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# Molecular Characterization of South Indian Insecticide Resistant *H. armigera* (Hubner)

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**ABSTRACT: Molecular characterization of the insecticide resistant *Helicoverpa armigera* (Hubner) larvae was done by cytochrome oxidase I (CO-I) primer. Based on the multiple nucleotide sequence alignment of cytochrome oxidase I (CO-I) region, the larval samples were divided in to three haplotypes. Haplotype I: A1 Cyper selected –MBNR; A2 Metho selected-MBNR; A3 Spino selected- MBNR; A4 Indo selected – MBNR and B1 Cyper selected –Raichur. Haplotype II: C1 Cyper selected- Nagpur; C2 Metho selected-Nagpur; C3 Spino selected- Nagpur; C4 Indo selected- Nagpur. Haplotype III: B2 Metho selected-Raichur; B3 Spino selected-Raichur and B4 Indoselected-Raichur. Development of resistance by *H. armigera* to insecticides, the high cost of insect control, environmental concerns, legal restrictions on the use of chemicals and frequent outburst of *Helicoverpa* suggest that efforts are now needed to understand the molecular diversity leading to identification of virulent biotypes, understanding molecular basis of insecticide resistance and to formulate management strategies accordingly.**

**Keywords :** Helicoverpa armigera, Insecticide Resistance, Molecular characterization, CO-I, Haplotypes.

## INTRODUCTION

The bollworm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) is a polyphagous pest of worldwide causing estimated global economic losses of over 3 billion US dollars annually. It is estimated that *H. armigera* alone is responsible for losses over Rs. 35000 million annually in India despite heavy pesticide inputs because of its high fecundity, migratory behavior, high adaptation to various climatic conditions (Kumar et.al 2003). In India this insect occurs as a major pest in many economically important crops, including cotton, pigeonpea, chickpea, tomato, okra, blackgram, maize, sorghum and many other crops, causing heavy crop losses every year (Reed and Pawar, 1982; Manjunath, 1990). *H. armigera* is also characterized by its high mobility and fecundity and it has shown great capacity to develop resistance to synthetic insecticides used in its management (Armes *et al*. 1996; Kranthi, 1997; Ramasubramanian and Regupathy, 2004). The versatility of this species may be due to the presence of a strong genetic variability governing the behavior of *H. armigera* making it a serious pest on several crops (Zhou *et al*., 2000).

Understanding the genetic variation among the *H. armigera* populations occurring on host plants has become essential to understand the variation in their susceptibility to different insecticides. The ability of insect species to thrive on diverse host plants is an adaptive advantage for their better survival in the ecosystem. Majority of field populations of *H. armigera* in Pakistan exhibited susceptibility close to the baselines for indoxacarb and spinosad having novel modes of action, there were, nevertheless, signs of resistance development to the new chemistries as demonstrated by a low level of tolerance in many populations. This may be due to a cross-resistance from the resistance mechanisms, particularly metabolic, already selected against older chemistries (Ahmad *et al.*, 2003).

The occurrence of insecticide resistant populations can be reduced or delayed by reducing the selection pressure, by using alternate insecticides with novel mode of action. Understanding of genetic variation within and between geographical populations of *H. armigera* in the cotton ecosystem and genome-fluxing patterns, coupled with estimating resistance folds to each insecticide can expectedly help in pinning down the exact causes for such frequent outbreaks and versatility in evolving resistance to insecticides at a faster rate. The genetic variation among geographic populations of *H. armigera* collected from the South Indian cotton ecosystem was analyzed using RAPD markers and 12 populations could be classified into two distinct groups (Fakrudin *et al.,* 2004a). In this regard a better understanding of the genetic differences of

polyphagous pest like *H. armigera* can be very useful to understand the structure and population dynamics, their behavior and response to various selection pressures. Elucidation of gene statements responsible for insecticide resistance in *H. armigera* would bring more light in understanding the phenomenon and management of the problem. In the Indian context, a systematic and concerted effort to view the problem of insecticide resistance and cross resistance from this perspective is important.

Despite its obvious agricultural importance, very little is known about its genetic population structure at molecular level. In India, development of tolerance by *H. armigera* to insecticides, the high cost of insect control, environmental concerns, legal restrictions on the use of chemicals and frequent outburst of *Helicoverpa* suggest that efforts are now needed to understand the molecular diversity and genomic flux leading to identification of virulent biotypes, understanding molecular basis of insecticide resistance and to formulate management strategies accordingly.

Genetic data obtained from molecular techniques allows us to infer geographic structure by estimating genetic similarities and population subdivision among populations or examining relationships among genotypes from several populations relative to this geographic location (phylogeography) (Roderick, 1996).

Molecular genetic data can also enhance our understanding of insect biosystematics and evolutionary trends that have enabled some insects to rapidly achieve pest status (Roehrdang and Degraugillier, 2000).

## MATERIALS AND METHODS

The larvae collected from Mahaboobnagar, Raichur and Nagpur were reared separately in the laboratory to obtain pupae (Fig. 1). Male and female pupae were separated and kept for single pair mating. At least four single pairs were maintained from each place. The eggs obtained from single pair were reared to obtain first generation larvae. Third instar *H. armigera* larvae from $F_1$ with an average weight of 30 mg ± 0.011 S.E. of Mahaboobnagar, Raichur and Nagpur strains were subjected separately to different concentrations of the test insecticides *viz.,* methomyl representing carbamates, cypermethrin representing synthetic pyrethroids, spinosad belongs to spinosyns and indoxacarb belonging to oxadiazine group of insecticides (Fig.2 & Plate 1&2). Mortality of the larvae was recorded at 24, 48 and 72 hours after treatment. The mortality at 72 hours after treatment was considered as end point for the assessment of toxicity of test insecticides as reported by Fisk and Wright (1992). Thus, concentrations of wide range initially and subsequently narrow range were tested so as to get mortality data in the range of 5-90 %. The moribund larvae also were considered as dead while recording the mortality data.

The survivals at $LD_{50}$ concentration in each test insecticide at first generation ($F_1$) were reared separately to next generation ($F_2$). Male and female pupae were separated and allowed for single pair mating as per standard procedure. Third instar larvae from single pair mating second generation ($F_2$) were subjected again to different doses of all the test insecticides. The survivals at $LD_{50}$ in each test insecticides treatment of second generation ($F_2$) were reared separately to next generation ($F_3$ in the same manner of single pair mating. Third instar larvae from single pair mating of third generation ($F_3$) were subjected again to different doses of all the test insecticides. DNA extracted from the survivals of F3 generation and subjected to molecular characterization.

The DNA was extracted from the selected survivals of $F_3$ generation of different areas using modified C-TAB method (Saghai Maroof *et al*., 1984).

**DNA extraction.** The insects were washed thoroughly in double distilled water and the genomic DNA was prepared using a modified C-TAB method (Saghai Maroof *et al*., 1984). The intact genomic DNA was visualized using in a 0.8% agarose gel and quantified using a nanovue following standard procedures. Depending upon the concentration, the DNA samples were diluted with sterile distilled water to get a working solution of 20–25 ng/µl.

**PCR amplification.** The genomic DNA from *H. armigera* of different insecticide selected strains were subjected to polymerase chain reaction (PCR) using 9 different RAPD primers ABA-01, ABA-10, ABA-12, $AB_2$-01, $AB_2$-11, $AB_3$-18, $AB_4$-06, $AB_4$-07, $AB_4$-17 and CO-I primer specific for CO-I region of mtDNA. PCR was carried out in 25 µl reaction mixture containing 20-30 ng DNA as the template. Genomic DNA 1.0 µ1 (25 ng), dNTPs 1.0 µ1 (100 µM), assay buffer 2.5 µl (1X), RAPD primer 1µ1 (20 µM), Taq polymerase 0.2 µl (1units), magnesium chloride 2.0 µl (2mM), sterile distilled water 17.3 µl were added and for Co-I region farward primer 0.5 µ1(10p moles) ( 5'-GCTCAACAAATCATAAAGATATT-3'), reverse primer 0.5 µ1(10p moles) (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') were added in addition to the earlier compounds instead of RAPD primer one micro litre and PCR was performed in a DNA thermal cycler (www.mjr.com) programmed for 2 min at 94° C for initial denaturation. Following the initial denaturation the thermal cycler was programmed for 38 cycles of 45 sec at 94° C for denaturation, 45 sec at 51° C for annealing and 1.5 min at 72° C for extension. An additional cycle of 10 min at 72° C was also used for primer extension .

**Electrophoresis of PCR products.** PCR products were analyzed by electrophoresis in 6 per cent polyacrylamide gel in Bangolore genei make gel electrophoresis unit run at 100 V/cm for 1.5 h in 0.5X TBE buffer. The banding pattern was visualized using Gel doc instrument and photographed. The molecular size of the amplified products was estimated using a 100 bp DNA ladder (Fermentas Inc.USA,www.fermentas.com). The samples were analyzed twice for all primers to test the reproducibility of bands.

None of the RAPD primers used in the present investigation was able to amplify the genomic DNA.

However CO-I primers amplified the DNA fragment at an expected size of 700bp and the sequencing of that region was done at Genetech.Labs, Kerala. Analysis of the nucleotide sequence was done using genetech software for alignment. Based on multiple nucleotide sequence alignment and single nucleotide polymorphism, the samples were divided into different haplotypes.

## RESULTS AND DISCUSSION

The larvae of *H. armigera* resistant to cypermethrin, methomyl, spinosad and indoxacarb at $F_3$ generation were selected from Mahaboobnagar, Raichur and Nagpur populations.

DNA was extracted from the samples as detailed in materials and methods. A total of 9 RAPD primers *viz.,* ABA-01, ABA-10, ABA-12, $AB_2$-01, $AB_2$-11, $AB_3$-18, $AB_4$-06, $AB_4$-07 and $AB_4$-17 were used for the amplification of genomic DNA but none of them amplified the genomic DNA ( Fig.3) hence no polymorphism was observed. Cytochrome oxidae I (CO-I) primers for CO-I region of mt-DNA was used to study the genetic differences of the selected insecticide resistant larvae, as these primers can detect any minute changes in the conserved regions of the mt-DNA which inherited maternally and spread rapidly within the population. CO-I primers (forward primer: 5'GCTCAACAAATCATAAAGATATT3' and Reverse primer: 5'TAAACTTCAGGGTGACCAAAAAATCA3') amplified the DNA fragment at an expected size of 700bp. Blank DNA, positive control and negative PCR controls were carried along with normal experiments to check against any cross contamination. The amplified product of DNA samples of the insecticide resistant larvae were shown in Fig.4

**Multiple Nucleotide Alignment.** Cytochrome oxidase I region of mitochondrial DNA (mt DNA) at expected size of 700bp was sequenced. The multiple nucleotide sequence alignment of cytochrome oxidase sub unit I (CO-I) was shown in Fig.5. A total of 360 base pairs of the CO-I region were sequenced. The results indicated the existence of considerable similarities, dissimilarities and substitutions of nucleotide sequences in multiple alignment. The nucleotide differences, substitutions and similarities results in to different haplotypes. Based on multiple nucleotide sequences alignment three haplotypes were formed.

Haplotype I: A1 Cyper selected MBNR; A2 Metho selected-MBNR; A3 Spino selected- MBNR; A4 Indo selected – MBNR and B1 Cyper selected–Raichur;

Haplotype II: C1 Cyper selected- Nagpur; C2 Metho selected-Nagpur; C3 Spino selected-Nagpur and C4 Indo selected- Nagpur;

Haplotype III: B2 Metho selected-Raichur; B3 Spino selected-Raichur and B4 Indo selected- Raichur

The results indicated that the insecticide resistant larvae showed similar nucleotide sequence at Cytochrome oxidase I (CO-I) region of mitochondrial DNA irrespective of the chemicals they were selected for. Further, the differences in the nucleotide sequences of Cytochrome oxidase I (CO-I) region may be attributed to be geographic in nature.

In nucleotide sequence alignment 92 per cent samples were similar even if they were selected for different insecticides indicating that there may not be any influence on nucleotide sequence at Cytochrome oxidase I (CO-I) region by insecticide selection. Further, when selection was done by log dose probit bioassay method the metabolic activity and detoxification mechanisms would be very active in insects.

Mitochondrial genome is maternally inherited, any change in the mitochondrial (mt) DNA is transmitted to the entire progeny. Evolutionary changes in conserved regions of mt DNA spread rapidly within populations. If the changes have a functional significance they are in evolutionary adaptation. Cytochrome oxidase I (CO-I) region of mt DNA is the most studied region of the insect mitochondrial genome. Sequencing of this CO-I region is used to study the intra specific, inter specific and any minute variations at genomic level in insects.

The inter specific differences among *H. armigera* and *H. assulta* was studied using the farward (C1-J-2090) and reverse (C1-N-2659) primers to amplify mid CO-I region. The partial CO-I sequences of *H. armigera* and *H.assulta* indicates that 18 nucleotide substitutions and 8 amino acid substitutions were evident between them (Kranthi *et al*., 2006). Hence, using cytochrome oxidase I sequencing is most advanced and reliable method to study the differences at molecular level in insects.

Genetic variability within and between geographic populations of cotton bollworm, *H. armigera* prevailing in South Indian cotton ecosystems using RAPD-PCR. The mean similarity coefficient across populations ranged from 0.75 to 0.82. Samples from Madhira had the highest (0.82) and those from Nagpur, Nanded and Nalgonda had the lowest similarity (0.75). Within populations also, it ranged from 0.73 to 0.86 indicating significant amount of genetic heterogeneity. Clustering analysis revealed two major groups, each comprising of six populations, corresponding to Southern and Northern parts of South Indian cotton ecosystem ( Fakrudin *et al,* 2004a)

In recent past the studies on polymorphic microsatellite loci for the cotton bollworm, *H. armigera* found five polymorphic tri and tetra nucleotide microsatellite loci suitable for population genetic analysis. *H. armigera* from two partial phage mid genomic libraries enriched for microsatellite inserts. The overall microsatellite cloning efficiency in *H. armigera* is 2.5%, which is approximately eightfold lower than that for the gadoid fishes (20%) employing the same enrichment protocol, supporting the notion of a relative low frequency of microsatellite sequences in lepidopteron genomes. In addition, a large proportion of cloned microsatellite sequences turned out to be repetitive DNA, thus further increasing the difficulty of developing such markers in butterflies and moths (Ji *et al.,* 2003)

Further, the molecular studies with the help of RAPD markers to assess the genetic variability among 12

geographical populations of the cotton bollworm, *H. armigera,* collected during peak incidence from each of the 12 locations of the South Indian cotton ecosystem, *i.e.*, Naded, Nagpur and Parbhani (Maharashtra), Guntur, Madhira and Nalgonda (Andhra Pradesh), Raichur, Dharwad and Mysore (Karnataka), Coimbatore, Madurai and Kovilpatti (Tamil Nadu) was done by Fakrudin *et al,* 2004b. A total of 497 amplicon levels resulting from 25 primers were used for the analysis. The highest number of 37 amplicon levels was produced by the primer OPA 01, followed by 32 levels each by OPD 07 and OPA 05. The lowest number of 7 marker levels was obtained with OPD 13. On average, there were 19.88 amplicon levels per primer, of which 19.80 were polymorphic, indicating high variability among *H. armigera* populations. All 25 primers selected for the study produced unique banding patterns that differentiated all 12 geographical populations of *H. armigera*.

The present findings indicate that differences in nucleotide sequences among different populations were evident and rapid changes in the nucleotide sequences of CO-I region of mt DNA were negligible. The method of sequencing of CO-I region is very accurate and expensive to know the differences at nucleotide level. The molecular studies will be used for genetic diversity, resistance mapping and to formulate the effective management strategies. The future scope of work will be exploiting the molecular analysis data to manage the pest and minimizing the yield losses.

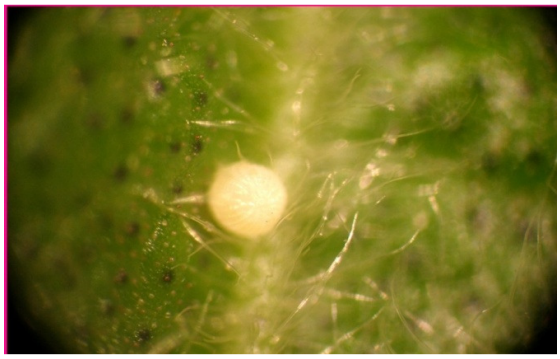


**Plate 1:** *Helicoverpa armigera* egg.

**Plate 2:** *Helicoverpa armigera* larvae on artificial diet.


**Fig. 1.** Map showing the *Helicoverpa armigera* collection sites.

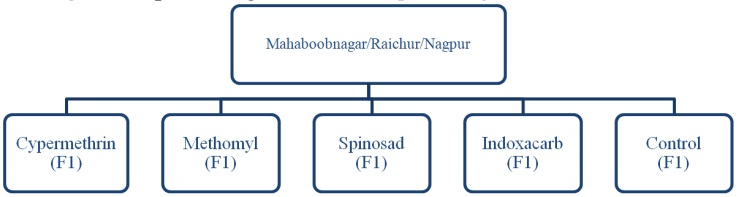



**Fig. 2.** Bio assay procedure for different populations

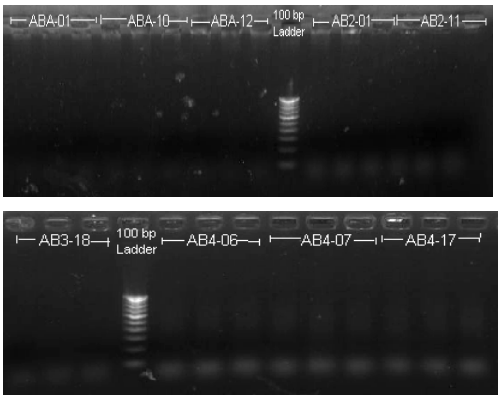
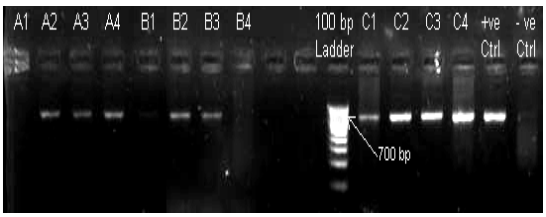



**Fig 3.** RAPD product of insecticide resistant *H.armigera* with 9 primers




A1 Cyper selected –MBNR;
A2 Metho selected-MBNR;
A3 Spino selected- MBNR;
A4 Indo selected – MBNR;
B1 Cyper selected –Raichur;
B2 Metho selected-Raichur;
B3 Spino selected-Raichur;
B4 Indoselected- Raichur;
C1 Cyper selected- Nagpur;
C2 Metho selected-Nagpur;
C3 Spino selected-Nagpur;
C4 Indo selected- Nagpur;
100 bp ladder;
+ve control;
-ve control;

**Fig. 4.** PCR-CO I product of *H.armigera.*

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          10        20        30        40        50        60        70        80        90       100
...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
A1 TGGTAATTGACTTGTACCTTTAATATTAGGAGCCCCTGATATA
   GCTTTCCCCCGAATAAATAATATAAGTTTTTGATTACTTCCCCCTTCTTTAACTTTA
A2 ..........................................................................................
A3 ..........................................................................................
A4 ..........................................................................................
B1 ..........................................................................................
B2 ...A......................................................................................
B3 ...A......................................................................................
B4 ...A......................................................................................
C1 ..........................................................................................
C2 ..........................................................................................
C3 ..........................................................................................
C4 ..........................................................................................

          110       120       130       140       150       160       170       180       190       200
...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
A1 CTTATTTCAAGAAGAATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTA
CCCCCCACTTTCATCTAATATTGCACATGGAGGAAGATCAGTAGACC
A2 ..........................................................................................
A3 ..........................................................................................
A4 ..........................................................................................
B1 ..........................................................................................
B2 .....................................................................................A....
B3 .....................................................................................A....
B4 .....................................................................................A....
C1 ..........................................................................................
C2 ..........................................................................................
C3 ..........................................................................................
C4 ..........................................................................................
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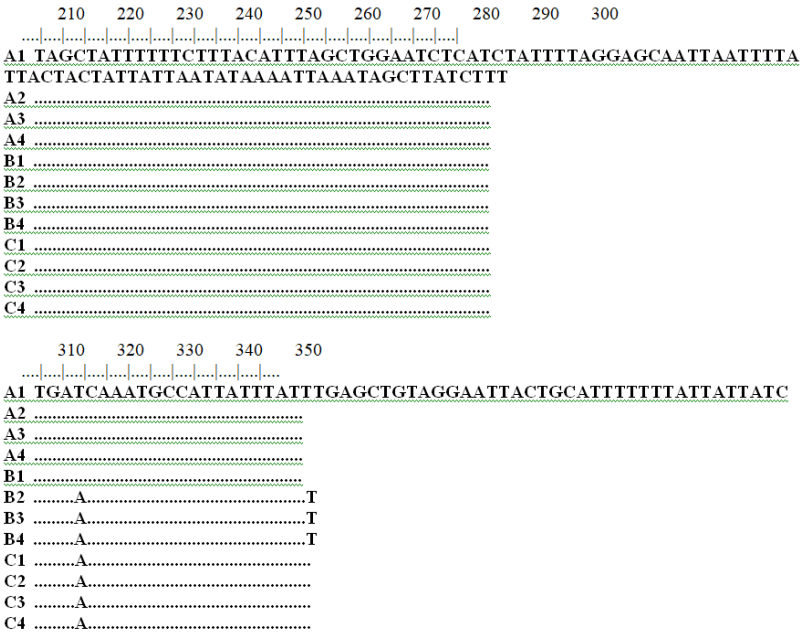


A1 Cyper selected –MBNR; A2 Metho selected-MBNR; A3 Spino selected- MBNR; A4 Indo selected – MBNR; B1 Cyper selected–Raichur;
B2 Metho selected-Raichur; B3 Spino selected-Raichur; B4 Indoselected- Raichur; C1 Cyper selected- Nagpur; C2 Metho selected-Nagpur;
C3 Spino selected-Nagpur; C4 Indo selected- Nagpur;

**Fig. 5.** Mulitiple nucleotide alignment of mitochondrial CO I region insecticide resistant *H.armigera* selected from Mahabbonagar, Raichur and Nagpur populations at $F_3$ generation.

## CONCLUSION

Molecular characterization by different molecular techniques allows us to study geographic structure, genetic similarities and population variations among different populations. Cytochrome oxidase I (CO-I) region of mt DNA is the most studied region of the insect mitochondrial genome. Sequencing of this CO-I region is used to study the intra specific, inter specific and any minute variations at genomic level in insects. In the present study three populations *viz.,* Mahaboobnagar, Raichur, Nagpur populations of insecticide resistant *H. armigera* are characterized by using CO-I sequencing.

## FUTURE SCOPE

Molecular characterization of different populations using Cytochrome oxidase I (CO-I) will give the accurate genetic variations and similarities among populations.

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**Conflict of Interest.** None.

## REFERENCES

Ahmad, M., Arif, M. I. and Zahoor Ahmad (2003). Susceptibility of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to new chemistries in Pakistan. *Crop Protection, 22*(3): 539-544.

Armes, N. J. Jadhav, D. R. and Desouza, K. R. (1996). A survey of insecticide resistance in *Helicoverpa armigera* in the Indian subcontinent. *Bulletin of Entomological Research*, *86:* 499-514.

Fakrudin, B. Prakash, S. H. Krishnareddy, K. B. Vijaykumar, Prasad, P. R. B. Patil,B.V. and Kuruvinashetti, M. S. (2004b). Genetic variation of cotton bollworm, *Helicoverpa armigera* (Hubner) of South Indian cotton ecosystem using RAPD markers. *Current Science*, *87*(12): 1654-1657.

Fakrudin, B. Vijaykumar, Krishnareddy, K. B. Patil, B. V. and Kuruvinashetty, M. S. (2004a). Status of insecticide resistance in geographical populations of cotton bollworm, *Helicoverpa armigera* in South Indian cotton ecosystem during 2002-03. *Resistant Pest Management Newsletter, 13*(2): 12-15.

Fisk, T. and Wright, D. J. (1992). Speed of action and toxicity of acylurea insect growth regulators against *Spodoptera exempta* (Walk.) and *Spodoptera littoralis* (Biosd.) larvae: effect of inter moult age. *Pesticide Science*, *35*: 331-337.

Ji, Y. J. Zhang, D. X. Hewitt, G. M. Kang, L. and Li, D. M. (2003). Polymorphic microsatellite loci for the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae) and some remarks on their isolation. *Molecular Ecology*, *3*(1): 102

Kranthi, K. R. Armes, N. J. Rao, N. G. V. Raj, S. and Sundaramurthy, V. T. (1997) Seasonal dynamics of metabolic mechanisms mediating pyrethroid resistance in *Helicoverpa armigera* in central India. *Pesticide Science,* 50:91-98

Kranthi, S. Kranthi, K. R. Bharose, A.A. Syed, S.N. Dhawad, C.S. Wadaskar, R.M. Behere, G.T. and Patil, E.K. (2006) Cytochrome oxidase I sequence of Helicoverpa ( Noctuidae:Lepidoptera) species in India – Its utility

as a molecular tool. *Indian Journal of Biotechnology*, *5*: 195-199

Kumar, H. and Kapur, A.(2003). Transgenic Bt crops as a component of Integrated Pest Management. In: *Biotechnological Strategies in Agro-Processing*, 85-104.

Manjunath, T. M. (1990). Controlling cotton pests. Deccan Herald, January 4, 1990 *Science and society* Pp: 1-3.

Ramasubramanian, T. and Regupathy, A. (2004). Laboratory and field evaluation of spinosad against pyrethroid resistant population of *Helicoverpa armigera* (Hub). *Journal of Biological Sciences, 4*(2): 142-145.

Reed, W. and Pawar, C. S. 1982 *Heliothis*,a global problem. In: *Proceedings of International Workshop on Heliothis Management, ICRISAT*, Patancheru, Andhra Pradesh, India pp: 9-14.

Roderick, G. K. (1996) Geographic structure of insect populations: gene flow, phylogeography and their uses. *Annual Review of Entomology*, *41*: 325-352.

Roehrdanz, R. L. and Degrugillier, M. E. (2000). Mitochondrial gene order and arthropod evolution. *Entomological Society of American North Central Branch Meeting*, March 23-26, Minneapolis.

Saghai Maroof, M. A. Soliman, M, K. Jorgenson, R. A. and Allard, R. W. (1984). Ribosomal DNA spacer length polymorphism in barley, Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences* (USA) *81*: 8014-8018.

Zhou, X. Fartor, O. Applebaum, S. W. and Coll, M. (2000). Population structure of the pestiferous moth *Helicoverpa armigera* in the Eastern Mediterranean using RAPD analysis. *Heredit, 85*: 251-256.

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