Resistant Pest Management Newsletter

A Biannual Newsletter of the **Center for Integrated Plant Systems (CIPS)** in Cooperation with the **Insecticide Resistance Action Committee (IRAC)** and the **Western Regional Coordinating Committee (WRCC-60)**

Vol. 13, No. 1 (Fall 2003)

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Michigan State University currently maintains an electronic arthropod pesticide resistance database via the Internet (www.cips.msu.edu/resistance). Documented resistance developed by arthropod species currently stands at 542 species (Figure 1 and Table1)

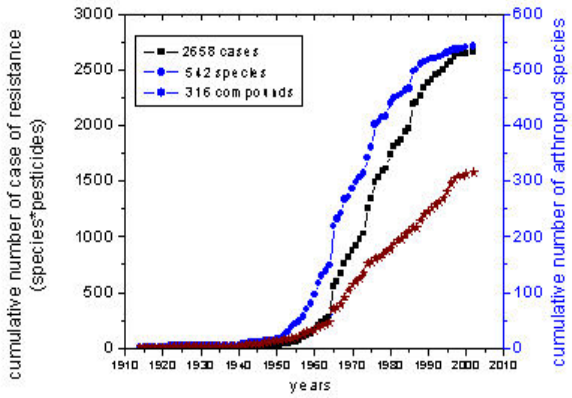

Figure 1. Cumulative newly-resistant arthropod species and reported cases of pesticide resistance around the world

Table 1. Current status of pesticide resistance (MSU database 2003)

Compounds	316
Species	542
Cases	2,658
Countries	168
National Cases	4,789
Regional Cases	5,774
References	1,478
Total documents	2,599

distributed in 168 countries around the world. The combination of species and compounds yields 2,658 resistance cases. However, the crossed combination of species, compounds and region totals 5774 cases of resistance in the world. The current database is based upon an examination of more than 2658 peer-reviewed journal articles. While this is a significant contribution, continuing support for locating and reviewing resistance information is necessary to report the current status of arthropod resistance. Despite the review of the recent information, there is a gap in time between the completion of monitoring studies and when the report is published. Often more than a year passes before the results of monitoring are published, and this delay dramatically reduces the effectiveness of resistance management. There is a critical need for a real-time reporting method for resistance cases. In addition to rapid reporting, baseline susceptibility data in transgenic crops and novel compounds is necessary to detect and compare the evolution of resistance over time. Finally, USEPA has just identified the Resistant Arthropod Database as one of the resources for official resistance criteria from Emergency State Exemptions (section 18).

We are requesting your resistance expertise and benevolent assistance in providing timely editorial reviews of our web-based electronic survey system that will rapidly update cases of resistance (and susceptibility to novel compounds and transgenic organisms) in the resistance database. We have designed the review process to be efficient and user-friendly to minimize your time commitment and maximize the application of your expertise. The submitted resistance cases will be labeled with an accession number (like the gene bank) and will be used as a citation as authors. National and international pest resistance specialists will submit their resistance cases and a panel of experts will review a limited number of cases annually. The electronic resistance survey system will be fully operational by the first week of the new year. If you agree to help serve as subject editor, please return your response and comments to us by the end of November. At the end of December you will receive an e-mail containing the website address to submit resistance cases. At the end of this month you will receive an email with the website address to complete the electronic survey system for the review and inclusion in the database. As a resistance expert note below the entry that describes the elements that will be required to submit a survey.

The goal of the Michigan State University Arthropod Resistance Database is to provide a seamless electronic means of collecting resistance information in a timely fashion. The searchable database will be made available to the entire scientific community through the Internet. Those of us at MSU involved with the resistance database look forward to your response to our request for your help. A number of noted experts in the resistance field have already

agreed to serve as editors. Therefore, we are confident that we will be able to utilize your editorial assistance perhaps 4-6 times annually. If you agree to serve in this capacity, we will include your name on the editorial assistants' page at the beginning of the web site. Thank you for considering this novel editorial request

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Data entry for the electronic resistance survey

The electronic survey system is going to be a novel feature of the database. Data gained from resistance specialists as expert opinion will be entered. We intend to incorporate as much data as possible, so as to maximize the utility for referencing emergent cases of resistance, as well as establish baseline effects of new chemistries.

The proposed elements in the survey are:

1. Arthropod classification (class, order, family, species, author, common name (ESA), other local common names)
2. Pesticide (chemical and mode of action)
3. Geographic location (country, state or province, site, coordinates (GPS) latitude, longitude, scale)
4. Collection size (agricultural crop, storage, urban, lab)
5. Stage collected (egg, larva, nymph, pupa, adult)
6. Sex: (male, female, both)
7. Bioassay (topical, residual, discriminating dose, stage, egg, larva, nymph, pupa, adult)
8. Type of resistance (field detection or resistance developed in the lab, cross-resistance)
9. Median lethal values (LD50, or LC50, KD50, LT50) and fiducial limits, and slopes plus SE, ratios of resistance (RR)
10. Impact of the resistance (a) non or low, b) medium, c) high, d) severe, and e) NA:
    a) No change in the efficacy of the compound to control the pest in the field/urban setting.
    b) Some reduction in the efficacy of the compound in the field.
    c) Significant reduction in the efficacy of the compound in the field.
    d) No control; pesticide is no longer effective.
    e) Lab-selected or not applicable
11. Reference (laboratory data, field test, peer review paper, not peer review paper, author, year, journal, volume, pages)
12. Editorial Review
13. Contact information of the specialist (name, institution, address, e-mail, phone, fax)

Resistance Management Reviews

Pyrethroid Resistance and Fitness Loss in Aphids

By Dr Terry Mabbett

Development of the first synthetic pyrethroid insecticides in the late 1970s heralded the dawn of a new era for insect pest control. Insecticides such as permethrin and cypermethrin, with a wide activity spectrum, high potency, and stability, stood to provide broad-spectrum control with residual activity at dosage rates of just a few grams per hectare.

But over-indulgence with this new and exciting insecticide chemistry rapidly gave rise to a series of problems as insects including Hemiptera (e.g. aphids) and Lepidoptera (e.g. bollworms) rapidly became insensitive (resistant) to the pyrethroid molecule's mode of action. Due to resistance, the pyrethroid insecticides have only realised a fraction of their full commercial potential.

Be that as it may, there are now many different pyrethroid products on the market possessing a chemistry and mode of action that still offers the most versatile and cost effective means of insect pest control with synthetic chemicals. Lambda-cyhalothrin, a pyrethroid insecticide with contact and stomach action, offering rapid knockdown and long residual activity, is valued for its broad-spectrum control that includes plant sucking bugs (e.g. aphids), beetle adults, and larvae and leaf eating lepidoptera.

It is used as a foliar spray on potato (*Solanum tubersoum*) in many countries, including the United Kingdom, for control of aphids such as *Myzus persicae* (peach-potato aphid) and *Macrosiphum euphoriae* (potato aphid). These aphids rarely cause terminal damage to mature plants through physical damage and solute removal, but carry and transmit a wide range of plant pathogenic viruses spread rapidly from plant to plant and field to field by the winged aphid forms.

Viruses like potato virus Y have the capacity to devastate yields and spell doom in the seed growing areas of Scotland and Northern Ireland where disease-free seed tuber stocks are essential to maintain home and export markets.

Spray applications of Lambda-cyhalothrin are a cornerstone in aphid control and plant virus management for United Kingdom potato growers. Following widespread and established resistance for lambda-cyhalothrin in *Myzus persicae*, caused by increased esterase activity, research teams at Institute of Arable Crops Research at Rothamsted (UK) have been screening populations of *Macrosiphum euphorbiae* for the existence of similar problems.

In May 2002 Despina Philippou, postgraduate student at Imperial College, joined the Rothamsted team to determine the status of lambda-cyhalothrin resistance in *M. euphorbiae*, for the research project component of her MSc degree in Integrated Pest Management.

By using two biochemical methods (polyAcrylamide gel electrophoresis and total esterase assay), routinely used to measure esterase activity in *M. persicae*, Despina identified variability in esterase activity within twelve different *M. euphorbiae* clones initiated from field samples collected on potatoes in the UK since 1998.

Leaf dip assays conducted in the laboratory showed esterase activity to be positively associated with resistance to lambda-cyhalothrin in ten out of twelve clones. In addition, all twelve clones produced cross-reactions in an immunoassay test previously developed to assess levels of E4/FE4 carboxylesterase resistance in *M. persicae*. This suggests, says Despina, that *M. euphorbiae* has developed a metabolic resistance mechanism to lambda-cyhalothrin based on over-production of an esterase enzyme analogous to that found in *M. persicae*.

More far reaching developments were indicated when Despina went on to study the response to alarm pheromone, an important component of aphid fitness, in controlled laboratory assays, using *M. euphorbiae* clones showing variability in esterase activity.

The data obtained by Despina provided strong evidence for an inverse relationship between the quality of response to alarm pheromone and total esterase activity. This indicates that an adverse selection mechanism may be acting against high esterase forms in the field, says Despina.

Furthermore, says Despina, it supports the hypothesis that an accelerated evolutionary process of resistance can produce mechanisms associated with fitness costs in the absence of insecticides, a situation similar to that seen in *M. persicae*.

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Insecticide Resistance in Cotton Bollworm, *Helicoverpa Armigera* (Hubner): A Mini Review

Productivity and quality of cotton crop plugged by serious insect pest problems. Among the pest complex invading the crop and limiting the productivity and quality of cotton, bollworms take a major toll, particularly *Helicoverpa armigera* takes a lions share (1). Though cultivated cotton occupies only in 5% of the total cultivable area in India, it consumes more than 55% of the total insecticides used in the country (2). It commonly destroys more than half the yield with an estimated of annual loss of over US $ 500 million in cotton and pulse crops. Among the options available to control *H. armigera,* insecticidal spray is considered as

the most practical option by the farmers in India, presently. The indiscriminate use of insecticides, particularly during 1980s and 1990s, has contributed to the emergence of *Helicoverpa armigera* as a primary pest of cotton in recent years. In India, resistance in the American bollworm, *Helicoverpa armigera* (Hubner), to almost every class of insecticides has been documented (3). This phenomenon is complex and reported to vary in time and space including mechanisms responsible for resistance development (4). Possible change in cropping pattern, insecticide resistance and suppression of natural enemies have made *H. armigera* a dominant pest in south Indian cotton ecosystem (5). The situation is exactly same in other ecosystems and crops of value where insecticide application intensity is high (6).

Organophosphates and organochlorines were first molecules to have recorded resistance in bollworm and it is only since one and a half decade to problem of pyrethroids (7, 8,9). Monocrotophos and quinalphos constitute 75% insecticide market in India, where, about 85% of quinalphos and 68% of monocrotophos are used solely on cotton (13). In India, high level of pyrethroid resistance was reported from cotton growing region of Andhra pradesh in *H.armigera* during 1980's (10, 11). Subsequently it has been shown that *H. armigera* developed resistance to virtually every insecticide group. In 1980's early 1990's to combat the unprecedented pest pressure, farmers resorted to application of heavy doses of insecticides and often used combination of two or three insecticides, thereby creating high selection pressure on the insect to develop resistance. Running behind molecules and concentrations to manage *H.armigera* is at best ad hoc and despracy, which is worsening the situation. Thorough understanding of the population flux and biology in each location and thereon resistance management strategies to each location is required.

DOCUMENTING RESISTANCE A thorough know-how about the mode of action of the insecticide molecules and the response pattern of insect populations is essential for measurement and a meaningful documentation of resistance. An efficient detection and monitoring in flux of resistance need to be achieved by pest management programs for each of the population.

Reliable, quick and effective techniques to distinguish between susceptible and resistant individuals are necessory (14). Once the status of resistance for a pest population is known, strategies to mitigation become easy. Monitoring methodology is key factor to evolve effective resistance management strategies, which are implementable and verifiable (13).

Thorough toxicological bioassays indicate to response of populations to insecticidal molecules, there will always be a gap between assay results and field performance. The methods chosen to determine resistance in field populations should be able to quickly detect shifts in the frequency of resistant individuals in the target population and provide estimates of resistance levels. Correlation of such results with field efficacy of the pesticides will be more informative for further use. Most of the techniques currently available can reveal responses of different magnitude to insecticides (15,16,17,18). In some cases, biochemical techniques have been employed that measure changes in sensitivity at the target site or metabolic changes that are associated with a resistance mechanism (19,20,21,22). Identification of resistance allele using specific molecular markers, if possible to each of the insecticides, will be of paramount use in detecting and monitoring such allele frequency within and between populations. Such technology could provide speed, sensitivity and accuracy of monitoring resistance. Different bioassay techniques generally used to document, monitor insecticide resistance are

- Topical application
- Leaf dip and leaf spray
- Vial method
- Diet incorporation
- Growth inhibition assay method
- Sticky card technique
- Biochemical methods

INSECTICIDE RESISTANCE MANAGEMENT Worldwide insecticide resistance management strategies for cotton pests are being developed where resistance monitoring is an important component (12,23) .of these strategies. There have been increasing efforts towards incorporating insecticide resistance management (IRM) strategies into the larger realm of IPM in recent years (24). As a matter of fact there is no IRM panacea, suggesting the fact not being any necessarily effective in all pest resistance situations (24). It is important to formulate practical guidelines for the design, implementation, and servicing of an IRM program. In IRM, emphasis is on the rational use of insecticides and on the restriction of treatments to prevent selection for resistance and thus increase to longevity of the molecules. Information on the insecticide molecules, mode of action of individual insecticide, nature of resistance build up, nature of species and its life cycle, synergists, cross resistance patterns, population dynamics will strengthen the IRM strategies. Current knowledge of insecticide resistance, allele frequency and population fluxing patterns should be made use in IRM (25). Some of the strategies and tactics which can have direct bearing on selection pressure are as follows (26).

1. Use of high doses to eliminate heterozygous or resistant homozygotes if such knowledge available by molecular markers and bioassays

2. Selection pesticides that show lower resistance
3. Treating the most susceptible life stage
4. Use of synergists to block metabolic mechanisms
5. Use of mixtures

Development of an inventory of insecticide resistance to all insecticides used in an ecosystem and year after year undertaking thorough continous monitoring is not only updates the status but also useful in IRM strategies. Such inventory has been developed for south Indian cotton ecosystems for *H.armigera* (32).

Minimize the amount of selection pressure applied by

1. Reduction the concentrations of insecticides to preserve a sufficient number of susceptible genotypes
2. Decreasing the frequency of insecticide applications
3. Selection of pesticides with short residual activity and eliminate slow release formulations
4. Avoiding area and wide applications for a pest; include spot applications
5. Use of rotations to avoid sequential exposure of each generation to a single pesticide; avoiding and spatial mosaic treatments

Identification of DNA fingerprints specific to individual populations (population fingerprints) could be of great use in studying and elucidating the population fluxing patterns. Such fingerprints can determine more accurately the extent and rate of migration of each population. In resistance terminology, movement population is movement of resistance allele vis-vis resistance. Hence, such information can be very useful in IRM strategic models. In an ecosystem like south India, where farmers are independent to choose an insecticide and are often influenced by availability of insecticides and market strategies, naturally spatial mosaic of not definitive pattern happens. This is also true with respect to concentration of each molecule used. Otherwise, as a thought-out strategy mosaics are not adopted (25).

A number of genetic models and computer simulations have been tested to provide insight into possible outcomes of use of different insecticide use strategy. In such models factors intrinsic to genetic systems relative fitness of genotypes, allelic frequencies and degree of dominance of resistant alleles along with operational factors such as dosage rates and type of insecticide etc. are considered. Ecological and life history parameters also figure importantly into such models (27,28,29). Generation time can drive the outcome of simulation models, with shorter generation times generally representing shorter times to resistance build (29,30).

INSECTICIDE ROTATIONS Logical rotation of insecticides is probably the most viable strategy in most circumstance. Viability of alternating insecticides rests on the pretext that resistant individuals possess lower fitness than susceptibilities in the absence of a selecting insecticide. Unless fitness levels in resistant individuals are not severely reduced. There is little difference between rotating insecticides or sequentially introducing different insecticides (25, 31).

SPATIAL MOSAICS Over an ecosystem, alternation of two or more insecticides is known as spatial mosaic. In practice, mosaics are logistically more difficult than temporal rotations and are not as strongly supported logically as insects populations will be in some degree of dynamics (25,31,32).

INSECTICIDE MIXTURES A number of workers have supported the practice of mixing two insecticides for the purpose of avoiding or delaying resistance (43,45,54,55). It is important that the insecticides being mixed have equal persistence and effective. It would be more logical if they have different modes of actions. However such strategies need much more testing. There were also a number of key strategy guidelines, which recommend additional non-chemical counter measures to reduce selection pressure (25)

- Grow early maturing crops to avoid dominant *H. armigera* populations late in the season
- Avoid growing certain alternative host crops near cotton, as they serve as early season nursery crops for resistant insects
- Avoid consecutive sprays of pyrethroids where *H. armigera* are emerging from neighboring early season alternative host crops, as resistance levels will be exacerbated by selection of moths before mating
- Target pyrethroids to egg hatch, to avoid selection of older established larvae
- Check crops frequently and thoroughly and spray on threshold. This can minimize the need for sprays
- Utilize host plant resistance whenever possible
- If a pyrethroid is used to control for a pest, do not follow up a pyrethroid for *H. armigera*

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Resistance Management from Around the Globe

Baseline Resistance Information

Native Insecticide Resistance in Spotted Bollworm, *Earias vitella* (Fabricius) in Western Vidarbha Region of India

ABSTRACT The results, after comparing the LD50 values of *Earias vitella* collected from different locations with the LD50 of the laboratory-reared susceptible strain to monitor the level of resistance against particular insecticide in this part of the country, revealed that the Akola strain was comparatively more resistant to all the insecticides tested except quinalphos when compared with Amravati, Washim and Nagpur strains. It was 3.63 and 6.05-fold resistant against endosulfan, 20.96 and 39.69-fold resistant against cypermethrin and 5.29 and 7.81-fold resistant against thiodicarb during 2000-01 and 2001-02, respectively. However, Washim strain was found more resistant to quinalphos (7.55 and 12.66-fold during 2000-01 and 2001-02 respectively) than other strains. Moreover, *E. vitella* population from all the locations was highly resistant to cypermethrin than the other three insecticides tested.

INTRODUCTION *Earias vitella* (Fabricius), synonymously known as spotted bollworm of cotton and shoot and fruit borer of okra, is the most dreaded pest of these two important cash crops of Vidarbha region of Maharashtra. As a bollworm of cotton, it causes 40 per cent losses in seed cotton at national level (Veeresh, 1980) and up to 45.57 per cent in Maharashtra state (Satpute et al. 1986) Similarly, *E. vitella* is devastating on okra causing 41.6 per cent crop losses in different parts of India (Krishnakumar and Srinivasan, 1987). Being devastating pest of cotton and okra with its high reproductive as well as damage potential, suppression of *E. vitella* becomes inevitable. Use of various chemical insecticides belonging to different classes is in vogue for suppression of this pest but only partial control of this pest could be achieved. On the contrary, the indiscriminate and extensive use of these insecticides since past few decades have led to many serious problems like development of insecticide resistance, resurgence of minor pests, destruction of beneficial fauna and environmental pollution (Mahapatro and Gupta, 1998). Over past few decades, management of *E. vitella* has become increasingly difficult due to its internal feeding habit and developed resistance to the most commonly used chemicals. In this context, very meager work on insecticide resistance studies against *E. vitella* seems to have been done in India. Inspite of this, possibility of resistance development cannot be ruled out considering the continued and indiscriminate use of insecticides. Keeping this in view, the present studies were planned to monitor the level of insecticide resistance in *E. vitella* in Akola and the adjoining areas.

MATERIALS and METHODS

Mass rearing of E. vitella

The eggs and larvae of *E. vitella* collected from the host plants viz. cotton and okra from the fields of Dr. PDKV, Akola as well as from the farmers' fields in adjoining areas were reared in the laboratory on semi-synthetic diet (Gupta et al., 1998) for further culturing. The diet was provided daily and the larvae were allowed to pupate in the rearing vials and containers. These pupae were transferred in adult emergence chamber by maintaining equal male female ratio (1: 1) and were provided with adult diet (10 % honey solution). Five pairs of moths were released in each mating chamber made of glass bell jar. The small strips of cotton cloth (nappy liners) and fresh okra fruits were used as oviposition substrate for the female moths. These substrates after egg laying were kept in incubator for hatching. The neonates emerging from the eggs were carefully released on small pieces of okra fruits for 3-4 days, then transferred on the freshly prepared semi-synthetic diet. The larvae from this 'F-1' generation were used for conducting the bioassay studies.

Laboratory Reared Susceptible Strain of E. vitella

Continuous rearing of *Earias* in the laboratory without selection pressure of insecticides was done for nine generations at the Insect Biotechnology Laboratory of Department of Entomology, Dr. PDKV, Akola (Maharashtra) to get relatively susceptible strain. The third instar larvae of ninth generation population were exposed to different insecticides to determine the LD50 and LD90 values. These values of median lethal dose (LD50) were compared with the field collected population from Akola and the adjoining areas for monitoring the prevalent level of insecticide resistance in *E. vitella*.

Insecticide Resistance Studies in E. vitella

Four technical grade insecticides belonging to different groups viz., cypermethrin, quinalphos, endosulfan and, thiodicarb were used for conducting resistance studies in *E. vitella* against them in Akola region. The *E. vitella* population were collected from okra growing fields of different locations viz., Akola, Amravati, Washim and Nagpur for two years during 2000-01 and 2001-02. The field collected third instar larvae were bioassayed with the help of Hamilton's microapplicator against these insecticides by dosing 1 µl insecticide per larva and the mortality data were subjected to Probit analysis as per the procedure of Finney (1977) and by using the computer software 'Indostat' entomological package. From this data, the resistance levels were computed by using following formula.

$$\text{Resistance level} = \frac{\text{LD50 of Field collected population}}{\text{LD50 of laboratory reared susceptible strain}}$$

RESULTS and DISCUSSION

Laboratory Reared Strain of E. vitella

The data on bioassays conducted on each generation of *E. vitella* reared in laboratory without selection pressure revealed that the mixed field culture of *Earias* still retained its resistance to endosulfan, cypermethrin, quinalphos and thiodicarb for four-five generations. The resistance tends to reduce after fourth-fifth generation and was found to be reduced 2.35-fold to endosulfan, 10.34-fold to cypermethrin, 3.12-fold to quinalphos and 6.90-fold to thiodicarb by the ninth generation. During ninth generation, the LD50 of these respective insecticides against *E. vitella* was 0.0666, 0.0026, 0.0116 and 0.0869 µg /larva (Table 1). The reduction in fold toxicity in successive generations is also presented graphically in Figure 1.

Table 1. Generation studies against *E. vitella* for monitoring the fold reduction in insecticide resistance

Generations (Unselected)	Endosulfan		Cypermethrin		Quinalphos		Thiodicarb	
	LD_{50} µg/larva	Reduction in fold toxicity	LD_{50} µg/larva	Reduction in fold toxicity	LD_{50} µg/larva	Reduction in fold toxicity	LD_{50} µg/larva	Reduction in fold toxicity
F1	0.157	-	0.0269	-	0.0362	-	0.6004	-
F2	0.2143	0.73	0.0279	0.96	0.0403	0.89	0.5614	1.06
F3	0.163	0.96	0.0265	1.01	0.0351	1.03	0.4881	1.23
F4	0.1327	1.18	0.0139	1.93	0.0275	1.31	0.5435	1.1
F5	0.1405	1.11	0.0112	2.4	0.0196	1.84	0.3142	1.91
F6	0.1226	1.28	0.0092	2.92	0.0141	2.56	0.2266	2.64
F7	0.1214	1.29	0.007	3.84	0.0125	2.89	0.1159	5.18
F8	0.0957	1.64	0.0043	6.25	0.0136	2.66	0.1017	5.9
F9	0.0666	2.35	0.0026	10.34	0.0116	3.12	0.0869	6.9

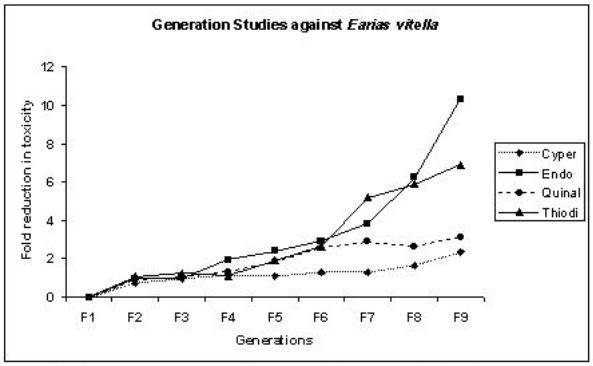

Fig. 1: Generation studies against *E. vitella* for monitoring the fold reduction in insecticide resistance

Insecticide Resistance Studies in E. vitella

The results of bioassay studies conducted on field population of *E. vitella* collected from infested okra plants at Akola, Amravati, Washim and Nagpur during 2000-01 and 2001-02 as well as on laboratory maintained strain of *E. vitella* revealed a higher LD50 values for Akola, Nagpur, Amravati and Washim strains than that of laboratory reared strain. The comparative resistance levels demonstrated higher resistance to cypermethrin (Akola-20.96-fold, Nagpur-15.38-fold, Washim-11.92-fold and Amravati-10.03-fold) followed by quinalphos (Akola-5.50-fold, Nagpur-1.93-fold, Washim-7.55-fold and Amravati-2.31-fold), thiodicarb (Akola-5.29-fold, Nagpur-3.04-fold, Washim-4.93-fold and Amravati-2.70-fold) and endosulfan (Akola-3.63-fold, Nagpur-2.79-fold, Washim-1.90-fold and Amravati-2.55-fold) (Table 2).

Similar situation existed during 2001-02 when the resistance was found to have increased exhibiting highest level of resistance against cypermethrin (Akola-39.69-fold, Washim -18.80-fold, Amravati-15.26-fold and Nagpur-17.03-fold) followed by quinalphos (Akola-7.21-fold, Nagpur-3.56-fold, Washim-12.66-fold and Amravati-4.12-fold), thiodicarb (Akola-7.81-fold, Nagpur-4.95-fold, Washim-5.96-fold and Amravati-4.72-fold) and endosulfan (Akola-6.05-fold, Nagpur-3.08-fold, Washim-2.93-fold and Amravati-2.65-fold). The corresponding LD50 values for each location and for both the years are given in Table 2.

Table 2: Level of insecticide resistance against *E. vitella* collected from different locations during 2000-01 and 2001-02

Locations	Endosulfan		Cypermethrin		Quinalphos		Thiodicarb	
	LD_{50} µg/larva	RR	LD_{50} µg/larva	RR	LD_{50} µg/larva	RR	LD_{50} µg/larva	RR
2000-01								
Akola	0.2423	3.63	0.0545	20.96	0.0639	5.5	0.4599	5.29
Washim	0.1269	1.9	0.031	11.92	0.0876	7.55	0.4288	4.93
Amravati	0.1699	2.55	0.0261	10.03	0.0268	2.31	0.2352	2.7
Nagpur	0.1859	2.79	0.04	15.38	0.0224	1.93	0.2648	3.04
2001-02								
Akola	0.4032	6.05	0.1032	39.69	0.0837	7.22	0.6789	7.81
Washim	0.1953	2.93	0.0489	18.81	0.1469	12.66	0.518	5.96
Amravati	0.1769	2.65	0.0397	15.27	0.0479	4.13	0.4103	4.72
Nagpur	0.2056	3.09	0.0443	17.04	0.0414	3.57	0.4302	4.95
Akola Susceptible*	0.0666	-	0.0026	-	0.0116	-	0.0869	-

* - Reared in laboratory for 9 generations at Akola without selection pressure.
RR - LD50 of reference strain / LD50 of laboratory reared strain

Thus, the present studies have clearly demonstrated that the *E. vitella* population has developed significant resistance to cypermethrin, quinalphos, thiodicarb and endosulfan. Akola population was found to have developed comparatively more resistance than other localities except against quinalphos to which Washim population was more resistant. Therefore, endosulfan, quinalphos and thiodicarb with comparatively lower resistance levels than cypermethrin holds promise of better control of *E. vitella*.

As such no authentic reports are available about the development of insecticide resistance in *E. vitella* population of these four localities but due to indiscriminate use of pyrethroids and the findings of present investigations clearly pointed out the possibility of resistance phenomenon operating in *E. vitella* population of these localities. This pest has already been found to develop resistance to insecticides in some parts of India as Saini et al., (1989) reported after 15 generations selected with fenvalerate and cypermethrin that Earias exhibited 7.8-fold resistance to fenvalerate and no cross resistance to cypermethrin. Hirano et al., (1993) reported that *Earias* from two different locations exhibited variable resistance. Similarly, Al-Betagy (2001) observed that the Spiny bollworm was in the tolerant stage with 3.31, 2.81, 2.75, 3.89 and 3.45-fold resistance to the population of *E. insulana* collected from different parts of Egypt and compared with the responses of susceptible strain. However, Undirwade (2001) recorded *E. vitella* population from Akola to be more resistant to cypermethrin, quinalphos and chlorpyriphos as compared to endosulfan, which is in agreement with the present findings. But very meagre work on the development of resistance to insecticides in *E. vitella* has been done.

Riskallah et al (1983) reported 33- and 27-fold resistance in *S. litura* strains to fenvalerate and deltamethrin respectively. Jensen et al (1984) found 37-fold resistance to cypermethrin in *H. virescens*. Similar results were obtained by Vines et al (1984) with *Diatraea saccharalis* and Chen and Sun (1986) with *Plutella xylostella*, Marugesan and Dhingra (1995) with *S. litura*, Chandler and Ruberson (1996) with beat armyworm, Burikam et al (1998) with *H. armigera* and Singh and Singh (1998) with *S. litura*. Gunning and Easton (1994)a & b found moderate resistance in field collected population of *H. punctigera* against deltamethrin, fenvalerate, endosulfan and DDT but the high levels of endosulfan resistance (>50-fold) were recorded in *H. armigera*. Wolfenbarger et al (1997) reported that all the pyrethroids tested were more toxic to the laboratory-reared strain of *S. litura* than any field collected strain in eastern and western Mexico, Louisiana or Georgia which corroborates present findings. Similarly, Patel et al (2000) found that the population of Vadodara and Sadarkantha were more than 10-fold resistant for both localities of *H. armigera* when tested against cypermethrin, quinalphos, chlorpyriphos and carbaryl.

The present findings indicate that the field strains of *E. vitella* have developed resistance to cypermethrin, a synthetic pyrethroid tested and accordingly pyrethroids should be applied only when needed, at rates sufficient to control infestations, and not routinely, for routine treatment is likely to accelerate the development of resistance. The present data also show that the organophosphorus (quinalphos), the carbamates (thiodicarb) and the cyclodienes (endosulfan) with comparatively lower field resistance can be effective in controlling larvae of *E. vitella* that have acquired resistance to pyrethroids.

Hence, considering very meagre work done on insecticidal resistance development in *E. vitella*, further elaborate studies on these aspects will provide detailed account of insecticide resistance and its probable mechanism in *E. vitella* population of this region.

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Differential Susceptibility of Field Collected Population of Redgram Pod Borer, *Helicoverpa armigera* Larvae to Recommended Insecticides on Redgram in Karnataka

ABSTRACT Field collected redgram pod borer, *Helicoverpa armigera* (Hb.) (Lepi:Noctuidae) larvae from high pesticide imposed area recorded low per cent mortality to recommended insecticides indicating the loss of susceptibility to insecticides/development of insecticide resistance. Similarly mortality of larvae collected from redgram areas varied significantly with commonly used/uncommonly used and new insecticides.

INTRODUCTION In recent years *Helicoverpa armigera* Hubner has emerged as a dominant pest of various crops, including cotton and pulses in the country. It has been claimed that annual lossess due to this pest on redgram and bengalgram alone amounts to Rs. 3000 million and if lossess on cotton, vegetables and other cereals are taken into consideration the total losses due to this pest are colossal (Mehrotra, 1992).

Before the probable existence of the pesticides resistance was reported in India by large scale crop failures in Andhra pradesh, it has been suggested that *H.armigera* obtained from various regions of the country differ significantly with respect to susceptibility/resistance to pesticides (Reed and Pawar, 1982, and Phokela and Mehrotra, 1985). The first out break of *H.armigera* was seen in the cotton belt of Guntur, Prakasham and parts of Krishna districts in Andhra Pradesh. This population showed high levels of resistance to various insecticides (Mc Caffery et al, 1989 and Mehrotra, 1992).

Since 1988, regular outbreaks of *H.armigera* was observed on redgram during November and December months. To understand the level of susceptibility /resistance of *H.armigera* to recommended commonly used insecticides, the present study was undertaken in redgram ecosystem where considerable amount of insecticides are used.

MATERIAL and METHODS Redgram pod borer, *H.armigera* larvae of different sizes viz. early instar larvae (1-3rd instars) and grown up larvae (4-5th instars) were collected from high insecticide imposed (Jewargi) and low insecticide imposed area (Lingasugur) during second fortnight of November. At Jewargi, the farmer had given ten sprays with various combinations and at Lingasugur one spray with endosulfan and one spray with quinalphos was given. Such field collected larvae were exposed to redgram pods sprayed with recommended insecticides at recommended dosage (Table 1) under laboratory conditions. Fifteen replications were maintained for each insecticide treatment. Observation on larval mortality was recorded.

Table 1.Susceptibility of field collected *H.armigera* larvae to recommended insecticides on redgram

Place of collection		Stage of larvae	Insecticides	Recommended dosage (ml/lit of water)	* Per cent mortality
I	High insecticide imposed area: (Jewargi)	High insecticide imposed area: (Jewargi)			
		(a) Early instar larvae (1-3 instars)	Endosulfan 35EC	2.0	46.50 (42.99)
			Monocrotophos 36SL	1.0	67.80 (55.43)
			Fenvalerate 20EC	0.5	62.50 (52.24)
		(b) Grown up larvae (4-5 instars)	Endosulfan 35EC	2.0	23.60 (29.06)
			Monocrotophos 36SL	1.0	34.20 (35.79)
			Fenvalerate 20EC	0.5	38.00 (38.06)
II	Low pesticide imposed area: (Lingasugur)	Low pesticide imposed area: (Lingasugur)			
		(a) Early instar larvae (1-3 instars)	Endosulfan 35EC	2.0	64.50 (53.43)
			Monocrotophos 36SL	1.0	83.80 (66.27)
			Fenvalerate 20EC	0.5	91.20 (72.74)
		(b) Grown up larvae (4-5 instars)	Endosulfan 35EC	2.0	56.50 (48.73)
			Monocrotophos 36SL	1.0	77.80 (61.89)
			Fenvalerate 20EC	0.5	84.20 (66.58)

*Mean of 15 replications Note: Figures in parantheses are transformed angular values

In another studies, *H.armigera* larvae of early instars were collected from different places of Gulbarga district (which is known as Tur bowl of Karnataka) viz., Sedum, Gulbarga, Chitapur and Chincholi where redgram was grown extensively and over years maximum application of insecticides was undertaken. Such field collected larvae were later exposed to redgram pods sprayed with commonly used insecticides on redgram (Endosulfan, Monocrotophos and Fenvalerate) uncommonly used insecticides on redgram (Chlorpyriphos and Quinalphos) and new insecticides (Spinosad, Indoxacarb and Fenpropathrin). Fifteen replications were maintained for each insecticide treatment and observation on larval mortality was recorded.

RESULTS and DISCUSSION The susceptibility of field collected *H.armigera* larvae to recommended insecticides on redgram varied considerably with regard to place of collection and age of larvae (Table 1). The per cent mortality of early instar larvae at Jewargi (high pesticide applied area) was 44.00, 53.00, 58.00, and 70.00 per cent in endosulfan, monocrotophos fenvalerate, and chlorpyriphos treatments respectively. Whereas in case of the grown up larvae mortality recorded was only 18.00, 25.00,

38.00, and 45.00 per cent in endosulfan, monocrotophos fenvalerate, and chlorpyriphos treatments respectively indicating the ineffectiveness of insecticides. At Lingsugur (low pesticide applied area) the early instar larvae recorded 60.00, 78.80, 81.00, and 92.40 per cent mortality and grown up larvae recorded 60.00, 75.80, 80.00, and 85.00 per cent mortality in endosulfan, monocrotophos fenvalerate and chlorpyriphos treatments respectively. The data clearly indicated that *Helicoverpa* strains collected from Jewargi has lost susceptibility to a greater extent to recommended insecticides than that of Lingasugur *Helicoverpa* strain. This is mainly because of indiscriminate application of insecticides at Jewargi area. This clearly indicated the probable development of insecticide resistance in *Helicoverpa* population collected from Jewargi area. Similar reports of *Heliothis* developing resistance to insecticides were also reported by Reed and Pawar, 1982; Phokela and Mehrotra, 1985 and McCaffere et al, 1989.

H.armigera larvae collected from different redgram growing places of Gulbarga distict, Karnataka showed varied level of mortality to different insecticides tested (Table 2). It is clear from the table that present mortality was low in all the places with endosulfan, monocrotophos and fenvalerate insecticides. Whereas uncommonly used insecticides in redgram ecosystem like chlorpyriphos and Quinalphos recorded on an average 80.25 and 76.42 per cent mortality respectively. This indicates their further potential use on *Heliothis* in redgram crop. New insecticides viz., Indoxacarb 14.50 SC, Spinosad 48 SC and Fenpropathrin 30EC have recorded on an average 95.67, 97.85 and 89.00 percent mortality (Table 2) indicating their superior effectiveness on *H.armigera*.

The study pinpoints that *Helicoverpa* has lost susceptibility/developed resistance to commonly used insecticides (endosulfan, monocrotophos and fenvalerate) and their further usage on redgram needs to be properly monitored. Whereas chlorpyriphos and Quinalphos usage should be encouraged on redgram crop till new insecticides are recommended on redgram for pod borer management. Similar suggestions were also made by Reed (1990) Mehrotra (1992) and Patil (1993) as a strategy to contain the development of insecticide resistance in insects.

Table 2.Mortality of field collected *H.armigera* larvae from different redgram areas to insecticides.

Sl.No.	Insecticide	Dosage (ml/Lit of water)	Per cent mortality* Different redgram areas				
			Sedum	Gulbarga	Chitapur	Chincholi	Av.
1	Endosulfan 35EC	2.00	45.2	48.3	50	42.6	46.52
			(42.25)	(44.03)	(45.00)	(40.74)	(43.80)
2	Monocrotophos 36SL	1.00	50	55	58	65	57
			(45.00)	(47.87)	(49.60)	(53.73)	(49.02)
3	Fenvalerate 20EC	0.50	66	58	65.3	60	62.32
			(54.33)	(49.60)	(53.91)	(50.77)	(53.03)
4	Chlorpyriphos 20EC	3.00	75	82	80	84	80.25
			(60.00)	(64.90)	(63.00)	(66.42)	(64.86)
5	Quinalphos 25EC	2.00	70	75.3	79.4	81	76.42
			(56.79)	(60.20)	(63.01)	(64.16)	(61.75)
6	Indoxacarb 14.50 SC	0.50	92	94.2	100	96.5	95.67
			(73.57)	(76.06)	(90.00)	(79.22)	(79.41)
7	Spinosad 48SC	0.20	95.2	100	99	97.2	97.85
			(77.34)	(90.00)	(84.26)	(80.37)	(82.75)
8	Fenpropathrin 30 EC	0.30	85	91	88	92	89
			(67.21)	(72.54)	(69.73)	(73.78)	(70.63)

*Mean of 15 replications Note: Figures in parantheses are transformed angular values

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Baseline Susceptibility and Quantification of Resistance in *Plutella xylostella* (L.) to Cartap hydrochloride

ABSTRACT Bioassays were conducted on a *Plutella xylostella* population with Cartap hydrochloride up to 7 generations. Significant variations in the LC50 values from 1st (0.0178) to 7th generation (0.0148) were observed. The resistance ratio for 7th generation came down to 0.83 from 1st generation which indicated that the insect was susceptible to Cartap hydrochloride. Cross resistance to commonly used insecticides viz. monocrotophos, malathion, endosulfan, dichlorvos, cypermethrin and carbaryl was studied at various discriminating concentrations. Comparison of LC50 values revealed that cypermethrin (0.0267) was the most toxic of all the insecticides where as monocrotophos (1.354) was the least toxic to Cartap

hydrochloride-selected strain. The relative toxicity of cypermethrin, dichlorvos, carbaryl, malathion and endosulfan revealed that these insecticides were 50.71, 32.77, 5.56, 5.18 and 2.32 times more toxic than monocrotophos.

KEY WORDS *Plutella xylostella*, baseline susceptibility, cross resistance, Cartap hydrochloride

INTRODUCTION The diamondback moth, *Plutella xylostella* (L.) is an oligophagous pest of both wild and cultivated crucifers including oilseeds and vegetable crops (Talekar and Griggs, 1986). It is cosmopolitan in distribution (CIE,1967), particularly dominant in south-east Asia (Robert and Wright,1996). In India, it is one of the major constraints in the profitable cultivation of cole crops. The extensive use of a number of commercial insecticides has led to the development of resistance in this insect across south-east Asia (Georghiou,1990). In India, the preponderance of reported resistance in this insect has increased due to the pronounced cultivation of early cauliflower with intensive use of conventional insecticides in order to get higher value of the crop during off-season (Bhatia, 1988; Mehrotra, 1989). With the introduction of new eco-friendly and more potent insecticides in India, it has been suggested that use of these chemicals would counter the menace of resistance development in *P.xylostella* for the time being. Cartap hydrochloride has been found effective against *P.xylostella* (Nagesh and Verma, 1997). It is a derivative of nereistoxin, a naturally occurring substance isolated from the marine segmented annelids, *Lumbrineris heteropoda* and *L. breviccira* (Sakai, 1969). It kills insects by blocking ganglionic transmission through competitive binding to receptors of neurotransmitters in the central nervous system. Though commercial use of this insecticide for past many years across the world has resulted in the development of resistance in *P.xylostella* (Hama,1992), yet the information on this aspect is not at hand from our country where it was introduced in 1987 and an effective dose of 200g a.i./ha was recommended for the control of this pest (Peter et al. 1989). Therefore, the present study was formulated to generate baseline toxicity data and quantify the potential of development of resistance in *P.xylostella* to this chemical.

MATERIALS and METHODS

I*nsect Rearing*

The culture of *P.xylostella* was initiated by collecting about 200 larvae from farmer's cauliflower fields and brought to the laboratory for further multiplication at a regularly maintained temperature of 24±10C. The larvae were reared on cauliflower leaves till pupation. The adult moths were allowed to lay eggs in oven-dried glass jars having cauliflower leaves to serve as substrate for oviposition. They were provided with 10% honey solution fortified with multivitamins for feeding on a cotton swab. The neonates were provided with fresh cauliflower leaves to feed upon. At every successive instar, the larvae were shifted to clean jars containing fresh leaves. The whole stock was divided into two lots. One lot was named as parental stock and the other was used for exposure to Cartap hydrochloride.

Preparation of Insecticidal Concentration

The proprietary products of all the insecticides were used to prepare one percent stock solution in acetone from which further dilutions were prepared subsequently.

Bioassay and Laboratory selection

FAO method No.21 (Busvine, 1980) for topical application of insecticide to the larvae with slight modification was followed. Instead of directly treating the larvae without any substrate, the larvae released on cut discs of cauliflower leaves of the size of a Petri dish (2.5 cm diameter) were treated to simulate the application of insecticide in the field. To facilitate the movement of the larvae on both sides of the leaf disc, the leaves bearing slightly thick midribs and veins were selected for cutting the leaf discs. Before spraying, ten 3rd instar larvae were released on the upper side of the leaf disc as one replication. One ml each of the insecticidal concentrations was sprayed on each side of the leaf disc with Potter's towers at 51lb/inch2 pressure. All three replications were maintained for each concentration. After the treatment, the petri dishes were shifted to BOD incubator maintained at 24±10C with 60-70% relative humidity. Larval mortality was recorded after every 72 hours of the exposure by counting the larvae as dead when they did not resume activity after repeated prodding. The survivals from the experiments, affording around 85% mortality, were reared to the next generation. The progeny of the first surviving lot was termed the F1 generation and the exposure and selections were conducted up to 7 generations. The parental strain was also maintained through without exposure.

Statistical Analysis

Data on mortality was subjected to Abbott's formula for correction wherever required (Abbott, 1925). LC50 values of all the insecticides were determined by probit analysis (Finney, 1971).

Quantification of Insecticidal Resistance

The degree of development of resistance through different generations was determined by working out LC50 values in each generation and thus computing the

resistance ratio by dividing the LC50 value for that generation with LC50 value of the F1.

Cross Resistance with Other Insecticides

The studies on cross-resistance of Cartap hydrochloride-selected strain of *P. xylostella* to commonly used insecticides viz. monocrotophos, malathion, endosulfan, dichlorvos, cypermethrin, and carbaryl were also made as per the procedure described.

RESULTS and DISCUSSION The selection pressure of Cartap hydrochloride to *P. xylostella* was given in every successive generation at various discriminating concentrations up to 7 generations. The mortality data and the insecticidal concentrations used to obtain insect kill around 85% is presented in Table 1. The regression equations, LC50 values, slope and resistance ratios for different generations are presented in Table 2. The chi-square values show a heterogeneity in the population of the test insect in 1st and 4th generation which depicts its differential response to the insecticide. The trend of the LC50 values was decreasing from 2nd generation onwards with an exceptional and marginal variation in 4th generation. The resistance ratio (0.83) in the 7th generation as compared to the 1st generation remained low. Thus, the results indicated no resistance development in the insect. There are several reports indicating development of resistance to Cartap hydrochloride in *P. xylostella* from Japan (Horikiri, 1989; Ozawa et al., 1989; Hama et al., 1990); Taiwan (Cheng et al., 1992); China (Chen et al., 1995) and Korea (Cho and Lee, 1994), where it has been in use for many years against this pest. The results of the present studies are in conformity with those obtained by Branco and Gatehouse (1997) in laboratory bioassays in Brazil who also did not encounter any resistance in *P. xylostella* to this insecticide. Cartap hydrochloride, since its introduction, is being reported to control *P. xylostella* effectively (Peter et al., 1989; Rai et al., 1992; Nagesh and Verma, 1997). Joia and Udeaan (1997) observed this insecticide to be highly toxic to the multi-resistant *P. xylostella* population in Punjab with LC50 values ranging from 0.015 to 0.020 per cent.

There was no cross resistance in the Cartap hydrochloride-selected strain of *P. xylostella* to the six insecticides, i.e. monocrotophos, malathion, endosulfan, dichlorvos, cypermethrin and carbaryl tested in the present studies (Table 3). Rather, the LC50 values of monocrotophos, dichlorvos and carbaryl for the selected strains were marginally lower that those against the parental strain (1st generation). The available literature gives contradictory picture. In one study, no cross resistance to malathion and carbaryl was observed in the insect which had been selected for 12 generations to Cartap hydrochloride (Cheng, 1986) where as in another study, a slight cross resistance in *P. xylostella*, selected for 35 generations to the selection pressure of Cartap hydrochloride, was observed to dichlorvos and malathion and no cross-resistance to cypermethrin (Chen et al., 1993). The results obtained in the present studies, by and large, fall in line with those obtained in earlier studies. The variations may be explained by the facts that the exposure of the insect to Cartap hydrochloride had

Table 1. Selection of residual population of *Plutella xylostella* (L.) in various generations exposed to Cartap hydro chloride.

Generation	Concentrations of Cartap hydrochloride used	Mortality (%)	Residual population Rejected (X) Selected (√)
F1	0.01	54.4	X
	0.05	62.7	X
	0.1	85.6	√
F2	0.075	73.55	X
	0.09	78.1	X
	0.1	83	√
F3	0.075	74.3	X
	0.1	84.1	√
F4	0.05	63.86	X
	0.075	74.53	X
	0.1	83.36	√
F5	0.025	62.1	X
	0.05	71.15	X
	0.1	83	√
F6	0.1	80.9	X
	0.15	82.9	√
F7	0.1	78.22	X
	0.2`	81.38	√

Table 2. Toxicity of Cartap hydrochloride to third instar larvae of *Plutella xylostella* in different generations

Generation	Heterogeneity $\chi^2_{(n-2)}$	Regression equation	LC50 (%)	Fiducial limits	Resistance ratio	Slope± S.E
I	$\chi^2_{(5)}$=4.3584	Y=2.6095+1.0625X	0.01783	0.01276	1	1.0625±0.07
				0.02477		
II	$\chi^2_{(5)}$=1.7126	Y=2.7894+1.0037X	0.01594	0.01255	0.89	1.0037±0.05
				0.02023		
III	$\chi^2_{(4)}$=0.7119	Y=3.0561+0.8762X	0.01654	0.00578	0.92	0.8762±0.23
				0.04733		
IV	$\chi^2_{(4)}$=1.8746	Y=3.0067+0.8946X	0.0169	0.01302	0.94	0.8946±0.05
				0.02193		
V	$\chi^2_{(4)}$=2.8304	Y=2.7379+1.0905X	0.01186	0.01004	0.66	1.0905±0.03
				0.01402		
VI	$\chi^2_{(4)}$=0.3867	Y=3.0507+0.9143X	0.01355	0.00158	0.75	0.9143±0.07
				0.01914		
VII	$\chi^2_{(4)}$=0.7252	Y=3.2250+0.8176X	0.01482	0.01306	0.83	0.8176±0.02
				0.01683		

Table 3. Toxicity of different insecticides to Cartap hydrochloride selected strain of *Plutella xylostella* larvae.

Insecticides	Heterogeneity $\chi^2_{(n-2)}$	Regression equation	LC50 (%)	Fiducial limits	Relative toxicity	Slope± S.E
Monocrotophos	$\chi^2_{(7)}$=1.42483	Y=2.8101+0.4267x	1.35472	0.32441	1	0.4267±0.31
				5.65727		
Malathion	$\chi^2_{(7)}$=0.56775	Y=2.0940+0.6578x	0.26131	0.10513	5.18	0.6578±0.20
				0.64955		
Endosulfan	$\chi^2_{(7)}$=2.64639	Y=2.7930+0.4630x	0.58367	0.14084	2.32	0.4630±0.31
				2.41889		
Dichlorvos	$\chi^2_{(7)}$=0.12642	Y=2.0486+0.8161x	0.04134	0.01608	32.77	0.8161±0.21
				0.10632		
Cypermethrin	$\chi^2_{(7)}$=0.14848	Y=2.5539+0.7138x	0.02671	0.0127	50.71	0.7138±0.16
				0.0562		
Carbaryl	$\chi^2_{(7)}$=0.22262	Y=2.7932+0.5031x	0.24327	0.0682	5.56	0.5031±0.28
				0.86771		

been given for much greater number of generations in other studies. It could, thus, be concluded that inspite of the reports, chances of development of resistance in *P.xylostella* to Cartap hydrochloride were bleak in India so far.

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Susceptibility of Two Ixodid Ticks of Punjab, India to Some Acaricides - Generation of Base-Line Data

ABSTRACT Susceptibility of two common ixodid ticks, *Boophilus microplus* (Canestrini) and *Hyalomma anatolicum anatolicum* (Koch) to five acaricides was studied for establishing base-line data for monitoring acaricide resistance in these species. The populations of ticks were collected from Ludhiana and adjoining districts of Punjab. Bioassays, using FAO technique No. 7, were conducted on laboratory reared F1 generation larvae of ticks from different populations. Out of 23 populations of *B. microplus*, the population from Bacchuana (*Santa di Goushala*) in Mansa district was found to be the most susceptible to all the acaricides tested. The LC50 values against this population for diazinon, lindane, deltamethrin, cypermethrin and malathion were 0.00176, 0.00306, 0.00349, 0.00407 and 0.01975 %, respectively. Out of seven populations of *H. anatolicum anatolicum*, the one from Kahorian, district Sangrur was found to be the most susceptible. The LC50 values for the respective insecticides were 0.00285, 0.00396, 0.00504, 0.00570 and 0.02360 %. Against both the species, diazinon was found to be the most and malathion the least toxic. The LC50 values obtained for these most susceptible populations were designated as base-line values. The base-line data thus generated can be used to monitor the development of acaricide resistance in these species in future.

KEY WORDS Ticks, *Boophilus microplus*, *Hyalomma anatolicum anatolicum*, base-line, resistance, acaricides, LC50

INTRODUCTION Ticks are the most important ectoparasites of livestock in tropical and subtropical areas and are responsible for substantial economic losses. Ticks not only cause direct effect on animals by sucking blood, they are also responsible for the transmission of several dreaded protozoan, rickettsial and viral diseases (Soulsby, 1982). Tick borne diseases account for an estimated 90% of all reported vector - born disease in the USA (Oliver, 1996). The most abundant and important tick pests of cattle and buffaloes in India are, *Boophilus microplus* and *Hyalomma anatolicum anatolicum*. The indigenous cattle are generally resistant to ticks but exotic and crossbred stock suffers heavy losses due to tick infestation. To maintain the health and productivity of the animals and to avoid economic losses, appropriate control measures must be taken for these ectoparasites. The non-acaricidal methods like host resistance to ticks, pasture spelling, immunization, hand picking, brushing etc. are generally uneconomical and impractical for many in a country like India. Therefore, use of acaricides is the only option available with the farmers, which they are practicing. However, continuous use of acaricides exerts selection pressure on the ticks, which may result in the development of resistance in the tick populations. In order to manage acaricide resistance, regular monitoring of the populations for their susceptibility to various acaricides should be carried out at regular intervals. For comparison of susceptibility through time and space, the base-line data for various acaricides must be generated and should be easily available to scientists for comparative studies. However, there seems to be no work done on these lines in India. The present investigations were, therefore, undertaken to generate base-line susceptibility data for diazinon, lindane, deltamethrin, cypermethrin and malathion, so that the monitoring for the development of resistance to these chemicals in both the species of ticks is possible in future. The study is the first of its kind in this part of India.

MATERIAL and METHODS

Test tick populations and location of work

The work was carried out in the laboratory of Veterinary Parasitology, Punjab Agricultural University, Ludhiana on populations of ticks collected from Ludhiana and adjoining areas on both the species. About 30 engorged female ticks of *B. microplus* or *H. anatolicum anatolicum* were collected from each selected dairy farm / *goushala*. A maximum of five ticks were collected from any one animal as per procedure suggested by Beugnet and Chardonnet (1995). Male ticks were collected for identification. Twenty-three populations of *Boophilus microplus* and seven of *Hyalomma anatolicum anatolicum* were collected from different dairy farms/ *goushalas* randomly selected (Annexure 1). While collecting ticks, history of acaricides used at that farm was recorded.

Annexure 1: Locations of dairy farms/ dairy complexes/ *goushalas* in Punjab from where tick populations were collected

a. *B. microplus*

I. **Ludhiana**
1. Cattle and Buffalo dairy farm, PAU, Ludhiana.
2. Go-raksha Samiti, Chaura Bazaar, Ludhiana.
3. Gurdayal Singh's dairy farm, Thrike, Ludhiana
4. Haibowal dairy complex, (No 14, South), Ludhiana
5. Haibowal dairy complex, (No 26, North), Ludhiana
6. Dandi Swami Dharm Goushala, Haibowal Kalan, Ludhiana
7. Singh's dairy farm, Malakpur bet, Ludhiana
8. Aulakh dairy farm, Humbran, Ludhiana
9. Cattle dairy farm, Mattewara, Ludhiana
10. Krishan Gopal Goushala, Mullanpur, Ludhiana
11. Sri Krishna Goushala, Jagraon, Ludhiana
12. Gowardhan Goushala, Khanna, Ludhiana
13. Exotic Cattle Farm and Sperm Station, Khanna, Ludhiana
14. Sri Krishna Goushala, Gobindgarh, Ludhiana

II. Moga
15. Nestle Dairy Farm, Moga
16. Truck Union Goushala, Moga

III. Patiala
17. Patiala Goushala, Kapra Market, Patiala
18. Kali Mandir Goushala, Bus stand, Patiala
19. Cattle Breeding Farm, Nabha, Patiala

IV. Sangrur
20 Malerkotla Goushala, Malerkotla, Sangrur
21 Go-rasha Mandal Samiti, Goushala Road, Sangrur

V. Mansa
22 Santa di goushala, Bacchuana, Mansa
23 Sri Panchayati Goushala, Budhladaq, Mansa

b. *H. anatolicum anatolicum*

I. Ludhiana
1. Haibowal Dairy Complex (No. 3, North. Haibowal, Ludhiana)
2. Churpur Dairy farm, Churpur, Ludhiana
3. Dairy farm, Vill. Payal, Doraha, Ludhiana.
4. Sanatan Dharam Goushala, Jagraon, Ludhiana

II. Moga
5. Dairy Farm, Vill. Bhangarian, Talwandi, Moga

III. Sangrur
6. Kalayan Goushala, Sunam, Sangrur
7. Dairy farm Vill. Kahorian, Sangrur

Bioassay

Engorged female ticks were kept in sterilized test-tubes for egg laying and hatching at 80-85% humidity and 27±1 o C temperature using 10% KOH solution at the base of the desiccator in the BOD incubator. Test for the susceptibility to different acaricides were carried out on one to three week old larvae using acaricide impregnated filter paper packet (FAO, 1984).

Acaricides (insecticides)

The following technical grade acaricides were used in the present investigation:

1. Cypermethrin (95.1%)
2. Deltamethrin (98.5%)
3. Diazinon (97.0%)
4. Lindane (99.5%)
5. Malathion (96.4%)

Filter paper (Whatman's No. 541) were cut into 85x75 mm size and impregnated with different concentrations

of test acaricides as per details described earlier (Kumar, 1999). The test concentrations of acaricides ranging from 0.00061 to 5% were prepared in olive oil: chloroform (1:2). The larvae (approximately 100) were exposed to acaricides impregnated test papers for 24 hours at 27±1 o C temperature and 80-85% humidity. The mortality was assessed after 24 hours exposure period and data were analyzed to calculate LC50 and other parameters using a computer program based on probit analysis (Finney, 1971). The LC 50 value of the most susceptible population was considered as base-line value.

RESULTS and DISCUSSION After computation the LC50 values of the test populations for both the species were compared and the LC 50 value of the most susceptible population was considered as base line value (Table 1 and2).

Susceptible strain of B. microplus and base-line data

Among 23 locations, at one place no acaricide had ever been used for the control of ticks since the *goushala* was established about 20 years back and only hand picking of ticks was common routine practice. This *goushala*, named *Santa di goushala*, was situated in village Bacchuana, district Mansa. The population of *B. microplus* collected from this *goushala* was found to

Table 1. LC50 values and log-dose probability regression (LD-PR) of different acaricides for larvae of *B. microplus* population from Bacchuana, Mansa

Acaricides	LC_{50} values (%)	Fiducial limits (95%)		Relative toxicity Index	Slope ±SE	χ^2 values	Degree of Freedom
		Lower	Upper				
Diazinon	0.00176	0.00162	0.00192	11.22	2.72±0.14	17.62	18
Lindane	0.00306	0.0028	0.00333	6.45	3.31±0.16	17.06	14
Deltamethrin	0.00349	0.0032	0.00381	5.65	2.75±0.12	16.79	16
Cypermethrin	0.00407	0.00372	0.00444	4.85	2.34±0.10	13.35	20
Malathion	0.01975	0.01773	0.02201	1	1.98±0.08	26.93	22

Table 2. LC50 values and log-dose probability regression (LD-PR) of different acaricides for larvae of *H. anatolicum anatolicum* population from Kahorian, district Sangrur

Acaricides	LC_{50} values (%)	Fiducial limits (95%)		Relative toxicity index	Slope ±SE	χ^2 values	Degree of Freedom
		Lower	Upper				
Diazinon	0.00285	0.00262	0.0031	8.28	2.43±0.11	18.38	20
Lindane	0.00396	0.00352	0.00446	5.96	2.41±0.11	34.23	20
Cypermethrin	0.00504	0.00456	0.00557	4.68	2.88±0.13	23.89	16
Deltamethrin	0.0057	0.00489	0.00663	4.14	1.96±0.08	46.72	20
Malathion	0.0236	0.02157	0.02583	1	2.59±0.11	24.46	22

be highly susceptible to all acaricides tested there. No acaricide had ever been used at this farm and hand picking of ticks and brushing the animals was the practice to get rid of the pests. Non exposure of ticks to any acaricide may be the reason for high susceptibility. The LC50 values (% concentration) relative toxicity index along with other associated data is presented in Table 1.

Diazinon was found to be the most and malathion the least toxic acaricide against *B. microplus* (Table 1). Diazinon was 11.22 times more toxic than malathion. The patterns of results were similar to that of Guyanese strain of Caribbean countries where diazinon was found to be more toxic as compared to several other organophosphorus insecticides and lindane (Rawlins and Mansingh, 1978a).

The LC50 values of diazinon, lindane, deltamethrin, cypermethrin and malathion were 0.00176, 0.00306, 0.00349, 0.00407 and 0.01975 per cent, respectively against this susceptible population. The LC50 value in susceptible population was more for diazinon i.e. 0.01429 per cent in Guyanese strain (Rawlins and Mansingh, 1978b), 0.012 in Yerrongpilly strain from Australia (Schnitzerling et al., 1974) and 0.01199 in St. Catherine strain of Jamaica (Rawlins and Mansingh, 1978a), than the present value with packet test method but was less than Z-strain of Queensland i.e. 0.00085% when immersion method was used (Shaw, 1965). The difference may be due to differential susceptibility of strains and method variations.

The LC50 values of lindane against this population i.e. 0.00306% was less than some other strains i.e. 0.05616% in St. Catherine strain of Jamaica and 0.01923 in Guyanese strain of Caribbean country by larval packet test (Rawlins and Mansingh 1978a, 1978b) and was more than 0.000070 in Z-strain of Queensland (Shaw, 1965), 0.00059-0.00080 in village strain of Kumaon district of Uttar Pradesh, India (Chaudhuri and Naithani, 1964) and 0.00011 percent in Z strain of Queensland (Shaw et al., 1968) when immersion method was used. This difference may be due to the strain specificity and method variations.

Results of base-line data of deltamethrin were in accordance with findings of Beugnet and Chardonnet (1995) and Nolan et al (1989). The LC50 value of deltamethrin in susceptible population of *B. microplus* in Yerrongpilly strain of Queensland was 0.0044 (Nolan et al ,1989, Beugnet and Chardonnet 1995). In the present study the LC50 value of deltamethrin in susceptible population was 0.00349%.

The LC50 value of cypermethrin was less than the findings of Nolan et al. (1989). They observed LC50 value of 0.037 for cypermethrin in Yerrongpilly strain of Queensland as compared to 0.00407 in the present investigation. The difference may be due to strain specificity and genetic tolerance.

The results showed higher susceptibility to malathion in *B. microplus* (Z strain, the susceptible strain) of Queensland (Shaw, 1965) than the present value (Table 1). He observed LC50 value of 0.045 in malathion (immersion technique), whereas LC50 values of malathion were found to be 0.01975 in the present study. This difference may be due to strain specific difference and variation in the method.

Susceptible strain of H. anatolicum anatolicum and base line data

The most susceptible population of *H. anatolicum anatolicum* was found to be from a dairy farm in village Kahorian, district Sangrur, where no acaricide were ever used and hand picking of the ticks was the practice. This population was susceptible to all acaricides used. The LC50 values, relative toxicity index with associated data are presented in Table 2. Again non chemical method of tick control may be the reason for high susceptibility of ticks to all acaricides tested. It was noticed that the *H. anatolicum anatolicum* population was most susceptible to diazinon and least susceptible to malathion as observed for *B. microplus* (Table 1). Literature regarding toxicity of different acaricides to *H. anatolicum anatolicum* was not available for comparison.

The LC50 values obtained during the present investigations can be taken as base-line values for monitoring development of acaricide resistance in these tick species. Keeping in view the economic importance of these ticks in dairy farming, data on the susceptibility of different populations to commonly used acaricides must be regularly generated. Substantial changes in susceptibility must be viewed seriously for timely management of acaricide resistance development.

ACKNOWLEDGEMENTS The authors are thankful to Head, Department of Veterinary Parasitology for providing laboratory facilities and Head, Department of Entomology for computer facilities. The financial assistance in the form of Junior Research Fellowship provided by Indian Council of Agricultural Research to the first author is gratefully acknowledged.

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Status of Insecticide Resistance in *Heliothis species* - An Update

INTRODUCTION There are three *Heliothis* species: *Helicoverpa (Heliothis) armigera* (Hubner), feeding on almost all host plants; *Heliothis peltigera*, mainly on safflower and the weed *Acanthospermum hispidum*; and *Heliothis assulta*, on the weed *Datura metal* (Jadhav et al., 1985). The gram caterpillar or American bollworm *Helicoverpa armigera* is a polyphagous and multigeneration noctuid pest. *H. armigera* has a wide host range of over 200 weed plants, 25 crop plants including cotton, maize, sorghum, sunflower, tomato, okra and legumes like pigeon pea, chickpea etc. This wide host range allows the pest to breed throughout the year causing extensive damage (Regupathy et al., 1997a). *H. peltigera* and *H. assulta* are seldom exposed to pesticides; the reason being one feeds on marginal crop on which insecticides are not used and another on weed host. Hence the most of studies on resistance is confined to *H. armigera*.

BIOEFFICACY OF INSECTICIDES Plant protection in India began with the introduction of DDT in 1947, followed by HCH in 1949, organophosphates and carbamates in 1953 and synthetic pyrethroids in 1972. Among the crops, cotton receives 55% of pesticide consumed and the maximum is targeted to control bollworms in general and *H. armigera* in particular. An

array of insecticides had been evaluated against bollworm complex including *H. armigera* such as fenvalerate (Sorathia and Chari, 1981; Mallik et al., 1985; Agnihotri et al., 1986 and Daly et al., 1988), cypermethrin (Deshpande et al., 1988 and Devaiah, 1990), permethrin (Deshpande et al., 1988 and Agarwal et al., 1983) deltamethrin (Sarag and Satpute, 1988; Devaiah, 1990 and Somasundaram and Regupathy, 1985), fluvalinate (Sarag and Satpute, 1988), fenpropathrin (Dhawan et al., 1991), fluvalinate (Brahmankar et al., 1990 and Devaiah, 1990), polytrin-C (Dhawan and Simwat, 1997 and Bhuvaneswari and Uthamasamy, 1994), RIL 006 (Lingappa et al., 2000), monocrotophos (Gaikwade and Deshpande, 1978 and Ali and Karim, 1990 and 1995), quinalphos (Gaikwade and Deshpande, 1978 ; Joginder Singh and Sidhu, 1985; Simwat and Dhawan, 1996 ; Sharma and Jaglan, 1997; Butter et al., 1995 and Clement, et al. 1990), triazophos (Brahmankar et al., 1990; Sharma and Jaglan, 1997 and Arora. et al., 1992), profenofos (Dhawan and Simwat, 2001 and Dhawan et al., 1991), fluvalinate (Somasundaram and Regupathy, 1985), endosulfan (Deshpande et al., 1988), methomyl (Manisegaran et al., 1991 and Mane et al., 2000), carbaryl (Gaikwade and Deshpande, 1978), analycarb and spinosad (Murray and Lloyd, 1997).

In India cotton is grown almost throughout the year; during May-October in Northern and central India and in two seasons *viz.*, winter (Aug-Feb) and summer cotton (Feb-July) in South India. Owing to the favourable environment and farming conditions like crop mosaic resulted in extensive and frequent use of pesticides (Regupathy et al., 1999). In view of continued predominance of *H. armigera* as a major pest in cotton pest complex, the farmers resorted to as high as 25-35 rounds of insecticide applications including tank mixtures.

HISTORY OF DEVELOPMENT OF INSECTICIDE RESISTANCE The pyrethoids which were considered most potent insecticides for its control lost their efficacy (Armes et al., 1992a and Ahmad et al., 1997). In India, the first case of control failure after spraying synthetic pyrethroids from suspected insecticide resistance in *H. armiger* (Hubner) was from Guntur in Andhra Pradesh (Reddy 1990). Pyrethroid resistance in this population was confirmed by Dhingra et al., (1988). However population from Hyderabad was not found resistant to pyrethroids during the same period (Mc Caffery et al., 1989) and was comparatively less resistant to pyrethroids than Guntur population (Venkataiah et al., 1990). Differential susceptibility of population in Andhra Pradesh and the severity of pyrethroid resistance were reported (Armes et al., 1992a,b and Armes et al., 1994). Kurnool population was more resistant to pyrethroids when compared to Srikakulam population (Reddy et al., 1991). Chittoor and Ranga Reddy populations were found to be the most and moderately susceptible to fenvalerate respectively when compared to least susceptible Guntur population (Reddy et al., 1993 and Nagesh et al., 1996). During 1997, many farmers in Andhra Pradesh could not achieve effective control of this pest, the reason being development of insecticide resistance (Jadhav and Armes, 1996).

Existence of resistance to pyrethroids, organophosphates, carbamate and cyclodienes was confirmed later in laboratory test (Dhingra et al., 1988; Mc Caffery et al., 1989; Phokela et al., 1989; Armes et al., 1992b, 1994 and 1995; Pasupathy et al., 1994; Pasupathy and Regupathy, 1994 ; Regupathy et al., 1994a, 1998a,b and 1999).

Sekhar, et al.,(1996) reported low degree of resistance to deltamethrin than to cypermethrin and fenvalerate when compared to susceptible Reading strain (UK). Synthetic pyrethroids exhibited high resistance factors when compared to organophosphates (Rosaiah et al., 1997).

Similar differential resistance reaction was reported from Gujarat (Patel and Koshiya, 1999), Uttar Pradesh (Tripathy and Singh, 1999), Haryana (Baruah and Chauhan, 1996) and Maharashtra (Kranthi et al., 1998, 2000).

MONITORING The nation-wide resistance monitoring programme since 1993 under NRI - ICRISAT - ICAR co-ordinated programme in different locations of India by the collaborating laboratories of Indian Council of Agricultural Research *viz.*, Central Institute for Cotton Research, (CICR) Nagpur, ICRISAT, Hyderabed, Tamil Nadu Agricultural University, (TNAU) Coimbatore, Punjab Agricultural University, (PAU) Ludiana and A.N.G.R.A.U, Lam farm, Guntur revealed that pyrethroid resistance is ubiquitous and stable at around 50 - 80 per cent in most areas. Organophosphate and endosulfan resistance is stable at around 20-50 per cent (Armes et al., 1994 and 1995, Regupathy et al., 1998a, b and 1999). Subsequently the programme was continued with the financial support from Common Fund for Commodities, Europe, International cotton advisory committee, USA , Natural Resources Institute,U.K, Indian Council of Agricultural Research involving

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(Regupathy et al., 2003) and the high level pyrethroid resistance was confirmed.

PATTERN OF INSECTICIDE RESISTANCE (NRI, 1998; Regupathy et al., 2003 and Kapoor et al., 2003)

South India

The level of resistance increased from 76.7 in 1995 to 90.7 percent in 2002 for fenvalerate and from 76.5 to 84.2 percent for cypermethrin 0.1µg. Pbo suppression of fenvalerate resistance was high. Endosulfan resistance frequency ranged between 30.7

and 57.3 percent. Resistance to quinalphos was 27.8 and 45.4. The level of resistance to chlorpyriphos was very low (26.4%).

Central India

Resistance frequencies to pyrethroids were high (71.6 - 98.8). This trend was observed throughout the season except in the month of October, when the frequencies were moderate. Pyrethroids were used mostly in October in cotton. Resistance to quinalphos and endosulfan was low till the end of February and increased to 50% by the end of April.

North India

The survival of *Helicoverpa armigera* larval population collected from cucurbits of non-cotton growing areas of Hoshiarpur when exposed to discriminating doses of fenvalerate, quinalphos, endosulfan, methomyl and cypermethrin was found to be 0, 10, 12.2, 13 and 22.2 percent respectively. The respective survivals of larvae from gram crop were found to be 30, 10, 10.5, 21.1 and 31.6 per cent. The per cent larval survival of pest population collected from different non-cotton crops of Ludhiana and Moga ranged from 47.1 to 83.3, 8.3 to 67.7, 12.5 to 47.5, 17.6 to 62.9 and 41.2 to 72.2, respectively.

The pest population from different cotton growing areas of Punjab showed (43.4 to 100 per cent) wide spread development of resistance to cypermethrin and Fenvalerate. The population collected from Bathinda area was found to be most resistant with 91.7 and 100 per cent survival at discriminating dose of cypermethrin and fenvalerate respectively. Population from Mansa, Khuban, Barnala and Ludhiana showed significant resistance with survival ranging from 43.4 to 89.5 per cent.

H. armigera populations showed varying level of resistance to quinalphos. The highest level of survival was observed in population from Bathinda (75%) followed by those from Barnala (55%) Mansa-I (54.2%), Mansa-II (64.7%), Ludhiana (44.8%) and Khuban (32.3%). The resistance to endosulfan was observed to be of the lower order at different location with per cent survival ranging from 7.7 to 58.3 per cent. Interestingly, the population collected from cotton growing area of Khuban was found to be almost as susceptible to endosulfan as that from non cotton growing area of Hoshiarpur. Though methomyl has never used for the control of insect in agriculture in Punjab, the resistance frequency was in the range of 14.2 to 60.0 per cent. The carbamates still show only incipient resistance (RF 2-30). Endosulfan resistance is still moderate at 20-50% but even these levels may result in control failures. Op resistance is likewise stable at the 20-50% level but seem to partly share the same major resistance mechanisms as Endosulfan.

The resistance factors based an LC50 indicated very high level of resistance to pyrethroids and underrate level to endosulfan and quinalphos (Kapoor et al., 2003).

RESISTANCE OF *H.armigera* TO ORGANOPHOSPHATES Until introduction of synthetic pyrethroids in 1982, compounds belonging to organophosphates and carbarmate groups were amongst the most widely used insecticides on cotton in India. In general, resistance to organophosphates across the country was low to medium.

In Andhra Pradesh, the population from Guntur exhibited appreciable resistance to quinalphos (i.e. >8 - fold) than monocrotophos over a period of four years (Kranthi et al., 2000). Guntur, Chittoor and Ranga reddy populations were most susceptible to monocrotophos when compared to other group of chemicals (Nagesh et al., 1996). Similarly Guntur populations exhibited low resistance factor (1.09-1.78) for organophosphates than synthetic pyrethroids (10.82-18.20) (Rosaiah et al., 1997).

In Tamil Nadu, quinalphos resistance was less than that of synthetic pyrethroids averaging 30.0 to 75.0 per cent (Regupathy et al., 1998b). Similarly profenofos resistance was low (20-45 per cent) (Tamil Selvi, 2001).

In Punjab and Haryana region, high resistance factor of 39 to 65 fold was recorded for monocrotophos from Sirsa, Dabwali and Bhatinda populations (Kranthi et al., 2000). Gujarat populations showed greater resistance to triazophos than to endosulfan, carbaryl, monocrotophos and quinalphos (Patel et al., 1992).

RESISTANCE OF *H. armigera* TO CHLORINATED HYDROCARBONS In 1986, *H. armigera* populations from Hyderabad was found to be resistant to DDT but not to pyrethroids or endosulfan. The DDT resistance increased in 1987 (Mc Caffery et al., 1989). Resistance to endosulfan was less when compared to monocrotophos, carbaryl, and fenvalerate (Nagesh et al., 1996) in three different populations of Andhra Pradesh.

Haryana population was more susceptible to endosulfan when compared to organophospates and synthetic pyrethroids (Baruah and Chauhan, 1996). In Tamil Nadu the survival of *H. armigera* for endosulfan (41-60 per cent) was less, indicating lower but still significant resistance when compared to pyrethroids (Regupathy et al., 1998a). The level was slightly higher in southern zone than western and cauvery delta zone.

The nation-wide monitoring of insecticide in *H.armigera* by the collaborating laboratories of Indian Council of Agricultural Research *viz.*, Central Institute for Cotton Research, Nagpur, ICRISAT, Hyderabed, Tamil Nadu Agricultural University, Coimbatore and Madurai, Punjab Agricultural University, Ludiana and A.N.G.R.A.U, Lam farm, Guntur revealed that

pyrethroid resistance is ubiquitous and stable at around 50 - 80 per cent in most areas. Organophosphate and endosulfan resistance is stable at around 20-50 per cent (NRI,1998)

MAGNITUDE OF RESISTANCE The resistance to cypermethrin was the highest in *H. armigera*. It varied from 115 fold in Warora to 34700 fold in Bhatinda. The similar trend has been observed for fenvalerate also (64 fold in warora to 32480 fold in Bhatinda). The resistance to endosulfan (2-25 folds) and quinalphos (3-29 folds) remained very low. The RR was highest in Bhatinda and lowest in Warora for the above insecticides except quinalphos.

LAB MEASURED RESISTANCE AND FIELD CONTROL One of the major themes of recent research on resistance monitoring has been to relate the results of laboratory bioassays to mortality in the field. To compare and obtain relationship between lab measured resistance and field control extensive field trial has been conducted with cypermethrin, fenvalerate, endosulfan, quinalphos and profenofos (Niranjan kumar, 2002) and thiodicarb, spinosad and indoxacarb (Ramasubramanian, 2003). Though the control was more in the field population at recommended dose when compared to monitoring studies done in the lab, the studies confirmed high level of resistance to the synthetic pyrethroids and moderate level to endosulfan, quinalphos and thiodicarb, low level to indoxacarb and susceptible to spinosad.

MECHANISM OF RESISTANCE

MFO Induction

MFO mediated pyrethroid resistance was shown to be specific for pyrethroids having a phenoxy benzyl or similar functional group (Scott and Georghiou, 1986) and involvement of cytochrome P450, a monooxygenase in insecticide resistance of *Helicoverpa* spp. had been suggested by Brown (1990). The trans and cis methyl positions of acid moiety and 4-phenyl position are the major sites susceptible to oxidative metabolism. Hydroxylation at fifth position may also occur due to presence of alpha-cyano moiety (Matsumara, 1985). MFO is involved in the metabolic conversion of pyrethroids.

Enhanced monooxygenase activity as a mechanism of resistance to pyrethroids in *H. armigera* had been reported from India (Phokela and Mehrothra, 1989, Kranthi et al.,1997 and Tamil Selvi,2001).

Monooxygenase was involved in *H. armigera* resistant to cypermethrin and fenvalerate (Kranthi et al., 1997; Regupathy et al., 1997b and Martin et al., 2000). Rajasekhar et al (1994) revealed that suppression of cypermethrin resistance by PBO was either very low or non-existent. Metabolic resistance mediated by mixed function oxidases was less significant in *H. armigera* populations of Andhra Pradesh.

In field fenvalerate (75 g.a.i./ha) and cypermethrin (50 g.a.i./ha) at recommended dose effected only 15.0 and 17.2 per cent reduction of *H. armigera* larval population 7 DAT. Co-application of pungamia oil (PO) improved the efficacy and reduced the pest population to 54.7 and 59.0 respectively while piperonyl butoxide (Pbo) reduced 37.5 and 36.3 per cent respectively (Sumathi,2002; Sumathi and Regupathy 2003) confirming the predominant MFO mechanism.

CE Induction

In insects carboxyl esterases (CE) are important in insecticide resistance and metabolic degradation of xenobiotics. Pasupathy et al. (1994) reported high carboxyl esterase activity in fenvalerate treated *H. armigera* larvae than those with fenvalerate plus quinalphos. Pyrethroid resistant *H. armigera* recorded higher amount of CE activity (1-9 fold) than susceptible populations (Manikandan and Ravisankar, 1999).

Induction of carboxyl esterase (CE) activity was observed in pyrethroid treated individuals of *H. armigera* populations (Pasupathy et al., 1994; Hua et al., 1996; Kranthi et al., 1997 and Clement, 1999; Tamilselvi 2001; Praisy Leithal, 2001; Sumathi, 2002, and Ramasubramanian, 2003).

RATE OF RESISTANCE DEVELOPMENT AND DECLINE The rate of development was high due to selection pressure and the rate of decline is slow when the pressure was removed. Selection by pyrethroids increased the level of resistance by 5.24 fold for fenvalerate, 4.91 fold to cypermethrin, 6.77 fold to lambdacyhalothrin and 7.14 fold to betacyfluthrin. In unselected population, the increase in susceptibility was to the extent of 1.91, 3.49, 2.99 and 3.01 fold to fenvalerate, cypermethrin, deltamethrin, lambdacyhalothrin and betacyfluthrin respectively by the end of fourteenth generation (Ramasubramanian, 2003).

PATTERN OF CROSS RESISTANCE Population selected for cypermethrin and fenvalerate showed cross resistance to deltamethrin and lambda cyhalothrin and the population selected for quinalphos showed cross resistance to other OP compounds chlorpyriphos and methyl parathion (Clement,1999). Populations selected to any one pyrethroid *viz.*, fenvalerate, cypermethrin, deltamethrin and lambda cyhalothrin showed cross resistance to all other pyrethroids, no cross resistance to endosulfan, thiodicarb a nd spinosad and negative cross resistance to indoxacarb (Ramasubramaniam,2003)

CONCLUSION Among the three *Heliothis* sp. in India *viz.*, *H. armigera*, *H. peltigera* and *H. assulta* resistance to insecticides is observed in *H. armigera* only due to its feeding habit. The resistance to synthetic pyrethroids, B.cyfluthrin , cyhalothrin, cypermethrin , deltamethrin , lambda cyhalothrin is ubiquitous and very high, moderate to thiodicarb, endosulfan and quinalphos, low to indoxacarb, chlorpyriphos and profenofos. And no resistance detected to spinosad. Populations selected to one pyrethroid showed cross resistance to all other pyrethroids, no cross resistance to endosulfan ,thiodicarb and spinosad and negative cross resistance to indoxacarb. The major mechanism of resistance to synthetic pyrethroids is induction of MFO and to some extent to CE enzymes. The presence of nerve insensitivity can not be ruled out.

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Arthropod Resistance

Inheritance of Resistance by a Strain of Beet Armyworm to Fenvalerate, Methomyl, Methyl Parathion and Permethrin

ABSTRACT In generation two the strain from Tifton (T), Georgia, was resistant to fenvalerate, but it was susceptible to methyl parathion, permethrin and methomyl. An LD50 of 20 µg/larva was used as a resistance threshold. Reciprocal crosses of the T strain were made with DOW-Zeneca reference (S) strain. S was susceptible to fenvalerate, methomyl, methyl parathion and permethrin. LD50s of methomyl and fenvalerate for T x S cross and S strain were equal. LD50s of all four insecticides for S x T and T were equal. LD50s of fenvalerate and methomyl for S x T were significantly greater than those for T x S indicating sex-linkage for male of T strain.

INTRODUCTION Inheritance of resistance by the beet armyworm, *Spodoptera exigua* (Hubner), to fenvalerate has been shown [Brewer and Trumble 1991]. They suggested that inheritance of resistance was polygenic for fenvalerate when a strain from Baja California Norte, Mexico was crossed with a susceptible strain from California.

Larvae of the beet armyworm feed on cotton and vegetable crops in the United States of America (USA). Larvae are more difficult to control in the eastern USA than they are in the western USA [Wolfenbarger and Brewer 1993]. No information has been found on inheritance of resistance factors by a strain of this pest from eastern USA. A strain from Tifton, GA was resistant to fenvalerate, methyl parathion and permethrin [Wolfenbarger and Brewer 1993]. Methomyl was susceptible based on a resistance threshold of 20 µg/larva [Wolfenbarger and Wolfenbarger [2001].

The objective was to determine inheritance of resistance factors of T resistant strain to fenvalerate, methomyl, methyl parathion and permethrin when crossed with S strain. Inheritance was determined with LD50s of reciprocal crosses of the T with the S strain.

MATERIALS and METHODS Larvae of the T strain were obtained from cotton near Tifton, GA. The S strain was obtained as eggs from Syngenta, Richmond, CA which was obtained from DOW, Inc. [Wolfenbarger and Brewer 1993 and Wolfenbarger and Wolfenbarger 2001].

Larvae were maintained on 12 to 15 ml artificial diet (Shaver and Raulston 1974) in 30 ml plastic cups (Wolfenbarger and Wolfenbarger 2001). Pupae were held for adult emergence as brother-sister or sexed for use with reciprocal crosses. Ten to 20 pairs/strain or cross were used. All viable moths were used as described by Wolfenbarger and Wolfenbarger (2001). The female is listed first in all crosses. A 5% sucrose solution was used as food for moths during their 6 to 18 d lifetime.

Technical of insecticides (93% to 100% purity) were obtained from manufacturers. Each insecticide was diluted in acetone and one µl was applied to the dorsum of the thorax of larvae weighing 15 ± 6 mg. Microgram doses of fenvalerate, methomyl, methyl parathion and permethrin ranged from 0.195 to 200, 0.0975 to 100, 0.195 to 200 and 0.0015 to 25, respectively.

Mortalities of larvae were determined after 72 h. Only larvae with no movement following probing were counted as dead. LD50, slope ± standard error (SE) and 95% confidence interval were determined by probit analysis of SAS (1988). LD50 and confidence interval were µg/larva. Overlapping CI values indicate LD50s which are equal. Significant differences between

LD50s were indicated by non-overlapping confidence intervals. Where slope ± SE ratios were <1.96 the regression was not significantly different from zero. Non-significant regression is shown because it represents a response by that insecticide.

RESULTS In the second generation LD50s of T strain of permethrin, methyl parathion and methomyl were 773, 70 and 5 fold greater and significantly different from LD50s of S (Table 1). LD50s of T were not resistant based on resistance threshold. LD50s of S strain by pyrethroids were < 0.01 µg/larva, while the anticholinesterase inhibitors showed LD50s of < 0.5 µg/larva.

In this same generation only fenvalerate was resistant to beet armyworm. The strain showed a non-significant regression, a slope ± standard error of 0.41 ± 0.22 for 112 larvae and 38% mortality was determined at 200 µg/larva.

Of interest was the obvious sex linkage with the male of the T strain by all four of the insecticides. This was determined in the S x T cross. LD50s of methomyl and fenvalerate of S x T and T were significantly greater than shown by T x S and S. LD50 for methomyl, permethrin and methyl parathion of T strain and the S x T cross were equal. LD50s of fenvalerate and methomyl for T x S and S were equal. Results suggest that resistance factors for T strain are associated with the two X chromosomes and not the Y chromosome of the female.

Table 1. Toxicity of insecticides to Tifton (T) and reference (S) strains and reciprocal crosses of beet armyworm larvae in second generation. 1990.

Larvae Treated	Slope ± SE	LD50 (µg/Larva)	95% Confidence Interval
		T strain	
		methyl parathion	
153	0.83±0.16	18.25	9.18-67.4
		permethrin	
167	0.77±0.17	7.5	3.67-14.06
		methomyl	
194	0.52±0.13	2.23	0.61-5.02
		S strain	
		methomyl	
191	1.77±0.35	0.42	0.24-0.57
		methyl parathion	
261	0.47±0.13	0.26	0.12-1.26
		permethrin	
133	1.57±0.14	0.0097	0.0075-0.012
		fenvalerate	
239	0.82±0.16	0.0031	0.00065-0.0071
		T x S	
		methomyl	
262	0.79±0.19	0.14	0.016-0.34
		fenvalerate	
276	0.33±0.1	0.058	0.0037-0.2
		S x T	
		methyl parathion	
241	0.47±0.09	11.2	4.68-45.23
		fenvalerate	
141	1.59±0.25	7.78	5.4-11.22
		methomyl	
270	1.33±0.15	6.48	4.74-8.73
		permethrin	
68	2.7±0.85	6.48	2.12-9.67

Slopes of regression of all insecticides for T strain were flat (<1). Slopes of regression of all insecticides of S strain were flat [50%] and intermediate (1-2). Slope of T x S was flat. Twenty-five percent (methyl parathion) of those for S x T were flat, 50% (fenvalerate and methomyl) were intermediate and 25% (permethrin) were steep [>2]. SE of slopes was not large; they ranged from 19% to 31% of the slope. An appropriate number of larvae (133 to 270) were used to treat strains and crosses.

Based on resistance threshold T strain reversion to susceptibility was shown for methyl parathion and permethrin in the first two generations, while methomyl was susceptible both generations. T strain was resistant to fenvalerate both generations.

ACKNOLEDGEMENTS Thanks are extended to G. A. Herzog, Georgia Agricultural Experiment Station, Tifton, GA for collecting and sending the larvae. Thanks are extended to A. Rhemke, Syngenta, Richmond, CA for the DOW-Zeneca reference strain. Thanks are extended to D. J. Wolfenbarger for the preparation and review of this manuscript.

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Effectiveness of *Helicoverpa armigera* Nuclear Polyhedrosis Virus Against Insecticide Resistant Strains of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae)

ABSTRACT Larvae of cotton bollworm, *Helicoverpa armigera* (Hubner) resistant and susceptible to Fenvalerate, Endosulfan, Monocrotophos, Chlorpyriphos, Quinalphos and Cypermethrin were tested for susceptibility to a nuclear polyhedrosis virus. The LC50 values were less in resistant strains than in susceptible. The resistance ratio to *Ha*NPV was highest in fenvalerate resistant strain (0.90) followed by cypermethrin resistant (0.72) strain.

Increased use of chemical pesticides has lead to several serious concerns in the management of cotton bollworm. This has resulted in development of alternative methods for pest control. The microbial insecticides are now used to control the insect pests and also to lessen pesticide residues in crops. Since use of pesticides cannot be dispensed with for suppression of cotton bollworm, it is imperative to know whether the insecticide resistant population of the insect pest is more, less or equally susceptible to an entomopathogen than in a non-insecticide selected population. Since the modes of action of entomopathogens are different from those of chemical insecticides, one would not expect physiological cross resistance between a chemical and a entomopathogen. Hence, the present investigation has been taken up to manage the insecticide resistance by employing entomopathogen, *Ha*NPV.

MATERIALS and METHODS

Maintenance of insect strain

About 300 larvae of *H.armigera* collected from cotton fields from Raichur during October 2002 were reared in glass vials on artificial diet individually following the procedure of NRI manual (1995). The susceptible strain obtained from unsprayed fields of upland cotton of Devdurga was reared in the laboratory following the similar procedure and used for comparison, with the field strain.

The population were selected for resistance to fenvalerate, endosulfan, monocrotophos, chlorpyriphos, quinalphos and cypermethrin by giving sub-lethal doses for five generations by applying 1µl of sub-lethal dose of each insecticide on the thorasic dorsum of 3rd instar larvae. In 6th generation they were bioassayed for NPV.

Entomopathogen bioassay

A diet surface treatment bioassay was used to determine the susceptibility of insecticide resistant and susceptible strains of *H.armigera* to nuclear polyhydrosis virus obtained from Bio-control unit, UAS, Dharwad. Fifty microlitres of *Ha*NPV was spread evenly on the surface of the artificial diet. After 15 minutes of air-drying, one third instar larva was released into each vial for microbial ingestion. Sixty larvae were treated with each concentration (treatment) in three replications of twenty each. Larval mortality was recorded from third day after treatment till pupation or death. LC50 values were determined using MLP software package for the pathogen in resistant and susceptible strains.

RESULTS and DISCUSSION The virus proved to be the most effective in mitigating the resistance to insecticide in *H.armigera*. It is surprising to note that the LC50 was less in fenvalerate resistant (0.90 times), cypermethrin resistant (0.72), endosulfan resistant (0.60), chlorpyriphos resistant (0.41), quinalphos resistant (0.39) and monocrotophos (0.35) strains than susceptible strain (Table 1). Though, there are several reports on the utility of NPV in the *H.armigera* management,only in 1989 NPV was demonstrated for *H.armigera* control in many crop in India (Jayaraj et

Table1.Pathogenicity of HaNPV in resistant and susceptible strains

H.armigera strain	LC_{50} (POB/ml)	Resistance Ratio	Fiducial limits (POB/ml)
I.Susceptible	$1.53x\ 10^7$	-	$6.80x10^6$-$4.52x10^7$
II.Resistant			
a.Fenvalerate	$1.39x10^7$	0.9	$5.92x10^6$-$4.10x10^7$
b.Endosulfan	$9.26x10^6$	0.6	$5.12x10^6$-$2.88x10^7$
c. Monocrotophos	$5.32\ x10^6$	0.35	$7.23x10^6$-$2.55x10^7$
d. Chlorpyriphos	$6.32\ x10^6$	0.41	$3.62x10^6$-$3.12x10^7$
e.Quinalphos	$5.94\ x10^6$	0.39	$4.80\ x10^6$-$3.92x10^7$
f.Cypermethrin	$1.10\ x10^7$	0.72	$7.20\ x10^6$-$3.45x10^7$

Resistance Ratio = LD50 of resistant strain/LD50 of susceptible strain

al.,1989). The present findings slightly differ from the work of Ignoffo and Roush (1986) who stated that insecticide resistant strains were as sensitive to the NPV as insecticide susceptible strains of *H.virescens*. The above findings indicate varied effectiveness of organisms in differentially responsive strain to pesticide action. The response to the action of entomopathogen in the test insect was altered by differetial level of pesticide resistance existing in the populations.

The practical utility of the present finding is in conformity in respect of NPV with the results of Listov and Nesterov (1976), Rud and Bellonik (1984), Rabindra and Jayaraj (1985) and Basavanagoud and Lingappa (2000) who reported that entomopathogens can break insecticide resistance when properly introduced into the population.

Based on the relative efficacy of *Ha*NPV in resistant and susceptible strains, it may be concluded that NPV, apparently becomes an obvious choice and hence its usage can become viable proposition in mitigating the pesticide resistant problem in *H.armigera*.

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Effect of Nuclear Polyhedrosis Virus Infection on the Insecticide Susceptibility of *Heliothis armigera* Larvae

Laboratory bioassay studies revealed that nuclear polyhedrosis virus infection in late stage larvae of *Heliothis armigera* increased their susceptibility to insecticides. The virus infection increased the susceptibility of final instar larvae of *H.armigera* to fenvalerate, cypermethrin, Endosulfan, quinalphos, chlorpyriphos and monocrotophos.

The notorious polyphagous pests *Heliothis armigera* (Hbn.) is known to have developed resistance to the commonly used insecticides (Mehrotra, 1989), with the result, it is becoming increasingly difficult to manage this pest. Fortunately, the insect is highly susceptible to its nuclear polyhydrosis viruses (Rabindra and Jayaraj, 1986; Santharam, 1985) and can be successfully controlled if the application coincides with the occurrence of early stages of the larvae. It is well known that late stage larvae are more tolerant to the virus (Rabindra and Subramaniam, 1974; Santharam, 1985), but the present investigations have shown that sublethaly infected late instar larvae are more susceptible to insecticides than their healthy counterparts.

MATERIALS and METHODS Disease-free colonies of *H.armigera* were maintained in the laboratory on a semi synthetic diet (Shorey and Hale, 1969). The susceptibility of healthy and NPV infected final instar larvae of *H.armigera* to fenvalerate ,quinalphos, Endosulfan, cypermethrin, monocrotophos and chlorpyriphos was studied by bioassay. Fifth instar larvae of uniform age and size were inoculated by oral feeding of one µl of NPV suspension to give a dose of

10^5 polyhedral occlsion bodies (POB)/larva. Half of the larvae of the same batch was maintained without virus inoculation. On the third day, both NPV-inoculated and healthy larvae reached the final instar and were bioassayed for insecticide susceptibility. The insecticides diluted to appropriate concentration with acetone were applied on the dorsal side of the larvae in one µl aliquots using a microsyringe and mortality was recorded after 24 hours of treatment and LC50 values are determined using MLP software package.

RESULTS and DISCUSSION In all the bioassays, it was observed that the NPV-inoculated larvae of *H.armigera* is more susceptible to the insecticides than their un-inoculated counterparts. The LC50 values were much lower in NPV-inoculated than in uninoculated larvae (Table1).

Comparison of the susceptibility ratios showed that the NPV-induced susceptibility in *H.armigera* was maximum in Endosulfan (2.45) followed by monocrotophos (2.36) and minimum in cypermethrin (1.52).

Table1: Effect of NPV infection on the susceptibility of final instar larvae of *Heliothis armigera* to some insecticide

Insecticides	NPV/control	Chi-square value (χ^2)	Slope (b)	LD_{50} (μg/larva)	Fiducial limits	Susceptibility ratio
Fenvalerate	NPV	1.58	2.05	5.375	3.9843 -7.1711	1.62
(final instar)	Control	8.14	0.39	8.728	7.1038 -10.7135	
Quinalphos	NPV	1.75	1.86	1.57	0.8566 -2.2309	1.86
(final instar)	Control	10.35	0.43	2.922	2.5382 -3.3196	
Endosulfan	NPV	3.67	2.19	5.35	4.0148 -7.0482	2.45
(final instar)	Control	12.38	0.58	13.156	11.1393-15.4543	
Cypermethrin	NPV	0.92	2.12	6.006	4.5180 -7.9798	1.52
(final instar)	Control	9.37	0.42	9.132	7.2087 -11.0628	
Monocrotophos	NPV	0.91	0.95	4.676	3.9971-5.2890	2.36
(final instar)	Control	0.9	2.67	11.07	8.5711 -5.04441	
Chlorpyriphos	NPV	6.93	0.46	2.233	1.7034 -2.8402	2
(final instar)	Control	5.86	2.37	4.458	3.3749 –5.7310	

The influence of subacute infection of polyhedrosis virus on the insecticide susceptibility has been reported in the cabbage looper *Trichoplusia ni* also by Girardeau and Mitchell (1968) who postulated that devitalization of the host by a disease may so alter its physiology, that stresses or toxins relatively minor to a healthy vigorous insect may have severe effects and even cause death in a diseased insect. Justin et al. (1989) have reported that *Bacillus thuringiensis* (BactospeineR) also increased the susceptibility of *H.armigera* and *S.litura* larvae to insecticide. Increased insecticidal susceptibility due to protozoan infection has been reported in coleopteran insects like the American boll weevil, *Anthonomus grandis* (Bell and McLaughlin,1970) and the flour beetles, *Tribolium confusum*, *T.destructor* (Listov and Nesterov,1976) and *T.castaneum* (Rabindra et al.,1988).

Oxidative metabolism of cyclodien compounds occur in fat body as reported in larvae of *Heliothis zea* and *Spodoptera eridania* (Krieger and Wilkinson,1969). Mixed function oxidases responsible for breakdown of monocrotophos was detected in fat body of *Spodoptera littoralis* (Dittrich et al.,1980) and *Heliothis virescens* (Bull and Whitten,1972). Esterases responsible for breakdown of synthetic pyrethroids have been found in the hypodermis and fat body in *S.littoralis* (Abdel-Aal and Soderbund,1980). It is known that NPV infects the vital organs like the fat body, blood cells and hypodermis apart from other organs. It may be postulated that infection of fat body, blood cells and hypodermis by the virus should have played a major role in the increased susceptibility of the larvae to the different insecticides.

The present findings are of immense importance in the context of insecticide resistance reported in *H.armigera*. Our findings, as well as other reports cited indicate that by properly integrating the use of NPV in the pest management programmes, *H.armigera* may be successfully controlled with optimum doses of chemical insecticides. The viruses may be applied alternatively or used in combination with chemical insecticide resistance management, as microbial infection can break insecticide-resistace in host insects (Listov and Nesterov,1976).

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Management of Spider Mite, *Tetranychus macfarleni* Baker and Pritchard on Pumpkin, *Cucurbita moschata* Dutch

ABSTRACT Pumpkin, *Cucurbita moschata* Dutch, is an important cucurbitaceas vegetable crop and is rich in energy and carbohydrates. During the months of summer spider mite, *Tetranychus macfarlanei* Baker and Pritchard is a potential pest of pumpkin, *Cucurbita moschata* Dutch, crop in eastern Uttar Pradesh, India. The indiscriminate use of pesticides results to outbreak of this mite pest due to kill the non target predatory mite. An experiment was carried out under polyhouse condition, plants were kept in two sets, for pesticides influence and biological management through predatory mites. Some acaricides/plant extracts/botanical pesticides viz. dicofol 5 % WP, ethion 50 % EC, abamectin 1.9 % EC, phosalone 35% EC, phosphamidon 85% SL, fluvalinate 25% EC, malathion 50% EC, fenvalerate 20 % EC, azadirachtin 0.03%, neem oil, mahua oil (extract of fruit of *Madhuca indica*), *Citronella* oil, NSKE, *Durance repines*, *Clerodendron inerme* and *Vitex negundo* were selected for test of efficacy against this pest on their recommendation. In the second trail predatory mite, *Amblyseius tetranychivorus* (Gupta) was used at different ratios of predatory: prey viz. 1:5,1:10,1:15,1:20,1:25, 1:30 and 1:35, to find out the ratio which is good for the management of spider mite population. The data were collected at pre treatment, 1, 3, 7, and 14th days after treatment. The results indicated that dicofol responded maximum mortality followed by abamectine. Whereas botanical pesticides and plant extracts were shown less mortality but having encauraging performance. The predatory and spider mite ratio 1: 20 showed more affective followed by 1: 25 and 1: 15 ratios. The ratio 1:20 is very much suitable for mite management. On the basis of this experiment we can advice to growers that the botanical pesticide and biological agent is the good component for integrated mite management and are eco-friendly to environment also.

KEY WORDS spider mite, predatory mite, pumpkin, acaricides, botanical pesticides, management.

INTRODUCTION The spider mite has been identified as detrimental mite pest of several vegetable crops like okra, cowpea, brinjal, cucurbits, cucumber and melons in India. This mite pest found very injurious through out tropical and sub- tropical parts of the world (Jeppson et.al 1975). In recent past years the outbreaks of spider mite, *Tetranychus macfarlanei* Baker and Pritchard on pumpkin, *Cucurbita moschata* Dutch, a common vegetable of summer months have attracted attention to the growers and acarologists in eastern part of Uttar Pradesh. Heavy population of mites built profuse webs on plants, which cover them. This mite pest problem has gained momentum because of rapid changes that have occurred in cultural practices to grow pumpkin crop, *Cucurbita moschata* Dutch. The spider mite causes direct damage like loss of chlorophyll, stunting of growth defoliation, reduction in size and quality of fruits, appearance of various types of plant deformities etc. and all these severely effect the yield. Besides causing direct damage few phytophagous mites inject toxic substances in to their hosts, which cause increased localized growth and disruption of tissue (Jeppson et al., 1975). The monoculture and indiscriminate use of insecticides/acaricides on pumpkin, *Cucurbita moschata* Dutch has been enhancing the resurgence of this mite. The utilization of natural enemies of mite pest is eco-friendly approach in the management of injurious spider mite, *Tetranychus macfarlanei* Baker and Pritchard on pumpkin, *Cucurbita moschata* Dutch used predatory mite, *Amblyseius tetranychivorus* (Gupta) are belongs to family Phytoseiidae. The predatory mite, *Amblyseius tetranychivorus* (Gupta) is sensitive to commonly used pesticides (Jagdish and ChannaBasavanna, 1983). The population of spider mite can be suppressed effectively without affecting *A. tetranychivorus* using some indigenous plant extacts. The seed extracts of neem, pongamia, mahogany; leaf extacts of *Clerodendron inerme* and *Vitex negundo* recorded 72 to 100% mortality of *T.urticae* (Anon., 1994-96; Chandrashekhera, 1997). Overmeer (1985) reported that phytoseiids are best known predators among the acari and may be mass reared easily and shipped. Some phytoseiids are currently reared and sold for the biological control of spider mite, *Teteranychus urticae* Koch (McMurtry, 1982). Our aim is to manage the spider mite population on pumpkin, *Cucurbita moschata* Dutch through biologically with the predatory mite, the botanical pesticide and some

acaricides / insecticides. These are the good component for integrated mite management.

MATERIALS and METHODS The present investigation was carried out under poly house condition, in the month of March to June 2000-2001. The experiment was conducted at 35 -38 oC temperature and relative humidity (R.H.) 55 % on the natural population of spider mite, *Tetranychus macfarlanei* Baker and Pritchard on pumpkin, *Cucurbita moschata* Dutch. The trial was replicated four times on potted plant of pumpkin under poly house. The sixteen formulations of acaricides, botanical pesticides and plant extracts were taken in this trial. The commercial grades formulation of the acaricides, botanical pesticides and was used in this experiment. The seed kernels of neem and leaves of *Duranta repens*, *Clerodendron inerme* and *Vitex negundo* were used for extraction and testing. The plant material was dried under shade for 8-10 days, later powdered using a blender, extracted in Soxhlet apparatus using methanol as solvent. Methanol was removed by steam distillation process. The amount of proprietary ingredient required was calculated.

The proprietary ingredient so determined was mixed with 1 liter of water and sprayed on plants. The actual amount of toxicant and water required for spraying in each plot. The control was treated with water+sandovit spray, the chemical solution were prepared just before the application. After spraying the subsequent readings were recorded at, 1, 3, 7, and 14th days after. The per cent mortality was also calculated. The corrected per cent mortality was calculated through Abbott's formula (1925).

In the biological trial predatory mite, *Amblyseius tetranychivorus* (Gupta) was cultured in laboratory on castor pollen (Krishnamoorthy, 1985). The predatory mite, *A. tetranychivorus* transferred to potted plant of pumpkin (where the prey spider mite already established), at different predator: prey ratios i.e. 1:5, 1:10, 1:15, 1:20, 1:25, 1:30 and 1:35 per plant for their management.

RESULTS and DISCUSSION The efficacy of pesticides viz. azadirachtin (0.03%), dicofol (5%WP), ethion (50%EC), phosphamidon (85% SL), fluvalinate (25% EC), malathion (50% EC), abamectin (1.9% EC), NSKE (5%), neem oil, mahua oil, phosalone (35% EC), *Citronella* oil, *Durance ripines, Cleodendron inerme* and *Vitex negundo* were evaluated at their recommendaed concentration against the adult spider mite, *Tetranychus macfarlanei* Baker and Pritchard. The result indicates that used pesticides showed significant variation in the mortality and observed at 1, 3, 7 and 14 days after the treatment (Table 1)

After one day less mortality was responded with *Citronella* oil (24.56%) followed by *Cleodendron inerme* (28.66%), *Durane ripines* (33.66%), *Vitex negundo* (40.88%), mahua oil (42.68%), neem oil (48.52%) and NSKE (50.26%). The moderate mortality was responded with azadirachtin (52.88%) followed by fluvalinate (58.94%) and fenvalerate (66.88%). Whereas the moderate mortality was responded with dicofol (78.55%) followed by phosalon (76.55%), abamectin (75.88%), ethion (72.66%) and phosphamidon (70.88%) (Table 1).

Table: 1. Efficacy of acaricides and botanical pesticides against *Tetranychus macfarlanei* Baker and Pritchard on pumpkin.

S. No.	Treatments	Conc.	Corrected per cent mortality after days of treatments				Mean
		(%)	1	3	7	14	
1	Dicofol (5 %WP	0.0125	78.55* (9.36)**	84.68 -9.7	98.56 (10.42)	80.85 (9.54)	85.66 (9.75)
2	Abamectin (1.9 % EC)	0.014	75.88 (9.21)	80.55 (9.47)	96.99 (10.34)	80.78 (9.48)	83.55 (9.64)
3	Ethion (50 % EC)	0.05	72.66 (9.02)	80.44 (9.46)	90.86 (10.03)	68.92 (8.80)	78.22 (9.34)
4	Fluvalinate 25% EC	0.005	58.94 (8.17)	78.55 (9.36)	88.44 (9.90)	46.95 (7.35)	68.22 (8.75)
5	Phosphamidon 85% SL	0.025	70.88 (8.91)	81.55 (9.53)	90.66 (10.02)	63.55 (8.47)	76.66 (9.25)
6	Phosalone (35 % EC)	0.07	76.55 (9.24)	85.22 (9.73)	90.22 (9.99)	74.21 (9.11)	81.55 (9.53)
7	NSKE (5 %)	5	50.26 (7.58)	64.55 (8.53)	73.88 (9.09)	21.07 (5.09)	52.44 (7.74)
8	Azadirachtin (0.03 % EC)	5	52.88 (7.77)	68.22 (8.75)	76.55 (9.24)	52.55 (7.74)	62.55 (8.40)
9	Neem oil	2	48.52 (7.46)	59.88 (8.23)	68.44 (8.77)	48.92 (7.49)	56.44 (8.01)
10	Malathion 50%EC	0.05	67.78 (8.73)	76.55 (9.24)	86.55 (9.80)	62.88 (8.42)	73.44 (9.06)
11	Mahua oil	2	42.68 (7.03)	54.66 (7.89)	68.22 (8.75)	32.88 (6.23)	49.66 (7.54)
12	Fenvalerate 20%EC	0.005	66.88 (8.67)	78.22 (9.34)	85.77 (9.76)	55.77 (7.96)	71.66 (8.96)
13	*Vitex negendo*	2	40.88 (6.89)	56.66 (8.02)	64.88 (8.55)	19.34 (4.89)	45.44 (7.24)
14	*Citronella* oil	2	24.56 (5.00)	42.55 (6.56)	68.88 (8.32)	28.55 (5.38)	41.13 (6.45)
15	*Durane ripines*	2	33.66 (5.84)	43.22 (6.61)	62.88 (7.96)	30.44 (5.56)	42.55 (6.56)
16	*Cleodendron inerme*	2	28.66 (5.40)	40.44 (6.39)	52.66 (7.29)	22.88 (4.83)	36.16 (6.05)
17	Control (Water+Sandovit)	16.36 (4.54)	25.11 (5.51)	34.28 (6.35)	19.04 (4.86)	23.69 (5.36)	23.69 (5.36)
18	Control	19.69 (4.93)	21.47 (5.13)	27.55 (5.74)	16.76 (4.59)	21.36 (5.12)	21.36 (5.12)
	SEM ±	-	12.23	10.7	10.23	12.62	
	CD (0.01%)	-	32.78	28.68	27.4	33.83	

* Mean corrected percent mortality is an average of four replications
** Figures in parenthesis are the ? X+0.5 transformed value, where X= mean corrected percent mortality.

After 3 days less mortality was observed with *Cleodendron inerme* (40.44%) followed by *Citronella* oil (42.55%), *Durane ripines* (43.22%), mahua oil (54.66%), *Vitex negundo* (56.66%) and neem oil (59.88%). The moderate mortality was observed with NSKE (64.55%), azadirachtin (68.22%), malathion (76.55%), fenvalerate (78.22%) and fluvalinate (78.55%) respctively. Whereas the maximum mortality was observed with phosalone (85.22%) followed by dicofol (84.68%), phosphamidon (81.55%), abamectin (80.55%), and ethion (80.44%) (Table 1).

After 7th day showed less mortality with *Cleodendron inerme* (52.66%) followed by *Durane ripines* (62.88), *Vitex negundo* (64.88%), mahua oil (68.22%), neem oil (68.44%) and *Citronella* oil (68.88%). The moderate mortality was observed with NSKE (73.88%), azadirachtin (76.55%), fenvalerate (85.77%), malathion (86.55%) and fluvalinate (88.44%). Whereas the maximum mortality was observed with dicofol (98.56%) followed by abamectin (96.99%), ethion (90.86%), phosphamidon (90.66%) and phosalone (90.22%) (Table 1).

After 14th day less mortality was responded with *Vitex negundo* (19.34%) followed by NSKE (21.05%), *Cleodendron inerme* (22.88%), *Citronella* oil

(28.55%), *Durane ripines* (28.55%), mahua oil (32.88%), fluvalinate (46.95%), neem oil (48.92%) and azadirachtin (52.55%). The moderate mortality was observed with fenvalerate (55.77%) followed by malathion (62.88%) phosphamidon (63.55%) and ethion (68.92%). Whereas the maximum mortility was shown with dicofol (80.85%) followed by abamectin (80.78%) and phosalon (74.21%) (Table 1).

Over all results indicated that dicofol responded maximum mortality followed by abamectin, phosalon, ethion and phosphamidon i.e 85.66, 83.55, 81.55, 78.22 and 76.66 per cent respectively. Where as the moderate mortality was responded with malathion followed by fenvalerate, fluvalinate azadirachtin neem oil and NSKE i.e.73.44, 71.66, 68.22, 62.55, 56.44 and 52.44 per cent respectively. The less mortality was responded with mahua oil followed by *Vitex negundo*, *Durane ripines*, *Citronella* oil and *Cleodendron* i.e 49.66, 45.44, 42.55, 41.13 and 36.16 respectively. Many worker, Singh et.al (1989), Singh and Singh (1992), Rai et.al (1993), Yathiraj and Jagadish (1999) and Kumar et. al. (2001) earlier supported the results.

In the second trail predatory mite, *Amblyseius tetranychivorus* (Gupta) was used at different ratios of predatory: prey viz. 1:5,1:10,1:15,1:20,1:25, 1:30 and 1:35, to find out the ratio which is good for the management of spider mite population. The results indicate that the predatory and spider mite ratio 1: 20 showed more affective followed by 1: 25 and 1: 15 ratios. The ratio 1:20 is very much suitable for mite management (Table 2).

Table:-2. Influence of predatory mite *Amblyseius tetranychivorus* (Gupta) on the population of *Tetranychus macfarlanei* Baker and Pritchard on pumpkin.

Predator : Prey	Corrected per cent mortality after days of treatments				Mean
	1	3	7	14	
1:05	1.44* (1.99)	2.66 (1.78)**	3.11	3.28	2.62
			-1.9	-1.94	-1.77
1:10	1.22 (1.31)	2.98	3.48	4.8	3.12
		-1.86	-1.99	-2.3	-1.9
1:15	2.64	2.86	3.64	4.48	3.4
	-1.77	-1.83	-4.48	-2.23	-1.97
1:20	3.48 (1.99)*	4.38	8.67	9.74	6.56
		-2.2	-3.02	-3.2	-2.65
1:25	2.48	3.8	4.6	4.88	3.94
	-1.77	-2.07	-2.25	-2.31	-2.1
1:30	2.55	2.9	3.28	3.86	3.14
	-1.74	-1.84	-1.94	-2.08	-1.9
1:35	2.88 (1.83)	3.22 (1.92)	3.56 (2.01)	3.88 (2.09)	3.38
					-1.96
Control	15.46	24.8	26.66	22.9	22.45
	-3.99	-5.02	-5.21	-4.83	-4.79

* Mean corrected percent mortality is an average of four replications

A strain of *Phytoseiulus persimilis* resistant to organophosphorus pesticides (Loginova et al., 1987 and Hoy, 1985) has been used in integrated management of two spotted mite. Chandrashekrappa et al. (1995) was also reported that higher temperature and higher humidities induced increased feeding in both the species. The maximum number of egg consumed by an adult *Amblyseius longispinosus* was 21.77 eggs at 30 ° C and 94 .5 to 96.5 per cent R.H., where as *Amblyseius tetranychivorus* consumed a maximum of 8.5 ± 1.2 adults at the same temperature and humidity level. The minimum number of prey consumed by *Amblyseius longispinosus* and *Amblyseius tetranychivorus* was 14.0 ± 1.58 eggs and 6.6 ± 1.91 adults respectively at 25 ° C and 32.5 - 33.0 per cent R.H. Therefore, the biological control is the only alternative to reduce the mite population. On the basis of this experiment be can advice to growers that the botanical pesticide and biological agent is the best component for integrated management of this mite, because both are eco-friendly to environment also.

ACKNOWLEDGEMENTS The authers are highly grateful to the Head of the Department of Entomology and Agricultural Zoology, Institute of Agricultural Sciences B.H.U. Varanasi -221005 UP, India for providing necessary facilities during the course of study.

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Negative Cross Resistance to Spinosad: An Interesting Observation in the Field Population of Cotton Leaf worm Larvae, *Spodoptera littoralis* in Egypt

ABSTRACT Field strain of the cotton leaf worm larvae, *Spodoptera littoralis* (known to be tolerant or resistant to most of the conventional insecticides) proved to be more susceptible to spinosad than the laboratory strain (known as susceptible to conventional insecticides) .

Within the same instar, LC 50 of the field larvae showed significantly lower figure than for the laboratory larvae. After 48 hours of feeding on spinossad-treated leaves, resistance ratio (RR) was 0.11, 0.11, 0.03, 0.04 , 0.05and 0.05 times for the 1st, 2nd, 3rd, 4th, 5th, and the 6th instars of CLW, respectively. The first three instars of field strain showed lower LC50 than the 1st instar of laboratory strain. This finding may offer a considerable advantage to spinosad for being unaffected by the existing resistance mechanism. So, this may help and solve the conventional-resistance problem in the field cotton leaf worm in Egypt.

KEYWORDS Field and laboratory strains, *Spodoptera littoralis* larvae, spinosad

INTRODUCTION The cotton leaf worm *Spodoptera littoralis* (Boisd) (CLW) is a major polyphagous key pest in Egypt. It is active all year round without hibernation period and attacking cotton as well as more than 29 hosts from other crops and vegetables. Its rate of infestation could reach more than 50,000 egg masses/ one acre attacking leaves, buds, flowers, and bolls. To combat this regular pest on cotton Ministry of Agriculture (MOA) use to spray through a rotation program (Sawicki and Denholm 1987) conventional insecticides extensively from organophosphates (OPs), pyrethroids (PYs) and carbamates (CARs) every year (Temerak 2002). This species has the ability to develop relatively fast resistance to most of the conventional insecticides.

Several products have showed field failure and cancelled from the official cotton spraying program against CLW (Temerak 2002). During launching of PYs., MOA restricted PYs to a single application/ season/ year on cotton only with no use outside cotton in order to prevent or delay resistance following Dr.R.M. Sawiki recommendation. Fold of resistance of CLW to most of pyrethroids was not exceeding 10 fold in 1980 (ElDahan et al 1985), however fold of resistance of most of pyrethroids were ranged between 25.5 up to 6667 fold, in 1990 (El-Barmaway et al 1991-92)

Several publications have confirmed the significant difference between the LD50 or LC50 of the field and laboratory strain (kept in the laboratory without exposure to any insecticide). These differences have indicated either tolerant or real resistance in the field strain to most of conventional insecticides from OPs or PYs or CARs (eg. El Dahan et al 1985, Yhia et al 1985, El-Said et al 1991, El-Barmawy et al 1991-92, Rashwan et al 1991-92, Maher 1975, El-Sebae et al 1993, Allam et al 2000a,b) Accordingly, Egypt is suffering from CLW resistance over 40 years. Trials were done to investigate the susceptibility of field CLW larvae (known as tolerant or resistant to conventional insecticides) versus the laboratory strain (known as susceptible to conventional insecticides)to the new natural product spinosad.

MATERIALS and METHODS In Egypt, laboratory (lab.) strain which kept for 30 years away from any exposure to conventional insecticides and known in all locally published work as susceptible used in this study as standard reference.

Field strain egg masses were collected from cotton fields in delta north Egypt at which CLW larvae have been exposed to field routine selection pressure of certain conventional insecticides that are usually applied every year from June-September. These insecticides were insect growth regulators, organophosphates (OPs) as Chlorpyrifos ethyl and profenfos, pyrethroids (PYs) as cypermethrin and carbamates (CARs) as carbaryl (Temerak 2002).

In this study around 50 000 egg-masses was collected during June. After mixing these egg masses sub sample of around 1000 ones were reared on castor bean leaves under 29 C and 54-59 % relative humidity.

Leaf residue dipping technique

Spinosad formulation utilized in this study was spintor 24 SC. It is trademark of Dow Agrosciences Co. It is a naturally occuring mixture of two active components (spinosyn A&D) produced by fermentation of the soil acctinomycete;

Sacharopolyspora spinosa Marz &Yao (Thompson et al 1997).

Spinosad were diluted with water to obtain a range of 7-8 different concentrations/ each instar. Leaves of castor bean were dipped for 5 seconds in different concentrations of spinosad and left dry under lab condition for one hour.

Four replicates with 10 larvae each per each concentration were used for each of the first three instars of CLW. The last three instars, 4 replicates with only 5 larvae each were used / each concentration. Total of around 1440 larvae was used for treated field strain. Untreated field strain was served by 4 x 10/each of the first three instars and 4 x 5 larvae /each of the last three instars (180 larvae).

Leaves for untreated were dipped in water only. Grand total of larvae used was 1620 larvae.

The same number of larvae was used for laboratory strain as in the field strain. Leaves of untreated larvae were dipped in water only. Petri-dishes were used to serve the first three instars but the last three instars were placed in large containers covered with muslin and rubber band.

Statistical analysis

Data on mortality after 48 hours was subjected to abbott formula for correction wherever required. Probit analysis was determined to calculate LC50 and LC90 (Finney, 1971).

RESULTS and DISCUSSION Probit analysis criteria of field and laboratory strain of all CLW instars are presented in table 1. Resistance ratio between the field and laboratory CLW larvae / respective instar was presented in table 2.

Table1:Probit analysis of all larvae instars of field cotton leaf worm versus laboratory strain

Line name	LC50	Lower limit	Upper limit	Index	folds	Slope	SE	LC90
FIELD1	0.545	0.444	0.673	100	1	2.237	0.239	2.038
FIELD2	1.192	0.938	1.489	45.721	2.187	1.965	0.219	5.352
FIELD3	1.866	1.424	2.363	29.207	3.424	1.847	0.219	9.219
LAB1	4.843	3.618	6.69	11.253	8.886	1.378	0.185	41.226
LAB2	10.896	8.293	14.851	5.002	19.993	1.495	0.193	78.489
FIELD4	12.378	7.125	19.086	4.403	22.712	1.365	0.281	107.565
FIELD5	29.03	18.626	42.454	1.877	53.266	1.539	0.275	197.567
FIELD6	48.209	27.895	73.647	1.13	88.457	1.386	0.28	405.433
LAB3	66.854	52.818	85.426	0.815	122.668	1.783	0.211	349.874
LAB4	351.042	246.029	502.876	0.155	644.114	1.747	0.287	1901.124
LAB5	583.677	409.42	845.403	0.093	1070.967	1.617	0.238	3619.623
LAB6	932.267	632.856	1369.258	0.058	1710.582	1.484	0.226	6808.889

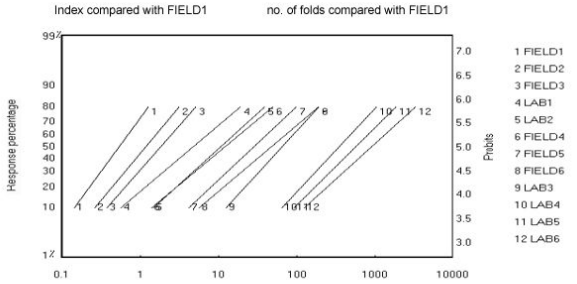

Table2: Resistance ratio between field CLW and laboratory strain

Larval instar	LC50 Field strain	LC50 Lab. strain	Rsist.ratio
1st instar	0.545	4.843	0.11
2nd instar	1.192	10.896	0.11
3rd instar	1.866	66.854	0.03
4th instar	12.378	351.042	0.04
5th instar	29.03	583.677	0.05
6th instar	48.209	932.267	0.05

Resistance ratio =LC50 of field÷LC50 of laboratory

Untreated larvae of both field and lab. strains showed no mortality during the 48 hours of feeding. After 48 hours of feeding on spinosad-treated leaves, LC50s of field larvae were 0.545, 1.192 , 1.866, 12.378, 29.03, and 48.209 for the 1st, 2nd, 3rd, 4th , 5th . and the 6th instars of CLW, respectively. However, in the laboratory strain these figures were 4.843, 10.896, 66.854, 351.042, 583.677, and 932.267 ppm, respectively. The results confirmed the greater LC50 in the laboratory strain than in the field strain. The laboratory strain of the 1st instar showed higher LC50 than any of the first three instars of field strain. Resistance ratio was 0. 11, 0.11, 0.03, 0.04, 0.05, and 0.05 for the 1st instar up to the 6th instar of CLW, respectively.

Results showed that spinosad was not affected by the existing situation of resistance which coming from the combined previously mentioned products.

Locally ,similar results were found by (Abo-Elghar and Radwan 1995) between field and lab. strains of CLW when they tested the natural product Abamectin which is a fermentation of the acctinomycete, *Streptomyces avermitililis*. From abroad, Mascarenhas et al (1998) indicated that LC50 of chlorpyrifos and thiodicarb of field strain of the beet army worm *S. exigua* had significantly high LC50 than the reference strain. However, with newer products eg spinosad was comparable .

Scott (1998) found similar result, the house flies, *Musca domestica* resistant to cyclodiene, was more sensitive to spinosad.

Gunning and Devonshire (2002) indicated that *Heliothis armigera* activates indoxacarb using the same estrase enzymes that are involved in pyrethroid resistance. Increased esterase activity leads to increased activation and therefore increased susceptibility to indoxacarb. They added that the data confirmed the negative cross resistance between pyrethroid and inoxacarb in *H. armigera*. Nannan et al (2000) was on line with gunning and Devonshire when they found that the housefly permethrin-resistant strain was more susceptible to spinosad.

Arora (2002) concluded that spinosad-selected strain of *Plutella xylostella* (L) did not show any cross resistance to certain conventional insecticides. Hendrix

et al (2001) concluded that results conducted across several years and numerous crops demonstrated that spinosad constantly provides beet army worm *Spodoptera exigua* (Hubner)control that is superior to most of other products labeled for this pest.

Further studies should be done to draw the base line of field CLW to spinosad in comparison to the traditional current insecticides by collecting samples from all governorates of cultivated cotton. In the laboratory, Py-resistant, OP-resistant, and Carb-resistant strains should be developed utilizing field strain and assessed their susceptibility to spinosad. Detoxification or activation enxymes should also measured to see which group is responsible for this negative cross resistance.

Spinosad is the one of the most promising new chemicals, which has a favourable mammalian toxicity and environmental profile (Sparks et al 1995). The availability of a novel chemical group, with a new mode of action that is different from conventional insecticides in current use, is an asset to insecticide resistance management programs (Horowitz and Ishaaya 1994).

Present results indicated that these field strain could exhibit more susceptibility to the unconventional insecticides such as Spinosad. This may be due to the differences in the mode and site of action of such compound from one side and the conventional insecticides from the other side (Salgado et al 1997). This finding may offer a great advantage to spinosad for being unaffected by the existing resistance mechanism in CLW for conventional insecticides.

Spinosad could play a significant role to combat the conventional-resistant insects due to its novel mode of action (Salgado et al 1997). Bret et al (1997) and Peterson et al (1997) indicated that the low toxicity of Spionosad to natural enemies allows it to be incorporated in most integrated pest management. We generally recommended this product to be used to control conventional-resistant CLW larvae or to be rotated with them through an integrated pest management program.

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Joint Action of Insecticides Against *Helicoverpa armigera* Hubner

ABSTRACT Co-toxicity analysis of ten insecticide mixtures indicated that pyrethroids when mixed with OP compounds (profenofos or chlorpyrifos) exhibited synergistic action (CTC > 100), but independent action was observed in combination with endosulfan (CTC < 100) against *Helicoverpa armigera*.

KEY WORDS: *Helicoverpa armigera*, insecticide mixtures, joint action

INTRODUCTION The cotton bollworm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) is one of the major threats to present day intensive agriculture in Asia because it has developed resistance to all the major group (synthetic pyrethroids, organo phosphates, organo chlorines and carbamates) of insecticides used against it (Regupathy et al., 2003). Over-dependence of a particular group of chemistry is one of the important reasons for rapid development of resistance. This is evidenced by very high level of resistance to synthetic pyrethroids, which occupied 50 - 70 per cent of the insecticides sprayed over the cotton in India (Jayaswal, 1989). Farmers resort to use tank mixtures of insecticides though not recommended to manage the resistant cotton bollworm, *H.armigera*. Insecticide mixtures were reported to delay the development of resistance in *H.armigera* (Yanchao et al., 1995; Vaissayre and Alaux, 1996). However no other scientific report is available in India with respect to joint action of insecticides against *H.armigera*. The CTC analysis is not only useful for the estimation of joint toxicity in the laboratory but also to decide the dosage of each toxicant in the mixture for preliminary field tests. Hence a study was carried out to assess the joint action of insecticides by co-toxicity coefficient analysis (CTC) and the results are reported.

MATERIALS and METHODS *H.armigera* population collected from field were reared to next generation on standard artificial diet (Armes et al., 1993) at 27± 2°C and 75 per cent RH in the laboratory. Third instar larvae (30-40mg) of F1 generation were used for the tests. The required insecticidal dilutions prepared from technical grade insecticides were applied on the thoracic dorsum of each insect @ 1µl using Hamilton repeating dispenser. Five concentrations were used for each toxicant.

The acute toxicity of each insecticide to *H.armigera* was assessed by establishing LDPM lines. The ratio of two insecticides in the mixture was decided on the basis of LD50 value of each insecticide and then co-toxicity coefficient (CTC) of mixture (M) was determined (Sun and Johnson, 1960) using non-pyrethroid insecticides (profenofos, chlorpyrifos and endosulfan) as standards.

RESULTS and DISCUSSION

Pyrethroids and OP mixtures

Synergistic action was noticed when pyrethroids were mixed with either profenofos (or) chlorpyrifos (Table 1). The CTC value was comparatively higher to fenvalerate and profenofos mixture (122.7629), whereas it was low to profenofos and cypermethrin mixture (106.052). Among the chlorpyrifos and pyrethroid mixtures, fenvalerate combination showed higher synergistic action (CTC=130.95) followed by deltamethrin (114.56), cypermethrin (112.58) and finally lambdacyhalothrin (101. 49).

The monitoring studies conducted since 1993 indicated that the predominant mechanism of pyrethroid resistance in *H.armigera* populations from Tamil Nadu was by the induction of monooxygenases as evidenced by the effective suppression of resistance by MFO inhibitors, PBO, propargyloxypthalimide and pungam oil (Regupathy et al.,1995; Gavigowda, 1996; Tamilselvi, 2001; Sumathi,2002; Ramasubramanian, 2003).

The synergistic mechanism of profenofos and chlorpyrifos with pyrethroids is probably due to competitive inhibition of MFO for their activation and leaving less MFO available for detoxification of pyrethroids. Both profenofos and chlorpyrifos come under the group of phosphorothionates, the latent

phosphorothionates need activation to become more toxic phosphates. This conversion is mediated through oxidation by MFO. Carboxyl esterases also play a considerable role in detoxification of synthetic pyrethroids in *H.armigera* population from Tamil Nadu. Profenofos being a specific inhibitor of carboxyl esterase generally enhances the toxicity of synthetic pyrethroids. These are the possible reasons for the synergistic property of synthetic pyrethroid + phosphorothionate mixtures.

Table1. Co-Toxicity co-efficient (CTC) for insecticide mixtures

Insecticide	LD_{50}	Fiducial limits	Regression equation	χ^2	CTC
Profenofos	1.4754	1.0959 - 1.9864	Y = 0.6279 + 1.3797x	0.3945	-
Chlorpyrifos	1.21	0.9041 - 1.6304	Y = 0.8969 + 1.3303x	0.2047	-
Endosulfan	6.3628	5.0715 - 7.9828	Y = 1.5397 + 1.7193x	1.1147	-
Fenvalerate	3.638	2.8818 - 4.5927	Y = 1.0231 + 1.6915x	2.5338	-
Cypermethrin	3.5266	2.8358 - 4.3857	Y = 1.4536 + 1.8193x	1.3296	-
Lambda cyhalothrin	1.5389	1.1213 - 2.1121	Y = 0.8572 + 1.2998x	0.1286	-
Deltamethrin	2.399	1.8750 - 3.0710	Y = 0.4750 + 1.6200x	0.226	-
Profenofos + Pyrethroid mixture					
Profenofos + Fenvalerate	2.071	1.6386 - 2.6173	Y = 0.6759 + 1.7116x	0.1649	122.763
Profenofos + Cypermethrin	2.3637	1.8321 - 3.0494	Y = 0.2837 + 1.5662x	0.4987	106.052
Profenofos + Lambda cyhalothrin	1.2974	0.9763 - 1.7241	Y = 0.6203 + 1.4069x	2.065	116.276
Chlorpyrifos + Pyrethroid mixture					
Chlorpyrifos+ Fenvalerate	1.8507	1.4239 - 2.4052	Y = 0.0725 + 1.5081x	0.3689	130.95
Chlorpyrifos + Cypermethrin	2.1195	1.6101 - 2.7900	Y = 0.2793 + 1.4192x	0.8635	112.58
Chlorpyrifos + Lambda cyhalothrin	1.3565	1.0205 - 1.8032	Y = 0.5615 + 1.4169x	0.0505	101.49
Chlorpyrifos + Deltamethrin	1.5775	1.2345 - 2.0157	Y = 0.288 + 1.6535x	0.1262	114.56
Endosulfan + Pyrethroid mixture					
Endosulfan + Fenvalerate	5.7445	4.3124 - 7.6522	Y = 0.3360 + 1.4194x	1.1618	87.47
Endosulfan + Cypermethrin	5.2889	4.6088 - 6.9779	Y = 0.4116 + 1.4534x	0.1571	93.59
Endosulfan + Lambda cyhalothrin	4.5203	3.5033 - 5.8324	Y = 0.5857 + 1.5282x	1.0264	54.87

Pyrethroids and Endosulfan mixtures

The low co-toxicity co efficient value (87.47, 93.59 and 54.87 for endosulfan + fenvalerate, endosulfan + cypermethrin and endosulfan + lambdacyhalothrin respectively) obtained with pyrethroids + endosulfan combination indicated the independent action of the two chemistries. The synthetic pyrethroids act principally on the voltage sensitive sodium channels (Narahashi, 1971; 1976; 1992.) whereas, the cyclodienes including endosulfan specifically attack the picrotoxinin receptor site (Ghiasuddin and Matsumura, 1982; Kadous et al., 1983) on GABA (gamma amino butyric acid) receptor. Thus, theoretically pyrethroids and endosulfan had differential site of action. The independent action of these two groups of chemistries was also confirmed by earlier studies with pyrethrins - organochlorine combination (dieldrin + pyrethrins - 67.2; aldrin + pyrethrins - 67.6; a - chlordane + pyrethrins - 64.6; ß - chlordane + pyrethrins - 67.6) (Sun and Johnson, 1960).

ACKNOWLEDGEMENT The financial support from the Common Fund for Commodities (CFC) Europe, the International Cotton Advisory Committee (ICAC) and Natural Resources Institute (NRI), UK is acknowledged.

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Monitoring Techniques for Detecting Insecticide Resistance in *Helicoverpa armigera* (Hubner)

ABSTRACT In the absence of susceptible strain of *Helicoverpa armigera* in India, indirect method was used to fix discriminating doses (DD) for other methods of bioassay *viz*., vial, bouquet, spray tower and larval dip for commonly used insecticides *viz*., endosulfan, quinalphos, chlorpyriphos, fenvalerate and cypermethrin. The DD for other methods were extrapolated by multiplying the DD available for susceptible cultures of NRI, UK and Australia by topical application with the factor of ratio of LD99 of topical and other methods. Validation of extrapolated DDs was done by testing on different *H. armigera populations*. The extrapolated DDs inflicted mortality with standard error (SE) varying from 3.9 to 7.1 when batches of 50 insects were used. The variation could be reduced with more number of insects per test.

KEY WORDS: Discriminating dose, insecticide resistance, *Helicoverpa armigera*.

In India, resistance was first recorded in major cotton growing region of Andhra Pradesh in 1987 (McCaffery et al., 1989). *Helicoverpa* resistance to different chemicals is widely recognized and documented (Phokela et al., 1989; Pasupathy and Regupathy 1993; Arms et al., 1992, Regupathy et al., 2003). The effective resistance management depends on the successful monitoring programme. In most practical situations the best monitoring method is the use of discriminating doses i.e. the dose that kills 99% of susceptible individual (Roush and Miller, 1986).

In the monitoring programme the discriminating doses for fenvalerate, cypermethrin, quinalphos and endosulfan have been fixed using the susceptible cultures available in Australia for topical application method (Forrester and Cahill, 1987; Forrester et. al., 1993; Gunning et al., 1984). The high-tech nature of topical application prevents many field level workers and marginal farmers adopting this due to low literacy rate in India.

In the absence of susceptible lines for *Helicoverpa* in India, extrapolated discriminating doses for other bioassay methods are to be arrived. With this in view an attempt was made to extrapolate the discriminating doses for other methods from that of topical application.

MATERIALS and METHODS

Mass culturing of H. armigera

Various populations of *H. armigera* larvae were reared in a semi-synthetic diet as described by Sathiah, (1987).

Preparation of Insecticide Solutions

The insecticide solutions required for the discriminating dose assays were prepared from technical grade insecticides (of known purity) diluted with analytical grade acetone. The technicals used for different bioassay methods were, endosulfan (92%), chlorpyriphos (95%), quinalphos (71.5%), cypermethrin (94.8%) and fenvalerate (96.2) obtained from Thudialur Co-operative Agro. Service Pvt. Ltd., Coimbatore, Coramandal Indag Pvt. Ltd., Chennai, Syngenta India Ltd., Mumbai, National Organic Chemical Industries Ltd., Mumbai and Rallis India Ltd., Bangalore respectively.

For larval dip method the respective formulations *viz*., endosulfan (Thiodan 35 EC), quinalphos (Ekalux 25 EC), fenvalerate (Sumicidin 20 EC), cypermethrin (BILCYP 25 EC) and chlorpyriphos (Durmet 20 EC) were used.

Bioassay methods

In all the methods third instar larvae (30-40 mg) were used.

Topical assay

Serial dilutions of technical grade insecticides in analytical grade acetone were prepared and 1ul repeating dispenser (PB 600-01, Hamilton Co Ltd) fitted with 50 µl syringe and "Rheodyne needle" was used to deliver 1 µl drop to the thoracic dorsum of each larva.

Vial residue bioassay/Contact toxicity method

Glass scintillation vials of 20 ml capacity were evenly coated with 1 ml acetone solutions of technical grade insecticides (Plapp 1971). Larvae were released into the vials individually and covered with a muslin cloth.

Foliar residue bioassay Bouquet method/ Terminal bud bioassay

Chickpea shoots were taken and terminal branches were removed retaining only five compound leaves on each shoot. These shoots were surface sterilized in 0.5% sodium hypochlorite, rinsed in sterile water and shade dried (Rose Victoria, 1991). These shoots were dipped in respective concentration of the chemical which was prepared with analytical grade acetone containing Triton-X 100 surfactant (0.25 g/lit) for about 30 sec. and the excess fluid was drained off. The petioles of the shoots with cotton swab were kept immersed in water in a penicillin vial and allowed for shade drying. Each penicillin vial having the treated shoots was kept separately in a large plastic (19x10 cm) container to which third instar larvae were released individually. The mouth of the container was covered with muslin cloth and the larvae were allowed to feed on the shoots. After allowing 24 h of feeding, the larvae were transferred to semi-synthetic diet. A control was maintained with chickpea shoots treated with acetone plus Triton-X 100 alone.

Spray (potter's) tower bioassay

One ml of various concentrations of the technicals prepared in acetone was sprayed at a pressure of 2 kg/cm2 with the help of a spray tower on to the *H. armigera* larvae in a Petri dish. Each time a batch of 10 larvae were anesthetized with the help of CO2 and sprayed. The Petri dishes containing the larvae were dried for 5 min and the treated larvae were transferred individually into vials containing semi-synthetic diet.

Larval dip bioassay

Aqueous dilutions of formulated (emulsion concentrates) insecticides were prepared and batches of third instar larvae were submerged for 5 sec. as described by Watkinson et al (1984). A group of 50 larvae were dropped into 100 ml of the appropriate dilution in 500 ml beaker and gently swirled for 5 sec. to ensure complete wetting. The solution plus larvae were then poured through a fine nylon mesh suspended over an empty beaker. The solution was decanted and larvae separated by this process. After shade drying for about 5 min. the treated larvae were then transferred individually into semi synthetic diet. Control insects were treated with water alone.

Bioassays were carried out at 26 ± 1° under approximately 12h: 12h LD Photo period. Mortality was recorded at 24h interval up to 6 days. The log dose/concentration probit mortality curve was fitted to extrapolate LC/LD99 after making Abbott's correction.

The tentative discriminating doses of endosulfan, quinalphos, cypermethrin, fenvalerate and chlorpyriphos for different methods were extrapolated considering the following discriminating doses calibrated for topical application.

Endosulfan: 10.0 µg/µl (Approximate LD99 for susceptible strains calibrated in Australia).
Cypermethrin: 1 µg/ml (LD99 for Delhi strain) (Phokela et al., 1989).
Quinalphos: 0.75 µg/ml (LD99 for NRI Lab susceptible strain).
Fenvalerate: 0.2 µg/ml (LD99 for susceptible strain in Australia) (Forester and Cahill, 1987).

The above discriminating doses for topical application were multiplied by the factor of ratio of LD99 of topical and other methods.

Validation of different methods

Various field populations or a particular location at periodic intervals were dosed with the extrapolated discriminating doses and mortality was recorded. The extrapolated discriminating doses for different methods *viz*., topical contact, foliar, spray tower and larval dip tested with batches 50-100 larvae and the mortality was recorded at an interval of 24 h for 6 days.

Corrected control mortality was calculated using Abott's formula (Abott, 1925) and binomial standard error was calculated by using the formula:

P (100 - P) /(n-1)
P = % of larva surviving in the discriminating dose
n = Number of larva tested

Linear correlation was worked out for the combination of different methods.

RESULTS and DISCUSSION

Lethal dose

The LD99 of the tested insecticides by topical application varied from 2.24 µg - 36.70 µg; (Tables 1,2, 3, 4, and 5) the lowest for chlorpyriphos and the highest value for endosulfan.

The LD99 values were higher than the discriminating doses of these compounds used for assessing the resistance nature indicating the high degree of resistance of *H. armigera* population evaluated for these compounds.

Lethal concentration

The LC99 for the above chemicals by vial method was 94.40 µg for endosulfan, 36.49 µg for fenvalerate, 32.13 µg cypermethrin, 17.90 µg for quinalphos and 4.50 µg for chlorpyriphos. In the case of bouquet method the LC99 varied between 59.35 and 2571.20 ppm. The LC99 obtained by larval dip method varied from 123.02-3706.50 ppm. The LC99 obtained by spray tower method varied from 363.08-14003.67 ppm (Tables 1,2, 3, 4, and 5). Irrespective of the methods the order of toxicity of the insecticides was same i.e.

chlorpyriphos > quinalphos > cypermethrin > fenvalerate > endosulfan.

Table 1. Toxicity of endosulfan to third instar *H. armigera* by different bioassay methods

Methods	Regression equation	χ^2 (df 3)	LD_{50}/ LC_{50}	Fiducial limits LL		UL	LD_{95}/ LC_{95}	Fiducial limits LL		UL
*Topical	1.79 + 2.16 x	3.55	3.02	2.63	-	347	36.7	24.32	-	54.21
Vial	2.57 + 2.41 x	2.5	10.2	9.01	-	11.57	94.4	67.15	-	133.34
Bouquet	-0.07 + 2.17 x	3.01	217.64	203.91	-	269.55	2571.2	1732.19	-	3851.73
Larval dip	-5.09 + 3.48 x	0.36	793.27	704.12	-	891.97	3706.5	2549.86	-	5388.69
Spray tower	-2.82 + 2.45 x	0.81	1548.82	1372.99	-	1747.16	14003.7	9538.41	-	19976.7

* .... logx. 101

Table 2. Toxicity of fenvalerate to third instar *H. armigera* by different bioassay methods

Methods	Regression equation	χ^2 (df 3)	LD_{50}/ LC_{50}	Fiducial limits LL		UL	LD_{95}/ LC_{95}	Fiducial limits LL		UL
*Topical	2.96 + 1.78 x	2.59	1.4	1.19	-	1.65	28.66	16.95	-	46.87
*Vial	2.70 + 1.80 x	3.74	1.87	1.58	-	2.19	36.49	22.91	-	57.54
Bouquet	1.47 + 1.92 x	3.91	68.95	55.46	-	82.46	127.4	507.54	-	2505.65
Larval dip	1.42 + 1.85 x	4.83	85.8	68.46	-	107.79	1559.46	821.64	-	2960.19
Spray tower	0.69 + 1.89 x	6.49	193.4	165.57	-	225.53	3330.78	1807.21	-	6123.61

* .... logx. 101

Table 3. Toxicity of cypermethrin to third instar *H. armigera* by different bioassay methods

Methods	Regression equation	χ^2 (df 3)	LD_{50}/ LC_{50}	Fiducial limits LL		UL	LD_{95}/ LC_{95}	Fiducial limits LL		UL
*Topical	3.32 + 1.92 x	0.38	0.75	0.45	-	1.27	12.43	6.35	-	23.85
*Vial	2.80 + 1.81 x	5.25	1.65	1.31	-	2.11	32.13	16.59	-	62.01
Bouquet	1.62 + 1.92 x	0.99	57.54	49.81	-	66.48	933.25	545.46	-	1596.76
Larval dip	1.39 + 1.87 x	2.61	85.2	67.89	-	106.71	1501.31	802.35	-	2816.02
Spray tower	0.90 + 1.83 x	0.91	175.98	138.39	-	223.98	3328.07	1729.02	-	6412.09

* .... logx. 101

Table 4. Toxicity of quinalphos to third instar *H. armigera* by different bioassay methods

Methods	Regression equation	χ^2 (df 3)	LD_{50}/ LC_{50}	Fiducial limits LL		UL	LD_{95}/ LC_{95}	Fiducial limits LL		UL
*Topical	4.01 + 1.61 x	1.96	0.42	0.36	-	0.51	12.2	6.13	-	22.58
*Vial	3.59 + 1.66 x	1.2	0.71	0.59	-	0.85	17.9	11.14	-	28.92
Bouquet	3.05 + 1.77 x	0.14	12.58	10.29	-	15.32	260.85	159.17	-	415.09
Larval dip	2.79 + 1.59 x	2.33	24.49	18.19	-	33.12	703.26	322.8	-	1471.68
Spray tower	2.39 + 1.57 x	0.69	45.65	36.89	-	56.51	1380.38	774.66	-	2459.69

* .... logx. 101

Table 5. Toxicity of chlorpyriphos to third instar *H. armigera* by different bioassay methods

Methods	Regression equation	χ^2 (df 3)	LD_{50}/ LC_{500}	Fiducial limits LL		UL	LD_{95}/ LC_{95}	Fiducial limits LL		UL
*Topical	0.19 + 3.04 x	0.82	0.38	0.33	-	0.44	2.24	1.49	-	3.36
*Vial	0.13 + 2.71 x	0.96	0.62	0.41	-	0.93	4.5	2.99	-	6.68
Bouquet	1.96 + 3.03 x	1.01	10.09	7.81	-	12.8	59.35	10.59	-	86.62
Larval dip	1.30 + 2.89 x	0.23	19.51	14.87	-	25.56	123.02	85.74	-	176.52
Spray tower	1.91 + 2.12 x	0.63	28.77	20.51	-	40.37	363.08	140.1	-	940.94

* .... logx. 101

The lowest values were observed for chlorpyriphos and highest for endosulfan in all the bioassay methods. The steepness of log - dose - probit - mortality (ldpm) lines for the various insecticides by different methods was more or less same as indicated from the slope function. The slope function was 1.78-1.92 for fenvalerate (Table 2), 1.81-1.92 for cypermethrin (Table 3) and 1.57-1.77 for quinalphos (Table 4). In the case of chlorpyriphos, the slope function was very low (2.12) for the spray tower method whereas for other methods the `b' value was 2.71 for vial, 2.89 for larval dip, 3.04 for topical and 3.03 bouquet method. In the case of endosulfan the slope function ranged from 2.16-3.48, the highest for larval dip and lowest for the topical (2.16) whereas that of the contact and spray tower were 2.41 and 2.45 respectively. The LC99 value was always the least in the vial method followed by bouquet, larval dip and spray tower method for all insecticides indicating the less variation in the response of *H. armigera* for different methods of bioassay.

Tentative Discriminating Doses

Provisional discriminating doses that kill 99 per cent were derived by extrapolation. The LD99: LC99 ratios between topical and other methods were worked out by taking topical method as unity (Table 6).

Table 6. Tentative extrapolated discriminating doses (DD) for different insecticides for different bioassay techniques

Methods	LD_{99}/ LC_{99}	Ratio	Estimated DD	Suggested DD
Endosulfan				
Topical	36.7	1	10	10
Vial	94.4	2.6	26	25
Bouquet	2571.2	70.05	700.5	700
Larval dip	3706.5	100.99	1009.9	1015
Spray tower	14003.67	781.6	3816	3800
Fenvalerate				
Topical	28.66	1	0.2	0.2
Vial	36.46	1.27	0.25	0.25
Bouquet	1123.99	39.33	7.84	8
Larval dip	1559.46	54.41	10.88	10
Spray tower	3330.78	116.22	23.24	23
Cypermethrin				
Topical	12.43	1	1	1
Vial	32.13	2.58	2.58	2.6
Bouquet	133.25	75.08	75.08	75
Larval dip	1501.31	120.78	120.78	125
Spray tower	3328.07	267.74	267.74	270
Quinalphos				
Topical	12.2	1	0.75	0.75
Vial	17.9	1.47	1.1	1.1
Bouquet	260.85	21.38	16.04	16
Larval dip	703.26	57.64	43.23	45
Spray tower	1380.38	113.15	84.86	85
Chlorpyriphos				
Topical	2.24	1	1	1
Vial	4.5	2.01	2.01	2
Bouquet	59.29	26.59	26.59	27
Larval dip	123.03	54.92	54.92	55
Spray tower	363.08	162.09	162.09	163

The LD99: LC99 ratio between topical and vial methods was 2.06 for endosulfan, 1.27 for fenvalerate, 2.58 for cypermethrin, 1.47 for quinalphos and 2.01 for chlorpyriphos. The ratios of LD99: LC99 of topical: bouquet methods were 70.05, 39.33, 75.08, 21.38 and 26.59 for endosulfan, fenvalerate, cypermethrin, quinalphos and chlorpyriphos respectively. The ratios of LD99: LC99 of topical: larval dip was 100.99, 54.41, 120.78, 57.64 and 54.92 for endosulfan, fenvalerate, cypermethrin, quinalphos and chlorpyriphos respectively. The ratios between topical and spray tower method were 781.60, 116.22, 267.74, 113.15 and 162.09 for endosulfan, fenvalerate,

Table 7. Insecticide resistance in different *H. armigera* populations based on percentage survival in different bioassay methods

Methods	Percentage resistance ± SE				
	Endosulfan	Fenvalerate	Cypermethrin	Quinalphos	Chlorpyriphos
TNAU, Lab cultured population					
Topical	8 ± 3.88	78 ± 5.29	44 ± 7.09	28 ± 6.41	8 ± 3.88
Vial	16 ± 5.24	84 ± 5.24	50 ± 7.14	34 ± 6.86	10 ± 4.29
Bouquet	10 ± 4.29	82 ± 5.48	46 ± 7.12	30 ± 6.55	10 ± 4.29
Larval dip	12 ± 4.64	84 ± 5.24	50 ± 7.14	30 ± 6.55	10 ± 4.29
Spray tower	8 ± 3.88	76 ± 6.10	44 ± 7.09	28 ± 6.41	8 ± 3.88
Madurai population					
Topical	10 ± 4.29	68 ± 6.66	30 ± 6.55	22 ± 5.92	10 ± 4.29
Vial	14 ± 4.96	72 ± 6.41	36 ± 6. 86	24 ± 6.10	14 ± 4.96
Bouquet	16 ± 5.24	68 ± 6.66	30 ± 6.55	22 ± 5.92	12 ± 4.64
Larval dip	14 ± 4.96	70 ± 6.55	34 ± 6.86	24 ± 6.10	12 ± 4.64
Spray tower	12 ± 4.64	68 ± 6.66	30 ± 6.55	20 ± 5.71	10 ± 4.29
Chidambaram population					
Topical	30 ± 6.55	76 ± 6.10	50 ± 7.14	32 ± 6.66	18 ± 5.49
Vial	34 ± 6.86	80 ± 5.71	54 ± 7.12	40 ± 6.99	20 ± 5.71
Bouquet	32 ± 6.66	78 ± 5.92	52 ± 7.14	34 ± 6.77	18 ± 5.49
Larval dip	34 ± 6.86	80 ± 5.71	54 ± 7.12	34 ± 6.77	18 ± 5.49
Spray tower	30 ± 6.55	76 ± 6.10	50 ± 7.14	32 ± 6.66	16 ± 5.24
Thalaivasal population					
Topical	20 ± 5.71	80 ± 5.71	28 ± 6.41	20 ± 5.71	-
Vial	24 ± 6.10	84 ± 5.24	34 ± 6.86	22 ± 5.92	-
Bouquet	22 ± 5.92	80 ± 5.71	30 ± 6.55	20 ± 5.71	-
Larval dip	24 ± 6.10	82 ± 5.48	32 ± 6.66	20 ± 5.71	-
Spray tower	18 ± 5.49	80 ± 5.71	28 ± 6.41	18 ± 5.49	-
TNAU field population					
Topical	10 ±4.29	78±5.92	44±7.09	30±3.66	8±3.88
Vial	16. ±5.24	82±5.48	48±7.14	36±6.86	12±4.64
Bouquet	12±4.64	82±5.48	44±7.09	32±6.66	10±4.29
Larval dip	14±4.96	82±5.48	46±7.12	32±6.66	10±4.29
Spray tower	8±3.88	80±5.71	44±7.09	30±6.55	8±3.88

Number of insects tested = 50

cypermethrin, quinalphos and chlorpyriphos respectively.

The discriminating doses of 1.0 µg, 0.75 µg, 0.2 µg, and 10 µg for cypermethrin, quinalphos, fenvalerate and endosulfan used for topical application were multiplied by the respective above ratios. The tentative discriminating doses derived for endosulfan were 25 µg, 700 ppm, 1015 ppm and 3800 ppm, for fenvalerate 0.25, 8 ppm, 10 ppm, 23 ppm, for cypermethrin 2.6 µg, 75 ppm, 125 ppm, 270 ppm and for quinalphos 1.1 µg, 16 ppm, 45 ppm and 85 ppm for the vial, bouquet, larval dip and spray tower method respectively.

The tentative discriminating doses were used to assess the resistance nature of different Helicoverpa populations *viz*., laboratory cultured TNAU (Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India) population and field collected populations from TNAU, Madurai, Chidambaram and Thalaivasal. In the case of chlorpyriphos LD/LC 99 values were used for comparing the different methods (Table 7).

Lab cultured TNAU population exhibited survival rate of 8-16% for endosulfan, 44-50% for cypermethrin, 76-84% for fenvalerate, 28-34% for quinalphos and 8-10% for chlorpyriphos. The survival rate of Madurai population for endosulfan was 10-16%, cypermethrin 30-36%, fenvalerate 68-72%, quinalphos

Table 8. Linear correlations (r) among different resistance monitoring techniques

Methods	Topical	Vial	Bouquet	Larvel dip	Spray tower
Endosulfan					
Topical	1	0.982	0.982	1	0.984
Vial	-	1	0.943*	0.986	0.961
Bouquet	-	-	1	0.982	0.993
Larval dip	-	-		1	0.984
Spray tower	-	-			1
Fenvalerate					
Topical	1	0.985	0.951	0.96	0.957
Vial	-	1	0.962	0.984	0.902
Bouquet	-	-	1	0.989	0.91
Larval dip	-	-		1	0.883*
Spray tower	-	-			1
Cypermethrin					
Topical	1	0.997	0.994	0.989	1
Vial	-	1	0.995	0.998	0.997
Bouquet	-	-	1	0.994	0.953
Larval dip	-	-		1	0.989
Spray tower	-	-			1
Quinalphos					
Topical	1	0.998	0.999	0.994	0.999
Vial	-	1	0.997	0.987	0.997
Bouquet	-	-	1	0.991	1
Larval dip	-	-		1	0.99
Spray tower	-	-			1
Chlorpyriphos					
Topical	1	0.97	0.999	0.999	0.999
Vial	-	1	0.978	0.978	0.978
Bouquet	-	-	1	1	1
Larval dip	-	-		1	1
Spray tower	-	-			1

Significant at p=0.01

* Significant at p=0.05

20-24% and chlorpyriphos 10-14%. The Chidambaram population showed the survival rate of 30-34% for endosulfan, 50-54% for cypermethrin, 76-80% for fenvalerate, 32-40% for quinalphos, and 16-20% for chlorpyriphos. The Thalaivasal population exhibited the survival rate of 18-24% for endosulfan, 28-34% for cypermethrin, 80-84% for fenvalerate and 18-22% for quinalphos. TNAU field collected population showed the survival rate of 8-16% for endosulfan, 44-48% for cypermethrin, 78-82% for fenvalerate, 30-36% for quinalphos, and 8-12% for chlorpyriphos.

Though the degree of resistance was found to be varying among the different populations and with the insecticides, the assessment made by different methods

did not indicate much variation except in one or two cases.

The linear correlation co-efficients (r) were worked out to establish the relationship among different assay technique (Table 8). The variation in the survival rate as assessed by different assays was not much; the `r' values being 0.943-1.0 for endosulfan, 0.953-1.0 for cypermethrin, 0.883-0.989 for fenvalerate, 0.987-1.0 for quinalphos and 0.970-1.0 for chlorpyriphos.

Perfect relationship (r = 1) was obtained in cases topical vs. larval dip for endosulfan, topical vs. spray tower for cypermethrin, bouquet vs. spray tower for quinalphos, bouquet vs. larval dip, bouquet vs. spray tower and larval dip vs. spray tower for chlorpyriphos. The `r' values obtained for vial vs. bouquet for endosulfan and larval dip vs spray tower for fenvalerate were significant at p=0.05. The `r' values for all other combination were significant at p=0.01.

It indicated that any one of the above methods could be used for monitoring the resistance by field level functionaries. Among these the larval dip method was found to be easy-to-adopt. A ready to use field kit has been designed and is being tested by different institution.

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Differential Susceptibility of Pink and Spiny Bollworms to the Ova-larvicidal Activity of Spinosad, a Natural Metabolite of the Actinomycete *Saccharopolyspora spinosa* with Special Reference to Solve the Field Failure of Thiodiocarb in the Current Resistance Rotation Spraying Program in Egypt

ABSTRACT Laboratory, field-laboratory and field trials were conducted to evaluate the response of the pink boll worm, *Pectinophora gossypiella* and the spiny bollworm, *Earias insulana*, to the natural product spinosad.

These trials confirmed that eggs and larvae of *E.insulana* were more susceptible to spinosad than eggs and larvae of the pink boll worm.

After dipping bolls in 0.05 ppm spinosad, successful penetration of neonate larvae% was 57 and zero for pink and spiny, respectively.

Field rotation trials showed that the best program for control of both bollworms utilized chlorpyrifos-ethyl mixed with hexaflumuron, followed by es-fenvalerate , profenfos and ending with spinosad+oil. This program showed 92% reduction. Other programs

were in descending order as reduction %; program ending with carbaryl (88.5),spinosad alone (77), and thiodiocarb (69). Spinosad could replace the old chemicals as thiodiocarb or carbaryl in the current official resistance rotation program in Egypt.

KEY WORDS Pink and spiny boll worms, spinosad, rotation program, cotton

INTRODUCTION Egypt produced more than 90 % of the global production of the tetraploid, *Gossypium barbadense* cotton. The author believed that this type of cotton is not nutritionally suitable for the establishment of *Helicoverpa* (Heliothis) spp, in Egypt. Over the last 50 years this pest never exceed the economic injury level on cotton although of its availability on other crops as tomato and maize. Cotton infestation by this pest is too low to be considered. However, almost all countries cultivating *Gossypium hirsutum* are suffering from this pest on cotton except Egypt.

The pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), and spiny bollworm (SBW), *Earias insulana* (Boisd), are considered to be the two most destructive pests of cotton bolls in Egypt. They are worldwide in distribution and cotton is considered to be the preferred host for these insects. Each species can affect cotton seed and lint yield as quality and quantity. Egypt spends 15-20 million dollars to combat cotton bollworms (CBW) on an area of about 800 000 acres every year.

The first nationwide program strategy of alternating different chemical classes to combat cotton leafworm and CBW was adopted by the Egyptian authorities in cooperation with Dr .R.M. Sawiki in 1979 (Temerak 2002). Accordingly, in 1979 Ministry of Agriculture (MOA) started the rotation resistance program of different insecticide groups, with severely restricted use of pyrethroids. Pyrethroids have to be applied only on cotton and restricted to one spray /season (Sawicki and Denholm 1987).

Spiny bollworm is considered to be more dangerous pest than PBW for several reasons: a) SBW has no diapause in the seeds to be controlled in the factories at 55-58 C as in case of PBW b) SBW can attack more than one boll and have one generation more than PBW (6 versus 5 generations) (Abdel-Hamid et al 1999,Hossain et al 1999) c) PBW larvae can not attack very old bolls (7-week-old) but spiny can (Kalifa et al 1980, Ragab 1999) d) One unit pink infestation in the early cotton growing season caused 5 % reduction of total yield weight while in case of spiny caused 6-9 % loss (El-Saadany et al 1975).

Pink bollworm is the dominant cotton pest, established throughout Egypt, and SBW is concentrated in Upper Egypt (1/3 of the total area), south of the delta. (Abul-Nasr et al 1971). Spiny bollworm usually attacks non-infested bolls by pink and vise versa in the field. In 1994-1998, rotation resistance program was almost stopped by the introduction of disruption pheromones for pink only (Temerak 2002). The new program of MOA was the application of disruption pheromone for pink only and apply PYs or OPs when infestation reach 3 %. As matter of doing this, bolls were saved for spiny which invaded new areas and almost established in most of the cotton governments, and became a key pest in the south of Egypt (Ragab 1999).

In 1998, MOA stopped completely the disruption pheromones of PBW and back again to rotation resistance program due to the great loss in the yield in 1998 (Temerak 2002). This great loss mostly due to the partial replacement and the establishment of SBW over PBW. MOA returned back carbaryl for the control of CBW specially spiny after being banned in 1996 due to the resistance field failure of thiodiocarb in most of governments. Disrupted functional ecosystem consequences of increased pesticide use induced by resistance (Hollingworth and Whalon 2002).

MOA believed that CARs were the best insecticides for spiny control at the last spray and holding only one CAR (Carbaryl) with no other promising products or better performance. Egypt is hoping to find a product safe, low dose in the environment, with satisfactory killing power specially for spiny bollworm. So, spinosad was chosen because it is classified by EPA as a reduced risk product and awarded the green chemical challenge award from the white house in the USA in 1999 (http://www.epa.gov/greenchemistry/past.htm).
Furthermore, Spinosad was undertaken in order to provide an alternative of other resource to control CBW mainly spiny bollworm late in the growing season.

MATERIALS AND METHODS Spinosad, spintor 24SC (Dow AgroSciences Co), was used for laboratory & field tests. Spinosad is a naturally occurring mixture of two active components (spinosyn A and D) produced by fermentation of the soil actinomycete. *Saccharopolyspora spinosa* Martz & Yao.

Laboratory trials /dipping eggs

Pink bollworm and SBW were both reared on an artificial diet as described by Rashed and Ammar (1984-85), under laboratory conditions (temp.= 25 C / R.H. = 50 %). Single eggs on piece of white clothes were removed on a daily basis in order to have different ages of eggs as 1-, 2-, and 3-day-old. Different concentrations of spinosad as: 0.1, 0.25, 0.5, 1, 2.5, 5, 10, and 15 ppm were prepared. Six eggs were used as one replicate. Four replicates were served / one treatment (24 eggs). Eggs were dipped in various concentrations of spinosad for two second and left to be dry for one hour under lab. condition. Check eggs

were dipped in water only and another check without dipping. Counts of unhatched eggs, dead and alive larvae were done when check eggs hatched.

Statistical analysis

Data were analyzed by f-test. L.S.DTest was used to compare among means.

Field-Lab. Trials / dipping bolls

Cotton naked bolls (without leaves) as 2-3 week-old were collected from the field in an isolated area where no CBW infestation in the middle of Cairo. Cotton variety was Giza 85. Bolls were dipped for two seconds in spinosad concentrations of 0.0125, 0.025, 0.05, 0.1, 0.25, 0.5, and 1.0 ppm and left to air dry under laboratory conditions for one hour. Two 1st instar larvae, less than one day old, were placed in a plastic container with a single cotton boll. This container measured 7 x7 by 5 height cm. Four bolls were served per one treatment. Inspection of bolls was done 8 days after dipping to count alive larvae inside, feeding signs, and successful penetration into the bolls. Containers were held under lab. conditions.

Field Trial

Field trials were conducted at the experimental research station at Bohira Governorate. Planting date was mid of April in 2002. Cotton variety was Giza 89. Insecticide spray was applied when about 30% of bolls attained their final size(mid of July). The MOA insecticide rotation program was used for the first 3 insecticide applications, but not the fourth or last spray that was the focus of our study.

Formulations and rates

First spray: Chlorpyrifos ethyl 75 WG (Dursban) at 640 g ./ acre. +Hexaflumuron 10EC (consult) at 200ml/acre

Second spray: Es-fenvalerate 5 EC (Sumialfa) at 600 ml / acre

Third spray: Profenfos 72 EC (Curacuron) at 750ml/acre

Fourth spray: Carbaryl 85WP (Sevin) at 1.5 kg. /acre orThiodiocarb 80SP (Larvin) at 0.5 kg. / acre or Spinosad 24 SC (Spintor) at 50 ml / acre or Spinosad 24 Sc (Spintor) at 50 ml + mineral oil 96 % at 1L / acre

Programs

Four different spray programs were used. In each program, four different insecticide spray regimes were applied at two weeks interval. The first, second, and the third insecticide spray were common to all programs as MOA way utilizing Dursban+consult, Sumialfa, and curacuron at their recommended rates, respectively. At the last spray, different manipulations were applied by spraying Sevin (standard) or Larvin or Spintor alone or Spintor mixed with oil.

Application

A Solo sprayer delivering 12 L water volume was used for all spray applications. One acre consumed 240 L of water. Plot size was 6 m wide and 7 m long. There were four replicates of each treatment.

Assessment

Twenty-five bolls were randomly collected from each replicate as 100 bolls / one treatment. Infestation counts of alive larvae for both PBW and SBW were done on weekly basis. Reduction % was calculated utilizing Abbot formula for the last count. Handerson and Tilton formula was not used because the pre-spray count were zero.

RESULTS AND DISCUSSIONS

Laboratory trials / dipping eggs

Hatchability and mortality % of 1-, 2-, and 3-day-old of pink single eggs after being dipped in different concentrations of spinosad (Spintor24 SC) are shown in Table 1. The same for spiny are shown in Table 2. The combined of 1-, 2-, and 3-day-old of both pink and spiny are summarized in Table 3.

Table 1: Hatchability and mortality % of different ages of pink bollworm eggs after being dipped in different spinosad concentrations

	1 DAY -OLD EGGS			2 DAY -OLD EGGS			3 -DAY-OLD EGGS		
Concent. PPM	unhatched eggs	dead larvae	% mortality	unhatched eggs	dead larvae	% mortality	unhatched eggs	dead larvae	% mortality
0.1	0	0	0	0	0	0	0	0	0
0.25	0	0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0
2.5	2	0	8.3	0	12	50	2	10	50
5	4	8	50	4	10	58.3	2	12	58.3
10	6	8	58.3	4	12	66.66	4	12	66.66
15	6	10	66.66	4	14	75	6	14	83.3
Average/rep	0.563a		1.375A	0.375b		1.875B	0.438ab		1.938B
CheckAorB	0	0	0	0	0	0	0	0	0
L.S.D 0.05	0.173		0.383	0.173		0.383	0.173		0.383

Check A = bolls dipped in water Check B = without water Figures followed by the same type letter are not significant

Table 2: Hatchability and mortality % of different ages of spiny bollworm eggs after being dipped in different spinosad concentrations

	1 DAY -OLD EGGS			2 DAY -OLD EGGS			3 -DAY-OLD EGGS		
Concent. PPM	unhatched eggs	dead larvae	% mortality	unhatched eggs	dead larvae	% mortality	unhatched eggs	dead larvae	% mortality
0.1	3	13	66.6	2	10	50	2	4	25
0.25	6	18	100	4	16	83.3	2	10	58.3
0.5	6	18	100	6	18	100	4	10	58.3
1	8	16	100	6	18	100	6	16	91.6
2.5	16	8	100	14	10	100	12	12	100
5	16	8	100	16	8	100	14	10	100
10	18	6	100	16	8	100	14	10	100
15	18	6	100	16	8	100	14	10	100
Average/rep	2.884a		5.75A	2.5b		5.5A	2.125c		4.688A
CheckAorB	0	0	0	0	0	0	0	0	0
L.S.D.0.05	0.295		0.437	0.295		0.437	0.295		0.437

Check A = bolls dipped in water Check B = without water Figures followed by the same type letter are not significant

Based on Table 1, no significant difference was recorded for the hatchability between 1 & 3-day-old and between 2 & 3-day-old of pink eggs. However, the younger eggs was significantly the most sensitive to spinosad in case of spiny eggs (Table 2) Based on the summary Table 3, at 2.5 PPM unhatched eggs were 58.3 % and 5.56 % for spiny and pink, respectively. The same dose revealed 100 and 36 % of total mortality for spiny and pink, respectively. Spiny eggs were significantly sensitive to spinosad at all ages than pink eggs. Total mortality of unhatched eggs plus dead larvae (ova-larvicidal mortality) was significantly greater in case of spiny than pink. Spinosad has good

ova-larvicidal effect on *Heliothis* eggs and most of mortality in treated eggs was from larvae ingesting spinosad as they fed on the chorion of the eggs (Nolting et al 1970).

Field- laboratory trial / dipping bolls

Successful penetration of first instar larvae of PBW or SBW into the bolls after being dipped in different spinosad concentrations are presented in Table 4. At 0.05 ppm spinosad, none of the SBW larvae successfully penetrate and lived within the cotton boll. At the same dosage of spinosad, 57 % PBW larvae successfully penetrated and lived within the treated cotton bolls. These results confirmed that SBW larvae were more susceptible to spinosad toxicity than PBW larvae. However, at the spinosad dosage rates of 1.0 ppm, no PBW larvae successfully penetrated and lived within the treated cotton bolls. SBW larvae were more susceptible to Abamectin, a natural product of another actinomycete *Streptomyces avermitilis* than PBW larvae (Sonia et al 1994).

In the check, there was only one SBW larva per boll while there were 1-2 larvae PBW per boll. These results reflect the normal behavior of SBW and PBW under field condition in Egypt. This may explain the low rate of successful penetration by SBW in the untreated control.

Table 3: Combined ova-larvicidal mortality of 1-, 2- , 3-day-old of pink and spiny bollworm eggs after dipping in different spinosad concentrations (summary of table 1 and 2)

PPM of Spinosad	pink bollworm/tr unhatched eggs %	pink bollworm/tr total mortality%	spiny bollworm/tr unhatched eggs %	spiny bollworm/tr total mortality%
0.1	0	0	9.7	47.2
0.25	0	0	16.6	80.5
0.5	0	0	22.2	86.1
1	0	0	27.8	97.2
2.5	5.56	36.1	58.3	100
5	13.89	55.5	63.8	100
10	19.4	63.88	66.7	100
15	22.2	75	66.7	100
Avg.No/rep	**1.375B**	**5.188b**	**7.469A**	**15.938a**
CHECK	0	0	0	0
L.S.D 0.01	0.399	0.387	0.399	0.387

Data presented all replicates
Total mortality= unhatched eggs + dead neonate larvae
Figures followed by the same type letter are not significant

Table 4: Succesful penetration of 1st instar larvae of CBW into cotton bolls after dipping bolls in various concentration of spinosad

	Pink larvae				Spiny larvae			
PPM	No.dead larvae outside bolls	No.alive larvae inside bolls	successful penetraion of larvae %	Corrected mortality %	No.dead larvae outside bolls	No.alive larvae inside bolls	successful penetraion of larvae %	Corrected mortality %
0.0125	2	6	**75**	85.7	6	2	**25**	50
0.025	4	5	**62.5**	71.4	7	1	**12.5**	25
0.05	4	4	**50**	57.1	8	0	**0**	0
0.1	5	3	**37.5**	42.9	8	0	**0**	0
0.25	6	2	**25**	28.6	8	0	**0**	0
0.5	7	1	**1.25**	1.4	8	0	**0**	0
1	8	0	**0**	0	8	0	**0**	0
CHECK	**1**	**7**	**87.5**		**4**	**4**	**50**	

Two larvae of less than 1-day-old accompanied each boll, 4 bolls / one tr.

ppm, no PBW larvae successfully penetrated and lived within the treated cotton bolls. SBW larvae were more susceptible to Abamectin, a natural product of another actinomycete *Streptomyces avermitilis* than PBW larvae (Sonia et al 1994).

In the check, there was only one SBW larva per boll while there were 1-2 larvae PBW per boll. These results reflect the normal behavior of SBW and PBW under field condition in Egypt. This may explain the low rate of successful penetration by SBW in the untreated control.

Comparing results of Table 3 and 4, it could conclude that eggs and larvae of spiny were more susceptible than of eggs and larvae of pink. Similar results were achieved by Ochou and Martin (2002) in West Africa. Also, larvae of both were more susceptible than eggs. Since the eggs are lasted in the field for 3 days for spiny and 4 days for pink approximately, while the first instar larvae of both remains out side bolls from few minutes to few hours until they penetrate (El-Sayed and Azab 1969, El-Sayed and Abder-Rahman 1960), any good product to control both must have ova-larvicidal activity and /or long residual effect in order to catch the moments of larval presence outside the bolls.

Field trial with 4 different rotation programs

Infestation % of spiny and pink, is presented in Table 5a and 5b, respectively. Both insect infestation were summarized in Table 5c. Very good performance of Dursban 75 WP+Consult, Sumi alfa, and curacuron was noticed during in the 1st, 2nd, and the 3rd spray, respectively. Based on the last count of the 4th spray which reflect the whole season accumulated effect the following were the major results:

Spiny infestation: Program ending with spintor plus oil was the best and performing 100% reduction. Spintor alone was equal to sevin and better than of larvin.

Pink infestation: Program ending with spintor plus oil was equal to sevin (89.5% reduction), and better than that of spintor alone or larvin.

Both spiny and pink: The best programs in descending order that showed good reduction % were that ending with spintor plus oil (92 %), sevin (88.5 %), spintor (77%) alone, and larvin (69%). Adding oil mostly protect spintor from UV light and consequently prolong the residually. No further infestation of spiny was noticed after spraying spintor mixed with oil. Adding mineral oil enhance performance and elongate residually of certain products (El-Deeb 1993, Mourad et al 1994) .No build-up of mites was noticed in all plots, especially spintor plots.

Chlorpyrifos had the best ovicidal activity against PBW (El-Sayed et al 1980). Based on the results of spinosad's activity against SBW eggs and larvae,

Table5a: Infestation % of spiny bollworm after using different four spray rotation program on cotton, 2002

Last spray program	Pre	1st spray Dursban+ consult (640g+200ml)		2nd spray sumialfa 5% at 600 ml/acre		3rd spray curacuron 72 at 750ml /acre		4th and last spray product #		Reduction %
Rate/ acre. #	spray count	1st week	2nd week	1st week	2nd week	1st week	2nd week	1st week	2nd week	Of last count
Sevin85WP at 1.5k	0	0	0	0	0	1	0	1	1	85.7
Larvin80SP at 0.5k	0	0	0	0	1	1	0	2	3	57.1
Spintor24SC at 50ml	0	0	0	0	0	0	1	0	1	85.7
Spintor24 50ml+1 Loil	0	0	0	0	0	1	0	0	0	100
Check	0	0	1	2	3	3	5	6	7	

Table 5b: Infestation % of pink bollworm after using different four spray rotation program on cotton, 2002

Last spray program	Pre	1st spray Dursban+ consult (640g+200ml)		2nd spray sumialfa 5% at 600 ml/acre		3rd spray curacuron 72 at 750ml /acre		4th and last spray product #		Reduction %
Rate/ acre. #	spray count	1st week	2nd week	1st week	2nd week	1st week	2nd week	1st week	2nd week	Of last count
Sevin85WP at 1.5k	0	0	0	1	2	1	2	1	2	89.5
Larvin80SP at0.5k	0	0	0	2	2	2	2	4	5	73.6
Spintor24SC at 50ml	0	0	0	1	2	2	2	3	5	73.6
Spintor24 50ml+1 Loil	0	0	0	1	2	2	1	2	2	89.5
Check	0	1	3	5	10	12	13	15	19	

Table5c: Infestation % of both bollworms after using different four spray rotation program on cotton, 2002

Last spray program	Pre	1st spray Dursban+ consult (640g+200ml)		2nd spray sumialfa 5% at 600 ml/acre		3rd spray curacuron 72 at 750ml /acre		4th and last spray product #		Reduction %
Rate/ acre. #	spray count	1st week	2nd week	1st week	2nd week	1st week	2nd week	1st week	2nd week	Of last count
Sevin85WP at 1.5k	0	0	0	1	2	2	2	2	3	88.5
Larvin80SP at0.5k	0	0	0	2	3	3	2	6	8	69.2
Spintor24SC at 50ml	0	0	0	1	2	2	3	3	6	76.9
Spintor24 50ml+1 Loil	0	0	0	1	2	3	1	2	2	92.3
Check	0	1	4	7	13	15	18	21	26	

Spintor could be alternated with Chlorpyrifos and other chemicals in the MOA rotation resistance program. Applying insecticides with different modes of action can help delaying insecticide resistance (Sawicki and Denholm 1987).

The existed rotation resistance program to combat CBW is dealing with 4 sprays only in comparison to > 7-11 sprays in Sudan and most of other cotton growing countries. As part of this success is the banned use of PYs outside cotton which still valid until now following Dr. Sawiki recommendation (Temerak 2002). It is generally recommended that using spinosad plus oil as terminal treatment in the current resistance rotation program to replace larvin might be a good plan. Also, these studies could offer another resource of sevin to control CBW specially SPW after the last pest being established in most of the growing cotton governorates.

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Insecticide Cross Resistance in Cypermethrin Selected Strains of *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera)

Helicoverpa armigera is a major polyphagous pest and has been reported to cause extensive economic losses. Resistance is a major problem with chemical control of this pest, and it is important to know about the insect resistance in which selection of resistant populations by one agent is accompanied by resistance to another compound that may or may not be either related chemically or in their mode of action. Hence, knowledge of resistance mechanisms involved in a particular insecticide will allow a more rational choice of a follow up compound, by avoiding one that is likely to be affected by the resistance mechanism operating (Basavana goud and Lingappa, 2001). Therefore in the present study development of cross resistance pattern to other insecticides in cypermethrin selected strain of *Helicoverpa armigera* was studied.

About 600 larvae of *Helicoverpa armigera* collected from cotton fields from Jewargi (High insecticide imposed area) were reared on artificial diet individually in glass vials to get the next generation larvae. The susceptible strain maintained in the laboratory was used for comparison. Fenvelarate, carbaryl, monocrotophos and endosulfan were assayed for both resistant and susceptible strains. Thirty third instar larvae in each of four replicates were administered with cypermethrin dissolved in acetone at five concentrations. One µl of each concentration was topically applied to the thorasic dorsum of each larvae with the help of Hamilton micro applicator. The treated larvae were transferred to glass vials containing the artificial diet. Dead and moribund larvae were counted at 72 hour after treatment and surviving larvae in the highest concentration were reared to get the next generation larvae which were reared again subjected to similar test. Thus the selection was made in eight successive generations to get cypermethrin resistant strain. The third instar larvae from the 9th generation were exposed to different insecticides as explained above to assess the development of cross resistance by computing the LD50 values for each insecticide. Mortality data were subjected to log dose probit analysis using MLP software package to obtain LD50 values for each insecticide. Cross resistance patterns in the cypermethrin selected strain was determined by the ratio between the LD50 value of insecticide for the cypermethrin selected strain and the corresponding value for the susceptible strain.

The resistance ratio computed from LD50 values to susceptible and cypermethrin selected strain reflected that cross resistance to fenvelarate (4.14) was distinct, moderate to monocrotophos (2.42) and endosulfan (2.33) and marginal to carbaryl (2.07) (Table 1).

Table 1.Development of cross resistance in cypermethrin selected strains of *Helicoverpa armigera* to different insecticides

	Susceptible strain			Cypermethrin selected strain			
Insecticide	LD_{50}	95% FL	Slope	LD_{50}	95% FL	Slope	R.R*
Fenvalerate	1.025	0.895	2.011	4.251	3.924	1.824	4.14
		1.061			5.642		
Carbaryl	0.825	0.628	1.71	1.254	0.954	1.699	2.07
		1.081			1.421		
Monocrotophos	1.324	1.054	1.772	3.214	2.794	2.145	2.42
		1.742			4.015		
Endosulfan	2.149	1.707	1.624	5.025	3.924	1.947	2.33
		2.854			6.874		

* LD50 for cypermethrin selected strain/ LD50 for susceptible strain

Priester and Georghiou (1980) and Golenda and Forgash (1985) were also of the view that the selection for resistance to one pyrethroid confer, at least, partial resistance to many other pyrethroids. Jenson et al. (1984) also reported high level of cross resistance in permethrin selected strain of *H.virescens* to cypermethrin. Leonard et al. (1988) also reported the spectrum of cross resistance in fenvalerate resistant strain to several other pyrethroids tested was wide in *H.virescens*. The present results do not form an exception to the generalized impact of resistance developed to one pyrethroid to other pyrethroids.

The cross resistance to monocrotophos was distinct. In line with the present findings regarding cross-resistance capacity in cypermethrin selected strain to monocrotophos, Campanahola and Plapp (1987) reported similar phenomenon in pyrethroid resistant strain of *H.virescens* to several organophosphorus insecticides. In contrast to 1.78 fold cross resistance to endosulfan noticed in cypermethrin strain of *H.armigera*, 5 fold increase in cypermethrin strain of *Earias vitella* is reported by Saini et al.(1989). In the latter case fenvelarate selected strain showed high cross resistance (2800fold) to endosulfan.

Almost similar comparison prevails between cypermethrin strain of *H.armigera* and other insects to cross resist carbaryl. The level of cross resistance to carbaryl in cypermethrin selected strain was 3 fold in *Earias vitella* (Saini et al., 1989) and 3.60 fold in *Spodoptera litura* to methomyl after 14 generations (EL - Sayed et al., 1985).

On the strength of limited information on cross resistance in pyrethroid resistant strain to representative four classes of insecticides, the scope of new pyrethroids to come, would be at stake in view of the above phenomenon operating uninterruptedly and accelerating steadily. Hence, new molecules of pyrethroids are not an answer to tackle pyrethroids resistant population of *H.armigera*. Conversely, the conventional insecticides like endosulfan, monocrotophos and carbaryl which were pushed aside for a while in the wake of expanded use of pyrethroid,

seems to be the better alternatives while blending chemical component in the management strategy for *H.armigera* in cotton ecosystem. It also means that if the pyrethroids are to be retained, in view of their increased effectiveness, their usage have to be contained to minimum.

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Status of Paddy Insect Pests in Unsprayed Fields of Tungabhadra Project Area of Karnataka

Rice (*Oryza sativa* L.) is one of the important cereal crops of the world and forms staple food for more than 50 per cent of the population and known as king of cereals. In India, rice is grown over an area of 42.04 million hectares with a production of 86.3 mt (Anonymous, 2002). Tungabhadra project, which is known as "Rice Bowl" of Karnataka, occupies a prominent place in the rice map of India accounting for nearly 3.62 lakh ha area under paddy.

A survey was undertaken during kharif season, of 2001-2002 in five districts representing paddy growing area of Tungabhadra project *viz*., Raichur, Manvi, Sindhanur, Sirguppa and Gangavati. Fixed plot survey and rowing survey was followed at fortnightly interval from July to December 2001 in both nursery and transplanted field. For fixed plot surveys, in each district ten farmers fields were marked and in each field, 25 hills were observed at random for the incidence of paddy insect pests after transplanting. Whereas, in nursery 25 clumps were observed at random for the incidence. Two unsprayed plots were maintained at agricultural research stations of Gangavati and Sirguppa.

Comparative study on the status of paddy insect pests in Tungabhadra Project revealed that only few insect species attacked the crop in unsprayed fields when compared to the farmers fields when regular spray was undertaken, (Table 1). Among the insect

Table 1. Population of paddy insect pests in farmers fields and unsprayed fields

Insect pests	Farmers field*	Unsprayed field**
1) Stem borer		
a) Dead heart (%)	2	1.2
b) White ear (%)	2.5	1
2) GLH/hill	0.78	0.71
3) BPH/hill	8.82	7.3
4) Leaf folder		
a) Larvae/hill	0.74	0.46
b) Cumulative leaf damage (%)	0.04-6.10	2.16-3.72
5) WBPH/hill	12	0
6) Skipper/hill	0.56	0

*Average of 50 locations **Average of two locations

pests observed, mean stem borer incidence of 1.20 per cent dead hearts during vegetative stage and 1.00 per cent white ears at harvest was recorded in unsprayed fields as against 2.00 per cent dead hearts and 2.50 per cent white ears in farmers field. The mean green leaf hopper population of 0.71 per hill and mean brown plant hopper counts of 7.30 (Adults and nymphs) per hill were recorded in unsprayed fields as against 0.78 green leaf hopper per hill and 8.820 brown plant hopper per hill in farmers field. Mean leaf folder larvae of 0.46 per hill and cumulative leaf damage ranging from 2.16 per cent to 3.72 per cent was recorded in unsprayed fields as against 0.74 larvae per hill and 0.04 to 6.10 per cent cumulative leaf damage in farmers field. There was no infestation of white backed plant hopper, thrips, paddy cut worm and skipper in unsprayed fields, whereas they were found in farmers fields in Tungabhadra Project.

The low populations of insect pests in unsprayed fields compared to the farmers field is due to the higher level of natural enemy complex especially spiders and mirids and also due to the application of recommended dose of fertilizers particularly Nitrogen. The present investigations on the lower pest population in unsprayed plots are in conformity with the findings of Katti et al. (2000). Natarajan and Scaria (1988) also reported higher leaf folder damage in maximum protection treatments.

The higher infestation of pests above ETL in farmers fields of TBP is probably due to improper cultivation practices. Farmers applied high fertilizer rates and practice double or continuous cropping. Insecticides may not be critical to insect outbreaks. Many farmers in TBP area spay maximum rounds of synthetic Pyrethroids and Quinalphos to their rice crop as early as one to two weeks after transplanting regardless of number of insect pests or beneficial arthropods. Quinalphos and Synthetic Pyrethroids have been reported to be a BPH resurgence inducing insecticides (Reissg et al., 1982)

Naturally occurring biological control has a potential role to play in the management of rice pests in tropical south and south East Asia and there is a need to emphasize the impact of indigenous natural enemies as an essential part of IPM programmes (Way and Heong, 1994; Ooi and Shephard, 1994).

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Differential Susceptibility in Field Population of *Helicoverpa armigera* (Hubner) to Some Conventional Insecticides at Jammu (India)

ABSTRACT Laboratory bioassays were conducted to observe the susceptibility level of field collected and susceptible strain of *Helicoverpa armigera* to some conventional insecticides viz., endosulfan, malathion, monocrotophos, cypermethrin, fenvalerate and carbaryl with various discriminating concentrations during 2002. The results revealed that the level of susceptibility in the third-instar larvae of the insect had decreased considerably in the field population as compared to the recommended concentrations of these insecticides. The highest resistance factor of 2.88 was observed with carbaryl followed by cypermethrin

(2.48). Malathion encountered lowest resistance factor of 1.51 which indicated that the insect was more susceptible to it than all other insecticides. It was concluded that the indiscriminate use of these insecticides in future could help this insect to increase its tolerance to the levels achieved in other parts of the country.

INTRODUCTION In India, *Helicoverpa armigera* (Hubner) is the most destructive polyphagous pest attacking cereals, pulses, vegetables, and other commercial crops. Due to the indiscriminate use of insecticides to control it, several reports of development of resistance in this pest to almost all classes of compounds in India (Reed and Pawar, 1982; Phokela and Mehrotra, 1985; Phokela et al., 1989; Armes et al., 1994) and other parts of the world (Goodyear et al., 1975; Ahmad and McCaffery, 1988; Gunning et al., 1989; Martin et al., 2000) have been documented. Several cash crops including the seasonal /off-season vegetables are grown abundantly in the state of Jammu and Kashmir for local consumption as well as for exporting them to other states. To combat the menace of this pest, farmers rely heavily on insecticides considering only high economic returns without paying any attention to their ill-effects. No reliable data are available regarding the consumption of insecticides against this particular pest. Some cumulative unofficial figures regarding availability of pesticides from the state government reveal that during 1999-2000, the technical grade pesticide consumption was 1384 metric tones (MT) in the horticulture sector alone and a demand of 1399 MT for 2000-2001 had been projected. Besides 453 sale outlets of the state government, there are 1150 private pesticide traders and 5 cooperatives/agro sale points. There are 22 registered pesticides available in the horticulture sector in the state, out of which 9 are insecticides and 13 are fungicides. At present, the rate of consumption of pesticides in the state is 37g /ha which is very low as compared to that at the national level. However, the state government had planned to increase it to 70g/ha in the succeeding plans. Keeping in view the pesticide consumption and its trade in the state and the propensity of *H. armigera* to develop resistance to insecticides, a need was realized to revisit the levels of susceptibility to some conventional insecticides recommended by various state agencies and the entomologists for the control of this pest resulting into the formulation of present study.

MATERIALS and METHODS

Insect rearing

The culture of *Helicoverpa armigera* was initiated by collecting about 200 larvae of different stages from the unsprayed farmers' fields of pea crop around Jammu and brought to the laboratory for further multiplication. Only the pre-pupae were retained to obtain adult moths. Five pairs of adults were released in glass jars covered with black muslin cloth for oviposition to rear F1 generation of the insect. Neonates were reared on fresh leaves of *Rumex* sp. and subsequent instars on artificial diet prepared as per Singh and Rembold (1992). A susceptible culture of the insect maintained on semi-synthetic diet for at least 22 generations was procured from Delhi. Third instar larvae were used for the bioassay studies.

Preparation of Insecticidal Concentrations

The proprietary products of all the insecticides were used to prepare one percent stock solution in acetone from which further dilutions were prepared subsequently.

Bioassay Method

Sub-lethal concentrations of insecticides were applied to the thoracic dorsum of each third instar larva of susceptible as well as field strain of the insect with MERCK micropipette @ 1.0 µl/larva. Ten larvae per replicate were treated with one concentration of each insecticide. A minimum of 4 concentrations for each insecticide excluding control were tested with three replications maintained for each concentration. The treated larvae were housed in Petri plates (9 cm.dia) individually and shifted to BOD incubator (26±10C; 12:12 L/D period). The mortality data were recorded 24 hours after the treatment.

Statistical analysis

Data were subjected to probit analysis for computing the regression equations and LC50 values with Statistical programme for social sciences (SPSS 8.0). Resistance factor (RF) was calculated by using the formula suggested by FAO, Rome (1979).

RESULTS and DISCUSSION The response of *H.armigera* larvae in terms of mortality was achieved up to 100 percent in the susceptible strain with fenvalerate (0.20%) followed by cypermethrin (96.66%) at 0.15 percent concentration as compared to field strain where 100 percent mortality was recorded with malathion at 0.25 percent followed by fenvalerate (80%) at 0.25 percent. Perusal of the toxicity data in Table 1 (Figures 1 & 2) clearly indicates the low susceptibility of the field collected strain of *H.armigera* compared to the susceptible one.

Table 1. Dose mortality response of *Helicoverpa armigera* to some commonly used insecticides

Insecticide	Population	Heterogeneity $\chi^2_{(n-2)}$	Regression equation	Slope	Fiducial limits 95% CF	LC_{50}	Resistance factor
Endosulfan	Susceptible	10.051	Y=-1.12+12.34x	12.34	0.003-0.13	0.09	1.75
	Field	8.246	Y=-0.95+ 6.04x	6.04	0.08-0.21	0.158	
Malathion	Susceptible	9.8	Y=-0.94+14.27x	14.27	0.02-0.10	0.066	1.51
	Field	8.193	Y=-1.14+11.13x	11.12	0.06-0.13	0.1	
Monocrotophos	Susceptible	10.733	Y=-0.95+13.87x	13.87	0.001-0.11	0.068	1.98
	Field	5.499	Y=-1.14+8.47x	8.47	0.05-0.21	0.135	
Cypermethrin	Susceptible	7.712	Y=-0.47+19.39x	19.39	0.005-0.01	0.05	2.48
	Field	7.37	Y=-0.88+7.08x	7.08	0.09-0.15	0.124	
Fenvalerate	Susceptible	14.283	Y=-0.62+16.06x	16.06	0.002-0.07	0.038	2.13
	Field	11.023	Y=-0.72+8.87x	8.87	0.03-0.13	0.081	
Carbaryl	Susceptible	6.798	Y=-1.17+19.87x	19.87	0.001-0.01	0.059	2.88
	Field	6.605	Y=-1.02+6.0x	5.99	0.14-0.20	0.17	

The field population recorded higher LC50 values for all the insecticides where as they were considerably low for the susceptible population. The increase in tolerance manifested to carbamate and both the synthetic pyrethroids was more as compared to organophosphates. The highest resistance factor of 2.88 with carbaryl was indicative of its increased use in the field for the control of this pest. The lowest RF (1.51) was recorded for malathion followed by monocrotophos (1.98).Our results are near to the lower limit of those obtained by Kapoor et al. (2000) in the neighbouring state of Punjab who recorded 3 to 5-fold increase in the level of resistance to cypermethrin in *H.armigera*. The differences in the results are obvious due to the fact that the insect experiences eight to ten sprays of different kinds of insecticides during cotton cropping season in Punjab which is not grown in our state. Reports from Haryana (Lal, 1998), Delhi (Kodandaram et al.2003), Gujarat (Patel et al., 1992; Lande and Sarode, 1993; 1995) also reveal differential susceptibility of *H.armigera* to all these insecticides. However, considering the low consumption of insecticides in our state, it could be inferred that *H.armigera* may exhibit its capacity to develop resistance to all these compounds if the promiscuous use of these insecticides is not curtailed and the farmers need to be intimidated to follow the pest management schedules devised to meet their requirements in various crops.

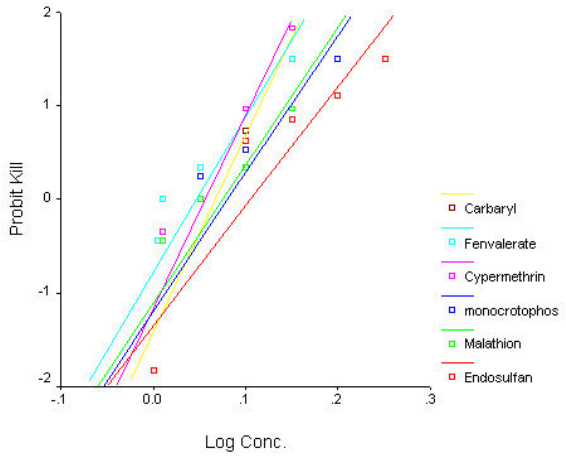

Fig. 1. Dose-mortality regression lines for various insecticides against susceptible strain of *Helicoverpa armigera*

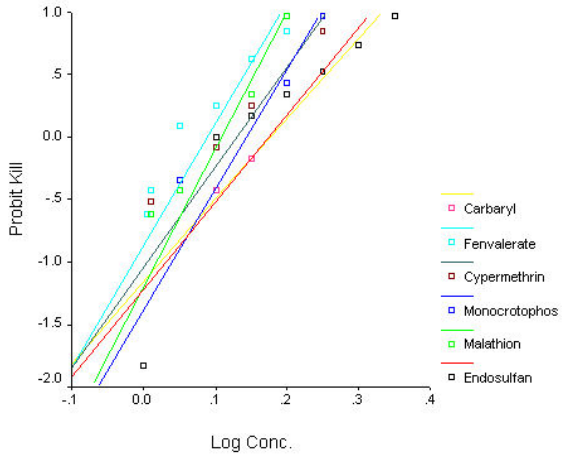

Fig. 2 Dose-mortality regression lines for various insecticides against field collected strain of *Helicoverpa armigera*

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Monitoring Insecticide Resistance in Whitefly, *Bemisia tabaci* (Genn) in Indian Cotton Ecosystems

INTRODUCTION The whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) has become one of the economically most important pests in the world. Epizootic outbreaks of pest populations began in the seventies in Sudan and in the Middle East (Dittrich et al 1985), reached the USA in the eighties (Johnson et al 1982) and are expanding to most tropical and subtropical countries, including Australia, and temperate countries like Japan, Canada and The Netherlands (Gerling and Mayer 1996). *B. tabaci* causes severe damage amounting to hundreds of millions of dollars annually (Menn 1996). Damage is due to feeding and deposition of honeydew (Schuster et al 1996), physiological disorders (Yokomi et al 1990) and transmission of gemniviruses (Bedford et al 1994, Markham et al 1996). No significant economic damage had been caused to crops in Asia until population outbreaks started in 1998 in cotton crops. Among the suggested causes of whitefly outbreaks were climatic factors (Jayaraj et al 1986, Byrne et al 1992), cropping practices (Byrne et al 1992), and use of broad-spectrum insecticides that induce resistance development (Dittrich et al 1985, Prabhaker et al 1985 & Prabhaker et al 1989) and/or disrupt control by natural enemies (Eveleens 1983).

To manage the pest efficiently, resistance management strategies should be implemented. This highlights need to assess and monitor the responses to insecticides in the target population, to enable the timely use of alternative control measures such as rotation of different insecticides, reduction in the number of applications, or the use of synergists. The use of chemicals, although necessary in multiple-pest crop systems such as cotton, should not compromise the activity of a diverse fauna of parasitoids and predators that attack and sometimes effectively control the pest (Stanley et al 1997, McAuslane et al 1993).

In order to assure a sustainable resistant management strategy, it was essential to survey the insecticide resistance levels in each cotton growing region (Nibouche 1994). Therefore, in the present study we planned to determine how widespread the problem of resistance in whitefly had been spread in cotton growing areas in India for most commonly used insecticides.

MATERIALS and METHODS The commercial formulations of three most commonly used insecticides viz. Hostathion 40 EC (triazophos), Thiodan 35 EC (endosulfan) and Confidor 200 SL (imidacloprid) were used. Two discriminating concentrations for each insecticide triazophos: 0.25 & 1.0 %, endosulfan: 0.25 & 1.0 % and imidacloprid: 0.025 & 1.0 % were used to investigate the level of resistance.

Bioassays were performed using Petri plates with adult population as per Sethi et al (2002) in cotton fields. Bioassays were conducted in the farmers' fields from the regions Guntur (Andhra Pradesh), Coimbatore (Tamil Nadu), Kolar (Karnataka), Ludhiana, Bathinda (Punjab), Sirsa (Haryana) and Sri Ganganagar (Rajasthan) in India. The cotton leaf discs were dipped in the aqueous solution of formulated materials for 10 seconds and allowed to dry for 1 hr. Thirty (unsexed) whitefly adults were used in five replications for each concentration. Control tests were also conducted with cotton leaf discs without any insecticide treatment in

Petri plates. Mortality was recorded after 24 hrs. The Resistance Factor (RF) was also calculated. The percent resurgence of whitefly population for each insecticide was calculated by Henderson and Tilton's formula

$$\text{Resurgence (\%)} = \left\{ \frac{(\text{Ts} \times \text{CF})}{(\text{Cs} \times \text{TF})} - 1 \right\} \times 100$$

- Ts = Number of live whitefly in post treatment count
- TF = Number of live whitefly in pre treatment count
- Cs = Number of whitefly in untreated check (Post-treatment)
- CF = Number of whitefly in untreated check (Pre-treatment)

RESULTS

Triazophos

The mortality at lower concentration (0.25 %) was recorded to be lower (58 %) in case of Bathinda population followed by Sirsa, Ludhiana, Sri Ganganagar, Kolar, Guntur and Coimbatore populations. (Fig.1). It was highest in Coimbatore population up to tune of 76.67 per cent. At higher concentration (1.0 %), mortality was lowest in case of Kolar and Bathinda population (90 %) (Fig.2). Bathinda whitefly population showed 1.32 times more resistance as Coimbatore population at 0.25 per cent concentration. Northern Indian whitefly population was more resistant that southern Indian population (Table 1). Populations from Coimbatore, Guntur and Kolar exhibited little variation in resistance factor.

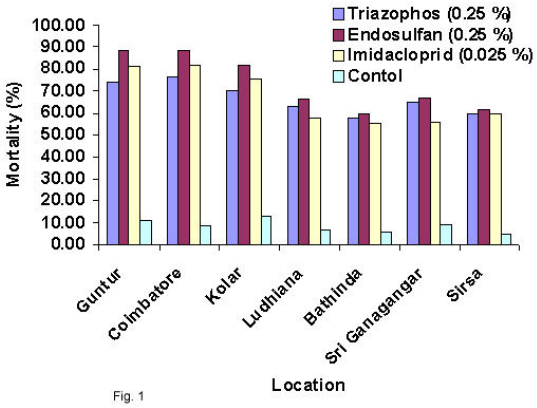

Fig. 1

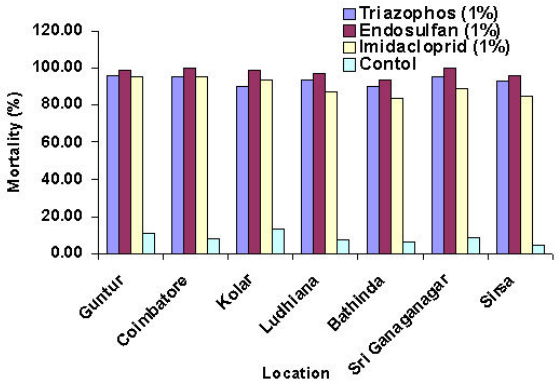

Fig.2

Table.1 Resistance factor (RF) of whitefly population against the most commonly used insecticides in India

	Resistance Factor (RF)		
	Triazophos (0.25 %)	Endosulfan (0.25 %)	Imidacloprid (0.025%)
Coimbatore	1	1	1
Guntur	1.04	1	1
Kolar	1.1	1.08	1.09
Ludhiana	1.22	1.34	1.41
Bathinda	1.32	1.47	1.48
Sri Ganaganagar	1.18	1.32	1.46
Sirsa	1.17	1.42	1.36

Endosulfan

The similar trend was also found in case of endosulfan. The mortality at lower concentration (0.25 %) was recorded to be lower (58 %) in case of Bathinda population followed by Sirsa, Ludhiana, Sri Ganganagar, Kolar, Guntur and Coimbatore populations. (Fig.1). It was highest in Coimbatore population up to tune of 88.33 per cent. Incase of higher concentration (1.0 %), mortality was almost 100 per cent in all populations (Fig. 2). There was no significant difference was found in mortality at higher concentration. Bathinda population showed higher resistance factor (1.47 fold) as compared to Coimbatore population (Table. 1).

Imidacloprid

The Bathinda population showed minimum mortality at lower concentration (0.025 %) in Bathinda population (58 %) followed by Sri Ganganagar, Ludhiana, Sirsa, Kolar, Guntar and Coimbatore populations. The Guntur and Coimbatore populations were almost at par with respect to mortality (81 %). Per cent mortality at higher concentration (1.0 %) was lowest in case of Bathinda population (83 %). The mortality incases of Guntur and Coimbatore populations were at par (81 %) (Fig. 2). Higher degree of resistance (1.48 fold) was found against imidacloprid in case of Bathinda population as compared to Coimbatore population (Table. 1).

The mortality in control population was maximum in Kolar population (13.33 %) followed by Guntur (11.0 %), Sri Ganganagar (9 %), Coimbatore (8.33 %), Bathinda (6 %) and Sirsa (5 %) populations (Fig. 1).

Resurgence was not observed with any insecticides in all whitefly populations (Table. 2). Values for resurgence for all three insectcides were negative in all the populations.

Table. 2 Resurgence of whitefly against the most commonly used insecticides in India

S. No.	Treatments	Conc. (%)	% Decrease						
			Coimbatore	Guntur	Kolar	Ludhiana	Bathinda	Sri Ganganagar	Sirsa
1	Triazophos	0.25	-75	-71	-65	-60	-55	-62	-58
		1	-95	-96	-88	-94	-89	-95	-93
2	Endosulfan	0.25	-87	-87	-79	-63	-57	-64	-60
		1	-100	-98	-98	-97	-9	-100	-96
3	Imidacloprid	0.025	-80	-79	-71	-55	-52	-52	-58
		1	-95	-94	-92	-86	-82	-88	-84

DISCUSSION Analysis of *B. tabaci* populations among different locations in India detected a high degree of resistance in virtually every location. The highest degree of resistance as measured by resistance factor (RF) was found for imadaclorid and endosulfan, followed by triazophos. The pattern of resistance closely followed the frequency of use, imidacloprid and endosulfan being the two of the most popular compounds with farmers for whitefly control. There was no resurgence occurred in all populations for all the insecticides. Results from these surveys so far indicate that whitefly resistance is common and likely to expand and intensify among cotton growing areas in India. Northern Indian whitefly populations from areas like Bathinda, Ludhiana, Sirsa and Sri Ganganagar showed a higher level of resistance against all the three most commonly used insecticides triazophos, endosulfan and imidacloprid in cotton crop. This shows that insecticide pressure is higher in case of north India as compared to south India. This explained that resistance levels were appropriate with the usage of pesticides. The study conducted by Forrester (1990) also clearly revealed that the resistance levels rose when insecticides were used but fell significantly when they were withheld. Thus, the pesticides were creating very high pressure for resistant genotypes. The susceptibility of pest populations to a given insecticide is an evolutionary phenomenon arising from changes in frequencies of resistant and susceptible alleles in pest populations (Dobzhanky 1951). These changes in allele frequencies result from the complex interaction of mutation, selection, gene flow and random genetic drift (Roush and Daly 1991). Once use of a pesticide has become wide spread against a pest, selection and gene flow have major influences on the evolution of the susceptibly of the populations to the pesticide, are the most likely factor that can be manipulated to achieve a desired goal. The manipulation of selection so as to influence evolution of susceptibility requires monitoring for resistance which traditionally involves comparisons of LC50, LC90 values or slopes of dose response curve between field populations (Roush and Daly 1991). This suggests that indiscriminate use and heavy dependence on pesticide will further complicate the already worsened situation and hints at aiming for insecticide resistant management strategies. As there are few alternative materials for farmers to use at present, insecticide resistance will likely become an increasing challenge for farmers and can jeopardize their ability to produce high quality export cotton in near future.

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A Modelling Approach of the Sustainability of Bt Cotton Grown by Small Farmers in West Africa

INTRODUCTION Transgenic cotton, that produces toxins from the bacterium *Bacillus thuringiensis*, kills the first instars of the bollworm *Helicoverpa armigera*, a key-pest of cotton in the Old World. The increasing difficulty of controlling the bollworm, due to its resistance to pyrethroid insecticides (Martin et al. 2000), could led to a large adoption of Bt Cotton in a near future by many West African countries.

However, many researchers are concerned that cotton bollworm might become resistant to Bt toxins without a crop management strategy adapted to the farming systems. Considering the results obtained in resistance mechanism heritability as well as the role of natural and cultivated host plants in the dynamics of the bollworm populations, modelling is a tool already used to develop such a strategy in diverse situations (Caprio, 1994; Gould, 1998; Ru et al. 2002; Vacher et al. 2003). In the US, it led to the 'High Dose Refuge' strategy (HDR) combining a high level of toxin expression in the cotton plant associated with a toxin-free refuge inside the cotton area (Gould, 1998). Our paper is a contribution in modelling as an approach of the resistance management strategy that could be adapted to Bt Cotton in the small farming systems found in West Africa.

METHODS

Modelling of H. armigera life history

Data concerning the life history of *Helicoverpa armigera* in Western Africa are available from the work carried out in Burkina Faso by Nibouche (1994). Considering the agronomic and ecological homogeneity of the Savannah region of Western Africa, these data from Burkina Faso can be extrapolated to the whole region.

Schematically, *H. armigera* colonizes two kinds of habitats: rainfed crops and irrigated vegetable crops. These two agrosystems are geographically distinct: vegetable crops are mainly located in the vicinity of towns and rainfed crops occupy the majority of agricultural landscapes. Colonization of these two agrosystems by the pest is asynchronous: rainfed crops are colonized from mid-July to end of October, vegetable crops are mainly colonized from the end of October to the end of April. During the rainy season (May to October), populations on vegetables crops are very small because surfaces of vegetable crops reduce dramatically (owing to high incidence of fungal diseases). During the transition period at the beginning of the rainy season (May to mid-July), the main host plant of *H. armigera* is *Cleome viscosa* L. (Capparidaceae), a weed very common in cultivated and non-cultivated areas.

Nibouche (1994) showed that diapause occurs in *H. armigera* in Western Africa, but this diapause does not allow the pest to bridge the six to seven months duration of the dry season. Survival of the pest during the dry season is allowed by the migration: rainfed crop areas are re-colonized at every beginning of rainy season (May) by moths produced by populations colonizing vegetable crops during the dry season. The reverse migration (from rainfed crops to vegetable crops) is likely to occur at the beginning of the dry season (November). This asynchronous colonization of two distinct agrosystems has also been reported by Bourdouxhe (1980) in Senegal. The lack of genetic structure and the amount of gene flow in West African populations of *H. armigera* support these findings (Nibouche et al., 1998).

The main rainfed host crops of *H. armigera* are cotton and maize. The main vegetable host is tomato. Although millet, groundnut and sorghum are known as host plants in several regions of the world, local varieties cultivated in Western Africa are totally free of any attack from the pest. The search for wild host

plants during the dry season has been unsuccessful (Nibouche, 1994). In addition to *Cleome viscosa*, five weeds are possibly colonized by the pest during the rainy season, but the densities of these plants are very low compared to cultivated hosts and the densities of the pest population on these plants are very weak.

According to these points, we modelled the population dynamics of *H. armigera* as described in Fig. 1.

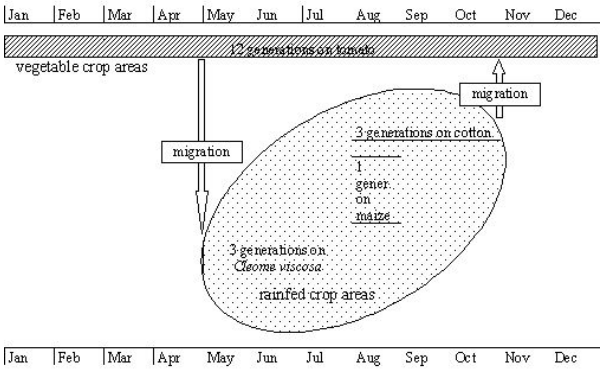

Figure 1. Modelling of the population dynamics of *H. armigera* in West Africa.

As cultivated landscapes are mainly organized in patchworks of small fields (the surface of fields seldom reach five hectares), the model assumes that reproduction is panmictic between cotton and maize populations. The model assumes that the rate of increase of pest populations (increase of population size from one generation to the next one) is the same whatever the host plant. The carrying capacities of crops (maximum pest population carried per crop surface unit) is assumed to be non-limiting.

Genetics of resistance to Bt toxin

Resistance is determined by a single gene with two alleles : R (resistant) and S (susceptible). Dominance is computed according to Bourguet et al. (1996) :

DLC = (log LCRS - log LCSS) / (log LCRR - log LCSS)

where LCRS , LCSS , LCRR are concentrations of toxin that cause a 50% mortality level respectively for RS, SS et RR genotypes. DLC value ranges between 0 (complete recessivity) and 1 (complete dominance).

Characteristics of the resistance mechanism are those obtained by Uraichuen (2002), after a selection pressure on a laboratory strain of *H. armigera* originating from Côte d'Ivoire (West Africa). The resistance factor RF = LCRR / LCSS is 164. The slope of the dose - mortality curve is the same for all genotypes. On the other hand, the value of dominance DLC was an entry parameter of the model.

Considering the lack of data, we assumed that the resistance mechanism causes no cost and that the fitness of the three genotypes is the same in absence of exposition to the toxin.

Parameters of simulations

Following Vacher et al. (2003), we considered that resistance was achieved when the frequency of the R allele reached 50% in the population colonizing rainfed crops. The number of generation to resistance is T50. As duration of a generation in the model is one month, T50 may be converted in the number of years to resistance.

The first Bt-cotton varieties have been created to control *Heliothis virescens* (F.) populations. The dose of Cry1Ac toxin produced by these plants are compatible with the HDR strategy (Gould, 1998). But the efficacy of first generation Bt-cotton might be different on *H. armigera*. Uraichuen (2002) showed in laboratory bioassays that Cry1Ac toxin was five-fold less toxic for *H. armigera* that for *H. virescens*. Forrester and Pyke (as quoted in Gould, 1998) showed that mortality of susceptible *H. armigera* on Cry1Ac Bt-cotton may be less than 90% in some conditions. Therefore, following the same process as Vacher et al. (2003), we simulated situations where the mortality level induced by the Bt-cotton may be far away from the level required by the HDR strategy.

Three toxicity levels were used: high, medium and low (Table 1). The equations of the dose (log) - mortality (probit) curves of each genotype allowed computation of the mortality level of RS and RR genotypes, given the mortality of SS genotype and given the dominance DLC.

Some studies (Uraichuen, 2002; Jackson et al., 2003a) have shown that resistance to Bt toxins might be not recessive, as assumed in the HDR strategy. For that reason, we did not considered that the resistance was systematically recessive and we therefore used DLC as an entry parameter of the model. According to 2002 FAO statistics (http://apps.fao.org/debut.htm), the ratio of superficies maize vs. cotton is 3.2 in Western Africa. The ratio used in our model was 3 (75% maize and 25% cotton).

Initial frequency of the R allele was fixed at 0.001, which is a value similar to the value determined by Gould et al. (1997) for a *H. virescens* population.

At the beginning of the dry season (November), during the migration from rainfed crops to vegetable

Table 1. Mortality level (ML %) of SS and RR genotypes as a function of the toxicity level of Bt cotton for *H. armigera*.

Toxicity of Bt cotton	ML_{SS}	ML_{RR}
high	99.99%	0.29%
medium	80%	< 0.01%
low	60%	< 0.01%

crops, moths emerging from cotton fields migrate and mate with moths emerging from tomato fields. The migration rate MR is the percentage of moths originating from cotton among the parents of the generation colonizing tomato in November. If MR = 0, no migration is assumed and rainfed crop colonization is followed by an extinction. If MR = 1, populations colonizing tomato during the rainy season are assumed negligible and the re-colonization of tomato is assumed to occur only with immigration of moths from cotton fields. No data allow an estimation of MR, and we used arbitrary values of 1, 0.1 and 0.01. Percentage of cotton superficies devoted to non-Bt refuges is Pref.

RESULTS

Role of non-cotton host plant as refuge

The only non-cotton host plant acting as refuge is maize. Tab. 2 and Tab. 3 allow an estimation of the refuge effect of maize. When no non-Bt cotton refuges are set up and in the absence of maize, time to resistance is very short, with a maximum value of 52 months (Tab. 2). The delaying effect of maize is weak (Tab. 3): the maximal delay allowed is 24 generations (two years).

Table 2. Time to resistance (T50) as a function of dominance (DLC) in the absence of cotton refuges (Pref = 0) and in the absence of maize. Migration rate from cotton to tomato (MR) = 1.

Toxicity of Bt cotton	$D_{LC} = 0.1$	$D_{LC} = 0.5$	$D_{LC} = 1$
high	3	3	3
medium	38	16	16
low	52	28	28

Table 3. Time to resistance (T50) as a function of dominance (DLC) in the absence of cotton refuges (Pref = 0) when maize occupies 75% of rainfed host crop superficies. Migration rate (MR) = 1.

Toxicity of Bt cotton	$D_{LC} = 0.1$	$D_{LC} = 0.5$	$D_{LC} = 1$
high	4	4	4
medium	51	27	27
low	76	50	50

The explanation lays in the short duration of the colonization of maize by the pest. The attractive period of the crop (flowering) is short and, as the sowing dates of maize fields are grouped together (at the beginning of the rainy season), the flowering periods are similarly grouped together. Maize acts as a refuge during the August generation. The two following generations (September and October) colonize exclusively cotton. Implementation of non-Bt cotton refuges appears therefore indispensable to ensure sustainability of Bt-cotton in Western Africa.

Optimal size of refuges

We studied the optimal size of non-Bt cotton refuges allowing an acceptable durability of Bt-cotton. The durability objective chosen here was 30 years, i.e. T50 = 360.

Three parameters influence the optimal proportion of refuges (Pref): toxicity of Bt-cotton for the pest (high, medium or low toxicity), dominance of the resistance (DLC) and migration rate from cotton to tomato (MR). Fig. 2 shows influences of these parameters. All parameters have a strong influence on the size of refuges Pref. In the less favourable hypothesis (MR = 1 and DLC ˜ 1), refuges have to cover 82 to 89% of cotton superficies, which implies a strong limitation of the development of Bt-cotton (11 to 18% of total cotton superficies).

In the most favourable hypothesis (MR = 0.01), the optimal size of refuges is below 17% if Bt cotton exhibits a high toxicity. For medium and low toxicities, T50 is above 360 generations even when no refuges are set up, whatever the dominance.

Discussion and conclusion

The results of simulations allow to conclude that, in the agro-ecological conditions of West Africa, the use of non-Bt cotton refuges is indispensable to ensure a minimum durability of Bt-cotton and to delay the appearance of Bt toxin resistance in *H. armigera*. The optimal size of these refuges depends on mortality levels that the pest will exhibit on Bt-cotton and on the amount of gene flow between rainfed crops areas and vegetable crop areas.

In the US (Jackson et al., 2003b ; Abney et al., 2003), India or South Africa (Head et al., 2003) alternative crops or weed hosts act as natural refuges for cotton bollworms and may avoid set up of non-Bt cotton refuges in some situations. Alternative host likely to act as natural refuges should have two characteristics: to carry large populations of the pest and to be colonized synchronously with cotton. The later condition is not satisfied for the two alternative hosts encountered in rainfed crop areas in Western Africa: the weed *Cleome viscosa* is colonized before cotton and maize is synchronously colonized with the first cotton generation only.

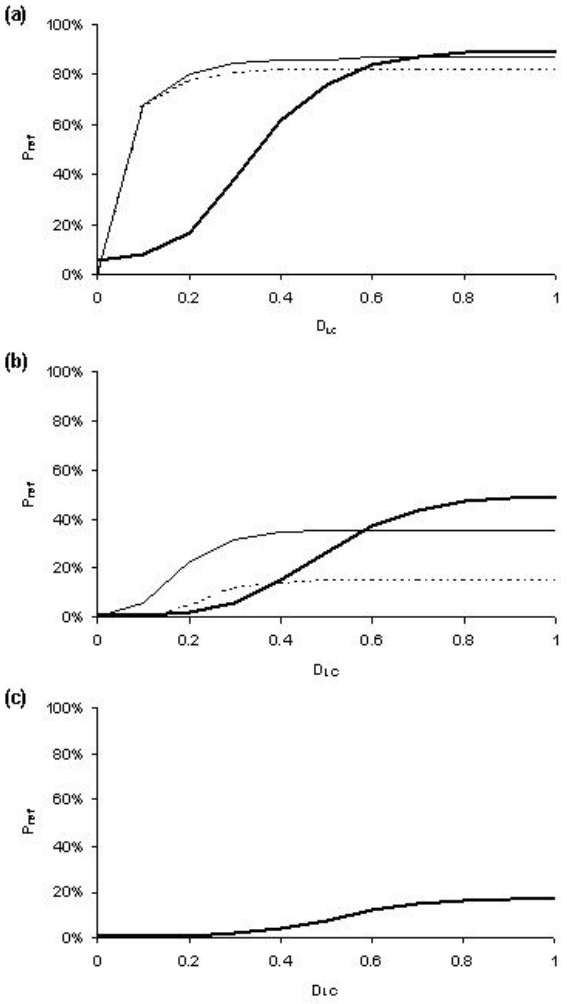

Figure 2: Minimum size of cotton refuges (Pref) allowing a time to resistance (T50) longer or equal to 360 generations (30 years) as a function of dominance DLC. High toxicity of Bt cotton = bold line, medium toxicity = thick line, low toxicity = dashed line. Migration rate from rainfed crops to vegetable crops MR = 1 (a), MR = 0.1 (b) or MR = 0.01 (c).

In West Africa, vegetable crops might act as natural refuges. But their efficiency as refuge depends on the gene flow between vegetable and rainfed crops. Further work is necessary for a better knowledge of the migration process involved.

Before achievement of work currently studying these aspects, results presented here suggest that the diffusion of transgenic Cry1Ac Bt cotton in West Africa should be limited to 20% of the cotton acreage.

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Identification of Factors Responsible for Insecticide Resistance in *Helicoverpa armigera* (Hubner)

ABSTRACT *Helicoverpa armigera* (Hubner) larvae collected from field crops and tested for resistance to cypermethrin, fenvalrate, endosulfan, monocrotophos and quinolphos. Larvae were treated with a dose of the pesticide that would kill 99% of the susceptible insects. The percent survival of the resistant strains was determined. Highest percent of survival was recorded to fenvelrate (64.96%) followed by cypermethrin (62,4%). Acetylcholinesterase of resistant larvae was less sensitive to monocrotophos and methyl paraoxon. Resistant larvae showed higher activities of esterases, phosphatases and methyl paraoxon hydrolase compared with susceptible larvae. The presence of high activity of esterases was attributed to appearance of extra bands of esterases in native PAGE. The presence of P-glycoprotein expression was detected in resistant larvae using P-gp antibodies and the same was not detected in the susceptible larvae. Our results indicate that the high level of resistance detected in the field pests could be because of a combined effect of decreased sensitivity to AChE, higher levels of esterases and phosphatases and the expression of P-gp.

KEYWORDS *Helicoverpa armigera*, esterases, phosphateses, p-glycoprotein, pesticide resistance

INTRODUCTION American bollworm, *Helicoverpa armigera* (Hübner, Lepidoptera; Noctuidae) is one of the major pests of cotton, legumes, and more than 100 other plant species (Bhatnagar et al., 1982). In India, crop losses due to *H. armigera* are commonly more than half the yield, and annual losses to cotton and pulses alone have been estimated at US $ 300-500 million (King, 1994). This pest has developed resistance to all the major insecticide classes and it has become increasingly difficult to control their population not only in India, (Mc Caffery et al, 1998; Armes et al., 1992; Dhingra et al., 1988 and Kranthi et al., 1997) but also in Australia (Gunning et al., 1991; Forrester et al., 1993), Indonasia (Mc Caffery et al., 1991) and Thailand (Ahmad et al., 1989).

Resistance to insecticides belonging to organophosphate and carbamate groups has been reported in the *H. armigera* and the leaf worm, *Spodoptera letura* (Fabricius) in India (Armes et al., 1996, 1997; Kranthi et al., 2001). Insensitive acetylcholinesterase (AChE) has been implicated as one of the mechanism of resistance to organophosphorous (OP) and carbamate insecticides in tobacco white fly, *Bemisia tabaci* (Genn.) (Dettrich et al., 1985; 1990) and *Heliothis virescense* (Brown and Bryson, 1992). Similar insensitive AChE variants have now been detected in heterogenous populations of many important pest species; in housefly, the cotton aphid *Aphis gossypiella* (Moores et al., 1988, 1989) and several Mosquitoe sps. (ffrench Constant and Bonning, 1989).

Model substrates such as naphthyl esters are commonly used in preference to insecticidal esters, which are normally more difficult to use as assaying agents (Byrne and Devonshire, 1991). Comparisions of total naphthyl esterase activity between susceptible and resistant strains of insects are now performed routinely, as one test for insensitive acetylcholinesterase alleles. A correlation between high naphthyl esterase activities and resistance to organophosphate and pyrethroid resistance was reported *B. tabaci* from Sudanese cotton (Dittrich et al., 1985). Many more cases of esterase mediated OP resistance have been reported for example in mosquitoe, *Culex tarselis* (Whyard et al., 1995), tobacco budworm, *H. virescens* (F.) (Goh et al., 1995; Harold and Offea, 2000), Colarado potato beetle, *Leptinotarsa decemlineata* (Say) (Anspaugh et al., 1995) and saw-toothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) (Conyers et al., 1998). Increased malathion carboxylesterase activity is responsible for malathion resistance in insects such as *L. cuprina* (Raftos, 1986) and the green rice leaf hopper, *Nephotettix cincticeps* (Miyata and Saito, 1976).

It has been reported (Lanning et al., 1996) that *Heliothis virescens* is capable of over expressing a protein called P-glycoprotein, primarily localized in the cuticle which is one of proteins responsible for mediating resistance. This glycoprotein was an analog of the multidrug resistance (MDR) protein expressed in mammalian cancer cells. The function of this protein has been explored completely and is involved in efflux of drugs, resulting in the decrease in intracellular drug accumulation (Michael et al., 1993; Prasad et al., 1996).

Several mechanisms of resistance have been identified in *H. armigera* populations in various parts of the world. These include reduced penetration (Gunning et al., 1991; Kennaugh et al., 1993; Armes et al., 1992; Kranthi et al., 1997, 2001), decreased nerve sensitivity (West and Mc Caffery, 1992) and enhanced metabolism (Ahmad and Mc Caffery, 1991).

It is clear from these studies that the esterases and p-glycoprotein are involved in mediating the resistance in several pests. However such information is lacking in *Helicoverpa armigera* of Indian populations. Here we report the activities of different esterases and the

presence of P-gp in the resistant strain of, *Helicoverpa armigera* in an attempt to explain the mechanism of pesticide resistance in field strain.

MATERIALS and METHODS

Chemicals

The C219 antibodies were purchased from Signet laboratories (Dedham, MA, USA). The ECL luminescence kit from Amersham. Methyl paraoxon from Sigma chemical company, USA. Technical grades of cypermethrin, fenvalrate; endosulfan, monocrotophos, quinolphos were gifted by Dr. B.V. Patil, Agriculture Research Station, Raichur. All other chemicals used were of reagent grade.

Assessment of Survival of Field Insects at a Single Lethal Dose

Adult pod borer larvae were collected from fields cultivating pigeonpea and chickpea around Gulbarga (India) during 1999-2000 at monthly intervals to get F1 population for bioassay. The *H. armigera* larvae were reared on soaked Bengal gram seeds individually in glass vials till the pre-pupal stage. Pupation was allowed in small plastic baskets filled with moist saw dust. After emergence, adult males and females were collected and released into cages for mating and egg laying. 1 % honey solution was provided in cotton swabs as food for adults. The hatched neonates were collected and reared on chickpea flour based semi-synthetic diet (Sathiah, 1987) till third/forth instar to assess the resistance level. Resistance to insecticides was assessed by using discriminating dose method, that is a dose which kills 99% of susceptible *H. armigera* (Roush and Miller, 1986).

The bio-assay was carried out on third/fourth instar larvae at monthly interval. Individual larvae weighing 30-40 mg were subjected to discriminating doses of test insecticides prepared by dissolving technical grade insecticides in acetone. The discriminating doses of different insecticides were fenvelrate 0.2 µg/µl, cypermethrin 1.0 µg/µl, endosulfan 10.0 µg/µl, quinolphos 0.75µg/µl, and monocrotophos 10 µg/µl (NRI manual 1993-95; Forrester and Cahill, 1987; Arms et al., 1996). One microliter of test solution was dispensed on thoracic region of each larvae with help of hand microapplicator and reared individually in glass vials on semi-synthetic diet for 144 hours after treatment under condition of 26 ± 10 C temperature and 14 h:10 h LD (14 hours of light : 10 hours of darkness) photoperiod. The number of larvae per insecticide were 20, each time replicated thrice. The mortality of larvae was recorded at every 24 hours interval. The level of resistance was computed as described in the NRI manual, (1993-95).

A laboratory susceptible strain of *Helicoverpa armigera* was obtained from Project Directorate of Biological control, ICAR, Bangalore. This strain was reared for several generations in the lab and used as a susceptible strain for comparative studies of enzymes.

Acetylcholinesterase (AChE) inhibition

The acetylcholinesterase activity was determined using fine nerve ganglia, dissected from the fifth to ninth segments of a fifth instar larva. Groups of 25 ganglia were homogenized in 1 ml 0.14 M phosphate buffer (pH 7.6) with a Teflan glass Potter -Elvehjem homogenizer. The homogenate was centrifuged for 20 min in a microfuge at 10,000 x g. The supernatant was immediately used as the enzyme source with acetylthiocholine as the substrate (Ellman et al., 1961). I50 values were calculated using several concentrations of monocrotophos and paraoxon.

Preparation of tissue homogenate

All the following experiments were carried out at 40 C. Fifth instar larvae of the resistant strains were homogenized in 0.1M Tris-HCl buffer, pH 7.6. The homogenate was filtered through a four layered musclene cloth and the supernatant was used as an enzyme source. Head portions were dissected and homogenized with an all-glass homogenizer in 20 mM Tris-HCl buffer pH 7.2, centrifuged at 10,000 x g for 10 min at 40 C and used as an enzyme source. Protein concentrations were determined using the method of Lowry et al., (1951) method.

Enzyme assays

Esterase activities were assayed by the method of Meghji et al., (1990). Reaction was carried out in a final volume of 3 ml containing 50 mM phosphate buffer (pH 7.4), 100 µM p-nitrophenyl acetate as substrate and 50 µl of enzyme extract. The tubes were incubated at 370 C for exactly 10 min. The reaction was terminated by the addition of 200 µl of 0.3 M sodium carbonate.

Alkaline phosphatase activity was assayed by the method of Garen and Levinthal (1960). The reaction was carried out in a final volume of 2 ml containing 50 mM glycine-NaOH buffer (pH 10.5), 5 mM magnesium chloride, 5 mM p-nitrophenyl phosphate (disodium salt) and aliquotes of enzyme (20µl) was incubated at 370 C for 20 min. The reaction was terminated by adding 1 ml of 0.1 M NaOH. The p-nitrophenol liberated was read spectrophotometrically at 405 nm. One unit of enzyme was defined as mmol of p-nitrophenol liberated per min per ml of enzyme. The specific activity was expressed units per mg of protein under standard conditions.

Paraoxon hydrolase activity was measured spectrophotometrically. The mixture contained an aliquot of enzyme, 1 mg methyl paraoxon in 10 µl of acetone in a total volume of 1 ml of 0.1 M phosphate buffer (pH 7.6). The reaction was recorded continuously at 405 nm at 300 C. The activity of the

enzyme was calculated from the rate of formation of the product. Non-enzymatic degradation of methyl paraoxon was corrected in the reference cell.

Electrophoresis

Esterases were separated by discontinuous, native polyacrylamide gel electrophoresis (PAGE) using the method of Hughes and Roftos (1985). Electrophoresis was carried out for 3-4 hrs at 25 V constant power per gel, at 40 C. The gels were stained for esterase activity at 370 C for 10 min using a solution containing 0.02% w/v a-naphthylacetate dissolved in acetone and 0.05% fast blue RR salt in 0.1 M phosphate buffer pH 6.8.

For phosphatase activity, the gel was incubated with a solution containing 0.2% lead nitrate and 0.1M sodium b-glycerophosphate in 50 mM acetate buffer (pH 5.0) for 10 min at room temperature. The reaction was terminated by transferring the gel into 2% acetic acid for one min. The gel was rinsed in distilled water and then incubated with dilute solution of ammonium sulfide for 1 min (Gomori, 1950).

Detection of p-glycoprotein by Immunoblot analysis

At least 50 fourth instar larvae were dissected and the gut contents were removed from each larva. The tissues were pooled and homogenised in 0.1 M phosphate buffer pH 7.6 containing 1 mM phenyl thiourea (PTU), 1 mM dithiothital (DTT) and 1 mM EDTA. The homogenate was centrifuged at 10,000 g for 10 min and the supernatant was centrifuged at 100,000 x g for 1 hr. The pellet was suspended in 0.1 M sodium phosphate buffer pH 7.8 containing 0.1 mM PTU, 0.1 mM DTT, 0.1 mM EDTA. 15 µg of membrane protein were heated briefly at 550 C for 3 min and resolved on 7.5% polyacrylamide gels by the method of Lammli (1970). The separated proteins were transferred for 1 hr to polyvinyldine difluoride (PVDF) membranes and incubated for two hrs in a Tris-buffered saline (TBS) blocking solution containing 2.5% carnation non-fat dry milk powder and 0.1% Tween-20. The p-gp antibodies (C219) were applied at a concentration of 1 µg/ml for 2 hrs. The membranes were washed and incubated with biotinylated goat antimouse IgG diluted 1:600 in TBS containing 0.1% Tween-20. After 1 hrs, the membranes were washed and incubated for 1 hr, with streptavidin horse radish peroxidase diluted 1:6000 in phosphate buffered saline (PBS). The membranes were treated with the ECL reagents and exposed to X-ray film for 5 mins.

RESULTS

Assessment of Resistance Levels

Our studies showed that during 1999-2000 season, *H. armigera* in the Gulbarga region had high resistance to insecticides. Among the different insecticides tested, the resistance level was high for pyrethroides (fenvelrate and cypermethrin) followed by quinolphos, monocrotophos and endosulfan. The rate of survival was about 40.8 percent during October and as season advanced the survival rate increased upto 75.4 percent as indicated by monthly mean survival across the insectides (Table-1). This was true with respect to individual insecticides also. There was very high percentage of survival to fenvelrate (65%) followed by cypermethrin (62.4%) as indicated by seasonal average survival to each insecticides. The highest level of survival (79.13%) was observed against fenvelrate during February. The results are consistent with the existence of moderate to high levels of resistance in *H. armigera* of Gulbarga ecosystem to almost all insecticides tested.

Acetylcholinesterase activity and inhibition

In view of the ability of the *H. armigera* developed resistance to various insecticides, the effects of monocrotophos and methyl paraoxon on acetylcholinesterase were tested. Inhibition of AChE by monocrotophos in resistant and susceptible larvae is presented in Table-2. These results indicate that there is almost 4 fold difference in the sensitivity of AChE to monocrotophos and methyl paraoxon between two strains.

Table 1. Insecticide resistance levels in *Helicoverpa armigera* (Hub.)

Month	Percent survival					Monthly mean
	Cypermerthrin	Fenvelrate	Endosulfan	Monocrotophos	Quinalphos	
Oct-99	43.27	46.9	35.71	38.11	40	40.8
Nov-99	51.69	55.65	49.5	45.71	48.25	50.16
Dec-99	68.5	69	53.9	58.65	60.41	62.09
Jan-00	73.29	74.15	62.52	68.05	69.71	69.54
Feb-00	75.25	79.13	72	74.52	76.25	75.43
Seasonal average 1999-2000	62.4	64.96	54.72	57	58.92	59.6

The figures indicate the percent survival of insects treated with a dose that kills 99% of susceptible insects.

Table 2. Effect of monocrotophos and methyl paraoxon on acetylcholinesterase activity

Chemical	I_{50} (M)	
	Susceptible	Resistant
1. Monocrotophas	$7.18 \pm 0.52 \times 10^{-3}$	$26.32 \pm 0.45 \times 10^{-3}$
2. Paraoxon	$6.28 \pm 0.42 \times 10^{-3}$	$28.14 \pm 0.46 \times 10^{-3}$

Figures indicate mean value ± standard deviation of three replicates. Other experimental details are given in the methods section.

Esterase activities

The presence of different esterases and phosphatases was detected in native PAGE. A total of eight esterases were observed in the resistant strain whereas three of these esterases E1, E7 and E8 were not detected in susceptible strain (Fig. 1). All esterases were able to use a-nephthyl acetate. Among all 8 esterases only one (E6) had shown phosphatase activity with sodium b-glycerophosphate as a substrate (Fig. 2). The phosphatase activity was high in the resistant strain (Table-3). No paraoxon hydrolase activity was observed in the susceptible pest.

Identification of P-gp

P-gp expression in whole larvae prepared from 4th instar larvae was examined using C219 antibody (Fig. 3). High levels of P-gp was observed in the resistant population whereas no P-gp was detected in the susceptible strain. This indicates resistant pest express a specific protein called P-gp, which helps in mediating the resistance.

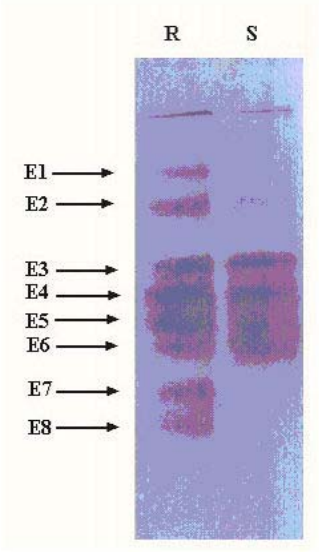

Fig. 1: Polyacrylamide gel electrophoresis of general esterases stained with β naphthyl acetate and fast blue RR salt.
Lane 1: 100 μg of enzyme extract of resistance pest
Lane 2: 100 mg of enzyme extract of sensitive pest.
Experimental details are given in methods and material section

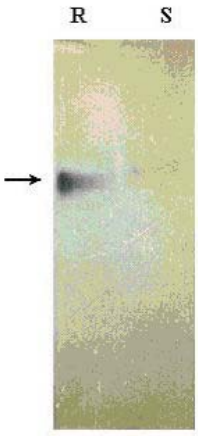

Fig. 2: Polyacrylamide gel electrophoresis of phosphatase stained with β-glecerophospate, lead nitrate, and ammonium sulfide.
Lane 1: 100 μg of enzyme extract of resistant pest and
Lane 2: 100 ug of enzyme extract of susceptible pest.
Other experimental details were given in the methods and material section.

Table 3. Specific activities of various enzymes in the fifth instar larvae of *H. armigera* (Hubner)

Enzyme [a.]	Susceptible strain	Resistant strain
1. Alkaline phosphatase	0.028 ± 0.045	0.156 ± 0.042
Head region	0.020 ± 0.015	0.160 ± 0.060
2. General esterases	0.027 ± 0.08	0.247 ± 0.012
3. Paraoxon hydrolase [b.]	Nil	0.195 ± 0.120
Head region	Nil	0.202 ± 0.210

Values represent the mean ± standard deviation, of three replicates.
Other experimental details are given in the methods section.
a. m mol. of p-nitrophenol liberated/min/mg protein.
b. n mol. of p-nitrophenol liberated/min/mg protein.

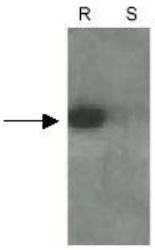

Fig. 3: P-gp expression in resistant and susceptible *Heliothis armigera* larvae. Immunoblot analysis of 15 μg of whole larvae prepared from susceptible and resistant population of fourth instar *H. armigera* larvae with the C219 P-gp antibody. This figure is representative of at least three trails.
Lane 1: Membrane extract of resistant pest
Lane 2: Membrane extract of susceptible pest.

DISCUSSION High levels of pyrethroid resistance were recorded in the intensive cotton and pulse growing regions of Central and Southern India where excessive application of insecticide is common (Armes et al., 1996). *H. armigera* had developed high resistance as the season advanced and reached highest between January-February (Table-1). A similar seasonal pattern of cypermethrin resistance frequencies was reported in the discriminating dose monitoring studies conducted by ICRISAT Asia Form (Armes et al., 1994). It is probable that the resistance to pyrethroid, or organophosphate and carbamate insecticides in the Indian subcontinent can be attributed to an inherited or inducible mixed function oxidase complex.

Insensitive AChE appears to play a role in the resistance and acts in conjunction with metabolic detoxification to confer overall resistance to pyrethroids, OPs and carbamates. Previous studies have shown a very strong correlation between AChE insensitivity and increased metabolism of insecticides (Oppenoorth, 1984). Insecticide sensitive and insensitive acetylcholinesterase variant in individual adult whitefly were identified on the basis of their sensitivities to inhibition by paraoxon and azamethiophos (Byrne and Devanshire, 1993). Brown and Bryson (1992) have reported that AChE activity from the tobacco budworm *Heliothis virescurs*, was 22

fold resistant to inhibition by methyl paraoxon in larvae of adults of a methyl parathion resistant woodrow strain when compared with a susceptible, Florence strain.

It has been shown that one major resistance gene for AChE insensitivity is common, and the toxicology data from Dittrich et al., (1985) suggest that the AChE variant contributes to the substantial resistance to monocrotophos and dimethoate. These date do not, however, take into account for other possible contributing factors in this resistance, such as hydrolysis or sequestration by esterases.

Our results indicate that AChE in resistaant *H. armigera* is less susceptible to monocrotophos compare with that in susceptible strain (Table-2). Monocrotophos, which has been extensively used to control the pests, proved to be ineffective at inhibiting the AChE. The modification of AChE which decreases their sensitivity to OPS are found in resistant strains of many insects including the cattle tick, *Boophilus microplus* (Roultson, et al., 1968) housefly, *Musca domestica* (Oppenoorth, 1984) and mosquitoe, *Anopheles albimanus* (Hemingway, et al,. 1983). *Nephotettix cincticeps* (the green rice leaf hopper) has developed resistance to N-methyl carbonates and the enzyme in the R-strain is about 17 fold resistant to these compounds than that of the S-strain (Yamamoto, et al., 1983).

Alternations in the levels of other esterases provide the second mechanism of resistance. Esterases, phosphatase and paraoxon hydrolase activities were high in the resistant strain compared with the susceptible strain (Table-3). The appearance of extra esterase bands [E1,E7 and E8 in Fig. 1] and one of the enzymes having high activities of both esterase and phosphatase activities supports their involvement in the development of resistance. High phosphatase and paraoxon hydrolase activities were observed in the head region (Table-3). Since the head contains the highest amount of acetylcholienesterase, which is the target for OP inhibition, the localization of the phosphoesterase in the head of the resistant larva may serve to protect the AChE, which is as sensitive as that of the susceptible larvae (Konno, et al., 1989). Phosphotriester hydrolases, including arylesterases, paraoxonase and DFPase are important enzymes for the detoxication of toxic phosphotriester compounds and they have been reported to be present in high levels in mammals and in low levels in birds (Brealey et al., 1980) and a few insect species (Dauterman, 1983). The hydrolase found in the methyl parathion resistant tobacco budworm is responsible in part for its resistance (Konno et al., 1989). This was the first enzyme reported to be responsible for resistance in insects.

Amplification of such esterase genes, resulting in increased enzyme production, is responsible for OP resistance in the peach potato aphid, *Myzus persicae* and mosquitoes of the *Culex pipiens* complex (Oppenoorth., 1984). In both species number of strains with different degrees of resistance occur, due to different levels of activity of an esterase hydrolyzing the OP analog of the insecticides. In the case of the aphid, Devonshire and Sawicki (1979) were able to show that the same enzyme was present in the various strains in different amounts and that these could be arranged in a geometrically processing series with factor 2 from 1 to 64 (possible gene frequency). The enzyme in the most resistant strain represented 3-4% of the total protein (Yamamoto, et al., 1983). This led them to the hypothesis that the geometrical series results from a series of tandem duplications of the structural gene coding the enzyme.

The presence of P-gp was detected in the resistant pest by using C219 antibodies, which are specific for MDR 1, P-gp. Lanning et al., (1996) used P-gp antibodies to detect P-gp expression in various resistant populations of tabacco budworms and found to be 2-6 times more than that of susceptible larvae. Tobacco budworm P-gp was glycosylated and localised primarily in the cuticle and fat body with little expression in the mid gut. They demonstrated the role of P-gp in pesticide resistance of tobacco budworm larvae, treated with P-gp inhibitor, quinidine and challenged with various doses of thiodicarb. Inhibition of P-gp decreased the LD50 for thiodicarb by a factor of 12.5. Quinidine treatment did not result in a significant inhibition of the P450 system nor did it alter the feeding of the larvae, suggesting the potential involvement of P-gp in pesticide resistance. An age dependent increase in P-gp expression was detected in resistant larvae compared with susceptible larvae. This correlates with reported age dependent increase in resistance and is consistent with the role of P-gp in the development of pesticide resistance.

Results from this investigation indicate that the pests collected from the field had developed high percentage of resistance and this is likely to be due to the combined effects of decreased sensitivity of AChE inhibition by monocrotophos and methyl paraoxon, high levels of esterase and phosphatase activities and expression of a specific protein called P-glycoprotein.

ACKNOWLEDGEMENT We thank Dr. J.V. Rao, IICT, Hyderabad for carrying part of this work in his lab. We also thanks Dr. C.L. Christian, A. Stephan, Dr. P. Werner, Medical University, Lubeck, Germany for their help in doing immunoblot. We thank Dr. V.H. Mulimani and T.B. Karegoudar, Department of Biochemistry, Gulbarga University, Gulbarga, for their support during this study and Dr. Cletus D'Souza Mysore University, Mysore for critical comments.

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Acaricide Resistance as Mediated by Detoxification and Target Site Insensitivity

ABSTRACT The paper reviewed the status of the resistance of acari to organochlorine, carbamate, organophosphorus, pyrethriod acaricides during recent 10 years. Generally, the resistance ratios of livestock acaricides range from 2 to 60 while those of crop acaricides is up to 4673750.The paper also reviewed acaricide resistance mechanisms during recent 30 years. The resistance mechanisms mainly involve detoxification of Cytochrome P450, esterase, glutathione transferase and insensitivity of target sites AchE and KDR II.

INTRODUCTION The acari comprise mites and ticks and form one of the largest and most biologically diverse groups of the arachnida. It is estimated there are 30,000 described species of acari and the number of underdescribed species of acari could exceed this total by up to twentyfold . They are distributed worldwide and rival the insects. The mite host-associations range from commensalism to parasitism while many species living in temporary habitats practice phoresy and use a variety of other arthropods as vehicles for dispersal. Ticks are parasitic on a wide range of vertebrate hosts, including man and domesticated animals and are vectors of a wider range of disease organisms than any other group of arthropods

Acari are an important pest affecting the agriculture and livestock industry across the world . The acari inflict heavy losses to the agriculture and livestock industry. Before the introduction of arsenic, physical methods were quite widely used to control acari. However, they were not very successful. Since the late 1800's , a number of pesticides have been used for control of acari, and were effective for a long time.

However, another problem has arisen: this is the resistance of acari to these acaricides. Acaricide resistance is the result of the selection of acari individuals in a population through the action of an acaricide that either kills or affects the reproduction of more susceptible individuals. Acaricide resistance has occurred wherever acaricides were used extensively. The first report of resistance to an acaricide was the resistance the to inorganic acaricide, arsenic in *Boophilus decoloratus* in Southern Africa in the 1930's (Keith,1983). Due to resistance and low toxicity, this acaricide is now less widely used. At present, the most popular acaricides in use are carbamates , organophosphates and pyrethroids. As these acaricides were more extensively used, more and more resistance was reported from all over the world. In this report, literature on acaricide resistance (10 years) and its resistance mechanisms (30 years) is reviewed.

ACARICIDE RESISTANCE

Organochlorinated Acaricides

In the past, much resistance to organochlorine acaricides was reported. Because of residual problems and environmental considerations, the organochlorine acaricides are no longer used widely and so,reports of resistance to them have decreased. Schroder (1987) reported *Amblyomma hebraeum* had developed resistance to toxaphene . Gough (1990) tested the field efficacy of dicofol, tetradifon and dienochlor to *Tetranychus urticae* in southern Queensland and found that they had become largely ineffective. This implies their resistance may have arisen there. Estrada,et al (1990) tested susceptibility of *Tetranychus urticae* in Mexico to chlorobenzylate , endosulfan and dicofol and found resistance to all. The resistance ratio of dicofol was the highest: 4673750.

Kagaruki (1991) used lindane and dieldrin to test 189 strains of 10 tick species from cattle in 17 regions of Tanzania and found 85 strains (45%) had developed resistance to these chemicals. Of the resistant strains, *V. boophilus* species occupied 36.5%, *R. appendiculatus* occupied 3.5%, *R.evertsi* occupied 36.5% of the total resistance cases.

The most extensively reported organochlorine resistance is that of *Sorcoptes scabie* to lindane (Purvis,et al,1991;Taplin, et al ,1991; Roth,1991; Judd,1993; Leibowitz, 1993; Orkin, et al ,1993). *S. scabei* leads to contagious skin disease. This epidemic could be treated with 1% lindane lotion. However, due to lindane resistance, doctors had to treat the epidemic with permethrin 5% cream.

Regassa, et al (1993) performed a survey of acaricide resistance on ticks from 18 dairy farms and 6 veterinary clinics in western Ethiopia and found *Boophilus decoloratus* had developed resistance extensively to toxaphene and dieldrin.

For the multi host ticks, *Rhipicephalus*, *Amblyomma* and *Hyalomma*, it was found that resistance is largely limited to cyclodienes and lindane (Nolan, et al, 1990).

Carbamate Acaricides

For carbamate acaricide resistance, Richter, et al(1990) reported that a population of *Tetranychus urticae* on cucumber in a greenhouse in Germany had cross-resistance to organophosphate, carbamate and formamidine acaricides. In Queensland, aldicarb and bromopropylate had largely become ineffective to *T. urticae* on roses (Gough,1990). This implies that this mite may develop resistance to these carbamate acaricides, but resistance level were not determined. In Brazil, mites from roses and strawberries were up to 4.08 times as resistant as those from native forest (Takematsu,1994).

Dusco, et al (1992) determined the resistance levels of Italian strains of the predatory mites *Typhlodromus pyri* and *Amblyseiu andersoni* with carbaryl and found that the LC50 of the *A. andersom*'s resistant strain to Carbaryl was 27.46 times that of a susceptible strain and the high level of resistance is often associated with a marked reduction in fecundity.

Organophosphate Acaricides

For organophosphate acarice resistacne, Brazil researchers used susceptible mites from native forest to test the resistance level of two spotted spider mites *Tetranychus urticae* from different crops in Brazil to dimethoate, naled and mevinphos. They found that the mites from different crops had slightly different levels of resistance to these OP acaricides: the mites from strawberries had from 0 to 45.4 times resistance (Sato, et al ,1994);mites from roses had from 1.8 to 5.2 times resistance (Suplicy , et al ,1994); and mites from grapes had from 1.24 times (to naled and mevinphos) to 5.02 times (to dimethoate) (Souze, et al, 1994).

Herron, et al (1998) tested the resistance level of *Tetranychus urticae* from cotton in Australia to all organophosphate acaricides used on cotton fields. The resistance ratios were very high: 650 times to demeton-s-methyl; 750 times to dimethoate; 78 times to parathion; 100 times to profenofos; and 400 times to monocrotophos.

In addition, Herron, et al (1998) also determined resistance levels of another cotton mite, *T.ludeni* in Australia to dimethoate, parathion and profenofos. Their resistance ratios were 375 times ,39 times and 6.5 times, respectively.

In China, cotton spider mite *Tetranychus cinnabarinus* and *T.truncatus* were found resistant to monocrotophos (Cao, et al,1993).

Pyrethroid Acaricides

For the resistance of pyrethroid acaricides, one with the largest scale problems is the resistance of *Varroa jacobsoni* to fluvalinate. The earliest report of *V. jacobsoni* resistance was from Italy. Large-scale use of fluvalinate apparently began in Italy around 1986-1987. Preliminary reports of resistance began in 1991--1993. In 1994, fluvalinate LD50s in *Varroa* mites from the Lombardy region were increased 10 times compared with susceptible mites. In the field, Apistan's effectiveness ranged from 4--89% (Frank ,1995). In 1996, the LD50 for mites from areas where treatments with fluvalinate were no longer effective was about 25--50 times higher than that for susceptible mites. The LD50 of flumethrin and acrinathrin for mites surviving Apistan (fluvalinate) treatments was 10--60 times higher than the LD50 for mites from the population susceptible to Apistan, indicating the presence of cross-resistance (Milani,1995).

In addition, many other countries also reported *Varroa* mite resistance to fluvalinate . For example, in 1996, resistance was reported in France. In the same year, Londzin, et al (1996) found the efficacy of fluvalinate was only 66.4% in Poland, which indicated possible resistance. In 1997, it was reported that the efficacy ranged only from 6.4% to 20.5% in almost all of Belgium (Bruneau,1997). In 1998, it was reported that *Varroa* mites had developed 5-fold resistance in China (Wu,1998) and this year, the resistance also was detected in the U.S.A.

Boophilus microplus,a common cattle tick of great economic importance, was found to have 5.7-fold resistance to deltamethrin in New Caledonia in 1992(Brun) and this resistance ratio increased to 97.7 in 1995 and all deltamethrin-resistant populations were found cross-resistant to fenvalerate (Beugnet, et al, 1995)

Typhlodromus pyri is a predatory mite in New Zealand apple orchards and it was found to have resistance to permethrin and cypermethrin in 1978. Continued field selection raised the level of resistance to 41 times that of a susceptible strain in 1990, which led to cross-resistance to other synthetic pyrethroids.

THE RELATIONSHIP OF ACARICIDE RESISTANCE TO DETOXIFICATION

Cytochrome P-450

Cytochrome P450 is a carbon-monoxide-binding pigment of microsomes, a kind of electron-transferring hemoprotein containing iron-porphyrin . It has a unique wavelength of the absorption maximum of the carbon monoxide derivative of the reduced form, namely, 450 nm. Its primary function is to facilitate elimination of lipophilic xenobiotics. As for its relationship with acaricide resistance, the mechanism of fluvalinate resistance in the *Varroa* mite is a good example.

In 1991, the first loss of efficacy of tau-fluvalinate against the honeybee ectoparasite *V. jacobsoni* was recorded in Sicily. Since then, diminshed efficacy with available pyrethroid treatment has been encountered in many regions and countries. Hillesheim, et al (1996) developed a test to examine possible metabolic resistance mechanisms in *V. jacobsoni* known to be resistant to tau-fluvalinate (apistan): when the acaricide was applied to the resistant mites with a synergist, piperonyl butoxide (PBO), a significant decrease in LC50 was observed, but resistance was not completely reversed. This indicated that the resistance of *V. jacobsoni* to tau-fluvalinate can partly be explained by an increased detoxification due to P450 monooxygenases, which are inhibited by PBO.

Esterase

A large amount of evidence shows that esterases are associated with acaricide resistance in many acari. In a study of the resistant mechanism of *Dermatophagoides farinae* and *D. pteronyssinus* to permethrim, the spectra of esterase complexes was examined by means of electrophoresis in PAGE; the zones of esterase activity were identified with the help of two substrates: 2-NA and ATI; Qualitative differences in the esterase complexes were found between resistant strain and susceptible strain in both species. The results showed the possible role of esterases in permethrin detoxification.

Kim and Lee (1990) obtained resistant strains of *Tetranychus urticae* by successive selection with the acaricides: carbophenothion, ethion, dicofol, cyhexation and bifenthrin. After selection, they separated esterase isoenzymes by polyacrylamide gel electrophoresis, and were able to detect different isoenzymes in the resistant and susceptible strains. For *T. urticae*, Weyda, et al (1984) found that there were different esterase patterns and band numbers in polyacrylamide gel electrophoresis between strains resistant and susceptible to thiometon. The difference suggested that esterases are related to the resistance mechanisms of the tested acaricides. This conclusion was verified by many similar studies (Capua, et al ,1990 and Sundukov, et al 1989).

The resistance of *Tetranychus kanzawai* Kishide (Axarina:Tetranychidae) is associated with an increase in esterase activity to naphthalenyl acetate, tributyrin and methyl butyrate (Kuwahara, et al, 1982). All these esters are hydrolysed by esterases (aliesterase). The resistance of this mite to malathion is associated with increased esterase activity at E3 and E4 bands resolved by agar gel electrophoreses, on which the main peaks of malathion degradation were detected. Why does the esterase activity in resistant acari increase? The reason is that selection of acaricides influences the allele frequency of an esterase locus, alpha-Est1 and so leads to increased amounts of esterase

(Osakabe,1993;Tanaka, et al,1972).The compound k-1(2-phenyl-4H-1,3,2-benzodioxaphosphorin 2-oxide) inhibits esterase activity, and also displayed synergism to malathion in the mite (Kuwahara,1984).

In an investigation in the laboratory on acaricide resistance in a stored-grain mite, *Caloglyphus berlesei*, Blan (1979) used polyacrylamide gel electrophoresis to separate the extracts of *C. berlesei* into 10 non-specific esterases, and found non-specific esterases in resistant strain were much less sensitive to organophosphorus inhibitors than those in a susceptible strain. This decreased sensitivity may imply the non-specific esterases are responsible in part for the organophosphorus acaricide resistance mechanism of *C. berlesei*.

Glutathione Transferase

For the role of glutathione transferase in acaricede resistance, Naoki, et al (1974) reported GST as the mechanism of azinphosmethyl resistance in the predacious mite, *Neoseiulus fallacies* based on higher degradative activity in a resistant strain. The activity was in the soluble fraction of the homogenate, enhanced by the addition of GSH, and the major metabolite found with the phosphorus-labeled compound was desmethyl azinphosmethyl. Since no differece was observed in all the other factors investigated, i.e., cholinesterase sensitivity and cuticular permeability, the resistance mechanism was attributed to the difference in glutathione S-alkyltranseferase activity. The substitution of the dimethoxy group of azinphosmethyl by other dialkoxy groups decreased the resistance level significantly, supporting this conclusion.

RELATIONSHIP OF ACARICIDE RESISTANCE WITH TARGET SITE INSENSITIVITY: AChE AND KDR

AChE Insensitivity

The major mechanisms of resistance to AChE inhibitors (i.e., OPs and Carbamates) are increased ability to metabolize the inbibitor to less toxic products and reduced sensitivity of acetylcholinesterase toward inhibition (Schnitzerling, et al,1974; Nolan and Schnitzerling,1975;Oppenoorth and Welling,1976). Reduced reactivity of acetylcholinesterase was believed to result from changes in the active site of the enzyme that reduce reaction rates with both inhibitors and normal substrate (AChE) (Nolan and Schnizerling,1976).

For *Tetranychus kanzawai* Kishida (Kuwahara, 1982) and *Caloglyphus berlesei* (Blank,1979), their main mechanism of resistance to organophosphorus compounds is the lowered sensitivity of the AchE. In these mites, a good correlation exists between in vivo resistance to the compounds and in vitro sensitivity of AChE.

The result of research on a Mexican strain of *Boophilus microplus* had a similar conclusion: altered AChE with decreased sensitivity to inhibition is an important defense mechanism used by *B. microplus* to cause resistance to coumaphos. But, in the Mt. Alford strain of *B. microplus*, the mechanism was a combination of increased detoxification and less sensitive AChE (Schuntner and Thompson,1978). In a earlier study, Stone, et al (1976) studied the biochemical genetics of resistance to organophosphorus compounds in three strains (B, M, R) of *B. microplus* and found that different strains had different resistant mechanisms: decreased AChE activity in adult brains of strain B and M; decreased AChE sensitivity to inhibitors in adult brains and larvae of strain B, M and R, and increased detoxification in larvae and adult females of strain M. The results of their research indicated decreased AChE activity in strains B and M is controlled by single autosomal gene; decreased sensitivity of AChE in strain B is incompletely dominant and is controlled by a single autosomal gene. And decreased sensitivity of AChE was a major mechanism of resistance to dimethoate. Domiance of increased degradative metabolism of coumaphos in strain M was variable, and this mechanism still waits to be studied more deeply.

KDR

During the past 30 years, there have been few report on the relationship of acaricides with KDR (Knock Down Resistance). Only one report on this is available, and documents that the resistance of *B. microplus* to synthetic pyrethroids is caused by KDR. This mechanism blocks the effect of these compounds on the nervous system (Briggs, et al, 1984). Sederlund and Bloomquist (1990) reviewed: " the possible existence of a KDR-like mechanism in the cattle tick is based principally on cross resistance to pyrthroids in DDT resistant strains. Although there is some evidence for reduced neuronal sensitivity in adult ticks, the interpretation of this finding is complicated by the existence of a strong hydrolytic detoxification mechanism in the strain employed" (p82).

DISCUSSION The review clearly shows that acari on livestock develop slower and lower resistance than those on crops. The resistance ratios of livestock acaricides generally range from 2 to 60 while those of crop acaricides is up to 4673750. This great difference must result from their different selection pressure and selection frequency. Because acaricides have completely different toxicity to acari and plant hosts, farmers may increase acaricide concentration and its application frequency to a great extent without hurting the hosts. But for livestock acaricides, due to similar toxicity of acaricides to acari and their hosts, the

acaricide concentration and application frequency are limited, resulting in reduced selection pressure,which must lead to slower and lower resistance development.

As known from the above paper, the situation of study on acaricide resistance and its mechanism lags behind that on insect resistance and its mechanism. The study of acaricide resistance is only concentrated on some species of acari and small number of acaricides, while the study of insecticide resistance involves thousands of insect species and insecticides. This situation may result from two reasons. The first reason is the difference of total number between acari and insects. Insects constitute about 90% of all known arthropods. Over 900,000 species have been described and the actual number of species are probably between 3-10 million. However, the number of described acari species is only around 30,000 and the actual number of species is only around 600,000. Due to more species diversity, insects naturally bring more serious losses for human beings. In return, they receive greater and more extensive pressure by chemical selection. Therefore, insecticide resistance will develop more widely and quickly and correspondingly be more extensively studied. The second reason is the different importance of acari and insect impacts to human beings. Insects mainly hurt agricultural crops while acari mainly hurt livestock. Obviously, the former has a closer and wider relationship with human life than the latter, and this important relationship naturally attracts more attention from researchers and results in intensive research on insect resistance.

So far, the study of acaricide resistance has mainly been limited to the detection of resistance. Although resistance mechanisms for some acaricides have been involved, these studies are rather incomplete. They usually involve synergism and enzyme assays, but rarely involved cuticular penetration and electrophysiology. This situation may result from the limit of acari size to a certain extent. Acari are usually small, averaging about 1 mm in length. So, in the future , specific techniques for the study of acari and the molecular basis for resistance mechanisms in acari will be very important to learning about acari resistance and its management.

ACKNOWLEDGMENTS Thank Blair Siegfried and Marion Ellis for their valuable suggestion. Special thanks are given to Mike Scharf for his detailed grammar correction.

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An Investigation of the Metabolic Detoxification of Fluvalinate in *Varroa destructor* Anderson and Truemann

ABSTRACT Detoxification enzyme activities including general esterase, glutathione S-transferases (GST) and cytochrome P450 monoxygenase, using model substrate assays were compared from fluvalinate resistant and susceptible strains of *Varroa* mites populations. No significant differences in activities were observed for the three enzyme systems tested. These results suggest that fluvalinate resistance in the population tested is not due to increased metabolic detoxification. Synergism bioassay with the cytochrome P450 monoxygenase inhibitor, piperonyl butoxide, also indicate that the fluvalinate resistance is not involved in the observed resistance. Our results suggest that other resistance mechanism(s) are responsible for fluvalinate resistance in *Varroa* population examined.

KEYWORDS *Varroa* fluvalinate resistance metabolic detoxification esterase glutathione s-transferases P450 monoxygenase

INTRODUCTION There are many pests on honey bees worldwide, although *Varroa destructor* Anderson and Trueman (*Varroa*) is perhaps the most damaging. *Varroa* infestation results in deformed , undernourished bees, reduced bee longevity and an increased incidence of bee pathogens.

The pyrethroid insecticide/acaricide, fluvalinate, has been used in the U.S. since 1987 to reduce *Varroa* populations in beehive (Ellis et al. 1988). When properly applied, it is 98% - 100% effective on susceptible mite populations (Hillesheim et al 1996), and can be effective for up to eight weeks or longer when formulated in plastic strips (Apistan ®). The extended activity of Apistan strips allows all mites in the hive to be exposed to the chemical when they emerge from sealed cells. However, as a result of the widespread use of fluvalinate, some *Varroa* populations have become resistant to fluvalinate: The earliest report of fluvalinate resistance came from Italy (Milani et al, 1994). Large-scale use of fluvalinate for *Varroa* control apparently began in Italy in 1986-1987.

Preliminary reports of resistance began to appear in 1992-1993, and by 1994, the LD50 of fluvalinate for *Varroa* from the Lombardy region of Italy had increased ten times relative to susceptible mite populations. At that time, the effectiveness of fluvalinate ranged from 4%-89% (Eischen,1995). In 1996, the LD50 for mites from areas where treatments with fluvalinate were no longer effective was 25-50 times greater than for susceptible populations. The LD50 of flumthrin and acrinathrin for mites surviving Apistan® treatments was 10-60 times higher than the LD50 for mites from the susceptible population, suggesting cross-resistance among pyrthriods (Milani, 1995). Subsequently, other countries also reported *Varroa* resistance to fluvalinate. In 1995, fluvalinate resistance was reported in France (Faucon et al., 1995), and in the same year, Londzin et al (1996) found the efficacy of fluvalinate was only 66.4% in Poland. In 1997, Bruneau (1997) reported that fluvalinate efficacy ranged from 6.4% to 20.5% in Belgium. In 1998, Wu (1998) reported *Varroa* had developed a five-fold resistance in China. Baxter et al. (1998) reported fluvalinate resistance in the U.S.A. in 1998.

Pesticide resistance results from repeated and prolonged exposure of a population to the selective agent and occurs most rapidly when the same chemical is used over an extended period of time and for multiple generations (Jeffrey, 1990). Alternative approaches to management of *Varroa* such as mite-tolerant bees is still in its infancy, and therefore, resistance management to protect chemical tools is an important aspect of successful beekeeping.

The objective of the present study was to attempt to identify the physiological mechanism of fluvalinate resistance in *Varroa* mites. Insects or mites usually have three basic resistance mechanisms: decreased cuticular penetration, increased metabolic detoxification and target site insensitivity (Jeffrey, 1990). This study focuses on the potential involvement of increased metabolic detoxification in resistant to fluvalinate among *Varroa* populations using both in vitro assays of detoxification enzyme and activity, and in vivo synergism of fluvalinate toxicity to susceptible and resistant strains of *Varroa* mites.

MATERIALS and METHODS

Mite Colonies

The susceptible mites used in our bioassay and assays originating from an apiary located at the University of Nebraska-Lincoln. The fluvalinate-resistant mites for PBO synergism experiment and P450 assay originating from Cable, Wisconsin. The fluvalinate-resistant mites for other assays originating from David Gainesville, FL (resistance ratio: 13.4 (Macedo, P.A, 2001)). Bioassays indicated mite's susceptibility to fluvalinate.

Chemicals

Technical grade tau-fluvalinate (88%) and the cytochrome P450 monooxygenase inhibitor piperonyl butoxide (PBO) (98% mix of Isomers) were purchased from Chem Service, Inc. (West Chester, PA). 2-Chloro-3,4-dinitrobenzene (CDNB) was obtained from Aldrich Chemical Co. Methoxyresorufin and b - Nicotinamide adenine dinucleotide phosphate,reduced form (b-NADPH) were purchased from Sigma Chemical Co.

Susceptibility Measurement

A glass vial residual bioassay was used to measure mite susceptibility to fluvalinate. Five different concentrations (0.5, 1, 2, 4, 8 µg/ml) and one control were used . Each concentration was replicated three times. A one half ml solution containing known quantities of fluvalinate in acetone or pure acetone was pipetted into each of 20 ml borosilicate glass scintillation vials. All open vials were then rolled for 6 min to obtain an even distribution of fluvalinate on vial inner surfaces (Plapp and Vinson,1977). Ten mites were used in each replicate.

To obtain mites for bioassays, frames of infested brood were brought to the lab. Individual brood cells were opened and adult mites were harvested from mature pupae with a brush (Elzen et al., 1999).

Ten mites were placed in each vial. Vials were kept in darkness at 24 C. Twenty four hours later, mite mortality was evaluated. Mites were considered dead if they did not exhibit leg movement when touched by a probe (Hillesheim et al., 1996).

LC50 or LD50 values of fluvalinate were calculated using POLO-PC .

PBO Synergism Experiment

All PBO synergism bioassays included two groups of mites: one group treated with only acetone and another group treated with PBO in acetone. Operation technique was similar to the above susceptibility measurement. Three application methods for PBO were evaluated:

Method 1: For the group of mites with PBO, 0.5 ml of 1mg/ml PBO (solvent: acetone) was pipetted into each of three vials. Two hundreds mites were placed in each vial for two hours in darkness at 24 C. After two hours, dead mites were removed, and the remaining mites were transferred to fluvalinate-coated vials and were kept in darkness at 24 C for 24 hours. For the group of mites without PBO, 0.5 ml of pure acetone was pipetted into each of another three vials. Another 200 mites were evenly placed in each vial for two hours in darkness at 24 C. Other operations were the same as the group of mites with PBO.

Method 2: One ml of 20 mg/ml PBO (solvent: acetone) or pure acetone was applied to filter paper (diameter = 90 mm) that was placed in each of three dishes for the group of mites treated with PBO, and for the group of mites without PBO, respectively. Other operations were the same as method 1.

Method 3: 0.2 µl of 500 µl /L PBO solution (solvent: acetone) or 0.2 µl of pure acetone was topically applied to each of the group of mites with PBO and without PBO, respectively. Other operations were the same as method 1.

Method 1 was included in the experiment because it was easily performed. But for method 1, PBO concentration must be restricted to or under 1 mg/ml. Higher concentration of PBO solution cannot be distributed evenly onto vials. Method 2 was used for higher PBO concentrations. Method 3 was used to further check the results of the above two methods in Elke Hillesheim et al's method (1996).

Enzyme assay

General esterase activity: The mites were obtained from adult bees using powder sugar (Macedo et al, 1999) and were stored at -80 C for late use (Gerson et al., 1991). Nonspecific esterase was measured by quantifying the production of V-naphthol following the technique described by Van Asperen (Van Asperen ,1962). Twenty mites were homogenized in 200 µl ice cold buffer A [0.1 M sodium phosphate buffer (PH 7.8) + 1% Triton X-100]. They were then centrifuged at 12,000 rpm for 20 minutes at 4 C . The supernatant was transferred to a clean tube without disturbing the pellet . The lipid layer formed on top of the supernatant. The reaction mixture consisted of 10 µl of five-fold diluted supernatant, 2 µl substrate V-naphthly acetate (25 mM) in acetone, and 188 µl buffer B [0.02 M sodium phosphate buffer (PH 7.0)] in a total volume of 200 µl. The reaction was initiated by addition of the substrate, and after a 15-min incubation at 30 C (with shaking), and was stopped by the addition of 33.2 µl 0.3 % Diazo blue B in 3.5% SDS. After allowing color to develop for 15 min, absorbency at 600 nm was measured in a microtiter plate reader , and the concentration of hydrolyzed substrate was calculated from standard curves of V-naphthol.

Native polyacrylamide gel electrophoresis (PAGE): PAGE was used to separate the above general esterase, using a 8 % separating gel and 4 % stacking gel with a discontinuous Tris-glycine buffer system. The enzyme extract was diluted two folds with dye solution (0.1 % Bromophenol Blue in 10 % sucrose solution). Individual wells were loaded with a volume of microsomal protein containing 60 µg. The gel ran at a constant voltage for 45 min, and protein bands were observed by staining the gels for 30 min with staining solution (49 ml 0.84 mM fast blue salt BN in 0.02 M sodium phosphate plus 1 ml 25 mM a-Naphthyl acetate). After the bands appeared, the gel was transferred from the staining solution into the destaining solution (7% acetic acid) for one night. Finally, they were transferred to a drying solution (40 % methanol, 10% acetic acid, 3% glycerol and 47% D.D. water).

Glutathione S-transferase activity: The method to obtain the mite was the same as in general esterase activity. Glutathione transferase activity was measured with CDNB as an electrophilic substrate according to Grant et al.'s method (1989). The reaction mixture (200 µl) consists of 150 µl sodium phosphate (0.1 M, PH7.6) and 20 µl 50 mM Glutathione(GSH), and 10 µl 40 mM CDNB and 20 µl enzyme extract. The enzymatic reaction was conduced at 30 C for 2 minutes. Absorbency of the conjugate formed was continuously monitored at 340 nm against a reference lacking enzyme (i.e. blank) using a Beckman DU-65 spectrophotometer, and GST activity was calculated using the extinction coefficient e (9.6 mM-1.cm -1) of the S-(2,4-dinitrophenyl)-glutathione conjugate and l = 0.8 cm, v = 200 µl / well. The formula to convert A340 to nmoles product / mg protein was: nmoles product/mg protein = A340 x 1000/1.0752.

P450 activity: The mites were obtained from adult bees: Adult bees were collected in a 500 ml jar with a screen lid. The jar was put in a plastic bag, and CO2 was introduced into the bag for 1-2 minutes. This procedure anesthetized both bees and mites and the mite released from their host. The jar was shaken to screen the mites out. After five minutes, the mites recovered. Live mites were used in P450 assays. Three hundreds resistant mites and three hundreds susceptible mites were homogenized in 1 ml 0.1 M PH7.6 sodium phosphate buffer containing 15 % glycerol (W/V), and centrifuged at 10,000 g for 20 min at 4 C, respectively. The resulting supernatant was used to measure P450 activity. The reaction mixture (200 µl) consisted of 143 µl 0.1 M PH7.6 sodium phosphate buffer without glycerol, 40 µl supernatant, 2 µl 0.5 mM methoxyresorufin and 15 µl 1.2 mM E-NADPH. Activity was measured against a control lacking E-NADPH in a 95 well fluorescent plate reader (Fluoroskan Ascent FL Series, Labsystems; Helsinki, Finland). The measurement conditions were : temperature 30.1 C; interval 10 seconds; measurement count 70.

RESULTS

Synergism

Results of bioassays with and without the synergists PBO are summarized in table 1. In method 1, the resistant ratio of resistant strain and susceptible strain 18.6. This resistant ratio indicates that resistant mites clearly respond differently than the susceptible

population. Table 1 shows that the LC50 or LD50 values for the mites treated with and without PBO in all three methods are not significantly different. Their synergistic factors ranged from 1.0 to 1.5. These results indicate that PBO does not have a significant effect of synergizing fluvalinate applied to *Varroa* mites. PBO is an inhibitor of monooxygenases in the P450 system. Our results suggest that resistance development in *Varroa* mites may be unrelated to the activity of P450 monooxygenases.

Table 1. Biological activity of fluvalinate applied with and withoout PBO to *Varroa* populations that were susceptible and resistant to fluvalinate

			without PBO			RR	with PBO			RR	SF
method	strain	N	LC 50 or LD 50	(95% CL)	Slope	N	LC50 or LD50	(95% CL)	Slope		
1	S	180	0.127	0.095-0.184	1.535	180	0.084	0.045-0.131	1.209		1.5
	R	180	2.357	1.763-3.111	2.196	18.6 180	2.249	1.635-3.014	2.109	26.8	1.0
2	R	180	2.628	1.630-4.442	1.251	181	1.770	1.011-2.840	1.183		1.5
3	R	121	0.777	0.461-1.794	1.356	181	0.761	0.455-1.833	1.028		1.0

S was susceptible strain
R is resistant strain
N is the number of tested mites
RR, i.e. resistance ratio, is equal to the LC50 of the resistant strain divided by the LC50 of the susceptible strain
SF, i.e. synergistic factor, is equal to the LC50 or LD50 of the unsynergized treatment divided by the LC50 or LD50 of the synergized treatment
The LC50 was used in method 1 and method 2 and its unit was µg/ml
The LD50 was used in method 3 and its unit was ng/mite

General esterase activity

For Table 2, the following statistic test is conducted:

H0 : SS^2 = SR^2,
i.e. there is no significant difference in esterase activity for susceptible mites and resistant mites.
Ha : SS^2 (not =) SR^2
i.e. there is significant difference in esterase activity for susceptible mites and resistant mites.

F= SS^2 / SR^2 = 0.911 / 0.279 = 3.265
F0.005,8,8 = 7.496
F9.995,8,8 = 0.133

So, fail to reject H0, that is , SS^2 = SR^2. Our results suggest that there is no significant difference in esterase activity for susceptible and resistant mites. It appears that *Varroa* resistance is not caused by elevated esterase activity.

Table 2. The esterase activity of *Varroa* mites of the susceptible and resistant strain

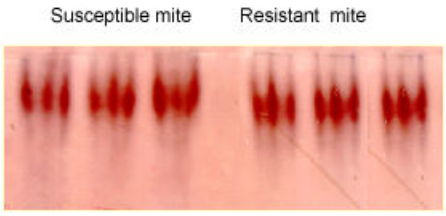
Treatment No.	Replicate No.	Susceptible Mite (nmoles naphthol/15/mgprn)	Resistant Mite (nmoles naphthol/15/mgprn)
1	1	6.127	5.854
	2	8.034	6.246
	3	8.642	6.141
2	4	6.570	6.701
	5	7.503	6.270
	6	5.967	5.015
3	7	6.505	6.407
	8	6.189	6.585
	9	7.596	6.090
$\bar{y} = \sum y / n$		7.015	6.090
$S2 = \sum (y - \bar{y})2$		0.911	0.279

n is the number of replicates

Native polyacrylamide gel electrophoresis (PAGE)

Esterase is divided into three classifications: A - Esterases, B - Esterases, C - Esterases. Each classification contains one or more types of esterase that have different structure and character. The component esterase can be separated through electrophoresis. Figure 1. shows that the esterase compositions are identical for susceptible and resistant strains. Our results suggest that resistance development is not caused by the qualitative change of esterase in *Varroa* mites.

Figure 1. Esterase bands from native PAGE



Glutathione transferase activity

In Table 3 and Figure 2, as incubation time increased, the average product of the GST reactions for the two strains increased. This indicates that the GST of both susceptible and resistant strains have activity.

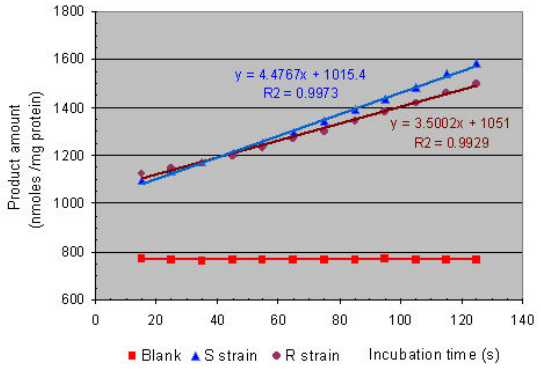
Table 3. The average product of the GST reaction at each reading interval for susceptible and resistant strains

Time (s)		Blank	S strain	R strain
15		770.1	1097.5	1127.2
25		768.2	1134.7	1144
35		762.6	1170.9	1167.2
45		765.4	1210.9	1200.7
55		767.3	1251.9	1232.3
65		767.3	1296.5	1270.5
75		766.4	1343	1303.9
85		765.4	1392.3	1342.1
95		770.1	1437.9	1380.2
105		765.4	1485.3	1422.1
115		764.5	1540.2	1462.1
125		767.3	1583.9	1500.2

The unit of average product of GST reaction is nmoles product / mg protein.

The average values came from the data of three treatments, each of which has three replicates for each strain of *Varroa* mites

Figure 2. The average product of GST reaction For S and R strains VS. incubation time



However, in Figure 2, the two regression lines for reaction product vs. incubation time for S strain and R strain are very close to each other. The results suggest

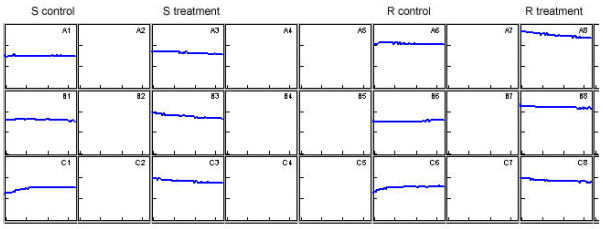
that their GST activities are not significantly different between S strain and R strain. Fluvalinate resistance in *Varroa* mites appears not due to an increased detoxification of glutathione s-transferase.

P450 activity

Most cases of monoxygenase-mediated resistance results from an increase in detoxification. When increased detoxification occurs, there is an increased level of P450 monoxygenase activity in resistant strains when compared with susceptible strains. However, Figure 3 shows the reaction curves are flat in control and treatment for both susceptible strain and resistant strain. This suggests that there are not any P450 monoxygenase differences in susceptible strain and resistant strains of *Varroa* mites.

When our results differed from those of Hillesheim et al. (1996), the first thing we checked was the sensitivity of the assay method. When European corn borer larvae, *Ostrinia nubilalis* (Hübner) (known to contain P450 monoxygenase) was used to repeat the assay, high activity was detected. This indicate that the

Figure 3. P450 monooxygenase activity measured as increased fluorescence from the conversion of methoxyresorufin to resorufin.



assay method was sensitive to P450 monoxygenase and that the above result is valid. It is also consistent with the result of the previous PBO synergism bioassay. So, it can be affirmative that fluvalinate detoxification in *Varroa* mites does not involve the P450 monoxygenase system.

CONCLUSIONS From the above analysis, several points become clear. First, *Varroa* resistance to fluvalinate is not due to the elevated detoxification of esterase or glutathione transferase because there are not any significant differences in esterase activity, esterase composition or in GST activity between susceptible and resistant strains of *Varroa* mites. Second, *Varroa* resistance to fluvalinate does not appear to be due to changes in P450 monoxygenase, since no P450 monoxygenase activity was detected in either susceptible or resistant strains of *Varroa* mites. PBO, the inhibitor of P450 monoxygenase also did not decrease the resistance level. Our results differ from the results of Hillesheim, et al. (1996) and R. Mozes-koch, et al (2000). To further test and verify our findings, the topical application method used by Hillesheim, et al. (1996) was tested for the PBO synergism bioassay and the assay method by R. Mozes-koch, et al's (2000) was tested for P450 activity. However, our results still were the same. The assays used by Hillesheim, et al. (1996) and R. Mozes-koch, et al (2000) may not be reliable for *Varroa*. For Hillesheim, et al's bioassay, topical application is difficult to consistently apply to a small organism like *Varroa* mites, which can move rapidly . We suggest that gluing the mites to double face tape for PBO application may affect reliability. For R. Mozes-koch, et al' assay, their paper states: "The substrates initially used in the monooxygenase microassay included p-nitroanisole (PNA), benzo(a)pyrence, methoxyresorufin and benzphetamine. However, as result were obtained solely with PNA, only this substrate was subsequently used. " Methoxyresorufin is the most sensitive of the four substrates. If methoxyresorufin didn't exhibit activity, naturally, p-nitroanisole also could not.

Two conclusions emerge from this study:(1) There may be more than one mechanism of resistance in *Varroa* population; (2) There must be other resistance mechanism that leads to *Varroa* mite resistance to fluvalinate. For example, decreased cuticular penetration or target site insensitivity.

ACKNOWLEDGEMENTS We thank Xugao Zhuo (Joe) and Diana Londona for their assistance in setting up assays and Bob Roselle and Paula Macedo for their assistance in conducting bioassay.

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Fungicide Resistance

Study of Induced Resistance in Chickpea Seedlings Due to Non-Pathogenic *Fusarium oxysporum*

INTRODUCTION Fusarium wilts, caused by formae speciales of *Fusarium oxysporum* are among the most severe diseases in the world. Fusarium wilts affected many plant species belonging to all the botanical families with the exception of Graminaceae. *F. oxysporum* f.sp. *ciceri* causing wilt of chickpea is a serious threat to cultivation of chickpea in India. Due to the hazardous effects of chemicals to human health and environment, there is need to promote use of biological control agents for control of serious soil borne diseases like fusarial wilts. With increasing awareness of deleterious effects of fungicides on the environment, growing interest in chemical free agricultural products and time consuming breeding programmes, the biological control of plant pathogens has achieved a considerable attention in crop production.

Biological control of Fusarium wilt diseases by formae speciales of *F. oxysporum* had shown potential as an alternative disease management strategy. Non-pathogenic Fusarium has successfully reduced the incidence of Fusarium wilt in numerous crops in green house and field trials (Alabouvette and Couteaudier 1992, Larkin and Fravel 1998). Application of Fo47 strains of non-pathogenic Fusarium isolated from wilt suppressive soils in France had been found highly effective in controlling Fusarium wilt tomato and other vegetable crops in green house tests (Alabouvette et al 1993 and Alabouvette and Lemanceau 1998).

It is well established that pre-inoculation of a plant with an incompatible strain of *F. oxysporum* (either a non-pathogenic strain or a pathogenic strain belonging to another forma speciales) resulted in the mitigation of symptoms when the plant was later inoculated with a compatible pathogen (Matta, 1989, Fuchs et al 1997, Larkin and Fravel 1999). The two isolates of non-pathogenic Fusarium i.e. Fo47, Fo47B10 (French isolate), were recognised as potential antagonists in suppression of Fusarial wilts (Alabouvette et al 1993). During the present studies one Indian Fo52, which showed highly antagonistic activity in preliminary studies (Paul 1997 and Kaur 2003) was used to study the induced resistance in chickpea seed lings.

MATERIALS AND METHODS Induced systemic resistance by three non-pathogenic strains of *F. oxysporum* i.e. Fo52 (Indian isolate) and Fo47 and Fo47B10 (French isolates) was studied in chickpea seedlings by using two methods:

Modified Split Root System

Chickpea (*Cicer aeritinum*) seeds were grown in sterilized (double autoclaved at 15 psi pressure for 20min at 121.7 C temperature) soil. After 5-6 days when radical of seed developed, the germ lings were removed from soil and washed in running water. The radicals were trimmed off with a sterilized sharp blade and again germ lings were planted in sterilized soil. Due to this cutting, the growth of tap rot was terminated and secondary roots developed profusely (Plate 1).

A B C D

Plate-1 Modified split root system in chickpea
A: tap root in chickpea seedling B: cutting of tap root of chickpea
C: initiation of secondary roots D: secondary roots in chickpea

Three-week-old seedlings were removed from soil and roots were divided into two equal parts. Plants were transferred to polythene bags. One of portion was planted in soil polythene bag containing biocontrol agent (Fo52, Fo47 and Fo47B10) @10^6 cfu/g soil (preinoculation with biocontrol agent) and other half of root system was sown in bags containing um amended soil i.e without any BCA. After 10 days pathogenic *F. oxysporum* f.sp. *ciceri* (Foc) was added to second half @ 10^3 cfu/g soil. Eight replicate plants were included per treatment. Plants were kept under controlled conditions of temperature and humidity and observations regarding incidence of wilt was taken at 15 days interval. Control comprised of uninocualed plants having half portion of roots in Foc and other half to unamended soil with antagonists.

Whole Plant Method

Chickpea seeds were grown in soil containing selected isolates of non-pathogenic Fusarium i.e. Fo52, Fo47 and Fo47B10 separately added (@10^6 cfu/g soil) to soil prior to sowing. Three week old seedlings were removed and transplanted to pots containing Foc @ 10^3 cfu/g soil. Ten plants were kept under each treatment. Plants were kept under controlled conditions of temperature and humidity. Observations were taken in terms of development of wilt in plants after transplanting to Foc inoculated pots.

RESULTS Pre-inoculation of chickpea plant with non-pathogenic isolates i.e. Fo52, Fo47 and Fo47B10 resulted in the mitigation of symptoms in them when, were later inoculated with pathogenic isolate (Foc). Modified split root system and whole plant method had been used to study the induction of resistance by non-pathogenic isolates of *F. oxysporum*. The isolate Fo52, Fo47 and Fo47B10 were able to induce the resistance in the pre inoculated chickpea plants and significantly reduced disease incidence as compared to uninoculated plants. In modified split root system disease incidence was reduced to 25-30 per cent in pre inoculated as compared to 75 per cent in uninoculated plants in form

Table 1. Study of induced resistance in non-pathogenic isolates of *Fusarium* and effect on disease incidence

System of inoculation	Antagonists	Disease incidence (%) after days			
		15	30	45	60
A. Modified Split	Fo52	0 (1.0)	0(1.0)	12.5(3.7)	25.0 (5.1)[a]
root system	Fo47	0(1.0)	0(1.0)	12.5(3.7)	25.0(5.1)[a]
	Fo47B10	0(1.0)	12.5 (3.7)	25.0 (5.1)	37.5 (6.2)[b]
	Un-inoculated	0(1.0)	25.0(5.1)	50.0(7.1)	75.0 (8.7)[c]
B. Whole Plant method	Fo52	0(1.0)	0(1.0)	10(3.3)	30(5.5)[a]
	Fo47	0(1.0)	0(1.0)	10(3.3)	30(5.5)[a]
	Fo47B10	10(3.3)	10(3.3)	20(4.6)	40 (6.4)[b]
	Un-inoculated	20 (4.6)	20(4.6)	40 (6.4)	70 (8.4)[c]

FACTOR MEANS							
Method of inoculation (A)			2.16	5.52			
Antagonists (B)			2.95	3.44	4.50	4.50	
Days of observations (C)			2.71	2.71	4.20	5.74	
C.D. (p=0.05)	A-1.3	B-0.18	AxB-0.23	C-1.8	AxC-0.23	BxC-0.3	AxBxC-0.52

Figures in the parenthesis are square root transformed values
C.D. critical difference between the values
Data was analysed using statistical package CPCS1

A(i) A(ii) B(i) B(ii)

Plate 2 : Modified split root in chickpea
A (i & ii)-preinoculation with non-pathogenic *F. oxysporum* isolate Fo52
A(i) soil inoculated with Fo52 A(ii) soil inoculated with pathogenic isolate
B (i & ii) uninoculated plants

of wilting of plants. The similar trend was also found in whole plant system. The incidence was 10-20 per cent in the preinoculated plants as compared to 40 per cent in the un-inoculated plants. The maximum of 30-40 per cent disease incidence was observed in case of whole plant method of inoculation (Table 1). The disease incidence was significantly (p=0.05) reduced after 45 and 60 days in case of both modified split root system and whole plant system. In whole plant system, the disease incidence was significantly reduced both after 45 and 60 days of inoculations in all three non-pathogenic isolates of *F. oxysporum* as compared to un-inoculated plants. Two isolates namely F052 and fo47 were found to reduce the disease incidence maximum of 40 per cent (c.d.=0.52, p=0.05) in both the systems.

Disease incidence in plants under modified split root system was significantly less as compared to whole plant method. However, in both the methods the isolates of non-pathogenic Fusarium were able to

induce resistance but efficacy in terms of modified split root system was higher. In terms of plant growth parameters the plants pre-inoculated with non-pathogenic strains gave better growth as compared to uninoculated plants (Plate 2)

DISCUSSION Induced systemic resistance had been extensively studied for non-pathogenic isolates of Fusarium. Biles and Martyn (1989) were the first to attribute to ISR the control of Fusarium wilt of watermelon achieved by several strains of non-pathogenic *F. oxysporum*. Many investigators like Biles and Martyn (1989), Mandeel and Baker (1991), Fuchs et al (1997) and Larkin and Fravel (1999) used split root method to study ISR in Fusarium and observed that non-pathogenic *F. oxysporum* actively colonized at least the upper layers of root cells. These observations were correlated with the fact that both Fo47 and CS-20 were able to induce ISR in some plant species (Larkin and Fravel 1999). ISR was correlated with both enzymatic changes in the plant often leading to induction of the physical barriers (Benhamou and Garand 2001).

Fuchs et al (1997) attributed the biocontrol activity of non-pathogenic strain Fo47 to induced resistance in tomato correlated with an increased activity of chitinase, beta 1-3 glucanase and beta 1-4 glucosidase. Duijff et al (1998) showed that the non-pathogenic strain Fo47 although not very effective in inducing systemic resistance in tomato induced increased PR proteins. When the main mode of action of a non-pathogenic strain was induction of systemic resistance, it was obvious that the phenomenon implies the physiological state of the plant & fluctuating environmental conditions may affect the ability of the plant to express its resistance to the pathogen, induced by the non-pathogenic *F. oxysporum*.

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Research in Resistance Management

RAPD-PCR Analysis to Monitor Imidacloprid Resistance in Cotton Whitefly

ABSTRACT RAPD-PCR analysis of imidacloprid treated whiteflies produced amplicons (amplified fragments) which were categorized into four groups namely fragments amplified i) in the control (untreated population) but disappeared or reappeared in subsequent generations due to insecticidal pressure, ii) in the imidacloprid treated population (both dead and alive) in different generations, iii) in the individuals surviving the effect of imidacloprid pressure and iv) in the imidacloprid treated dead individuals but were

absent from the treated live individuals. Analysis of amplicons indicated that imidacloprid treatment produced distinct genetic alterations in surviving whiteflies. The potential markers for imidacloprid resistance in whitefly were identified from F6, F9 and F11 generations.

KEY WORDS: *Bemisia tabaci*, molecular markers, insecticide, generations

INTRODUCTION Cotton whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera : Aleyrodidae) is one of the most devastating agricultural pests worldwide as it affects the yield of a broad range of agricultural, fiber, vegetable and ornamental crops (Cahill et al., 1996). Whitefly infestation affects growth, flowering, boll formation, seed and lint development, and thus causes loss of quality and quantity of cotton produce. Worldwide, in many agricultural systems high levels of resistance in whitefly has been reported for many insecticides such as organophosphates, carbamates, synthetic pyrethroids, chlorinated hydrocarbons, several insect growth regulators and neonicotinoids (Prabhaker et al., 1985, Ahmed et al., 1987, Prasad et al., 1993, Mohan and Katiyar 1995, Cahill et al., 1996, Elbert and Nauen 2000).

Random amplified polymorphic DNA-Polymerase chain reaction technique (Williams et al., 1990) has been used previously for population genetic studies of a number of insects including aphids (Black et al., 1992), grasshoppers (Chapco et al., 1992), fruit flies (Haymer and McInns, 1994) and blow fly (Stevens and Wall, 1995). RAPDs are viewed as having several advantages over other molecular markers and DNA fingerprints as the technique randomly samples the genome and hence multiple amplifiable fragments are present for each primer (Lynch and Milligan, 1994). Amplification of genomic DNA by the RAPD-PCR was used to differentiate between deltamethrin resistant and susceptible *Culex pipiens pallens* (Zhu et al., 1998) to study nucleotide divergence and insecticide resistance in aphids (Komazaki et al., 1998).

In spite of the work done on various insecticides, information on the molecular diagnostic techniques for monitoring imidacloprid resistance in whitefly populations is lacking. In view of the above, attempts have been made to identify such molecular markers by exposing whiteflies to imidacloprid selection pressure for eight generations (F0 and from F5 to F11). After the selection, resistance ratio increased by 11.7 folds and this was correlated with the markers amplified in the survivors.

MATERIALS AND METHODS

Selection procedure

Standard leaf disc dip method of bioassay with slight modifications (Elbert and Nauen 2000) was used to treat whiteflies with imidacloprid (0.1%, 0.05%, 0.025%, 0.0125%, 0.00625%, 0.00312% with five replications each). Adults of mixed sex (25-30) collected with mouth aspirators were released in each ventilated Petri dish. Petri dishes were held at 27±2ºC, R.H. 75±10% and photoperiod of 12:12 (light: dark). After 24 hours concentration-mortality regression was worked out using Probit analysis package POLO-PC (Le-Ora software 1987 based on Finney 1971) and living whiteflies of control and treated tests were released separately in rearing cages on untreated young plants. Sampling was done both from dead and live whiteflies of treated and untreated control populations and were stored in 70 per cent ethanol in 1.5 ml Eppendorf tubes in deepfreezer at -35ºC for further analysis.

Living whiteflies (F0) after treatment were released on a fresh plant to obtain a batch of next generation while maintaining discrete generations. Adults that emerged from these plants were considered as F1. Similar procedure was followed for all the succeeding generations. The number of adults subjected to selection in each generation varied depending upon the number and vigor of the adults of the preceding generation. Whiteflies of generation F6-F9 and F11 were exposed to single selective concentration chosen equivalent to LC50 (Bloch and Wool 1994).

DNA extraction and Quantification

For total DNA isolation, 8-14 whiteflies (dead and live) in each generation were thoroughly macerated individually with micro pestle in a 1.5 ml Eppendorf microcentrifuge tube containing 50µl lysis buffer (50 mM KCl, 10 mM Tris-Cl pH 8.4, 60 µg ml-1 proteinase K (Merck, >30 mAnson units/ mg), 0.45% Nonidet NP-40 and 0.45%Tween 20). DNA extraction procedure involved 65ºC for 45 min, 95ºC for 10 min, centrifugation at 13200 rpm for 3 min, two folds dilution with Mili-Q quality autoclaved water, -20ºC for storage. Prior to RAPD characterization DNA quality assessed by agarose gel electrophoresis (0.7% prepared in TAE buffer) was of high molecular weight with DNA band near the wells and no streaking or RNA band. DNA concentration assessed at 260 nm in Biophotometer® was ~15-30 ng/ µl on an average.

PCR and RAPD analysis

Forty Operon primers (Operon Technologies, Almeda, U.S.A.) belonging to OPA, OPB, OPC, OPE, OPF and OPH series were initially screened and out of those, 10 primers showing good amplification with discrete fragments and polymorphism were selected for studying insecticide resistance (Table 1).

The PCR reaction was performed in a 500 µl PCR tube with a reaction volume of 25 µl containing final concentrations as 30-60 ng/ µl DNA, 1.6 nM Primer

(Operon Technologies, Almeda, U.S.A.), 1X Reaction Buffer (Biogene U.S.A.), 1.5 mM MgCl2 (Biogene, U.S.A.), 0.2 mM dNTP mix (MBI, Fermentas), 2.5 U Taq polymerase enzyme (MBI, Fermentas). To avoid evaporation one drop of sterilized mineral oil was overlaid and the reaction was set in the Thermal Cycler with following thermal profile using 40ºC as the annealing temperature [found to be optimum from amplified comparative profiles obtained for different polymerization experiments set at different annealing temperatures (37-40ºC) (data not shown)]: 95ºC - 5 min, 94ºC - 1 min, 40ºC - 1.5 min, 72ºC - 2 min (94ºC - 1 min, 40ºC - 1.5 min, 72ºC - 2 min, 40 cycles), followed by 72 ºC for 20 min as final elongation step and 4ºC for storage until use.

The DNA fragments in the PCR amplified products were separated in 1.4% agarose gel (in TAE buffer having 3 µl (of 1 mg ml -1) ethidium bromide/ 100 ml) run in 1X TAE buffer at 5V/cm for 1 hr with a 100 bp molecular weight standard run along the samples. Gels were photographed by Ultralum Gel Documentation system for scoring.

RESULTS

Total and polymorphic fragments amplified by selected primers

The primers amplifying more than 50 per cent polymorphic fragments were used in all subsequent studies on treated whiteflies in each generation. Per cent polymorphic amplicons (amplified fragments) by each primer are listed in Table 1.

Table 1. Total number of amplified fragments and percent polymorphism

Primer	Sequence (5'-3')	Polymorphism (%)
OPF 12	ACGGTACCAG	84.6
OPH 16	TCTCAGCTGG	54.5
OPB 20	GGACCCTTAC	79.7
OPF 01	ACGGATCCTG	72.9
OPE 02	GGTGCGGGAA	66.1
OPB 05	TGCGCCCTTC	92.3
OPB 10	CTGCTGGGAC	80.6
OPC 04	CCGCATCTAC	76.9
OPC 10	TGTCTGGGTG	85.7
OPA 10	GTGATCGCAG	84.6
Mean		77.8

On an average, each primer amplified 54 fragments in all the generations studied and the number of polymorphic amplicons were 42 with 77.8 per cent polymorphism. Maximum number of polymorphic fragments were amplified using primer OPB 05 (92.3%). Total number of PCR amplified fragments by each primer in all generations ranged from 39 (OPC 04) to 67 (OPB 10).

The number of amplicons per primer and the number of polymorphic fragments in each generation are shown in Table 2. Maximum number of fragments were amplified in the field population (F0) and maximum polymorphism in F5 (83.1%). More than 65 per cent polymorphism in the amplified fragments was obtained in all the generations. Increased polymorphism increases the chances of effective detection of genetic variation that has arisen due to insecticidal pressure.

Table 2. Number of amplicons and polymorphism in different generations

Generation	Total no. of bands per primer (range)	Polymorphism (%)
F_0	3-12	76.9
F_5	4-11	83.1
F_6	3-10	80
F_7	3-12	75.4
F_8	3-9	82
F_9	2-9	69.2
F_{10}	2-10	76.7
F_{11}	3-12	78.7

Identification of PCR based markers

The specific amplicons were divided into the four categories. First category included fragments amplified in the control (untreated) population but disappeared or reappeared in subsequent generations after the insecticidal treatment with imidacloprid. These fragments indicated a genetic change in the whiteflies due to insecticide treatment in the form of alteration in the nucleotide sequences that resulted in disappearance of fragments which were originally present in the untreated population. Second category included the PCR products which appeared in imidacloprid treated population (both dead and alive) in different generations. This category indicated a significant deviation in the genetic organization of whiteflies resulting from the imidacloprid selection pressure and the change was depicted in variation in the banding pattern of treated whiteflies from that of control. Third category was of the fragments amplified in imidacloprid treated dead individuals but were absent from the treated living individuals. These amplicons indicated that the selection pressure created by insecticidal treatment has led to mutation in the genome of whitefly and due to these alterations in the genetic structure of the survivors, the primer does not anneal and hence, leads to disappeared band. Most important category included the PCR amplified products that appeared only in the individuals surviving the effect of imidacloprid selection. The potential molecular (RAPD) markers for imidacloprid resistance in whitefly were identified from F6, F9 and F11 generations as they showed distinct genetic variation among the imidacoprid treated survivors and the susceptible insects which died after the treatment (Table 3).

As evident from the table OPB 05 primer amplified maximum polymorphism (92.3%) along with maximum polymorphic distinguishing fragments that could serve to develop SCAR (Sequence characterized amplified regions) markers for imidacloprid resistance followed by OPB 10, which had lesser polymorphism (80.6%) in the amplified fragments but amplified three potential markers.

Table 3. Potential RAPD markers for insecticide resistance

Primer	Polymorphic bands in imidacloprid treatment surviving individuals only
OPF 12	1100, 800
OPH 16	1200
OPB 20	—
OPF 01	1031
OPE 02	—
OPB 05	2000, 1500, 1031, 900
OPB 10	1150, 1000, 900, 850
OPC 04	—
OPC 10	1100
OPA 10	1200, 950, 850

DISCUSSION The amplicons that were either amplified or those that disappeared in the individuals surviving the effect of imidacloprid selection pressure can serve as the potential RAPD markers for the identification of resistance at an early stage and could help in the pest management programmes. Early detection of resistance is helpful in the identification of effective insecticides to manage the pest. Present work indicated that RAPD fragments are useful as genetic markers to identify insecticide resistance, but a number of factors can complicate their use and interpretation e.g. DNA quality, primer sensitivity and co-migration of non homologous fragments (Stevens and Wall, 1995).

Significance of the fragments amplified only in the survivors is that these show the development of variants in the resistant whitefly population after the selection pressure and hence can be used as possible markers to identify resistant individuals from a field population. In the generations except F6, F9 and F11, the variability was not stable and inconsistency may be due to the random changes in the genome after selection of whiteflies to the insecticide so were not included to identify markers for resistance. Complete segregation of the survivors from the dead individuals in these generations indicated that a distinct variation occurred in the living individuals, which separated them from dead individuals indicating possibility of development of resistance in the survivors.

For developing a repetitive marker, selection should be carried out till the resistance stabilizes as the first few generations developed resistance to imidacloprid at a slow rate followed by a rapid increase in the succeeding generations (Prabhakar et al., 1997). Gradual increase in the initial stage may be due to the fact that the resistance genes are rare in the population but subsequently by selection, as the expression of these genes increases, resistance also increases (Prabhakar et al., 1997). Earlier research indicated that the inheritance of insecticide resistance, which is known to involve gene amplification, could be unstable in the absence of selection and insecticide resistance is also believed to arise from selection acting at random variation i.e. it is pre-adaptive (Devonshire and Field, 1991). Sethi et al. (2002) suggested that insecticide resistance in whitefly against imidacloprid is controlled by more than one gene. Also, dominance estimates indicated that insecticide resistance was completely recessive. Moreover, whiteflies are arrhenotokous and in haplodiploidy fertilized eggs give rise to females, which are heterozygous and unfertilized eggs produce males, which are haploid. This method of reproduction allows rapid selection at later stages and fixation of resistance genes (Denholm et al., 1998). Thus, these factors along with the random changes in the genome affect the amplification of a repetitive RAPD marker. So, the selection pressure carried out for more number of generations can result in increase in the homogeneity of whitefly response and also in discreet markers.

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Resistance Management News

CAST Report on Integrated Pest Management Sets Out a Safer Future for Everyone

July 9, 2003
Washington, D.C

The Council for Agricultural Science and Technology (CAST) is releasing *Integrated Pest Management: Current and Future Strategies*, a report that offers a comprehensive, insightful, and up-to-date analysis of the issues involved in pest control. Integrated Pest Management (IPM) is about making informed choices that impact positively on crops, animals, and the environment, as well as on society. The CAST report is designed to offer policymakers, opinion makers, and educators an informed overview of the changing nature of these choices in the twenty-first century.

This new report sets out the scientific, environmental, and political contexts in which IPM has developed over the last two decades. The document represents a thorough re-evaluation of this approach to pest management, which was addressed in an earlier CAST report on the same topic in 1982.

"Many of the technologies that now impact IPM simply did not exist two decades ago," the report's chair, Dr. Kenneth R. Barker, North Carolina State University, explains. "In preparing this latest assessment, the successful integration of these new and improved tools was considered a critical priority. The authors also stress that the concept of IPM has extended beyond crops, animals, and rangelands to include homes, businesses, schools, and other public buildings. It is a topic that directly affects most citizens." Dr. Barker led the multidisciplinary task force of 20 academic contributors who examined the availability of these new pest management tools and considered how integrated approaches will maximize their benefits.

There are many definitions of IPM. The working definition of the report is "a sustainable approach to managing pests by combining biological, cultural, physical, and chemical tools in a way that minimizes economic, health, and environmental risks." "Essentially, it is a system of practices that leads to the most economical and environmentally friendly actions being taken to control pests," Dr. Barker comments.

The launch of the report is timely for a number of reasons. At an environmental level, invasive native and nonnative pests-including many insects, pathogens, and weeds-are posing new threats to the environment while at a management level, pest challenges must be met within the context of increasingly restrictive regulatory controls. The political context of IPM has evolved over the last decade. In 1993, the U.S. government set a national goal of 75% IPM implementation on U.S. crop acres, and in 1996 the Food Quality Protection Act

established IPM as the pest control paradigm for federal properties and programs. The recently published *Roadmap for Integrated Pest Management* sets out the Federal Government's current strategy. Objective, science-based information is essential for policymakers to address effectively the complex issues involved.

"The pace of development has meant that a complete revisiting of the topic was appropriate," comments Dr. Teresa A. Gruber, Executive Vice President of CAST. "Although others have addressed aspects of IPM, we feel that the CAST approach represents the most comprehensive contribution to the subject."

The new report examines the environment as a series of distinct but interlinked ecosystems, from crop and animal production systems to rangeland, pastures, and forests to aquatic ecosystems and urban environments, and assesses for each the most appropriate interventions in terms of pest control techniques. Cultural practices, biological controls, conventional and new chemistry pesticides, transgenic pesticidal plants, precision application techniques, and diagnostic tools all are considered.

The authors identify seven key issues that future IPM strategies must address. These include

- impact of biotechnology on agriculture;
- genetic diversity and pest adaptability;
- ecology-based management systems;
- increased understanding of microflora/fauna in the environment;
- training and technology transfer;
- government policies and regulations; and
- need for continuous assesment of strategy.

The new CAST report is essential reading for anyone participating in debate and/or making decisions about pest management strategies and tools, including the applications of pesticides in the environment. It also makes an excellent textbook resource for students seeking a grounding in IPM issues. "CAST's mission statement is to bring the most objective, insightful, and relevant scientific information to the widest possible audience. This new report represents a valuable and unique contribution to the ongoing debate about pest management," Dr. Gruber comments.

The complete report, *Integrated Pest Management: Current and Future Strategies*, 246pp., is available online at http://www.cast-science.org/ along with many of CAST's other scientific works, or may be purchased ($50.00 plus shipping) by contacting CAST at 515-292-2125. CAST is an international consortium of 38 scientific and professional societies. CAST assembles, interprets, and communicates science-based information regionally, nationally, and internationally on food, fiber, agricultural, natural resource, and related societal and environmental issues to its stakeholders-legislators, regulators, policymakers, the media, the private sector, and the public.

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Abstracts

Monitoring Resistance in Mexican Populations of TBW, CBW and PBW for Response to CryIA(c) Toxin of *Bacillus thuringiensis*

Cotton production in Mexico has decreased drastically in the last five years, from 300, 000 hectares planted in the middle 1990´s, this season there were planted only 63,595 ha. There are several factors involved in the decrease of cotton production in Mexico, some of them are: low fiber price, high production costs, draught, lack of real federal subsides, and the impact of cotton importation from USA to Mexico. Transgenic cotton was introduced to Mexico in 1996. This technology has been well accepted for pink bollworm (PBW) *Pectinophora gossypiella* control, and now is increasing its popularity against tobacco budworm (TBW) *Heliothis virescens* and cotton bollworm (CBW) *Helicoverpa zea*. Due to the mode of action of the toxin and its expression in cotton plants, the insect populations affected are subject to a high selection pressure. Resistance is a concern and the Mexican government has made obligatory to develop

data on the response of populations under selection pressure. The objective is to detect any change that could indicate resistance problems. Monsanto (line owner of this technology) has established a resistance management strategy based on leaving refuges close to planted areas of transgenic cotton. In order to evaluate the success of the strategy and detect any shift in response to the *Cry* IA (c) protein, tobacco budworm *Heliothis virescens* (F:) cotton bollworm *Helicoverpa zea* and pink bollworm *Pectinophora gossypiella* populations are monitored in three different laboratories each year, for response to the toxin contained in BOLLGARD® transgenic cotton. Bioassays are carried out on field-collected populations and compared to a laboratory susceptible colony. Overlay concentrations of 0.05 µg/ml for TBW and 5 µg/ml for CBW of the toxin are used as diagnostic dosages applied overlay in lepidoptera diet. More than 500 neonate larvae are used in each bioassay, for each colony representing a different cotton producing area. Data on mortality, larvae reaching 3rd instar and percent growth inhibition is obtained 5 days after treatment. Previous results have indicated that these dosages prevented larvae from reaching the 3rd instar, which is an estimator of susceptibility to the toxin. Percent growth inhibition is another parameter to measure susceptibility. In the case of pink bollworm the diagnostic dosage used is 1.0 µg/ml mixed with Lepidoptera diet 21 days later data was obtained including fourth instars, and weight. The resistance monitoring program up to now has not detected any shift in response to this toxin in TBW, CBW or PBW populations from Mexico, even though, transgenic cotton was introduced commercially in Mexico since 1996. This indicates either that the resistance management strategy (refuges close to transgenic cotton) is working well or that selection pressure from transgenic cotton has not been enough to produce resistant genotypes.

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Resistance of *Botrytis cinerea* to Pyrimethanil in Tomato

Seventy *Botrytis cinerea* single-spore isolates were obtained from diseased tomato fruits or leaves collected in 2001 from protected fields in several regions of Liaoning Province. Sensitivity of these isolates to anilinopryimdime fungicide pyrimethanil was determined by the methods of mycelial growth inhibition. The results showed that 20.0% isolates were moderately resistant. High resistant isolates were induced by the fungicide in laboratory but not detected in field, the highest resistant times reached to 35.7. There was no cross-resistance between pyrimethanil and carbendazim as well as between pyrimethanil and procymidone. The wild-type resistant isolates have a good inherit stability, no decreasing of resistant level appeared after transferred 9 times. Significant differences in mycelial growth, fresh weight and osmotic sensitivity were observed among isolates of *B. cinerea*, but no correlation could be drawn between these biological differences and sensitivity. It also was found that there were no differences between resistant isolates and sensitive ones in pathogenetic ability.

KEY WORDS tomato, *Botrytis cinerea*, pyrimethanil, fungicide resistance

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Evaluation of Antixenosis and Antibiosis Resistance Mechanism of Rice Varieties to Striped Stem Borer in the Laboratory Condition

Evaluation of antixenosis and antibiosis resistance of rice varieties to Striped Stem Borer (SSB), *Chilo suppressalis* Wlk. was carried out in the laboratory condition during 1996-97. Antixenosis was determined

by number of oriented and settling larvae on the tested varieties after 1 and 24 hours respectively. There was significant correlation between number of oriented and settling larvae on the experimental varieties (r=0/513*). Antibiosis was determined by larval feeding severity and studies on the survival rate and developmental period of SSB larvae on different rice genotypes.

The results showed that there are significant differences (P< 0.05) between rice varieties due to settling larvae and feeding severity. The Khazar and Sepid roud varieties influence the insect development initially and reduce the survival rate of insect. The Neamat variety had partial resistance in all trials which confirms the field test. Antixenosis and antibiosis resistance are rather similar together and often their identifications are difficult.

KEY WORDS Antixenosis, Antibiosis, rice varieties, Striped Stem Borer.

From the 7th Iranian Crop Sciences Congress, Aug. 24-26, 2002, Karaj, Iran

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Laboratory assays for evaluating Guilan´s rice germplasms resistance to Asiatic rice borer, *Chilo suppressalis* (Walker)

Three resistance screening assays were developed for evaluating rice germplasms, *Oryza sativa* (L.), resistance to the Asiatic rice borer (ARB), *Chilo suppressalis* (Walker), one of the most intractable pest problems of this important food crop during 1996-97. The first assay, the no-choice screening test using larval feeding rate on stem pieces of host plant tested for 48 h. A preference hierarchy representing the resistance ranking of test varieties was obtained using larval feeding rate on stem pieces of host plants tested.

The second and third assays were no-choice test using orientation and settling responses of 3-4 instars of ARB larvae on stem pieces of tested varieties for 1-2h. and 24-48h. respectively. The rice germplasms were significantly different in their degree of resistant to the pest. In this test the variety Neamat showed resistance similar to that determined by the no-choice screening test and field screening. The three assays are complementary and provide useful information on antixenosis and antibiosis, and can therefore be used in sequence. Various attributes of the choice and no-choice tests are discussed.

From the Iranian Journal of Agricultural Sciences, Vol. 32, No. 4, 2001

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Announcements and Submission Deadlines

Thank you to those who contributed to this issue - you have really made the newsletter a worthwhile reading experience! Our contributors truely increase the newsletter's success at sharing resistance information worldwide.

We encourage all of our readers to submit articles, abstracts, opinions, etc (see the newsletter online at http://whalonlab.msu.edu/rpmnews/general/rpm_submission.htm for submission information).

The Newsletter is a resource to many around the globe. It is also a wonderful and effective way to enhance the flow of ideas and stimulate communication among global colleagues. We appreciate your efforts to support the newsletter and we look forward to your continued contributions.

The next two **submission deadlines** are:
Monday, March 15th, 2004
Monday, September 20th, 2004

We hope you continue to consider the newsletter as a forum for displaying your ideas and research.

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