflower and Tracer[™] for the management of *Helicoverpa armigera* in several field crops.

Spinosad was one of the most commonly used insecticides for the management of *P. xylostella* during early 2005 when the test insects were collected (Table 15; Kamala, 2006). Therefore, spinosad was selected as one of the chemicals to understand the susceptibility levels of the five field collected populations and also for understanding the potential cross resistance levels in insecticide selected *P. xylostella*.

3.2.2 Indoxacarb

Indoxacarb, (S) – methyl – 7 – chloro – 2,5 – dihydro – 2 (methoxycarbonyl) (4 – (trifluoromethoxy) phenyl) amino) carbonyl) – indeno (1,2-e) (1,3,4) oxadiazine – 4a (3H) – carboxylate, belongs to oxadiazine group and works as a sodium channel blocker. It is expected that once an insect ingests indoxacarb, it gets bio-activated to more toxic metabolite DCJW (N- decarbomethoxylated JW62). DCJW is a highly potent voltage dependent blocker of Sodium (Na⁺)-dependent compound action potential than indoxacarb itself (Wing *et al.*, 2000).

It is produced by DuPont, and marketed as Avaunt™ and formulated as a 14.5 % a.i. suspension concentrate (SC), a liquid formulation, for use on cotton and many vegetables including the crucifers. This was introduced mainly targeting the lepidopteran insect pests. The chemical was found very effective during the early 2002 (Jayadevi, 2002) and had cornered the lion's share of the insecticide market associated with DBM around Bangalore (Kamala, 2006) in cabbage and cauliflower ecosystem. The profligate use of the chemical led to its quick replacement by other alternatives such as Spinosad, Eamectin benzoate and Novaluron and by the end of 2004 it was in the declining phase of its use in crucifers in the State of Karnataka. Hence in order to understand the potential reasons for its quick decline in the usage, the chemical was selected for resistance development studies.

3.2.3 Cypermethrin 25 EC

Pyrethroids show extremely rapid knockdown effect on insects. Cypermethrin is a synthetic pyrethroid that acts on voltage gated Na^+ channel in the nerve membrane. The molecules bind to these Na^+ channels and keep them open for long periods than required resulting in hyper excitation, convulsion, paralysis and death of the treated insect.

It is both a stomach and a contact insecticide. It is very effective against a wide variety of crop pests and is the most widely used pyrethroid against crop pests in the country. However, because of its mode of action, as also other pyrethroids available in the market, it is anticipated that the cypermethrin could be a potential candidate for a negative cross resistance with indoxacarb. Hence being the most widely used pyrethroid, the insecticide was selected for resistance development studies with a view to understand the potency of development of negative cross resistance to indoxacarb.

3.2.4 Neem Seed Kernel Extract (NSKE)

Crude extracts of neem provides excellent control of insect pests in field (Schmutterer, 1992; Srinivasan and Krishnamoorthy, 1993; Sannaveerappanavar, 1995; Jayadevi, 2002). Hence, neem seed kernel extract (NSKE) at 4 % by w/v is widely recommended as the best management strategy for the lepidopteran pests of vegetable cabbage in India (Srinivasan and Krishnamoorthy, 1993; Jayadevi *et al.*, 2003). Methodology for the preparation of high quality NSKE was also standardized and

recommended by UAS, Bangalore (Anonymous, 2000; Jayappa, 2000). Therefore in order to verify susceptibility of DBM to a botanical insecticide and also to understand the possible strength of NSKE in avoiding the development of resistance in insects, it was used for selecting the resistant strains of *P. xylostella*.

Neem seed kernel extract (NSKE) was prepared afresh at the time of each bioassay and selection. Seeds were collected from neem trees in Botanical Garden, UAS, GKVK, Bangalore. From among the 15 labeled trees (Jayappa, 2000; Kumar *et al.*, 2001), seeds of four trees, viz., 1, 6, 7 and 14 were collected during July and August of 2004- 2006. The seeds were washed, cleaned, shade dried and treated with Dithane -M-45 to prevent fungus development and stored in plastic boxes separately under room condition. Fresh seeds collected in a year were used until the next stock of fresh seeds became available, as seeds are known to lose their insecticidal property during storage (Jayappa, 2000; Ashoka, 2001; Ashoka and Kumar, 2002). For the bioassays, equal numbers of seeds from all the four trees were mixed, shelled, and the kernels used for preparation of NSKE. The kernels were ground to a fine powder, and soaked in water in a ratio of 1 : 6 (wt to volume) overnight. The resultant solution was then filtered using multilayered fine muslin cloth. The volume was made up after repeated washings of the soaked powder to get the required per cent aqueous extract of the neem kernels by wt. to volume.

3.2.5 Neemazal-F

Azadirachtin (=Azadirachtin-A) is the major chemical component of the neem seeds that is responsible for insecticidal property (Schmutterer, 1995; Kraus, 1995) of neem, both by its activity against insects and also by its relative content in the seeds (Rembold, 1996). Consequently, many commercial neem formulations (CNFs) based on neem are currently available in the market in India and can serve as potential replacements for NSKE (Ermel and Kleeberg, 1995; Gahukar, 1996; Gopinathan, 2002). However, the concentration of the active ingredient, azadirachtin, varies greatly in these products ranging from 300 to 50,000 ppm. Neemazal- F is a highly concentrated formulation of azadirachtin available in India and is also one of the CNFs readily available as an alternative to crude extract of neem (Gopinathan, 2002; Kleeberg, 2002). The product is now widely recommended for pest management in a wide variety of crops and insect pests. It was also found quite effective for the management of DBM in cabbage (Jayadevi,

2002). Hence, Neemazal-F, was used for testing the susceptibility of the DBM larvae from around Bangalore.

Azadirachtin is expected to have a wide range of activity against insects. Thus unlike the synthetic insecticides, the chemical becomes an example of a situation where in a single compound will have multiple effects on the insects. As a result, if used for resistance selection studies, would provide a different and higher degree of selection intensity than the single molecule based synthetic insecticidal products, where the rule would be one chemical and one mode of action. Neemazal-F is a highly concentrated azadirachtin (5 %) based formulation available in the market. Major active molecule in the product is azadirachtin. Other neem molecules are in a relatively lower concentration than Azadirachtin – A. Therefore, in the absence of pure azadirachtin, the concentrated azadirachtin based product, Neemazal-F was used for resistance selection studies in DBM.

3.2.6 Insecticides used in cross resistance

The above five insecticides were used in testing the susceptibility of DBM larvae from five locations around Bangalore and four of them in selection studies. In addition, five more insecticides representing different groups were also used for ascertaining the cross resistance among the populations selected for resistance. These included one each of organo chlorine (Endosulfan), organophosphate (Chlorpyrifos) and carbamate (Cabaryl), neonicotinoid (Imidacloprid) and chitin synthesis inhibitor (Novaluron) (Table 16). All the five chemicals are in low levels of usage in cabbage system against DBM around Bangalore (Kamala, 2006).

3.3 Bio-assays

Bioassays were conducted by following the leaf dip method of treatment (Matsumura, 1975; Perry *et al.*, 1998; Kumar *et al.*, 2001). The methodology adapted for the purpose is the primary method of bioassay followed in our lab, since almost a decade. The method has been found to give consistent results that could be compared across several different studies carried out at different times in the lab (Jayappa, 2000; Kumar *et al.*, 2001, Ashoka and Kumar, 2002, Kumar *et al.*, 2003, Jayappa and Kumar, 2003). However, the methodology is suitable only for the purpose of ascertaining the mortality and IGR effects and not suitable for other biological effects.

Based on the published results 5-6 concentrations in geometrical proportion were selected for the preliminary trials. The mortality data of the larvae obtained in the bracketing studies of the preliminary trials were used for determining the concentrations for detailed bioassays. The concentration that gives more than 90 per cent mortality at around 96 h in the preliminary bioassay was worked out and fixed as the highest concentration for the actual bioassay. Log or arithmetic proportions or a combination of the two were used to fix the remaining concentrations required for the assay. Separate bracketing studies were carried out before each assay for each insecticide on the susceptible JJ-Lab strain. Stock solutions of highest concentration were made initially and then serially diluted to get the required concentration. Generally nine such concentrations were used in each assay along with a control. Soap solution at the rate of 0.05 per cent was used in all the concentrations and in the control as emulsifier.

Fresh and clean mustard leaves were cut into bits of approximately 10 sq cm areas along with the leaf stalk. The stalk was wrapped in wet cotton to maintain the turgidity of the leaf bits. The leaf bits were then dipped in the solutions of the predetermined concentrations for ten seconds, air dried for 15-20 minutes and placed in a clean petri plate. Each such leaf bit received ten freshly moulted second instar larvae of DBM and served as a replication. Three such replications were maintained for each concentration. Petri plates were then maintained at room temperature (25 ± 2 °C). After 48 h of treatment, the treated leaves were replaced with fresh and similar but untreated leaf bits (Jayappa, 2000).

Observations on the larval mortality were made and data recorded at 24 h intervals. All larvae were touched with a fine hairbrush to ascertain their activity. Larvae that did not respond despite pressing were considered dead (Jayadevi, 2002). The mortality data recorded for each replication were corrected depending upon the mortality in the control following Abbot (1925). The corrected per cent mortality data were subjected to probit analysis (Finney, 1971) for determination of median lethal concentrations for each observation.

The LC_{50} values thus obtained from the assays were utilized for comparing the susceptibility of the different field strains collected from around Bangalore with respect to

susceptible JJ-Lab strain. Bioassays were also carried out at different generations of selection for insecticide resistance to selected insecticides to know the rate of development of resistance.

3.4 Selection for resistance

After the bio-assay in different field strains of DBM against selected insecticides, during F₁ generation, the pupae from each location were pooled and placed in a single cage. The different strains were pooled before selection to create wider genetic base for selection (Fig. 9). Adults emerged from these were allowed to mate randomly. The eggs were collected on mustard seedlings as indicated earlier. The III instar larvae from this F₂ progeny were sub divided into five sub colonies. Each of the sub colonies were subjected to selection with indoxacarb, cypermethrin, Neemazal-F or NSKE. However, the fifth one was maintained without treatment with any insecticide under laboratory condition (25 ± 2 °C) as described above. Each selected strain was correspondingly named JInd-R, JCyp-R, JNzF-R or JNSKE-R and unselected susceptible stock, JField. All the cultures were maintained in isolation to ensure lack of inter-breeding.

3.4.1 Unselected field strain (JField)

Unselected field strain, JField strain was maintained on mustard seedlings without treatment with any insecticide. The culture was used as a control for comparison of development of resistance and for other life history parameters. Further, change in susceptibility of the field strain on relaxation to 4 insecticides were studied by carrying out the bio-assay at 5 and 10th generations of rearing under laboratory conditions. LC₅₀s of the field strain (JField) at the 10th generation against 10 insecticides were used to compare with the LC₅₀s of selected strains to know the cross resistance to these insecticides on selection.

3.4.2 Selection for resistance

It is possible that the second instar larvae were too small and they can remain without feeding and die or recover for feeding after 48 h after treatment, when the fresh leaf becomes available. On the other hand, the fourth instar larvae, even though feed vigorously, can go for pupation truncating their feeding. As a result, both these instars were considered less useful for imposing the selection. However, the III instar being in an

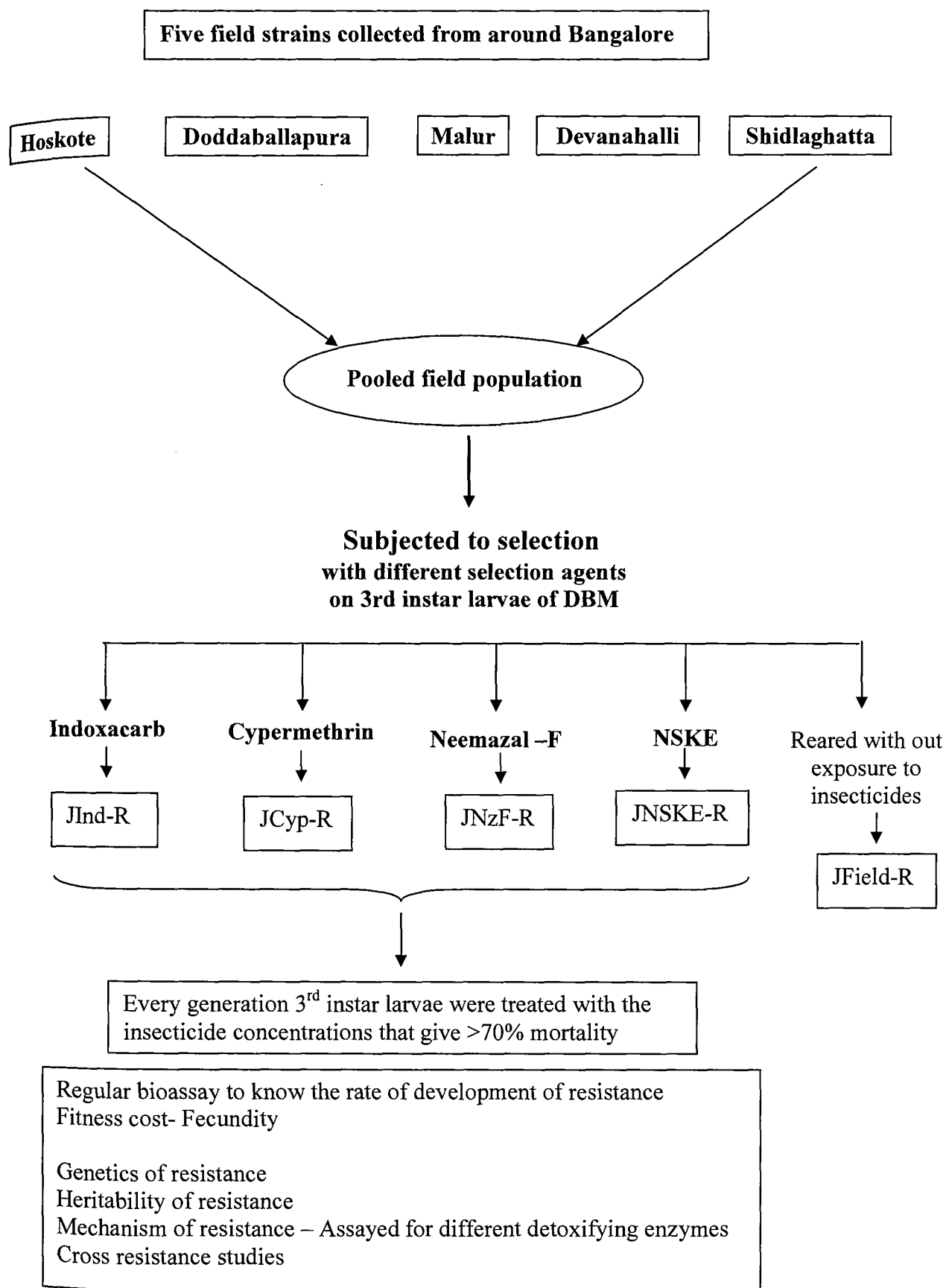


Figure 9: The different steps involved in selection for resistance to different insecticides, indoxacarb, cypermethrin, Neemazal-F and NSKE against III larvae of *P. xylostella*

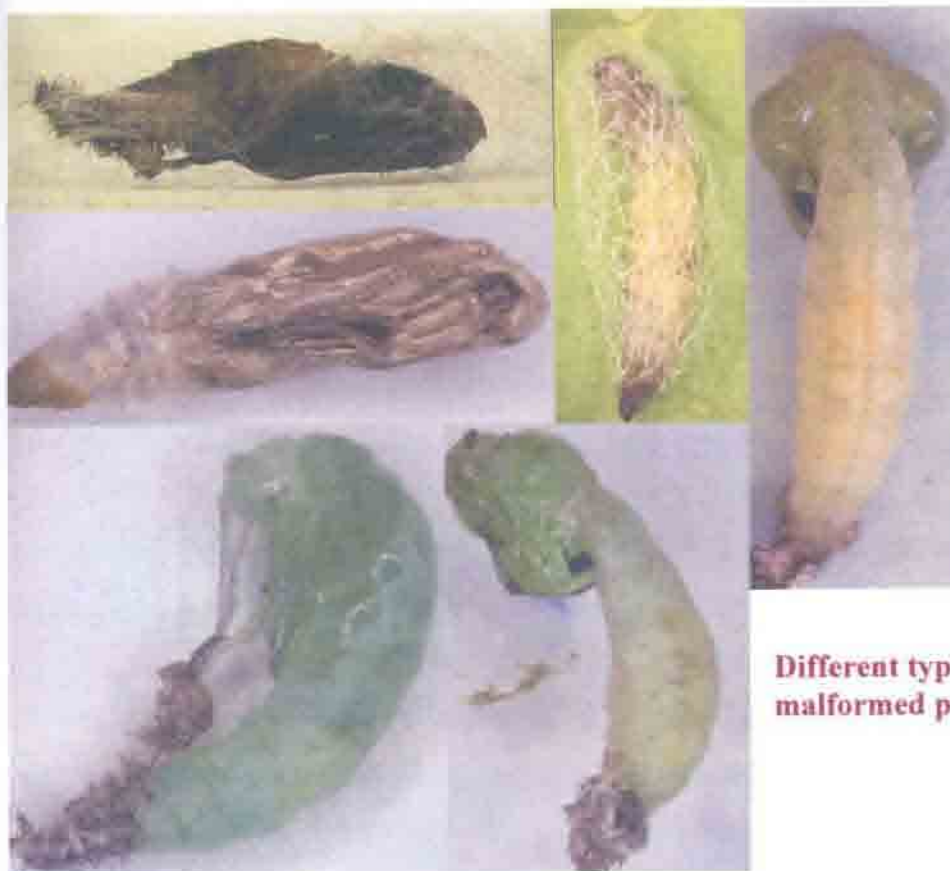


Unhatched egg with
formed larva inside

Deformed adult



Mortality at
larval stage



Different types of
malformed pupae

Plate 2 : Different types of mortality observed in *Plutella xylostella*
treated with NSKE and Neemazal-F

actively growing period, are in a must feed condition. As a result, III instar larvae were used for all selection treatments (Brown and Payne, 1988).

Selections for resistance to four selected insecticides were conducted on mustard leaves. Whole leaves were treated with selection concentrations with their respective insecticides by dipping for 30 seconds (Plate 1). Then the treated leaves were air dried for 10-20 minutes or until all the surface water disappeared. In each generation, the third instar larvae were released on to these treated leaves. Insecticide treated leaves were provided at this stage for 48 h and later substituted with fresh untreated leaves at 24 h interval. Observations were recorded daily on the number of larvae treated, number surviving, number dead, and number pupated until all the treated larvae pupated or died. Pupae formed every day were preserved live up to a maximum of six days in a refrigerator for synchronizing the emergence time.

During the course of selection, it was observed that pupal malformation, lack of adult emergence from apparent normal pupae, poor hatching rate of eggs, or malformed adults (Plate 2) were commonly found in NSKE and Neemazal-F selected strains. In these two cultures, the mortality of the treated insects extended at a relatively higher rate up to the adult emergence stage. As a result, the cut off value of 70 per cent mortality for selection pressure was considered only at the adult emergence uniformly for all cultures.

In every generation, a maximum of 100 pupae were placed individually into homeopathic vials and monitored for per cent adult emergence and sex ratio. Normally the adults started emerging from third day onwards after pupation. Emerging adults were counted and sexed twice a day. Sexing was done based on the slender abdomen and the presence of a dark slit at the caudal tip on the ventral side of the abdomen that demarcated the males (Plate 1). In females, the abdominal tip was covered with a tuft of black hairs and the slit was absent. Comparatively female abdomen almost always appeared swollen.

All emerged adults were released into a cage provided with 20% honey as adult food. The eggs were collected on three day old mustard seedlings and reared on mustard seedlings up to the III instar as described above. Third instar larvae in every generation were subjected to selection with their respective selection agents.

3.4.3 Selection concentrations and Resistance Ratios

Selection concentrations in each generation were chosen with a view to obtain roughly 70 per cent mortality by adult emergence. The selection concentrations were changed, when ever the per cent survival increased > 30 % at adult emergence. However, on continuous treatment with the same concentration, there was an increase in per cent survival over generations.

Resistance ratios were worked out as the ratios of LC₅₀ of selected strain by LC₅₀ of JField strain to a particular insecticide. To establish the base line LC₅₀ values for each insecticide, the pooled LC₅₀ values were calculated. Pooled LC₅₀ values were calculated by pooling all the mortality data at each concentration of the insecticide of five field strains and then the percentages were reworked for probit-analysis. This pooled LC₅₀ value was used for further comparison over generations of selection and to work out the change in resistance ratio on selection or relaxation. Resistance ratios over generations of selection were worked out with respect to these pooled baseline LC₅₀ values of field strain (JField) in respective insecticide.

$$\text{Resistance ratio} = \frac{\text{LC}_{50} \text{ of selected strain}}{\text{Pooled LC}_{50} \text{ of JField strain}}$$

3.5 Genetics of insecticidal resistance : analysis

After 10 generations of selection in JInd-R, JCyp-R and JNSKE-R strain, the LC₅₀s were found significantly different from base line LC₅₀ values (F₁ generation, JField). Hence, the selected populations were considered as resistant and were used for working out the genetics of resistance to their respective insecticides. These studies aimed at understanding the pattern of inheritance, degree of dominance, and number of genes.

3.5.1 Pattern of inheritance

Passing of genes for a specified trait from one generation to the next through chromosomes is known as inheritance. Inheritance may take place either through autosomal chromosomes or through sex chromosomes. In case the sex chromosomes (allosomes) are involved in the transmission of a trait from parents to progeny, then, the trait may either be passed on from a mother or a father to the progeny. Such sex linked inheritances are correspondingly called maternal or paternal inheritance.

The nature of inheritance of a trait is therefore, ascertained through a series of studies of the parents and the progeny of reciprocal crosses between resistant and susceptible populations, in the case of insecticidal resistance.

3.5.1.1 Reciprocal and back crosses

Fundamentally, for the purpose of ascertaining the inheritance and the nature of the trait (degree of dominance) the homozygous strains representing both the resistant and the susceptible populations are required. In the present study, the homozygosity of the resistant strains was assumed for all the resistant strains, as the LC_{50} values were significantly different (see below for ascertaining the significance) from the base line values at the 10th generation. The JJ-Lab strain being reared in the laboratory for over 100 generations was considered as homozygous susceptible. Using the males and females from these cultures, the reciprocal crosses were made and the progenies obtained.

The resistant lines for the purpose of clarity are designated as ‘R’ strains and the susceptible culture is designated as ‘S’ strain for all reciprocal crosses, in congruence with the phenotypic nature of the strains. For the purpose of crossing the pupae of both the resistant and the susceptible strains (JJ-Lab) were individually separated into vials. Emerging adults were then sexed and grouped according to the need of reciprocal crosses. All the reciprocal crosses were mass crosses. For the R (♀) X S (♂) cross, 50 females of the R strain and 50 males of the S strain were pooled and released into a single cage. For the S (♀) X R (♂) cross, 50 females of S strain, and 50 males of the R strain were pooled and released into a single cage. Eggs were collected daily for five days to obtain the larvae for further studies.

Bioassays were then carried out on the II instar larvae of the susceptible strain, resistant strain and the F₁ progeny of the two reciprocal crosses using the insecticide used for the selection of the resistant strain. The four LC_{50} values were then compared to understand the nature of inheritance and the degree of dominance (Bourguet *et al.*, 2000). It is anticipated that the LC_{50} values for the two progenies of the reciprocal crosses will be equal, if the resistance is inherited autosomally. The equality of the LC_{50} values was ascertained by comparison of the fiducial limits of the LC_{50} s ($p = 0.05$). If the fiducial limits of the LC_{50} values overlapped then the two median lethal values of the reciprocal

crosses were considered equal. On the contrary, if the fiducial limits did not overlap and the two LC_{50} s differed, then the sex linked effects are ascribed. Further, if the progeny of a cross whose mother is resistant and its LC_{50} value is higher than the other reciprocal cross, then the trait is considered maternally inherited. Similarly, if the progeny of a cross whose father is resistant and its LC_{50} value is higher than the other reciprocal cross, then the trait is paternally inherited (See Fig. 10 for details).

3.5.2 Nature of resistant trait (Degree of dominance)

There are two different ways of knowing the nature of resistance (dominance of the resistance)

1. Based on Probit mortality lines
2. By calculating the Stone's D (Stone, 1968)

For both the methods it is necessary that the four populations are assayed for the LC_{50} values and the log dose-probit mortality lines are available. Therefore, the data generated for the purpose of understanding the nature of inheritance was used even for the calculation of Degree of dominance of the resistant trait.

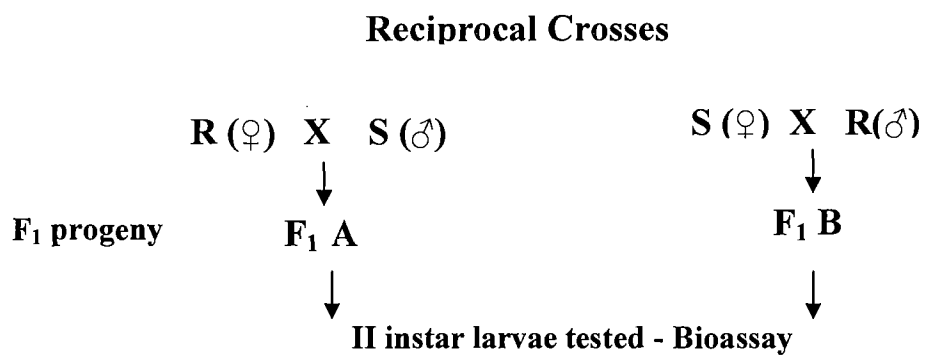
3.5.2.1 Based on Probit mortality lines

Brown (1958) proposed the following simple model for understanding the degree of dominance of the genes controlling for insecticidal resistance. The same is adopted here. Probit mortality line of the progeny of the cross between the resistant and the susceptible strains (F_1) is plotted and compared with those of the resistant (RR) and susceptible (SS) homozygous populations. If the F_1 log probit mortality line falls towards the probit mortality line of the resistant population then it is expected to be controlled by Dominant gene or genes (Fig. 11b). Alternately, if the trait is regulated by recessive gene or genes then the F_1 probit mortality line would fall closer to that of the susceptible population (Fig. 11a). However, if it is midway between that of the resistant and the susceptible populations then the trait is considered to be regulated by co-dominant gene or genes (Fig. 11c).

3.5.2.2 Stone's Degree of Dominance

The nature of the gene controlling the resistance can also be estimated in terms of a comparative analysis of the LC_{50} values of the parents and the progeny. This model

Inheritance of resistance



If F₁ A (LC₅₀) > F₁B(LC₅₀) Maternal effect } Sex linked
 F₁A (LC₅₀) < F₁B (LC₅₀) Paternal effect }

F₁A (LC₅₀) = F₁B (LC₅₀) Autosomal inherited

Figure 10: Reciprocal cross between resistant (R) and susceptible (S) strains of *P. xylostella* to know the genetics of inheritance of resistance to insecticide.

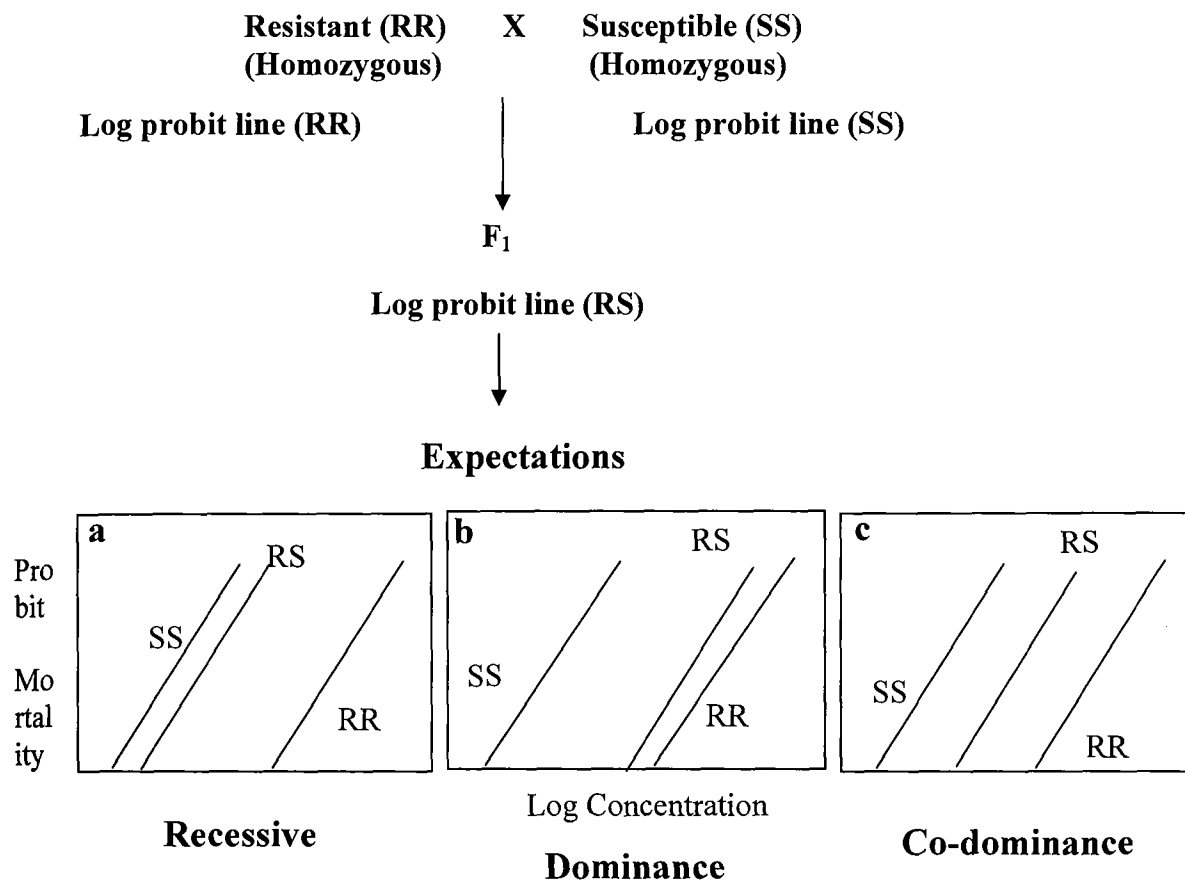


Figure 11: Relative position of the probit mortality line of F₁ with respect to parental population (Brown, 1958).

developed by Stone (1968) does not however specify the sex of the parents from resistant and susceptible strains for obtaining the progeny. Therefore, whenever the detailed data on the sex of the parents and their source is available, 'D' the degree of dominance can be calculated for both the reciprocal progeny independently. Hence in all our studies, two values of 'D' were calculated.

The index of Degree of Dominance, 'D' is calculated by using the following equation :

$$D = \frac{2Y_3 - Y_2 - Y_1}{Y_2 - Y_1}$$

Where,

Y_1 = Log LC50 of susceptible (SS) strain

Y_2 = Log LC50 of resistant strain after 'n' generations of selection (F_n)

Y_3 = Log LC50 of F_1 (S X R) or F_1 (RXS) progeny

Once the 'D' values are available, the Degree of dominance is inferred by the following guidelines :

D = -1 Completely recessive

0 No, dominance

1 Completely dominant

However if the perfect values are not available, then negative values indicate incompletely recessive and if positive, incompletely dominant.

3.5.3 Number of loci governing resistance

3.5.3.1 Based on predetermined expectations for the number of genes

Number of loci governing resistance can be determined through the response of the F_2 progeny of the back cross of the F_1 progeny (Resistant X Susceptible or *vice versa*) to parental strains (F_1 X Parent). Logically the expectations are that the F_1 (R X S or S X R) in an autosomally inherited situation are expected to be heterozygous (genotype) for the resistant allele. But phenotypically they may vary from the parent lines depending on the type of dominance expected for the resistant allele. Thus if the resistance is recessive, it is expected that the susceptibility will be relatively high among the F_1 compared to the homozygous resistant parent line. In other words its behaviour will be similar to the

dominant susceptible parent. Alternatively, if the resistance is by a dominant allele, the F_1 progeny will be as tolerant to the insecticide as the homozygous resistant parent line. However, if the trait is governed by more than one gene, then it is anticipated that the above mentioned simple expectations may be quite misleading. Therefore, in the absence of the knowledge of the number of genes governing the trait, it would be best to develop a segregating population so that the response of the segregating population can then be verified in the light of the Mendelian expectations for single, or multiple gene inheritance. Therefore, in order to develop such a segregating population, the heterozygous F_1 will be further crossed with one of the parent lines to obtain the segregating F_2 progeny.

Such an F_2 progeny is expected to be highly segregating and the phenotypic expectations would then match the genotypic representations in the population. In a monogenic condition, therefore, the F_2 progenies of the back crosses are expected to be segregating into 1:1 ratio genotypically but the phenotypes may behave differently depending on the nature of the resistant allele (See Fig. 12). As a consequence, the two standard backcrosses between the F_1 progeny with either resistant or susceptible parental strain both may not give the desired phenotypic segregation. Hence, it is preferable to choose the right parent line to get the best phenotypic segregation in the F_2 progeny. Under such situations, the backcross is best carried out between the F_1 and the parental strain that differs most from the F_1 (Roush and Daly, 1990). Therefore, if resistance is partly or completely recessive, F_1 offspring's were crossed with the resistant parental strain (Roush *et al.*, 1986) and if resistance is partially or completely dominant, then F_1 is crossed with the susceptible strain (Tabashnik, 1991). However, in the present study, in order to determine the number of genes governing the resistance to indoxacarb and cypermethrin, both the backcrosses ($R \times S$) ($\varnothing$) $\times$ R (σ) and ($S \times R$) ($\varnothing$) $\times$ S (σ) were carried out and comparisons made.

Then, the observed mortalities of the F_2 progeny at different doses will be compared with the expectations for mortalities at that concentration. Given that the expected mortalities at any given concentration is half of the mortality of the F_1 plus half of the mortality of the parental strain (the parents of the F_2 progeny- Fig. 13), the Mendelian monogenic autosomal inheritance of the resistance was tested by checking for congruence of the expected and the observed values for the mortality at each of the concentrations using chi-square, following Tabashnik (1991). If the sum of the chi-square

Resistant allele is Dominant

F₁ Cross

Resistant (RR) X **Susceptible (ss)**
(Dominant) (Recessive)

↓
F₁

Heterozygous (Rs)

F₁ – Dominant allele is expressed

Backcross I

F₁(Rs) X **Susceptible (ss)**
(Dominant) (Recessive)

↓

F₂

Rs:ss::1:1

Resistant

Susceptible

F₂ progeny segregate into Resistant: Susceptible on treatment of insecticide

Backcross II

F₁ (Rs) X **Resistant (RR)**
(Dominant) (Dominant)

↓

F₂

RR:Rs::1:1

Both are Resistant

All the F₂ progeny behave as resistant. Because the resistance is governed by dominant allele and they will not be phenotypically segregating into Resistant: Susceptible and hence difficult to test the monogenic model.

In F₂ progeny segregation was measured in terms of mortality at each dose.

Observed mortality : % mortality of F₂ progeny

Expected mortality : At particular concentration =

$$\frac{1}{2} * (\% \text{ mortality of F}_1) + \frac{1}{2} * (\% \text{ mortality of SS})$$

Figure 12: Importance of back crosses in estimating number of gene/s governing resistance when resistance is governed by dominant allele/s

Resistant allele is Recessive

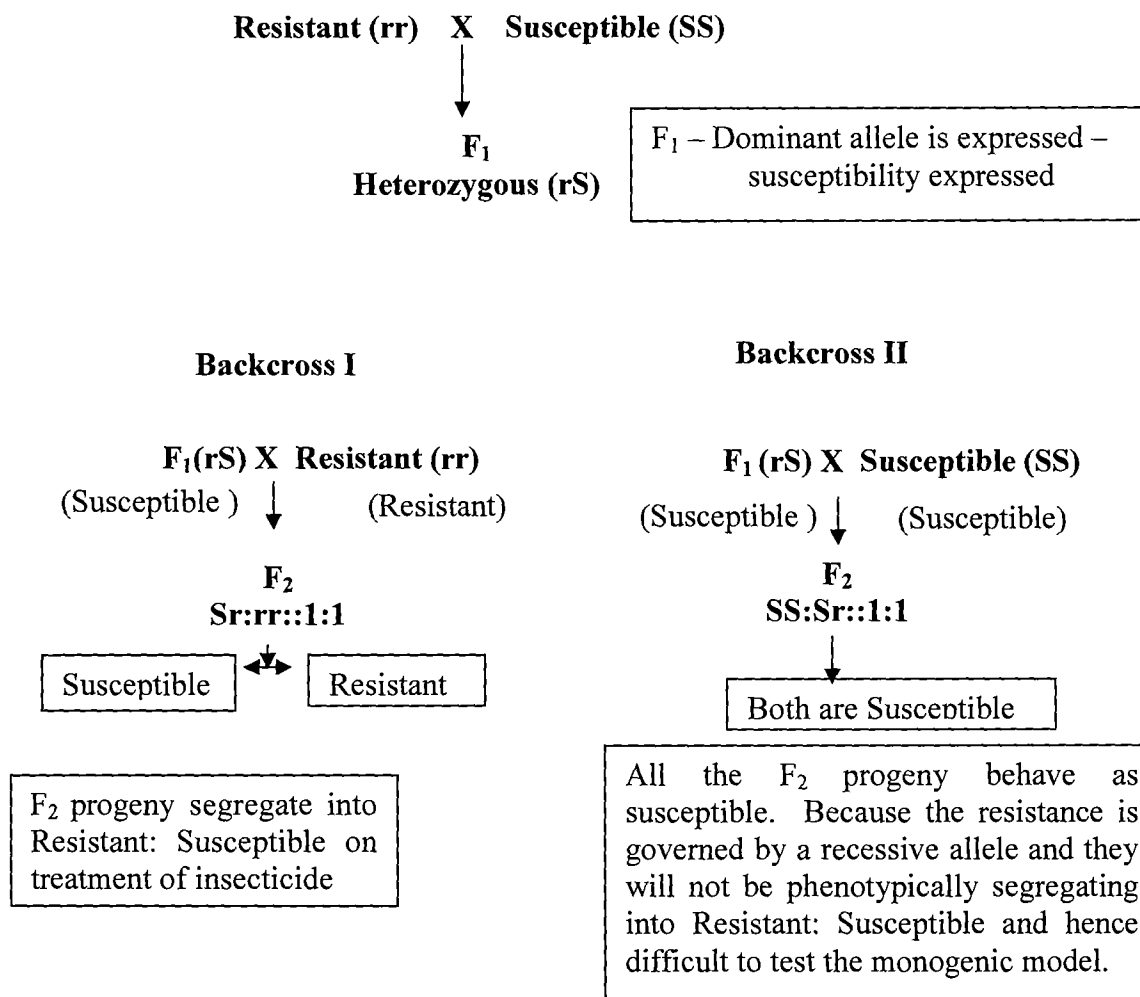


Figure 13: Importance of back crosses in estimating number of gene/s governing resistance when resistance is governed by recessive allele/s

calculated was less than the table chi-square value for the given degrees of freedom, then it fits the monogenic model of inheritance and if not then it is considered to be a polygenic trait. Alternatively, when the monogenic model does not fit the expectations, it is also possible to recalculate the expected mortality values for each concentration tested using suitable Mendelian genotypic segregations corresponding to the desired numbers of genes (Tabashnik, 1991). However, this was not attempted in the present study.

3.5.3.2 Lande's (1981) method of calculation of number of loci for resistance

An alternative approach to calculate the numbers of loci governing insecticide resistance trait was provided by Lande (1981). Basically, the method involves an adoption of the Wright's method (Wright, 1952) of ascertaining the number of quantitative trait loci. An inherent assumption is the neo-Darwinian idea that all traits are quantitative and that the numbers of loci can vary anywhere between 5 to 20 loci for a given trait. Further large evolutionary changes seen in artificially selected populations can occur through Darwinian process of accumulation of numerous genetic factors with individually small effects (Falconer, 1989, Wright, 1968). The method essentially requires parental heterogeneous (susceptible) wild type and the inbred (selected insecticide resistant) lines and their F_1 and back cross F_2 progenies. Procedure involves estimation and comparison of phenotypic means and variances of parental populations and their F_1 hybrids and backcross F_2 progeny.

Estimation of minimum number of genetic factors based on segregation of variance (Lande, 1981) was given as

$$n_E = (R-S)^2 / 8\sigma_s^2$$

Where,

R= logarithm of LC_{50} of resistant strain

S = logarithm of LC_{50} of susceptible strain

$$\sigma_s^2 = (\sigma_{B1}^2 + \sigma_{B2}^2) - (\sigma_{F1}^2 + (0.5 * \sigma_R^2) + 0.5 * (\sigma_s^2))$$

Where,

σ_{B1}^2 = Variance of Backcross -I

σ_{B2}^2 = Variance of Backcross -II

σ_{F1}^2 = Variance of F_1

σ_R^2 = Variance of resistant parent

σ_s^2 = Variance of susceptible parent

and

Variance = $(1/b)^2$ where b is the slope of the log dose-probit mortality line.

By using the above formula numbers of loci were estimated in JInd-R strain against indoxacarb and JCyp-R strain against cypermethrin.

3.6 Realised Heritability (h^2) :

The estimation of heritability and related parameters is fundamental to understanding the heredity and evolution of any trait in an organism. Insecticide resistance in insects is the most common example of evolution of a trait in an organism. Thus a study of these parameters also provides an insight to understand the risk of development of resistance in a target insect for a specified insecticide (Knight and Norton, 1989; National Research Council, 1986). Insecticide resistant field populations are ideal for such studies. In the absence of suitable field data, the laboratory selection experiments are expected to be the best alternatives for resistance risk assessment studies in crop pests (Keiding, 1986 and Brown and Payne, 1988). Therefore, laboratory selection studies are routinely carried out to develop insecticide resistant populations.

Heritability is the proportion of phenotypic variance accounted by additive genetic variation (Falconer, 1989). It is also defined as the proportion of the genes or character transmitted by parents to their offspring. The method described by Tabashnik (1992) that was adopted from Hartl (1988) and Falconer (1989) for estimating the realized heritability to insecticide resistance was employed to estimate the heritability of the resistance in different selected strains to their respective insecticides.

Essentially the method uses the information on per cent selection pressure imposed over generations during selection and LC_{50} s and their slopes for calculation of the extent of heritability of the resistant trait. Further, estimated LC_{50} s from every generation against cumulative selection differential were regressed.

Realized heritability (h^2) (Hartl, 1988; Falconer, 1989) was estimated as

$$h^2 = R/S \quad (1)$$

where,

R = response to selection

S = selection differential

Response to selection (R), the difference in mean phenotype between the offspring of the selected parents and the whole parental generation before selection (Falconer 1989) was estimated as

$$R = \frac{\log(\text{final } LC_{50}) - \log(\text{initial } LC_{50})}{n} \quad (2)$$

where final LC_{50} is the LC_{50} of offspring after 'n' generations of selection and initial LC_{50} is that of the parental generation in the initial generation before selection. The difference between LC_{50} s was calculated on a logarithmic scale because the logarithm of tolerance was assumed to be normally distributed. The numerator of equation 2 estimates the cumulative response to selection over 'n' generations of selection; dividing this cumulative total by 'n' estimates the average response to selection per generation (R).

Selection differential (S), is the difference in mean phenotype between the selected parents and the entire parental generation (Hartl, 1988), was estimated as:

$$S = i \sigma_p$$

where i is the intensity of selection and σ_p is the phenotypic standard deviation. Intensity of selection (i) was estimated from 'p' the percentage surviving the selection using

Appendix A of Falconer (1989), which is based on the properties of the normal distribution (Appendix IV).

Phenotypic standard deviation (σ_p) was estimated as the reciprocal of the mean of the estimated slopes of the probit regression lines (Finney, 1971) from the parental generation before selection (initial slope) and the offspring after 'n' generations of selection (final slope):

$$\sigma_p = [1/2 (\text{initial slope} + \text{final slope})]^{-1}$$

Once we know the heritability of resistance, we can also estimate the projected rate of resistance development. The response to selection (R) can be estimated as the product of heritability (h^2) and selection differential (S) (Falconer, 1989).

$$R = h^2 S,$$

which is a rearrangement of equation (1). The number of generations required for a 10-fold increase in LC_{50} , G, is the reciprocal of R:

$$G = R^{-1}$$

3.7 Life history parameters

The effect of selection and development of resistance to different selection agents (indoxacarb, cypermethrin and NSKE) on life history parameters were assessed in almost every generation of selection up to 10-15 generations. These included sex ratios, gross reproductive rates (GRR), net reproductive rates (NRR), mean generation times (T) and intrinsic rates of increase (r). All these parameters were computed following Wilson & Bossert (1971), Krebs (1985) and Gotelli (1995).

3.7.1 Sex Ratio

For ascertaining the sex ratios, at every generation after pupation (surviving pupae), at least 100 pupae were separated into individual vials. The adults emerged after three days were sexed into male or female (Plate 3).



Male



Female

Plate 3 : Sex differentiation in males and females of *Plutella xylostella*.

3.7.2 Gross Reproductive Rate (GRR)

Ten individual pairs of single ♂ and single ♀ were released into oviposition cages. Approximately 2,000 ml capacity plastic boxes served as the oviposition cages (Plate 4). Each pair was provided with 20 % honey in a cotton swab and with fresh mustard leaves. The leaves were changed daily with fresh leaves and the numbers of eggs laid were counted until the adults died. Male and female mortality patterns were also recorded. Using this data, mean number of eggs laid by all females living at age x measured in days after eclosion from the egg was computed and summed over all the days until the last female died. This summation provided the total potential fecundity of a female living till end of the maximum life span of the insect in each generation and is equated to gross reproductive rate- GRR.

Therefore,

$$GRR = \sum m_x$$

Where

m_x = number of eggs laid on an average by a female living at age x calculated over all living females at age x

3.7.3 Net Reproductive Rate (NRR = R_0)

Net reproductive rate was calculated as the mean number of offspring produced per female over her life time after adjusting for the mortality schedule. In other words, it is the sum of products of number of offspring born to an individual female of a particular age (m_x) and survivorship of a cohort of organism (l_x expressed as proportion living) at that age.

Therefore,

$$R_0 = \sum l_x \cdot m_x$$

Where

l_x = proportion of females living at age x counted from the date of eclosion from the egg. For calculation of this parameter, if the pupae were placed in the refrigerator for bringing synchrony in phenology these number of days were discounted



Plate 4 : Plastic container used as cage for single pair mating studies.

m_x = number of eggs laid on an average by a female living at age x calculated over all living females at age x

3.7.4 Mean generation time (T)

The mean generation time is defined as the average age of parents of all the offspring produced by a single cohort and is given by the equation

$$T = \sum l_x \cdot m_x \cdot x / \sum l_x \cdot m_x$$

Where,

$l_x \cdot m_x \cdot x$ represents the realized fecundity at age x multiplied by the age at the time of realizing this fecundity and $\sum l_x \cdot m_x$ represents the NRR (R_0).

3.7.5 Intrinsic rate of increase (r)

Intrinsic rate of increase represents the overall per capita growth rate per unit time. It is the number of progenies per female per day. An approximate estimate of the 'r' value is given by

$$r \approx \ln R_0 / T$$

However, exact 'r' value is calculated by iterative method using the **Euler equation**

$$1 = \sum e^{-rx} \cdot l_x \cdot m_x$$

By substituting the approximate 'r' value in Euler equation, through MS excel solver, exact 'r' value was calculated for each generation for all the cultures.

3.8 Mechanisms of resistance

Insects may acquire resistance against insecticides by varied mechanisms. For example, the mechanisms may involve reduced penetration, target site insensitivity and/or metabolic detoxification. However, additionally and mostly under field situations, a variety of behavioural resistance mechanisms may also evolve (Moore and Tabashnik, 1989). Under laboratory selection conditions this possibility is very low. During the present study, it was not possible to study every one of these possibilities. Therefore, only

some of the potential detoxification mechanisms primarily based on enzyme activity were assessed.

3.8.1 Enzyme activity assays

Primarily, esterases, glutathione-S-transferases and mixed function oxidases (MFOs, monooxygenases or cytochrome P₄₅₀s) are the primary enzymes involved in the detoxification mechanisms of stressful chemicals including insecticides (Scharf *et al.*, 1998). Therefore, some preliminary studies were done to assess the relative activity levels of these enzymes in unselected JJ-lab, JField and insecticide selected JInd-R, JCyp-R, JNzF-R and JNSKE strains.

Broadly the methodology involved, obtaining the larval homogenates, extraction of proteins, and ascertaining the activity of different groups of enzymes using suitable substrates. The enzymatic activity was then expressed as substrate equivalence of conversion by enzymes per unit protein for different substrates. Specific substrates were used for each class of enzymes. All assays were replicated three times. Assay results were quantified spectrophotometrically in a UV spectrophotometer or visible spectrophotometer.

3.8.1.1 Preparation of Larval homogenates

After 20 generations of selection, well grown fourth instar larvae surviving the insecticide treatments were used for enzyme assay. Selected larvae were separated and starved for 6 hours and 20 each were put into 2.5 ml eppendorff tubes and labeled. Further to prevent opening of the caps, the caps were sealed with wax paper, which were then transferred for a short while to liquid nitrogen and then on to a deep freezer and stored till the assay at -80 °C.

At the time of enzyme assay, 20 larvae from each strain were taken and weighed. The larvae were homogenized in 0.1M sodium phosphate (pH = 7.5; Appendix V) buffer in 2 ml eppendorff tube with a micro pestle. The homogenates were then centrifuged at 10,000 rpm for 10 minutes at 4°C, supernatant collected and the pellet with tissue was discarded. The supernatant was further centrifuged at 15,000 rpm at 4°C for 15 minutes to remove the remaining debris from the supernatant. The volume was made up to 5 ml finally by adding another 3 ml of 0.1 M sodium phosphate buffer containing 30% glycerol.

Resulting supernatant was used for esterase and glutathione-S-transferases activity estimation. Remaining supernatant was stored at -80°C for protein estimation and isozyme assay through NATIVE PAGE. Protein content was estimated by following Lowry's (1951) method.

3.8.1.2 Preparation of microsomes

Extraction of membrane bound microsomes from larval homogenates for estimation of MFOs activity was done following Mayer *et al.* (1977). Approximately 20 fourth instar larvae were weighed and homogenized in 300mM Potassium phosphate buffer ($\text{pH} = 7.8$) containing 0.1 mM EDTA with Teflon coated micro pestle and mortar at 4°C (ice in tray). The resultant homogenate was centrifuged at 750 g for 10 minutes to remove cellular debris. Supernatant was then centrifuged at 1000 g (3,600 rpm) for 10 minutes and again the supernatant separated, centrifuged at 10,000 g (11,500 rpm) for 20 minutes to remove all the cellular debris present in the supernatant. This final supernatant was centrifuged at 1,00,000 rpm for 1 h (4°C). Mixed function oxidases are membrane bound enzymes and they are present in endoplasmic reticulum of the cells, hence to know their activity they have to be removed from membrane. Therefore, larval supernatant containing microsomes were centrifuged at high speed (1,00,000 g) to separate them from membranes. After 1 h, the pellet formed was taken and the supernatant discarded. The pellet was dissolved in 500 μl of 300 mM Potassium phosphate buffer ($\text{pH} = 7.8$) containing 0.1 mM EDTA, protein estimated by Lowry *et al.* (1951) method. The activity of MFOs was determined by following Harold and Ottea (1997) with some modifications. All this work was done at PDBC, Hebbal, Bangalore.

3.8.1.3 Esterase assay

To know the possible involvement of esterase enzymes in detoxification of the insecticides in selected insecticide resistant strains they were assayed for non specific esterase activity using the two common substrates, α - naphthyl acetate (α -NA) and β -naphthyl acetate (β -NA).

NA esterase assay

The method followed was similar to that of Scharf *et al.* (1998) developed for estimation of esterases. Similar conditions were maintained for both α -NA and β -NA metabolism assay.

Larval homogenates prepared by using the standard methods (section 3.8.1.1) were used for developing the required three ml of the reaction mixture containing 500 μ l of NA working solution and 50 μ l of larval supernatant. The mixture was thoroughly stirred to mix the contents. The vials were incubated at 37 °C for 6 minutes for the reaction to take place. Then the reaction was stopped by adding 200 μ l of fast blue containing stop solution (see Appendix VI). The 2.3 ml of 0.2 M sodium phosphate buffer was added to make up the value to 3 ml. After ten minutes the dark pink or bluish pink colour formed is measured at 570 nm wavelength using a spectrophotometer. The activity of esterases was measured through standard curve of naphthol 0- 50 μ moles and expressed in terms of specific activity (μ g of naphthol/mg protein/min).

NA working solution contained 50 μ l of 31 mM NA in acetone in 5.0 ml of 0.2 M sodium phosphate (pH = 6.8) buffer. Fast blue stop solution was prepared afresh by dissolving 15 mg of fast blue B salt in 1.5 ml distilled water and 3.5 ml of 5 per cent SDS.

PNPA Esterase assay

Three ml of reaction mixture contained 2.87 ml of 0.2 M sodium phosphate buffer (pH = 7.4) and 100 μ l of larval supernatant in a cuvette. The same mixture also served as the blank. 30 μ l of PNPA stock substrate was added to the cuvette and the reaction was measured for 2 min at 405 nm at room temperature of 30 °C. PNPA stock substrate contained 200 mM p-nitrophenyl acetate (PNPA) in acetonitrile (Appendix VII). The enzymatic activity was measured in terms of the p-nitrophenol product formed over 2 minutes duration through an extinction coefficient of $6.53 \text{ mM}^{-1} \text{ cm}^{-1}$.

3.8.1.4 Glutathione -S- transferase (GST) assay

In order to assess the activity levels of glutathione-S-transferases, the conjugation of the substrate to the glutathione through the glutathione-S-transferases mediated activity was measured in terms of their activity to substrates under *in vitro* conditions. Two

commonly used substrates, 1-chlorodinitrobenzene (CDNB) and 2-dichloronitrobenzene (DCNB) were also used in the present study.

Dichloro nitrobenzene (DCNB)

0.1M Tris HCl buffer pH 9.5 containing 15 mM glutathione was used as the DCNB assay buffer. DCNB stock of 63 mM, 2-dichloronitrobenzene (DCNB) in methanol served as the substrate. Activity of the GST was measured by adding 50 μ l of larval supernatant to a spectrophotometer cuvette containing 2.91 ml of DCNB assay buffer (0.1 M Tris-HCl (pH = 9.5) buffer containing 15 mM glutathione). The mixture without the substrate was used as the control. Reaction was started by adding 40 μ l of 63 mM DCNB to the cuvette and the reaction was measured for 2 minutes at 334 nm using a UV spectrophotometer (see Appendix VIII). The enzymatic activity in terms of the substrate conjugated through extinction coefficient of $10.0 \text{ mM}^{-1}\text{cm}^{-1}$ was used to calculate the activity.

Chlorodinitrobenzene (CDNB)

For CDNB assay, 0.1M Sodium phosphate (SP) buffer (pH = 6.5) containing 15 mM glutathione was used as CDNB assay buffer. 63 mM, 1-chlorodinitrobenzene (CDNB) in methanol was the substrate.

A mixture containing 2.910 ml of 0.1 M Sodium phosphate buffer (pH = 6.5) with 15 mM glutathione and 50 μ l of DBM larval homogenate (enzyme source) in a cuvette served as the blank. To this, 40 μ l of 63 mM CDNB substrate was added to initiate reaction. After 2 minutes the absorbance of the product conjugated with glutathione was measured in terms of $9.5 \text{ mM}^{-1}\text{cm}^{-1}$ of extinction coefficient (Appendix IX).

3.8.1.5 Assay to measure mixed function oxidases activity : Para-nitroanisole (PNA)

Activities of MFOs towards para-nitroanisole (PNA) were measured with slight modification of the methodology developed by Kinoshita *et al.*, (1966) following Harold and Ottea (1997).

Three replications each of 600 μ l reaction mixture were prepared. The mixture contained 0.1 mM of NADPH, 0.1 M sodium phosphate buffer (pH= 7.8), 100 μ l of larval homogenate and PNA substrate.

Reaction mixture was developed by adding 100 μ l of larval homogenate to 469 μ l of 0.1 M sodium phosphate buffer (pH = 7.8). Then 10 μ l of 0.1 mM NADPH was added to this mixture. The reaction was started by adding of 21 μ l of 10 mM of para-nitroanisole (PNA in ethanol) as substrate and incubated at 37 °C for 30 minutes in water bath. Reactions were stopped by addition of 30 μ l of Trichloro acetic acid (2.5 % of final concentration). Reaction mixtures were then mixed vigorously and centrifuged at 10,000 g for 5 minutes at 4 °C. Supernatants were taken to fresh eppendorff tubes that contained 30 μ l of 5 N sodium hydroxide. Then the absorbance was measured at 405 nm using a UV spectrophotometer. The results were corrected for non-enzymatic activity using incubation without enzyme source (blank) as the control. The enzymatic activity was measured as the pico-moles of p-nitrophenol formed per minute.

3.8.2 Esterase Native PAGE

The NATIVE-PAGE for esterase isozymes of larval homogenates was conducted using slab discontinuous gels containing 10 per cent resolving and 5 per cent stacking gels. The method was based on the procedure described by Scharf *et al.*, (1998). The reagents for NATIVE-PAGE are described in Appendix X. A volume of larval homogenate containing 50 μ g protein was mixed with 15 μ l of native PAGE sample buffer and loaded onto gels. Electrophoresis was carried out in NATIVE PAGE running buffer, initially at 100 V, till it reached resolving gel. Once it reached the resolving gel, voltage was increased to 150 V and electrophoresis continued until the marker reached the base of the gel (Approximately 8 h). The whole process of electrophoresis was carried out at 4 °C temperature, to avoid degradation of enzymes. After electrophoresis, gels were incubated in 100 ml of NA assay buffer containing 2.0 ml each of α -NA and β -NA stock solutions. After 10 minutes, 1.0 ml of freshly prepared Fast blue B salt solution was added to visualize the esterase bands. After 5 minutes, gels were removed and fixed in 10 % acetic acid. Gel was photographed and the observation on the number of bands and intensity of bands formed were recorded.

3.9 Statistical analyses

For all bio-assays, probit analysis was carried out following Finney (1971). The LC₅₀ and slope values were worked out for all observations made at 96 h post treatment.

The median lethal values generated for 96 h post treatment were utilised for all further analyses. Two LC_{50} values were considered significantly different if their 95 per cent fiducial limits did not overlap ($p = 0.05$). Based on this response, field populations were compared with the susceptible JJ-Lab strain. If field populations differed from the lab population, pair wise comparison between the field strains were carried out to know the differential susceptibility to a given insecticide.

Resistance ratios were used to compare the levels of resistance between the different field strains across different insecticides. Resistance ratio is the ratio between the LC_{50} value of field strain divided by LC_{50} of susceptible, JJ-Lab strain to a given insecticide. However, the rate of increase or change in resistance levels on selection over generations was monitored through bioassays at intermittent generations. The LC_{50} values worked out by probit analyses were used for calculation of resistance ratios. Resistance ratio of selected strains is the LC_{50} value of selected strain divided by pooled LC_{50} of field strain (JField at F1) before selection.

Analyses of variance (ANOVA) were carried out following Sokal and Rohlf (1995) and where necessary, Pearson's Product moment correlations were worked out following Snedecor and Cochran (1967) or MS Excel-2003. The slopes of the linear or best fit non-linear (exponential) curves were compared between the selected strains through ANCOVA (Sokal and Rohlf, 1995).

IV. EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The diamondback moth (DBM), *Plutella xylostella* is a cosmopolitan pest of cruciferous vegetables. It is known to acquire resistance to any new insecticide alarmingly quickly. The present study is aimed at understanding the relative rates of development of resistance to four different selection agents, indoxacarb, cypermethrin, Neemazal-F and NSKE under laboratory conditions in *P. xylostella*. The selected strains were also examined to understand the genetics of resistance, biochemical mechanisms of resistance and effect of selection on their life history parameters. The results of these studies have been presented under suitable headings here under.

4.1 Relative susceptibility of five field collected strains of *Plutella xylostella*

Diamondback moth larvae were collected from five different locations around Bangalore. Response of the II instar larvae of F₁ generation from these five strains were studied against five selected insecticides through leaf dip method of bioassays (Table 16).

4.1.1 Variation between locations

4.1.1.1 Relative susceptibility of field strains to indoxacarb

Indoxacarb was one of the major insecticides used for the management of DBM during 2002. Towards the end of 2005, however, it was in the declining stage of its use. Among the five strains tested, Malur strain was the most susceptible ($LC_{50} = 0.00001$ % a.i.), than even the JJ-Lab strain (0.00002 % a.i.), while Devanahalli strain was the most tolerant to the chemical with an LC_{50} of 0.00023 per cent a.i. The overall mean susceptibility for all the cultures tested indicated an LC_{50} value of 0.00005 per cent a.i.

On the basis of overlapping of fiducial limits, the LC_{50} values of the five field strains were significantly different from each other ($p < 0.05$). Hoskote, Doddaballapura and Malur strains were on par with the susceptible lab (JJ-Lab) strain (Table 17). However, Shidlaghatta and Devanahalli strains were significantly different from the susceptible (JJ-Lab) and other three strains. The highest resistance ratio (RR) of 11.5 fold was recorded in Devanahalli strain followed by 6.5 fold in Shidlaghatta strain (Fig. 14).

Table 17: Relative susceptibility of second instar larvae of *Plutella xylostella* collected on cabbage or cauliflower crop from five different locations around Bangalore to selected insecticides during January, 2005

Strains	n	LC ₅₀ (% a.i.)	Fiducial limits @95% (%a.i.)	Y= a+bx	LC ₉₉ (% a.i.)	χ^2	RR*
Indoxacarb							
Lab culture	300	0.00002	0.00001-0.00003	6.35755+1.36525x	0.16659	6.339	1.00
Hoskote	300	0.00003	0.00001-0.00006	5.02052+1.09899x	0.00354	0.57	1.50
Doddaballapura	300	0.00002	0.00001-0.00004	4.61553+0.96742x	0.0043	1.028	1.00
Malur	300	0.00001	0.00000-0.00003	5.99178+1.22332x	0.00101	0.766	0.50
Devanahalli	300	0.00023	0.00015-0.00040	6.67806+1.83836x	0.00429	1.394	11.50
Shidlaghatta	300	0.00013	0.00006-0.00025	4.81731+1.23662x	0.00968	2.58	6.50
Pooled		0.00005	0.00003-0.00006	4.55692+1.05156x	0.00756	1.503	
Cypermethrin							
Lab culture	300	0.0047	0.00390-0.00554	6.62631+2.84622x	0.031	6.666	1.00
Hoskote	300	0.03169	0.02341-0.04208	5.17350+3.45119x	0.150	8.726	6.74
Doddaballapura	300	0.0249	0.01610-0.03387	4.70737+2.93497x	0.154	4.183	5.30
Malur	300	0.0311	0.02128-0.04248	4.17100+2.76743x	0.216	6.162	6.62
Devanahalli	300	0.05718	0.03837-0.08400	2.60228+2.09401x	0.738	0.279	12.17
Shidlaghatta	300	0.04513	0.03447-0.05912	5.02682+3.73580x	0.189	1.931	9.60
Pooled		0.03639	0.03133 -0.04183	3.80898+2.64685x	0.031	7.466	

RR* = LC₅₀ (strains from different location)/LC₅₀ (lab strain, JJ-Lab)

Contd ...

Table 17 contd....

Strains	n	LC ₅₀ (%a.i.)	Fiducial limits @95% (%a.i.)	Y= a+bx	LC ₉₉ (%a.i.)	χ^2	RR
Spinosad							
Lab culture	300	0.0000035	0.2498-0.5086 x 10 ⁻⁵	2.71095+1.86918x	0.00006	36.933	1.00
Hoskote	300	0.00002	0.00001-0.00003	9.50833+2.02008x	0.00028	3.344	5.64
Doddaballapura	300	0.00001	0.00001-0.00002	7.25463+1.48139x	0.00047	3.828	2.82
Malur	300	0.00003	0.00002-0.00005	9.32523+2.06141x	0.0004	2.426	8.46
Devanahalli	300	0.00004	0.00003-0.00006	8.12643+1.85733x	0.00075	1.563	11.28
Shidlaghatta	300	0.00003	0.00002-0.00004	8.31609+1.80893x	0.00049	0.453	8.46
Pooled		0.00002	0.00002-0.00003	8.32423+1.77972x	0.00043	5.534	
Neemazal-F							
Lab culture	300	0.01498	0.01095-0.01921	4.178+2.289x	0.155	0.806	1.00
Hoskote	300	0.01659	0.00716 - 0.03302	1.99061+1.11826x	1.996	2.17	1.11
Doddaballapura	300	0.01319	0.00683-0.02338	2.77206+1.47460x	0.499	0.591	0.88
Malur	300	0.00973	0.00572-0.01582	4.03641+2.00646x	0.141	0.567	0.65
Devanahalli	300	0.01495	0.00899 - 0.02369	2.92230+1.60095x	0.424	1.137	1.00
Shidlaghatta	300	0.01128	0.00414-0.02244	2.15555+1.10679x	1.427	3.92	0.75
Pooled		0.01251	0.00895 - 0.01689	2.49759+1.31261x	0.155	2.645	
NSKE							
Lab culture	300	0.03836	0.02263-0.05679	1.626+1.1484x	4.07	5.401	1.00
Hoskote	300	0.08315	0.05534 - 0.14039	2.58167+2.27383x	0.772	1.826	2.17
Doddaballapura	300	0.07457	0.04727 - 0.12795	2.06752+1.83386x	1.384	2.91	1.94
Malur	300	0.11899	0.07311 - 0.20594	1.58381+1.71317x	2.713	1.209	3.10
Devanahalli	300	0.02918	0.01710 - 0.04818	2.51341+1.63744x	0.769	5.373	0.76
Shidlaghatta	300	0.06181	0.02940-0.11566	1.54069+1.27442x	4.135	2.845	1.61
Pooled	300	0.06652	0.05266-0.08516	2.01626+1.71302x	1.517	2.307	

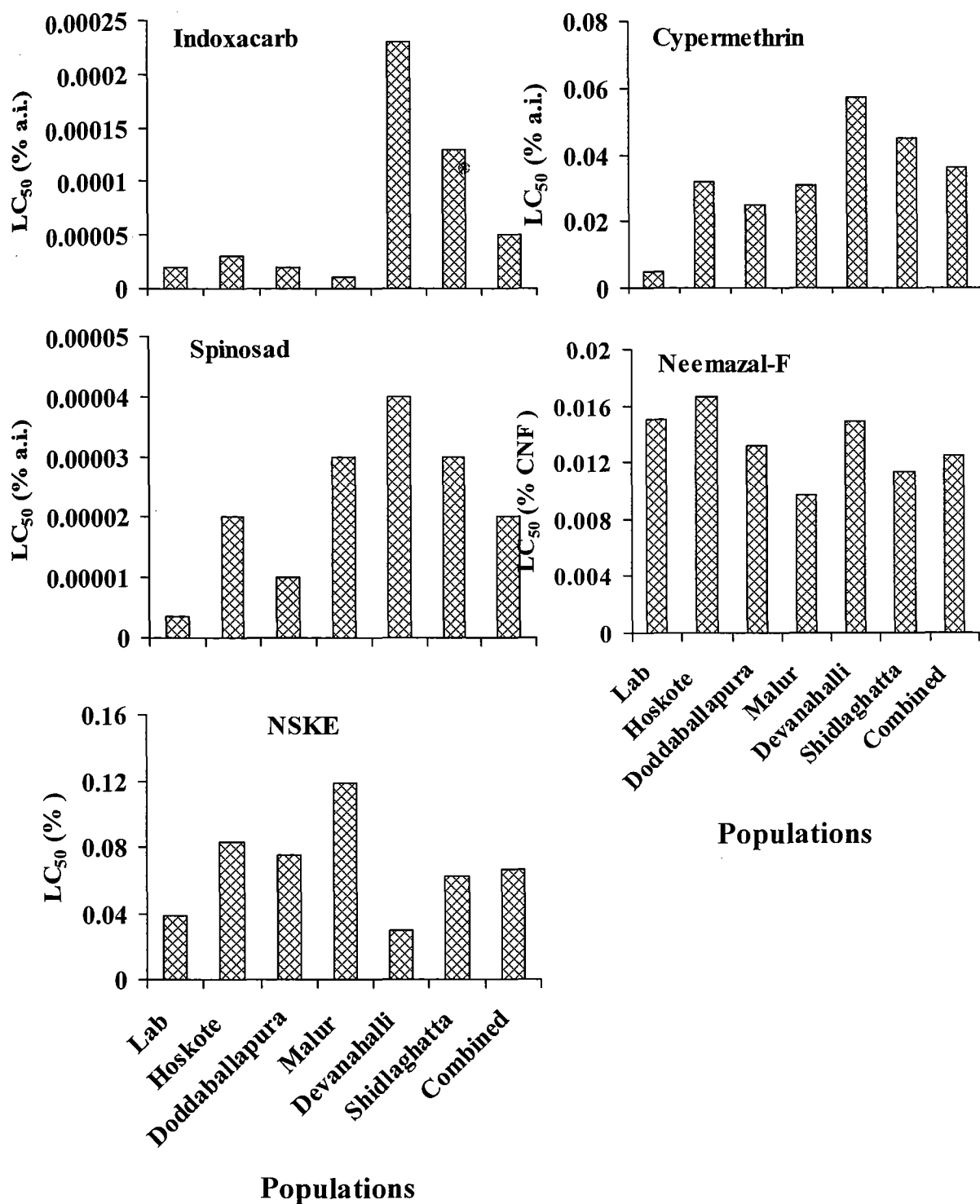


Figure 14: LC₅₀ values of the five different strains of *Plutella xylostella*, collected from around Bangalore during 2005 tested against selected insecticide, indoxacarb, spinosad, cypermethrin, neemazal-F and NSKE.

The results revealed a high degree of variability between the field strains *vis-a-vis* susceptibility to indoxacarb. Interestingly, the populations of DBM from Malur (0.00001 % a.i.) was more susceptible than even the JJ-Lab strain, used as the reference susceptible population.

4.1.1.2 Relative susceptibility of field strains to cypermethrin

Pyrethroids were widely and intensively used insecticides in the cabbage ecosystem, during the nineties. Cypermethrin, among them, is still in use either singly, or in combination with other insecticides. Therefore, this was chosen for evaluation as a representative of the insecticide with a history of past usage around Bangalore.

The LC_{50} values of the five field strains based on non-overlapping fiducial limits, were significantly ($p < 0.05$) different from susceptible JJ-Lab strain (0.0047 % a.i.; Table 17; Fig. 14). However, the field strains did not differ from one another in their susceptibility to cypermethrin. The highest resistance ratio of 12.17 fold was recorded in Devanahalli (0.05718 % a.i.) strain followed by Shidlaghatta (0.04513 % a.i.; RR = 9.6 fold) strain. Doddaballapura (0.0249 % a.i.) strain with 5.3 fold resistance was the most susceptible among the field strains. Hence, it can be concluded that almost all field strains are more or less equally tolerant to this insecticide of long usage, compared to the highly susceptible lab strain.

4.1.1.3 Relative susceptibility of field strains to spinosad

Spinosad was one of the most widely used insecticides for the management of DBM at the time of survey (Table 15; January 2005). Therefore, this insecticide was selected for ascertaining the susceptibility of different field populations.

Devanahalli strain was highly resistant (LC_{50} =0.00004 % a.i.; 11.28 fold) among the five strains tested. Malur and Shidlaghatta strains with equal LC_{50} of 0.00003 per cent a.i. (8.46 fold) were next to Hoskote population (0.00002 % a.i.) in their level of resistance. The lowest LC_{50} value was recorded in Doddaballapura (0.00001 % a.i; 2.82 fold RR) strain. However, LC_{50} values of all the five field collected strains were significantly different from the susceptible JJ-Lab strain (0.0000035 % a.i; $p < 0.05$; Table 17; Fig. 14). Devanahalli population being the most resistant was significantly different

from Doddaballapura strain. Hoskote, Malur and Shidlaghatta strains were all on par with Devanahalli strain.

4.1.1.4 Relative susceptibility of field strains to NSKE

Neem Seed Kernel Extract (NSKE) is a widely recommended bio-pesticide for insect pest management in India. It has been shown to be of excellent pest management value against DBM in cabbage and has been recommended in Karnataka since 1993. Therefore, the populations from around Bangalore were tested to know their relative susceptibility to NSKE.

Highest LC_{50} of 0.119 per cent of NSKE was recorded in Malur strain which is 3.10 fold higher than the JJ-Lab strain and was followed by Hoskote (0.083 % NSKE; RR = 2.17 fold). The lowest of 0.0292 per cent of LC_{50} was recorded in Devanahalli (RR=0.76 fold) strain (Table 17; Fig. 14), which was lower than that of JJ-Lab (0.03834 % NSKE) population. LC_{50} values of the field strains were not significantly ($p > 0.05$) different from the susceptible, JJ-Lab, strain suggesting lack of differences among the field populations for their susceptibility to NSKE. Therefore, all the field strains tested remain as susceptible as the lab strain and are yet to develop resistance even after a decade of recommended use in cabbage ecosystem.

4.1.1.5 Relative susceptibility of field strains to Neemazal-F

Azadirachtin is one of the main active components of neem seeds. Many commercial neem formulations (CNF) are available in the market with varying levels of azadirachtin. Neemazal-F with 50,000 ppm azadirachtin is the most concentrated product available in the market and can thus serve as an ideal replacement for NSKE to be used as an off-the-shelf biopesticide. Hence, Neemazal-F was used as one of the insecticides for testing susceptibility of DBM, in lieu of the pure azadirachtin.

LC_{50} values for Neemazal-F ranged from 0.0097 in Malur to 0.01659 per cent in Hoskote strain that were 0.65 and 1.11 fold higher than the LC_{50} of susceptible JJ-Lab (0.01498 % CNF) strain, respectively (Table 17; Fig. 14). LC_{50} values of Doddaballapura (0.01319 % CNF), Malur (0.00973 % CNF) and Shidlaghatta (0.01128 % CNF) were lower than the susceptible, JJ-Lab LC_{50} values. However, LC_{50} values of the field collected strains were not significantly different from susceptible, JJ-Lab strain ($p > 0.05$).

As a result, the susceptibility levels of all the field collected strains of DBM were on par with the lab strain, which was also the case with NSKE. Thus it appears that both lab and field populations of DBM behave similarly with both NSKE and Neemazal-F.

4.1.2 Comparison of relative susceptibility of field strains of DBM against five selected insecticides: comparison across insecticides and populations

Owing to the difficulty of using the median lethal concentrations of the different insecticides, the five field collected strains were compared using the resistance ratios calculated on the basis of the values of the susceptible lab culture (JJ-Lab). This provided a uniform platform for comparing the strains.

It was observed that the mean resistance ratios of all the field strains to different insecticides varied considerably (Fig. 15A). The field strains were more resistant to cypermethrin with the mean resistance ratio of 8.086 ± 2.770 fold. This was followed by spinosad (7.332 ± 3.215 fold) and indoxacarb with 4.2 ± 4.74 folds of RR. However, the lowest RR (0.878 ± 0.185) was recorded against Neemazal-F. Against NSKE, the mean ratio (1.916 ± 0.851) was next to Neemazal-F.

Mean resistance ratio of all insecticides in each field strain was computed to know the most resistant field strain among the five strains tested. Devanahalli strain was highly resistant with the mean RR of 8.77 ± 4.49 folds, followed by Shidlagahatta (6.65 ± 2.56), Malur (5.25 ± 2.96) and Hoskote (5.24 ± 2.6) strains. Thus the overall susceptibility of Doddaballapura strain (3.87 ± 2.17 ; Fig. 15B) was the highest among the five populations of DBM compared. Despite the number of insecticides tested being quite low, it was interesting to observe that a strong trend in the relative susceptibility/tolerance of the field cultures was visible among the populations studied.

4.1.3 Relation between insecticides based on the population responses

It is possible that the population responses can vary greatly for each tested insecticide as indicated above. However, this variation could be related among the insecticides tested, meaning the response patterns of different populations are similar for each tested insecticide so that the variation in the susceptibility to one insecticide can be gauged by the relative susceptibility to other. As a result, the positive and negative correlations between any two insecticides suggest the potential corresponding cross

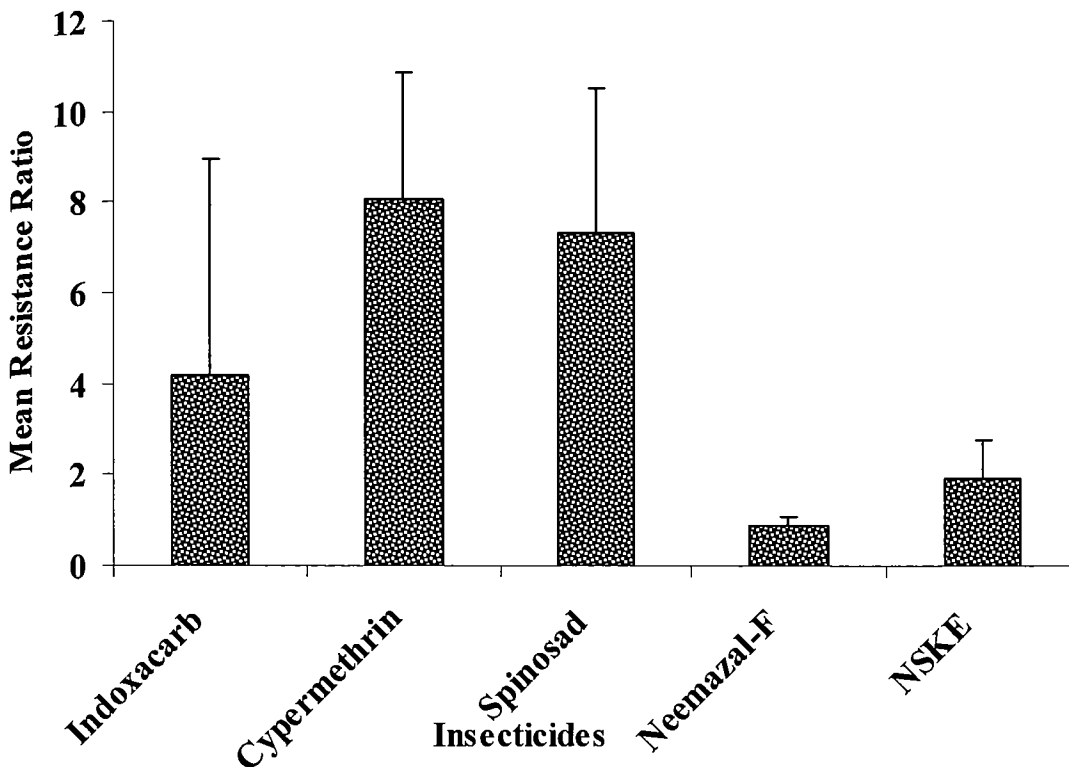


Figure 15A: Mean resistance ratios for different insecticides calculated on the basis of the response pattern of the five field collected strains compared to the lab strain (JJ-Lab) of *P. xylostella*. Vertical bars represent one standard deviation. RR is the ratio of LC_{50} of field collected strain divided by LC_{50} of lab strain JJ-Lab. Mean is the mean RR of all field collected strains for each insecticide tested.

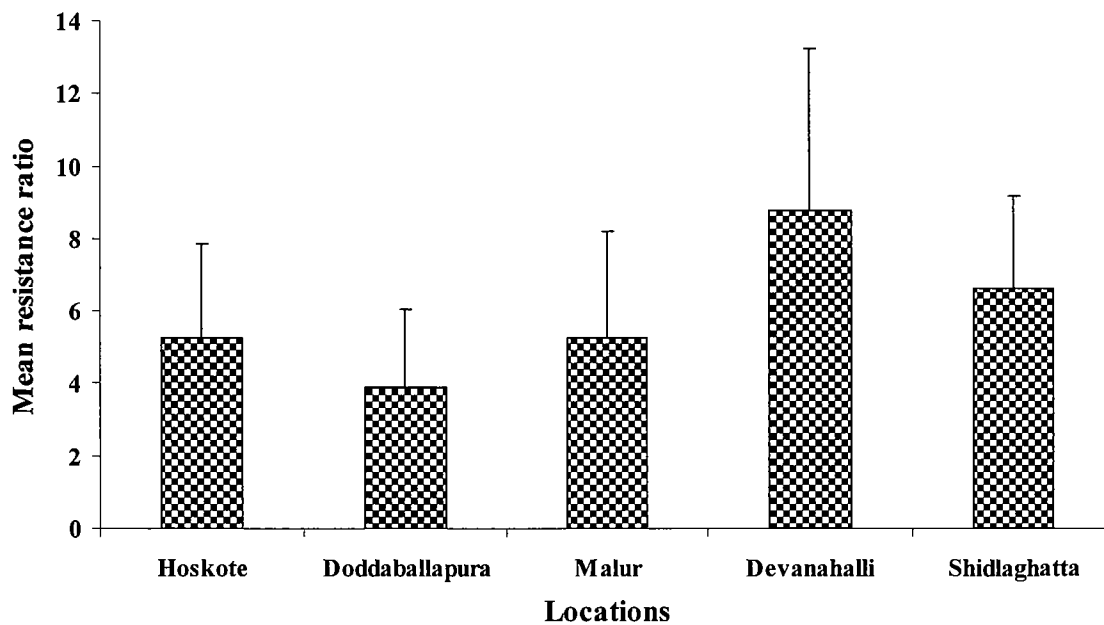


Figure 15B: Mean resistance ratio of the different strains collected from different locations around Bangalore. Resistance ratio are the mean of the resistance ratios of the five insecticides, indoxacarb, spinosad, cypermethrin, neemazal-F and NSKE in a particular field strain. Vertical bars represent one standard deviation

resistance. Hence, correlations between all possible combinations between five insecticides were carried out (Table 18; Fig. 16). Among the 10 possible combinations only three provided significant correlations. Significant positive correlations were observed between indoxacarb and cypermethrin ($r = 0.976$; $p = 0.004$) and between cypermethrin and spinosad ($r = 0.871$; $p = 0.05$). However, the relation between indoxacarb and NSKE was strongly negative ($r = -0.886$; $p = 0.045$).

Further, indoxacarb and spinosad demonstrated positive correlation, although was not significant ($p > 0.05$). Neemazal-F did not have any significant correlation with any of the other four insecticides tested. Therefore, the study indicated that DBM populations show similar response patterns across synthetic insecticides like indoxacarb, cypermethrin and spinosad, while they may behave differently between a plant based insecticide and a synthetic insecticide. This was evident by the fact that NSKE largely indicated negative associations with synthetic insecticides, while Neemazal-F was indifferent to other tested chemicals.

4.1.4 Comparison between locations to their susceptibility across five insecticides

Associational studies were also carried out between pairs of locations using pairs of LC_{50} values for each insecticide as the replicates. In general, different populations were strongly associated in respect of their LC_{50} values for different tested insecticides. Hoskote, Doddaballapura, Malur and Shidlaghatta populations demonstrated, significant positive correlations (' r ' values ranged from 0.896 to 0.999; $p < 0.05$; Table 19) between any two pairs. However, Devanahalli strain behaved entirely differently and did not show any association (' r ' value varied from 0.447 to 0.797; $p < 0.05$) with other four strains. Thus the results suggest that the overall behaviour of the populations is largely similar for the five insecticides tested, showing related patterns of tolerance for different insecticides tested. Devanahalli strain being more resistant among the strains tested to synthetic insecticides and being more susceptible to NSKE, was not strongly associated with other populations. Which, in essence, reflect differential selection pressures being experienced by the various populations of DBM studied.

4.2 Studies on selection for insecticidal resistance in *Plutella xylostella*

Five field populations of DBM collected from around Bangalore were pooled at the pupal stage and emerging adults were allowed to inter-mate. The pooling, it was

Table 18: Inter correlation matrix for the LC_{50} values of different insecticides tested against the second instar larvae of *Plutella xylostella* collected from different locations around Bangalore during January, 2005. LC_{50} s of different populations (Collected from different locations) were used as the pairs of data for calculation of correlation coefficient.

Insasecticides	Indoxacarb	Cypermethrin	Spinosad	Neemazal-F	NSKE
Indoxacarb	1.00				
Cypermethrin	0.976 (0.004)	1.00			
Spinosad	0.759 (0.137)	0.871 (0.055)	1.00		
Neemazal-F	0.232 (0.708)	0.158 (0.8)	-0.137 (0.826)	1.00	
NSKE	-0.886 (0.045)	-0.778 (0.122)	-0.376 (0.532)	-0.502 (0.389)	1.00

*Values in parentheses indicate the p value ; $p \leq 0.05$ = significant at 5%

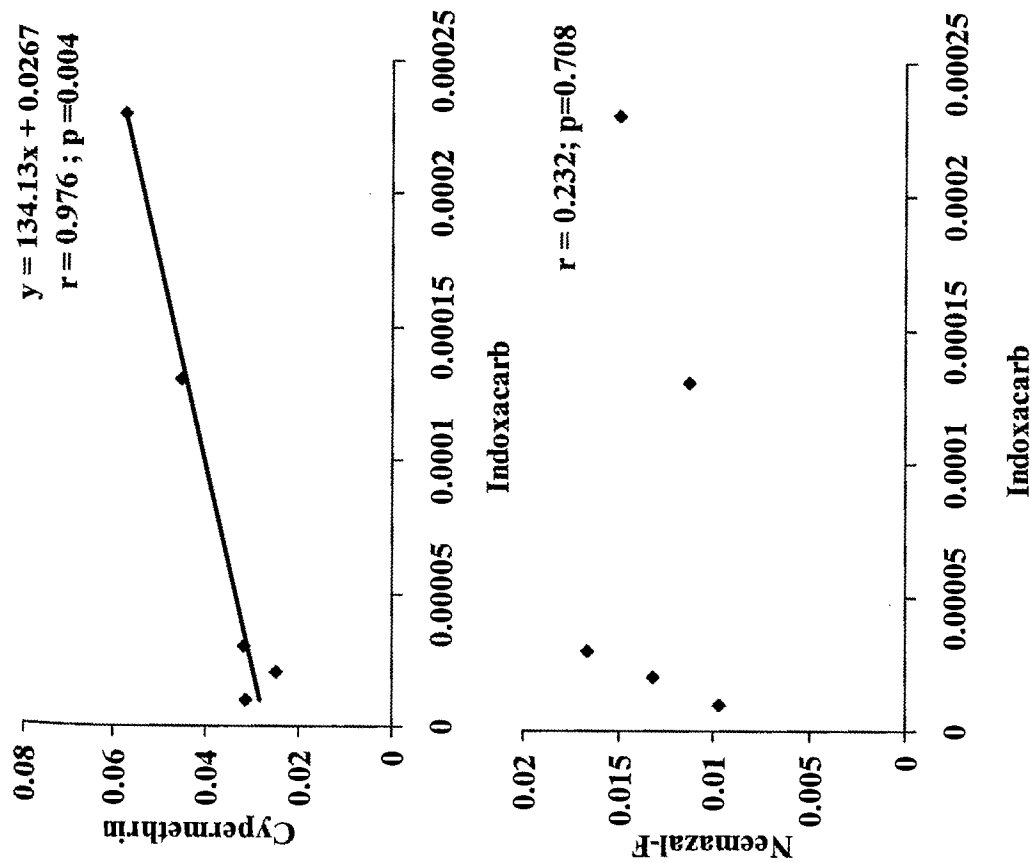


Figure 16: Interrelation between the pairs of five different insecticides (indoxacarb, cypermethrin, spinosad, neemazal-F and NSKE) tested against the five field populations of *Plutella xylostella* collected from around Bangalore during January 2005. Each point represents a pair of LC₅₀ values for two insecticides under scrutiny for a given population.

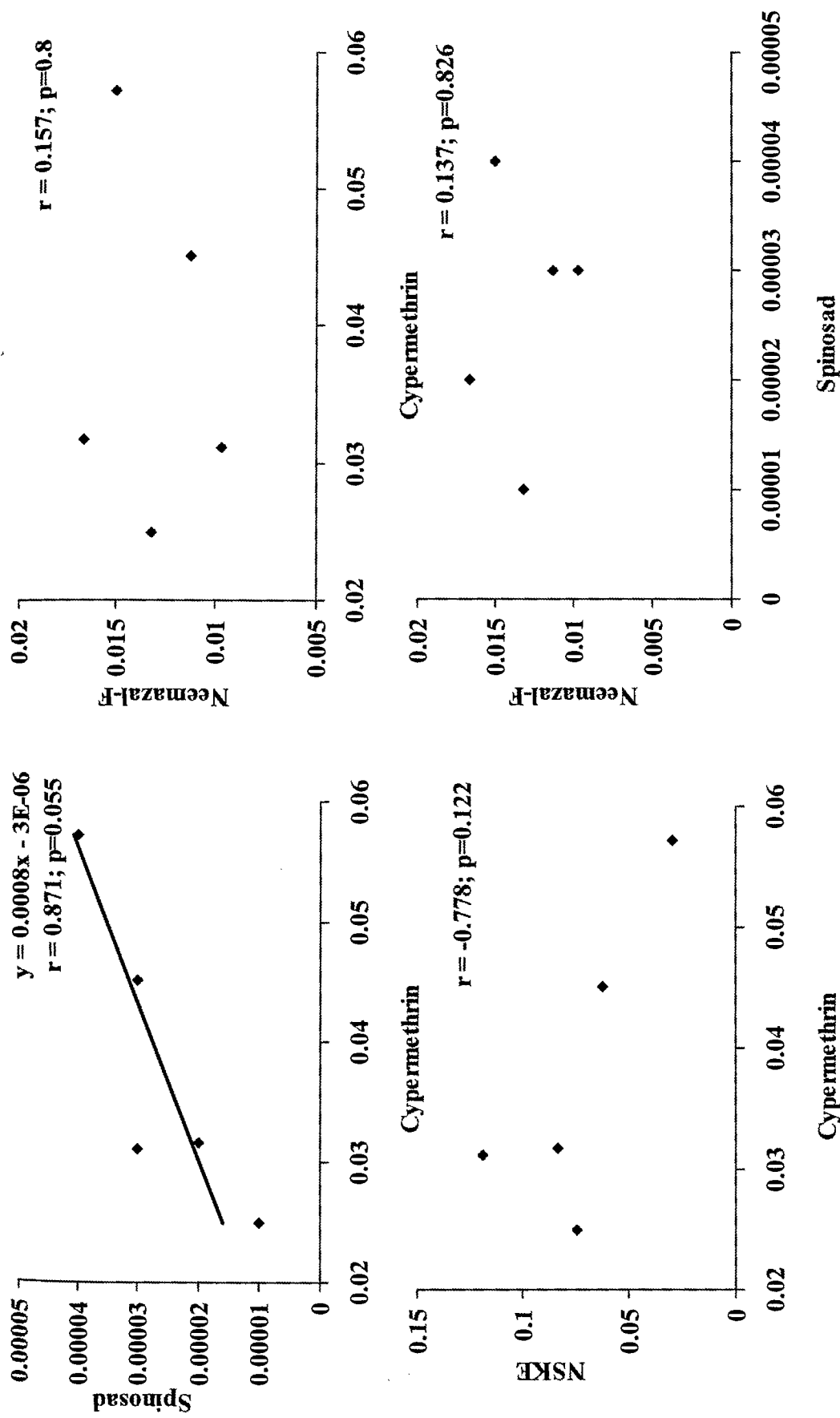


Figure 16 contd... Interrelation between the pairs of five different insecticides (indoxacarb, cypermethrin, spinosad, neemazal-F and NSKE) tested against the five field populations of *Plutella xylostella* collected from around Bangalore during January 2005. Each point represents a pair of LC₅₀ values for two insecticides under scrutiny for a given population.

Figure 16 Contd...

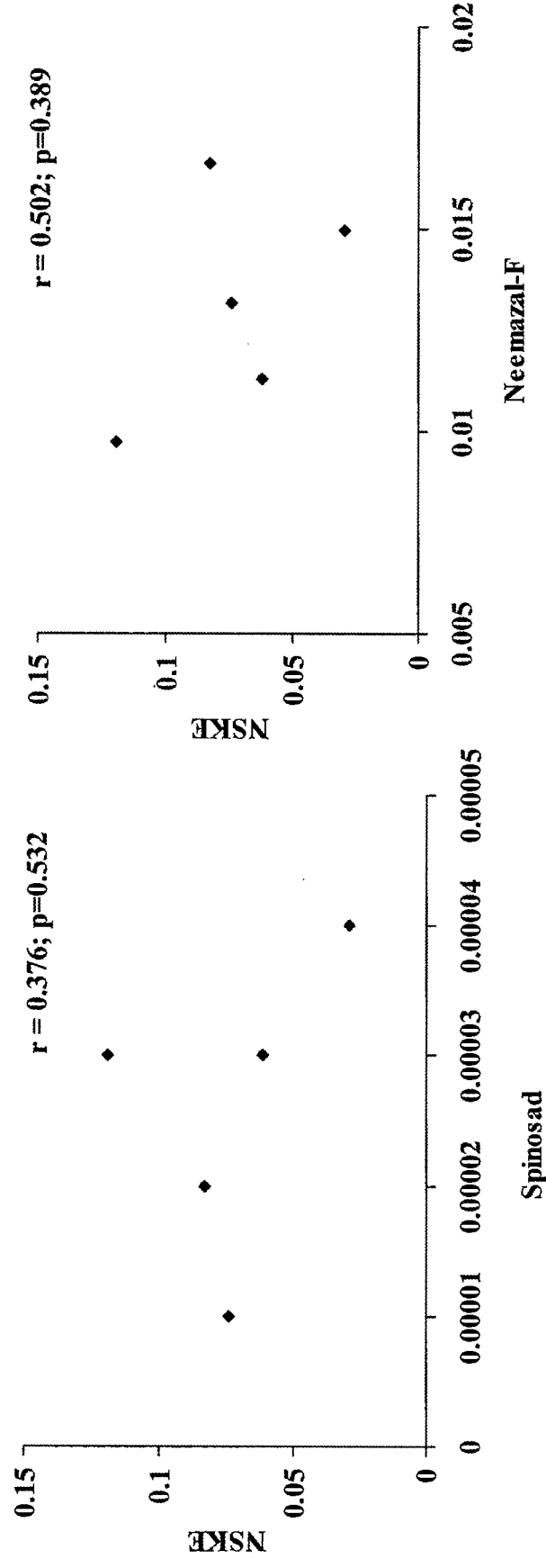


Figure 16: Interrelation between the pairs of five different insecticides (indoxacarb, cypermethrin, Spinosad, neemazal-F and NSKE) tested against the five field populations of *Plutella xylostella* collected from around Bangalore during January 2005. Each point represents a pair of LC_{50} values for two insecticides under scrutiny for a given population.

Table 19: Association between the strains of *Plutella xlostella* collected from different locations from around Bangalore for LC₅₀ values of five different insecticides tested during January, 2005. Data represent the pearson's product moment correlation between pairs of studies.

Locations	Hoskote	Doddaballa pura	Malur	Devanahalli	Shidlaghatta
Hoskote	1.00				
	0.999				
Doddaballapura	(0.000)**	1.00			
	0.988	0.994			
Malur	(0.002)**	(0.001)	1.00		
	0.552	0.511	0.447		
Devanahalli	(0.334)	(0.379)	(0.450)	1.00	
	0.939	0.922	0.896	0.797	
Shidlaghatta	(0.018)*	(0.026)*	(0.039)*	(0.106)	1.00

Values in parentheses indicate the p value ;

hoped, would provide a wider genetic base for imposing the selections. Third instar larvae obtained from this pooled stock were exposed to indoxacarb, cypermethrin, Neemazal-F and NSKE for selection to resistance. Each selected strain was correspondingly named JInd-R, JCyp-R, JNzF-R or JNSKE-R. One unselected susceptible stock (JField) from the pooled population was also maintained.

4.2.1 Selection concentrations and per cent mortality

During the course of selection for resistance to insecticides, at each generation data were recorded on the number of larvae treated, per cent mortality, per cent pupation, per cent adult emergence *etc.*, in all the selected strains.

4.2.1.1 Selection for resistance to indoxacarb in JInd-R strain

Selection for resistance to indoxacarb was initiated with treatment of the larvae at a concentration of 0.00007 per cent a.i. during the F₁ generation of field collected populations. At the 24th generation, the larvae were selected at 0.00725 per cent a.i. that was 103.57 fold higher than the initial concentration (Appendix XIa; Fig. 17a) of selection. Selections were imposed in all the generations of selection. However, selection concentrations were changed once in 2-3 generations, whenever there was an increase in percent survival of adults reaching closer to 50 per cent of the number of the larvae treated. Usually, the changed dose was twice the previous selection concentration. Increase in concentration over generations in terms of number of folds compared to the initial concentration was observed to be exponential in growth ($y = 1.075e^{0.2383x}$; $r = 0.919$; $n = 24$; $p < 0.05$; Fig. 18a). These concentrations, on an average, over generations, imposed a selection pressure of 72.72 ± 15.64 per cent which was measured as the extent of mortality (Fig. 19a).

4.2.1.2 Selection for resistance to cypermethrin in JCyp-R strain

Selection for resistance to cypermethrin in JCyp-R strain of DBM was carried out with an average of 75.26 ± 14.92 per cent (Fig. 19b) of selection pressure at adult emergence over 24 generations. However, due to very high mortality (87.26 %) at adult emergence at the tenth generation of selection, larvae were not treated during the 11th generation of rearing. From 12th generation onwards regular selection was resumed. Then onwards response to selection was observed to be quite high (Appendix XIb).

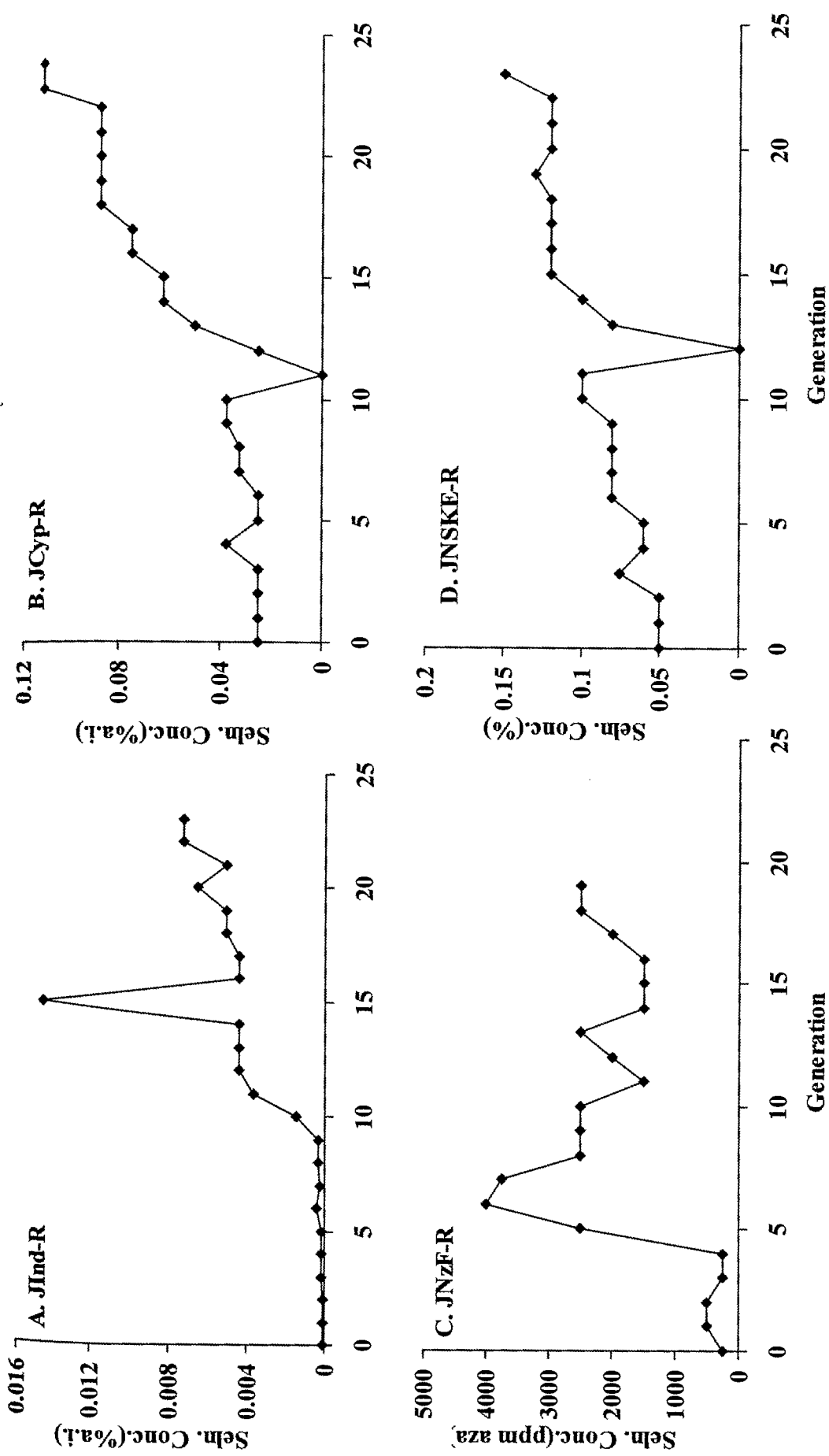


Figure 17: The concentration of insecticides used over generations for selecting *Plutella xylostella* for resistance to indoxacarb, cypermethrin, neemazal-F and NSKE, against III instar larvae of JInd-R, JCyp-R, JNzF-R and JNSKE-R strains, respectively during 2005-2006.

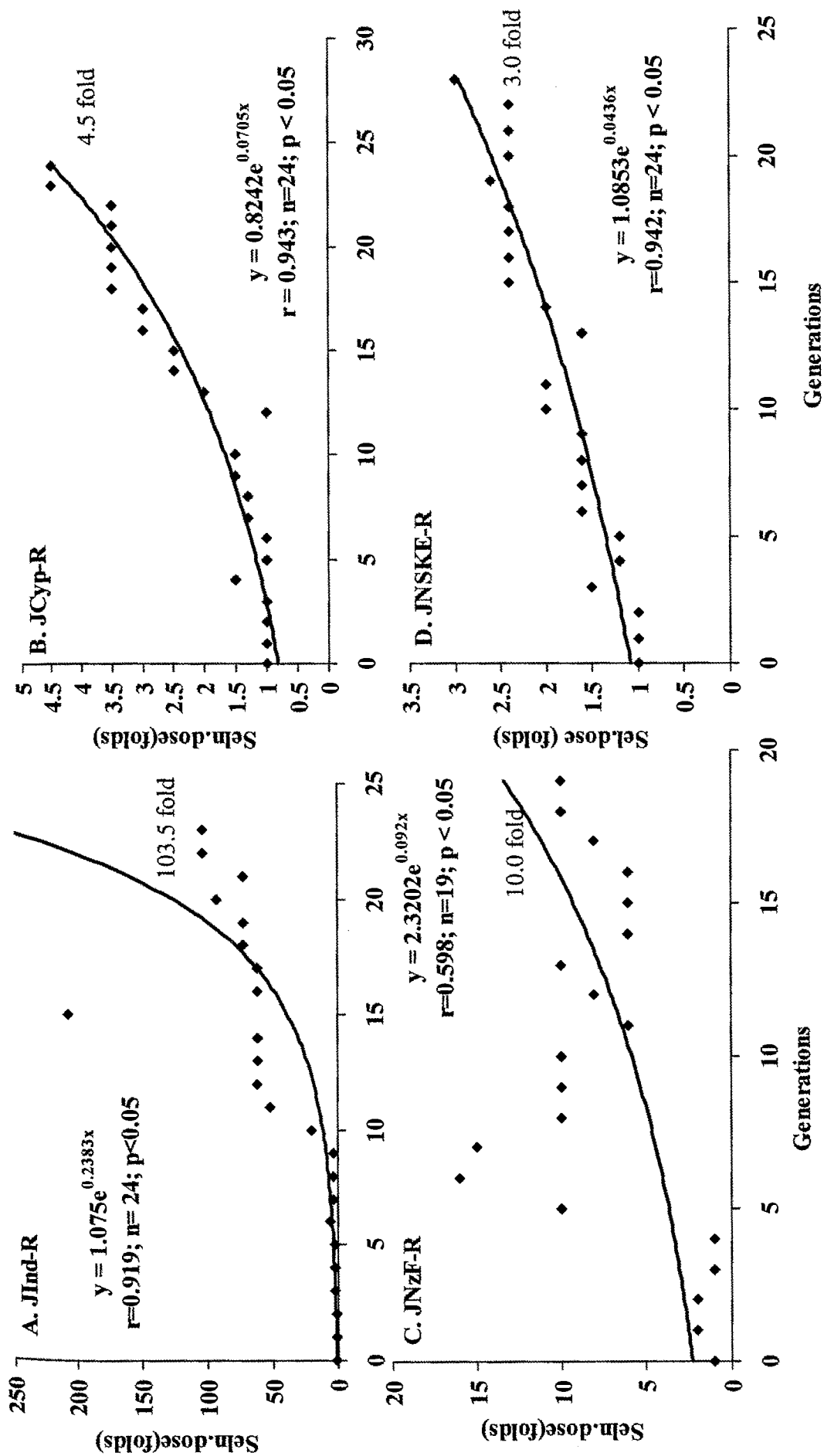


Figure 18: Change in dosage of the selection agents, indoxacarb, cypermethrin, neemazal-F and NSKE represented as folds of the initial value through the generations in four strains JInd-R, JCyp-R, JNSKE-R and JNzF-R, respectively of *Plutella xylostella*.

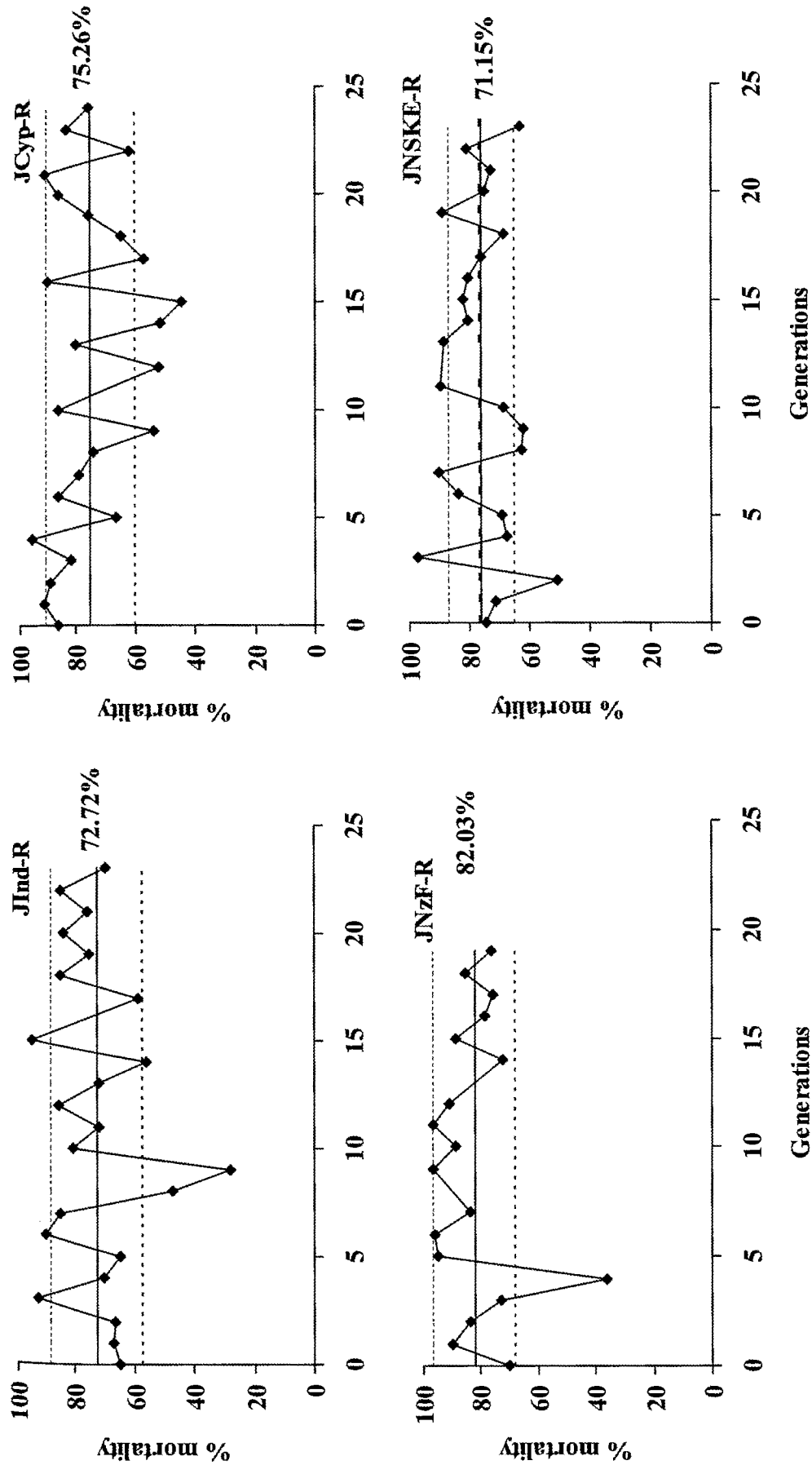


Figure 19: The per cent selection pressure (%mortality at adult emergence) imposed on 3rd instar larvae of JInd-R, JCyp-R, JNzF-R and JNSKE-R strains of *Plutella xylostella* to selection for resistance to four insecticides, indoxacarb, cypermethrin, neemazal-F and NSKE respectively. Solid line (—) indicates mean % percent selection pressure imposed, dotted lines (- - -) indicate one standard deviation on either side of the mean value.

Initial selection concentration imposed on the F_1 population was 0.025 per cent a.i. By the end of 24 generations of selection, the concentration was increased to 4.5 (0.1125 per cent a.i.) folds the initial concentration of selection (Fig. 17b) by changing the selection concentrations once every 3-4 generations. The changed concentrations over generations (in terms of number of folds) increased exponentially ($y = 0.8242e^{0.0705x}$; $r = 0.943$; $n = 24$; $p < 0.05$; Fig. 18b).

4.2.1.3 Selection for resistance to Neemazal-F in JNzF-R strain

As Neemazal-F was a commercial neem formulation with known concentration of azadirachtin, it was possible to ascertain the concentration of the azadirachtin during the selection treatments. The JNzF-R strain experienced an average of 82.03 ± 14.38 per cent (Appendix XIc; Fig. 19c) selection pressure during the course of selection over 19 generations of selection. To maintain this selection pressure, the III instar larvae were treated with 250 to 2500 ppm of aza over the selection period (Fig. 17c), by scaling up the selection gradient at suitable intervals. This increase, which was equivalent to 10 folds of the initial selection concentration by the nineteenth generation, roughly followed an exponential trajectory in growth. However, the relationship did not fit the exponential growth ($y = 2.3202e^{0.092x}$; $r = 0.598$; $n = 19$; $p < 0.05$; Fig. 18c).

4.2.1.4 Selection for resistance to NSKE in JNSKE-R strain

The III instar larvae of JNSKE-R strain were subjected to selection with NSKE at different concentrations over generations for developing resistance. NSKE being a crude aqueous extract of the neem seeds and as the seeds are known to vary in the concentration of azadirachtin with time, the exact concentration in terms of the azadirachtin content could not be ascertained. Therefore, all concentrations are worked out in terms of seed kernels (wt/vol). The selection concentration ranged from 0.05 to 0.15 per cent of NSKE (three folds higher than the initial selection concentration; Fig. 17d) over 24 selection events. Concentrations were changed over generations to maintain a mean selection pressure of 71.15 ± 20.41 per cent (mortality at adult emergence; Fig. 19d). However, during the 11th generation the selection concentration used resulted in 89.55 per cent mortality at adult emergence. Combined with reduced fecundity, this mortality level resulted in very low number of larvae in the 12th generation (Appendix XId). Hence, during the 12th generation, in order to sustain the culture, no selection pressure was imposed.

Barring this exception, the change in selection concentrations was observed to grow exponentially over the 24 generations of selection ($y = 1.0853e^{0.0436x}$; $r = 0.942$; $n = 24$; $p < 0.05$; Fig. 18d).

The synthesis:

In summary the increase in selection concentration was highest in JInd-R strain for selecting against indoxacarb (103.57 fold) and was followed by Neemazal- F in JNzF-R strain (10 fold). However, increase was only 4.5 folds in JCyp-R strain against cypermethrin and 3 folds in JNSKE-R strain against NSKE. On an average 72 to 82 per cent of selection pressure was maintained in all the strains during the course of selection for development of resistance in different cultures.

Despite this enormous variation, a strong uniformity that emerges is in the growth trajectories of the selection concentrations for the four insecticides. All the strains experienced an exponential growth in the selection concentrations over the generations, although, there were apparent strong differences in the slopes of the curves fitted (Fig. 18). Although, exponential trend was the best fit function for all the cultures, Neemazal-F provided the poorest fit. What is more striking is the emergence of the uniform trend despite various errors during the course of selection that at times resulted in the usage of overdoses causing the populations to reach the near extinction stages.

4.2.2 Survivorship patterns when selection concentrations are held constant over generations

During the course of selection for resistance to four selected insecticides, on occasions, the selection concentrations were held constant for two to four generations. This was largely to sustain the cultures going and was generally carried out whenever the survival rates substantially decreased (Fig. 17). As a result, it would be interesting to know the pattern of survival rates over generations whenever the selection concentrations were held constant for two to four generations. This was difficult to test due to lack of uniformity in the data in respect of generations, survival rates and also in terms of concentrations. Therefore, in order to bring all this data on a common platform, an attempt was made to represent the starting generation for any constant selection concentration as one and the subsequent generations were counted linearly. Secondly, the

per cent survival in the first of these generations was used as the base value, irrespective of the absolute value and the survival percentages in subsequent generations were calculated as the ratios of the values of this generation. Data of many such episodes were treated as independent replications irrespective of the number of generations for which the concentrations were held constant. These were plotted against the number of generations over which the selection concentration was held constant to know the pattern of change in survivorship.

It was observed that the change in survivorship increased exponentially as a ratio of survivorship in the first generation in JInd-R strain on selection with indoxacarb whenever the selection concentration was held constant ($y = 0.7784e^{0.485x}$; $r = 0.782$; $n = 11$; $p < 0.05$; Fig. 20a). Similarly, the survivorship ratios increased through generations in an exponential pattern whenever the selection concentrations were held constant, even among JCyp-R strain ($y = 0.6149e^{0.3102x}$; $r = 0.718$; $n = 15$; $p < 0.01$; Fig. 20b) and JNSKE-R strain ($y = 0.7713e^{0.213x}$; $r = 0.713$; $n = 16$; $p < 0.01$; Fig. 20d). However, a check on JNzF-R strain for similar behaviour suggested a lack of significant fit for exponential function ($r = 0.325$; $n = 12$; $p > 0.05$; Fig. 20c). Nevertheless, the correlation coefficient was highest for exponential function rather than other potential linear and non-linear functions verified. Thus the results overwhelmingly suggest an exponential pattern of increase in survivorship rates for populations under selection by an insecticide whenever the selection intensity measured as the dose or concentration remained constant over generations. But the slopes (b values) of these functions appeared to vary among the selection agents (insecticides). Highest 'b' ($b = 0.485$) value was recorded in JInd-R strain and lowest slope ($b = 0.1447$) was in JNzF-R strain against Neemazal-F. A check on the extent of variation among the four cultures suggested lack of significant differences in the slopes of the four exponential functions (ANCOVA; $F_{3,44} = 1.519$; $p > 0.05$). Thus the study further suggested a great uniformity among the cultures under insecticidal selection to show similar relief-response from repression whenever the selection intensity remained constant over generations.

4.3 Response of DBM to selection for insecticide resistance

Bio-assays were carried out at different generations during selection for resistance to monitor changes in the level of resistance over generations. Median lethal

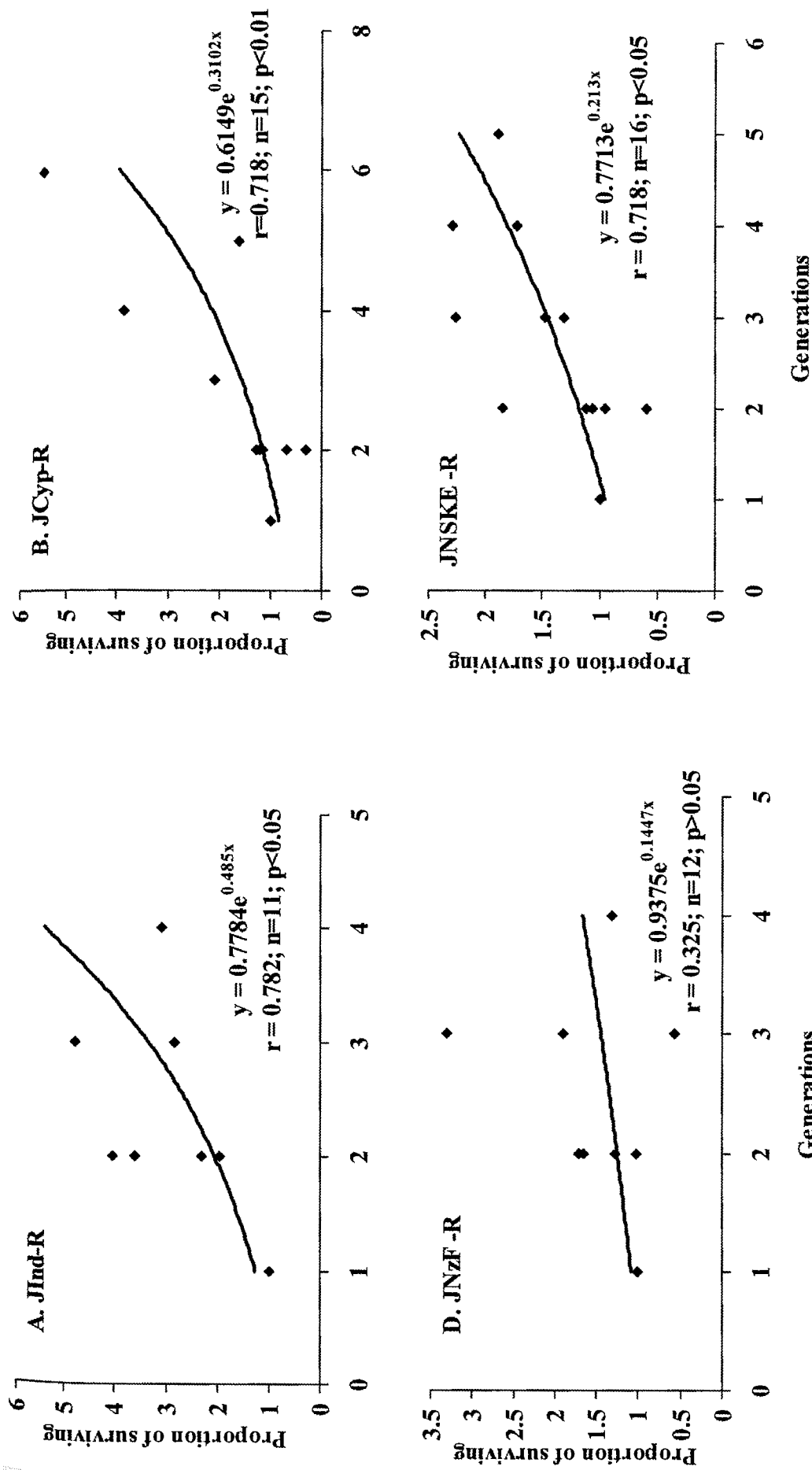


Figure 20: Change in proportional survival of treated individuals when selection dosage is kept unchanged through successive generations of four insecticide selected strains of *Plutella xylostella*. (JInd-R -Indoxacarb; JCyp-R- Cypermethrin; JNzF-R- Neemazal-F and JNSKE-R -NSKE).

concentrations calculated from bioassays were used for working out the resistance ratios (RR) as a measure of development of resistance. Resistance ratios were the ratios of median lethal concentrations of selected populations to that of the base line values generated before the imposition of selection (Pooled LC₅₀s of F₁ generations, JField strain) in different insecticide resistant strains.

Slope of the probit mortality curve plotted against the log concentrations can also be used as an alternative indicator of the change in population response. However, slopes indicate the homogeneity of the population. Flatter slopes with lower values are indicative of the heterogeneous population (before selection). However, when ever the selections are imposed and the population is moving towards the homogeneity with respect to that character (insecticide resistance), slope of the log concentration-probit mortality line becomes steeper. However, this needs to be viewed at times in conjunction with the intercept of the probit mortality line.

4.3.1 Response to selection for insecticidal resistance – the LC₅₀s

4.3.1.1 Response to selection for indoxacarb resistance in JInd-R strain

Pooled field populations with a mean (pooled) LC₅₀ of 0.00005 per cent a.i. of indoxacarb was subjected to selection over 14 generations with a mean selection pressure of 72.72 ± 15.65 per cent in JInd-R strain. After three generations of continuous selection there was a significant increase in LC₅₀ value (0.00084 % a.i.; $p < 0.05$). Then onwards, there was continuous increase in LC₅₀ values (Table 20A) through generations. At 14th generation of selection an LC₅₀ of 0.01245 per cent a.i. was recorded, that was equivalent to 249 fold shift from the base line value.

A pertinent question here would be the nature of trend in the way the resistance ratios changed through the generations of selection. Both selection concentrations and the changes in survivorship whenever the selection concentration was held constant, demonstrated exponential growth. In consonant with these results the LC₅₀ values over generations also demonstrated a strong exponential growth ($y = 0.0001e^{0.3379x}$; $r = 0.931$; $n = 9$; $p < 0.01$; Fig. 21a) under selection.

Table 20: Response measured as LC_{50} values of insecticide selected resistant *Plutella xylostella* cultures to corresponding insecticides during the course of selection, 2005-2006.

Sl.No.	Gener ation	LC_{50} (%a.i.)	Fiducial limit @95% (UL-LL - %a.i.)	$Y = a + bx$	LC_{99} (%a.i.)	χ^2	RR*
A. JInd-R strain							
1	0	0.00005	0.00003-0.00006	4.55692+1.05156x	0.00756	1.503	1.00
2	2	0.00084	0.00062-0.00122	5.06599+1.64689x	0.02170	4.725	16.80
3	4	0.00058	0.00044-0.00073	6.49073+2.00368x	0.00835	9.217	11.60
4	6	0.00129	0.00044-0.00512	4.38164+1.51590x	0.04407	12.279	25.80
5	9	0.00360	0.00265-0.00504	6.44291+2.63658x	0.02746	8.715	72.00
6	10	0.00858	0.00762-0.00966	15.43052+7.46767x	0.01759	1.760	171.60
7	11	0.00346	0.00253-0.00479	6.06514+2.46412x	0.03039	6.637	69.20
8	12	0.00842	0.00459-0.02012	4.22767+2.03789x	0.11668	15.800	168.40
9	14	0.01245	0.01072-0.01468	6.47511+3.39914x	0.06018	8.492	249.00
B. JCyp-R strain							
1	0	0.03639	0.03133 -0.04183	3.80898+2.64685x	0.031	7.466	1.000
2	7	0.03954	0.03051- 0.05249	4.34290+3.09559x	0.22314	5.143	1.087
3	8	0.02824	0.02194- 0.03433	4.51129+2.91201x	0.1777	8.257	0.776
4	10	0.08513		0.84557+0.79032x	74.7576	2.591	2.339
5	12	0.01547	0.01052- 0.02258	2.18638+1.20769x	1.30586	9.602	0.425
6	13	0.02887	0.02428 - 0.03362	3.97059+2.57894x	0.23037	2.313	0.793
7	14	0.03551	0.03073- 0.04093	3.89064+2.68384x	0.2613	6.384	0.976
8	15	0.03901	0.03339- 0.04571	5.85522+4.15609x	0.14155	4.919	1.072
9	16	0.02385	0.02075- 0.02670	7.43556+4.58291x	0.07676	2.587	0.655
10	17	0.02353	0.01984- 0.02683	6.24299+3.83374x	0.09514	3.291	0.647
11	19	0.03079	0.02650- 0.03504	5.18270+3.42876x	0.14687	3.766	0.846

RR* = LC_{50} (R strain)/ LC_{50} (lab strain, JJ-Lab)

Table 20 Contd...

Sl.No.	Generation	LC ₅₀ (%a.i.)	Fiducial limits (%a.i.)	Y = a + bx	LC ₉₉ (%a.i.)	χ^2	RR*
C. JNzF-R strain							
1	0	0.01251	0.00895 - 0.01689	2.49759+1.31261x	0.74052	2.645	1.00
2	1	0.00552	0.00121 - 0.01102	2.32812+1.03107x	0.99606	0.453	0.44
3	5	0.02231	0.01283 - 0.03865	1.82889+1.10737x	2.81335	5.516	1.78
4	7	0.01307	0.00623 - 0.02342	1.74699+0.92748x	4.21367	6.765	1.04
5	16	0.02808	0.01889-0.04238	2.55831+1.64889x	0.72331	5.020	2.24
D. JNSKE-R strain							
1	0	0.06652	0.05266-0.08516	2.01626+1.71302x	1.51712	2.307	1.00
2	3	0.05462	0.03492 - 0.07822	1.77653+1.40702x	2.45907	3.173	0.82
3	5	0.07758	0.04497 - 1.2942	1.52046+1.36945x	3.87679	2.893	1.17
4	6	0.09286	0.04986-0.15653	1.52495+1.47741x	3.48685	0.826	1.40
5	9	0.10973	0.07359-0.15989	1.71102+1.78289x	2.21375	5.777	1.65
6	10	0.12887	0.10023 - 0.16551	1.39065+1.56280x	3.96942	22.545	1.94
7	11	0.08351	0.04910-0.13645	1.25172+1.16086x	8.42793	6.132	1.26
8	13	0.05668	0.03589-0.08381	1.86896+1.49928x	2.01870	37.361	0.85
9	14	0.06349	0.04103-0.09271	1.92665+1.60918x	1.77167	8.556	0.95
10	16	0.05817	0.04164 - 0.07914	2.49218+2.01743x	0.82756	6.526	0.87
11	16	0.06450	0.04872 - 0.08397	2.57944+2.16683x	0.76418	25.681	0.97
12	19	0.23312	0.16174-0.33631	1.08234+1.71140x	5.33210	39.356	3.50
13	21	0.12370	0.08004-0.18219	1.21667+1.34052x	6.72659	4.627	1.86
14	23	0.14437	0.11896-0.17359	0.96201+1.14455x	15.5608	8.678	2.17

RR* = LC₅₀ (R strain)/LC₅₀ (lab strain, JJ-Lab)

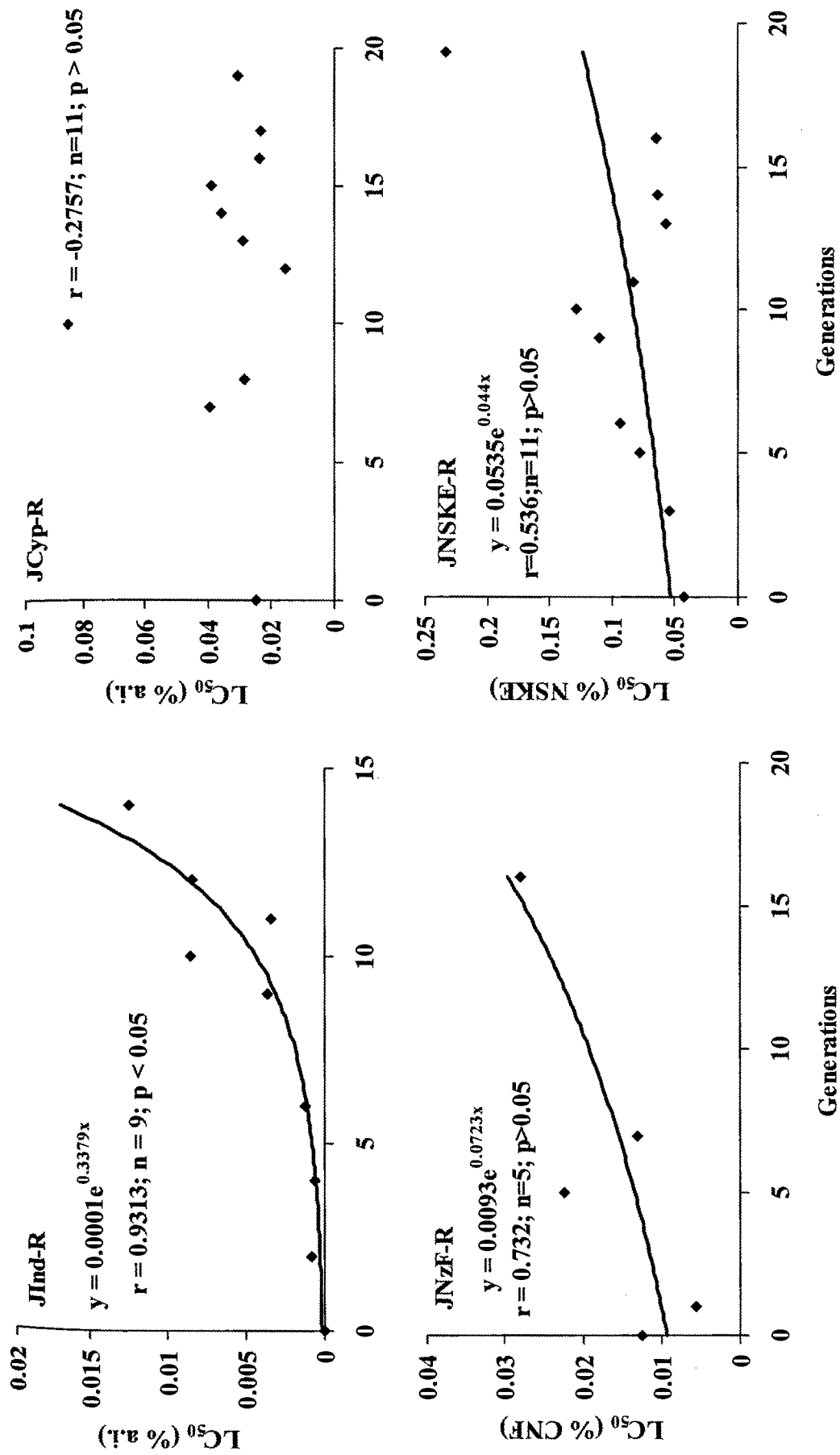


Figure 21: The response to selection measured as LC_{50} values over generations in four resistant, JInd-R, JCyp-R, JNzF-R and JNSKE-R, strains of *Plutella xylostella* selected with indoxacarb, cypermethrin, neemazal-F and NSKE, respectively during 2005-2006.

4.3.1.2 Response to selection for cypermethrin resistance in JCyp-R strain

Bioassay carried out after seven generations of continuous selection with cypermethrin in JCyp-R strain, an LC_{50} value of 0.039 per cent a.i. (1.08 fold) was recorded. This was slightly higher than the base line value (F_1 generation, JField strain, LC_{50} = 0.03639 per cent a.i.). Then, after ten generations of selection LC_{50} of 0.08513 per cent a.i. was recorded and was highest among the 11 bioassays conducted during 20 generations of selection (Table 20B). However, at 12th generation there was a sudden decrease in LC_{50} value to 0.01547 per cent a.i. and was lowest among all the assays (0.425 fold RR) and was less than the base line value. At the end of twenty generations of selection, an LC_{50} of 0.03079 per cent a.i. (0.846 fold RR) was recorded. Even after continuous increase in selection concentration over generations, the LC_{50} s at different generations fluctuated around the base line value.

Most surprisingly, the trend in LC_{50} values across generations did not indicate any significant change through generations, although in fact, the 'r' values indicated a possible insignificant negative trend ($r = -0.2757$; $n = 11$; $p > 0.05$; Fig. 21).

4.3.1.3 Response to selection for Neemazal-F resistance in JNzF-R

Selection was imposed over a period of 20 generations for selection of Neemazal resistant strain of DBM. During 20 generations of selection the changes in LC_{50} values were measured five times. Immediately after one generation of selection with Neemazal-F, LC_{50} of 0.00552 per cent of CNF was recorded and was lower than the base line value (0.01251 % CNF). However, the LC_{50} values were observed to be more than the base line values at 5th (LC_{50} = 0.0223 % CNF; 1.78 folds of base line value) and at 7th generations (0.01307 % of CNF). After fifteen generations of selection in the 16th generation, an LC_{50} of 0.028 per cent of CNF was recorded and was 2.24 fold higher than the base line value. Despite an apparently low increase, this LC_{50} value was significantly different from the base line LC_{50} value ($p < 0.05$).

Further, the changes in LC_{50} values in terms of resistance ratios over generations roughly followed an exponential function ($y = 0.0093e^{0.0723x}$; $r = 0.732$; $n = 5$; $p > 0.05$; Table 20C; Fig. 21c).

4.3.1.4 Response to selection for NSKE resistance in JNSKE-R strain

Changes observed in the median lethal concentrations of JNSKE-R strain during selection for resistance to NSKE matched those of Neemazal-F and remained relatively small. After three generations of selection, the LC_{50} (0.054 per cent of NSKE) value assessed was similar to the base line value (Base line LC_{50} = 0.066 per cent NSKE; $p > 0.05$). Further selection resulted in a gradual increase in LC_{50} values through generations. At the 10th generation (1.94 folds of RR) a maximum of 0.129 per cent NSKE was recorded as the LC_{50} . However, there was a drop in LC_{50} value in the 13th generation to 0.057 per cent of NSKE following the relaxation in selection during the 12th generation. But after resuming the selection, there was a gradual increase in LC_{50} values. At 23rd generation 0.144 per cent NSKE was recorded as the LC_{50} that represented an RR of 2.17 fold. But yet the recorded LC_{50} value was significantly different ($p < 0.05$) from the base line LC_{50} value.

The changed LC_{50} values over generations although increased steadily, did not fit a significant exponential function ($r = 0.536$; $n = 11$; $p > 0.05$; Fig. 21d). However, the scatter plot of the LC_{50} values over generations indicated a possibility of two different trends being followed by the LC_{50} values representing one prior to and the other post relaxation period. A check on this possibility revealed LC_{50} values before relaxation from 0-11th generation ($y = 0.047e^{0.0837x}$; $r = 0.869$; $n = 7$; $p < 0.05$; Fig. 22a) to follow a significant exponential function. On resuming the selection, the LC_{50} values increased over generations in a strong exponential pattern ($y = 0.0024e^{0.2307x}$; $r = 0.912$; $n = 4$; Fig. 22b). However, despite the small sample size of only four values, the equation fitted (not tested for the goodness of fit) indicated a substantial difference in the value of the slope during the post-relaxation period ($b = 0.2307$) relative to the pre-relaxation period ($b = 0.0837$).

4.3.2 Comparison of response trends among the four selected strains

Selection for resistance to indoxacarb in JInd-R strain resulted in an exponential increase ($y = 0.0001e^{0.3379x}$; $r = 0.931$; $n = 9$; $p < 0.01$; Fig. 21a) in LC_{50} values over generations. After fourteen generations of selection, 249 fold RR was recorded. However, in JCyp-R strain, even after continuous increase in selection concentration, LC_{50} values fluctuated around the baseline value (0.03639 % a.i). At the end of 20 generations of selection LC_{50} value of 0.03079 per cent a.i was recorded and was lower than the

baseline value. The LC_{50} values across generations did not follow any trend ($r = -0.2757$; $n = 11$; $p > 0.05$; Fig. 21b).

Similarly, in both JNzF-R ($r = 0.732$; $n = 5$; $p > 0.05$; Fig. 21c) and JNSKE-R ($r = 0.536$; $n = 11$; $p > 0.05$; Fig. 21d) strains the increased LC_{50} values did not fit the exponential functions. However, in both these strains, there was an apparent trend indicating the exponential function. This was evident when the JNSKE-R strain was checked for the trends before and after relaxation at the 12th generation. As a result, the overall development of resistance over generations indicates a possibility of exponential pattern in Resistance Ratios in any selected insecticide resistant strain. The lack of fit in JNzF-R appears to be more a consequence of small sample size.

Broadly considering that three of the four cultures demonstrated a possible exponential function, one can raise the question of the nature of trends, do slopes differ for different selection agents? Therefore an attempt was made to verify the congruence of the slopes of RRs of the three cultures. The relative rate of development of resistance among the strains to different selection agents were compared based on the slopes of the best fit functions of the three selected strains excluding the JCyp-R (In all it was exponential). ANCOVA carried out to compare the slopes of the three selected populations, indicated significant differences among the 'b' (slope) values of the three selected strains compared ($F_{2,19} = 21.364$; $p < 0.05$). Slope ($b = 0.3379$) of the exponential function of LC_{50} values over generations in JInd-R strain was significantly different from those of the other two strains, whereas the slopes of JNzF-R and JNSKE-R were on par for the two potential exponential functions.

Hence it can be concluded that, the rate of development of resistance was highest ($b = 0.3379$) in JInd-R strain to indoxacarb, followed by JNzF-R ($b = 0.0723$) strain to Neemazal-F and in JNSKE-R strain the slope was 0.044. Further, significant increase in LC_{50} values in JInd-R strain against indoxacarb are strongly indicative of the selection operating in DBM. However, in other three selected strains, JCyp-R, JNzF-R and JNSKE-R, shallow slopes and poor fit of the trend lines indicated the inability of the insect to respond to these selection agents. Moreover, the low levels of resistance ratios in JNzF-R, only 2.24 (0.028 % CNF) fold after 15 generation of selection, and in JNSKE-R strain

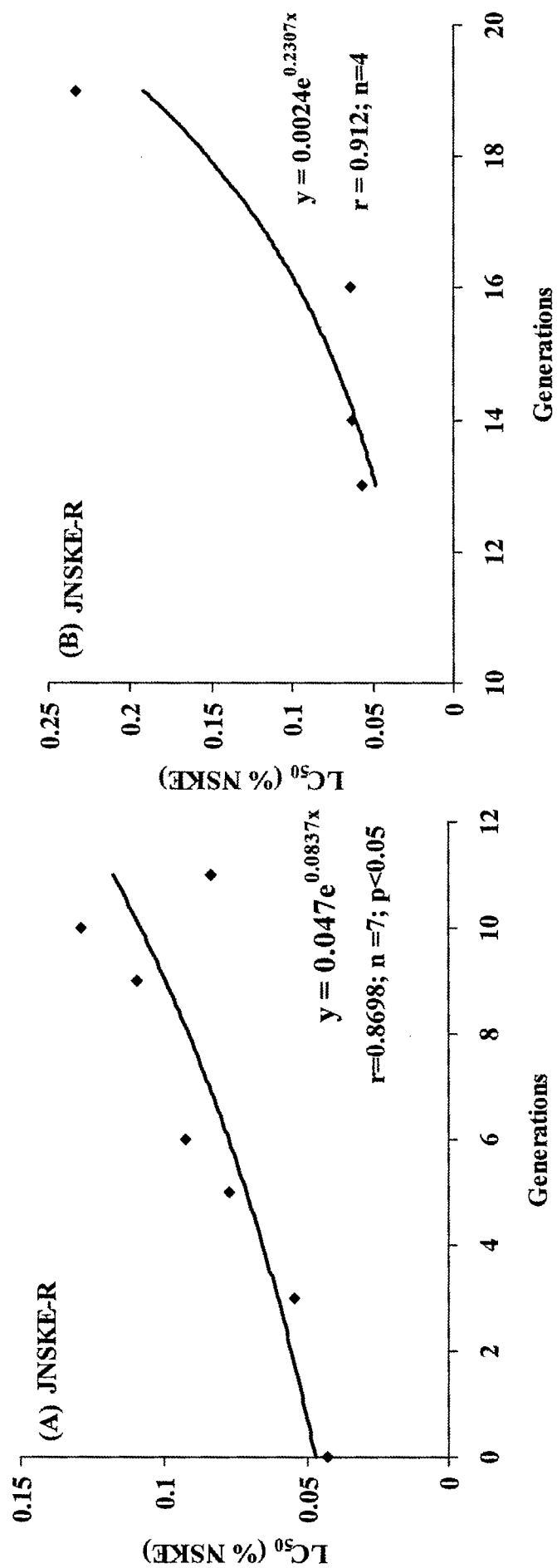


Figure 22: The response (LC_{50}) of JNSKE-R strain of *Phutella xylostella* larvae on selection with NSKE from 0-11th generation (A) and again on resuming selection (13-23) (B) after relaxation at the 11th generation.

only 2.17 (0.14437 % NSKE) fold after 23 generations of selection, indicated the possibility of neem and its products to resist the development of resistance in insects.

4.3.3 Rate of increase in resistance : A comparison using the slopes of the log concentration probit mortality lines

Slope of the log concentration probit mortality line of the probit analysis is expected to indicate the relative phenotypic (and as a consequence genetic) homogeneity of the cultures under scrutiny. As a result, the values of the slope are commensurate with the relative homogeneity of the population for their response to a selection agent such as an insecticide. Hence, slopes of the probit analyses can be used as the indicators of strains under selection attaining homogeneity in resistance.

JInd-R strain, slope values increased exponentially ($y = 1.282e^{0.0771x}$; $r = 0.662$; $n=9$; $p < 0.05$; Fig. 23a) over generations on selection with indoxacarb and the fit was significant. Even in JNSKE-R strain the slope values increased exponentially and the trend was significant ($y = 1.247e^{0.0202x}$; $r = 0.644$; $n=11$; $p < 0.05$; Fig. 23d). However, in JCyp-R and JNzF-R strains there was an increase in slope value but they did not show significant fit for the exponential functions (Fig. 23b; Fig. 23c). To be noted here is the fact that the fit perhaps was poor for JNzF-R ($r=0.5567$; $n=5$; $p>0.05$; Fig. 23c) due to the smaller sample size. JCyp-R culture on the other hand indicated a strong exponential pattern in the trends of the slope when the first three points were excluded, indicating that the pattern sustained across cultures.

Thus in essence, the trends in the slopes of the probit analyses indicated strong tendency among all the four selected cultures to be moving towards higher values with generations and consequently demonstrating increased levels of genetic homogeneity. Thus the trends in slopes also indirectly suggest the increased levels of insecticide resistance in the selected cultures.

4.4 Genetics of insecticide resistance

During the process of selection, once the selected population achieved an LC_{50} value that was significantly different from the initial base line value, the strain was used for characterizing the genetic mechanism involved in the resistance to the insecticide used for selection. The resistant culture was crossed with the susceptible JJ-Lab strain to obtain

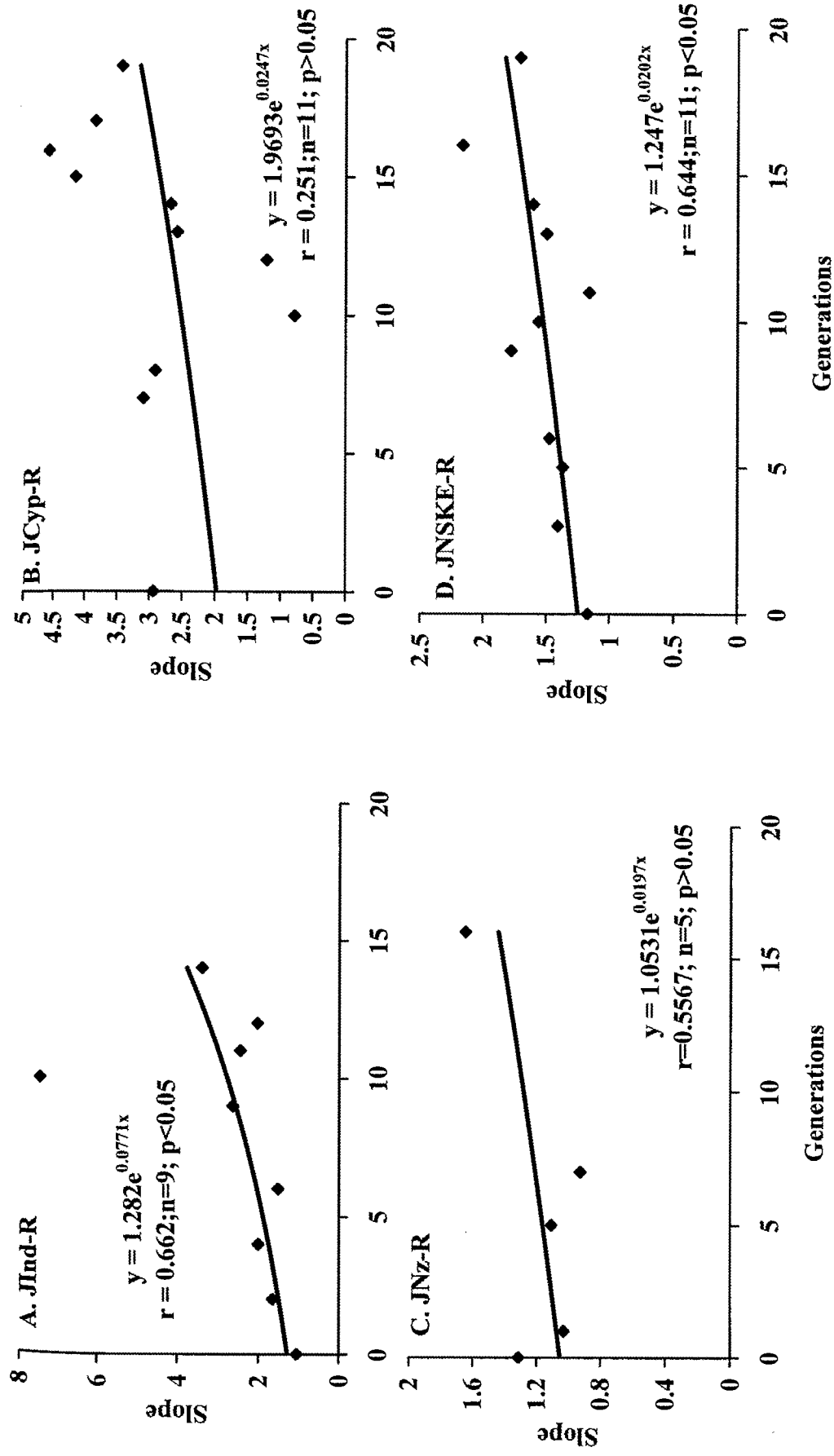


Figure 23: Relationship between the slope of the probit mortality line (indicator of homogeneity of the population) over generations of selection neemazal-F and NSKE, respectively during 2005-2006.

the F_1 population. Further the F_1 population was back crossed with susceptible or the resistant parent cultures to obtain the F_2 progenies. Based on the response (LC_{50} values) of F_1 , F_2 progenies and their parents, genetic parameters of resistance, such as mode of inheritance, degree of dominance of resistance governing gene and number of genes were worked out. JInd-R, JCyp-R and JNSKE-R strains were treated as resistant for the purpose after 10 generations of selection.

4.4.1 Mode of inheritance and degree of dominance of resistance

4.4.1.1 Genetics of indoxacarb resistance in JInd-R strain

Response of the reciprocal crosses between the indoxacarb selected JInd-R strain and susceptible JJ-Lab strains were measured in terms of LC_{50} value. The LC_{50} values of the F_1 progeny were 0.00435 (JInd-R (♀) x JJ-Lab (♂)) and 0.00474 per cent a.i. (JJ-Lab (♀) x JInd-R (♂)) (Table 21a) that were not significantly different from each other (Table 21b). Hence, the resistance to indoxacarb in JInd-R strain was observed to be independent of sex and is autosomally inherited.

Further, based on the response of the F_1 progenies, the degree of dominance (D) was also calculated. The 'D' values for the two crosses were 0.673 in JInd-R (♀) x JJ-Lab (♂) cross and 0.699 in JJ-Lab (♀) x JInd-R (♂) cross. Since these values of 'D' were more than 0.5 and were towards +1, the resistance to indoxacarb in *P. xylostella* can be considered as being regulated by incompletely dominant gene/s.

Again, based on the position of the Log concentration – probit mortality lines of the parents JInd-R, JJ-Lab and F_1 progenies of their reciprocal crosses, the dominance of the gene was inferred. The log concentration - probit mortality lines of the F_1 progenies were more towards that of the selected JInd-R strain (Fig. 24) rather than that of the susceptible JJ-Lab strain. Hence the results reinforce the idea that the resistance to indoxacarb in JInd-R strain is governed by incompletely dominant gene/s.

4.4.2 Genetics of cypermethrin resistance in JCyp-R strain

Mode of inheritance of resistance to cypermethrin in JCyp-R strain was measured through the response of F_1 progenies of reciprocal crosses. In JCyp-R (♀) x JJ-Lab (♂) cross the LC_{50} of 0.01889 per cent a.i. was recorded and in JJ-Lab (♀) x JCyp-R (♂) cross progenies it was 0.02187 per cent a.i. (Table 22a). The two LC_{50} s of the reciprocal crosses

Table 21a: Response (LC_{50}) of resistant (JInd-R), susceptible (JJ-Lab) parental strains, their F_1 progeny from reciprocal crosses and F_2 progeny from back crosses with resistant and susceptible parents after 14 generations of selection against indoxacarb.

Crosses	Strains Name	N	LC_{50} (%a.i.)	Fiducial limits (%a.i.)	$Y = a + bx$	LC_{99} (%a.i.)	χ^2
Parent – Susceptible (S)	JJ –Lab	300	0.00002	0.00001-0.00003	6.35755+1.36525x	0.16659	6.339
- Resistant (R)	JInd-R @ 14 gen.	300	0.01245	0.01072-0.01468	6.47511+3.39914x	0.06018	8.492
F_1 – Reciprocal crosses							
R ($\varnothing$) x S (σ)	JInd-R x JJ-Lab	300	0.00435	0.00338-0.00520	8.02499+3.39796x	0.02103	5.126
S ($\varnothing$) x R (σ)	JJ-Lab x JInd-R	300	0.00474	0.00352-0.00585	6.08136+2.61631x	0.03671	4.301
F_2 - crosses							
(S x R) ($\varnothing$) x S (σ)	[JJ-Lab x JInd-R] x JJ-Lab	300	0.00113	0.0074 -0.00154	4.37643+1.48463x	0.04160	23.304
(R x S) ($\varnothing$) x R (σ)	[JInd-R x JJ-Lab] x JInd-R	300	0.00300	0.00235-0.00373	6.16794+2.44468x	0.02683	11.679

Table 21b : Mode of inheritance and nature of dominance of resistance in indoxacarb selected *Plutella xylostella*, JInd-R strain calculated based on (Stone, 1968).

Crosses	LC_{50} (% a.i.)	Log of LC_{50}	Degree of dominance (D)*	Mode of Inheritance	Nature of dominance
JJ –Lab	0.00002	-4.70			
JInd-R @ 14 gen.	0.01245	-1.90			
JInd-R x JJ-Lab	0.00435	-2.36	0.673		
JJ-Lab x JInd-R	0.00474	-2.32	0.699	Autosomal	Incompletely dominant

*D (see Materials and Methods) = 1 Completely dominant; 0 No, dominance ; and -1 Completely recessive

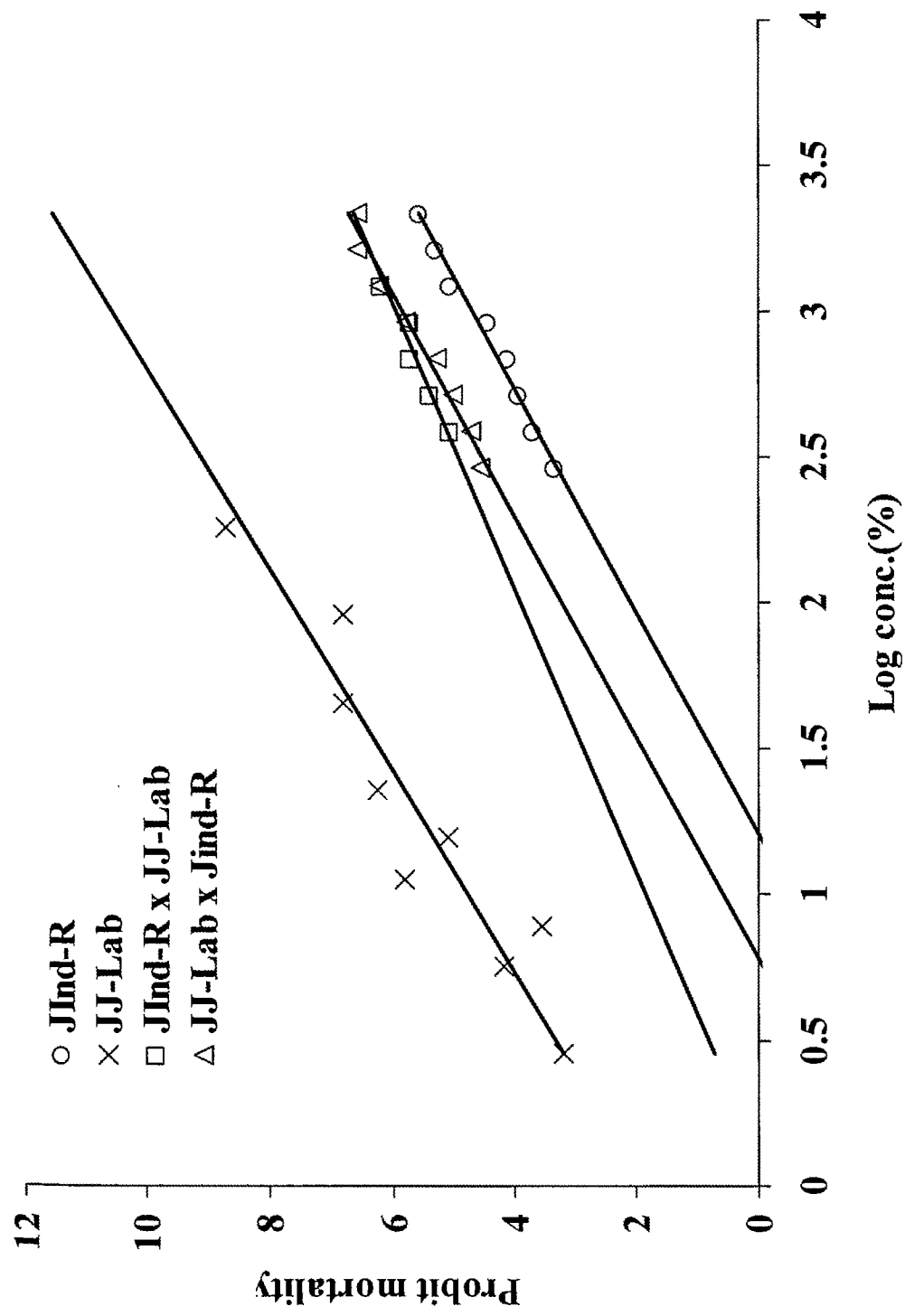


Figure 24: Log concentration-probit mortality line for resistant (JInd-R), susceptible (JJ-Lab) and F₁ progeny of the reciprocal crosses of *Plutella xylostella* against indoxacarb.

Table 22a: Response (LC_{50}) to cypermethrin in parental resistant JCyp-R, susceptible JJ-Lab strains, and their F_1 progeny of reciprocal crosses and F_2 progeny from back crosses of *Plutella xylostella*.

Crosses	Strains Name	n	LC ₅₀ (% a.i.)	Fiducial limits (% a.i.)	Y = a+bx	LC ₉₉ (% a.i.)	χ^2
Parent -Susceptible (S)	JJ-Lab	300	0.00470	0.00390-0.00554	6.62631+2.84622x	0.03085	6.666
-Resistant (R)	JCyp-R	300	0.02887	0.02428-0.03362	3.97151+2.57957x	0.23027	2.331
F₁ – Reciprocal crosses							
R (♀) x S (♂)	JCyp-R(♀) x JJ-Lab(♂)	300	0.01889	0.01540 - 0.02208	6.22122+3.60932x	0.08335	5.993
S (♀) x R(♂)	JJ-Lab(♀) x JCyp-R(♂)	300	0.02187	0.01843 -0.02526	6.33532+3.81630x	0.08903	12.024
F₂ - crosses							
(S x R) (♀) x S(♂)	(JJ-Lab x JCyp-R) x JJ-Lab	300	0.02265	0.01995 -0.02555	5.85239+3.55784x	0.10208	2.729
(R x S) (♀) x R(♂)	(JCyp-R x JJ-Lab) x JCyp-R	300	0.02739	0.02443 -0.03066	6.15457+3.93897x	0.10669	7.162

Table 22b: Mode of inheritance and nature of dominance of resistance in cypermethrin selected *Plutella xylostella*, JCyp-R strain calculated based on response of F_1 progeny (Stone, 1968)

Crosses	LC ₅₀ (%a.i.)	Log of LC ₅₀	Degree of dominance (D)*	Mode of Inheritance	Nature of dominance
S	JJ-Lab	0.0047	-2.328		
R	JCyp-R @ 13gen	0.02887	-1.539		
S (♀) x R(♂)	JJ-Lab(♀) x JCyp-R(♂)	0.02187	-1.661	Autosomal	Incompletely dominant
R (♀) x S (♂)	JCyp-R(♀) x JJ-Lab(♂)	0.01889	-1.724	0.533	

*D (see Materials and Methods) = 1 Completely dominant; 0 No, dominance ; and -1 Completely recessive

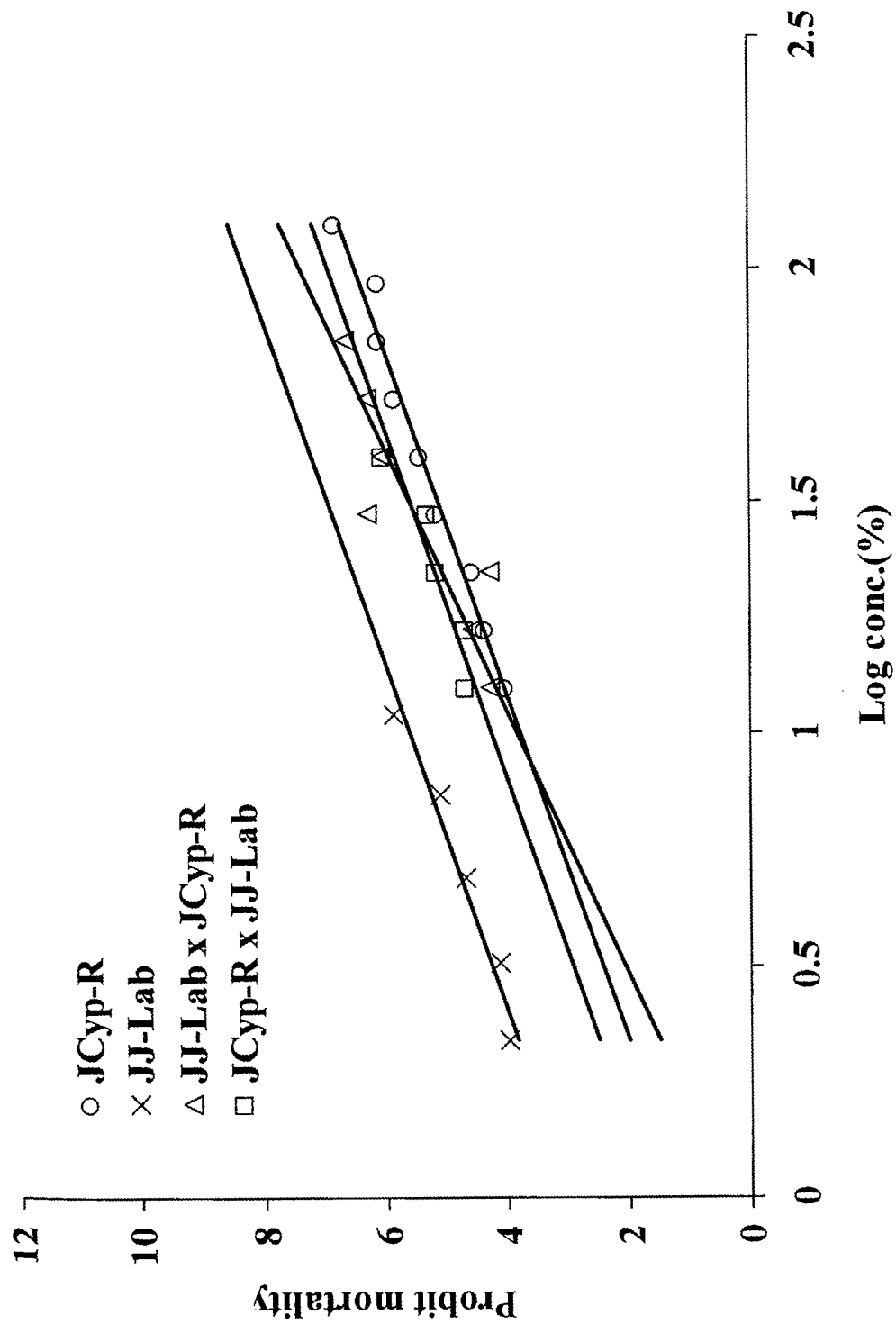


Figure 25: Log dose-probit mortality line for resistant(JCyp-R), susceptible(JJ-Lab) and F1 progeny of the reciprocal crosses of *Plutella xylostella* against cypermethrin.

were not significantly different from each other suggesting the resistance to cypermethrin to be inherited through autosomal chromosomes.

Even in JCyp-R strain resistance to cypermethrin was inherited as an incompletely dominant trait as the degree of dominance (D) calculated were 0.694 for F_1 reciprocal cross of JJ-Lab (♀) x JCyp-R (♂) and 0.533 for the reciprocal cross JCyp-R (♀) x JJ-Lab (♂) (Table 22b). In addition, log concentration - probit mortality lines of the F_1 progenies were more towards the probit lines of cypermethrin selected JCyp-R strain (Fig. 25) rather than the JJ-Lab strain. Therefore, the resistance to cypermethrin in JCyp-R strain was inherited by an incompletely dominant gene (Table 22b).

4.4.3 Genetics of NSKE resistance in JNSKE-R strain

Although the LC_{50} values of the NSKE selected JNSKE-R strain and that of the susceptible JJ-Lab strain for NSKE were different by only 2.17 fold, the two were observed to be significantly different from each other. Therefore the reciprocal mass crosses were carried out between the resistant and susceptible strains and the F_1 progenies obtained for understanding the mode of inheritance and the degree of dominance of resistance governing gene/s for NSKE.

The LC_{50} s for F_1 progenies were 0.04147 and 0.04357 per cent NSKE from the reciprocal crosses JNSKE-R (♀) x JJ-Lab (♂) and JJ-Lab (♀) x JNSKE-R (♂), respectively (Table 23a). These values were not significantly different from each other (Overlapping Fiducial Limits; $p > 0.05$). Therefore, the resistance to NSKE in JNSKE-R strain is inherited autosomally.

Based on LC_{50} values of the F_1 -progenies, the degree of dominance (D) values calculated for JNSKE-R (♀) x JJ-Lab (♂) and JJ-Lab (♀) x JNSKE-R (♂) crosses were observed to be -0.334 and -0.267 respectively. As these were between 0 to -1 *i.e.* negative, it can be inferred that the resistance to NSKE in *P. xylostella* is inherited as an incompletely recessive trait.

Even the position of log concentration-probit mortality lines of F_1 -progenies of reciprocal crosses, with respect to those of the JJ-Lab and JNSKE-R strains (Fig. 26) also supported the idea that the resistance to NSKE in JNSKE-R strain was inherited as an

Table 23a: Response (LC_{50}) of parental strains, resistant JNSKE-R strain, susceptible JJ-Lab strain, and F_1 progeny from reciprocal crosses to NSKE in *Plutella xylostella*.

Crosses	Strains Name	n	LC_{50} (%a.i.)	Fiducial limit (%a.i.)	$Y = a + bx$	LC_{99} (%a.i.)	χ^2
Parent – Susceptible (S)	JJ-Lab	300	0.02047	0.01538-0.02613	3.67484+2.17600x	0.24004	6.438
- Resistant (R)	JNSKE-R at F_{11}	300	0.08351	0.04910-0.13645	1.25172+1.16086x	8.42793	6.132
F_1 – Reciprocal crosses							
R (♀) x S (♂)	JNSKE-R(♀) x JJ-Lab(♂)	300	0.04147	0.02189 -0.06857	1.57905+1.1423x	4.51007	6.058
S (♀) x R (♂)	JJ-Lab(♀) x JNSKE-R(♂)	300	0.04357	0.03115-0.05923	2.16899+1.5939x	1.25523	4.257

Table 23b: Mode of inheritance and nature of dominance of resistance in NSKE selected *Plutella xylostella*, JNSKE-R strain calculated based on Stone (1968) method

Strain	Crosses	LC_{50} (%a.i.)	Log of LC_{50}	Degree of dominance (D)*	Mode of Inheritance	Nature of dominance
S	JJ-Lab	0.02047	-1.689			
R	JNSKE-R at F_{11}	0.08351	-1.078			
S (♀) x R (♂)	JJ-Lab(♀) x JNSKE-R(♂)	0.04357	-1.361	-0.267		
R (♀) x S (♂)	JNSKE-R(♀) x JJ-Lab(♂)	0.04147	-1.382	-0.334	Autosomal	Incompletely recessive

*D (see Materials and Methods) = 1 Completely dominant; 0 No, dominance ; and -1 Completely recessive

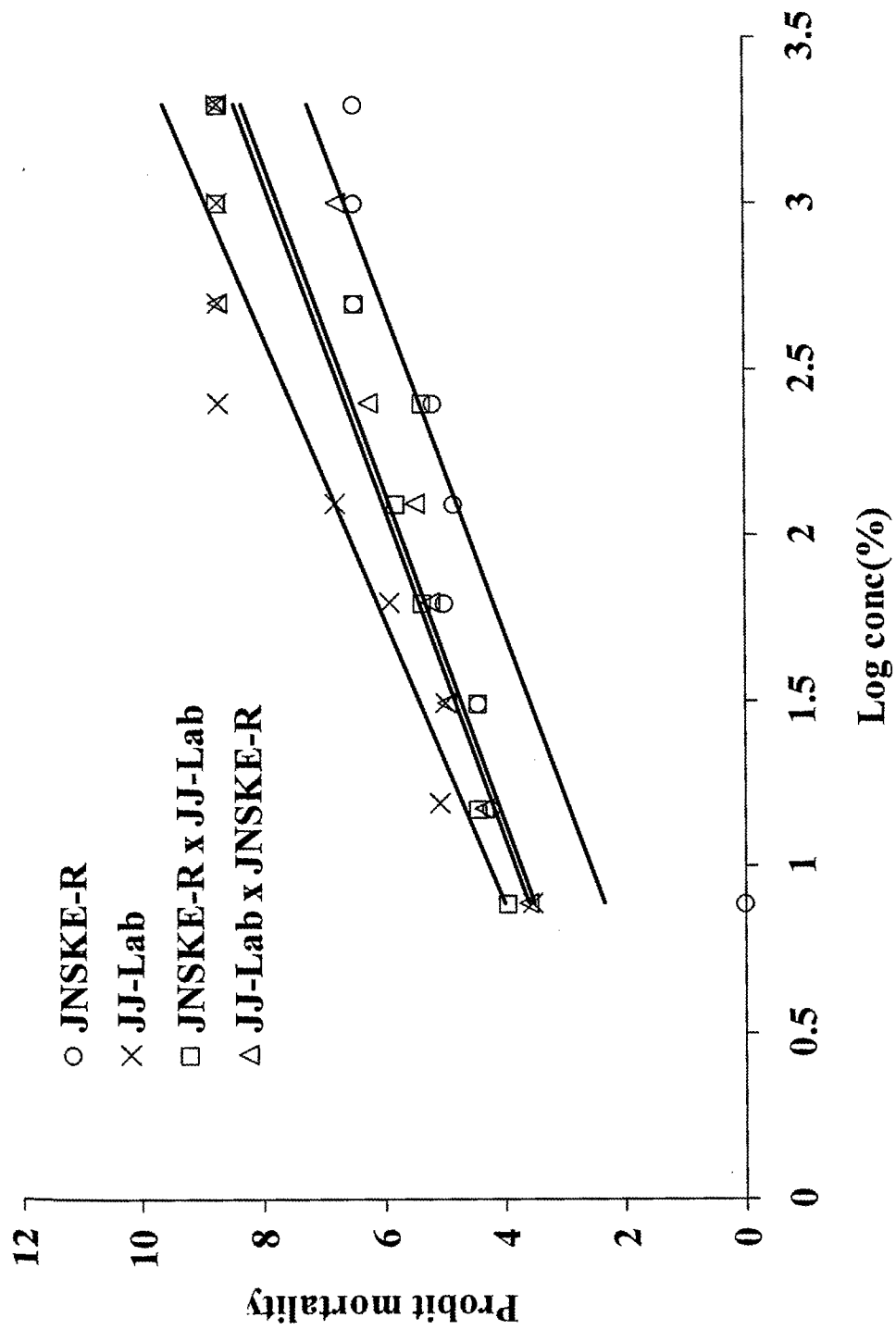


Figure 26: Log dose-probit mortality line for resistant (JNSKE-R), susceptible (JJ-Lab) and F1 progeny of the reciprocal crosses of *Plutella xylostella* against NSKE.

incompletely recessive trait. Both the lines of the F_1 - progenies were closer to that of the susceptible JJ-Lab strain.

Thus in summary, in all the selected strains, JInd-R, JCyp-R and JNSKE-R, the resistance to their respective selection agents, indoxacarb, cypermethrin and NSKE was inherited autosomally. Further, resistance to indoxacarb in JInd-R and to cypermethrin in JCyp-R strains were inherited as incompletely dominant traits while resistance to NSKE in JNSKE-R strain was inherited as an incompletely recessive trait.

4.4.2 Number of genes governing resistance

Numbers of genes influencing resistance to indoxacarb in JInd-R strain and to cypermethrin in JCyp-R strain were assessed through two methods of analyzing backcross data. Based on best fit monogenic model (Tabashnik *et al.*, 1992) through chi-square test and second method was to calculate the number of loci based on Lande's (1981) method with slight modifications (Tabashnik *et al.*, 2002).

4.4.2.1 Indoxacarb

Test of monogenic model of inheritance of resistance to indoxacarb by comparing the mortality of (JInd-R x JJ-Lab) x JInd-R back cross progeny suggested significant deviation of the observed from the expected mortality values at different concentrations ($\chi^2 = 70.641$; $n = 9$; $p < 0.05$). However, notwithstanding the above results, similar comparison for the progeny of the backcross of F_1 (JJ-Lab x JInd-R) with susceptible JJ-Lab strain, suggested strong congruence of the mortality pattern for observed with those expected from the monogenic model of resistance ($\chi^2 = 11.663$; $n = 10$; $p > 0.05$; Table 24) at different concentrations. Therefore, considering that the trait is governed by a dominant allele, resistance to indoxacarb in JInd-R strain was governed by single gene.

Further, number of loci governing resistance to indoxacarb was also calculated based on LC_{50} values and slope of the parent, F_1 progenies and backcross progenies by Lande's (1981) method. Based on F_1 cross of JInd-R x JJ-Lab strain, the n_E was equal to 4.379 loci and in JJ-Lab x JInd-R cross it was 5.973 (Table 24C) loci that are responsible for resistance to indoxacarb in JInd-R strain. The mean number of loci was 4.906.

Table 24: Test of monogenic model of inheritance of resistance to indoxacarb based on the response of the back crossed F_2 populations of the *Plutella xylostella* against indoxacarb

A) Backcross I – (JInd-R x JJ-Lab) x JInd-R

Sl.No.	Conc (%a.i.)	Expected	Observed	χ^2
1	0.0029	10.644	33.333	48.364
2	0.0039	28.852	38.889	3.492
3	0.0052	37.115	50.000	4.473
4	0.0069	54.902	61.111	0.702
5	0.0092	59.664	77.778	5.499
6	0.0122	72.689	94.444	6.511
7	0.0163	83.333	94.444	1.481
8	0.0218	85.714	88.889	0.118
9	0.0290	100.000	100.000	0.000
			Total	70.641

B) Back cross II– (JJ-Lab x JInd-R) x JJ-Lab

Sl.No.	Conc (%a.i.)	Expected	Observed	χ^2
1	0.0004	48.276	28.571	8.043
2	0.0007	48.276	42.857	0.608
3	0.0015	50.000	50.000	0.000
4	0.0029	66.667	64.286	0.085
5	0.0039	69.444	78.571	1.200
6	0.0052	75.000	85.714	1.531
7	0.0069	80.556	82.143	0.031
8	0.0092	88.889	88.095	0.007
9	0.0122	97.222	100.000	0.079
10	0.0163	97.222	100.000	0.079
			Total	11.663

C) Lande's (1981) method of calculation of number of loci for resistance to Indoxacarb in JInd-R strain

	LC ₅₀	Slope	RR	D	Variance	n_E
R	0.01245	3.399	622.500		0.087	
S	0.00002	1.365	1.000		0.537	
R x S	0.00435	3.398	217.500	0.673	0.087	4.379
S x R	0.00474	2.616	237.000	0.700	0.146	5.973
Pooled	0.0045	3.007	227.250	0.687	0.111	4.906
RS x R	0.003	2.445	150.000		0.167	
SR x S	0.00113	1.485	56.500		0.454	

Thus the results of the two studies do not provide a unifying answer for the number of genes regulating the resistance to indoxacarb in *P. xylostella*.

4.4.2.2 Cypermethrin

The expected and the observed mortality values were found to differ significantly in both the backcrosses of the F_1 with the susceptible ($\chi^2 = 35.246$; $n = 8$; $p < 0.05$; Table 25b) and the resistant parents ($\chi^2 = 68.039$; $n = 9$; $p < 0.05$; Table 25a). As the expected mortality levels are calculated on the assumption of trait being governed monogenically, the results overwhelmingly suggest the polygenic nature of the resistance in JCyp-R strain to cypermethrin.

An alternative approach taken for the calculation of number of genes using the LC_{50} values and slopes of the parent, F_1 progenies and backcross progenies by Lande's (1981) method was also attempted. However, the Lande's n_E expects that the calculated values be positive for drawing any inference. Unfortunately, the calculations made for estimating the n_E provided values (-0.696 and -0.804) below 0 and smaller than -1. Hence it is extremely difficult to draw any conclusion based on Lande's method to identify the potential number of genes that confer the resistance to cypermethrin in *P. xylostella*.

4.5 Resistance Risk Assessment

During the process of selection for resistance in different strains of *P. xylostella*, based on their response (LC_{50} values) at different generations, various parameters that determine the rate of development of resistance were estimated.

4.5.1 Response to selection (R)

Response to selection (R) measured as the difference in log concentration per generation of selection in three resistant strains of *P. xylostella* over generations of selection with indoxacarb, cypermethrin and NSKE were measured.

4.5.1.1 Cumulative R at different generations

Response to selection (R) was found to decrease over generations in JInd-R strain against indoxacarb. The R was 0.41 between 0-2nd generations of selection. However, it was 0.16 after 14 generations of selection (Table 26). Change in cumulative 'R' values

Table 25 : Test of monogenic model of inheritance of resistance to cypermethrin based on the response of the back crossed F₂ population of the *Plutella xylostella* against cypermethrin

A) Back cross I - (JCyp-R x JJ-Lab) x JCyp-R

Sl.No.	Conc.	Expected	Observed	χ^2
1	0.013	27.381	0.000	27.381
2	0.017	32.381	24.138	2.098
3	0.022	45.238	44.828	0.004
4	0.030	59.286	51.724	0.964
5	0.040	76.190	58.621	4.052
6	0.053	90.000	86.207	0.160
7	0.070	93.333	96.552	0.111
8	0.094	93.333	100.000	0.476
			Total	35.246

B) Back cross II - (JJ-Lab x JCyp-R) x JJ-Lab

Sl.No.	Conc.	Expected	Observed	χ^2
1	0.009	26.923	10.000	10.637
2	0.013	52.289	23.333	16.035
3	0.017	66.667	30.000	20.167
4	0.022	61.905	46.667	3.751
5	0.030	95.238	56.667	15.621
6	0.040	92.857	80.000	1.780
7	0.053	95.238	93.333	0.038
8	0.070	97.619	96.667	0.009
9	0.094	100.000	100.000	0.000
			Total	68.039

C) Lande's (1981) method of calculation of number of loci for resistance to cypermethrin

	LC ₅₀	Slope	RR	D	Variance	n_E
R	0.02887	2.580	6.143		0.388	
S	0.005	2.846	1.000		0.351	
R x S	0.019	3.609	4.019	0.533	0.277	-0.696
S x R	0.022	3.816	4.653	0.694	0.262	-0.804
Pooled	0.020	3.713	4.336	0.616	0.269	-0.748
RS x R	0.02739	3.939	5.828		0.254	
SR x S	0.02265	3.558	4.819		0.281	

over generations followed a significant exponential decay ($y = 0.3619e^{-0.0619x}$; $r = -0.823$; $n = 8$; $p < 0.05$; Fig. 27a) function.

In JCyp-R-strain, 'R' values over generations fluctuated (range 0.005 to 0.049) but yet demonstrated a declining trend with generations. However, unlike in the case of JInd-R, the decline did not follow the exponential decay function ($r = -0.496$; $n = 7$; $p > 0.05$; Fig. 27b).

Despite low levels of changes in the LC_{50} values through generations, in JNSKE-R strain selected with NSKE, the response to selection, the cumulative 'R' values, varied from 0.010 to 0.048. The highest 'R' value of 0.048 was recorded between 0-6th generations of selection. Lowest 'R' value (0.01) was recorded after 16 generations of selection (0-16th generation; Table 26). The trend in 'R' values over generations followed a significant exponential decay function ($y = 0.0893 e^{-0.1265x}$; $r = -0.812$; $n = 9$; $p < 0.01$; Fig. 27c).

Thus in all the three selected strains, JInd-R, JCyp-R and JNSKE-R, the cumulative response to selection, the 'R', values were found to decrease over generations and followed a significant exponential decay function in JInd-R and JNSKE-R strains. Considering that the JCyp-R did not provide substantial evidence of the development of resistance over generations, the observed trend in 'R' values over generations appears to be a general phenomenon. However, the 'R' values of the JInd-R strains were relatively high (0.16 to 0.41) compared to JNSKE-R strain, which ranged from only 0.010 to 0.048 (Table 26). Higher cumulative 'R' values in JInd-R are indicative of their ability of rapid development of resistance to indoxacarb while JNSKE-R strain with low cumulative 'R' values demonstrated the relatively poor performance of *P. xylostella* in responding to the selection by NSKE.

4.5.1.2 Between intermittent generations of selection

Considering that the 'R' can be calculated for values of any two generations among the different generations of a strain under selection, it is even possible to calculate the values for intermittent generations during the course of selection. This was also therefore, attempted for all the three cultures under selection. In JInd-R strain, selected with indoxacarb, the 'R' value was found to be high during the initial generations (I_0 - I_2) of

Table 26 : Estimates of response to selection (R), selection differential (S), no. of generations required for 10 fold increase in LC₅₀ (G) and realized heritability (h^2) for JInd-R, JCyp-R and JNSKE-R strains of *Plutella xylostella* selected with indoxacarb, cypermethrin and NSKE respectively.

Generation	No. of gen. seld. (n)	R	G	h^2
JInd-R				
Over generations				
I ₀ -I ₂	3	0.41	2.45	0.511
I ₀ -I ₄	5	0.21	4.70	0.271
I ₀ -I ₆	7	0.20	4.96	0.207
I ₀ -I ₉	10	0.19	5.38	0.306
I ₀ -I ₁₀	11	0.20	4.92	0.760
I ₀ -I ₁₁	12	0.15	6.52	0.237
I ₀ -I ₁₂	13	0.17	5.84	0.228
I ₀ -I ₁₄	15	0.16	6.26	0.307
JCyp-R				
Over generations				
C0-C7	8	0.025	39.83	0.056
C0-C8	9	0.006	164.64	0.013
C0-C10	11	0.049	20.60	0.071
C0-C12	13	-0.016	-62.89	-0.027
C0-C13	14	0.005	217.91	0.011
C0-C14	15	0.010	97.31	0.025
C0-C15	16	0.012	82.06	0.038
C0-C16	17	-0.001	-908.56	-0.004
C0-C17	18	-0.001	-732.38	-0.004
C0-C19	20	0.005	216.90	0.0131
JNSKE-R				
Between generations				
I0-I2	3	0.408	2.448	0.511
I2-I4	3	-0.054	-18.651	-0.076
I4-I6	3	0.116	8.642	0.160
I6-I9	4	0.111	8.974	0.227
I9-I11	3	-0.006	-174.151	-0.015
I11-I12	2	0.193	5.178	0.317
I12-I14	3	0.057	17.662	0.130
JNSKE-R				
Between generations				
C0-C7	8	0.025	39.83	0.056
C7-C8	2	-0.073	-13.68	-0.179
C8-C10	3	0.160	6.26	0.274
C10-C12	3	-0.247	-4.05	-0.237
C12-C13	2	0.135	7.38	0.266
C13-C14	2	0.045	22.25	0.118
C14-C15	2	0.020	48.99	0.087
C15-C16	2	-0.107	-9.36	-0.433
C16-C17	2	-0.003	-340.92	-0.010
C17-C19	3	0.039	25.69	0.155

Table 26 contd...

JNSKE-R				
Generation	No. of gen. seld. (n)	R	G	h^2
Over generations				
N0-N3	4	0.026	38.54	0.025
N0-N5	6	0.043	23.42	0.043
N0-N6	7	0.048	20.94	0.050
N0-N9	10	0.041	24.58	0.048
N0-N10	11	0.043	23.08	0.047
N0-N11	12	0.024	41.64	0.022
N0-N13	14	0.009	116.80	0.009
N0-N14	15	0.011	88.69	0.012
N0-N16	17	0.010	96.60	0.0130

Generation	No. of gen. seld. (n)	R	G	h^2
Between generations				
N0-N3	4	0.026	38.54	0.025
N3-N5	3	0.051	19.69	0.052
N5-N6	2	0.039	25.62	0.043
N6-N9	4	0.018	55.17	0.023
N9-N10	2	0.035	28.64	0.054
N10-N11	2	-0.094	-10.61	-0.094
N11-13	3	-0.056	-17.82	-0.044
N13-N14	2	0.025	40.59	0.025
N14-N16	3	0.002	437.68	0.003

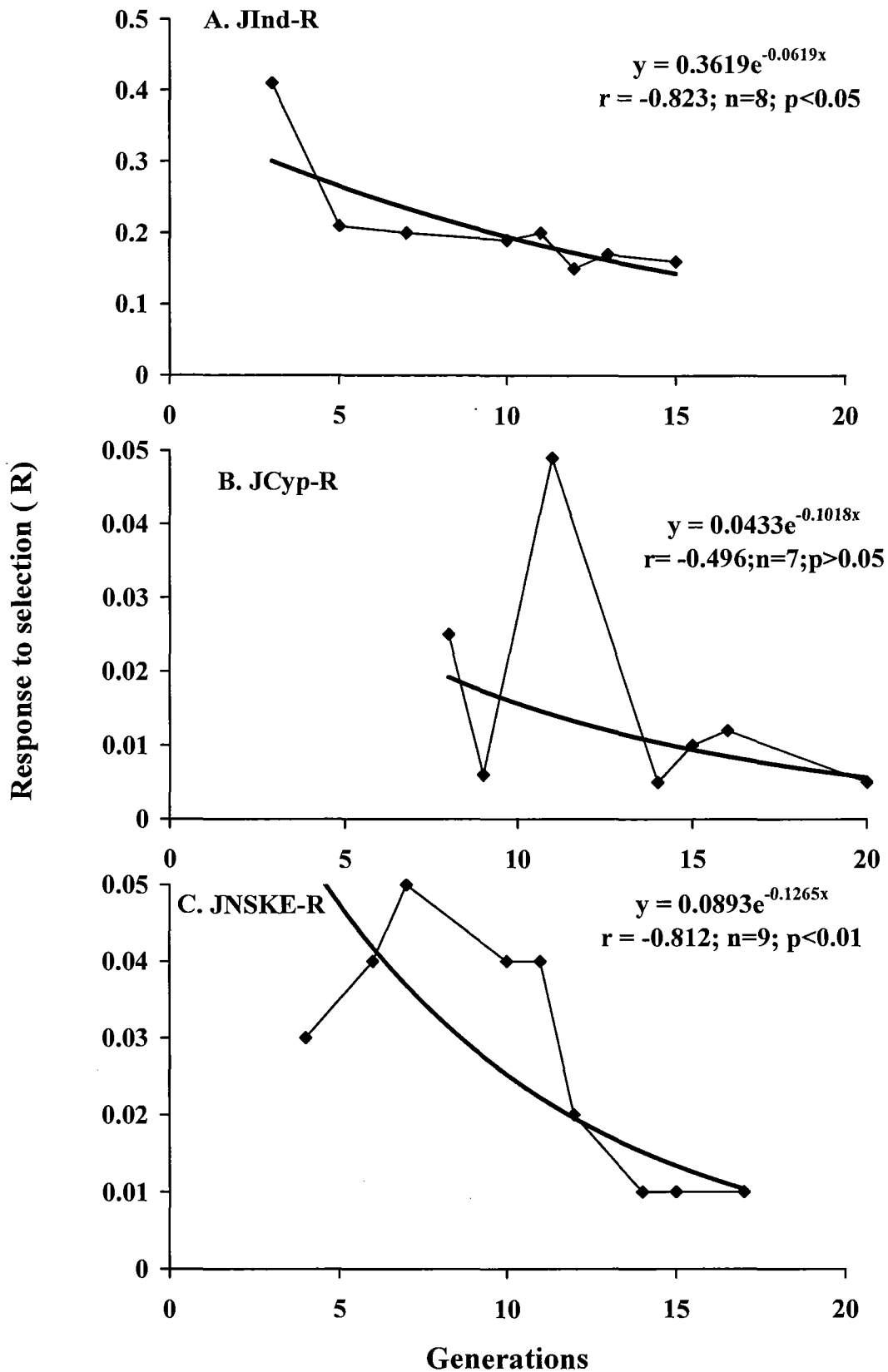


Figure 27: Response to selection (R) measured as the difference in log concentration per generation in indoxacarb, cypermethrin and NSKE selected strains of *Plutella xylostella* over different generations of selection.

selection ($R = 0.35$; Table 26; Fig. 28a). However, between 9th to 11th generations of selection, the 'R' value was negative (-0.11) (Table 26). The lowest (0.05) was during 2nd and 4th generations of selection followed by 4th to 6th generation (0.06) and 12th to 14th generations of selection. On the whole, the 'R' values calculated over intermittent generations suggested a lack of trend, but broadly indicated a possibility of reduction over generations ($r = -0.2805$; $n = 7$; $p > 0.05$).

The intermittent 'R' values in JCyp-R strain selected with cypermethrin were found to vary through generations of selection. The higher 'R' value recorded between 8th and 10th generation of selection were mixed with several negative values of 'R' that were observed between 7th and 8th (-0.073), 10-12th (-0.247), 15-16th (-0.107) and 16- 17th (-0.003) generations (Table 26; Fig. 28b). The overall decreasing trend in 'R' values with generations was not however, substantiated ($r = -0.2468$; $n = 6$; $p > 0.05$).

Even in NSKE selected JNSKE-R strain, the highest 'R' value of 0.051 was recorded between 3-5th generations of selection. The lowest positive 'R' value was observed between 14 and 16th generations of selection. The intermittent 'R' values varied from 0.002 to 0.051 between different intermittent generations of selection. However, negative 'R' values were also recorded between 10-11th (-0.094) and 11-13th (-0.056) generations of selection (Table 26; Fig. 28c). But yet, excluding the negative value, the pattern of decline followed an exponential function ($y = 0.0838e^{-0.1536x}$; $r = -0.7059$; $n = 6$; $p > 0.05$).

Thus, similar to the cumulative 'R' values calculated over a number of generations, even those values calculated for intermittent generations during the course of selection indicated a general declining trend, though not statistically substantiated in almost all the cultures of DBM selected for insecticidal resistance (Table 26; Fig. 28).

4.5.2 Estimates of realized heritability (h^2)

Heritability is the measure of the proportion of genetic variance that contributes to the total phenotypic variance. The realized heritability (h^2) calculated as R/S , depends on the response to selection (R) and selection differential (S).

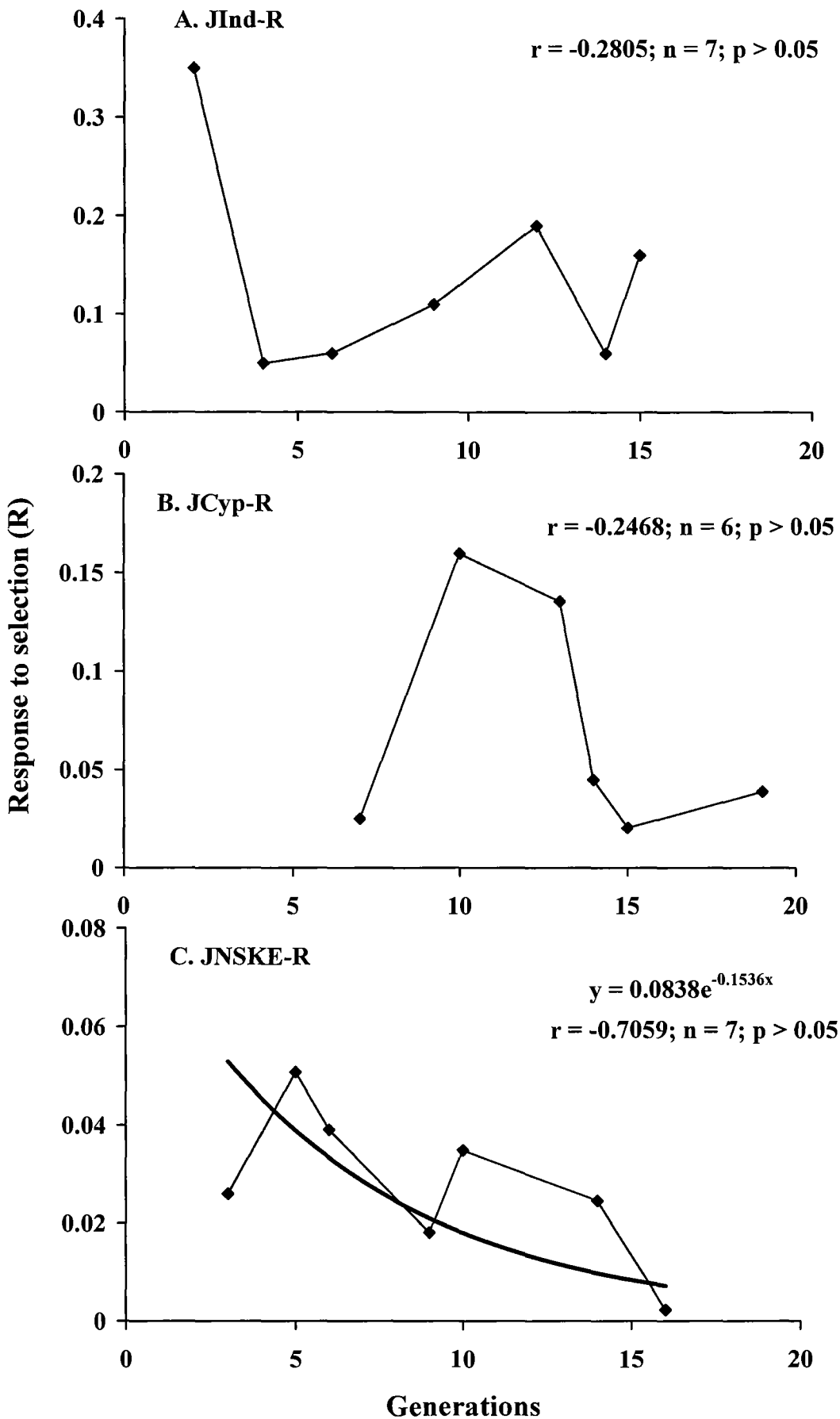


Figure 28: Response to selection (R) measured as the difference in log concentration per generation of selection in different strains between intermittent pairs of generations of selection in indoxacarb, cypermethrin and NSKE selected strains of *Plutella xylostella*. Note: Negative values were excluded from calculations

4.5.2.1 Cumulative realized heritability

Cumulated realized heritability (h^2) was found to be high in JInd-R strain. Between 0-2 generations, 0.511 of h^2 was recorded. Then onwards, there was a decrease in the h^2 , but between 0-10th generation, an h^2 of 0.760, the highest, was recorded. However, over all the generations of selection, the mean h^2 from base population to 14 generation of selection (0-14), an h^2 value of 0.307 was recorded (Table 26; Fig. 29a). Further, cumulative h^2 values over generations were found to decrease as the selection process continued (Fig. 29a), without a defined trend ($r = -0.162$; $n = 8$; $p > 0.05$).

Even in cypermethrin selected JCyp-R strain cumulative h^2 values were high during initial generations with 0.056 between 0 to 7th and 0-10 (0.071) generations. However, the negative h^2 values were also recorded between 0-12 (-0.027), 0-16 (-0.004) and 0-17 (-0.004) generations. Considering all the generations of selection, the mean h^2 of 0.013 was recorded between 0-19 generations (Table 26). Even though, cumulative h^2 values in JCyp-R strain over generations varied, they demonstrated a broad declining trend with generations that was however, not found significant ($r = -0.1598$; $n = 7$; $p > 0.05$; Fig. 29b).

Estimates of cumulative realized h^2 of resistance to NSKE in JNSKE-R strain varied over generations of selection. Initially after 4 generations, an h^2 of 0.025 was recorded; further after 7 generations of selection (0-6) the highest h^2 of 0.050 was recorded. Over all the generations (0-17) of selection, a mean h^2 of 0.013 was recorded for NSKE in JNSKE-R strain (Table 26). Despite these variations, the h^2 values over generations of selection were found to decrease exponentially ($y = 0.0777e^{-0.1065x}$; $r = -0.690$; $n = 9$; $p < 0.05$; Fig. 29c).

In all the three selected strains, over generations of selection, the mean realized heritability (h^2) values were observed to decrease in general (Fig. 29). These results thus strongly indicate an overall reduction in the phenotypic variation with generations on selection for a trait and in the present case selection for insecticidal resistance.

4.5.2.2 Realized heritability (h^2) between intermittent generations

Similar to the R values, as h^2 can also be calculated for values of any two generations among the different generations of a strain under selection, it is even possible

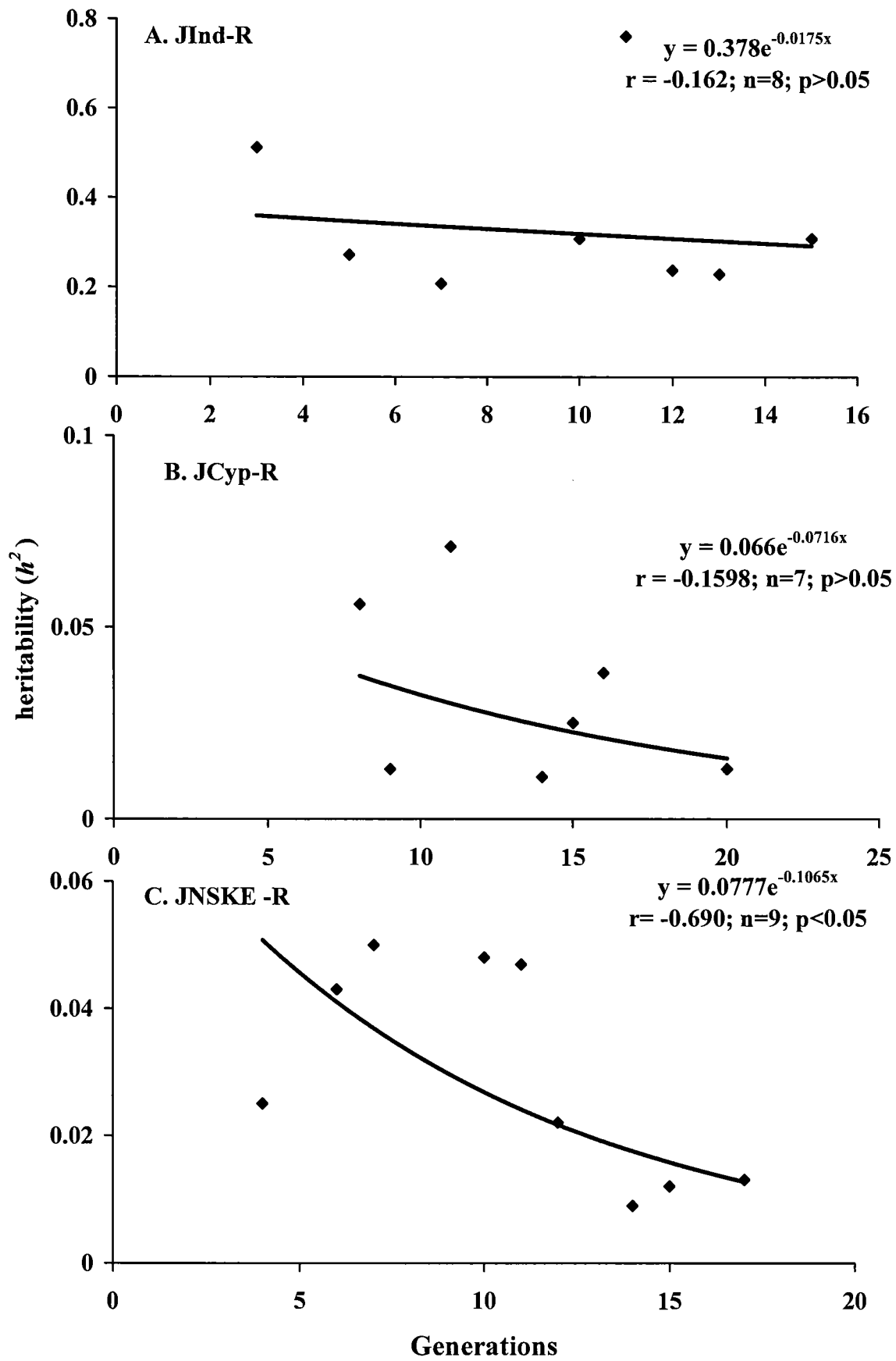


Figure 29: Trends in realized heritability (h^2) of resistance to insecticides, indoxacarb, cypermethrin and NSKE in resistant strains of *Plutella xylostella*, JInd-R, JCyp-R and JNSKE-R, respectively over generations of selection. (Note: (-) ve values were excluded from calculation in JCyp-R strain)

to calculate the values for intermittent generations during the course of selection. This was also therefore, attempted for all the three cultures under selection.

In JInd-R strain, the h^2 value was found to be high (0.511) during initial phase of selection (0-2 generation). Between different intermittent generations, the h^2 values varied from 0.130 to 0.503, excluding two negative h^2 values recorded (Table 26). There was no definitive trend with the generations for these h^2 values calculated for intermittent generations (Fig. 30a).

Similarly in JCyp-R strain against cypermethrin resistance, h^2 values varied between 0.056 to 0.266 at different intermitted generations of selection (Table 26; Fig. 30b), excluding some negative values. Even in this case there was no discernible trend for the h^2 values with generations.

The heritability of NSKE resistance (h^2 values) in JNSKE-R strain varied between 0.003 to 0.054 at different intermittent generations of selection (Table 26; Fig. 30b). However, as the selection process continued, the h^2 values between 13-14th (0.025) and 14-16th generation were very low (0.003) (Table 26; Fig. 30c). The h^2 values were observed to broadly decrease over generations with out a significant trend ($y = 0.0838e^{-0.137x}$; $r = -0.656$; $n = 7$; $p > 0.05$).

Hence, in all these strains, the heritability of resistance, the h^2 values, broadly indicated a tendency to decrease over generations of selection (Table 26; Fig. 30). However, none of the trends were significant.

4.5.3 Predicted rates of resistance development: Number of generations required for ten fold increase in LC₅₀ value (G)

The theoretical number of generations required for a ten fold increase in LC₅₀ values (G) is calculated as reciprocal of the response to selection (R) value ($G = R^{-1}$). Hence, 'G' values are determined by the h^2 values and selection differential (S). Therefore, whenever the h^2 values are high, the 'G' values will be low because 'R' is directly proportional to h^2 ($h^2 = R/S$).

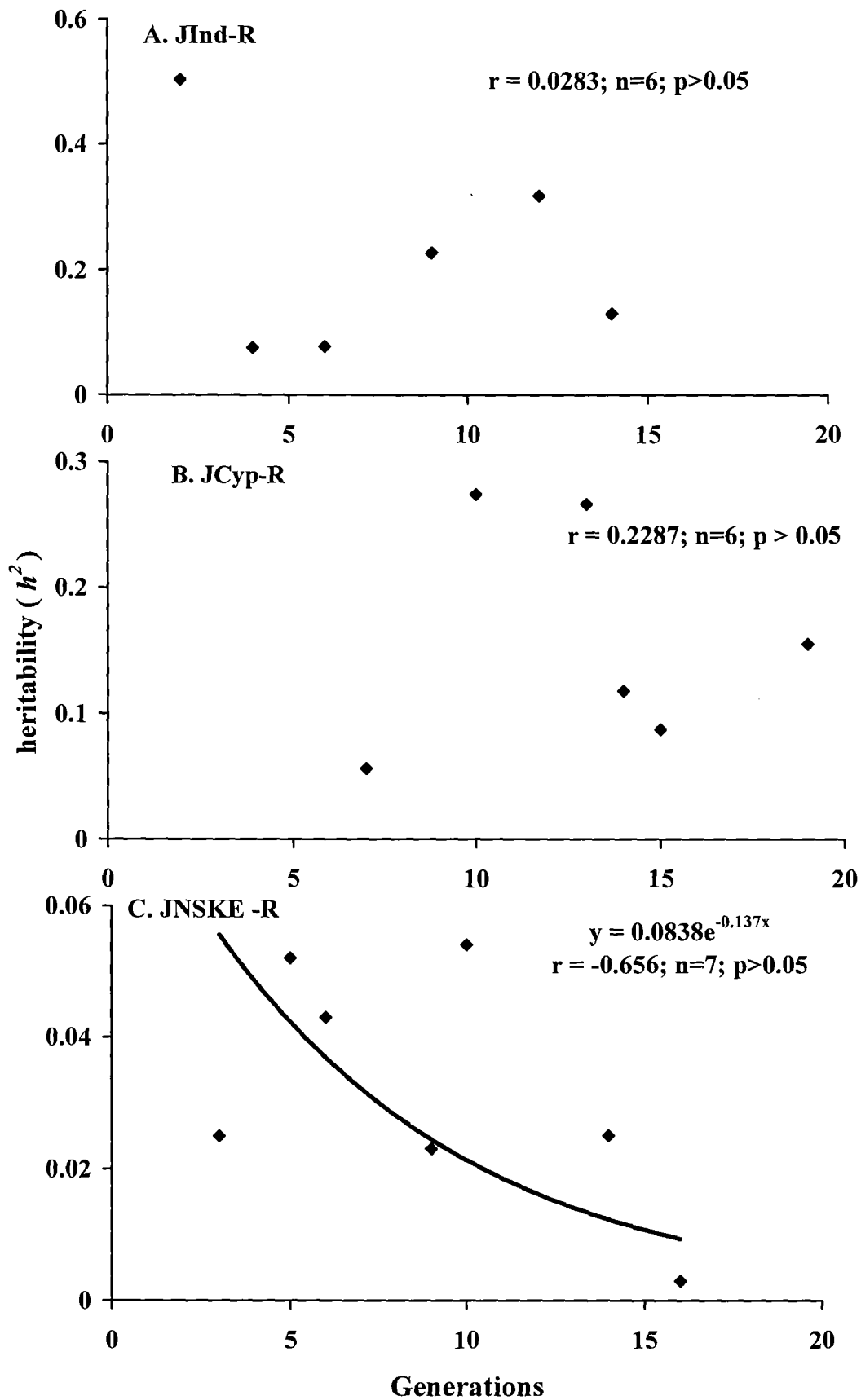


Figure 30: Changes in realized heritability (h^2) of resistance to insecticides, indoxacarb, cypermethrin and NSKE in JInd-R, JCyp-R and JNSKE-R strains of *Plutella xylostella*, respectively between intermittent generations of selection. JNSKE-R. (Note: Negative value were excluded from calculation)

4.5.3.1 Trends in G values

In case of JInd-R strain selected for resistance to Indoxacarb, the 'G' values varied between 2.47 (0-2 generation) to 6.52 generations between 0-11th generation of selection. The 'G' values increased exponentially over generations ($y = 2.7573 e^{0.0616x}$; $r = 0.829$; $n = 8$; $p < 0.05$; Fig. 31a). After 15 generations of selection to indoxacarb resistance in JInd-R strain at 0.307 of h^2 value, the number of generations (G) required for a ten fold increase in LC_{50} values was 6.26 generations.

The best fit function was a logarithmic function ($y = 2.1057\ln(x) + 0.6246$; $r = 0.915$; $n = 8$; $p < 0.05$) that clearly indicated a potential maximum value for the calculated 'G'. As a result, the rate of increase in resistance will be asymptotic to x -axis. In essence, the lower most bounds of heritability reached due to the high levels of resistance in the selected population sets a maximum number of generations for achieving the 10 fold increase in resistance levels. Nevertheless the increasing trend in 'G' values with generations was substantiated.

In JCyp-R strain selected with cypermethrin, the theoretical number of generations (G) required for 10 fold increase varied over generations of selection. It ranged from 20.60 generation during 0-10 generation to 217.91 generations after 14 generations (0-13) of selection (Table 26; Fig. 31b). However, after 20 generations of selection, the mean number of generations required for 10 fold increase in LC_{50} value was 216.90 with a realized heritability (h^2) value of 0.013. The 'G' values thus indicated an increasing trend with generations for resistance to cypermethrin in DBM. However, the trend was not statistically significant, probably due to low number of samples ($y = 21.874e^{0.1072x}$; $r = 0.508$; $n = 7$; $p > 0.05$).

The 'G' values varied over generations of selection for resistance in NSKE selected JNSKE-R strain of DBM. After 7 generations of selection, the number of generations (G) required for ten fold increase in LC_{50} values was 20.94 generations, which increased to 116.8 generation after 14 generations of selection (Table 26; Fig. 31c). However, after 17 generations of selection, 96.60 generations were required for ten fold increase. Further, the 'G' values over generations were increasing exponentially over generations of selection and the fit was significant ($y = 12.068e^{0.1179x}$; $r = 0.748$; $n = 9$; $p < 0.05$; Fig. 31c). Thus the data for JNSKE-R overwhelmingly suggests the low risk of

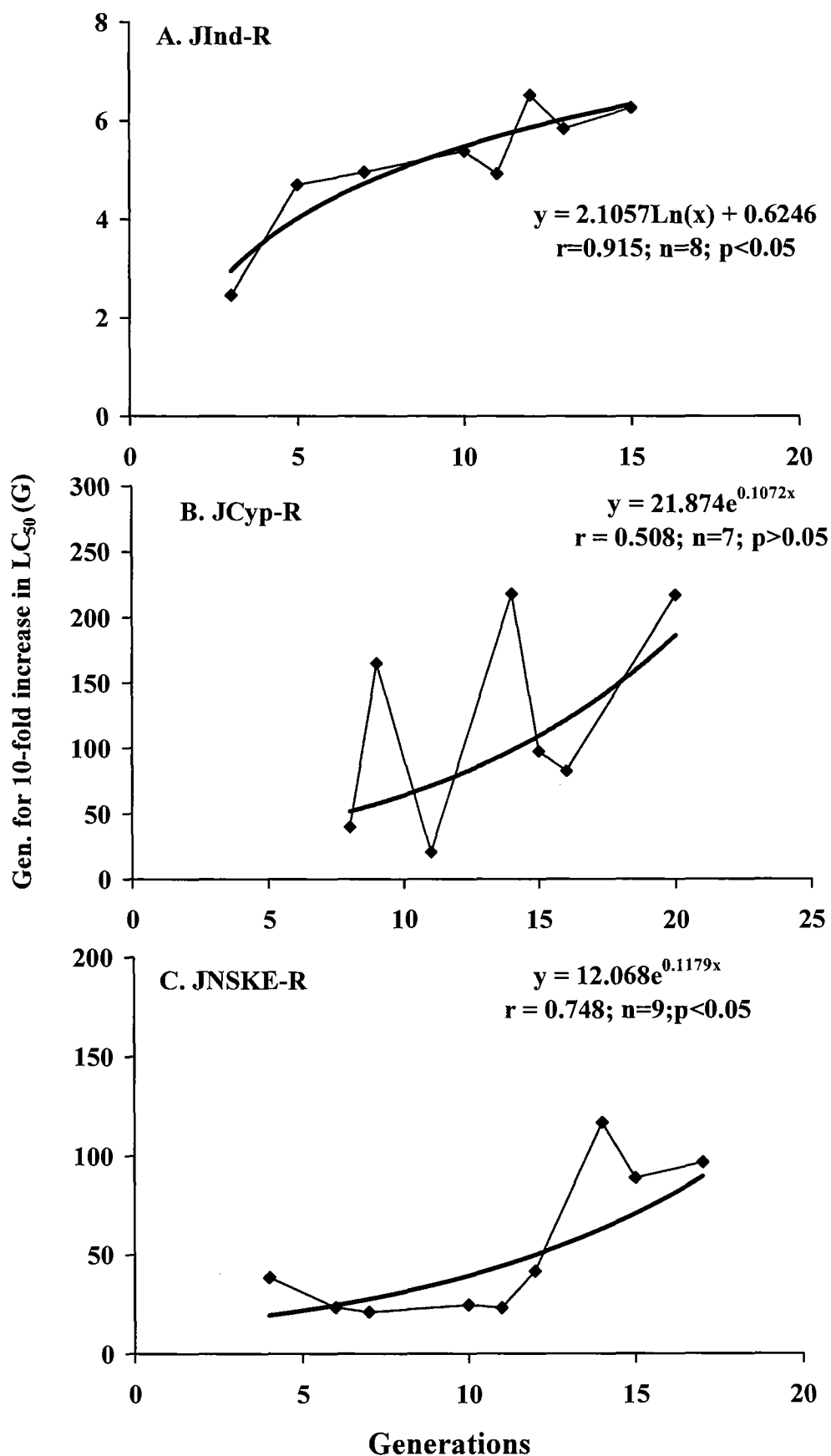


Figure 31: Trends in number of generations required (G) for 10 fold increase in LC_{50} at different generations of selection in indoxacarb, cypermethrin and NSKE selected strains (JInd-R, JCyp-R and JNSKE-R, respectively) of *Plutella xylostella*. (Note: Negative “G” values were excluded from calculation)

resistance development for NSKE. Further, an overall increasing trend in the resistance levels with reduced heritability as a consequence, contributed for the observed results.

In summary, on continuous selection with any insecticide, it appears that the G values tend to increase over generations. This could possibly because, the realized heritability values demonstrate a decreasing trend with generations, due to increased resistance levels. Interestingly, however, the G values tend to show a limit for growth in JInd-R strain, while in JNSKE-R strain the nature of increase seems to be unlimited – exponential. This difference is crucial for accumulation of resistance.

4.5.3.2 Effect of selection pressure at constant h^2

The importance of h^2 and their relation with the ‘ G ’ were assessed at different selection pressure. Wherein, at a constant h^2 value, by changing the selection pressure, ‘ G ’, the number of generations required for ten fold increase in LC_{50} values were computed out for different resistant strains. It is expected that these theoretical calculations will provide an insight into the nature of development of resistance when a population is selected by different selection agents.

The mean realized heritability values (h^2) after 14 generations of selection in JInd-R strain was 0.307, for JCyp-R it was 0.013 and for JNSKE-R strain h^2 was 0.0113. Keeping their respective slope values, before and after selections constant, the responses to selection (R) were worked out over different degrees of selection pressure. Based on the slopes of the base line values and changes in slopes on selection (final value) that were kept constant, the selection differential (S) and then R values were worked ($h^2 = R/S$) out. From these ‘ R ’ values, ‘ G ’ values were calculated ($\bar{G} = R^{-1}$) at different levels of selection pressure. These results (Fig. 32) were plotted across selection pressure.

In JInd-R strain selected for indoxacarb resistance, the ‘ G ’ values were found to decrease with increased selection pressure. The calculated ‘ G ’ values varied from 9.09 to 4.13 generations over 50 and 90 per cent selection pressure (Fig. 32), respectively. Resistance to cypermethrin in JCyp-R strain of DBM required about 117.02 and 53.21 generations for ten fold increases in resistance level at 50 and 90 per cent selection pressure respectively. However, in case of the resistance to NSKE in JNSKE-R strain, the

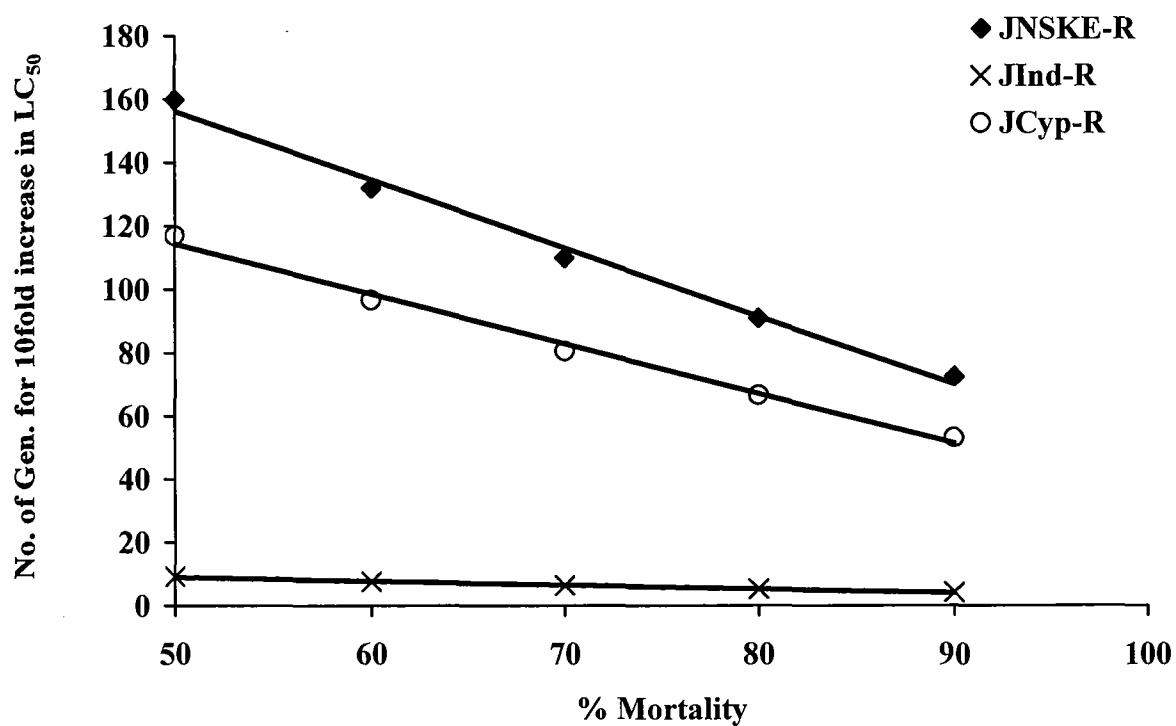


Figure 32: Effect of heritability (h^2) and percent mortality ($100\%-p$; where p = % surviving exposure) caused by different selection agents (indoxacarb, cypermethrin and NSKE) on generation required for a 10 fold increase in LC_{50} ($G=R^{-1}$) in resistant strains of *Plutella xylostella*.

number of generations required varied from 159.78 generation at 50 per cent selection pressure to 72.65 generations at 90 per cent selection pressure.

The results thus indicated that in general, the development of resistance to NSKE is extremely slow compared to cypermethrin or indoxacarb. Even at 90 per cent selection pressure it required >70 generations for 10 fold increase in LC_{50} value. However, for indoxacarb it was <10 generations even at 50 per cent selection pressure and only 4-5 generations were required for 10 fold increase in LC_{50} value at 90 per cent selection pressure (Fig. 32). Cypermethrin took the intermediate values.

4.5.3.3 Relation between selection pressure and realized heritability (h^2)

Considering the above theoretical calculations, it would be pertinent to understand, what happens to the 'G' values across selection pressures actually experienced by the population during the course of selection. This aspect was verified considering the values of selection pressure recorded at different generations over the course of selection. It was observed that, by and large, the observed trends for h^2 indicated a decreasing trend with the selection pressure in both JInd-R and JNSKE-R strains, while the cypermethrin resistant culture did not indicate any trend.

In case of JInd-R strain, selected with indoxacarb, the realized heritability (h^2) values showed a tendency to decrease with per cent selection pressure (Table 26; Fig. 33a) with a significant exponential relationship ($y = 152.91e^{-0.0901x}$; $r = -0.7836$; $n = 7$; $p < 0.05$). In case of JCyp-R, the h^2 values were observed to vary randomly with selection pressure (Table 26; Fig. 33b). Similar, to JInd-R strain, in JNSKE-R strain the h^2 values were found to follow a negative exponential trend, ($y = 0.00011e^{-0.4069x}$; $r = -0.7404$; $n = 9$; $p < 0.05$; Fig. 33c) with selection pressure.

Thus, on the whole, it appears that the h^2 values vary negatively with the selection pressure. This is contrary to the expectation that h^2 should be directly proportional to the selection pressure (See materials and methods). Such an aberration therefore, could be because, the data points represent the values across the entire gamut of selection episodes over generations. Thus the theoretical calculations notwithstanding, the h^2 values can be very different from the expectations. The expectations in fact represent the trends under constant genetic composition of the populations under selection. But in a population that

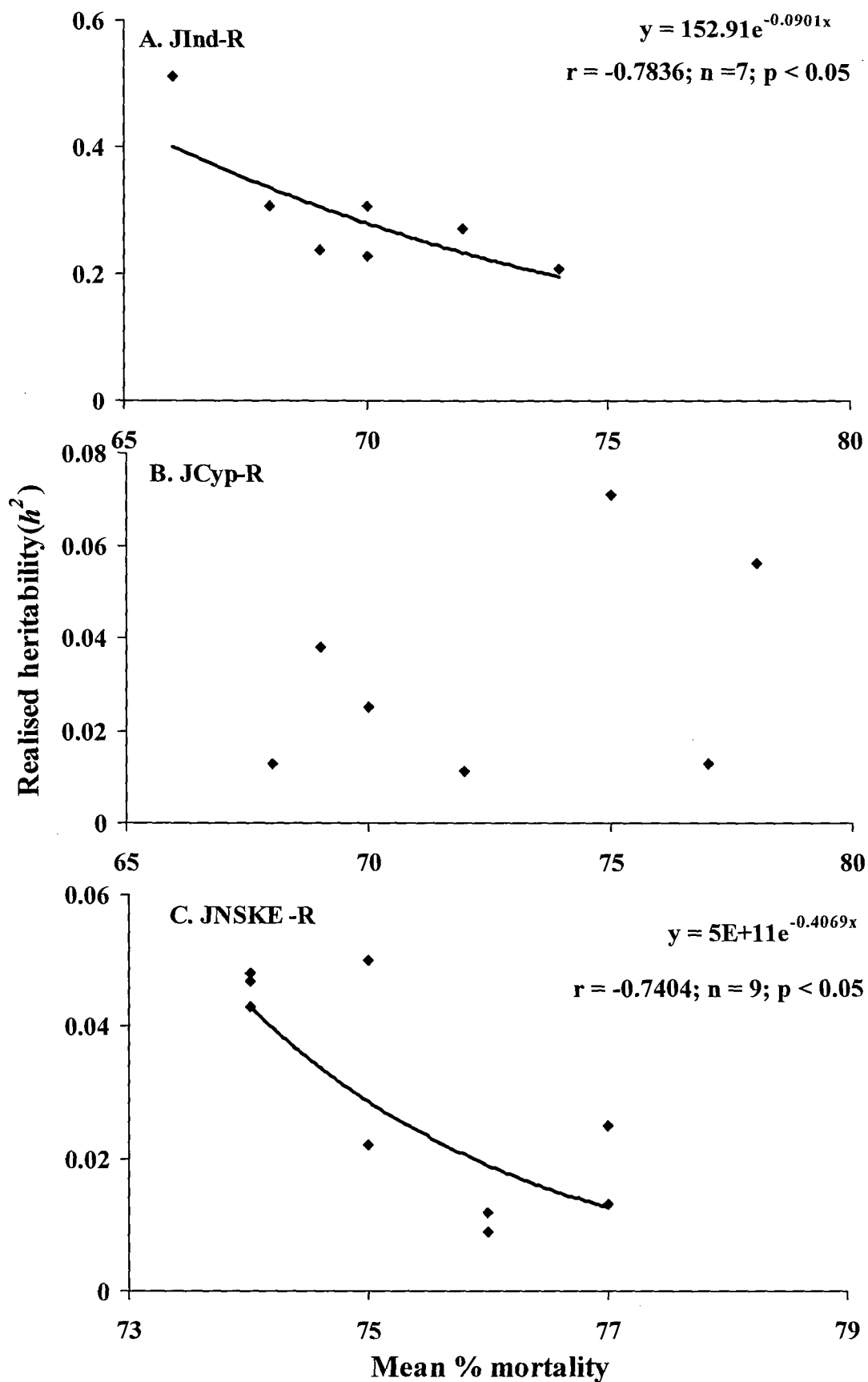


Figure 33: Interrelation between mean % mortality (at adult emergence) and realized heritability (h^2) between different intervals of selection in resistant strains of *Plutella xylostella*. (Indoxacarb-JInd-R; Cypermethrin-JCyp-R and NSKE-JNSKE-R)

is undergoing selection, it is likely that the selection pressures keep varying as is the case in the present set of selections. Hence, the observed results may be a more valid situation for the populations that are changing in genetic composition. However, given this situation, it is anticipated that the 'G' values should increase and was therefore tested.

4.5.3.4 Effect of selection pressure on 'G' over generations

Further, to the above observations, keeping the theoretical expectations at the back of mind, an attempt was made to understand the overall changes in 'G' values with selection pressure over the period of selection.

In JInd-R strain, number of generations (G) required for 10 fold increase in LC_{50} values were found to indicate an increasing trend with selection pressure but the relationship was not significant ($y = 0.107e^{0.055x}$; $r = 0.4351$; $n = 8$; $p > 0.05$; Fig. 34a). Further, in JCyp-R strain, 'G' values did not show any relation with the selection pressure (Fig. 34b). However, the NSKE selected JNSKE-R strain indicated the number of generations required (G) for 10 fold increase in LC_{50} values to increase exponentially with mean percent mortality (selection pressure) over the course of selection ($y = 1E-12e^{0.4126x}$; $r = 0.7355$; $n = 9$; $p < 0.05$; Fig. 34c).

Hence, contrary to the theoretical expectations, the 'G' values did not show a steady declining linear trend with selection pressure. However, this trend is commensurate with the trend observed for the h^2 over selection pressure (section 4.5.3.3). As a result, the differential genetic composition through the selection episodes coupled with the selection pressures that varied randomly contributed for the observed situation.

4.6 Effect of selection with insecticides on Life history parameters of *Plutella xylostella*

Resistance to an insecticide may incur certain cost. Perhaps these are best expressed in terms of the life history parameters. Hence, in order to know the possible costs involved in developing resistance to an insecticide, different life history parameters were studied in different resistant strains of *P. xylostella*, vis-à-vis unselected population. Sex ratio, gross reproductive rate (GRR), net reproductive rate (NRR), intrinsic rate of increase (r) and generation time (T) were worked out through life table calculations, at different generations during the course of study.

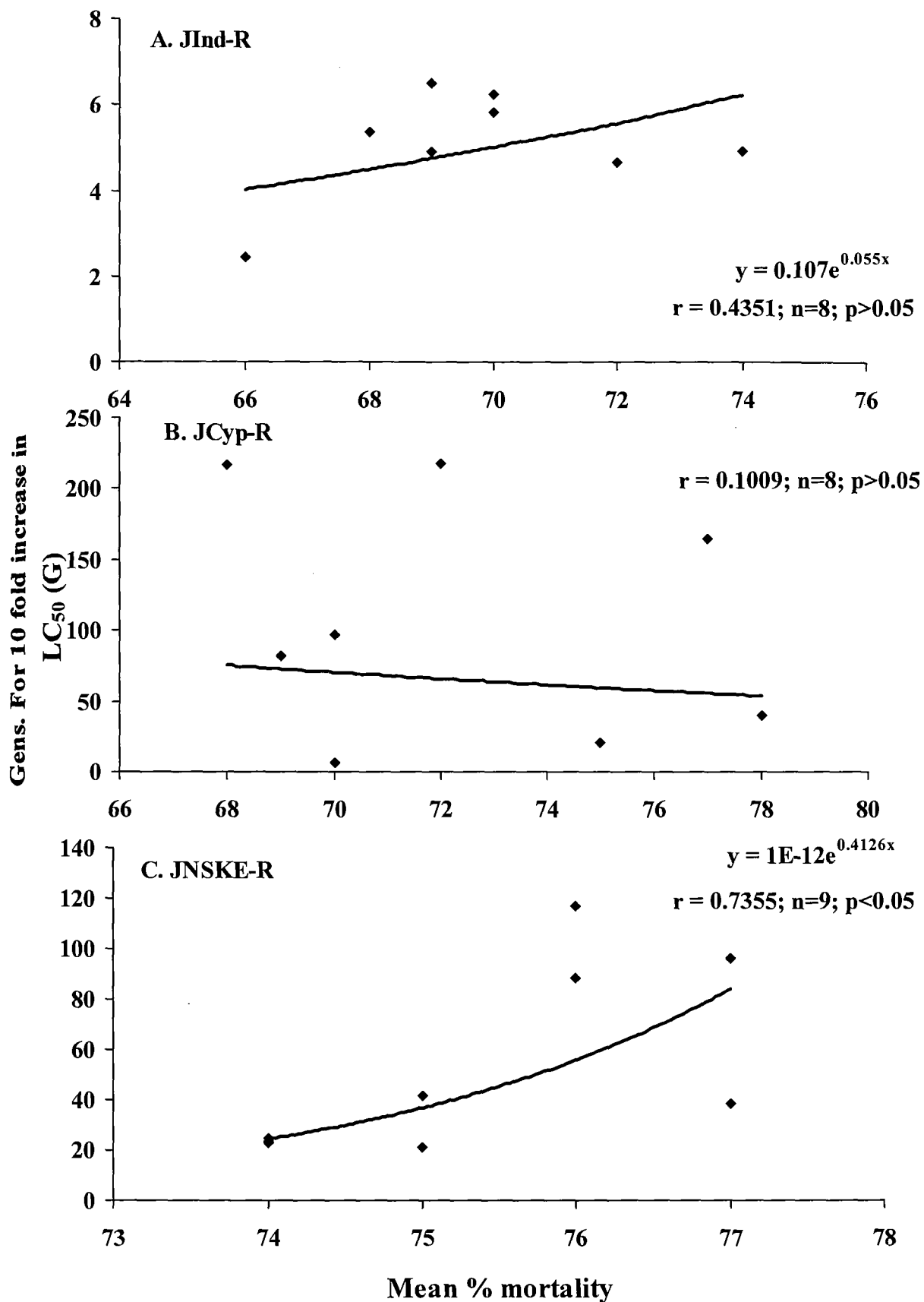


Figure 34: Interrelation between number of generations (G) required for 10 fold increase in LC_{50} and % mortality (at adult emergence) between different intervals of selection with indoxacarb, cypermethrin and NSKE in JInd-R, JCyp-R and JNSKE-R strains of *Plutella xylostella* on selection.

4.6.1 Sex ratio

When an insect is selected for resistance to an insecticide, selection may also act differentially on either sex. As a result there could be a deviation from the normal sex ratio. Therefore, during the selection for resistance in four strains of DBM, 100 pupae from each generation were followed for adult emergence and they were sexed in each generation.

In indoxacarb selected JInd-R strain, 0.52: 0.48 of male: female sex ratio was recorded. That is number of males formed were more than females but in all other strains it was female biased sex ratio. In JCyp-R a ratio of 0.45: 0.55 (Male to Female) was observed. JNzF-R with 0.45: 0.55 and JNSKE with 0.49: 0.51 of male: female ratio also recorded female biased sex ratios (Fig. 35). Average sex ratio in JField strain was 0.52: 0.48 (male: female) that was same as JInd-R strain. Therefore, it appears that except in JInd-R strain, all other strains developed for resistance with cypermethrin, Neemazal-F and NSKE, the impact appears to be stronger on males, resulting in female biased sex ratios.

4.6.2 Gross Reproductive Rate

Gross reproductive rates (GRR—mean number of eggs per female) in different strains of DBM were found to vary over generations of selection (Fig. 36). In indoxacarb selected JInd-R strain, on an average 85.44 ± 39.77 number of eggs/female were recorded (Fig. 36A). Whenever selection pressure or selection concentrations were increased, there was a reduction in the number of eggs laid per female. Further, when selection continued with the same concentration, there was an increase in the number of eggs laid per female over generations (Appendix XIII; Fig. 36A). However, on the whole there was no strong directional linear trend in the number of eggs laid per female.

Similarly, in JCyp-R strain, a mean number of 108.57 ± 36.08 eggs per females were recorded over generations of selection (Fig. 36B). Even in this strain the number of eggs laid varied over the generations of selection, failed to indicate any clear pattern. Further, in NSKE selected JNSKE-R strain, a mean number of 78.98 ± 33.99 eggs per female was recorded over generations (Fig. 36D). The overall behavioural trend was similar to that of the indoxacarb resistant strain.

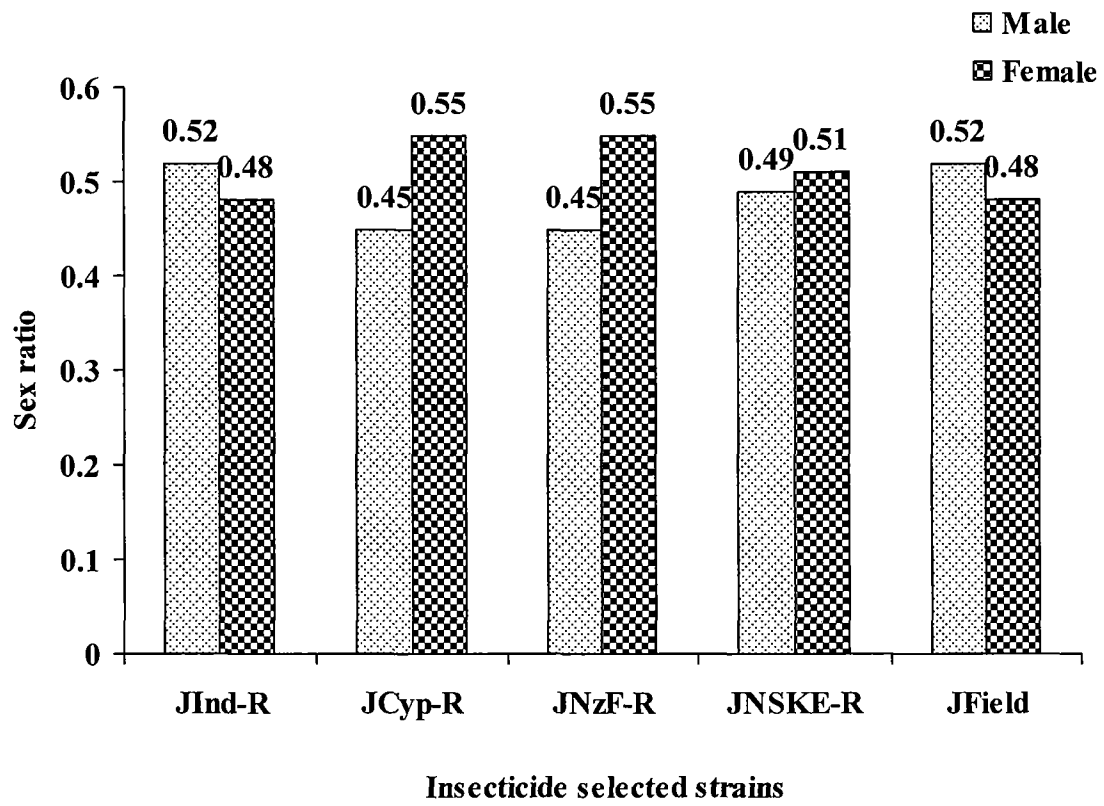


Figure 35: Mean sex ratio during selection for resistance in different strains of *Plutella xylostella*.

However, in case of JNzF-R strain selected for Neemazal-F resistance, the pattern was very different. As the observations made on the fecundity were few relative to other strains, no definite conclusions could be drawn. But yet the overall trend was similar to the indoxacarb and NSKE resistant strains. However, the mean number of 69.53 ± 42.32 eggs per female recorded during the ten generations of observation provided the lowest GRR values for any strain (Fig. 36C).

In the unselected field population (JField) the number of eggs per female recorded six times during the 25 generations of laboratory rearing, revealed the mean to be 126.92 ± 15.78 eggs per female (Appendix XIIIe) and this was the highest among the five cultures studied.

The numbers of eggs laid per female were found to vary significantly among the five strains studied ($F_{4,55} = 4.010$; $p < 0.05$; Table 27). DMRT identified three clusters. Unselected field strain, JField with 126.92 ± 15.78 eggs per female with JCyp-R, 108.57 ± 36.08 eggs per female formed the best clusters. This was followed by JCyp-R strain and JInd-R strain with 85.43 eggs per female formed the second best cluster. The cluster with the lowest mean values, JInd-R, JNzF-R (69.53 ± 42.32 eggs per female) and JNSKE-R (78.98 ± 33.99 eggs per female) strains rightfully formed a distinct cluster. Thus these results overwhelmingly suggest a strong negative association between the development of resistance to insecticide molecules and the GRR- expressed as number of eggs per female, indicating a potential biological cost in the development of insecticide resistance in *P. xylostella*.

4.6.3 Mean Generation time (T)

Another life history parameter that gets affected due to selection for resistance is the mean generation time. The mean generation time of the DBM during selection for resistance were compared with the unselected, JField-R strain. The longest developmental period of 22.73 ± 1.56 days was recorded in JNzF-R and the shortest in JCyp-R (20.89 ± 1.28 days) strain. A comparison of the five strains in the developmental period of DBM suggested the strains to differ significantly with one another ($F_{4,52} = 5.964$; $p < 0.05$). The JNzF-R strain was significantly different from other strains and was on par with JNSKE-R (22.04 ± 0.93 days) strain and the two formed a distinct cluster. JNSKE-R and the JField strains formed the second least set of values. JCyp-R (20.89 ± 1.28 days) and JInd-R

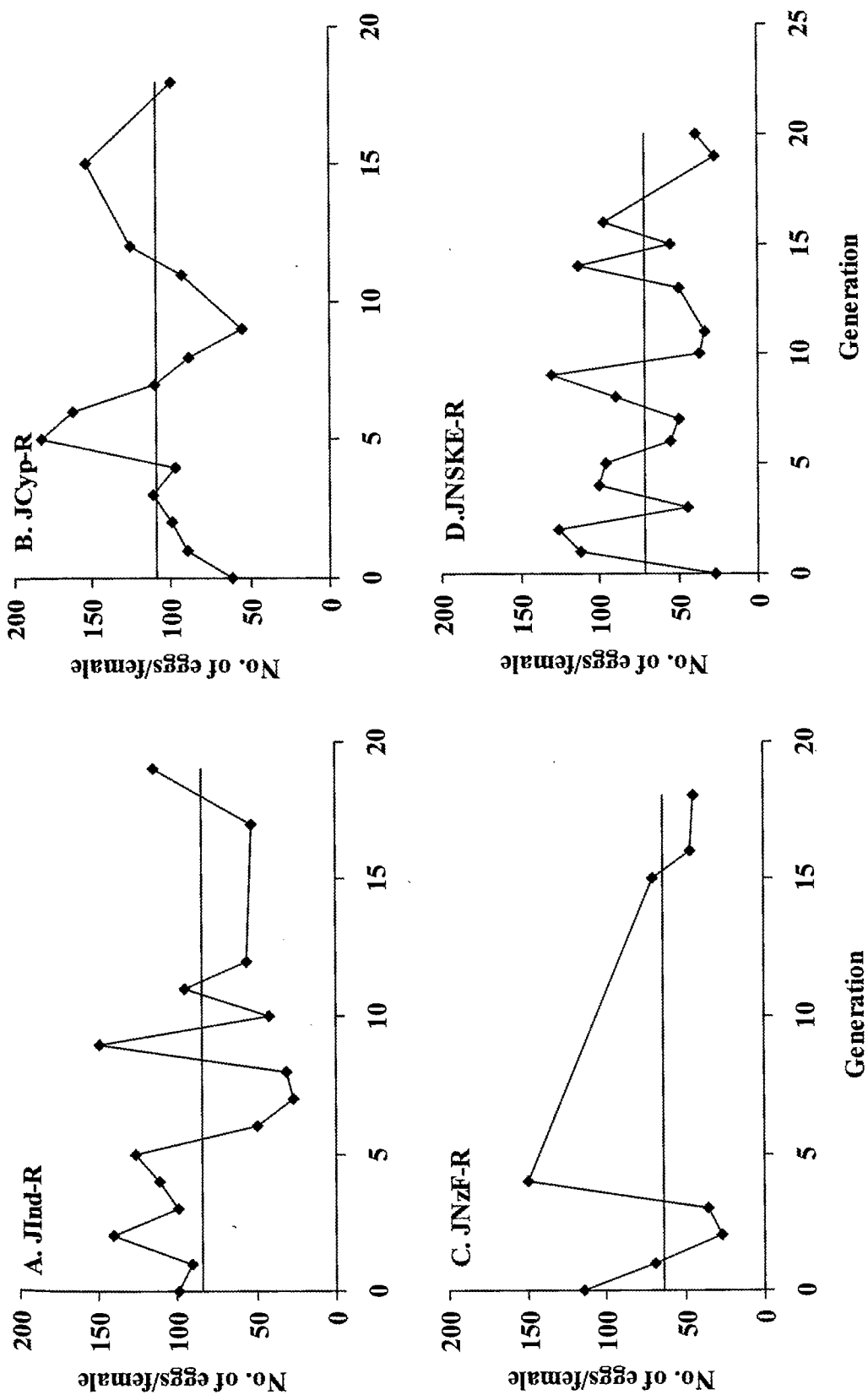


Figure 36: Gross reproductive rate (mean number of eggs / female) over generations in resistant JInd-R, JCyp-R and JNzE-R and JNSKE-R strains of *Plutella xylostella*.

Table 27 : The effect of selection to resistance on different life history parameters in four resistant strains (JInd-R, JCyp-R, JNZF-R and JNSKE-R) and in the unselected (JField) strain of *Plutella xylostella*.

Generation	GRR (No. of eggs/female)	NRR (No. of eggs/female)	T (Mean generation time - days)	Intrinsic rate of increase (r)*
JField	126.92 ± 15.78 ^a	46.67 ± 6.81 ^a	21.19 ± 0.68 ^{bc}	0.19 ± 0.01 ^a
JInd-R	85.44 ± 39.77 ^{ab}	13.65 ± 11.09 ^b	21.02 ± 0.65 ^c	0.11 ± 0.04 ^b
JCyp-R	108.57 ± 36.08 ^{bc}	15.06 ± 11.63 ^b	20.89 ± 1.28 ^c	0.12 ± 0.04 ^b
JNZF-R	69.53 ± 42.32 ^c	12.68 ± 16.08 ^b	22.73 ± 1.56 ^a	0.09 ± 0.05 ^b
JNSKE-R	78.98 ± 33.99 ^c	11.22 ± 8.47 ^b	22.04 ± 0.93 ^{ab}	0.10 ± 0.04 ^b
F Calculated	4.010	12.868	5.964	5.505
p	0.006	0.000	0.000	0.001

*No. of eggs/female/day

Note: Means followed by the same letters along the column are not significantly different by DMRT.

(21.02 ± 0.65 days) strains, were on par with the JField strain and formed the cluster with the least number of days for the mean generation time. Thus it can be noticed that two of the resistant strains had values lower than the JField (21.58 ± 0.59 days) strain while the two other resistant strains (both based on neem) had higher values than the JField strain (Table 27). Hence, the exact nature of the impact of the development of resistance in *P. xylostella* to the developmental duration does not seem to show a pattern.

4.6.4 Intrinsic rate of increase (*r*)

Intrinsic rates of increase (*r* - the mean number of eggs/female/day) were also worked out at different generations during the course of selection for resistance in different strains of *P. xylostella*. Intrinsic rate of increase represents the biotic potential of a population to grow. This factor subsumes the various growth, development, survival and reproductive parameters of a population. As a result, the overall impact of any selection agent on a population on its life history can best be gauged by the impact on the *r* values of that population. Negative changes associated with *r* values then represent the cost of response to selection.

Indoxacarb selected, JInd-R strain demonstrated the *r* values to vary over generations from 0.037 to 0.160 eggs / female/ day (Appendix XIII; Fig. 37A) with an overall mean of 0.11 ± 0.04 eggs / female / day. Even though a definite trend over generations of selection was lacking, there appeared to be an apparent reduction in the *r* values over generations. Similarly, in JCyp-R strain selected with cypermethrin, *r* values varied with a maximum of 0.19 eggs / female/ per day at 15th and minimum (0.06 eggs / female/ day) at 5th generation (Appendix- XIIIa; Fig. 37B). Mean of all the *r* values over generations of selection was 0.12 ± 0.04 eggs/ female / day. Even in NSKE selected JNSKE-R strain *r* values were decreasing over generations and they varied from 0.03 to 0.16 eggs / female/ day over generations (Fig. 37D) of selection. Mean intrinsic rate of increase over generations of selection with NSKE was 0.097 ± 0.04 eggs / female / day in JNSKE-R strain. In Neemazal-F selected JNzF-R strain, a mean intrinsic rate of increase of 0.0913 ± 0.054 eggs / female / day was recorded.

The *r* values at different generations of selection in selected strains and in unselected JField strain were compared using ANOVA. There was a significant difference ($F_{4,51} = 5.505$; $p < 0.05$; Table 27) in the mean values of *r*. Unselected JField strain

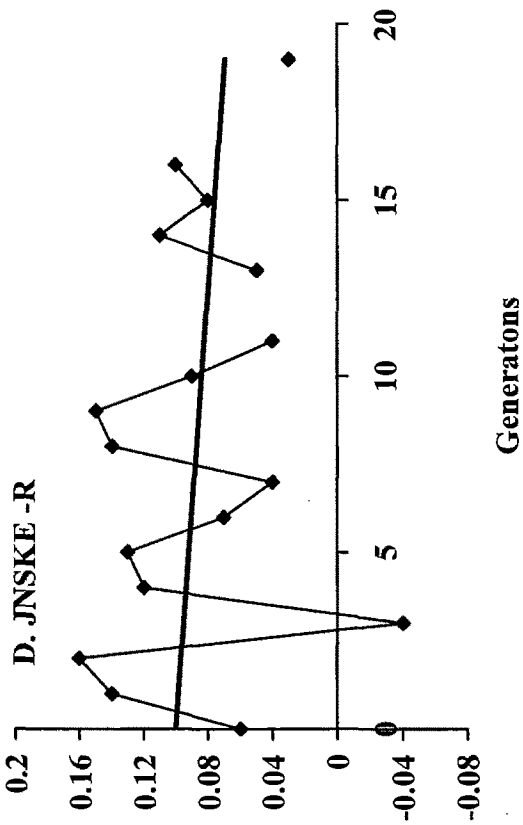
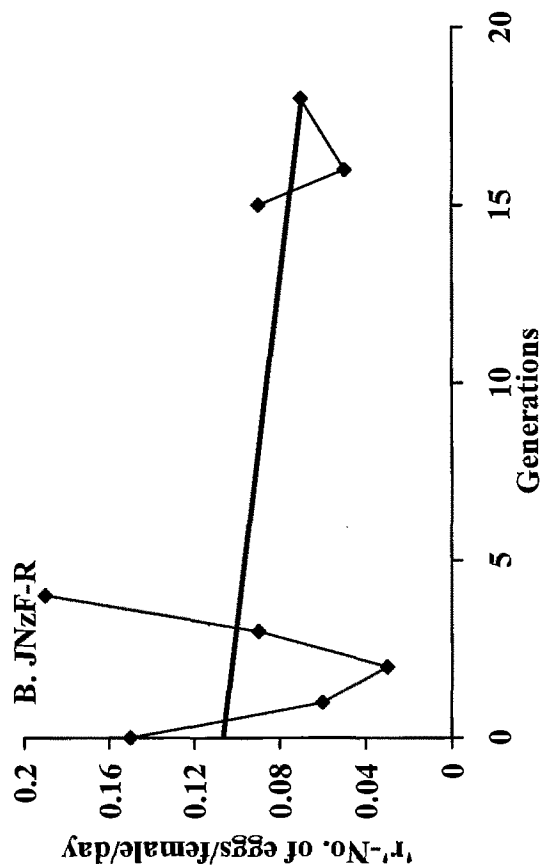
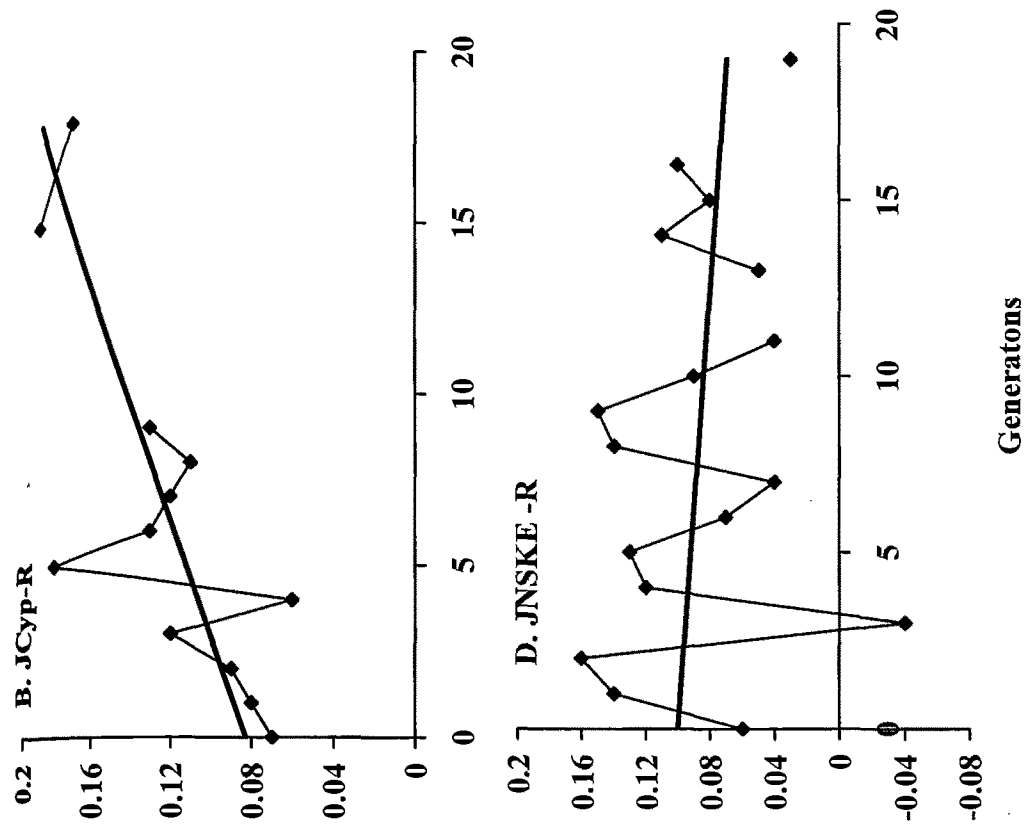
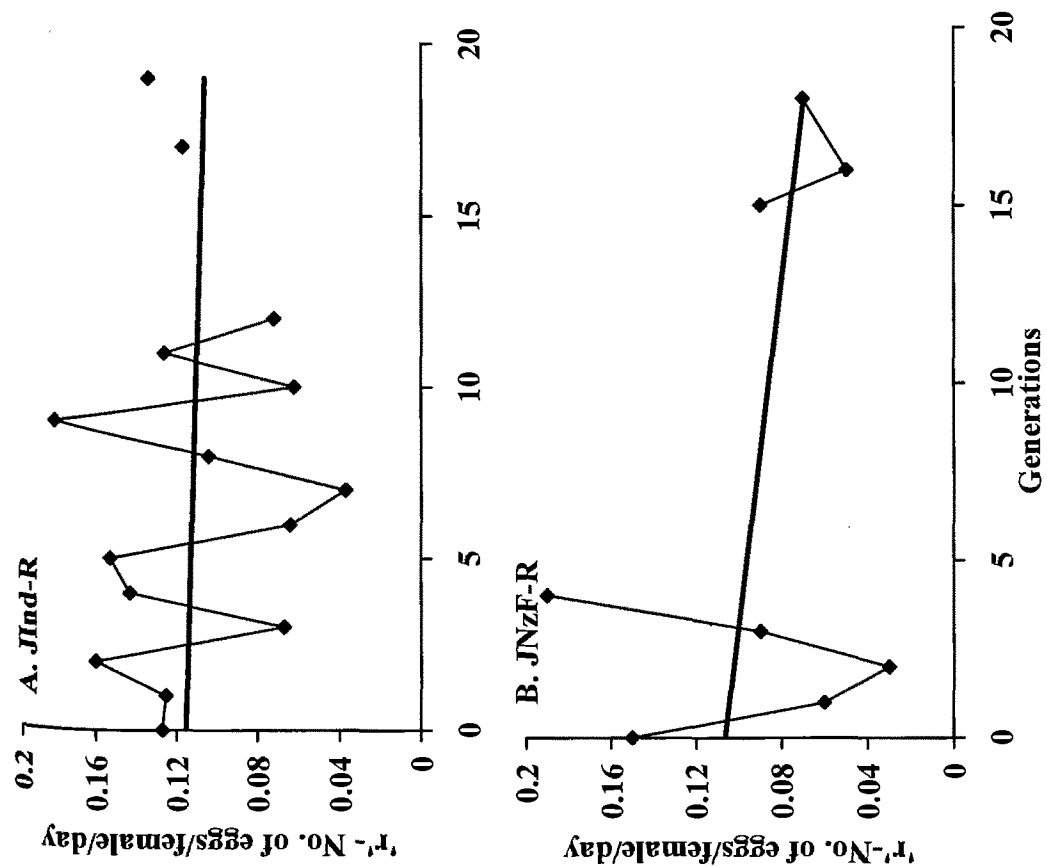


Figure 37: Intrinsic rate of increase "r" (mean number of eggs / female/day) over generations in resistant JInd-R, JCyp-R and JNz-R and JNSKE-R strains of *Plutella xylostella*.

recorded significantly higher r value (0.185 ± 0.01 eggs / female / day: DMRT) and was superior to all the other strains that have experienced the selection pressure from insecticides. Clearly, the r value serves as a strong indicator of the cost of insecticidal resistance among the selected populations.

As a corollary to the above, it was also observed that the r values tended to increase with generations whenever the selection concentrations were held constant in all the four insecticide selected strains. The trends were all significant and demonstrated an exponential growth (Fig. 38) in the ratios of r values with the generation. Exponential increase was highest in JNzF-R strain ($y = 0.419e^{0.8869x}$; $r = 0.9972$; $n = 5$; $p < 0.05$). The next best was JInd-R ($y = 0.8137e^{0.3243x}$; $r = 0.6529$; $n = 9$; $p < 0.05$), followed by JNSKE ($y = 0.8546e^{0.2108x}$; $r = 0.5584$; $n = 13$; $p < 0.05$) and JCyp-R ($y = 0.8033e^{0.1788x}$; $r = 0.9069$; $n = 8$; $p < 0.05$) strains. The trends were all significant and demonstrated an exponential growth in ratios of r values with the generation. Thus the results overwhelmingly support the idea of cost of insecticidal resistance in the first place and secondly suggest the potency of the insect to surge back whenever the selection pressures were held constant, with a greater vigour.

4.6.5 Inter-relationship between GRR, NRR and ' r '

4.6.5.1 Relation between GRR vs NRR

Net reproductive rate (NRR) is the realized number of eggs per female in a generation. The NRR considers the number of eggs laid at every age x by the proportion of females living at that age. Hence all effects on adult longevity and the overall fecundity get expressed in NRR calculations. Given this, what could be the relationship between GRR and NRR?

As can be expected, positive relations between GRR and NRR were evident in JInd-R ($y = 0.2236x - 5.4559$; $r = 0.802$; $n = 15$; $p < 0.01$), JCyp-R ($y = 0.206x - 7.305$; $r = 0.684$; $n = 12$; $p < 0.01$), JNzF-R ($y = 0.3508x - 11.71$; $r = 0.923$; $n = 8$; $p < 0.01$) and JNSKE-R ($y = 0.2093x - 5.0281$; $r = 0.899$; $n = 17$; $p < 0.01$). The significant correlations between NRR and GRR (Fig. 39) suggest the overall similarity in the mortality patterns of adults over generations during the course of selection in all the selected strains. Thus, few extraneous factors have played a role in the determination of NRR in all the resistant strains.

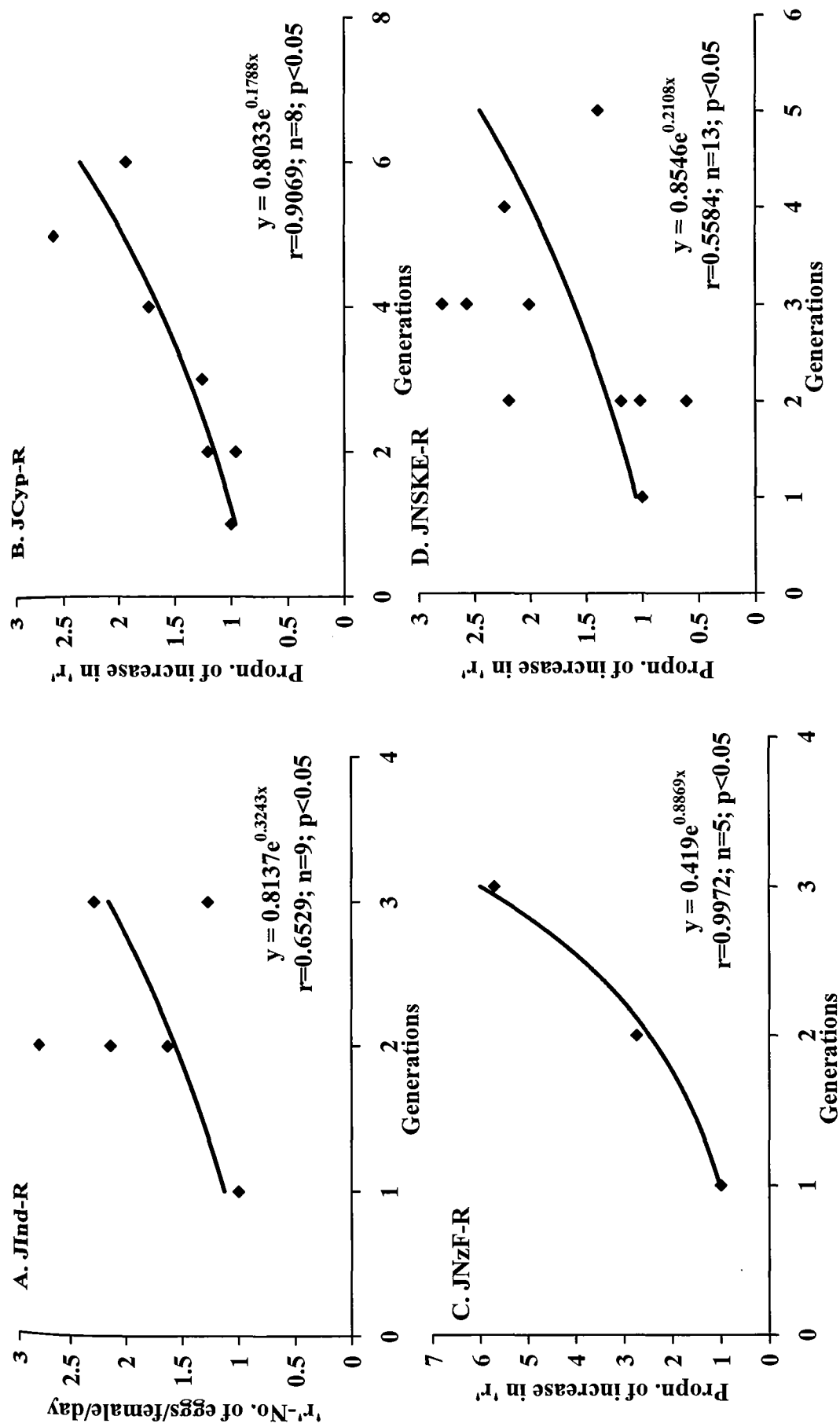


Figure 38: Proportional change in values of Intrinsic rates of increase " r " values (mean number of eggs / female/day) over generations on selection for resistance at constant dose in JInd-R, JCyp-R and JNzF-R and JNSKE-R strains of *Phutella xylostella*.

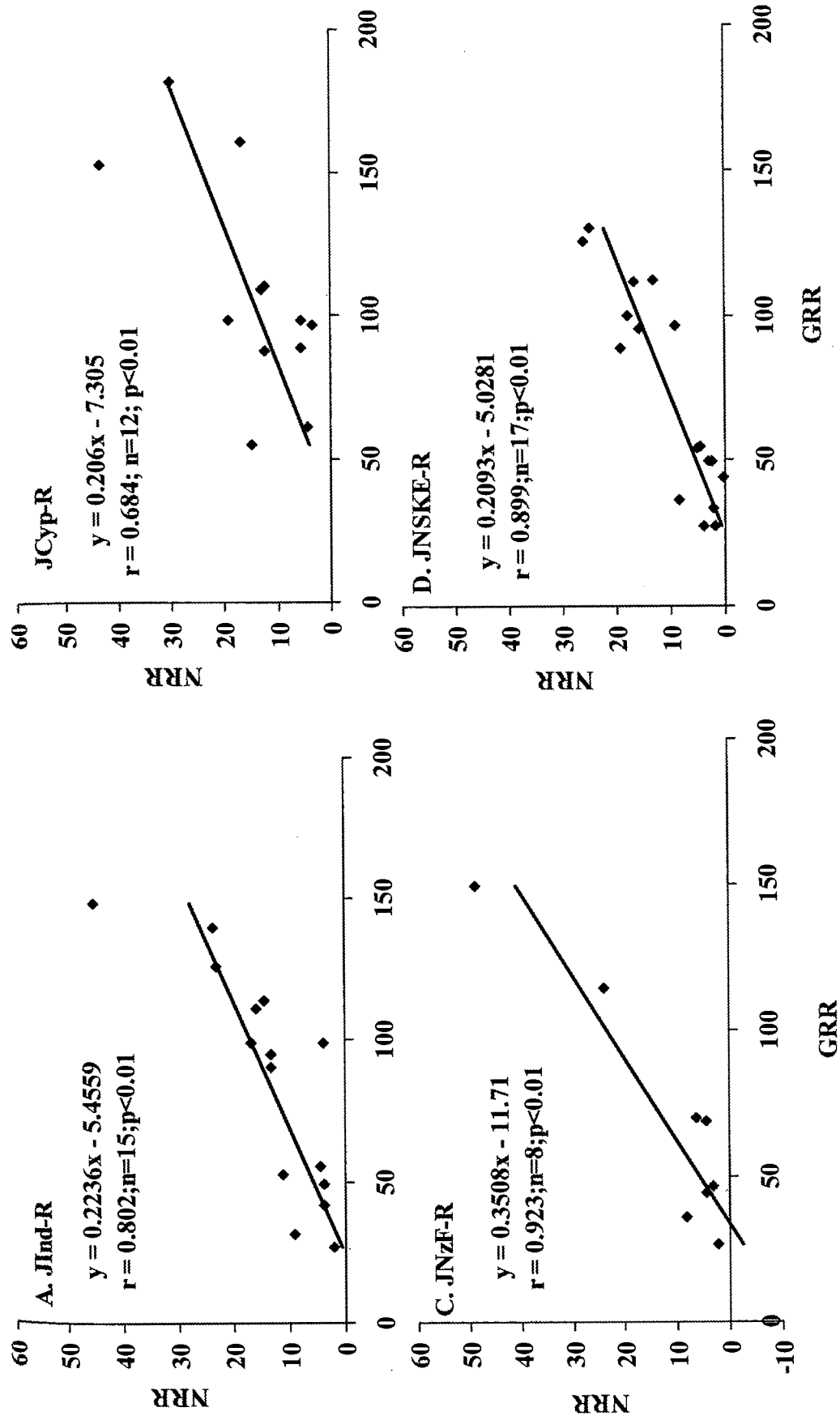


Figure 39: Relationship between the Gross reproductive rate (GRR- No. of eggs/female) and Net reproductive rate (NRR-No. of eggs/female/generation) of four resistant strains of *Plutella xylostella* at different generations. (Indoxacarb- JInd-R; Cypermethrin- JCyp-R; Neemazal-F- JNzF-R; NSKE- JNSKE-R).

4.6.5.2 Relation between GRR vs Intrinsic rate of increase (r)

Study on the relation between GRR and r gives the extent of effect on GRR that influences the intrinsic rate of increase. Strong positive linear relations were observed in all the selected strains between GRR and r . However, the impact of GRR on r can be best estimated through slopes of the linear relations. The influence of changed GRR was maximum on r in JNzF-R ($y = 0.0012x + 0.0097$; $r = 0.926$; $n = 8$; $p < 0.01$) and JNSKE ($y = 0.0011x + 0.0052$; $r = 0.872$; $n = 17$; $p < 0.01$). Followed by JInd-R ($y = 0.0009x + 0.0355$; $r = 0.836$; $n = 15$; $p < 0.01$) and JCyp-R ($y = 0.0007x + 0.0455$; $r = 0.630$; $n = 12$; $p < 0.01$) strain (Fig. 40). Therefore, above results indicate that, any effect on fecundity in all the selected strains will affect the biotic potential of the population and hence, the population growth.

4.6.5.3 Relation between NRR vs Intrinsic rate of increase (r)

Relationship of the NRR with intrinsic rate of increase (r) in general and the effect of changing NRR on r values in selected strains in specific were studied. When checked for their relation through fitting different linear and non-linear functions, it was observed that the logarithmic functions fitted best for the relationships. The relationships were significant in all the selected strains, JInd-R ($y = 0.0496\ln(x) - 0.0027$; $r = 0.9947$; $n = 15$; $p < 0.05$), JCyp-R ($y = 0.0534\ln(x) - 0.01$; $r = 0.9732$; $n = 12$; $p < 0.05$), JNzF-R ($y = 0.0509\ln(x) - 0.0101$; $r = 0.9959$; $n = 8$; $p < 0.05$) and JNSKE-R ($y = 0.0403\ln(x) + 0.0139$; $r = 0.9759$; $n = 17$; $p < 0.05$). Logarithmic relations, essentially indicate a maximum limit for the ' y ' values. Therefore the rate of change of r reduces with increasing NRR values so that after some limit, the increasing NRR will not result in increased r values. Hence, there is a limit for biotic potential that can potentially be achieved by a population that was evident in all the selected strains.

The slope of the logarithmic function was lowest in JNSKE-R ($b = 0.0403$) and highest in JCyp-R ($b = 0.0534$) strain (Fig. 41).

4.6.6 Effect of selection pressure on fitness parameters

Selection pressure is largely represented as the per cent mortality at adult emergence due to treatment with insecticides. Whenever the organisms (insects) were exposed to insecticides, the surviving individuals were supposed to incur some cost in

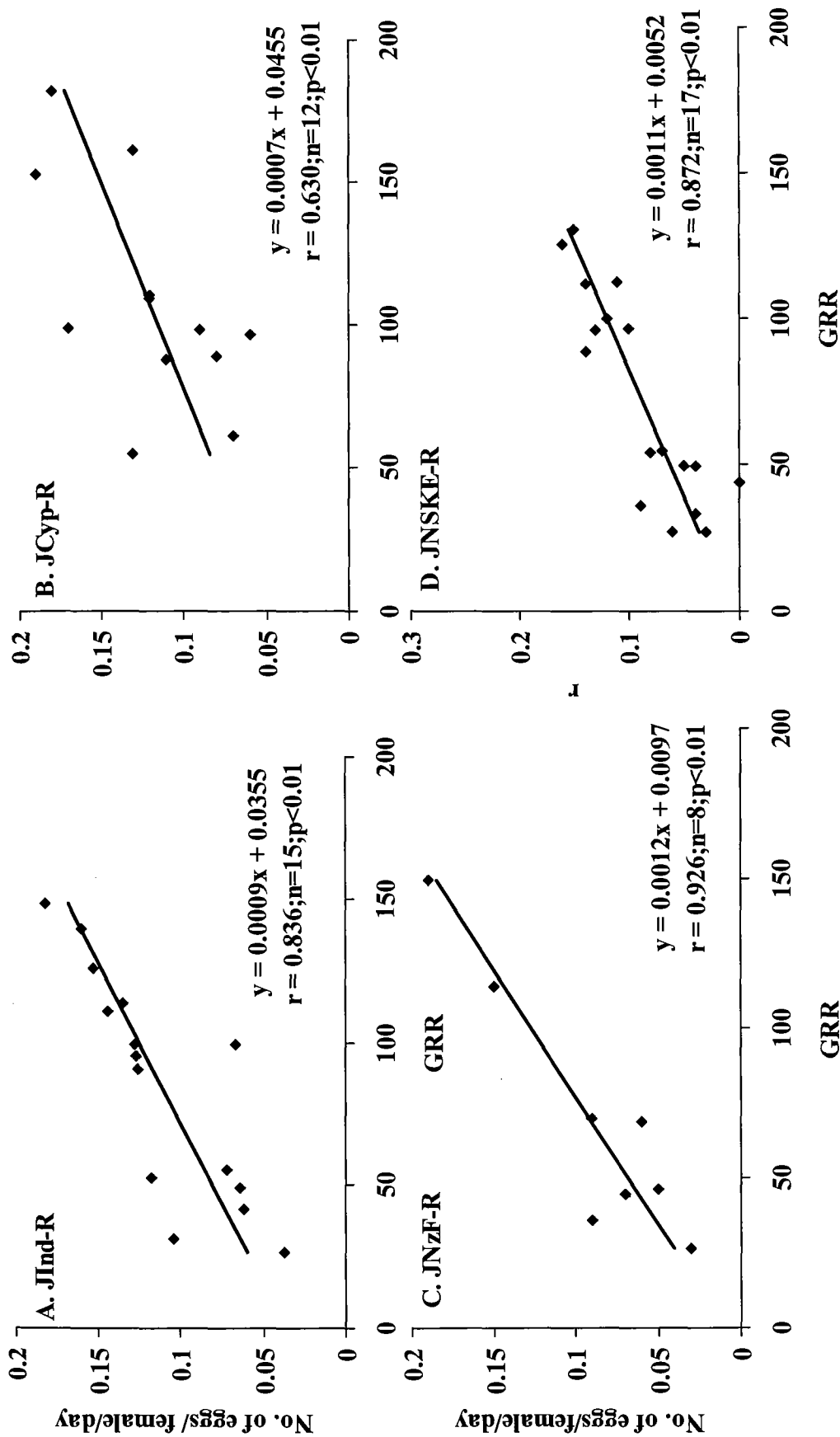


Figure 40: Relationship between the Gross reproductive rate (GRR- No. of eggs/female) and intrinsic rate of increase (r -No. of eggs/female/day) in four resistant strains of *Plutella xylostella* at different generations of selection. (Indoxacarb- JInd-R; Cypermethrin-JCyp-R; Neemazal-F- JNzF-R; NSKE- JNSKE-R)

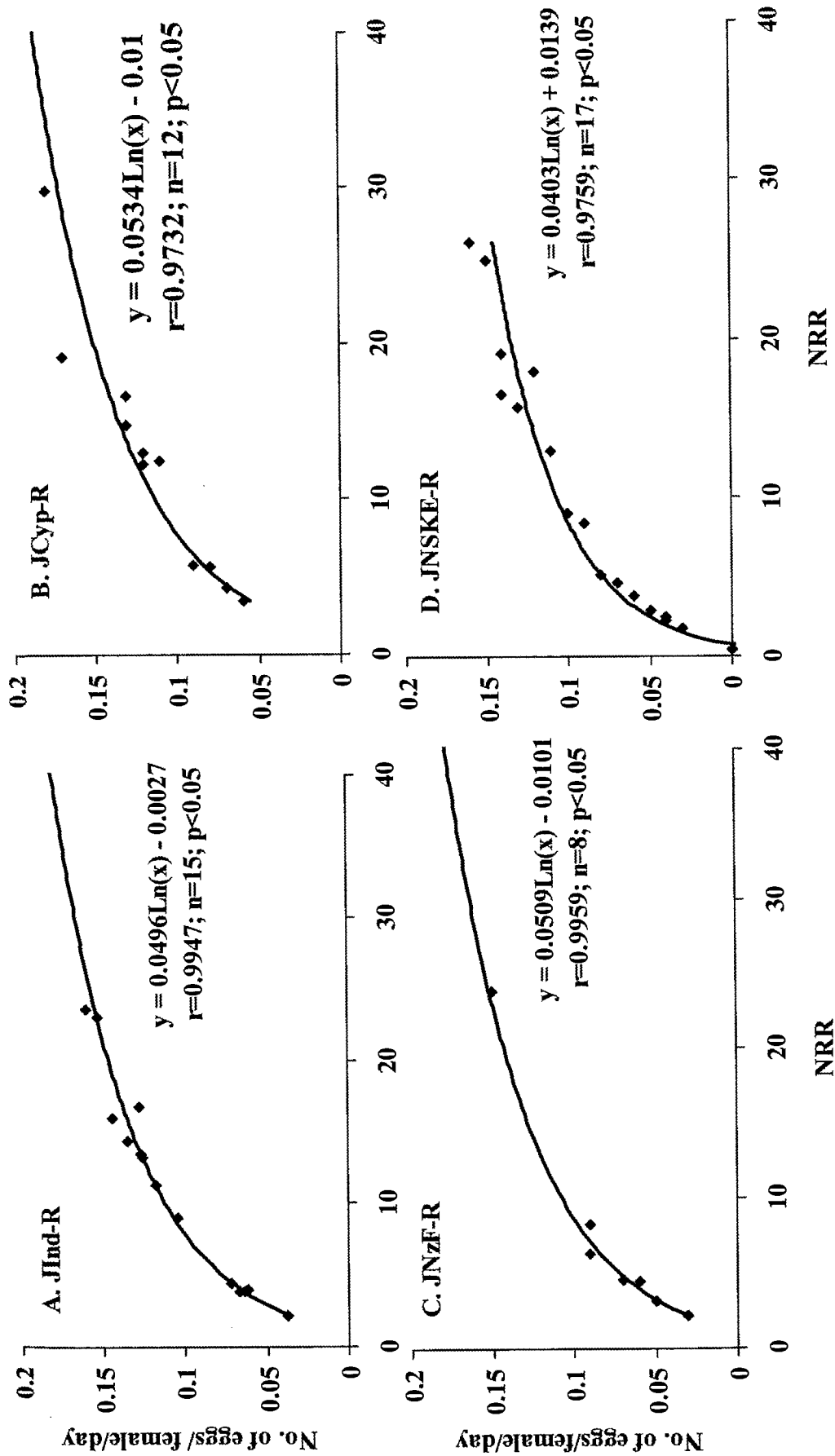


Figure 41: Relationship between the Net reproductive rate (NRR- No. of eggs/female/day) and intrinsic rate of increase (r -No. of eggs/female/day) in four resistant strains of *Phutella xylostella* at different generations of selection. (Indoxacarb- JInd-R; Cypermethrin-JCyp-R; Neemazal-F- JNzF-R; NSKE- JNSKE-R).

overcoming the effect of insecticides. Therefore, the best indicators of cost of resistance development are the different life history parameters (GRR, T , r , *etc.*). Hence, the different parameters, mean fecundity (GRR), NRR and r were compared with the selection pressure.

4.6.6.1 Gross reproductive rate (GRR)

Effect of selection pressure (per cent mortality at adult emergence) imposed over generations during the selection on gross reproductive rate (GRR) expressed as the number of eggs per female were assayed. The GRR appeared to decrease with selection pressure in both JInd-R ($r = -0.384$; $n = 15$; $p > 0.05$; Fig. 42A) and JCyp-R ($r = -0.248$; $n = 14$; $p > 0.05$; Fig. 42B) strains. However, the trends were not significant.

On the contrary, in both JNzF-R ($y = -1.8705x + 210.87$; $r = -0.767$; $n = 8$; $p < 0.05$; Fig. 42C) and JNSKE-R ($y = -1.9391x + 220.12$; $r = -0.652$; $n = 18$; $p < 0.05$; Fig. 42D) strains the relationships were very strong. Thus in both these strains, the GRR decreased with increasing selection pressure.

Thus on the whole, the selection pressure was observed to impose a commensurate reduction in the GRR values in all the selected cultures. Clearly the selection agents applied are impacting the overall biological parameters much beyond the imposition of the mortality.

4.6.6.2 Net Reproductive Rate (NRR)

Net reproductive rate (NRR) is the actual mean number of eggs laid per female in a generation. The effect of the selection for resistance on the NRR in different selected strains over the generations was assessed.

Selection pressure (per cent) imposed over generations was found to affect the NRR in indoxacarb selected JInd-R strain. Wherein, the NRRs were found to decrease with increasing selection pressure ($y = 50.667 - 0.5282x$; $r = -0.808$; $n = 15$; $p < 0.01$; Fig. 43A). Similarly, in JCyp-R strain selected with cypermethrin ($y = 61.682 - 0.6125x$; $r = -0.830$; $n = 14$; $p < 0.05$; Fig. 43B), JNzF-R selected with Neemazal-F ($y = 78.836 - 0.8755x$; $r = -0.845$; $n = 8$; $p < 0.01$; Fig. 43C) and JNSKE-R selected with NSKE ($y =$

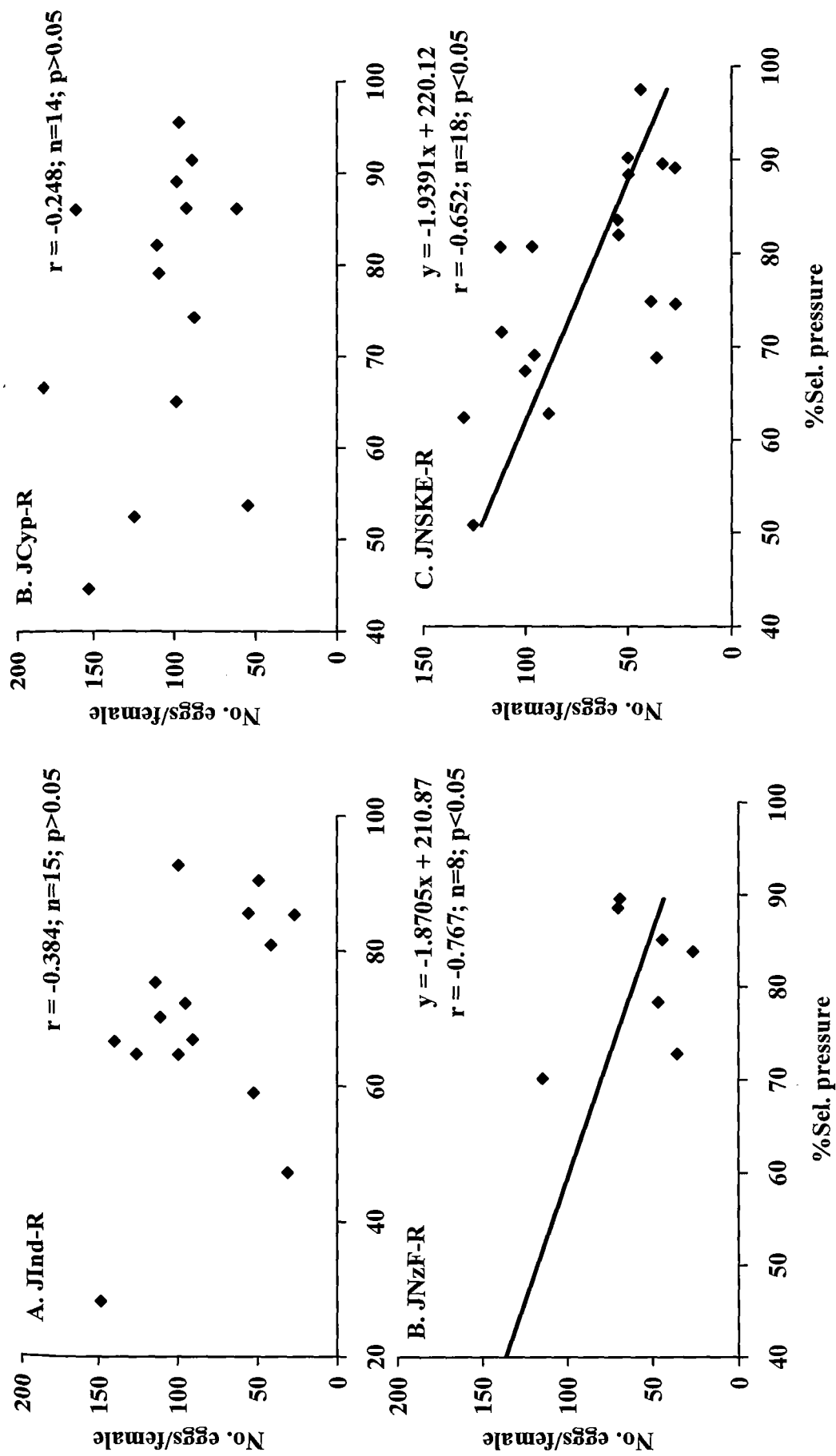


Figure 42: Interrelation between the % selection pressure imposed (% mortality at adult emergence) during selection and gross reproductive rate (GRR-No. of eggs/female) in four resistant strains of *Plutella xylostella*. (Indoxacarb-JInd-R; Cypermethrin-JCyp-R and NSKE-JNSKE-R).

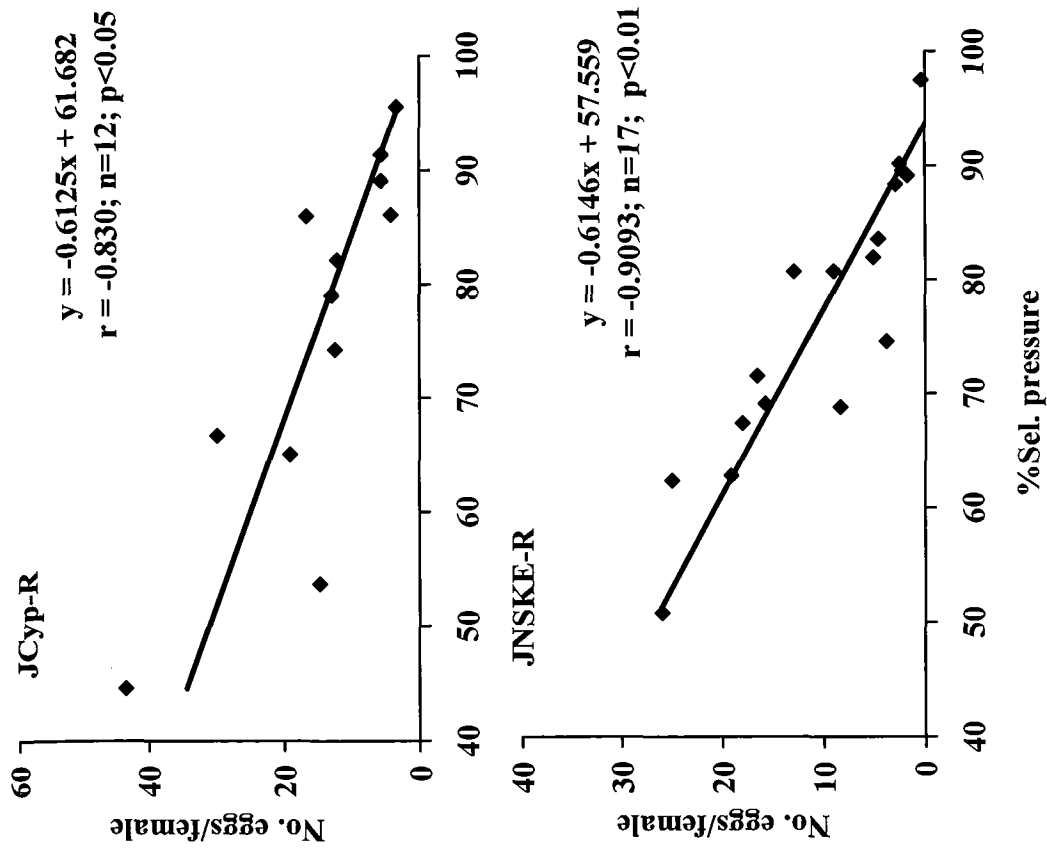
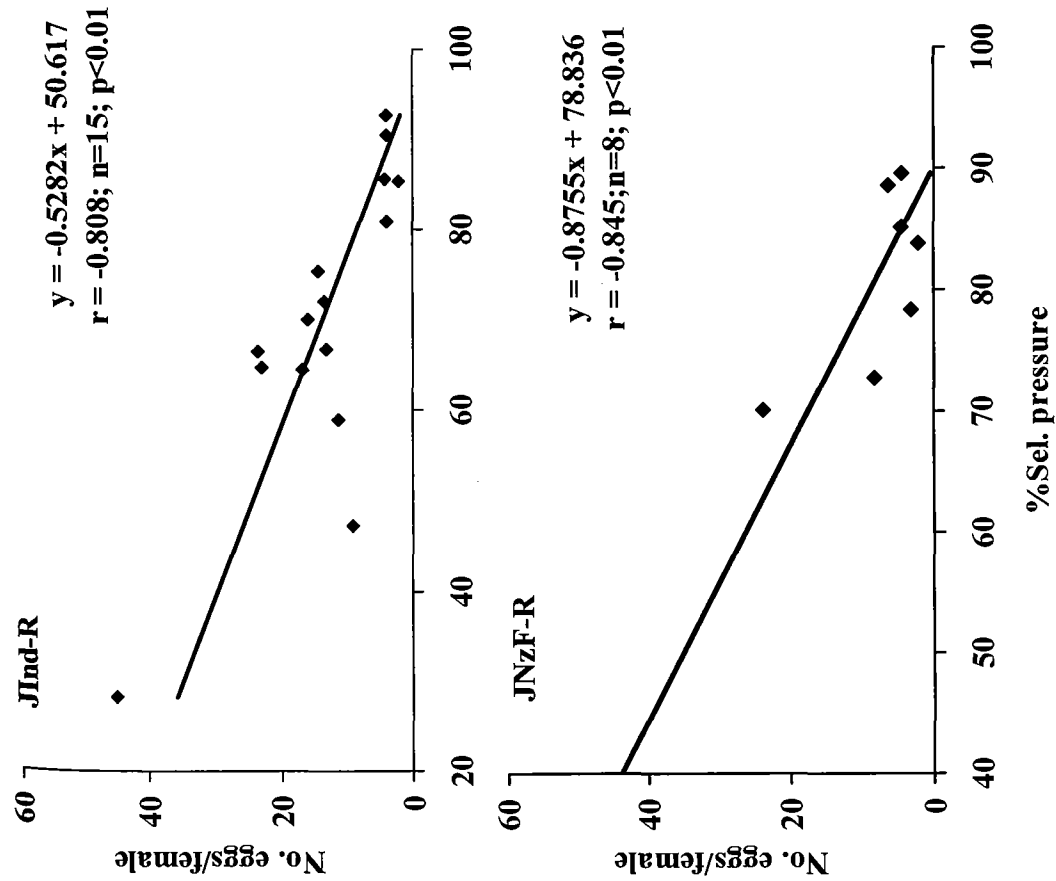


Figure 43: Inter relationship between the % selection pressure (% mortality at adult emergence) imposed during selection and net reproductive rate (NRR=No. of eggs/female) in JInd-R, JCyp-R and JNzF-R and JNSKE-R strains of *Plutella xylostella* on selection with indoxacarb, cypermethrin, neemzaal-F and NSKE, respectively.

$57.559 - 0.6146x$; $r = -0.909$; $n = 17$; $p < 0.01$; Fig. 43D), the NRRs were negatively correlated with selection pressure imposed over generations.

Further, to know the impact of selection pressure on NRRs in different strains, the slopes were compared across strains. The steeper the slope, higher the impact of selection pressure on NRR. The slope of the regression line was steepest (-0.8755) in Neemazal-F selected JNzF-R strain, followed by JNSKE-R ($-0.614.6$), JCyp-R (-0.6125) and JInd-R (-0.5282) strains. However, the slopes were not significantly different ($F_{3,44} = 1.719$; $p > 0.05$) from one another.

The above result indicates that as the intensity of selection pressure increases there was an increased fitness cost involved during development of resistance to insecticides. The above trend in the values of the slopes was commensurate with the degree of intensity of selection, where the intensity of selection was gauged in terms of number of selection agents and the modes of action.

4.6.6.3 Intrinsic rate of increase (r)

Intrinsic rate of increase (r) is the mean number of eggs / female / day, which is the direct indicator of the overall population growth potential. Therefore, effects of selection pressure on the intrinsic rates of increase (r) over generations were compared in different selected strains of *P. xylostella*.

The r values were also found negatively correlated with the selection intensity in all the four selected strains (Fig. 44). It was observed that the slopes of the relation between the intrinsic rates of increase (r) and the selection pressure followed the descending order of JNSKE-R ($y = -0.0038x + 0.3792$; $r = -0.894$; $n = 17$; $p < 0.01$), JNzF-R ($y = -0.0026x + 0.2871$; $r = -0.840$; $n = 8$; $p < 0.01$), JCyp-R ($y = -0.0022x + 0.2908$; $r = -0.827$; $n = 12$; $p < 0.01$) and JInd-R ($y = -0.0019x + 0.2414$; $r = -0.741$; $n = 15$; $p < 0.01$) strains (Fig. 44). The impact of increased selection pressure on ' r ' is more in JNSKE-R, followed by JNzF-R, JCyp-R and JInd-R strains.

4.6.7 Effect of selection intensity on different life history parameters

The selection agents used for selecting for insecticide resistance in four strains of DBM demonstrated differential selection intensity. Indoxacarb and cypermethrin being

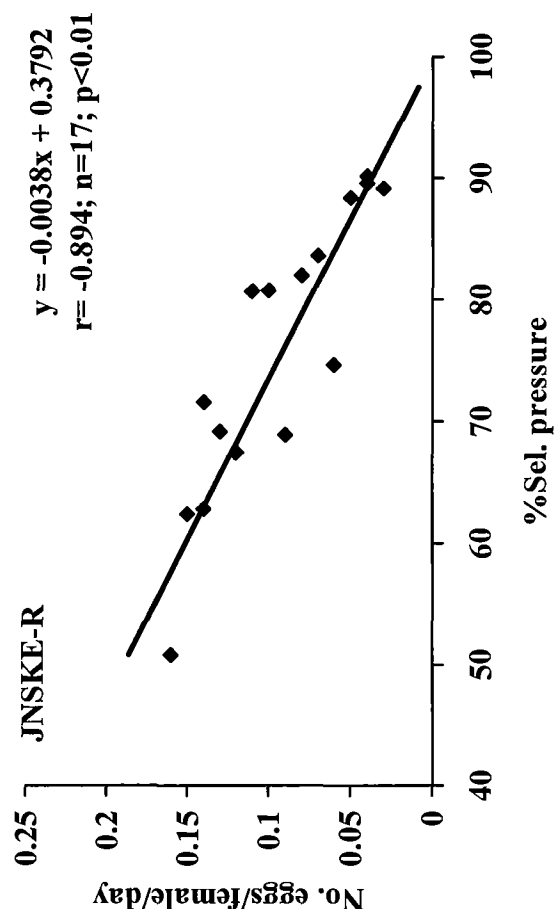
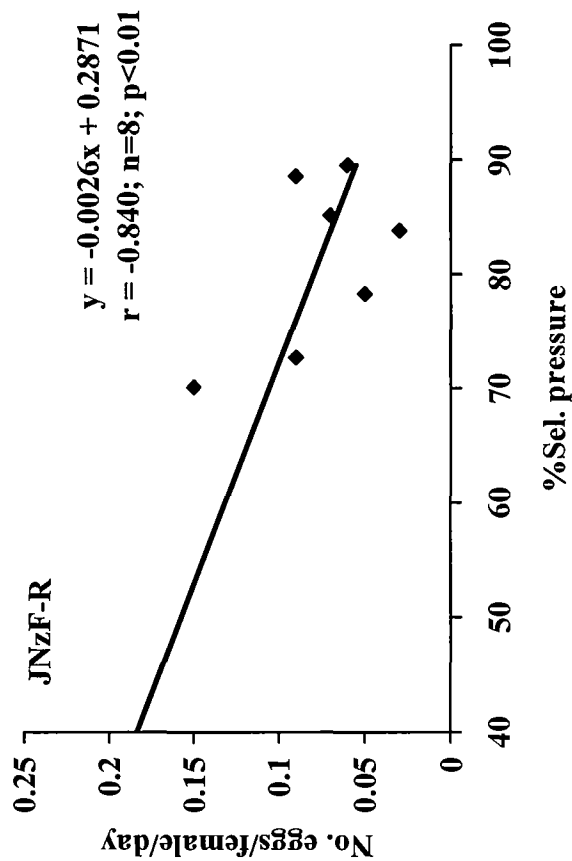
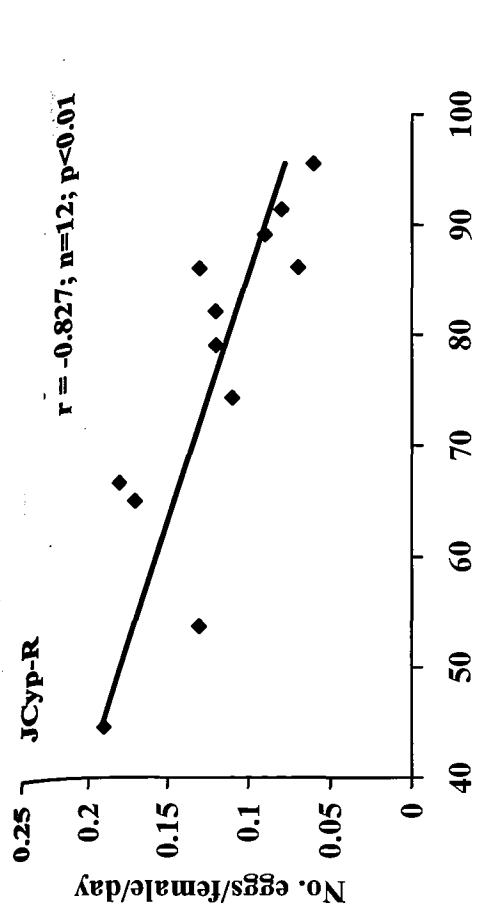
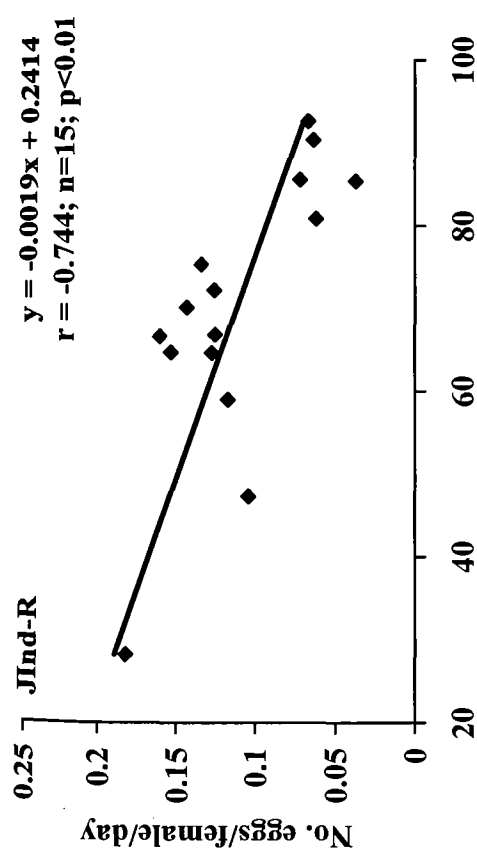


Figure 44: Inter relationship between the % selection pressure (% mortality at adult emergence) imposed and intrinsic rate of increase (r' -no. of egg/female/day) in JInd-R, JCyp-R and JNzF-R and JNSKE-R strains of *Plutella xylostella* on selection with indoxacarb, cypermethrin, neemza-F and NSKE, respectively.

synthetic insecticides are single chemicals with single modes of action. However, Neemazal-F, largely based on azadirachtin-A, is essentially a single chemical with multiple modes of action on the insect. On the contrary, the crude extract of neem – NSKE is a conglomeration of large number of chemicals (over 50 terpenoids are expected to have insecticidal property – Kraus, 1995) with multiple modes of action. If we thus rank the degree of intensities of selection on this basis, the unselected JField strain with a rank of 0, the two synthetic insecticides get a rank of 1, followed by rank 2 for Neemazal-F and rank 3 for NSKE. We made an over all comparison of the various parameters of insecticidal resistance and life history of the five strains across these ranks – the degree of intensity of selection.

The intensity of selection was found negatively associated with GRR ($y = -20.113x + 118.98$; $r = -0.8491$; $n=5$; $p > 0.05$; Fig. 45A), NRR ($y = -2.0109x + 16.409$; $r = -0.9501$; $n = 5$; $p < 0.05$; Fig. 45B) and by and large a positive association with the mean generation time (T) ($y = -20.113x + 118.98$; $r = -0.8491$; $n=5$; $p > 0.05$; Fig. 45C). Perhaps due to the low sample sizes, except the NRR and the selection intensity, others were not found significant. Nonetheless, the overall trends reflect the general expectations of the impact of the selection intensity. The sum effects of these trends was also evident even in the association of the intensity of selection with the intrinsic rate of increase ($y = -0.029x + 0.1582$; $r = -0.8826$; $n=5$; $p > 0.05$; Fig. 45D). Again despite the lack of statistical significance, it can be clearly seen that the trend appears to hold strong meaning commensurate with the general expectations.

On the whole, decreasing trends in life history parameters (GRR, NRR, and r values) clearly indicate the effect of increased degree of selection intensity. A consequent impact therefore, is expected to get reflected in the overall ability of the insect to acquire resistance against these selection agents. A comparison of this aspect revealed that the development of resistance decreased with the intensity of selection (Fig. 21), albeit the lack of statistical significance.

4.7 Relaxation from selection – the Response of JField to selection agents

Five field populations of *P. xylostella* once brought from field during January 2005 were combined and reared under laboratory (JField) condition without exposure to any insecticide. At 5th and 10th generations, bioassays were carried out to know the changes in

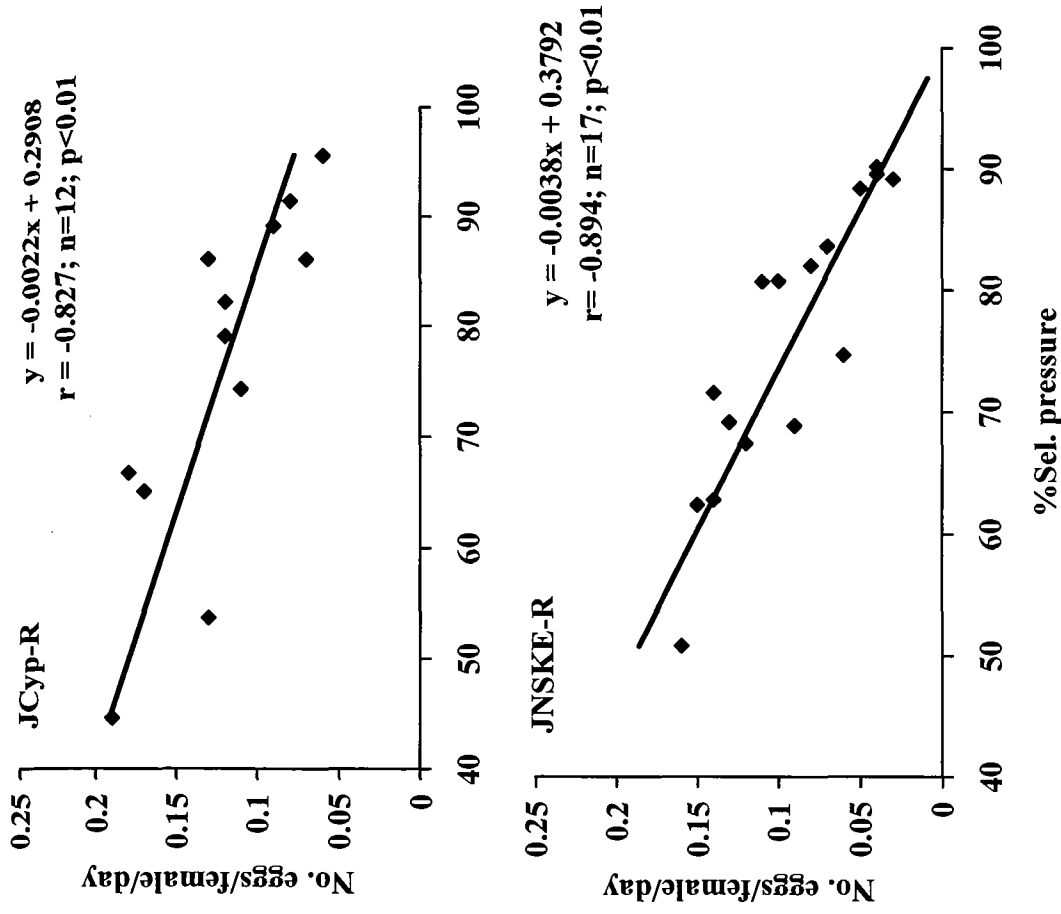
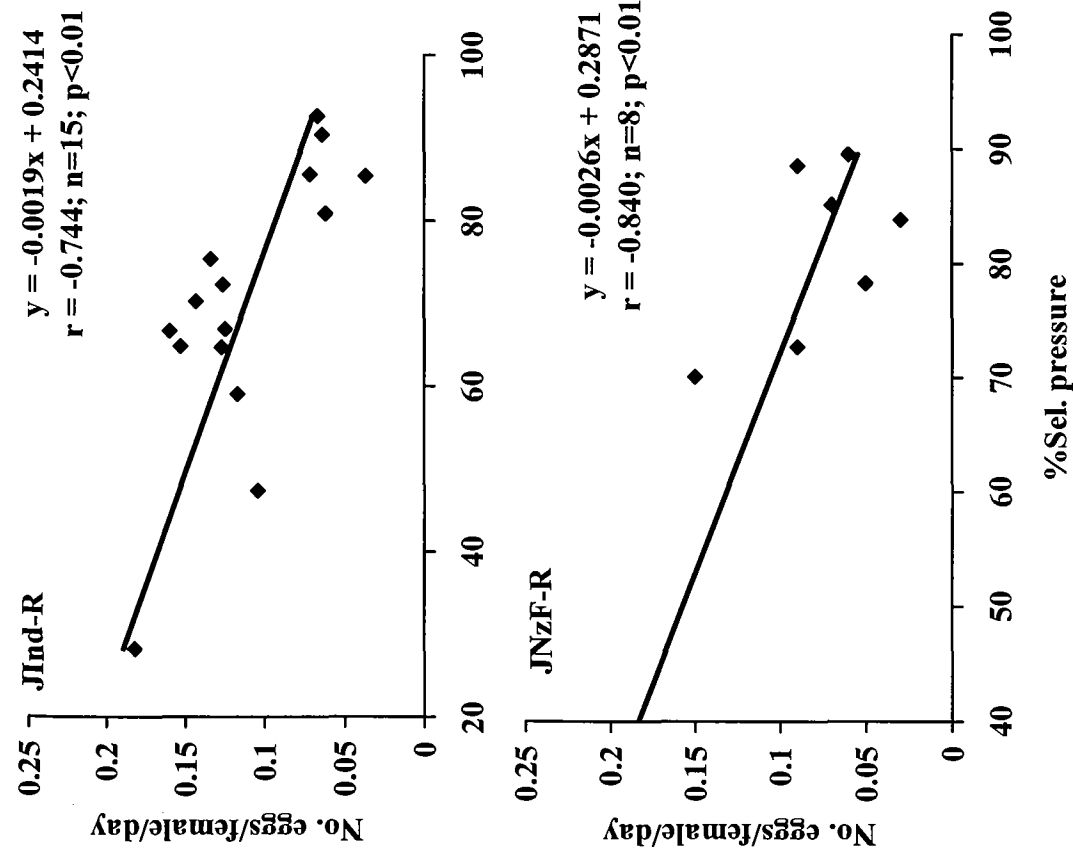


Figure 44: Inter relationship between the % selection pressure (% mortality at adult emergence) imposed and intrinsic rate of increase ('r'-no. of egg/female/day) in JInd-R, JCyp-R and JNz-R and JNSKE-R strains of *Plutella xylostella* on selection with indoxacarb, cypermethrin, neemza-F and NSKE, respectively.

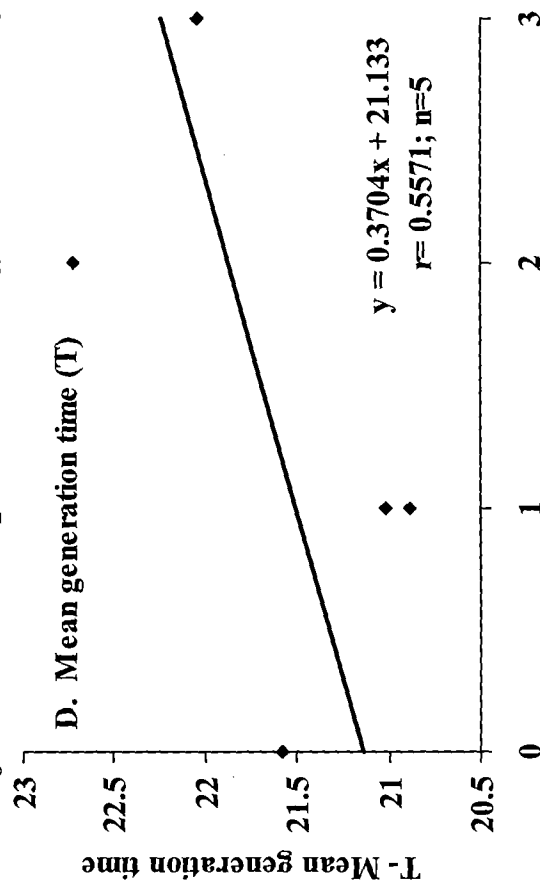
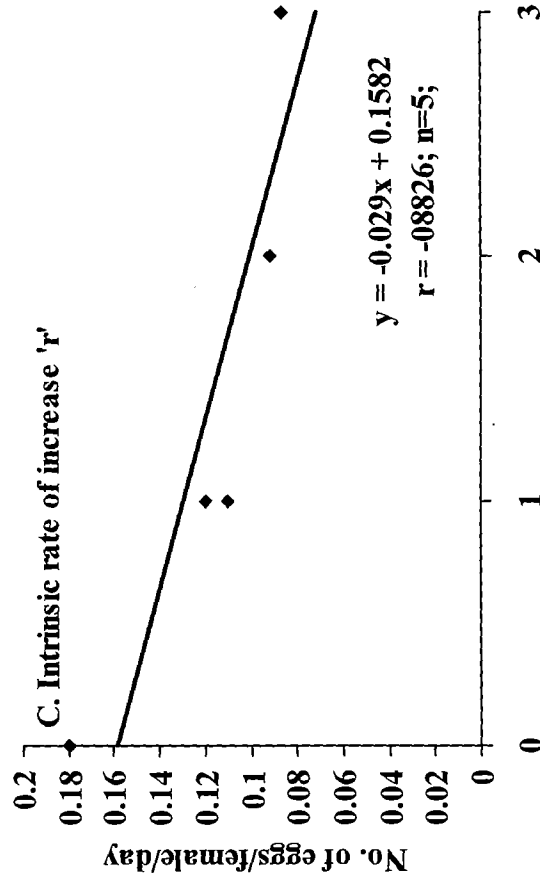
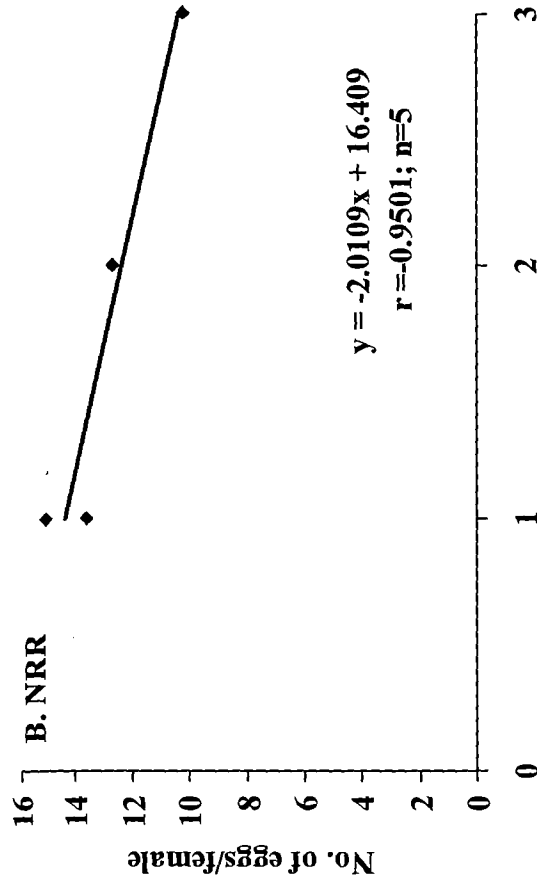
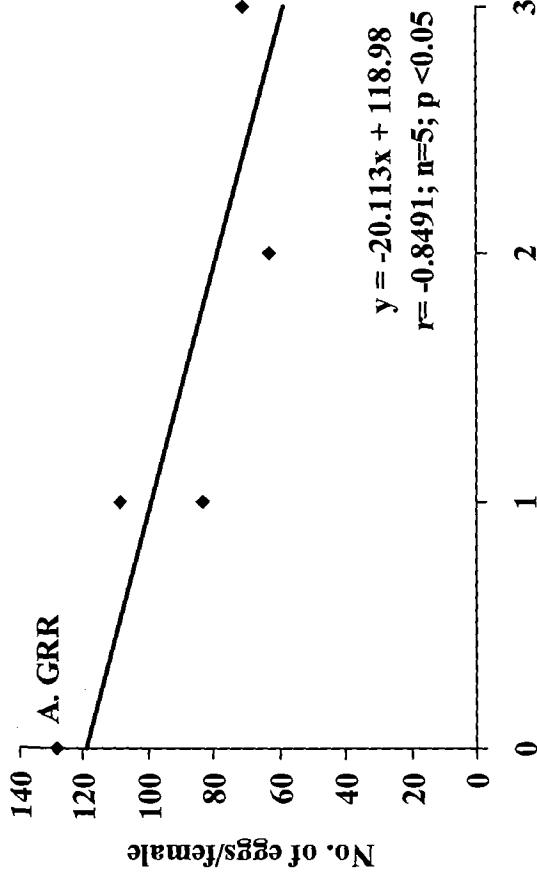


Figure 45: Relationship between the different life table parameters of *Plutella xylostella* with the selection agents (0-JField; 1-Indoxacarb; 1-cypermethrin; 2-Neemazal-F; 3-NSKE).

susceptibility on relaxation to the four selected insecticides, for which the resistant strains were selected separately, from the very same stock. LC_{50} values against indoxacarb increased at 5th generation (0.0004% a.i.) from pooled LC_{50} (0.00005 per cent a.i.) of baseline population and decreased to 0.00007 per cent a.i. at the 10th generation. However, LC_{50} values of the cypermethrin decreased over generations of laboratory rearing from the initial 0.03639 per cent a.i. at F1 generation to 0.0162 (5th generation) and 0.00638 per cent a.i. at the 10th generation. The LC_{50} values for the Neemazal-F was 0.0125 per cent CNF at F1 which decreased to 0.00766 by the 5th and later increased to 0.0101 per cent CNF at the 10th generation. With NSKE, the trend was similar to that of the indoxacarb. The base line value of 0.06652 per cent increased to 0.18116 in the 5th generation which then demonstrated a decline to 0.03251 at the 10th generation. Thus on the whole, there was a general tendency for the LC_{50} values to decrease by the 10th generation, although the trend was not strongly evident by the 5th generation.

The data despite being inadequate sufficiently provides an idea that the LC_{50} values under unselected conditions tend to decrease for all the insecticides – natural or synthetic, tested (Table 28).

4.8 Cross resistance studies

After 10 generations of selection for resistance with the insecticides JInd-R, JCyp-R and JNSKE-R strains of DBM were tested for their cross resistance to 10 insecticides available in the market including those used for selection. These represented different groups of insecticides. The cross resistance patterns were measured with respect to field strain that was also assayed after 10 generations to all the 10 insecticides along with the other susceptible lab strain (Tables 29 & 30) available. By and large it is anticipated that an RR value of more than 1 is indicative of potential positive cross resistance while a value of less than 1 is indicative of the potential negative cross resistance.

4.8.1 Cross resistance of the insecticide selected resistant strain

4.8.1.1 JInd-R strain

In the indoxacarb resistant, JInd-R strain, RRs of more than one were recorded in cypermethrin (4.998 fold), NSKE (2.929 fold), imidacloprid (1.172) and Neemazal-F (1.12 fold). However, against chlorpyrifos (LC_{50} = 0.029 % a.i.; 0.975 fold RR) and novaluron (LC_{50} = 0.000001 % a.i.; 0.25 fold RR), RRs were lower than that of the

Table 28: Response (LC_{50}) of unselected field strain, JField of *Plutella xylostella* to indoxacarb, cypermethrin, Neemazal F and NSKE at F_0 , F_5 and F_{10} generations of laboratory rearing with out exposure to any insecticides.

Generation	LC_{50} (%)	Fiducial limits at 95 % (% a.i.)	$Y = a + bx$	LC_{99} (% a.i.)	χ^2
Indoxacarb					
0	0.00005	0.00003-0.00006	4.55692+1.05156x	0.00756	1.503
5	0.00040	0.00020-0.00102	3.79038+1.11667x	0.04886	1.878
10	0.00007	0.00005-0.00010	7.19081+1.73500x	0.20424	11.139
Cypermethrin					
0	0.03639	0.03133 -0.04183	3.80898+2.64685x	0.27533	7.466
5	0.01620	0.01051-0.02241	4.55117+2.54169x	0.13325	7.566
10	0.00638	0.00518-0.00775	6.55631+1.29711x	0.03835	6.96
Neemazal-F					
0	0.01251	0.00895 - 0.01689	2.49759+1.31261x	0.74052	2.645
5	0.00766	0.00341-0.01326	3.22182+1.52284x	0.25821	1.951
10	0.01013	0.00640 -0.01543	2.86710+1.43764x	0.4206	8.071
NSKE					
0	0.06652	0.05266-0.08516	2.01626+1.71302x	1.51712	2.307
5	0.18116	0.12288-0.30455	1.36349+1.83773x	3.34148	3.081
10	0.03251	0.01680-0.05361	1.77495+1.19286x	2.89902	7.156

Table 29 : Response (LC₅₀) of susceptible JJ-Lab strain of *Plutella xylostella* larvae to ten different insecticides representing different chemical groups of insecticides.

Sl. No.	Insecticides	LC ₅₀ (%a.i.)	Fiducial limits (%a.i.)	Y= a+bx	LC ₉₉ (%a.i.)	χ^2
1	Indoxacarb 14.5SC	0.00002	0.00001 - 0.00003	=6.35755+1.36525x	0.00112	6.339
2	Cypermethrin 25EC	0.0047	0.00390-0.00554	=6.62631+2.84622x	0.03085	6.666
3	Neemazal-F-5%	0.002	0.00048 - 0.00391	=3.29070+1.21905x	0.16178	2.939
4	NSKE	0.02047	0.01538-0.02613	=3.67484+2.17600x	0.24004	6.438
5	Endosulfan 35EC	0.0391	0.01953 - 0.09168	=2.00891+1.42695x	1.66901	24.567
6	Carbaryl 50WP	0.08121	0.06829 - 0.09674	=2.43160+2.23003x	0.89702	2.163
7	Chlorpyrifos 20EC	0.02378	0.01446- 0.04199	=2.61983+1.61338x	0.6578	15.284
8	Novaluron 10EC	0.0000171	0.01178-0.02417x10 ⁻³	=1.96063+1.10959x	2.136	3.589
9	Spinosad 2.5SC	0.03545 x 10 ⁻⁴	0.02498 - 0.05086 x 10 ⁻⁴	=2.71095+1.86918x	0.62264 x 10 ⁻⁴	36.933
10	Imidacloprid 17.8SL	0.00424	0.00345-0.00517	=6.76943+2.85337x	0.02772	3.412

Table 30: Response (LC_{50}) of unselected field strain, (JField) of *Plutella xylostella* larvae to ten different insecticides representing different chemical group of insecticides after ten generations of laboratory rearing with out exposure to any insecticides.

Sl. No.	Insecticides	LC_{50} (%a.i.)	Fiducial limit (%a.i.)	$Y = a + bx$	LC_{99} (%a.i.)	χ^2
1	Indoxacarb14.5SC-I	0.00007	0.00005-0.00010	$=7.19081+1.73500x$	0.20424	11.139
2	Cypermethrin25EC	0.00638	0.00518-0.00775	$=6.55631+1.29711x$	0.03835	6.96
3	Neemazal-F-5%	0.01013	0.00640 -0.01543	$=2.86710+1.43764x$	0.4206	8.071
4	NSKE	0.03251	0.01680-0.05361	$=1.77495+1.19286x$	2.89902	7.156
5	Endosulfan35EC	0.05079	0.04450 -0.05775	$=4.14370+3.20161x$	0.27062	12.773
6	Carbaryl 50WP	0.08143	0.06698-0.09925	$=2.12363+1.94969x$	1.2705	6.803
7	Chlorpyrifos 20EC	0.03075	0.02256-0.04539	$=1.94588+1.28679x$	1.97547	7.688
8	Novaluron 10EC	0.000004058	$0.01504-0.10085 \times 10^{-4}$	$=1.20614+.86669x$	19.61104×10^{-4}	17.589
9	Spinosad 2.5SC	0.00001	$0.00604-0.01738 \times 10^{-3}$	$=1.89200+0.94592x$	0.13421×10^{-3}	7.045
10	Imidacloprid 17.8SL	0.0011	0.00079-0.00157	$=3.35472+1.13404x$	0.12393	15.431

unselected JField strain. There was no change in the RR of the spinosad ($LC_{50} = 0.000010$ % a.i). Thus, JInd-R strain appears to have a very low RR value for novaluron (Table 31).

4.8.1.2 JCyp-R Strain

JCyp-R strain demonstrated the highest positive cross resistance to imidacloprid (3.736 fold RR), followed by indoxacarb (3.285 fold RR), endosulfan (1.537 fold), NSKE (1.332 fold) and spinosad (1.1 fold RR). Only towards carbaryl a lower LC_{50} (0.0549 % a.i.; 0.674 RR) value than JField strain were recorded. However, LC_{50} values against novaluron (0.000004 % a.i.) were same as JField strain (Table 32).

4.8.1.3 JNSKE-R strain

After 10 generations of selection, the JNSKE-R strain was 3.964 fold resistant to NSKE. The increased RRs were recorded towards novaluron (6.00 fold), cypermethrin (4.76 fold), indoxacarb (2.00 fold), spinosad (2.00 fold), imidacloprid (1.782 fold) and chlorpyrifos (1.51 fold). Highest cross resistance was recorded towards novaluron (6.00 fold RR). Lower than one resistance ratios were recorded to Neemazal-F (0.831 fold), endosulfan (0.404 fold) and carbaryl (0.578 fold; Table 33).

4.8.1.4 JField vs JJ-Lab

An attempt was also made to verify the LC_{50} values of the two lab cultures that have not been exposed to any insecticide. JField was started as the check population along with the selected strains, from the same stock. However, JJ-Lab culture was started during September, 1997 from the larvae and pupae collected from around Devanahalli. Roughly >100 generations have been completed by this population in the lab. A comparative assessment of the LC_{50} values for the 10 insecticides between these strains suggested that in all but imidacloprid the JJ-Lab population had lower LC_{50} values than the JField culture (Table 29 and 30). Clearly this data suggests that the overall susceptibility of the lab cultures increase with time, irrespective of the chemical.

4.8.2 Inter relation between resistant strains

An interesting question would be to know whether there is any pattern in the way the selected strains behave among themselves. Therefore, an attempt was made to correlate the LC_{50} values for different insecticides between pairs of these cultures. It was observed that the trends in LC_{50} values, for different insecticides, of JJ-Lab, JField, JInd-R

Table 31: The response (LC_{50}) of Indoxacarb selected JInd-R strain of *Plutella xylostella* larvae to ten different insecticides representing different chemical group of insecticides. (JInd-R strain after 10 generation of selection with indoxacarb)

Sl. No.	Insecticides	LC_{50} (%a.i.)	Fiducial limits at 95 % (%a.i.)	$Y = a + bx$	LC_{99} (%a.i.)	χ^2
1	Indoxacarb 14.5SC-I	0.00858	0.00762-0.00966	15.43052+7.46767x	0.01759	1.760
2	Cypermethrin 25EC	0.03189	0.02773-0.03654	8.11820+5.42547x	0.08560	6.199
3	Neemazal-F-5%	0.01135	0.00740-0.01656	2.94087+1.51193x	0.39224	3.688
4	NSKE	0.09524	0.07477-0.12653	2.32024+2.27216x	1.00621	8.335
5	Endosulfan 35EC	-	-	-	-	-
6	Carbaryl 50WP	-	-	-	-	-
7	Chlorpyrifos 20EC	0.02998	0.02470-0.03527	4.66386+3.06196x	0.17242	3.926
8	Novaluron 10EC	0.01135 x10 ⁻⁴	0.00740-0.01656 x10 ⁻⁴	2.94087+1.51193x	0.39224 x10 ⁻⁴	3.688
9	Spinosad 2.5SC	0.01000 x10 ⁻³	0.00604-0.01738 x10 ⁻³	1.89200+0.94592x	2.87859 x10 ⁻³	7.045
10	Imidacloprid 17.8SL	0.00129	0.00085-0.00184	6.25810+2.16568x	0.01529	15.613

Table 32: The response (LC_{50}) of Cypermethrin selected JCyp-R strain of *Plutella xylostella* larvae to ten different insecticides representing different chemical groups of insecticides after 10 generations of selection.

Sl. No.	Insecticides	LC_{50} (%a.i.)	Fiducial limits at 95 % (%a.i.)	$Y = a + bx$	LC_{99} (% a.i.)	χ^2
1	Indoxacarb 14.5SC-I	0.00023	0.00018-0.00031	5.40263+1.48758x	0.00855	5.487
2	Cypermethrin 25EC	0.01547	0.01052- 0.02258	2.18638+1.20769x	1.30586	9.602
3	Neemazal-F-5%	0.00585	0.00358 -0.00855	2.65641+1.18983x	0.52795	4.65
4	NSKE	0.04333	0.03423 -0.05351	1.43395+1.05190x	7.05286	13.427
5	Endosulfan 35EC	0.0781	0.06094 -0.10354	3.04144+2.74659x	0.54909	17.063
6	Carbaryl 50WP	0.05491	0.04615-0.06389	3.28117+2.60336x	0.42977	13.558
7	Chlorpyrifos 20EC	-	-	-	-	-
8	Novaluron 10EC	0.03523×10^{-4}	(0.02588- 0.04812) $\times 10^{-4}$	2.17980+1.50017x	1.2522×10^{-4}	26.93
9	Spinosad 2.5SC	0.10748×10^{-4}	(0.05995 - 0.22297) $\times 10^{-4}$	1.28559+1.32714x	6.08425×10^{-4}	23.538
10	Imidacloprid 17.8SL	0.00411	0.00328 - 0.00502	7.44005+3.11802x	0.02291	2.613

Table 33: Response (LC_{50}) of JNSKE-R strain of *Plutella xylostella* larvae to ten different insecticides representing different chemical groups of insecticides after 10 generations of selection with NSKE.

Sl.No.	Insecticides	LC_{50} (%a.i.)	Fiducial limits at 95 % (%a.i.)	$Y = a + bx$	LC_{99} (%a.i.)	χ^2
1	Indoxacarb14.5SC	0.00014	0.00010-0.00020	6.30161+1.63559x 0.20023	0.00085 0.00371	14.052
2	Cypermethrin25EC	0.03038	0.02588 -0.03549	4.67107+3.07845x	0.17312	4.191
3	Neemazal-F-5%	0.00842	0.00556-0.01194	2.93413+1.41434x	0.37177	9.028
4	NSKE	0.12887	0.10023 -0.16551	1.39065+1.56280x	3.96942	22.545
5	Endosulfan35EC	0.02054	0.01776-0.02308	4.49356+2.66291x	0.15351	11.823
6	Carbaryl50WP	0.04709	0.03762-0.05638	3.97922+2.99860x	0.12600	8.041
7	Chlorpyrifos20EC	0.04646	0.04302-0.05010	3.74123+2.80670x	0.31325	13.461
8	Novaluron10EC	0.02428 x10 ⁻³	0.01134 -0.04855 x10 ⁻³	1.90745+1.18131x	2.26257 x10 ⁻³	78.617
9	Spinosad2.5SC	0.01981 x10 ⁻³	0.01467 -0.02716 x10 ⁻³	3.73287+2.19183x	0.22819 x10 ⁻³	3.773
10	Imidacloprid7.8SL	0.00196	0.00144 -0.00271	5.71254+2.10902x	0.00793	16.893

and JCyp-R were very similar. As a result, strong correlations were observed between any pair among them (Table 34). However, JNSKE-R strain, behaved very different from all other strains except the JInd-R strain ($r = 0.989$; $p = 0.0001$). Clearly the results suggest that the overall trends in LC_{50} values of above four cultures are similar. However, the neem selected cultures may behave very differently towards different insecticides.

Further, one can also raise a question of whether there was any trend among the insecticides tested across cultures. It was observed that 41 of the 45 possible combinations tested did not indicate any relationships among themselves (Table 35). Significant positive correlations were recorded between cypermethrin vs NSKE ($r = 0.940$; $p = 0.017$); chlorpyrifos vs novaluron ($r = 0.956$; $p < 0.044$) and chlorpyrifos vs spinosad ($r = 0.989$; $p < 0.011$) and negative correlation between Neemazal-F to imidacloprid ($r = -0.926$; $p < 0.024$) was observed.

4.9 Biochemical mechanisms of insecticidal resistance

The activity of the different groups of enzymes involved in detoxifying an insecticide in the selected strains were assayed in terms of their increased activity against specific substrates. For this purpose, activity levels of MFOs, esterases, glutathione-S-transferases were assayed across different resistant and susceptible cultures.

4.9.1 Mixed Function Oxidases (MFOs) : Para-nitroanisole

The activity of the MFOs was measured in terms of the η mole of para-nitrophenol formed per mg of protein per min from para-nitroanisole through NADPH mediated activity. The activity was highest in JCyp-R (4.26 ± 0.77 η mole/mg protein/minute) strain and the lowest (2.83 ± 0.45 η mole/mg protein/minute) in JJ-Lab strain. The observed differences in activity between the strains were significant ($F_{5,48} = 6.218$; $p < 0.05$; Table 36; Fig. 46a). The JCyp-R strain was significantly different from all other strains and was followed by JNzF-R (3.66 ± 0.93 η mole/mg protein/minute), JInd-R (3.65 ± 0.44 η mole/mg protein/minute) and JNSKE-R (3.32 ± 0.20 η mole/mg protein/minute) strains. All these three were on par with one another. JField (3.08 ± 0.57 η mole/mg protein/minute) strain had the next highest MFOs activity. The lowest activity of 2.83 ± 0.45 η mole/mg protein/minute was recorded in the susceptible, JJ-Lab strain that was also on par with JField and JNSKE-R strains.

Table 34: Inter-correlation matrix for the three selected (JInd-R, JCyp-R and JNSKE-R) and two unselected susceptible, (JField, and JJ-Lab) strains based on the LC_{50} values against ten different insecticides tested.

	JJ-Lab	JField	JInd-R	JCyp-R	JNSKE-R
JJ-Lab	1.000				
	0.981				
JField	(0.00)*	1.000			
	0.745	0.819			
JInd-R	(0.034)*	(0.013)*	1.00		
	0.799	0.879	0.991		
JCyp-R	(0.010)*	(0.002)*	(0.000)*	1.000	
	0.381	0.478	0.989	0.512	
JNSKE-R	(0.277)	(0.162)	(0.000)*	(0.159)	1.000

Values in parentheses indicate the significance levels (p values)

* Correlation is significant at $p \leq 0.05$.

Table 35: Inter correlation matrix for the LC₅₀ values of different insecticides tested against the second instar larvae of *Plutella xylostella* selected with insecticides and two unselected strains. LC₅₀s of three selected (JInd-R, JCyp-R and JNSKE-R) strain and two unselected strains (JJ-Lab and JField) strain were used as the pairs of data for correlation of coefficient.

Insecticides	Indoxacarb	Cypermethrin	Neemazal-F	NSKE	Endosulfan	Carbaryl	Chlorpyrifos	Novaluron	Spinosad	Imidacloprid
Indoxacarb	1.000									
Cypermethrin	0.622 (0.263)	1.000								
Neemazal-F	0.574 (0.311)	0.581 (0.304)	1.000							
NSKE	0.385 (0.523)	0.940 (0.017)*	0.539 (0.348)	1.000						
Endosulfan	0.530 (0.470)	-0.404 (0.596)	-0.085 (0.915)	-0.598 (0.402)	1.000					
Carbaryl	-0.799 (0.201)	-0.932 (0.068)	-0.220 (0.780)	-0.814 (0.186)	0.073 (0.927)	1.000				
Chlorpyrifos	-0.179 (0.821)	0.638 (0.362)	0.382 (0.618)	0.841 (0.159)	-0.766 (0.444)	-0.952 (0.198)	1.000			
Novaluron	-0.329 (0.589)	0.486 (0.406)	0.141 (0.821)	0.739 (0.154)	-0.696 (0.304)	-0.729 (0.271)	0.956 (0.044)*	1.000		
Spinosad	-0.066 (0.916)	0.690 (0.198)	0.500 (0.391)	0.842 (0.074)	-0.385 (0.615)	-0.818 (0.182)	0.989 (0.011)*	0.886 (0.046)	1.000	
Imidacloprid	0.455 (0.442)	-0.437 (0.462)	-0.926 (0.024)*	-0.489 (0.403)	0.398 (0.602)	-0.036 (0.964)	-0.403 (0.597)	-0.217 (0.725)	-0.440 (0.459)	1.000

Values in parentheses indicate the significance level (p values)

* Correlation is significant if $p \leq 0.05$ (2-tailed).

Thus in essence, all the resistant strains were having relatively higher MFOs activity than the susceptible strain – JJ-Lab.

4.9.2 Esterase activity

Esterase activity was measured by using both α -naphthyl acetate and β -naphthyl acetate in both selected and unselected cultures. The activity of esterases was measured in terms of the μ mole of α - or β -naphthol formed per mg of protein per minute from the substrate, α or β - naphthylacetate respectively.

4.9.2.1 α -naphthyl acetate (α -NA)

When α -NA was used as a substrate, the activity of esterases was observed to be the highest in JInd-R strain (0.82 ± 0.14 μ mole/mg protein/minute). The activity of the esterases varied significantly among the different strains of *P. xylostella* tested ($F_{5,48} = 28.817$; $p < 0.05$; Table 36; Fig46a). JInd-R strain was significantly different from all other strains. The lowest activity of esterases was observed in JNzF-R (0.47 ± 0.04 μ mole /mg protein/minute) strain which was significantly different from all other strains. The other strains recorded intermediate values with JJ-Lab recording 0.62 ± 0.04 μ mole /mg protein/minute, followed by JField (0.63 ± 0.01 μ mole /mg protein/minute), JCyp-R (0.58 ± 0.06 μ mole /mg protein/minute) and JNSKE-R (0.57 ± 0.01 μ mole /mg protein/minute) strain. All these together formed a separate group.

Interestingly neem and the product recorded the lowest values, much below the values for the two unselected strains.

4.9.2.2 β -naphthyl acetate (β -NA)

The activity of the esterases towards β -NA was measured in different strains in terms of μ mole of β -naphthol produced per mg of protein per minute. Even with β -NA, the highest activity was recorded in JInd-R (0.55 ± 0.18 μ mole/mg protein/minute) strain. The significant differences ($F_{5,48} = 15.59$; $p < 0.05$) among the strains in their activity towards β -NA were recorded. The activity of esterases in JInd-R formed an independent group by DMRT. JField (0.42 ± 0.01 μ mole /mg protein/minute) and JJ-Lab (0.41 ± 0.05 μ mole /mg protein/minute) formed the next group. However, the lowest activity of 0.25 ± 0.02 μ mole /mg protein/minute was recorded in JNzF-R strain that was on par with

Table 36: Activity levels of different detoxifying enzymes measured in terms of their activity towards different substrates in different strains of *Plutella xylostella*.

Strains	PNA* (μ mole / mg of protein/min)	α -NA* (μ mole/mg of protein/min)	β -NA* (μ mole/mg of protein/min)	PNPA* (μ mole / mg of protein/min)	CDNB* (μ mole / mg of protein/min)	DCNB* (μ mole / mg of protein/min)
JJ-Lab	2.83 $\pm$ 0.45 ^{c**}	0.62 $\pm$ 0.04 ^b	0.41 $\pm$ 0.05 ^b	2.40 $\pm$ 0.61	56.22 $\pm$ 4.24 ^{ab}	37.75 $\pm$ 3.42 ^b
JField	3.08 $\pm$ 0.57 ^{bc}	0.63 $\pm$ 0.01 ^b	0.42 $\pm$ 0.01 ^b	2.76 $\pm$ 0.26	60.06 $\pm$ 8.62 ^a	38.86 $\pm$ 3.97 ^b
JInd-R	3.65 $\pm$ 0.44 ^b	0.82 $\pm$ 0.14 ^a	0.55 $\pm$ 0.18 ^a	2.30 $\pm$ 0.30	66.38 $\pm$ 10.68 ^a	56.18 $\pm$ 16.12 ^a
JCyp-R	4.26 $\pm$ 0.77 ^a	0.58 $\pm$ 0.06 ^b	0.33 $\pm$ 0.02 ^c	2.38 $\pm$ 0.40	45.51 $\pm$ 8.40 ^b	40.16 $\pm$ 1.48 ^b
JNzF-R	3.66 $\pm$ 0.93 ^b	0.47 $\pm$ 0.04 ^c	0.25 $\pm$ 0.02 ^d	2.28 $\pm$ 0.70	59.53 $\pm$ 13.41 ^a	38.53 $\pm$ 3.85 ^b
JNSKE-R	3.32 $\pm$ 0.20 ^{bc}	0.57 $\pm$ 0.01 ^b	0.31 $\pm$ 0.01 ^{cd}	2.33 $\pm$ 0.35	55.71 $\pm$ 16.04 ^{ab}	33.93 $\pm$ 1.59 ^b
Mean $\pm$ sd	3.47 $\pm$ 0.50	0.62 $\pm$ 0.12	0.38 $\pm$ 0.10	2.41 $\pm$ 0.18	57.24 $\pm$ 6.90	40.90 $\pm$ 7.77
F calculated	6.218	28.817	15.593	1.274	3.597	7.087
Significance level (p)	0.000	0.0001	0.0001	0.290	0.008	0.0001

* Substrates

PNA- Para-nitroanisole for MFOs

β -NA- β -naphthyl acetate (2-NA) for esterases.

CDNB 1-chloro-2,4-dinitrobenzene for GST ; α -NA - ; α -naphthyl acetate (1-NA) for esterases

** Figures indicated by the same letters are not significantly different from one another ; PNPA- p-nitrophenyl acetate (PNPA) for both esterases and GSTs ; DCNB 2-dichloronitrobenzene for GST

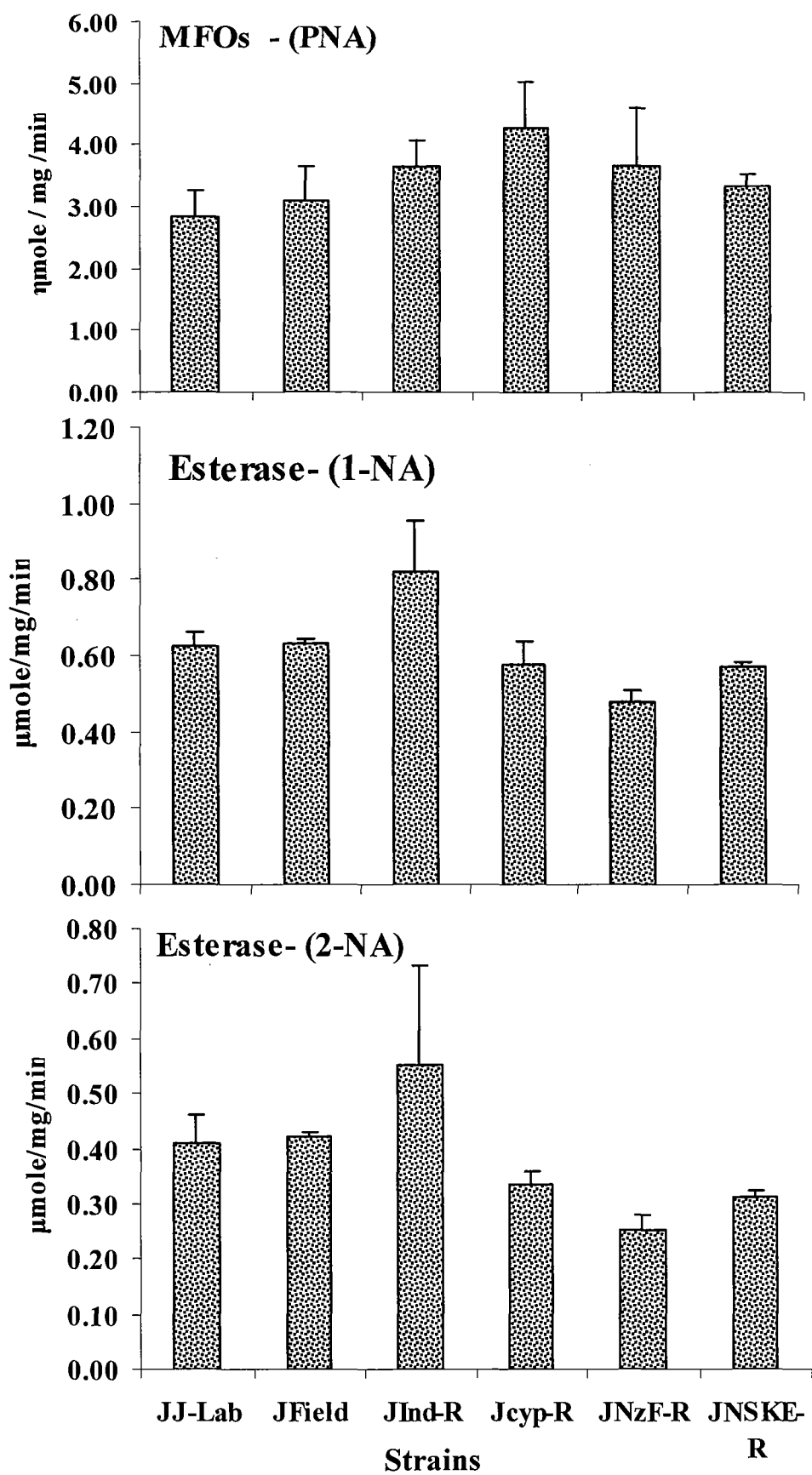


Figure 46a: Activity of three major detoxifying enzymes measured through their activity towards specific substrates (MFOs – PNA; Esterase- 1-NA & 2-NA) in different strains of *P.xylostella*.

JNSKE-R (0.31 ± 0.01 $\mu\text{mole /mg protein/minute}$). JNSKE-R was however on par with JCyp-R (0.33 ± 0.02 $\mu\text{mole /mg protein/minute}$) strain (Table 36; Fig.46a).

It was also observed that the activity of the esterases towards substrates α -NA and β -NA were strongly correlated ($y = 0.8918x - 0.1701$; $r = 0.975$; $n = 6$; $p < 0.05$). Hence, either of the substrates can be used in ascertaining the esterase activity in insects. Most surprisingly, the esterases activity was observed to be the lowest with the JNzF-R and the JNSKE-R strains selected respectively with the Neemazal-F and the NSKE. Both these recorded the esterase activity at much lower values than even the two unselected populations.

4.9.2.3 Isozyme assay

Five esterase isozyme bands were observed in different strains of DBM larvae. They were accordingly named as E_1 , E_2 , E_3 , E_4 and E_5 in the descending order of their molecular weight (Plate 5). E_1 and E_2 were observed in all the strains and differences were observed in the other 3 lower bands. All the five bands were observed in JJ-Lab, JField, JInd-R and JCyp-R strains. Banding pattern was similar in both JInd-R and JCyp-R strains. However, E_3 band in JNzF-R strain was reduced, while E_4 and E_5 bands were prominent compared to other strains. Similar to JNzF-R, even in JNSKE-R strain only two prominent bands were observed in place of E_3 , E_4 and E_5 . Although, further studies were not done, it is possible that the bands observed in the latter two strains could be quite different from the three corresponding bands observed in the other four cultures (Plate 5).

4.9.3 Glutathione –S-transferases (GSTs)

Two different substrates, viz., Chlorodinitrobenzene and Dichloro nitrobenzene were used for ascertaining the activity levels of GSTs in the six cultures studied.

4.9.3.1 Chlorodinitrobenzene (CDNB)

Activity of GST in terms of $\mu\text{mole of CDNB conjugated per mg of protein per min}$ was found to be highest in JInd-R (66.38 ± 10.68 $\mu\text{mole/mg protein /minute}$) strain while the lowest was observed in JCyp-R (45.51 ± 8.40 $\mu\text{mole/mg protein /minute}$) strain. While these two were significantly different from each other ($F_{5,48} = 3.597$; $p < 0.05$), JField (60.06 ± 8.62 $\mu\text{mole/mg protein /minute}$), JNzF-R (59.53 ± 13.41 $\mu\text{mole/mg protein$

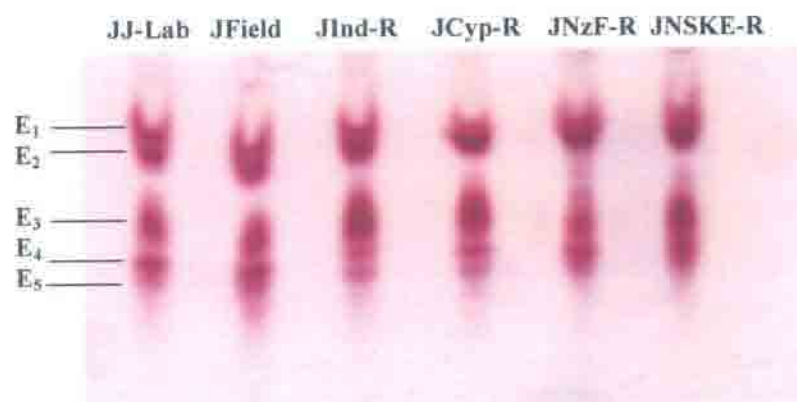


Plate 5: Isozymes of the Esterases in different strains of *P. xylostella*.

/minute) and), JJ-Lab (56.22 ± 4.24 $\mu\text{mole/mg protein /minute}$) and JNSKE-R (55.71 ± 16.04 $\mu\text{mole/mg protein /minute}$) were on par with JInd-R strain while the latter two were also on par with JCyp-R strain.

4.9.3.2 Dichloro nitrobenzene (DCNB):

The activity of GSTs was found to be highest in JInd-R (56.18 ± 16.12 $\mu\text{mole/mg protein /minute}$) and lowest in JNSKE-R (33.93 ± 1.59 $\mu\text{mole/mg protein /minute}$) strain when DCNB was used as the substrate. JInd-R strain was significantly different from all the other strains ($F_{5,48} = 7.087$; $p < 0.05$), while all others including JCyp-R (40.16 ± 1.48 $\mu\text{mole/mg protein /minute}$), JField (38.86 ± 3.97 $\mu\text{mole/mg protein /minute}$), JNzF-R (38.53 ± 3.85 $\mu\text{mole/mg protein /minute}$), JJ-Lab (37.75 ± 3.42 $\mu\text{mole/mg protein /minute}$) and JNSKE-R formed a distinct group.

Further, the two methods of assay employed for ascertaining the activity of GSTs were observed to be uncorrelated ($r = 0.567$; $n = 6$; $p > 0.05$). It is therefore, likely that different groups of GSTs may be acting on different substrates.

4.9.4 Para- nitrophenol acetate (PNPA)

The substrate, p-nitrophenol acetate (PNPA) was used in identifying the activities of both esterases and glutathione-S-transferases (GST) enzyme activity. The activity is measured in terms of the conversion of PNPA to pico-moles of p-nitrophenol per mg of protein per minute. The activity was highest in the JField strain (2.76 ± 0.26 $\mu\text{mole/mg protein /minute}$) and lowest (2.28 ± 0.70 $\mu\text{mole/mg protein /minute}$) in the JNzF-R strain. In JCyp-R, JJ-Lab, JNSKE-R and JInd-R strains 2.40 ± 0.61 , 2.38 ± 0.40 , 2.33 ± 0.35 and 2.30 ± 0.30 $\mu\text{mole/mg protein /minute}$, respectively were recorded. However, the differences in the activity of the enzymes between the strains were not significantly different ($F_{5,48} = 1.274$; $p > 0.05$; Table 36).

Anticipating that the PNPA based assay has potential relationship with the activity levels of esterases and GSTs, an attempt was made to relate the observed activity levels measured independently with that of the PNPA. It was seen that the PNPA based activity levels had no strong relationship with either the α -NA ($r = 0.06$) or with the β -NA ($r = 0.202$), suggesting that the PNPA is not an ideal test for the measurement of the activity of

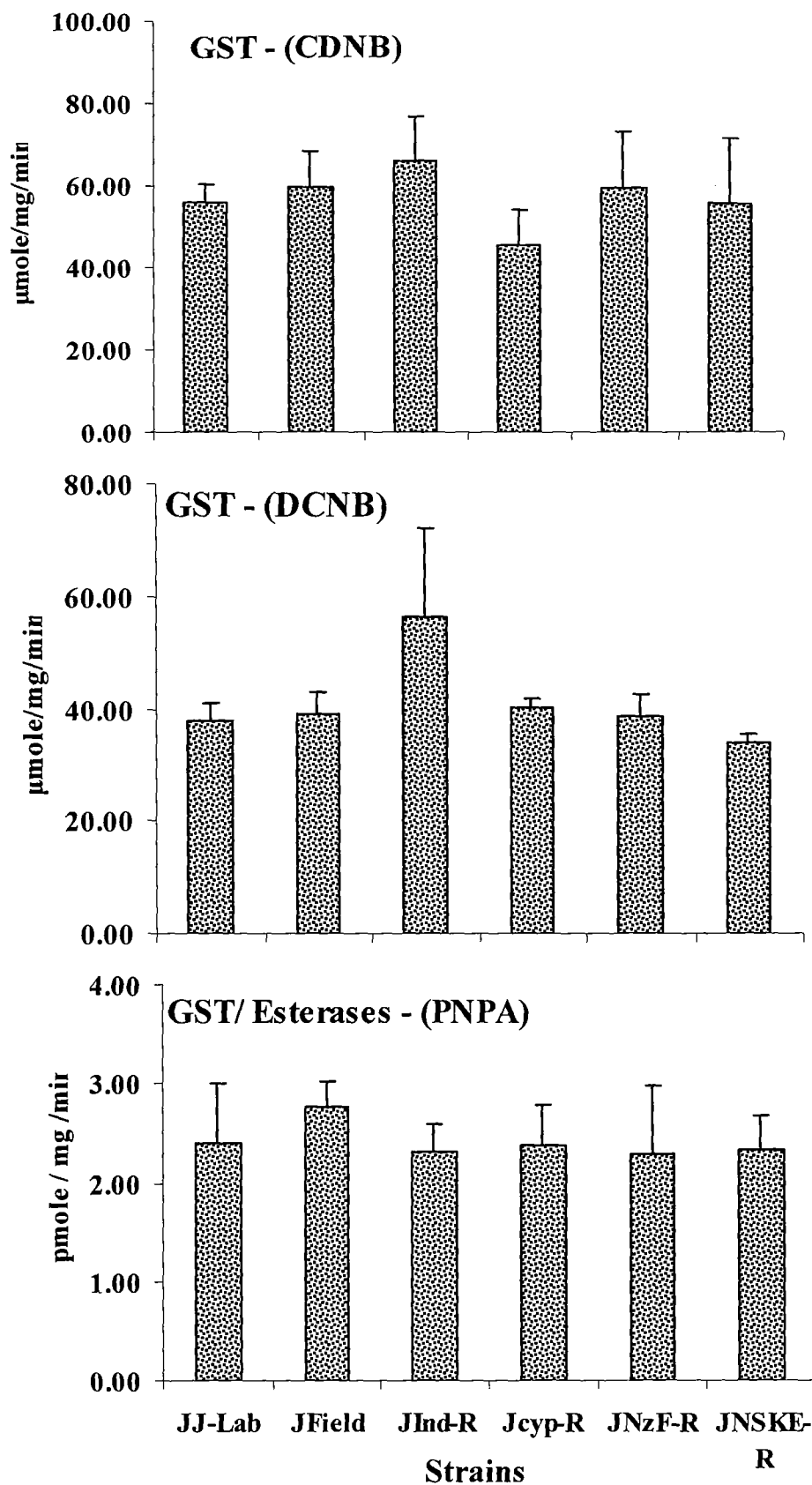


Figure 46b: Activity of three major detoxifying enzymes measured through their activity towards specific substrates (GSTs- CDNB, DCNB; GSTs/Esterases-PNPA) in different strains of *P.xylostella*.

esterases. This is further established by the fact that the association between the assessments of activity levels based on the α -NA and β -NA were strongly correlated.

Similarly, an estimate of the utility of the PNPA for the assessment of the GSTs activity using PNPA indicated that there was no relationship between either the CDNB ($r = -0.028$) or the DCNB ($r = -0.213$) based activity levels with those of the PNPA.

V. DISCUSSION

V. DISCUSSION

Diamondback moth (DBM), *Plutella xylostella* is the most important of all insect pests of cabbage and other crucifers, world wide. DBM among insect pests has developed resistance to maximum number of insecticides representing different groups of insecticides. Any new insecticide introduced for its management is expected to last only for two to three years (Talekar and Shelton, 1993). It is the first insect to develop resistance to *Bt* spray in field condition (Tabashnik *et al.*, 1990; Shelton and Wyman, 1992). DBM is a hardy insect that is easy to maintain under laboratory condition (Liu and Sun, 1984). As a result, it has been extensively used as a model insect for understanding different principles of pest management. In particular, it is an ideal insect for studies on insecticidal resistance (Tabashnik and Croft, 1982; Tabashnik, 1994). Therefore, in the present study DBM was used to understand the rate of development of resistance to four insecticidal chemicals/products. The primary aim was to check on the rate of development of insecticidal resistance against neem as compared to synthetic insecticides. In essence, synthetic chemicals represent single chemicals with single modes of action while the neem product largely represents a situation of single chemical with multiple modes of action and the crude extract of neem – NSKE represents multiple chemicals with multiple modes action. It is felt that these differing selection agents would provide different intensities of selection on the test insect and thus would help check on the relative rates of development of resistance when an insect is subjected for selection using these differing selection agents.

In the process an attempt was also made to understand the other governing factors of resistance such as genetics, some selected fitness parameters, cross resistance and biochemical mechanisms of resistance. The results of these studies are discussed here under suitable headings.

5.1 Relative susceptibility of five field collected strains of *Plutella xylostella* to five insecticides

5.1.1 Variation in relative susceptibility to five insecticides

Relative susceptibilities of different populations of DBM collected from five different locations around Bangalore were assessed. It was observed that the mean

resistance ratios of all the field strains to different insecticides varied considerably (Fig. 15A). The field strains were more resistant to cypermethrin, followed by spinosad and indoxacarb, perhaps reflecting the historical patterns of usage. Not surprisingly, the lowest RRs were recorded against Neemazal-F and NSKE. The LC_{50} s of all the field populations against NSKE and Neemazal-F were not significantly different from (Table 17) susceptible, JJ-Lab strain. This indicates that, even after a decade of recommendation in cabbage ecosystem, the field strains still remain susceptible to NSKE and have not developed resistance. However, this could also be because of low use pattern of neem products for pest management by the farmers (Pillai, 2002; Kamala, 2006).

DBM populations around Bangalore at its peak usage period were more than 25,000 fold resistant to cypermethrin (Sannaveerappanavar, 1995). However, due to decades of sparse usage, the resistance ratio has now dropped to a maximum of 12.17 fold in Devanahalli strain. This result is of particular importance as cypermethrin has made its way back into the pest management options against DBM around Bangalore in many combination products with some selected OPs (Kamala, 2006). Chop (Chlorpyrifos + Cypermethrin) and Rocket (Profenophos + Cypermethrin) are two such products that are in fairly wide usage as of now against DBM around Bangalore (Table 1). Further, the observed RR values for cypermethrin are, on the whole, quite meaningful compared to other studies (Luo *et al.*, 1995) in DBM.

Indoxacarb was introduced for the management of lepidopteran insect pests in cotton and cabbage ecosystem in South India during 2000. It was one of the major synthetic insecticides being used in the management of DBM around Bangalore during 2002 to 2004 (Kamala, 2006) and was also quite effective as a management tool against Lepidoptera affecting cabbage (Jayadevi, 2002). However, by 2005 none of the fields from which the five DBM populations were collected from around Bangalore were using indoxacarb for the management of DBM (Table 15), clearly indicating its fall from the farmers optional chemicals. The situation is an obvious indicator of repeated control failures when indoxacarb was used by the farmers. As a result, a high degree of resistance was expected among the populations collected. But the results indicated the situation to be very different with a mean resistance ratio of just 4.2 ± 4.74 fold while the highest RR of 11.5 fold was recorded in Devanahalli strain. In essence, it is interesting to know whether indoxacarb resistance is highly unstable resulting in quick loss of resistance leading to

records of low RRs in different populations. Nevertheless, indoxacarb appears to trigger high rates of resistance in DBM from different parts of the world (Joia *et al.*, 2003; Liu *et al.*, 2003; Sayyed and Wright, 2004), so much so, that some field strains have recorded 330 fold resistance (Sayyed and Wright, 2004). In principle, therefore, as of now, indoxacarb can still be used as an effective insecticide in the management of DBM in cabbage ecosystem around Bangalore.

Spinosad was the most commonly used insecticide during the time of collection of DBM field populations (Table 15). All the field populations were more tolerant to spinosad than indoxacarb and were next only to cypermethrin (Fig. 15A). The mean RR of five field strains was 7.332 ± 3.215 fold and highest RR of 11.28 fold was recorded in Devanahalli strain. DBM populations elsewhere in the world are known to have registered very high resistance to spinosad under field condition reaching up to 1,170 fold (Sayyed and Wright, 2004). Relatively high level of RR in Devanahalli population might be due to continuous application of spinosad (Table 15), where only Spinosad and Rocket (Profenophos + Cypermethrin) were used 8 times before the collection of the insects.

Resistance level recorded against different insecticides varied among the five field populations of DBM. Devanahalli strain was highly resistant with a mean RR of 8.77 ± 4.49 folds, followed by Shidlaghatta (6.65 ± 2.56), Malur (5.25 ± 2.96) and Hoskote (5.24 ± 2.6) strains. Doddaballapura strain (3.87 ± 2.17 ; Fig. 2B) was the most susceptible among the five strains. However, the populations from different locations varied in their response to insecticides. Despite the observed variations, the overall resistance levels for the insecticides tested were not quite alarming for any of the insecticides tested. This in fact is surprising due to two reasons. First the insecticide usage pattern in general around Bangalore is known to be unreasonably high (Khan, 1995; Kamala, 2006) and secondly, in India, DBM populations from South are considered more tolerant than North Indian populations (Mohan and Gujar, 2003). In addition the variation observed, though not very high, suggest the populations to be reasonably genetically well isolated. Observed variation may be a product of differences in local management practices (Tabashnik *et al.*, 1987; Mohan and Gujar, 2003) so that the populations studied despite their relative proximity to one another may have yet remained 'genetic islands'. However, this is not expected to be unusual as populations separated by less than 10 km may still demonstrate significant differences in their responses to insecticides indicating potential genetic

segregation of the populations; although, DBM can show great active and passive mobility (Tabashnik *et al.*, 1987).

5.1.2 Relation between insecticides based on the population responses

Associational studies carried out using locations as replications and insecticides as the variables suggested DBM populations to show similar response patterns across synthetic insecticides like indoxacarb, cypermethrin and spinosad, while they may behave differently between a plant based insecticide and a synthetic insecticide. This was evident by the fact that NSKE largely indicated negative associations with synthetic insecticides, while Neemazal-F was indifferent to other tested chemicals. Although, the sample sizes were small and the strength of the correlations were not quite strong, the results were indicative of two possibilities.

First the populations behave similarly across synthetic insecticides. This may indicate a multiple resistance across synthetic insecticides that are moving similarly in the populations studied. This possibility can not be discounted as each population is exposed to a wide variety of chemicals every season and as the crop is available through out the year, a continuous selection with large many synthetic insecticides (47 between 2002 and 2004 : Kamala, 2006). As a corollary to this possibility, one can even argue in terms of potential cross resistance across the synthetic insecticides tested. Tabashnik *et al.* (1987) through correlation of LC_{50} values of the insecticides across different populations has demonstrated the possibility of cross resistance between DDT-permethrin and diazinone-permethrin. Towing this line, positive correlation recorded between indoxacarb and cypermethrin is indicative of the positive cross resistance between the two as against the expectation that pyrethroid resistant insects are negatively cross resistant to oxadiazine group of compounds either due to increased esterase activity or allosteric effect of *kdr* resistance mechanism (Khambay *et al.*, 2001; Wing *et al.*, 2000; Gunning and Devonshire, 2002). But other studies disagree with this contention of potential negative cross resistance between the two insecticides (Anonymous, 2006; Yu and McCord, 2006). However, indoxacarb selected *M. domestica* with >118 fold resistance ratio also recorded positive cross resistance to spinosad (Shono *et al.*, 2004). DBM populations of Malaysia which were 1,170 fold RR to spinosad also exhibited 330 folds of resistance to indoxacarb (Sayyed and Wright, 2004). Therefore, observed positive associations in DBM

populations among the synthetic insecticides tested, indoxacarb, cypermethrin and spinosad are reasonably indicative of the positive cross resistance among them.

Second important possibility is the relationship between neem extract/product and the synthetic insecticides. It was observed that NSKE provided a reasonably good negative association between the RRs for synthetic insecticides across populations of DBM studied. However, the RRs for Neemazal-F were indifferent to the synthetic insecticides tested. On this count, do neem and/or its products show negative association with the synthetic insecticides? Present study is not sufficiently tuned to answer this particular question, nor there are other studies to address this issue. Nevertheless, if that is so, then neem will have enormous potential in boosting the field utility of synthetic insecticides, as interspersing neem in spray schedules helps reduce the resistance development in target insects. Neem thus may have multiple potential roles in insect pest management- direct use as insecticide and a role in IRM.

5.1.3 Comparison between locations to their susceptibility across five insecticides

Associational studies were also carried out between pairs of locations using pairs of LC₅₀ values for each insecticide as the replicates. In general, different populations were strongly associated in respect of their LC₅₀ values for the insecticides tested. Hoskote, Doddaballapura, Malur and Shidlaghatta populations demonstrated significant positive correlations (Table 19) between any two pairs. However, Devanahalli strain behaved entirely differently and did not show any association with other four populations. Thus the results suggest that the overall behaviour of the populations is largely similar for the five insecticides tested, showing related patterns of tolerance for the chemicals tested.

Reading these results along with the results of association among chemicals, suggests that the high resistance levels and the deviation from other populations of Devanahalli population seem to go beyond the current insecticide load (Table 15). In that context one can assume that the historical background in the immediate past may be of immense importance in deciding the nature of resistance to different insecticides by a population. The results however, further substantiate the 'genetic islands' concept, as Devanahalli population falls in between the other populations sampled. More interestingly, this population also showed very low tolerance to NSKE and Neemazal while being highly tolerant to cypermethrin, indoxacarb and spinosad (Table 17). As a

result, extending the earlier logic, NSKE, Neemazal-F or other neem based products may have significant role in future in contributing for pest management in general and IRM strategies in particular. To the best of our knowledge similar attempts to verify the interrelationship among the populations in the light of their response to insecticides or other toxins are lacking.

5.2 Studies on selection for insecticidal resistance in *Plutella xylostella*

Field populations of DBM collected from around Bangalore were subjected to selections with indoxacarb, cypermethrin, Neemazal-F and NSKE for resistance. Each selected strain was correspondingly named JInd-R, JCyp-R, JNzF-R or JNSKE-R. One unselected susceptible stock (JField) from the pooled population was also maintained.

5.2.1 Selection concentrations and per cent mortality

The increase in selection concentration was highest for indoxacarb against JInd-R strain (103.57 fold) and was followed by Neemazal-F in JNzF-R strain (10 fold). However, increase was only 4.5 fold in JCyp-R strain against cypermethrin and 3 fold in JNSKE-R strain against NSKE. These differences were despite an average 72 to 82 per cent selection pressure maintained in all the strains during the course of selection for development of resistance in different cultures.

The difference in the rate of increase in selection concentration depends on the population response to selection, *i.e.*, selection concentrations increased only when populations respond to selection and when there is a perceptible increase in per cent survival (Jensen *et al.*, 1984; McKenzie and Byford, 1993; Gould *et al.*, 1995). Extent of change in selection concentrations indicates the rapidness of response to selection.

Despite this variation among the insecticides used for selection, a strong uniformity that emerges is in the growth trajectories of the selection concentrations for the four insecticides. All the strains experienced an exponential growth in the selection concentrations over the generations, although, there were apparent strong differences in the slopes of the curves fitted (Fig. 18). What is more striking is the emergence of the trend despite various errors during the course of selection that at times resulted in the usage of overdoses causing the populations to reach the near extinction stages.

5.2.2 Survivorship patterns when selection concentrations are held constant over generations

During the course of selection for resistance to four selected insecticides, on occasions, the selection concentrations were held constant for two to four generations. This was largely to sustain the cultures going and was generally carried out whenever the survival rates substantially decreased (Fig. 19). It was observed that the change in survivorship increased exponentially as a ratio of survivorship in the first generation in three of the four cultures selected, JInd-R, JCyp-R and JNSKE-R strain (Fig. 20a,b&d). In JNzF-R strain, the best fit trend was also exponential but was not significant ($r = 0.325$; $n = 12$; $p > 0.05$; Fig. 20c). Thus the results overwhelmingly suggest an exponential increase in survivorship rates for populations under selection by an insecticide whenever the selection doses or concentrations remained constant over generations with minimum variations in the values of slopes (ANCOVA; $F_{3,44} = 1.519$; $p > 0.05$). As a consequence, considering that the response to selection is likely an exponential function, the relief-response indicates a reversal of the trend in similar fashion. Such a relief-response from repression could indicate potential fitness and genetic consequences (Groeters *et al.* 1993; Tabashnik *et al.*, 1995). If resistance is associated with fitness costs, then the above exponential response can be anticipated. Alternatively and also as a corollary, the proportion of heterozygotes may increase in the population with relief, leading to the above response (Fig. 20). A practical consequence of the result is the indirect evidence that the populations are yet to stabilize for resistance levels that can be best gauged by the homozygosity. Secondly, if such a response is natural to field populations then, a) changes in field dosages are not going to sustain the efficacy of chemicals for long and b) the chemicals that have lost their utility may gain an alternate or cyclical market life in course of time. Considering cypermethrin itself, it can be noted that DBM around Bangalore had shown as high as 26,000 fold resistance during early 90's (Sannaveerappanavar, 1995) had found its utility life back in the cabbage system, albeit in the form of combination products with OPs (Kamala, 2006). The present study only substantiates these possibilities and the potential reasons for this trend (see section 5.1).

5.3 Response of DBM to selection for insecticide resistance

Bio-assays were carried out at different generations during selection for resistance to monitor changes in the level of resistance over generations. Median lethal

concentrations calculated from bioassays were used for working out the resistance ratios (RR) as a measure of development of resistance.

5.3.1 Response to selection for insecticidal resistance – the LC₅₀s

JInd-R strain of *P. xylostella* on selection with indoxacarb demonstrated significant increase in resistance levels over generations. After 14 generations of selection the strain recorded a 249 fold increase over the base line value (JField). On the contrary, selection attempts with cypermethrin in JCyp-R strain provided just 0.846 fold RR over 20 generations. Considering neem, JNzF-R strain on selection with Neemazal-F, demonstrated a resistance level of 2.24 fold at 16th generation and for NSKE, the increase was only 2.17 fold after 23 generations of selection in JNSKE-R strain.

Even though, both indoxacarb and cypermethrin were synthetic insecticides, the rates of resistance development were significantly different. Good response to indoxacarb was anticipated. Selection studies have not been carried out with indoxacarb in DBM. However, selection with indoxacarb in *M. domestica* for 3 generations resulted in >118 fold of RR (Shono *et al.*, 2004). But one study on field population suggested comparable RR value (330 fold) to indoxacarb in DBM (Sayyed and Wright, 2004). Lack of response to selection for cypermethrin was however, unusual. Cypermethrin is perhaps the most widely used pyrethroid in pest management, at least in India. But yet, similar studies on development of resistance to cypermethrin under lab conditions are apparently wanting against any insect, unlike other pyrethroids (Kim *et al.*, 1990; Takeda *et al.*, 1992; Liu *et al.*, 1995). This contrasts strongly with the field resistance levels to cypermethrin in both DBM and other insects (Ovalle and Cave, 1989; Luo *et al.*, 1995; Sannaveerappanavar, 1995; Branco and Gatehouse, 1997). It is not clear therefore, whether cypermethrin poses some problem under lab conditions for insects to develop resistance. Alternatively, it is also possible that as in many other studies, it is just that laboratory insects fail to respond to some selection agents when a number of them are tested (Prabhaker *et al.*, 1998; McKenzie and Byford, 1993). This is not surprising as genetic bottlenecks may hamper successful selection of resistant populations under lab conditions (Roush and Hoy, 1981; McKenzie, 1985, 1996, Roush and McKenzie, 1987). Such a possibility can potentially be strong when defense strategy for the xenobiotic in the insects is multipronged and a wider genetic base is a necessity to find positive responses to selection (Brown and Payne,

1988). Similar failures in the development of resistance to insecticides in insects are not however, uncommon (Vines *et al.*, 1984; Rodriguez *et al.*, 2001).

Alternatively, cypermethrin being a chemical in use since over two decades against DBM in and around Bangalore, the resistance levels seen may reflect a reasonably good homogeneity in the genetic constitution of the populations. This less probable explanation may indirectly suggest two things. First is that the base line values for cypermethrin have changed over years and as a consequence the response levels tend to be extremely low, as hypothesized by Brown and Payne (1988). Secondly, the populations are quite homogeneous, that is expected to be reflected in the steeper slopes (Hoskin and Gordon, 1956). With a caveat for the probability that the slopes may not fully reflect the genetic constitution (Chilcutt and Tabashnik, 1995), all the field populations that were used in the selection for resistance against cypermethrin demonstrated relatively higher slopes than for all the other insecticides tested (Table 17).

Ever since neem attracted the attention of mankind for its use in insect pest management, a large number of studies have demonstrated the potential of this tree in insect pest management against a wide variety of insects (Schmutterer, 1995). Consequently tens of dozens of products based on neem are also available in the market and are being widely recommended against a variety of crop pests and cropping systems (Gahukar, 1996; Gopinathan, 2002). But yet there have been few studies to understand the limitations of neem or its products as potential pesticides, more so from the point of view of development of resistance. Green peach aphid, *Myzus persicae* after 40 generations of selection developed only nine fold resistance to pure azadirachtin (Feng and Isman, 1995). However, at equivalent concentrations of azadirachtin in crude neem seed extract it did not develop any resistance. Vollinger (1995) attempted to select DBM with pure azadirachtin and crude extract of neem. There was slight increase in LC_{50} values of the NSKE selected strains of DBM and also there was change in the fitness parameters like fecundity. Hence, he concluded that insects can develop resistance to neem but the rate may be just as low as 1.7 fold in 35 generations. These represent hardly any development of resistance against neem or their products. Present study despite being not so rigorous, could register 2.24 fold resistance in 16 generations of selection against Neemazal-F and only 2.17 fold against NSKE in JNSKE-R strain after 23 generations of selection. Slightly higher figures noted in the present study could be because of the use of NSKE that was not standardised

for the content of azadirachtin. This raises the simple question of why insects almost always fail to develop resistance against neem. Neemazal-F is largely a concentrated product based on azadirachtin-A with few additional azadirachtins (Kleeberg, 2002). Thus the marginal higher value of resistance to this chemical as against NSKE is validated *sensu* Feng & Isman and Vollinger (1995). Equating Neemazal-F to azadirachtin, the chemical is a multipronged arsenal against insect physiology and biology, unlike almost all synthetic insecticides. Most synthetic insecticides consist of single active compounds and possess one mode of action against the target insects. Azadirachtin is unusual in that it acts in a variety of ways against insect biology, behaviour, and physiology (Saxena *et al.*, 1981; Singh, 1985; Vollinger, 1987; Jagannadh and Nair, 1992; Saxena *et al.*, 1993 and Rembold, 1996) suggesting a possible array of target sites in insects. Thus, use of azadirachtin is almost equivalent to use of a mixture of large array of insecticides and behaviour modifying chemicals. Hence, a consideration of insect responses to selection for a mixture of insecticides should provide the pattern that one can anticipate for azadirachtin, and hence the present proxy, Neemazal-F. Many studies have indicated the possibility of reduction in the development of resistance when mixtures of insecticides were used as against single insecticides for selection (McKenzie and Byford, 1993; Prabhaker *et al.*, 1998). This is largely attributed to a reduction of selection pressure on individual among a set of target sites that ultimately contributed for a reduced response to selection (Lagunes, 1980; Georgiou, 1983; MacDonald *et al.*, 1983; Curtis, 1985). Neemazal-F, appears to go a step beyond, for we are yet to know the number of target sites for azadirachtin in insects. By the same logic, in fact reading the results the opposite way, extremely low levels of resistance development substantiates the earlier suggestions of a wide array of target sites for azadirachtin. An extension of these results further, are best represented in poor development of resistance to NSKE in *P. xylostella* (present study and Vollinger, 1995). While azadirachtin represents a situation of one chemical and many actions, NSKE represents a mixture of chemicals and a multiplicity of target sites. Consequently a great dilution in the selection pressure on individual target sites results, which ultimately leads to lack of overall response to selection. Could it be that, the insect physiologically fails to recognise the stress? Thus in conjunction with earlier studies (Feng and Isman, 1995; and Vollinger, 1995), the present study only substantiates the contention of Rice (1993) that neem, especially the crude extract, NSKE, is a product with multiple compounds and multiple modes of action that has a “built in resistance prevention mechanism (BIRP)” against insects.

5.3.2 Trends in resistance development : LC_{50} values

The review presented earlier explored the nature of the trends in the development of resistance to insecticides in different insects. Essentially almost all the trends demonstrated an exponential nature (See Table-5). The question then is, whether the trend is inescapable and would it remain eternally growing. Biologically, a selection procedure is expected to sieve out the unfit individuals. Hence considering a normal distribution for a particular trait in a population, the selection events should in course of time move towards an asymptotic condition to abscissa. Thus the normal distribution of the trait indicates the rate and the response, a cumulative function, suggesting a sigmoid response. Although, the studies reviewed did not suggest a sigmoid function in any of them, earlier long term and intensive selection studies have indicated this possibility of the populations attaining an asymptotic condition for resistance levels (Decker and Bruce, 1952; Brown, 1958). Assuming that the above argument is logically meaningful, the exponential function represents a portion of the sigmoid function. Therefore, if none of the studies compiled were long enough to reach the limits of selection responses, then the exponential trend becomes the rule in principle.

The results for the trends were evaluated with this background. JCyp-R strain on selection did not develop resistance to cypermethrin and hardly there was any change in LC_{50} values. Hence, LC_{50} values over generations did not fit any function (Fig. 21b). As discussed earlier, various factors might contribute for this situation. However, the trend in JInd-R strain, which responded very well to selection and developed 249 fold RR in 14 generations, for the LC_{50} values followed a strong significant exponential fit (Fig. 21a). But both in JNzF-R (Fig. 21c) and JNSKE-R (Fig. 21d) strains the increased LC_{50} values did not fit the significant exponential functions, yet the best fit functions were exponential. Thus the resistance development in the three strains is by and large validated albeit at different rates. The slopes of the three selected populations (Except JCyp-R strain), were significantly different ($F_{2,19} = 21.364$; $p < 0.05$), with indoxacarb having the highest slope. In essence, keeping in tune with the earlier idea, the growth trend of resistance to an insecticide is essentially an inherent feature of all insects. The observed exponential trend is the consequence of genetic change in the population. Resistance develops slowly in the beginning when most of the susceptible population is yet to be removed. As the selection progresses, the susceptible alleles will be removed at a greater rate and the response to selection picks up. Continued selection results in near homozygous condition for the

resistance factors in almost the entire population when the response attains a plateau, assuming a sigmoid function (Crow, 1956). JInd-R strain at the present recorded RR is yet to reach this state, as higher levels of RR are known for this insecticide in both DBM and other insects (Shono *et al.*, 2002; Sayyed and Wright, 2004).

Some of the studies compiled indicated lower response rate when selections were carried out with mixtures of insecticides (McKenzie and Byford, 1993; Prabhaker *et al.*, 1998; Table 5). Shallow nature of the exponential fit observed in the trends of RR in JNzF-R and JNSKE-R strains by and large fit this condition. However, despite the best fit function being exponential, shallow slopes suggest very low levels of selection operating in them. Potential reasons were discussed earlier.

Further, the rate of development of resistance is known to be faster after relaxing from selection than before relaxing the selection (Motoyama *et al.*, 1992; Tabashnik *et al.*, 1994; McKenzie, 1996; Zhao *et al.*, 2000). In NSKE selected JNSKE-R strain, the selection was relaxed after the 11th generation. The LC₅₀ values before (0-11th) and after (13 to 23rd) relaxation followed significant exponential functions over generations (Fig. 22). The slope of the fit was higher during the post-relaxation period ($b = 0.2307$) relative to the pre-relaxation period ($b = 0.0837$). Thus the results substantiate both the exponential trend in the development of resistance and secondly the change of gear in the response after relaxation. This is of particular significance, when low levels of resistance are observed, the development of resistance, if at all has to be substantiated should be discounted from being a chance factor (Tabashnik *et al.*, 1998) and the significant trend lines suggest the deviation from such a random possibility. An earlier study in DBM using fenvalerate has demonstrated a change to a higher rate of development after relaxation of selection (Motoyama *et al.*, 1992). Such a response is largely attributed to changed resistance allelic frequencies at the initial phases (McKenzie, 1996). Considering the same possibility, it would be of interest to reexamine the experience with cypermethrin. Field populations had shown a reasonably high level of resistance to pick up in the lab selections, as per the above expectations. However that was not to be the case calling for repeat attempts with wider genetic base.

5.3.3 Rate of increase in resistance: A comparison using the slopes of the probit lines

Slopes of the log concentration-probit mortality lines (ld-p lines) are expected to indicate the relative phenotypic (and as a consequence genetic) homogeneity of the cultures under scrutiny. As a result considering insecticidal resistance as a single trait, homogeneity to susceptible condition moves with the selection towards homogeneity for resistance passing through a phase of high heterogeneity. Thus the trends are expected to assume a 'U' shaped curve (Hoskins and Gordon, 1956). As a result, the values of the slope are commensurate with the relative homogeneity of the population for their response to a selection agent such as an insecticide. Hence, slopes of the probit analyses can be used as indicators of strains under selection attaining homogeneity in resistance. But the assumption of the U shape in the trajectories for slopes of ld-p lines was contested with strong evidences to the contrary (Chilcutt and Tabashnik, 1995). If phenotypic expression of susceptibility or otherwise to an insecticide in a population is not a reasonably good representation of the genotype then the U shaped trajectory for slopes of ld-p lines through generations of selection are not necessarily expected. But then, Chilcutt and Tabashnik (1995) suggested the possibility of insecticide-insect combination specific trends. As a result it is worth examining the trends for the slopes of ld-p lines in a population being selected for resistance.

The trends in the slopes of the ld-p lines of JInd-R and JNSKE-R strains increased exponentially (Fig. 23a & 23d) over generations. Although, the trend for the JNzF-R strain demonstrated an increase over generations, the fit was poor for an exponential function ($r=0.5567$; $n=5$; $p>0.05$; Fig. 23c). An examination similarly for the trend in the slopes of ld-p lines of JCyp-R strain over generations rightly indicated a lack of pattern (Fig. 23b). After all, the selection was not taking place for resistance to cypermethrin in this strain.

Considering the above results in the light of the argument by Hoskins and Gordon (1956) or that of Chilcutt and Tabashnik (1995), the latter's argument appears to be substantiated. However, the discernible trends were all exponential. As the selection proceeded with field collected populations with varying resistance levels for different insecticides, it is not improbable that we are only exploring one part of the 'U' shaped function as expected by Hoskins and Gordon. More importantly, Chilcutt and Tabashnik (1995) did not evaluate the trends for a possibility of being exponential. In any selection

study, it is anticipated that the genotypes do move towards increasing homogeneity. As a consequence, in principle, however low the relationships between the slopes and the genotypes are, an increasing trend of the slopes for Id-p lines is expected over generations or over LC_{50} values. Thus the present results, reading in conjunction with the trends for the LC_{50} values, for three of the four selection studies did indicate the potential movement of the populations towards homogeneity. In other words, all these cultures developed resistance to the selection agents tested.

5.4 Genetics of insecticide resistance

5.4.1 Mode of inheritance and degree of dominance of resistance

All examples of resistance to insecticides are governed by autosomal gene/s in DBM (Zhang *et al.*, 2001; Tabashnik *et al.*, 1992; Table 7), as also other insects with two exceptions (Wolfenbarger *et al.*, 1982; Kotze and Sales, 2001; Table 6) where resistance is sex linked. Even in the present study, resistance to indoxacarb, cypermethrin and NSKE in different selected strains of DBM were observed to be inherited autosomally. Considering the nature of the resistance, both dominant and recessive alleles are equally probable (Table 8). In case of DBM, resistance to indoxacarb and cypermethrin was governed by incompletely dominant alleles. However, to NSKE, resistance was governed by incompletely recessive alleles. It is expected that the type of allele governing the trait influences the rate of development of resistance (Roush and McKenzie, 1987), although the nature of the impact is not very clear. Rate of development of resistance in indoxacarb appears to suggest the possibility of faster development of resistance when the alleles conferring the resistance are dominant (McKenzie and Whitten, 1982). As a corollary, JNSKE-R strain developed resistance at a much slower rate for NSKE, probably due to the recessive nature of the alleles governing the resistance.

Resistance to indoxacarb in JInd-R strain of DBM was governed by single gene. By Lande's method, however, a mean number of 4.906 loci are governing the resistance. Resistance to cypermethrin in JCyp-R strain was also expected to be of polygenic nature. Methodology is therefore a contentious issue here. Resistance to *Bt* in *P. xylostella* and *H. armigera* are expected to be monogenic traits both by Lande's method and by test of monogenic model (Sayyed *et al.*, 2000; Kranthi *et al.*, 2003). In the present study considerable difference in the results of the two methods are evident for indoxacarb resistance in DBM. This difference may be due to the theoretical assumptions behind the

methods employed (Lande, 1981 and Tabashnik, 1991). As not many studies have verified both the methods simultaneously, it would be difficult to say which of the two methods provides a better estimate of the number of loci involved in insecticidal resistance. However, the Mendelian ratios are simple, easy to follow and perhaps the reason why it is also the most popular method.

5.5 Resistance Risk Assessment

5.5.1 Response to selection (R)

Response to selection was found to decrease over generations in all the three strains, JInd-R, JCyp-R and JNSKE-R on selection for resistance to different insecticides. Change in 'R' values over generations followed a significant exponential decay function in JInd-R (Table 26; Fig. 27a) and JNSKE-R (Fig. 27c). Similar to the 'R' values calculated over a number of generations, even those calculated for intermittent generations during the course of selection indicated a general but weak declining trend (Table 26; Fig. 27).

The decreasing trends in the 'R' values are indicative of the populations responding to selection. 'R' is expected to be an indicator of the extent of genetic variation, so that a decreasing trend over generations under selection indicates the increasing homogeneity for resistance alleles in the population (Falconer, 1989). The trend for 'R' values of the JInd-R strain thus demonstrated a good response of the population to selection. On the contrary, despite, the trend being strong, extremely low values observed in JNSKE-R strain are indicative of the poor performance of *P. xylostella* for selection with NSKE (Table 26). In the same vein, albeit negative, the non significant trend for 'R' values over generations in JCyp-R strain clearly indicated the inability of the population to develop resistance to cypermethrin even after 23 generations of selection. Further, considering the three populations together, the slopes of the trends varied with the extent of resistance accumulated in the population.

5.5.2 Estimates of realized heritability (h^2)

Heritability is the measure of the proportion of genetic variance that contributes to the total phenotypic variance. The realized heritability (h^2) calculated as R/S , depends on the response to selection (R) and selection differential (S). Rate of evolution of resistance under given selection pressure is influenced by heritability. Higher the heritability of a

given trait (greater variation among individuals of the same population is due to heredity) more rapidly it will evolve in the population under the influence of a given intensity of selection (Falconer, 1989).

Highest mean heritability (0.307) was recorded in JInd-R strain followed by JCyp-R and JNSKE-R strains. Heritability values tended to decrease over generations in all the three strains and the best fit functions were exponential decay functions. Nevertheless, two of these were not significant and only for JNSKE-R strain the trend was observed to be significant (Fig. 29). Even at intermittent generations of selection also the h^2 values decreased over generations in all the three strains (Table 26; Fig. 30), however, none of the trends were significant. Nevertheless, the results strongly indicate an overall reduction in the phenotypic variation with generation on selection for insecticidal resistance. The decreasing trends overwhelmingly suggest a change in genetic constitution of the population towards homozygosity, indicating the development of resistance (Falconer, 1989).

Considering the change in resistance levels, the JInd-R strain with 249 fold at 14th generation after selection, JCyp-R strain with lack of resistance development even after 20 generations of selection and JNSKE-R strain with 2.17 fold RR over 23 generations of selection had mean heritability values of 0.307, 0.0131 and 0.0130 respectively. These values match the earlier expectations that the higher the heritability higher will be the rate of development of resistance (Falconer, 1989). Only against phenthoate (0.42) a h^2 value higher than indoxacarb ($h^2 = 0.307$) observed in the present study was recorded (0.42; Bliss *et al.*, 1989). Other values are similar to what was known earlier generally for the insecticidal resistance in DBM and other insects (Tabashnik and Cushing, 1989; Li *et al.*, 1998; Sayyed and Wright, 2004; see Tables 6 and 7). On a similar note, heritability of resistance to NSKE was the lowest ever recorded for an insecticide. As a result it is not unusual that the development of resistance to NSKE was extremely low and it is not improbable that at this low level the insect may fail completely to develop resistance.

5.5.3 Predicted rates of resistance development: Number of generations required for ten fold increase in LC_{50} value (G)

Number of generations required for a ten fold increase in LC_{50} values (G) is calculated as reciprocal of the response to selection (R; where $G = R^{-1}$). Therefore, at

higher h^2 values the 'G' values will be low because R is directly proportional to h^2 ($h^2 = R/S$). As a consequence, 'G' values were found to increase exponentially over generations in JInd-R strain selected for resistance to indoxacarb (Fig. 31a). However, the best fit function was a logarithmic function ($y = 2.1057\ln(x) + 0.6246$; $r = 0.915$; $n = 8$; $p < 0.05$) that clearly indicates a potential maximum value for the calculated 'G'. As a result, the rate of increase in resistance will become asymptotic to x-axis as the selection progresses. At this level, heritability reaches its lower most bounds due to the high levels of resistance that reflects the high degree of homozygosity in the selected population. Increased homozygosity reduces the heritability consequently leading to low 'R' values and high 'G' values. In essence the overall response to selection reaches a maximum leading to a sigmoid response.

However, in JCyp-R strain selected with cypermethrin, the number of generations (G) required for 10 fold increase did not follow any trend ($y = 21.874e^{0.1072x}$; $r = 0.508$; $n = 7$; $p > 0.05$). Lack of development of resistance is thus substantiated in this strain.

In NSKE selected JNSKE-R strain the 'G' values increased exponentially over generations of selection (Fig. 31c). Thus the data for JNSKE-R overwhelmingly suggests the development of resistance in the strain for NSKE, in the first place. Further, an overall increasing trend in the resistance levels with reduced heritability as a consequence, contributed for the observed results. But what is more striking is the fact that despite extremely low h^2 values recorded, the 'G' values tended to increase exponentially. Meaning that as the selection progressed the expectation of lower heritability is met. But as the values of 'G' are so very high even at the 23rd generation, any further increase in the 'G' values would greatly reduce the rate of development of resistance further. In essence, as such with a very low RR of just around 2.17, lowest ever recorded h^2 and the function being an exponential function for 'G' against generations, the probability of further accumulation of resistance to neem would potentially decrease as an exponential decay function with generations. Thus a ten fold increase from 2.17 may become an almost impossibility to achieve even in a closed population. In conjunction with the results already discussed above, built in resistance prevention mechanism in neem is further substantiated.

Superiority of neem in reducing the resistance development in DBM is further tested by predicting the resistance risk to different insecticides at constant mean h^2 values (JInd-R = 0.307; JCyp-R=0.0131 and JNSKE-R = 0.0130 h^2 value; Table 26; Fig 32) at different selection pressures. It is to be noted that the 'G' is a function of both h^2 and the selection pressure. Both in essence are expected to move the population towards the homozygosity. The results suggested an extremely low rate of resistance development to NSKE compared to cypermethrin or indoxacarb. Even at 90 per cent selection pressure, it required >70 generations for 10 fold increase in LC_{50} value at a constant h^2 value calculated as the mean over all generations. However, for indoxacarb 'G' was <10 generations even at 50 per cent selection pressure and only 4-5 generations were required for 10 fold increase in LC_{50} value at 90 per cent selection pressure (Fig. 32). Cypermethrin took the intermediate values. These results only reinforce the idea that it is extremely difficult to develop strong NSKE resistant DBM populations compared to other insecticides. The results were similar to those expounded by Tabashnik, (1992). Only one other study on development of resistance in *P. xylostella* addressed this issue. Resistance to fipronil required 17 generations (G) to achieve a ten fold increase in RR (Sayyed and Wright, 2004).

5.5.4 Relation between selection pressure and realized heritability (h^2)

Considering the above theoretical calculations, it would be pertinent to understand, what happens to the 'G' values across selection pressures actually experienced by the population during the course of selection. This aspect was verified considering the values of selection pressure recorded at different generations over the course of selection. It was observed that, by and large, the observed trends for h^2 indicated a decreasing trend with the selection pressure in both JInd-R and JNSKE-R strains, while the cypermethrin resistant culture did not indicate any trend.

Thus, on the whole, it appears that the h^2 values vary negatively with the selection pressure. This is contrary to the expectation that h^2 should be directly proportional to the selection pressure (See materials and methods). Such an aberration therefore, could be because, the data points represent the values across the entire gamut of selection episodes over generations. Thus the theoretical calculations notwithstanding, the h^2 values can be very different from the expectations. The expectations in fact represent the trends under constant genetic composition of the populations under selection. But in a population that

is undergoing selection, it is likely that the selection pressures keep varying as is the case in the present set of selections. Hence, the observed results may be a more valid situation for the populations that are changing in genetic composition. However, given this situation, it is anticipated that the 'G' values should increase and was therefore tested.

Further, to the above observations, keeping the theoretical expectations at the back of mind, an attempt was made to understand the overall changes in 'G' values with selection pressure over the period of selection. Contrary to the theoretical expectations, the 'G' values did not show a steady declining linear trend with selection pressure; but varied positively. This trend, however, is commensurate with the trend observed for the h^2 over selection pressure. As a result, the differential genetic composition through the selection period coupled with the selection pressures that varied randomly contributed for the observed situation. Similar explorations for associations between h^2 , 'G' and selection pressure are not available in the literature for comparison, except for the work of Tabashnik (1992), which was discussed earlier.

5.6 Effect of selection with insecticides on life history parameters of *Plutella xylostella*

Resistance to an insecticide may incur certain fitness costs. Perhaps these are best expressed in terms of the life history parameters. Hence, in order to know the possible costs involved in developing resistance to an insecticide, different life history parameters were studied in different resistant strains of *P. xylostella*, *vis-à-vis* unselected population. Sex ratio, gross reproductive rate (GRR), net reproductive rate (NRR), mean generation time (T) and intrinsic rate of increase (r) were worked out through life table calculations, at different generations during the course of study.

5.6.1 Sex ratio

Sex ratio in JInd-R strain was 0.52: 0.48 (male: female) same as that of unselected JField strain. However sex ratios in JCyp-R (0.45: 0.55:: Male to Female), JNzF-R with 0.45: 0.55 and in JNSKE 0.49: 0.51 were female biased (Fig. 35). Although this aspect was not rigorously tested, it appears that except in JInd-R strain, in all other strains developed for resistance with Cypermethrin, Neemazal-F or NSKE, the males are affected much more than the females, resulting in female biased sex ratios. However, *H. virescens* on selection with spinosad resulted in reduced male developmental period (Wyss *et al.*,

2003). More detailed studies would provide better insights into the sex linked impacts on the biology due to acquisition of resistance against neem and its products.

5.6.2 Effect of selection on different life history parameters

Mean of GRR over generations of selection in different resistant strains were found to be significantly affected ($F_{4,55} = 4.010$; $p < 0.05$; Table 27). The GRR of JCyp-R strain was on par with that of JField. The number of eggs per female was significantly reduced in JInd-R, JNzF-R and JNSKE-R strain relative to JField strain. NRR, realized numbers of eggs per female in a generation in selected strains were also significantly different from unselected, JField strain ($F_{4,51} = 12.868$; $p < 0.05$). NRR of JNSKE-R strain was the lowest, followed by JNzF-R, JInd-R and JCyp-R strain (Table 27). Loss of fecundity as a consequence of resistance development was known previously in both DBM (Groeters *et al.*, 1994; Shirai *et al.*, 1998) and other Lepidoptera (Carriere *et al.*, 2001).

Another significant impact is the changes in developmental time in insecticide selected populations. JInd-R and JCyp-R strain selected for indoxacarb and cypermethrin resistance, respectively, resulted in decreased generation time than unselected JField strain. In JNSKE-R and JNzF-R strain mean generation times were more than that of unselected JField strain. Both increase and decrease in mean generation time are known in insecticide resistant populations of DBM (Shirai *et al.*, 1998; Yamada *et al.*, 1993). It is generally anticipated that any stress – biotic or abiotic should in essence induce a tendency among the organisms to move towards faster multiplication especially by sexual means. Given this, a general expectation is that the insecticide resistant populations should perceive the stress in terms of resource limitation and should aim at completing the life span early. Therefore, it is surprising that in many situations, the insecticide resistance leads to increased life span. Although there could be alternative explanations possible for the observed situation, JNSKE- and JNzF-R strains recording higher mean generation time may be due to this lack of perception of the stress on a ground similar to the argument presented in section 5.3.1. Further, the strains resistant to both synthetic insecticides thus provide better idea of reaction to a perceived stress in reducing the generation time. Physiologically, increased developmental period may also be due to the effect of neem (or azadirachtin) on the hormonal system leading to an increased larval period (Rembold, 1995).

Changes in GRR, NRR, and mean generation time, due to acquisition of resistance would get reflected in the intrinsic rate of increase (r '-number of eggs/female/day). All resistant cultures were significantly affected in terms of r ($F_{4,51} = 5.505$; $p < 0.05$; Table 27) compared to the unselected JField strain. More importantly, in all the resistant strains the r values were much below those of the JField culture. Results therefore, overwhelmingly uphold the idea that the resistance to insecticides involves fitness costs best expressed in terms of the biotic potential (Yamada *et al.*, 1993; Sota *et al.*, 1998). Exceptionally Motoyama *et al.* (1992) could not identify any associated fitness costs in a resistant strain of DBM.

A further substantiation of the involvement of the fitness costs was discovered in ascertaining the trends of r values, whenever the selection concentrations were held constant. The r values tended to increase with generations whenever the selection concentrations were held constant in all the four insecticide selected strains. The trends were all significant and demonstrated an exponential growth (Fig. 38) in the ratios of r values with the generation in all the four resistant cultures. Such a possibility exists only when the resistance involves some fitness costs. More over, results demonstrate the potency of the insect to surge back whenever the selection pressures were held constant, with a greater vigour. This observation fits the earlier idea of high impact of fitness cost during early stages of resistance evolution (Groeters *et al.*, 1993; Tabashnik *et al.*, 1995).

Logically, this argument, however, raises the question of why at all should there be a change in costs with increasing levels of resistance? There can be several potential explanations for this. One could be the nature of resistance. If the metabolic resistance is costly in one form to the insect, in the face of continued threat of selection pressure, it is likely that the alternative defence strategies evolve in the population. Alternatively, mechanisms of offsetting the fitness costs evolve with the same mode of metabolic defence. In any case, the favourable changes in overcoming the fitness costs becomes a necessity to reach the base line biological potential, as the ultimate biological requirement. Inability to do so should eventually shut out the species! Therefore, it would be pertinent to understand the inherent proximate mechanisms that lead to such a reversion at least to the base line biotic potential values.

5.6.3 Interrelation between GRR, NRR and intrinsic rate of increase (r)

5.6.3.1 Relation between GRR vs NRR

It was observed that there were significant correlations between NRR and GRR in all the four strains selected for insecticidal resistance over generations (Fig. 39). The associational study was carried out primarily to understand if there are any extraneous factors operating in influencing NRR apart from mortality patterns. However, significant trend lines suggest lack of any such extraneous effects on the NRR and the mortality patterns that remained more or less constant across generations contributed for the observed relationship.

5.6.3.2 Relation between GRR vs intrinsic rate of increase (r)

Study on relation between GRR and the r provided positive linear relation in all the selected strains. While the results were anticipated, the impact of change in fecundity on the r was particularly high (due to higher slope values) in Neemazal-F and NSKE selected strains (Fig. 40). Therefore, above results indicate that, any effect on fecundity in all selected strains will affect the biotic potential of the population and hence, population growth. Further, high values of slope for neem or its products suggest the great impact of these chemicals on the fecundity of insects.

5.6.3.3 Relation between NRR vs intrinsic rate of increase (r)

A similar study on the relationship between NRR and the intrinsic rate of increase (r) suggested all the best fit functions to be non-linear logarithmic functions for all the four resistant strains (Fig. 41). Logarithmic fit, indicates a potential limit for the r values against the NRR. Given all other things constant, a strong positive association was expected between the two parameters. However, results yet make biological sense in that the r can not increase indefinitely with NRR as other impinging factors such as viability, sex ratio and mean generation time affect the r values. More importantly, the slopes of the logarithmic function were the lowest for JNSKE-R ($b = 0.0403$) and highest for JCyp-R ($b = 0.0534$) strain (Fig. 41). Thus the enormous impact of neem on fecundity in particular and biology in general on the consequent intrinsic rate of increase are brought out.

5.6.4 Effect of selection pressure on GRR, NRR and r

Selection pressure is largely represented as the per cent mortality at adult emergence due to treatment with insecticides. Whenever the organisms (insects) were

exposed to insecticides, the surviving individuals were supposed to incur some cost in overcoming the effect of insecticides. Therefore, the best indicators of cost of resistance development are the different life history parameters (GRR, T , r , *etc.*). Hence, the different parameters, mean fecundity (GRR), NRR, r and mean generation time were compared with the selection pressure.

Changes in selection pressure were inversely related with GRR, NRR and intrinsic rate of increase (r) in all the four selected strains (Fig 42, 43 & 44). However, the impact of selection (decreased) pressure was more on JNSKE-R strain selected for NSKE resistance, followed by JNzF-R, JInd-R and JCyp-R strain.

Thus the results suggest that the selection agents applied are impacting the overall biological parameters, much beyond the imposition of the mortality. The above result indicate that as the intensity of selection pressure increases there was an increased fitness cost involved during development of resistance to insecticides. The above trend in the values of the slopes was commensurate with the degree of intensity of selection, where the intensity of selection was gauged in terms of number of selection agents and the modes of action.

5.6.5 Effect of selection intensity on different life history parameters

The agents of selection used for selecting DBM for insecticide resistance in four strains of DBM demonstrated as already argued, differential selection intensity. Indoxacarb and cypermethrin being synthetic insecticides are single chemicals with single modes of action. However, Neemazal-F, largely based on azadirachtin-A, is essentially a single chemical with multiple modes of action on the insects. On the contrary, the crude extract of neem – NSKE is a conglomeration of large number of chemicals (over 50 terpenoids are expected to have insecticidal property – Kraus, 1995) with multiple modes of action. If we thus rank the degree of intensities of selection on this basis, the unselected JField strain with a rank of 0, the two synthetic insecticides get a rank of 1, followed by rank 2 for Neemazal-F and rank 3 for NSKE. An attempt was made to check on changes in the various parameters of insecticidal resistance and life history parameters of the five strains across these ranks – the degree of intensity of selection.

The intensity of selection was found to have negative associations with GRR (Fig. 45A), NRR (Fig. 45B) and by and large a positive association with the mean generation time (T) (Fig. 45C). Perhaps due to the low sample sizes, except the NRR and the selection intensity, others were not found significant. Nonetheless, the overall trends reflect the general expectations of the impact of the selection intensity. The sum effect of these trends was also evident even in the association of the intensity of selection with the intrinsic rate of increase (Fig. 45D). Again, despite the lack of statistical significance, it can be clearly seen that the trend appears to hold strong meaning commensurate with the general expectations. Greater the intensity of selection greater will be the adverse impacts on the parameters of fitness.

A consequent impact therefore, is expected to get reflected in the overall ability of the insect to acquire resistance against these selection agents. A comparison of this aspect revealed that the development of resistance decreased with the intensity of selection (Fig. 21), albeit the lack of statistical significance.

Thus the sum and substance of all the above studies reflect the potential of neem products and the crude extracts in demonstrating their ability of “Built in Resistance Prevention” mechanism of neem (Rice, 1993) against insects in general and *P. xylostella* in particular.

5.7 Relaxation from selection – the Response of JField to selection agents

After establishing the base line values for insecticidal resistance in the five field populations collected, the insects were pooled and continued as a set of unselected JField population in the laboratory for purposes of comparative studies. This pooled population was evaluated for the LC₅₀ values of indoxacarb, cypermethrin, Neemazal-F and NSKE over time. It was observed that the susceptibility (LC₅₀ values; Table 28) of the unselected, JField population to all the four insecticides *viz.*, indoxacarb, cypermethrin, NSKE and Neemazal-F decreased by 10th generation. Against cypermethrin there was steady decline in LC₅₀ values, after 10th generation leading to 5.7 fold loss in resistance under relaxation. In the other three insecticides the decrease was not significant but varied around the base line LC₅₀ values.

Against Neemazal-F and NSKE decrease in LC_{50} values was very low, as the base line LC_{50} values remained similar to susceptible JJ-Lab strain, almost through out the period of evaluation. Essentially, such a situation can arise when the populations remain susceptible through out. It is likely that the small variations observed are just the expressions of the phenotypic variations for Neemazal-F and NSKE, without any significant change in the genetic constitution of the unselected population as related to the response to these chemicals.

The LC_{50} value estimated at the 10th generation for indoxacarb suggested the potential stability of the resistance up to these many generations. However, as the JField population represented the pooled populations of five different locations. Two of these had much greater LC_{50} values than the other three leading to an intermediate base line value used for comparison. As the populations were mixed in equal proportions, a general dilution effect might take much longer time to express compared to a situation of a single resistant population responding to the same relaxation. Considering this aspect, it is possible that the overwhelming impact of the resistant populations in the mixture of JField might delay the rate of lowering of resistance. As a corollary, the LC_{50} value for indoxacarb at 10th generation after relaxation was lower than the LC_{50} values of the two most tolerant populations, Devanahalli and Shidlaghatta, while being higher than that of the baseline value considered.

Thus in essence, the observed results tally with the earlier contention that the loss of resistance is commensurate with the extent of resistance in a population so that the loss of resistance in moderately tolerant populations takes much longer time (Tabashnik *et al.*, 1994). Neem and Neemazal-F serve as examples of situation where the low levels of resistance led to near no change situation in the relaxed state. Further, a general reduction pattern in LC_{50} values seen for different insecticides tested also followed the expectations, as resistance to both synthetic insecticides (Parrella and Trumble, 1989) and biopesticides (Gould *et al.*, 1995) are expected to decline under relaxation and the trends fitted an exponential decay function when analysed (Table 5).

Contrary to this expectation, it is possible that the relaxation may not reduce the resistance as seen in certain situations (Brown, 1958; Georghiou, 1962; Tang and Shelton, 1995). These situations represent extreme genetic homozygosity in the population for

resistance. Various other genetic and fitness parameters may have a say in such results and were discussed earlier.

5.8 Cross resistance studies

Cross resistance is expected to occur only when the resistance mechanisms are same or of similar mode of action (Oppenoorth, 1985). However, there are evidences to suggest that the cross resistance can be negative due to various factors (Table 14). An important factor that is expected to contribute for such a negative association is the similarity in the detoxifying and activating mechanisms being involved in the two xenobiotics in an opposing way.

With this background an examination of the different selected populations for their response to 10 different insecticides was carried out after 10 generations of selection. RR values were worked out as the ratios of the LC₅₀ value of the unselected JField strain. Therefore a value of >1 suggests tolerance, 1 no change and less than one indicates over sensitivity.

5.8.1 JInd-R

Indoxacarb belong to new group of insecticide, hence selected strain was assessed for possible cross resistance to different insecticides. Indoxacarb resistant strain exhibited positive cross resistance to cypermethrin (4.998 fold), NSKE (2.929 fold), imidacloprid (1.172) and Neemazal-F (1.12 fold) while increased sensitivity to chlorpyrifos (0.975 fold RR) and novaluron (0.25 fold RR; Table 31) and remained invariant to spinosad. Surprisingly the results especially in terms of response to cypermethrin defied earlier expectations of pyrethroid being negatively cross correlated to indoxacarb (Khambay *et al.*, 2001; Wing *et al.*, 2000; Gunning and Devonshire, 2002). However, other studies support the findings (Yu and McCord, 2006). Although potential negative cross response of indoxacarb and pyrethroids are being proposed on the basis of the relative modes of action ((Khambay *et al.*, 2001; Wing *et al.*, 2000), unequivocal evidences are lacking. On the contrary, evidences to the opposite are accumulating. Present study adds to this. Increased resistance to indoxacarb may result in enhanced activity of esterases and MFOs (Ahmad and Hollingworth, 2004). Even in the present study similar results were obtained. Esterases are involved in the detoxification of pyrethroids (Perry *et al.*, 1996). Thus the

observed results are quite meaningful and alternatives are possibly just the random population responses.

5.8.2 JCyp-R Strain

Cypermethrin selected strain also recorded higher LC_{50} value to indoxacarb (3.29 fold RR). As a result the above arguments hold much meaning. Further the results suggest a potential positive cross resistance between the two groups of chemicals. This aspect however, needs further verification. JCyp-R strain also recorded an RR value of more than one to imidacloprid (3.736 fold RR; Table 32), endosulfan (1.537 fold), NSKE (1.332 fold) and spinosad (1.1 fold RR) and lower than one RR to carbaryl (0.674 RR). Novaluron had the same LC_{50} values for both JCyp-R and JField. However, the recorded values are not too high to consider them as cross resistant.

5.8.3 JNSKE-R strain

JNSKE-R strain resistant to NSKE showed positive cross resistance to novaluron (6.00 fold), cypermethrin (4.76 fold), indoxacarb (2.00 fold), spinosad (2.00 fold), imidacloprid (1.782 fold) and chlorpyrifos (1.51 fold). Resistance ratios of less than one were recorded against Neemazal-F (0.831 fold), endosulfan (0.404 fold) and carbaryl (0.578 fold; Table 33).

One of the several modes of action of the neem is to affect the moulting in insects. It is likely that novaluron being a chitin synthesis inhibitor, the observed positive cross resistance was found due to similar modes of action between neem and novaluron. This aspect needs further verification both from the point of view of responses and also from the point of view of modes of action of the two chemicals.

5.8.4 JField vs JJ-Lab

An attempt was also made to verify the LC_{50} values of the two lab cultures that have not been exposed to any insecticide. JField was started as the check population along with the selected strains, from the same stock. However, JJ-Lab culture was started during September, 1997 from the larvae and pupae collected from around Devanahalli. Over 100 generations have been completed by this population in the lab. A comparative assessment of the LC_{50} values for the 10 insecticides between these strains suggested that in all but imidacloprid the JJ-Lab population had lower LC_{50} values than the JField culture (Table

29 and 30). Clearly this data suggests that the overall susceptibility of the lab cultures increase with time, irrespective of the chemical.

5.8.5 Inter relation between resistant strains

An interesting question would be to know whether there is any pattern in the way the selected strains behaved among themselves. Therefore, an attempt was made to correlate the LC_{50} values for different insecticides between pairs of these cultures. It was observed that the trends in LC_{50} values, for different insecticides, of JJ-Lab, JField, JInd-R and JCyp-R were very similar among themselves. As a result, strong correlations were observed between any pair among them (Table 34). However, JNSKE-R strain, behaved very differently from all other strains except the JInd-R strain ($r = 0.989$; $p = 0.0001$). Clearly the results suggest that the overall trends of LC_{50} values of above four cultures are similar across insecticides and the neem selected cultures may behave very differently compared to others.

Further, one can also raise a question of whether there were any trends among the insecticides tested across cultures. It was observed that 41 of the 45 possible combinations tested did not indicate any relationships among themselves (Table 34). Significant positive correlations were recorded between cypermethrin vs NSKE ($r = 0.940$; $p = 0.017$); chlorpyrifos vs novaluron ($r = 0.956$; $p < 0.044$) and chlorpyrifos vs spinosad ($r = 0.989$; $p < 0.011$) and negative correlation between Neemazal-F to imidacloprid ($r = -0.926$; $p < 0.024$) was observed. It is to be noted that the sample sizes were quite small ($n=5$) and larger number of populations would have provided better associations. However, good relationship between chlorpyrifos with two other chemicals of entirely new groups of insecticides and the negative cross resistance between Neemazal-F and imidacloprid need further verification. Considering similar studies, it was observed earlier that neem and its products may have negative association with some insecticides. Although, this was not completely substantiated, indications do exist in the form of only one negative association observed. A large number of associations being indifferent suggests that the behaviour of individual populations can be quite different towards individual insecticides. Similar studies to compare the associations for response to insecticides across populations or populations in terms of response to insecticides were not encountered during the course of review. However, one study by Tabashnik *et al.* (1987) did consider this kind of an associational approach for understanding the response patterns

of populations where in DDT resistant populations tended to be positively correlated to pyrethroids. Sample sizes, however, were quite low in the present study.

5.9 Biochemical mechanisms of insecticidal resistance

Increased activity of MFOs, esterases or the Glutathione-S-transferases being associated with insecticide resistance is a widely recognized phenomenon. Different groups of these enzymes are generally associated with resistance to different groups of chemicals. However, on occasions, more than one group of enzymes is also known to be associated with the resistance to certain insecticides. Different methods of assay were employed. Among these the α -NA and β -NA method employed for the assessment of esterase activity suggested that the two methods are similar in providing results ($r = 0.975$; $n = 6$; $p < 0.05$). Thus it is possible that only one of the two methods is sufficient to provide the desired activity levels of esterases in the selected insect strains. Further, on similar grounds, the DCNB and CDNB method of assays employed to ascertain the activity levels of GSTs suggested the two methods provide different results. If the two methods in fact differentiate the sub-groups of the GSTs, then, it is important that the results are accordingly interpreted.

An assessment of the activity of the MFOs by using para-nitroanisole through NADPH mediated activity revealed that the activity of MFOs is of higher level than the susceptible unselected cultures in all the selected strains of DBM. Considering the esterases, their activity was highest in JInd -R strain by both the methods of assay. JNSKE-R and the JNzF-R strains however recorded the lowest activities for the esterases, much below the susceptible strains. Similarly, both by CDNB and DCNB methods of assay, the GSTs activities were observed to be highest in JInd-R strain. Further, keeping in tune with the esterases activity, by and large, even the GSTs activity in neem and Neemazal-F selected strains, was below that of the unselected populations

Therefore on the whole the most striking results emerging are that the Indoxacarb selected JInd -R strain recorded much higher activity of MFOs, esterases and GSTs. Thus there is a possibility that all the three groups of enzymes being involved in the metabolism of indoxacarb. Only other study with oblique leaf miner resistance to indoxacarb also indicated increased activity of all the three enzymes (Ahmad and Hollingworth, 2004).

However, it is possible that the increased activity observed for a group of enzymes need not necessarily indicate an association with detoxification of the chemical, as esterases are also known to be involved in the bio-activation of indoxacarb (Wing *et al.*, 2000).

Considering the JCyp-R strain, MFOs activity in this strain was found to be the highest among all the strains verified. It is known that the pyrethroids are detoxified by MFOs and esterases (Oppenoorth, 1985). However, esterases activity in JCyp-R strain was far below those of even the unselected cultures by both the methods of assay. Thus, the contention of Oppenoorth (1985) needs to be verified. Nevertheless, it is also possible that as JCyp-R strain did not develop significant resistance to cypermethrin, it might have led to the present results. GSTs activity in cypermethrin selected strain however was on par with the unselected strain, by both the methods of assay. Clearly, the GSTs do not seem to have any role with cypermethrin in specific and pyrethroid metabolism in general.

The activity levels of various groups of enzymes in JNzF-R and JNSKE-R strain did not match what is generally expected of response to a xenobiotic. General belief that one or more groups of metabolic enzymes are found in insects tolerant to xenobiotics in general is quelled by the present results concerning these two strains. In both these strains, by and large, the activities of the esterases and GSTs decreased below the reference values of JJ-Lab strain. However, the MFOs were slightly higher than the reference value in the two strains. These results only uphold the earlier contention of Vollinger (1995), that the neem and its constituents have a great negative impact on the general activity levels of these groups of enzymes. However, the activity levels calculated as the activity per mg of protein did not come down below that of the reference values. Nevertheless, it is possible that the total MFO activity per insect could be lower than the reference value, which was however, not examined. Chemical constituents of neem in general and azadirachtin in particular are known to significantly affect the protein synthesis in general and hormonal systems in particular (Fritzsche and Cleffmann, 1987; Rembold, 1995). Even in the present study, it was observed that the mg of protein per g of insect was the lowest in the JNSKE-R (0.42 ± 0.05 mg/g body wt) followed by JNzF-R 0.48 ± 0.12 mg/g body wt) strain (results not reported; JJ-Lab, 0.53 ± 0.06 mg/g body wt; $F_{5,48} = 2.836$; $p = 0.02$). Thus the contention that the neem or azadirachtin has significant impact on the protein production, and possibly as a consequence, on the general strength of the various groups of metabolic enzymes is upheld (Fritzsche and Cleffmann, 1987; Vollinger, 1995).

Further, why at all should this happen and what are the consequence to the biology of the insect? If the protein production decreases, and the hormonal levels come down followed by the metabolic enzymes necessary for the detoxification of xenobiotics, then the biology of the insect is likely to be affected in a variety of ways. The resulting impacts may be seen over the entire generation or perhaps beyond that (?). As was rightly pointed out by Vollinger (1995), it is possible that the small impacts of a variety of chemicals or the same chemical (azadirachtin in this case) on several physiological and behavioural traits would potentially make the insect difficult to respond to any one effect in a concerted way. As a result, the insect may fail to show a significant response to even the single chemical- azadirachtin or the multitude of chemicals that affect the biology (as in the case of mixtures of chemicals in neem). The resulting impact – as was hypothesized, failure to recognize the physiological stress on the part of the insect followed by the dilution of the selection pressure, that ultimately impacts the biology but fails to make the insect to surge back. This is what is essentially the ‘Built-in Resistance Prevention’ mechanism of Neem (Rice, 1993).

5.9.1 Isozyme assay

The banding pattern of the JInd-R and JCyp-R strain were similar to JField and JJ-Lab strains, while the number of bands has reduced in JNzF-R and JNSKE-R strains. Of the three bands in the lower group only two bands were observed, but with relatively higher intensity. Further to loss of a band in the zymogram, even the activity of esterases towards NA substrates was reduced compared to the unselected strains. However, the esterase activity could be independent of the number of bands in the zymogram (Sannaveerappanavar, 1995). Resistance to malathion in ST strain of DBM was associated with the presence of Est8b and Est 9b esterases while the presence of Est 4b band was unique to susceptible strain (Maa *et al.*, 1992; Maa and Liao, 2000). Thus it is possible that despite the loss of some peptides, what remains could be crucial in conferring the resistance. Neem is known to affect the production of many peptides and the overall protein production could get affected, as a consequence loss of a band in the esterase zymogram only substantiates this possibility.

5.10 The Power of neem

In summary the study has brought out the following

- a. Populations from Devanahalli were most resistant to most of the insecticides tested among the five field strains
- b. The level of resistance to cypermethrin was highest compared to other test insecticides
- c. Field populations of DBM have remained susceptible to NSKE and its formulation Neemazal-F over decades of its recommendation for field use
- d. Indoxacarb can induce high levels of resistance in DBM
- e. Cypermethrin did not produce any measurable impact of resistance development in DBM. But, various other factors, such as the increasing trends in the selection concentrations over generations, increased levels of activity of MFOs *etc.* indirectly suggest the possibility that the strain has in fact acquired resistance to the chemical, which could not be measured on concrete terms of LC_{50} values.
- f. Neemazal-F, with high levels of azadirachtin –A, was able to cause development of resistance in neem
- g. Even the crude extract of neem, NSKE, could induce development of resistance in DBM
- h. The levels of resistance developed in the two strains selected for neem and Neemazal-F are so very low that the insect in principle, can be rejected as having really developed some meaningful levels of resistance to warrant caution in the use of neem
- i. Studies on genetics of resistance suggested resistance to indoxacarb and cypermethrin to be governed by dominant alleles while that of NSKE to be governed by recessive allele in DBM
- j. Resistance to indoxacarb is apparently a monogenic trait in DBM, while for cypermethrin it was polygenic
- k. All resistant strains demonstrated some fitness cost in terms of GRR, NRR, mean generation time and ultimately in terms of intrinsic rates of increase.
- l. In all the selected strains r value increased exponentially when ever the selection concentrations were held constant
- m. Heritability (h^2) and response to selection (R) values recorded in JInd-R are very high and very low values were recorded in JNSKE-R strain, indicating its poor response to selection
- n. As a result, at mean heritability values, JInd-R strain only requires 4-5 generations and JNSKE-R more than seventy generations for ten fold increase in resistance level. However, at very low h^2 value which decreases over generations, it becomes impractical to develop 10 fold resistance to neem over generations
- o. Cypermethrin and indoxacarb selected strains did not show over sensitivity to each other
- p. Higher MFOs, esterase and GSTs have been recorded in JInd-R strain suggesting their role in metabolism of indoxacarb.
- q. In JNSKE-R and JNzF-R strains slightly higher activity of MFOs and reduced levels of esterases and GSTs than reference strains was found
- r. As intensity of selection increased the rate of resistance development decreased as a negative exponential function

Power of Neem

Selection for resistance to indoxacarb developed 249 fold resistance in DBM. Similar selection attempts with NSKE and Neemazal-F in DBM resulted in increase of 2.17 and 2.24 fold by LC_{50} values, respectively. Fitness cost incurred even for this low level of increase was much higher than that of indoxacarb. In addition, the heritability values recorded were the lowest for NSKE selected strain, which decreased over generations. Therefore, the ability to further accumulate resistance decreases commensurate with the decrease in heritability. Genetically, the resistance was also governed by recessive alleles in neem. As a result, one can anticipate quick elimination of the heterozygotes on selection. If that is so, then the resistance level would have increased tremendously. However, the fact that the insects did not accumulate resistance despite selection with NSKE suggests that the insect does not seem to have the ability to eliminate the heterozygotes. This could be because of involvement of a large number of alleles or loci and it could even be the case of opposing alleles being involved. This aspect was however not verified in the present study.

On biochemical considerations, it was noted that both JNzF-R and the JNSKE-R strains produced much lower protein compared to those of the unselected or other selected strains. This was further reinforced by the fact that the zymograms for esterases indicated a complete loss of specific proteins. Low activity levels of esterases and GSTs relative to the unselected populations also reinforce this possibility. MFOs were the only set of enzymes that remained more active than in the unselected strain per unit protein. However, considering that the overall protein levels were reduced, this may not hold much meaning on per insect basis.

All these factors reinforce that development of resistance to neem and its formulations is at very low levels in insects. However, this low level of accumulation of resistance might be due to action of neem at different levels. Multiple components of neem affect insect behaviour, physiology and biology in a variety of ways, which is termed here as the 'physiological inability to recognize the stress (PIRS)' leading to a demonstration of significant built-in resistance prevention mechanism against insects. Therefore, neem is potentially a safe bet for long term use in insect pest management.

This contradicts the views of Vollinger (1995), who felt that the accumulated resistance to various insecticides in an insect population might make the insect tolerant to neem. Although, some indications to this effect did emerge in the present study, contrary results were also evident. Other studies suggest that the neem, as is available at present can hardly replace a requirement of 10 per cent of the overall insecticidal needs of the country (Kumar, 2003). Therefore, irrespective of Vollinger's suggestions, the supply rates will ensure limited rates of application. Nevertheless, interspersing NSKE or other neem products efficiently in schedule of sprays may greatly help overcome the problem of insecticidal resistance management. Thus neem remains the most powerful tool in the management of a large number of insect pests, especially the Lepidoptera, Diptera, Coleoptera and Hemiptera. Studies have shown, NSKE to be superior to any commercial neem product (Jayadevi, 2002 and Kumar *et al.*, 2003) in pest management and also that only 50 % of the neem seeds produced in the country are being harvested (Kumar, 2003). Given this, it is only that the farming community should learn to best exploit this powerful pest management tool in an efficient way, but, more as a local resource.

VI. SUMMARY

VI. SUMMARY

The diamondback moth (DBM), *Plutella xylostella* is one of the cosmopolitan insect pests of cruciferous vegetables. It is known to acquire resistance to any new insecticide at an alarming rate. The present study was aimed at understanding the relative rates of development of resistance to four different insecticides, indoxacarb, cypermethrin, Neemazal-F and NSKE under laboratory conditions in DBM and their genetic, physiological and fitness consequences.

Five different populations of DBM from around Bangalore were assessed for their differential susceptibility in F₁ generation by leaf dip method of bioassay. Highest resistance ratio (RR) was recorded in Devanahalli (11.5 fold) strain followed by Shidlaghatta to indoxacarb. Hoskote, Doddaballapura and Malur strains were on par with the susceptible lab (JJ-Lab) strain. Considering cypermethrin, the five field strains were significantly different from JJ-Lab strain and were not different from one another. The highest resistance ratio of 12.17 fold was recorded again in Devanahalli strain followed by Shidlaghatta (9.6 fold) strain. Doddaballapura (5.3 fold) was the most susceptible to cypermethrin among the field strains. Hence, it can be concluded that almost all field strains are more or less equally tolerant to this insecticide of long usage, compared to the highly susceptible lab strain. Even against spinosad, highest RR was recorded in Devanahalli (11.28 fold) strain followed by Malur, Shidlaghatta and Hoskote populations. The lowest LC₅₀ value was recorded in Doddaballapura (2.82 fold) strain.

Highest LC₅₀ of 0.119 per cent of NSKE was recorded in Malur strain, followed by Hoskote and Devanahalli strain. LC₅₀ values of the field strains were not significantly ($p > 0.05$) different from the susceptible, JJ-Lab strain. Even against Neemazal-F, LC₅₀ values of the field collected strains were not significantly different from susceptible, JJ-Lab strain ($p > 0.05$). As a result, all the field collected strains of DBM were as susceptible as the lab strain to NSKE and Neemazal-F.

Among all the field strains, Devanahalli strain was highly resistant with the mean RR of 8.77 ± 4.49 folds, followed by Shidlaghatta (6.65 ± 2.56), Malur (5.25 ± 2.96) and Hoskote (5.24 ± 2.6) strains. Doddaballapura strain (3.87 ± 2.17) was the most susceptible

among the five. Among the insecticides, mean RR was highest for cypermethrin (8.086 ± 2.770 fold) followed by spinosad (7.332 ± 3.215 fold) and indoxacarb with 4.2 ± 4.74 folds of RR probably reflecting the usage pattern of the three insecticides. Lowest RR (0.878 ± 0.185) was recorded against Neemazal-F and NSKE (1.916 ± 0.851).

When tested for possible cross resistance between insecticides, significant positive correlations were observed between indoxacarb and cypermethrin ($r = 0.976$; $p = 0.004$) and between cypermethrin and spinosad ($r = 0.871$; $p = 0.05$). The relation between indoxacarb and NSKE was strongly negative ($r = -0.886$; $p = 0.045$).

DBM was subjected to selection with indoxacarb, cypermethrin, Neemazal-F and neem seed kernel extract (NSKE). The increase in selection concentrations was highest in JInd-R strain against indoxacarb (103.57 fold), followed by JNzF-R strain selected against Neemazal-F (10 fold). However, the increase was only 4.5 folds in JCyp-R strain against cypermethrin and 3 folds in JNSKE-R strain against NSKE. On an average 72 to 82 per cent of selection pressure was maintained in all the strains during the course of selection for development of resistance in respective cultures. Change in survivorship increased exponentially whenever the selection concentrations were held constant.

Development of resistance was monitored through bio-assays at different generations. Indoxacarb selected JInd-R strain reported an LC_{50} value of 0.01245 per cent a.i. during the 14th generation and was equivalent to 249 fold of base line value. The levels of resistance ratios were only 2.24 fold in JNzF-R (0.028 % CNF) at 16th generation and in JNSKE-R strain is 2.17 fold (0.14437 % NSKE) after 23 generations of selection. JCyp-R strain of DBM however, did not develop resistance to cypermethrin even after 25 generations of selection, although there was an apparent increase in selection concentration over generations. Considering the rate of development of resistance, it was highest ($b=0.3379$) in JInd-R strain to indoxacarb, followed by JNzF-R ($b=0.0723$) strain to Neemazal-F and in JNSKE-R strain over generations.

After 10 generations of selection, selected strains were tested for genetic mechanism of resistance to their respective insecticides. In the three selected strains, JInd-R, JCyp-R and JNSKE-R, the resistance was observed to be inherited autosomally. Literature survey also indicated autosomal inheritance to be the major mode of inheritance

in insects for insecticidal resistance. Further, resistance to indoxacarb in JInd-R and in JCyp-R strain to cypermethrin were inherited as incompletely dominant traits while resistance to NSKE in JNSKE-R strain was inherited as an incompletely recessive trait. Both dominant and recessive modes of inheritance were found to be equally probable for insecticidal resistance in insects, based on the published information.

Considering that in JInd-R strain, the resistance was governed by dominant gene, the number of genes governing resistance to indoxacarb was by a single gene (Chi-square test for monogenic model with dominant allele). Lande's (1981) method, however, provided a different picture indicating as many as 4.906 resistance factors to be involved. Resistance to cypermethrin in JCyp-R strain was of polygenic nature (test of monogenic model).

The response to selection (R) values of the JInd-R strains were relatively high (0.16 to 0.41) compared to JNSKE-R (0.010 to 0.048) and JCyp-R (0.005 to 0.049) strains. Higher ' R ' values in JInd-R are indicative of DBM's ability to rapidly develop resistance to indoxacarb while JNSKE-R strain with low ' R ' values demonstrated the relatively poor performance of the insect in responding to the selection by NSKE. Change in ' R ' values over generations followed significant exponential decay in both JInd-R and JNSKE-R strains. ' R ' values calculated over intermittent generations suggested a lack of trend, but for broad declining trends in all the three strains.

Realized heritability (h^2) values decreased over generations of selection in all the three selected strains. However, mean heritability over all the generations was highest in JInd-R (0.307) followed by JCyp-R (0.0131) and JNSKE-R (0.0130) strains. The observed h^2 values for JNSKE-R strain is perhaps the lowest recorded for any insecticide. Even between intermittent generations the heritability of resistance to the respective selection agents in JInd-R, JCyp-R and JNSKE-R strains, decreased over generations of selection. However, none of the trends were significant.

Prediction for the rate of development of resistance in terms of ' G ', the number of generations required for ten fold increase in LC_{50} values, 'suggested an increase in ' G ' over generations of selection. Theoretical expectations of ' G ' values at constant h^2 values decreased with increasing selection pressure. JNSKE-R strain at 90 per cent selection

pressure required >70 generations and JInd-R strain, <10 generations for 10 fold increase in LC_{50} values. Cypermethrin took the intermediate values. Thus, these results reinforce the idea that NSKE has a strong ability to resist the development of insecticidal resistance in insects compared to other synthetic insecticides.

Practically, h^2 values showed a tendency to decrease with increased per cent selection pressure in both JInd-R and JNSKE-R strains and in JCyp-R, there was no observable trend. The number of generations (G) required for 10 fold increase in LC_{50} values were increased with selection pressure but the relationships were not significant in all the three strains.

Possible involvement of the fitness costs in accruing resistance to different insecticides was verified in terms of sex ratio, gross reproductive rate (GRR), net reproductive rate (NRR), mean generation time (T) and intrinsic rate of increase (r) in the four selected strains. In JInd-R strain the number of males formed was more than that of females and was similar to JField. In the other three strains, JCyp-R, JNzF-R and JNSKE-R, female biased sex ratios were recorded. In these strains, the impact of the selection agents appear to be stronger on males, resulting in female biased sex ratios.

The number of eggs/female (Gross Reproductive Rate) in JInd-R strain was on an average 85.44 ± 39.77 , in JCyp-R strain 108.57 ± 36.08 , in JNSKE-R strain 78.98 ± 33.99 and in JNzF-R strain 69.53 ± 42.32 eggs/female compared to the unselected JField (126.92 ± 15.78 eggs/female). Mean of GRR over generations of selection in different resistant strains were found to be significantly affected ($F_{4,55} = 4.010$; $p < 0.05$). The GRR of JCyp-R strain was on par with that of JField. The number of eggs per female was significantly reduced in JInd-R, JNzF-R and JNSKE-R strains relative to JField. NRRs, realized number of eggs/female in a generation, in selected strains were also significantly different from unselected, JField strain ($F_{4,51} = 12.868$; $p < 0.05$). NRR of JNSKE-R strain was the lowest, followed by JNzF-R, JInd-R and JCyp-R strain. Mean generation time (T), in insecticide selected JInd-R and JCyp-R strain decreased relative to the unselected JField strain. In JNSKE-R and JNzF-R strain mean generation times were more than that of unselected JField strain.

A combined impact of all the above factors can be best gauged in terms of biotic potential, the intrinsic rate of increase, (r '-number of eggs/female/day). In all the resistant strains r values were significantly affected ($F_{4,51} = 5.505$; $p < 0.05$) compared to the unselected JField strain. Lowest r value was the JNSKE-R, followed by JNzF-R, JInd-R and JCyp-R strain. The r values tended to increase with generations whenever the selection concentrations were held constant in all the four insecticide selected strains. The trends were all significant and demonstrated an exponential growth in the ratios of r values with the generation in all the four resistant cultures. Results demonstrate the potency of the insect to surge back whenever the selection pressures were held constant, with a greater vigour.

Significant correlations between NRR & GRR and GRR & r were observed in all the four strains. However, the relationship between NRRs and the intrinsic rates of increase (r) suggested all the best fit functions to be non-linear logarithmic functions for all the four resistant strains. Logarithmic fit, indicates a potential limit for the r values against the NRR.

Whenever there was an increase in selection pressure, there was a decrease in the GRR, NRR and intrinsic rate of increase (r) in all the four selected strains. However, the impact of selection (decreased) pressure was more on JNSKE-R strain selected for NSKE resistance. This was followed by JNzF-R, JInd-R and JCyp-R strains in a decreasing order.

The agents of selection used for selecting DBM for insecticide resistance in the four strains of DBM demonstrated differential selection intensity. Indoxacarb and cypermethrin being synthetic insecticides are single chemicals with single modes of action. Neemazal-F, largely based on azadirachtin-A, is essentially a single chemical with multiple modes of action on the insects. On the contrary, the crude extract of neem – NSKE is a cocktail of large number of chemicals with multiple modes of action. On this basis the intensity of selection was ranked as follows to understand the impact of intensity of selection on the various insecticidal resistance and life history parameters : 0 = unselected JField strain, 1 = two synthetic insecticides ; 2 = Neemazal-F and 3 = NSKE.

The intensity of selection was found to have negative associations with GRR, NRR and intrinsic rate of increase. A positive association was evident with the mean generation time (T). The trends indicate that, greater the intensity of selection greater will be the adverse impacts on the parameters of fitness. As a consequence development of resistance decreased with the intensity of selection. Thus above studies reflect the potential of neem products and the crude extracts in demonstrating their ability to resist development of resistance in insects in general and *P. xylostella* in particular.

Unselected, JField population, the LC_{50} values of cypermethrin were found to decrease over generations significantly, however, against indoxacarb, Neemazal-F and NSKE the LC_{50} values almost remained unchanged over time.

Selected strains were tested for cross resistance to other insecticides. Indoxacarb selected JInd-R strain exhibited positive cross resistance to cypermethrin (4.998 fold), NSKE (2.929 fold), imidacloprid (1.172) and Neemazal-F (1.12 fold) while increased sensitivity to chlorpyrifos (0.975 fold RR) and novaluron (0.25 fold RR) and remained invariant to spinosad.

JCyp-R strain selected for cypermethrin resistance showed increased LC_{50} than unselected, JField strain to imidacloprid (3.736 fold RR), followed by indoxacarb (3.285 fold RR), endosulfan (1.537 fold), NSKE (1.332 fold) and spinosad (1.1 fold RR). Only towards carbaryl a lower LC_{50} (0.0549 % a.i.; 0.674 RR) value than JField strain were recorded. However, LC_{50} values against novaluron (0.000004 % a.i.) were same as JField strain.

JNSKE-R with 3.964 fold resistance to NSKE, RR towards novaluron (6.00 fold), cypermethrin (4.76 fold), indoxacarb (2.00 fold), spinosad (2.00 fold), imidacloprid (1.782 fold) and chlorpyrifos (1.51 fold) were more than one. Lower than one resistance ratios were recorded to Neemazal-F (0.831 fold), endosulfan (0.404 fold) and carbaryl (0.578 fold).

When tested for interrelationship between LC_{50} values of different insecticides in different strains, JJ-Lab, JField, JInd-R and JCyp-R were very similar to one another in their response to different insecticides. However, JNSKE-R strain, behaved very different

from all other strains except the JInd-R strain. The neem selected cultures may thus behave very differently towards different insecticides.

In order to know the mechanisms of resistance the activity of MFOs, esterases and glutathione-S-transferases were assayed in the selected and unselected cultures. The activity of the MFOs was measured using para-nitroanisole as a substrate. The activity was highest in JCyp-R (4.26 ± 0.77 nmole/mg protein/minute), followed by JInd-R (3.65 ± 0.44 nmole/mg protein/minute), JNzF-R (3.66 ± 0.93 nmole/mg protein/minute) strain and were on par with JNSKE-R (3.32 ± 0.20 nmole/mg protein/minute) and JField (3.08 ± 0.57 nmole/mg protein/minute) strain. The lowest activity was in JJ-Lab strain. This variability suggests the importance of MFOs in conferring resistance against indoxacarb and cypermethrin. It is also possible that MFOs have a role in overcoming the impact of NSKE or azadirachtin based neem products.

Activity of the esterases when α - or β -NA was used as a substrate was highest in JInd-R followed by JCyp-R Strain. The activity of the esterases varied significantly among the different strains of *P. xylostella* tested. The activity levels of esterases recorded in JNzF-R and JNSKE-R strains indicated a reduction below that of the unselected strain. Esterase isozyme bands of JInd-R and JCyp-R strains were similar to JJ-Lab and JField strains. However, one zymogram band was reduced in JNzF-R and JNSKE-R strains. Therefore it is likely that this loss of a peptide may have a significant impact on the ability of the insect to develop resistance against neem and its products.

Higher activity was recorded in JInd-R strain to DCNB as well as to CDNB as substrates indicating higher activity of GSTs in the strain. On the whole, the general activity levels of neem selected strains were quite low.

In essence the study overwhelmingly demonstrated that indoxacarb resistance in DBM may be due to any and all of the three groups of detoxifying enzymes. That of cypermethrin was largely governed by MFOs. Unlike synthetic insecticides, neem or its products may greatly influence the activity levels of GSTs and esterases leading at times to loss of peptides. Thus, the negative impact of these chemicals on the enzyme activity may have significant role to play in thwarting the development of resistance in insects.

Neem and its products may have an extraordinary impact on the biology, physiology and behaviour of the insects in general and DBM in particular. As a consequence of these impacts, and a variety of genetic, physiological and biological parameters, herein demonstrated, the development of resistance in insects to neem or the products based on neem is unlikely to be a threat for pest management.

Thus on the whole the study demonstrated the extraordinary power of neem from the various angles of resistance risk associated with this chemical. Built in resistance prevention (BIRP) mechanism found in neem has been overwhelmingly demonstrated through genetic, biological and biochemical studies, using DBM as a model system. Further, BIRP in neem is hypothesized as a factor emanating from the dilution of selection pressure on individual target sites, leading to 'physiological inability to recognize the stress (PIRS)' of the xenobiotic in insects.

In summary, the results demonstrate that NSKE and other neem products being powerful tools in the management of many groups of insect pests, with their ability to resist development of resistance in insects, might potentially have an eternal practical life in insect pest management. It is therefore, a necessity that the farming community of India should use this powerful tool with greater zeal.

VII. REFERENCES

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VIII. APPENDIXES

Appendix I : History of development and use of insecticides and some major events

Year	Chemical/ Scientist	Country	Insect
	Chalk	Chinese	
	Woodash		
	Botanical	Middle & Near East	
	Chrysanthemum		
	Neem -Grain bins	India	
900AD	Arsenites	China	
1690	Tobacco	Europe	
1787	Soaps	Europe	
1800s	Pyrethroids	Caucasus	
1845	Phosphorus	Germany	
1848	Derris root Powder	Malaya	
Era Of Fumigates, Inorganic And Petroleum Products			
1854	CS ₂ for fumigation	France	
1867	Paris green	US	
1868	Petroleum products	US	
1874	DDT synthesized	Zeidler	
1877	HCN (Fumigant)		
1880	Lime sulphur	US	
1883	Bordeaux mixture	France	
1886	Pine resin		Scales
1892	Lead arsenate	US	Gypsy moth
		First report of resistance	San Jose scale
1908	Lime sulphur		
1910	Insecticide Act		
	-Label efficacy of the insecticide		
	-Inert ingredients		
	-Standards of purity & Strength –to be given		
1918	Chloropicrin	France	
1932	Methyl bromide	France	
1925	Dinitro compounds		
1926	Thiocyanates		
	DDT insecticidal property discovered	Geigy, Switzerland	Muller
1939			
1941	BHC	France	
1942	BHC	UK	

1944	Parathion	Germany
1945	Chlordane	Germany
1947	Carbamates	
1949	Allethrin	First synthetic pyrethroid
1950	EPN	
1952	Malathion	
1953	Aldrin, Dieldrin	
1958	Carbaryl	USA
1962	Rachel Carson	Silent spring USA
Hormones and pheromones		
1967	J.H. Insecticides	USA
1970	<i>Bt</i> development	USA
1970s	UK and Japan	
1972	Diflubenzuron	
1973	Methoprene	
1980s	USA	
1981	Abamectin	
1981	Phenoxycarb	
1986	Tebufenozide	
1988	Difenthiuron	
1990	Imidacloprid	
1991	Fenazaquin	
1992	Chlorfenvinphos, Fipronil	
1995	Spinosad	
1997	Indoxacarb	

Source : Matsumura, 1975; other

Appendix II : Number of insect species resistant to insecticides at different years

Sl. No.	Year	# of Species
1	1908	1
2	1918	3
3	1928	5
4	1938	7
5	1945	12
6	1948	14
7	1954	25
8	1970	224
9	1975	364
10	1980	428
11	1984	447
12	1987	490
13	1990	504
14	2004	530

Source: Forgash, 1984; Georghiou, 1990 and Bills *et al.*, 2004

Appendix III : Some of the insect pests which are resistant to maximum number of insecticides (Bills *et al.*, 2004).

Sl. No.	Species	Order	Family	No. of Chemicals
1	<i>Meligethes aeneus</i>	Coleoptera	Nitidulidae	20
2	<i>Panonychus citri</i>	Acari	Tetranychidae	20
3	<i>Psylla pyricola</i>	Homoptera	Psyllidae	21
4	<i>Rhizoglyphus robini</i>	Acari	Acaridae	22
5	<i>Sodoptera exigua</i>	Lepidoptera	Noctuidae	22
6	<i>Helicoverpa armigera</i>	Lepidoptera	Noctuidae	25
7	<i>Aphis gossypii</i>	Homoptera	Aphididae	30
8	<i>Phorodon humuli</i>	Homoptera	Aphididae	32
9	<i>Spodoptera littoralis</i>	Lepidoptera	Noctuidae	32
10	<i>Bemisia tabaci</i>	Homoptera	Aleyrodidae	33
11	<i>Tribolium castaneum</i>	Coleoptera	Tenebrionidae	34
12	<i>Heliothis virescens</i>	Lepidoptera	Noctuidae	37
13	<i>Leptinotarsa decimlineata</i>	Coleoptera	Chrysomelidae	40
14	<i>Panonychus ulmi</i>	Acari	Tetranychidae	40
15	<i>Myzus persicae</i>	Homoptera	Aphididae	68
16	<i>Plutella xylostella</i>	Lepidoptera	Plutellidae	69
17	<i>Tetranychus urticae</i>	Acari	Tetranychidae	72

Appendix IV

Table from Falconer book

<i>p</i> %	<i>x</i>	<i>i</i>		<i>p</i> %	<i>x</i>	<i>i</i>		<i>p</i> %	<i>x</i>	<i>i</i>
0.01	3.719	3.96		0.8	2.409	2.74		10	1.282	1.755
0.02	3.54	3.79		0.85	2.387	2.72		11	1.227	1.709
0.03	3.432	3.687		0.9	2.366	2.701		12	1.175	1.667
0.04	3.353	3.613		0.95	2.346	2.683		13	1.126	1.627
0.05	3.291	3.554		1	2.326	2.665		14	1.08	1.59
0.06	3.239	3.507						15	1.036	1.554
0.07	3.195	3.464		1	2.326	2.665		16	0.994	1.521
0.08	3.156	3.429		1.2	2.257	2.603		17	0.954	1.489
0.09	3.121	3.397		1.4	2.197	2.549		18	0.915	1.458
0.1	3.09	3.367		1.6	2.144	2.502		19	0.878	1.428
				1.8	2.097	2.459		20	0.842	1.4
0.1	3.09	3.367		2	2.054	2.421		21	0.806	1.372
0.12	3.036	3.317		2.2	2.014	2.386		22	0.772	1.346
0.14	2.989	3.273		2.4	1.977	2.353		23	0.739	1.32
0.16	2.948	3.234		2.6	1.943	2.323		24	0.706	1.295
0.18	2.911	3.201		2.8	1.911	2.295		25	0.674	1.271
0.2	2.878	3.17		3	1.881	2.268		26	0.643	1.248
0.22	2.848	3.142		3.2	1.852	2.243		27	0.613	1.225
0.24	2.82	3.117		3.4	1.825	2.219		28	0.583	1.202
0.26	2.794	3.093		3.6	1.799	2.197		29	0.553	1.18
0.28	2.77	3.07		3.8	1.774	2.175		30	0.524	1.159
0.3	2.748	3.05		4	1.751	2.154		31	0.496	1.138
0.32	2.727	3.03		4.2	1.728	2.135		32	0.468	1.118
0.34	2.706	3.012		4.4	1.706	2.116		33	0.44	1.097
0.36	2.687	2.994		4.6	1.685	2.097		34	0.412	1.078
0.38	2.669	2.978		4.8	1.665	2.08		35	0.385	1.058
0.4	2.652	2.962		5	1.645	2.063		36	0.358	1.039
0.42	2.636	2.947						37	0.332	1.02
0.44	2.62	2.932		5	1.645	2.063		38	0.305	1.002
0.46	2.605	2.918		5.5	1.598	2.023		39	0.279	0.984
0.48	2.59	2.905		6	1.555	1.985		40	0.253	0.966
0.5	2.576	2.892		6.5	1.514	1.951		41	0.228	0.948
				7	1.476	1.918		42	0.202	0.931
0.5	2.576	2.892		7.5	1.44	1.887		43	0.176	0.913
0.55	2.543	2.862		8	1.405	1.858		44	0.151	0.896
0.6	2.512	2.834		8.5	1.372	1.831		45	0.126	0.88
0.65	2.484	2.808		9	1.341	1.804		46	0.1	0.863
0.7	2.457	2.784		9.5	1.311	1.779		47	0.075	0.846
0.75	2.432	2.761		10	1.282	1.755		48	0.05	0.83
								49	0.025	0.814
								50	0	0.798

*p%- % surviving

Appendix V : Phosphate Buffer

X solution - 0.2M Monobasic sodium phosphate H_2O (27.8g in 1000ml)

Y solution- 0.2 M dibasic sodium phosphate (53.65g $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$) or (71.7g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ in 1000ml)

Sl.No.	pH	X (ml)	Y(ml)
1	5.7	93.5	6.5
2	5.8	92	8
3	5.9	90	10
4	6	87.7	12.3
5	6.1	85	15
6	6.2	81.5	18.5
7	6.3	77.5	22.5
8	6.4	73.5	26.5
9	6.5	68.5	31.5
10	6.6	62.5	37.5
11	6.7	56.5	43.5
12	6.8	51	49
13	6.9	45	55
14	7	39	61
15	7.1	33	67
16	7.2	28	72
17	7.3	23	77
18	7.4	19	81
19	7.5	16	84
20	7.6	13	87
21	7.7	10.5	90.5
22	7.8	8.5	91.5
23	7.9	7	93
24	8	5.3	94.7

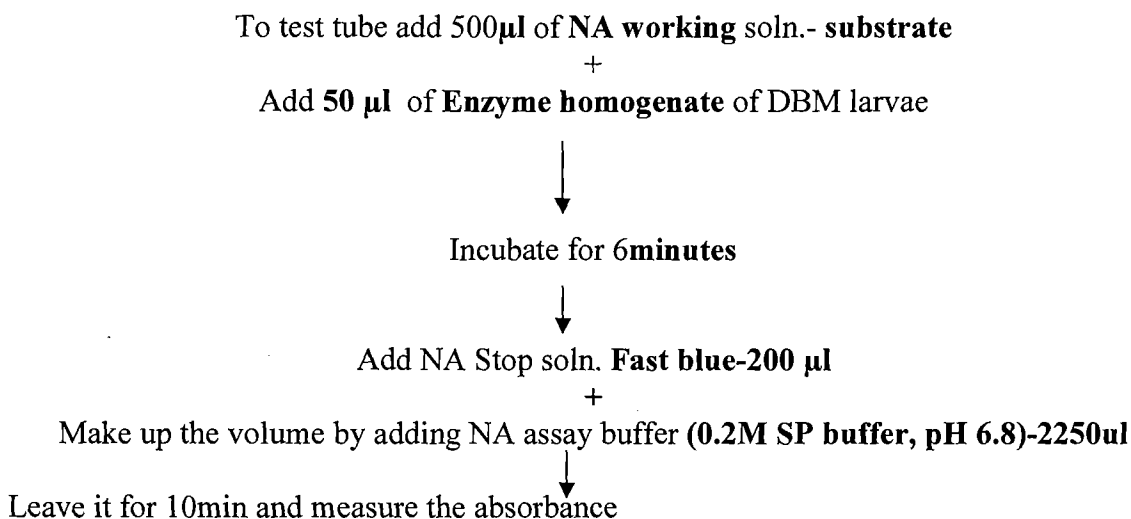
x ml of X + y ml of Y diluted to a total of 200 ml

Appendix VI: Quantification of Activity of Esterase Enzyme from 4th instar DBM larvae

Substrate : α -NA, β -NA

Wavelength : 570nm

Procedure



Details :

NA assay buffer: 0.2M Sodium phosphate (SP) buffer pH 6.8

NA stock : 31 mM α -Naphthyl acetate (α -NA) in acetone

NA Working solution : 50ul of 31mM NA stock solution in 5ml of assay (0.2M SP) buffer

NA stop solution : 15mg of Fast blue B salt in 1.5ml of water + 3.5ml of 5% SDS

31mM 1-NA:

Mol. Wt of 1-NA : 186.21gm/mole

186.2g 1000ml 1M

	Gm	ml of acetone	M
1M	186.21	1000	1M
1M	1.8621	10	1M
31mM	0.057722gm	10	0.031M
	0.028861gm	5ml	0.031M

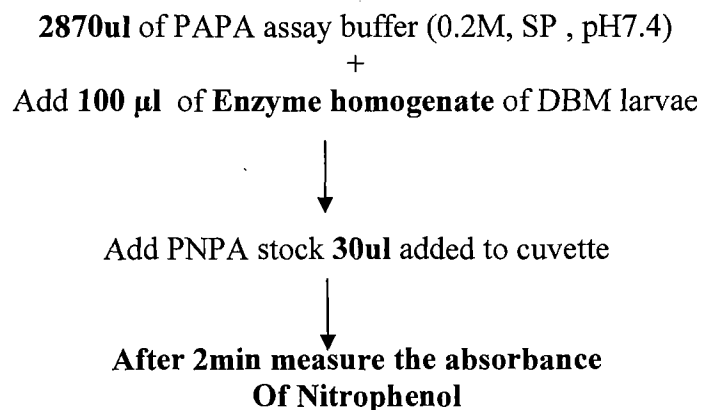
Same method also holds good for esterase using β -NA as a substrate

Appendix VII : Quantification of Esterase Enzyme from 4th instar DBM larvae using PNPA as a substrate

Procedure

Substrate : PNPA

Wave length : 405nm



Details :

PNPA assay buffer: 0.2M Sodium phosphate (SP) buffer pH 7.4

PNPA stock : 200mM p-nitrophenyl acetate dissolved in acetonitrile solvent

Enzyme homogenate

Extinction coefficient : $6.53\text{mM}^{-1}\text{cm}^{-1}$

200mM p-nitrophenyl acetate (PNPA)

Mol. Wt of 1-NA : **181.15gm/mole**

181.15gm 1000ml 1M

	Gm	ml of acetonitrile	Molarity
1M	181.15	1000	1M
1M	1.8115	10	1M
200mM	0.3623	10ml	0.2M
200mM	0.18115	5ml	0.2M

Appendix VIII: Quantification of activity of Glutathione S-transferase Enzyme from 4th instar larvae of *Plutella xylostella*. Using DCNB as a substrate.

Substrate : DCNB

Wave length : 334 nm

Procedure

To spectrophotometer cuvette add 0.1M Tris HCl buffer pH 9.5, with 15mM glutathione-
2910ul

+

Add **50 µl** of **Enzyme homogenate** of DBM larvae



Add substrate –DCNB-**40ul**



After **2min** measure the absorbance of the product conjugated with glutathione

Details :

DCNB assay buffer: 0.1M Tris HCl buffer pH 9.5 containing 15mM glutathione

DCNB stock : 63mM 2-dichloronitrobenzene (DCNB) in methanol

Enzyme homogenate

Extinction coefficient: 10.0mM⁻¹cm⁻¹

63mM DCNB

Mol. Wt : 192gm/mole

Glutathione

Mol.wt = 307.43gm

	Gm	ml of methanol	M
1M	192	1000	1M
1M	1.92	10	1M
63mM	0.12096	10ml	0.063M
63mM	0.06048	5ml	0.063M

	gm	ml of 0.1M Tris HCl buffer pH 9.5	Molarity (M)
1M	307.43	1000ml	1M
1M	3.0743	10ml	1M
15mM	0.046115	10ml	0.015M
15mM	0.230573	50ml	0.015M

Appendix IX: Quantification of activity of Glutathione S-transferase Enzyme from 4th instar larvae of *Plutella xylostella*. Using CDNB as a substrate

Substrate : CDNB

Wave length : 334 nm

Procedure

To spectrophotometer cuvette add 0.1M Sodium phosphate buffer with 15mM glutathione
2910ul

+

Add **50 µl** of **Enzyme homogenate** of DBM larvae



Add substrate -CDNB - **40ul**



After **2min** measure the absorbance of the product conjugated with glutathione

Details :

CDNB assay buffer: 0.1M Sodium phosphate (SP) buffer pH 6.5 containing **15mM glutathione**

CDNB stock : 63mM 1-chlorodinitrobenzene (CDNB) in methanol

Enzyme homogenate

Extinction coefficient : 9.5mM⁻¹cm⁻¹

63mM CDNB

Mol. Wt : 202.55gm/mole

202.55gm 1000ml 1M

	Gm	ml of methanol	M
1M	202.55	1000	1M
1M	2.0255	10	1M
63mM	0.127607	10ml	0.063M
63mM	0.063803	5ml	0.063M

Glutathione

Mol.wt = 307.43gm

	Gm	ml of 0.1M Sodium phosphate buffer	Molarity (M)
1M	307.43	1000	1M
1M	3.0743	10	1M
15mM	0.046115	10ml	0.015M
15mM	0.230573	50ml	0.015M
15mM	0.27669	60ml	0.015M

Appendix X : NATIVE PAGE**Resolving gel**

1. Acrylamide -30%	10ml
2. Resolving gel buffer (pH 8.8)	7.5ml
3. 10% APS	100ul
4. TEMED	30ul
5. Distill Water	12.425ml
Total	30ml

Stacking gel

1. Acrylamide -30%	0.8ml
2. Stacking Gel buffer (pH 6.8)	0.6ml
3. 10% APS	50ul
4. TEMED	10ul
5. Distill Water	3.9ml
Total	5ml

Native PAGE running buffer

0.192M Glycine -14.4gm

0.025M Tris -3.0gm

Make up the volume to 1000ml with distilled water, pH- 8.3

Native PAGE sample buffer

0.05g xylene cyanol in 1ml water + 2.5 ml glycerol and 2.5 ml Native PAGE running buffer

Fast blue B salt

26mg fast blue salt in 1.0ml water prepared fresh

Appendix XIa : Number of III instar larvae of *Plutella xylostella* JInd-R strain exposed , % survived and selection pressure (%) imposed at different generations of selection for resistance to indoxacarb during 2005-2006.

Sl. No.	Genera- tion	Conc. Treated (%a.i.)	No treated	No. dead	% mortality@ pupation	No. pupated	%pupation	% adult emergence	% Seln. Pressure @ adult emergence
1	0	0.00007	1006	582	57.853	424.00	42.147	35.356	64.644
2	1	0.00007	973	550	56.526	423.00	43.474	33.144	66.856
3	2	0.00007	940	559	59.468	381.00	40.532	33.366	66.634
4	3	0.00014	960	853	88.854	107.00	11.146	7.376	92.624
5	4	0.00014	502	327	65.139	175.00	34.861	29.836	70.164
6	5	0.00014	537	260	48.417	277.00	51.583	35.267	64.733
7	6	0.000435	1049	873	83.222	176.00	16.778	9.617	90.383
8	7	0.0002175	532	376	70.677	156.00	29.323	14.605	85.395
9	8	0.00029	304	68	22.368	236.00	77.632	52.721	47.279
10	9	0.00029	492	109	22.154	384.00	77.846	71.805	28.195
11	10	0.00145	818	457	55.868	361.00	44.132	19.084	80.916
Mean			737.55	455.82	57.32	281.82	42.68	31.11	68.89
±	0-10		±	±	±	±	±	±	±
s.d.			266.02	264.43	21.10	117.40	21.10	19.00	19.00
12	11	0.003625	1040	708	68.077	332.00	31.923	27.819	72.181
13	12	0.00435	1223	982	80.294	241.00	19.706	14.424	85.576
14	13	0.00435	874	518	59.268	356.00	40.732	28.110	71.890
15	14	0.00435	1875	916	48.853	959.00	51.147	44.198	55.802
16	15	0.0145	1288	1111	86.258	177.00	13.742	4.706	95.294
17	16	0.00435	NR	NR	NR	NR	NR	NR	NR
18	17	0.00435	466	231	49.571	235.00	50.429	41.024	58.976

Appendix XIa Contd...

19	18	0.005075	430	346	80.465	84.00	19.535	14.748	85.252
20	19	0.005075	823	587	71.324	236.00	28.676	24.661	75.339
21	20	0.006525	715	567	79.301	148.00	20.699	16.037	83.96
Mean			970.44	662.89	69.27	307.56	30.73	23.97	76.03
$\pm$	11-20		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
s.d.			451.46	294.03	13.90	258.51	13.90	12.95	12.95
22	21*	0.005075	551	379	68.78	172.00	31.22	24.18	75.82
23	22*	0.00725	1272	1026	80.66	246.00	19.34	14.98	85.02
24	23*	0.00725	816	495	60.66	321.00	39.34	30.48	69.52
25									
26									
Grand			847.22	560.00	63.65	287.26	36.35	27.285	72.72
Mean			$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
$\pm$ s.d.			361.58	290.74	17.90	177.43	17.90	15.65	15.64

NR- Not recorded

* - For which % adult emergence information was not there, based on pooled % emergence further calculations were carried out.

Appendix XIb : The number of III instar larvae of *Plutella xylostella* JCyp-R strain exposed , % survived and selection pressure (%) imposed at different generations of selection for resistance to cypermethrin during 2005-2006.

Sl. No.	Genera- tion	Conc. (%a.i.)	No treated	No. dead	% mortality@ pupation	No. pupated	%pupation	% adult emergence	% Seln. Pressure @ adult emergence
1	0	0.025	1038	874	84.20	164	15.80	13.90	86.10
2	1	0.025	674	578	85.76	96	14.24	8.63	91.37
3	2	0.025	756	630	83.33	126	16.67	10.93	89.07
4	3	0.025	697	500	71.74	197	28.26	17.87	82.13
5	4	0.0375	625	574	91.84	51	8.16	4.48	95.52
6	5	0.025	198	101	51.01	97	48.99	33.31	66.69
7	6	0.025	515	397	77.09	118	22.91	14.01	85.99
8	7	0.0325	732	560	76.50	172	23.50	20.96	79.04
9	8	0.0325	718	502	69.92	216	30.08	25.71	74.29
10	9	0.0375	548	244	44.53	304	55.47	46.31	53.69
11	10	0.0375	663	560	84.46	103	15.54	13.84	86.15
Mean			651.27	501.82	74.58	149.45	25.42	19.09	80.91
$\pm$	0-10		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
s.d.			202.39	202.69	14.79	70.92	14.79	12.09	12.09
12	11	0	Selection not imposed			246			
13	12	0.025	1361	589	43.28	772	56.72	47.55	52.45
14	13	0.05	2252	1743	77.40	509	22.60	19.59	80.41
15	14	0.0625	749	343	45.79	406	54.21	48.31	51.69
16	15	0.0625	1639	687	41.92	952	58.08	55.42	44.58
17	16	0.075	2146	1913	89.14	233	10.86	9.68	90.32
18	17	0.075	1144	547	52.19	597	47.81	42.62	57.38

Appendix XIb Contd....

19	18	0.0875	1273	779	61.19	494	38.81	34.98	65.02
20	19	0.0875	1490	1092	73.29	398	26.71	26.71	75.60
21	20	0.0875	1277	906	70.95	371	29.05	13.90	86.10
Mean			1481.22	955.44	61.68	497.80	38.32	33.20	67.06
$\pm$	11-20		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
s.d.			475.71	540.56	16.93	225.94	16.93	16.48	16.61
22	21	0.0875	1520	1211	79.67	309	20.33	8.63	91.37
23	22*	0.0875	1190	642	53.95	548	46.05	37.77	62.23
24	23*	0.1125	2228	1778	79.80	450	20.20	16.57	83.43
25	24*	0.1125	871	612	70.26	259	29.74	24.39	75.61
26	25								
Mean			1096.00	765.08	69.13	327.52	30.87	25.51	75.26
$\pm$	0-24		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
s.d.			561.51	470.91	15.83	225.20	15.83	14.38	14.38

Appendix XIc : The number of III instar larvae of *Plutella xylostella* JNzF-R strain exposed , % survived and selection pressure (%) imposed at different generations of selection for resistance to Neemazal-F during 2005-2006.

Sl. No.	Gener ation	Conc. Treated (ppm of aza)	No treated	No. dead	% mortality@ pupation	No. pupated	%pupation	% adult emergence	% Seln. Pressure @ adult emergence
1	0	250	682	385	56.45	297	43.55	29.90	70.10
2	1	500	814	630	77.40	184	29.21	10.45	89.55
3	2	500	429	335	78.09	94	21.91	16.19	83.81
4	3	250	218	96	44.04	122	55.96	27.28	72.72
5	4	250	359	81	22.56	276	76.88	63.68	36.32
6	5	2500	491	375	76.37	116	23.63	5.11	94.89
7	6	4000	386	347	89.90	39	10.10	4.15	95.85
8	7	3750	1045	874	83.64	171	19.57	16.36	83.64
9	8	2500	4 th instar treated	NR	NR	NR	NR	NR	NR
10	9	2500	1019	939	92.15	80	7.85	3.34	96.66
11	10	2500	1058	941	88.94	117	11.06	11.06	88.94
Mean			650.1	500.3	70.95	149.6	29.97	18.75	81.248
±	0-10		±	±	±	±	±	±	±
s.d.			300.28	310.2	21.66	79.04	21.25	17.29	17.29
12	11	1500	1102	920	83.48	182	16.52	3.54	96.46
13	12	2000	309	200	64.72	109	35.28	9.03	90.97
14	13	2500	NR	NR	NR	NR	NR	NR	NR
15	14	1500	714	518	72.55	196	27.45	27.45	72.55
16	15	1500	1493	1220	81.71	273	18.29	11.44	88.56
17	16	1500	1181	925	78.32	256	21.68	21.68	78.32

Appendix XIc : Contd....

18	17*	2000	1009	603	59.76	406	40.24	24.26	75.74
19	18*	2500	1932	1455	75.31	477	24.69	14.88	85.12
20	19*	2500	823	501	60.87	322	39.13	23.59	76.41
Mean	11-19		1070.38	792.75	72.09	277.63	27.91	16.98	83.0163
$\pm$			$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
s.d.			461.78	387.74	8.69	113.43	8.69	7.96	7.96
Grand									
Mean	0-19		836.889	630.278	71.4589	206.5	29.0556	17.9661	82.0339
$\pm$			$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
s.d.			446.73	386.93	17.66	118.38	17.39	14.38	14.38

NR- Not recorded

Appendix XIId: The number of III instar larvae of *Plutella xylostella* JNSKE-R strain exposed, % survived and selection pressure (%) imposed at different generations of selection for resistance to NSKE during 2005-2006.

Sl. No.	Genera- tion	Conc. (%a.i.)	Treated No	No. dead	% mortality@ pupation	No. pupated	%pupation	% adult emergence	% Seln. Pressure @ adult emergence
1	0	0.05	652	377	57.82	275	42.18	25.37	74.63
2	1	0.05	650	272	41.85	378	58.15	28.43	71.57
3	2	0.05	740	249	33.65	491	66.35	37.60	50.79
4	3	0.075	790	745	94.30	45	5.70	2.52	97.48
5	4	0.06	267	128	47.94	139	52.06	32.58	67.42
6	5	0.06	750	353	47.07	397	52.93	30.86	69.14
7	6	0.08	760	558	73.42	202	26.58	16.42	83.58
8	7	0.08	584	440	75.34	144	24.66	9.83	90.17
9	8	0.08	390	166	42.56	224	57.44	37.19	62.81
10	9	0.08	814	329	40.42	485	59.58	37.62	62.38
11	10	0.08&0.1	1023	574	56.11	449	43.89	31.13	68.87
Mean			674.55	381.00	55.50	293.55	44.50	26.32	72.62
$\pm$	0-10		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
s.d.			206.84	186.44	18.47	154.67	18.47	11.82	13.35
12	11	0.1	559	331	59.21	228	40.79	10.45	89.55
13	12	0	NR	NR	NR	NR	NR	NR	NR
14	13	0.08	3305	2322	70.26	983	29.74	11.62	88.38
15	14	0.1	2033	1218	59.91	815	40.09	19.33	80.67
16	15	0.12	1701	983	57.79	716	42.09	18.03	81.97
17	16	0.12	2325	1415	60.86	910	39.14	19.26	80.74

ppendix XIId Contd...

18	17	0.12	2231	1406	63.02	825	36.98	23.54	76.46
19	18	0.12	1209	616	50.95	593	49.05	31.22	68.78
20	19	0.13	2025	1683	83.11	342	16.89	10.86	89.14
21	20	0.12	998	672	67.33	326	32.67	25.11	74.89
Mean			1820.67	1182.89	63.60	637.56	36.38	18.82	81.18
$\pm$	11-20		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
s.d.			819.29	612.44	9.17	278.49	9.16	7.09	7.09
22	21	0.12	1709	995	58.22	714	41.78	26.96	73.04
23	22	0.12	2228	1578	70.83	650	29.17	18.83	81.17
24	23	0.15	1470	634	43.13	836	56.87	36.70	63.30
Grand			1212.38	755.81	55.62	464.12	39.01	22.43	71.15
Mean	N_0-N_{23}		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
$\pm$ s.d.			771.59	552.99	18.25	268.55	15.51	10.18	20.41

NR- Not recorded

Appendix XIIIa: Estimates of realized heritability (h^2) of resistance in JInd-R strain of *Plutella xylostella* on selection with Indoxacarb (a) at different intervals of selection and (b) between intermittent pairs of generations.

a) Over all generations

No. of generations selected	n	Initial		Final		LC ₅₀ (log)	Initial	Final	LC ₅₀ (log)	R	G	p	i	Slope		σ ²	S	h ²
		LC ₅₀	LC ₅₀	LC ₅₀ (log)	LC ₅₀ (log)									Initial	Final			
I ₀ -I ₂	3	0.00005	0.0008	-4.30	-3.08	0.41	2.45	34	1.078	1.05	1.65	0.74	0.80	0.511				
I ₀ -I ₄	5	0.00005	0.0006	-4.30	-3.24	0.21	4.70	28	1.202	1.05	2.00	0.65	0.79	0.271				
I ₀ -I ₆	7	0.00005	0.0013	-4.30	-2.89	0.20	4.96	26	1.248	1.05	1.52	0.78	0.97	0.207				
I ₀ -I ₉	10	0.00005	0.0036	-4.30	-2.44	0.19	5.38	32	1.118	1.05	2.64	0.54	0.61	0.306				
I ₀ -I ₁₀	11	0.00005	0.0086	-4.30	-2.07	0.20	4.92	31	1.138	1.05	7.47	0.23	0.27	0.760				
I ₀ -I ₁₁	12	0.00005	0.0035	-4.30	-2.46	0.15	6.52	31	1.138	1.05	2.46	0.57	0.65	0.237				
I ₀ -I ₁₂	13	0.00005	0.0084	-4.30	-2.07	0.17	5.84	30	1.159	1.05	2.04	0.65	0.75	0.228				
I ₀ -I ₁₄	15	0.00005	0.0125	-4.30	-1.90	0.16	6.26	30	1.159	1.05	3.40	0.45	0.52	0.307				

b) Between intermittent pairs of generations

No. of generations selected	n	Initial LC ₅₀		Final LC ₅₀		Initial LC ₅₀ (log)		Final LC ₅₀ (log)		R	G	p	i	Slope		σ ²	S	h ²
		LC ₅₀	Initial LC ₅₀	LC ₅₀	Final LC ₅₀	LC ₅₀ (log)	Initial LC ₅₀ (log)	Initial	Final									
I ₀ -I ₂	3	0.00005	0.00084	-4.301	-3.076	0.408	2.448	33.955	1.078	1.052	1.647	0.741	0.799	0.511				
I ₂ -I ₄	3	0.00084	0.00058	-3.076	-3.237	-0.054	-18.651	23.526	1.295	1.647	2.004	0.548	0.709	-0.076				
I ₄ -I ₆	3	0.00058	0.00129	-3.237	-2.889	0.116	8.642	24.906	1.271	2.004	1.516	0.568	0.722	0.160				
I ₆ -I ₉	4	0.00129	0.0036	-2.889	-2.444	0.111	8.974	37.187	1.020	1.516	2.637	0.482	0.491	0.227				
I ₉ -I ₁₁	3	0.0036	0.00346	-2.444	-2.461	-0.006	-174.151	39.569	0.966	2.637	2.464	0.392	0.379	-0.015				
I ₁₁ -I ₁₂	2	0.00346	0.00842	-2.461	-2.075	0.193	5.178	21.121	1.372	2.464	2.038	0.444	0.610	0.317				
I ₁₂ -I ₁₄	3	0.00842	0.01245	-2.075	-1.905	0.057	17.662	28.910	1.180	2.038	3.399	0.368	0.434	0.130				

Appendix XIIb: Estimates of realized heritability (h^2) of resistance from JCyp-R strain of *Plutella xylostella* on selection with Cypermethrin (a) Over all generations and (b) between intermittent pairs of generations.

a) Over all generations													
No. of generations selected	n	Initial LC ₅₀	Final LC ₅₀	Initial LC ₅₀ (log)	Final LC ₅₀ (log)	R	G	p	i	Initial slope	final slope	σ^2	h^2
C ₀ -C ₇	8	0.0249	0.03954	-1.60	-1.40	0.025	39.83	22	1.346	2.93	3.10	0.33	0.056
C ₀ -C ₈	9	0.0249	0.02824	-1.60	-1.55	0.006	164.64	23	1.32	2.93	2.91	0.34	0.013
C ₀ -C ₁₀	11	0.0249	0.08513	-1.60	-1.07	0.049	20.60	25	1.271	2.93	0.79	0.54	0.071
C ₀ -C ₁₂	13	0.0249	0.01547	-1.60	-1.81	-0.016	-62.89	28	1.202	2.93	1.21	0.48	-0.027
C ₀ -C ₁₃	14	0.0249	0.02887	-1.60	-1.54	0.005	217.91	28	1.202	2.93	2.58	0.36	0.011
C ₀ -C ₁₄	15	0.0249	0.03551	-1.60	-1.45	0.010	97.31	30	1.159	2.93	2.68	0.36	0.025
C ₀ -C ₁₅	16	0.0249	0.03901	-1.60	-1.41	0.012	82.06	31	1.138	2.93	4.16	0.28	0.038
C ₀ -C ₁₆	17	0.0249	0.02385	-1.60	-1.62	-0.001	-908.56	30	1.159	2.93	4.58	0.27	-0.004
C ₀ -C ₁₇	18	0.0249	0.02353	-1.60	-1.63	-0.001	-732.38	31	1.138	2.93	3.83	0.30	-0.004
C ₀ -C ₁₉	20	0.0249	0.03079	-1.60	-1.51	0.005	216.90	32	1.118	2.93	3.43	0.31	0.0131
b) Between intermittent pairs of generations													
No. of generations selected	n	Initial LC ₅₀	Final LC ₅₀	Initial LC ₅₀ (log)	Final LC ₅₀ (log)	R	G	p	i	Initial slope	final slope	σ^2	h^2
C ₀ -C ₇	8	0.0249	0.03954	-1.60	-1.40	0.03	39.83	22	1.346	2.93	3.10	0.33	0.056
C ₇ -C ₈	2	0.03954	0.02824	-1.40	-1.55	-0.07	-13.68	27	1.225	3.10	2.91	0.33	-0.179
C ₈ -C ₁₀	3	0.02824	0.08513	-1.55	-1.07	0.16	6.26	34	1.078	2.91	0.79	0.54	0.274
C ₁₀ -C ₁₂	3	0.08513	0.01547	-1.07	-1.81	-0.25	-4.05	36	1.039	0.79	1.21	1.00	-0.237
C ₁₂ -C ₁₃	2	0.01547	0.02887	-1.81	-1.54	0.14	7.38	40	0.966	1.21	2.58	0.53	0.266
C ₁₃ -C ₁₄	2	0.02887	0.03551	-1.54	-1.45	0.04	22.25	38	1.002	2.58	2.68	0.38	0.118
C ₁₄ -C ₁₅	2	0.03551	0.03901	-1.45	-1.41	0.02	48.99	56	0.798	2.68	4.16	0.29	0.087
C ₁₅ -C ₁₆	2	0.03901	0.02385	-1.41	-1.62	-0.11	-9.36	34	1.078	4.16	4.58	0.23	-0.433
C ₁₆ -C ₁₇	2	0.02385	0.02353	-1.62	-1.63	0.00	-340.92	29	1.18	4.58	3.83	0.24	-0.010
C ₁₇ -C ₁₉	3	0.02353	0.03079	-1.63	-1.51	0.04	25.69	43	0.913	3.83	3.43	0.28	0.155

Appendix XIIc: Estimates of realized heritability (h^2) of resistance from JNSKE-R strain of *Plutella xylostella* on selection with NSKE (a) Over all generations and (b) between intermittent pairs of generations.

a) Over all generations														
Generation	n	Initial LC ₅₀	Final LC ₅₀	Initial LC ₅₀ (log)	Final LC ₅₀ (log)	R	G	p	i	Initial slope	final slope	σ ²	S	h ²
N ₀ -N ₃	4	0.043	0.055	-1.37	-1.26	0.03	38.54	23	1.32	1.17	1.41	0.78	1.02	0.025
N ₀ -N ₅	6	0.043	0.078	-1.37	-1.11	0.04	23.42	26	1.248	1.17	1.37	0.79	0.98	0.043
N ₀ -N ₆	7	0.043	0.093	-1.37	-1.03	0.05	20.94	25	1.271	1.17	1.48	0.75	0.96	0.050
N ₀ -N ₉	10	0.043	0.110	-1.37	-0.96	0.04	24.58	26	1.248	1.17	1.78	0.68	0.84	0.048
N ₀ -N ₁₀	11	0.043	0.129	-1.37	-0.89	0.04	23.08	26	1.248	1.17	1.56	0.73	0.91	0.047
N ₀ -N ₁₁	12	0.043	0.084	-1.37	-1.08	0.02	41.64	25	1.271	1.17	1.16	0.86	1.09	0.022
N ₀ -N ₁₃	14	0.043	0.057	-1.37	-1.25	0.01	116.80	24	1.295	1.17	1.50	0.75	0.97	0.009
N ₀ -N ₁₄	15	0.043	0.063	-1.37	-1.20	0.01	88.69	24	1.295	1.17	1.61	0.72	0.93	0.012
N ₀ -N ₁₆	17	0.043	0.065	-1.37	-1.19	0.01	96.60	23	1.32	1.17	2.17	0.60	0.79	0.0130

b) Between intermittent pair of generations														
Generation	n	Initial LC ₅₀	Final LC ₅₀	Initial LC ₅₀ (log)	Final LC ₅₀ (log)	R	G	p	i	Initial slope	final slope	σ ²	S	h ²
N ₀ -N ₃	4	0.043	0.055	-1.37	-1.26	0.03	38.54	23	1.32	1.17	1.41	0.78	1.02	0.025
N ₃ -N ₅	3	0.055	0.078	-1.26	-1.11	0.05	19.69	22	1.346	1.41	1.37	0.72	0.97	0.052
N ₅ -N ₆	2	0.078	0.093	-1.11	-1.03	0.04	25.62	24	1.295	1.37	1.48	0.70	0.91	0.043
N ₆ -N ₉	4	0.093	0.110	-1.03	-0.96	0.02	55.17	25	1.271	1.48	1.78	0.61	0.78	0.023
N ₉ -N ₁₀	2	0.110	0.129	-0.96	-0.89	0.03	28.64	34	1.078	1.78	1.56	0.60	0.64	0.054
N ₁₀ -N ₁₁	2	0.129	0.084	-0.89	-1.08	-0.09	-10.61	21	1.372	1.56	1.16	0.73	1.01	-0.094
N ₁₁ -N ₁₃	3	0.084	0.057	-1.08	-1.25	-0.06	-17.82	11	1.709	1.16	1.50	0.75	1.28	-0.044
N ₁₃ -N ₁₄	2	0.057	0.063	-1.25	-1.20	0.02	40.59	15	1.554	1.50	1.61	0.64	1.00	0.025
N ₁₄ -N ₁₆	3	0.063	0.065	-1.20	-1.19	0.00	437.68	19	1.428	1.61	2.17	0.53	0.76	0.003

Appendix XIIIa: Different life table parameters of JInd-R strain of *Plutella xylostella* at different generations of selection for resistance to Indoxacarb under laboratory conditions.

Sl. No.	No. of generation	Mean fecundity GRR	NRR (R ₀)	Intrinsic rate of increase (r)	Mean gen. time (T)	Sex ratio		Larval period (days)	Pupal period (days)
						Male	Female		
1	0	99.333	16.746	0.127	22.254	0.488	0.512	7.856	3.587
2	1	90.556	13.293	0.125	21.099	0.567	0.433	7.510	4.657
3	2	139.900	23.673	0.160	20.188	0.516	0.484	6.912	4.731
4	3	99.333	3.908	0.067	20.543	0.467	0.533	7.168	3.911
5	4	111.100	16.057	0.143	19.837	0.505	0.495	7.684	3.629
6	5	126.156	23.071	0.153	20.909	0.536	0.464	8.209	3.750
7	6	49.400	3.907	0.064	21.292	0.529	0.471	8.084	4.164
8	7	26.800	2.208	0.037	21.429	0.517	0.483	8.090	3.448
9	8	31.300	9.057	0.104	21.190	0.544	0.456	8.085	4.133
10	9	148.591	45.112	0.182	20.944	0.576	0.424	7.835	4.163
11	10	41.600	3.969	0.062	22.136	0.5	0.5	8.351	3.938
12	11	95.100	13.445	0.126	20.602	0.492	0.508	7.951	3.820
13	12	55.571	4.431	0.072	20.887	0.489	0.511	7.913	3.689
14	13					0.53	0.47	8.249	4.373
15	14					0.505	0.495	8.091	3.892
16	15					0.564	0.436	7.764	3.821
17	16								
18	17	52.800	11.361	0.117	20.969	0.522	0.478	7.812	3.701
19	18								
20	19	114.000	14.444	0.134	20.236	0.558	0.442	8.045	3.279
Mean		83.40	13.59	0.11	21.02	0.523	0.478	7.86	3.97
±		±	±	±	±	±	±	±	±
sd		40.44	11.50	0.04	0.65	0.031	0.031	0.38	0.36

Appendix XIIIb : Different life table parameters of JCyp-R strain of *Plutella xylostella* at different generations of selection for resistance to cypermethrin under laboratory conditions.

Sl. No.	Generation	Mean fecundity (GRR)	NRR (R ₀)	Intrinsic rate of increase (r)	Mean gen. time (T)	Sex ratio		Larval period (days)	Pupal period (days)
						Male	Female		
1	0	61.40	4.27	0.07	21.45	0.50	0.50	7.86	3.93
2	1	89.00	5.68	0.08	21.22	0.42	0.58	8.05	3.98
3	2	98.40	5.72	0.09	20.53	0.51	0.49	7.59	3.94
4	3	110.68	12.27	0.12	21.45	0.47	0.53	8.15	3.83
5	4	96.67	3.42	0.06	20.65	0.50	0.50	7.56	4.14
6	5	181.93	29.86	0.18	19.45	0.51	0.49	8.14	3.27
7	6	161.16	16.68	0.13	21.62	0.38	0.62	8.19	3.18
8	7	109.40	12.97	0.12	22.29	0.43	0.57	8.37	3.33
9	8	87.70	12.47	0.11	22.81	0.45	0.55	8.31	3.72
10	9	54.80	14.78	0.13	20.86	0.42	0.58	8.04	4.05
12	11	92.40				0.39	0.61	8.19	3.72
13	12	124.60				0.49	0.51		
14	13					0.35	0.65		
16	15	153.00	43.42	0.19	20.36	0.49	0.51		
19	18	98.80	19.17	0.17	17.98	0.45	0.55		
Mean		108.57	15.06	0.12	20.89	0.45	0.55	8.04	3.74
±			±	±	±	±	±	±	±
s.d.		36.08	11.63	0.04	1.28	0.05	0.05	0.27	0.33

Appendix XIIIc : Different life table parameters of JNzF-R strain of *Plutella xylostella* at different generations of selection for resistance to Neemazal-F under laboratory conditions.

Sl. No.	Generation	Mean fecundity (GRR)	NRR (R ₀)	Intrinsic rate of increase (r)	Mean gen. time (T)	Sex ratio			Pupa period (days)
						Male	Female	Larva period (days)	
1	0	114.30	23.83	0.15	21.79	0.37	0.63	8.30	3.59
2	1	68.90	4.49	0.06	24.48	0.49	0.51	8.54	3.97
3	2	26.60	2.15	0.03	22.68	0.42	0.58	8.23	3.90
4	3	35.90	8.33	0.09	23.08	0.36	0.64	8.47	4.04
5	4	149.50	48.62	0.19	20.50	0.49	0.51	7.98	3.49
6	5					0.50	0.50	8.22	4.00
7	6					0.54	0.46		
8	15	70.14	6.33	0.09	21.57	0.33	0.67		
9	16	46.50	3.13	0.05	25.29	0.60	0.40		
10	18	44.40	4.55	0.07	22.47	0.36	0.64		
Mean		63.07	12.68	0.09	22.73	0.45	0.55	8.29	3.83
±		±	±	±	±	±	±	±	±
Sd		44.07	16.08	0.05	1.56	0.09	0.09	0.20	0.23

GRR-Gross reproductive rate - No. of eggs/female/generation (Potential)

NRR-Net reproductive rate- - No. of eggs/female/generation (Realized)

r- intrinsic rate of increase -No. of eggs/ female/day

Appendix XIII d : Different life table parameters of JNSKE-R strain of *Plutella xylostella* at different generations of selection for resistance to NSKE under laboratory condition.

Sl. No.	Generation	Mean fecundity (GRR)	NRR (R ₀)	Intrinsic rate of increase (r)	Mean gen. time (T)	Sex ratio		Larval period (days)	Pupal period (days)
						Male	Female		
1	0	27.20	3.82	0.06	21.89	0.45	0.55	7.92	3.66
2	1	111.89	16.54	0.14	20.90	0.47	0.53	8.39	4.13
3	2	125.69	26.01	0.16	20.70	0.51	0.49	8.42	3.60
4	3	43.90	0.38	-0.04	23.11	0.58	0.43	8.13	4.66
5	4	100.20	17.99	0.12	23.53	0.48	0.52	8.14	4.01
6	5	95.81	15.72	0.13	22.07	0.51	0.49	8.38	3.83
7	6	55.00	4.60	0.07	22.55	0.56	0.44	8.34	4.08
8	7	49.88	2.49	0.04	22.16	0.47	0.53	8.34	3.85
9	8	88.80	19.07	0.14	21.89	0.50	0.50	8.20	4.05
10	9	130.40	24.97	0.15	21.73	0.50	0.50	8.34	4.25
11	10	36.40	8.37	0.09	22.72	0.31	0.69	8.21	3.76
12	11	33.29	2.24	0.04	20.51	0.58	0.43	8.25	3.50
13	12								
14	13	49.80	2.89	0.05	22.76	0.50	0.50	8.34	4.25
15	14	112.40	12.91	0.11	23.54	0.40	0.60	8.38	3.61
16	15	54.40	5.12	0.08	21.02	0.43	0.57	8.33	3.66
17	16	96.80	9.00	0.10	22.05	0.48	0.52	8.34	3.51
18	17								
19	18								
20	19	27.29	1.73	0.03	21.52	0.53	0.48		
21	20	39.00				0.57	0.43		
Mean $\pm$		71.01 $\pm$	10.23 $\pm$	0.09 $\pm$	22.04 $\pm$	0.49 $\pm$	0.51 $\pm$	8.28 $\pm$	3.90 $\pm$
S.d.		35.97	8.41	0.05	0.93	0.07	0.07	0.13	0.32

Appendix XIIIe : Different life table parameter of unselected field susceptible JField strain of *Plutella xylostella* at different generations during laboratory rearing from 2005-2006.

Sl.No.		GRR	GRR	R0	T	r	Male	Female
1	3/3/2005	March 05	79.7					
2	23/3/05	March -Ap05	131.9	44.846	21.82	0.178	0.53	0.47
3	14/5/05	May05	138.9	52.782	20.91	0.194	0.49	0.51
4	4/6/2005	June05	113.33	38.99	22.018	0.170	0.52	0.48
5	5/8/2005	Aug 05	71					
6	25/8/05	Aug-Sept 05	112.66	42.81	21.1	0.18	0.53	0.47
7	16/9/05	Sept05	150.10	57.04	21.18	0.20	0.48	0.52
8	5/12/2005	Dec05	114.60	43.55	20.13	0.19	0.59	0.41
Mean			107.43	45.54	21.58	0.18	0.52	0.48
± s.d.			±33.49	±6.92	±0.59	±0.01	± 0.04	±0.04