INTERNATIONAL JOURNAL OF PLANT PROTECTION
VOLUME 7 | ISSUE 2 | OCTOBER, 2014 | 281-286 ● e ISSN-0976-6855 | Visit us : *www.researchjournal.co.in*

IJPP

RESEARCH PAPER

DOI : 10.15740/HAS/IJPP/7.2/281-286

Genetic variability of brown lacewing, *Micromus igorotus* Banks (Haemerobidae: Neuroptera) and *Dipha aphidivora* (Meyrick) (Pyralidae: Lepidoptera) through RAPD markers in sugarcane ecosystem

■ P.V. MATTI*, C.P. MALLAPUR, K.A. KULKARNI AND D.N. KAMBREKAR

Department of Entomology, University of Agricultural Sciences, DHARWAD (KARNATAKA) INDIA

ARITCLE INFO

Received : 02.08.2013
Revised : 16.07.2014
Accepted : 02.08.2014

KEY WORDS :
Genetic similarity, Molecular chacterization, *Micromus igorotus*, *Dipha aphidivora*, RAPD, PCR

***Corresponding author:**
Email: poornimamatti@gmail.com

ABSTRACT

This study used 10 RAPD markers, to provide insight into the genetic variability through, molecular characterization of brown lacewing and *Dipha aphidivora* from different geographical locations under laboratory conditions. There existed variation in the DNA profiling among all samples. The dendrogram constructed using symmetric matrix of different samples resulted into two major clusters. The similairity matrix pertaining to different isolates of *Micromus igorotus* and *Dipha aphidivora* revealed that the similarity co-efficient ranged from 0.5-0.80 and 0.57-0.80, respectively. The highest genetic similarity index (0.80) of *Micromus igorotus* was seen between the isolates from Bangalore and Poona followed by 0.6 between the isolates from Bangalore and both Dharwad and Bagalkot. However, the highest genetic similarity index of 0.8 was seen between the isolates *Dipha aphidivora* from Assam and Poona followed by 0.57 was seen between Bangalore and Dharwad and also from Bangalore and Sameerwadi (Bagalkot).

How to view point the article : Matti, P.V., Mallapur, C.P., Kulkarni, K.A. and Kambrekar, D.N. (2014). Genetic variability of brown lacewing, *Micromus igorotus* Banks (Haemerobidae: Neuroptera) and *Dipha aphidivora* (Meyrick) (Pyralidae: Lepidoptera) through RAPD markers in sugarcane ecosystem. *Internat. J. Plant Protec.*, **7**(2) : 281-286.

INTRODUCTION

Sugarcane woolly aphid (SWA), *Ceratovocuna lanigera* (Zehntner) a quick proliferating and fast spreading pest is prevalent in Tamil Nadu, Maharashtra and Karnataka. Of the carnivorous insect fauna feeding on SWA, predators play a significant role in suppressing its population. Of the 30 species of predators reported in the world (Joshi and Viraktmath, 2004), *Dipha aphidivora* (Meyrick) ranks the top in SWA suppression thanks to its host specificity, predatory potential (Arkaki and Yoshiyasu, 1988) and wider adaptability (Lingappa *et al.*, 2004). In China, *D. aphidivora* is one of the most abundant and important aphid suppressing factors (Cheng *et al.*, 1994). The unique feature of this predator are it is specific to SWA and feeds on all its stages. It is one of the two species of aphidiphagous pyralidae. Life cycle is completed in 35.2 days (Mote and Puri, 2003). Feeding always occurred inside the galleries only.

Even under natural conditions where, bioagents can breed in plenty, their number is not sufficient to keep pace

with that of sugarcane woolly aphid which is viviparous by reproduction, continuously laying individuals without any resting stage in its life cycle. The commonly found natural enemies of SWA are predators namely, the coccinellid, *Microaspis*, brown lacewing, *Micromus igorotus*, syrphid, *E. confrater*, lepidoptrean predator, *Dipha aphidivora* and the hymenopteran parasitoid, *Encarsia flavocinctum* of which the major one is *D. aphidivora* occurring always, during the surveys conducted in many parts of India (Anonymous, 2005).

Micromus igorotus commonly called as brown lacewing, is a voracious feeder found naturally in most of the SWA infested sugarcane fields. Of the three species of *Micromus, M. igoratous* (Banks) and *M. timidus* are prevalent in India (Patil, 2003). Among them, *M. igoratous* (Lingappa *et al*., 2004) is very effective and found feeding on all the stages of the pest. Both grubs and adults are predatory in nature, do not show cannibolism and co-exists with another predator, *Dipha aphidivora* Lingappa *et al*. (2004) have reported that both grubs and adults have a feeding potential of 20-25 aphids/day.

It is often difficult to separate morphologically similar species and there is evidence that some species could in fact be complexes of cryptic species. Recently, due to unreliability of morphological character, molecular techniques have been investigated to identify markers that differentiate closely related species. Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) using a random primer amplifies many regions of genomic DNA (Williams *et al.*, 1990). By using different primers polymorphisms at many loci can be detected between species and populations. Therefore, RAPD-PCR analysis can increase the resolution of genetic differences. This method has been widely used in the determination of population structure without prior knowledge of DNA sequences, on the basis of RAPDs, genetic polymorphism in natural populations (Haag *et al.*, 1993) and geographically isolated populations.

Molecular genetic data can also enhance out understanding of insect biosystemics and evolutionary trends that have enabled some insects to rapidly achieve pest status (Roehardang and Degrauiller, 2000). With advent of molecular markers has been introduced over the last two decades which has revolutionized the entire scenario of biological sciences. Among the repertoire of molecular markers tools that are available today to molecular Entomologists, two approaches Restriction fragment legnth polymorphisms (RFLPs) and (PCR) Polymerase chain reaction based markers which encompasse a variety of techniques such as microsatllites, RAPD, Inter-simple sequences repeates (ISSR) and Amplified fragment length polymorphic DNA (AFLP) seem to be indispensable genetic analysis with RAPD markers is more rapid and simpler than RFLP analysis and requires smaller amounts of DNA.

Polymerase chain reaction (PCR) refers to the *in vitro* amplification of particular DNA sequence using arbitrarily or specific primers and a thermostable DNA polymerase enzyme. Karry mullis discovered the PCR in the year 1985, PCR based RAPD is widely being adopted to detect the polymorphism among populations/individuals including insects ever since its discovery. The analysis of nucleiotide sequence variability has been revolutionized by the development of the polymerase chain reaction (PCR). It is very sensitive and powerful tool to detect the differences in the sequences of DNA depending on the primers employed. This technique is widely used to fingerprint the genotypes to develop DNA markers (RAPD markers) specific to races and biotype (Harry *et al.*, 1998). This RAPD method of genome analysis is fast and allows the examination of genomic variation without prior knowledge of DNA sequqnces (Williams *et al.*, 1990 and Welsh and McClelland, 1990). The resulting collection of amplified products can be separated by electrophoresis to generate a "fingerprint". Genetic variation can be useful for strain identification (Welsh *et al.*, 1991). This technique can detect cryptic changes and or differences between the individuals. The results of the RAPD analysis would give precise information on molecular nature of the pest and as well would also be useful in applied entomology and plant breeds in general (Henry, 1998).

MATERIAL AND METHODS

Sample collection :

In the present study, the genetic variability has been revealed by RAPD in different geographical populations. *Micromus igorotus* were collected during the peak incidence from each of the 4 locations of sugarcane ecosystem: Dharwad, Sameerwadi, Bangalore (Karnataka) and Poona (Maharastra). *Dipha aphidivora* were collected during the peak incidence from each of the 5 locations of sugarcane ecosystem: Dharwad, Sameerwadi, Bangalore (Karnataka), Poona (Maharastra) and Johrt (Assam).

Genomic DNA isolation and PCR analysis :

About 50 grubs (3rd instars) of *Micromus igorotus* and 3rd instar larvae of *Dipha aphidivora* from each location were randomly picked for isolation of genomic DNA isolation. The digestive system of the larvae was removed to avoid contamination. The starved grubs/larvae were desensitized using formalin swab. To prepare genomic DNA modified CTAB method (Murray and Thompson, 1980) 750 µl of lysis buffer (1% CTAB, 5% polyvinyl pyrrolidane (PVP), 1.4 M NaCl, 20mM mercapthethanol) and mixed gently and 0.5 µl of protease was added and kept in water bath for 3 hrs. DNA was further purified by (750 µl) phenol: chloroform: Iso amyl alcohol (IAA) (25:24:1) treatment. Totally 9 DNA samples were used for PCR-RAPD analysis. A set of 10 random

decamer primers was randomly selected from OPA kits obtained from Operon Technologies Inc. USA was tested across all the DNA samples. PCR was carried out for each primer in 20 µl standard mixtures consisting of 100 ng of templates DNA, 2 µl/reaction primer, 25 mM Magnesium Chloride ($MgCl_2$), 2 mM each dATP, dTTP, dGTP and dCTP (Bangalore Genie Pvt. Ltd.), 0.2 U Taq polymerase (Bangalore Genie Pvt. Ltd.) on an Eppendorf master gradient cycler (Eppendorf AG Germany) in 0.5 ml microcentrifuge tubes. Mineral oil was not added to the reaction mixture as the thermal cycler had hot lid facility. PCR started with a 2 min initial demonstration at 94^0C followed by 35 cycles of 1 min at 94°C for denaturation, 1 min at 36°C for annealing, 2 min at 72°C for extension at 72°C. These reaction products were kept overnight at 4°C prior to electrophoresis. Agarose gels (40mg agarose in 50 ml of TAE buffer) at 5V/cm for 3hrs, which resolved DNA fragments ranging from 1904bp to 564bp. All the 10 primers were tested at least twice for the reproducibility of banding pattern. A set of 10 primers *viz.*, OPA01(CAGGCCCTTC),OPA02(TGCCGAGCTG), OPA03(AGTCAGCCAC),OPA04(AATCGGGCTG), OPA05(AGGGGTCTTG),OPA06(GGTCCCTGAC), OPA07(GAACGGGTTG),OPA08(GTGACGTAGG), OPA09(GGGTAACGCC) and OPA10(GTGATCGCAG) were used. All the bands in the range of resolution were scored except very faint and ghost bands. The gel pictures acquired through a gel documentation system into a computer were processed and scored to get binary data.

Statistical analysis :

The presence or absence data (1,0) matrix was analyzed using the standard procedure in NTSYS Pc2 package (Rohlf, 1998). Similarity matrix was computed for each individual population. Genetic distance or similarity was determined by Jacquard similarity. The resultant similarity matrix was used to generate a tree by UPGMA, Unweighted pair group method with arithmetic average (Sneath and Sokal, 1973) in NTSYS Pc2 package.

All the 10 gels resulting from short listed primers had maximum number of clear and scorable amplicons in each DNA sample with few ghost or minor bands, which were ignored. Sample gel resulting from OPA9 and OPA10 random primers across individually pooled genomic DNA of all the 5 geographical populations of *Dipha aphidivora* is depicted in Fig. B and C isolates of *Micromus igorotus* is depicted in Fig. A.

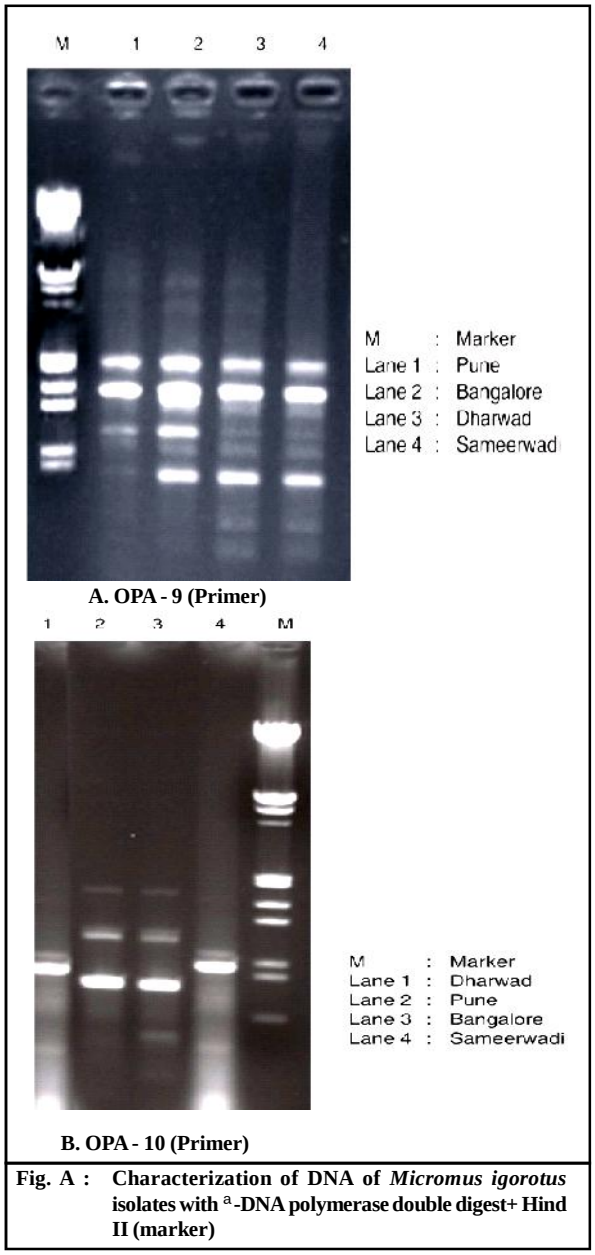

Fig. A : Characterization of DNA of *Micromus igorotus* isolates with [a]-DNA polymerase double digest+ Hind II (marker)

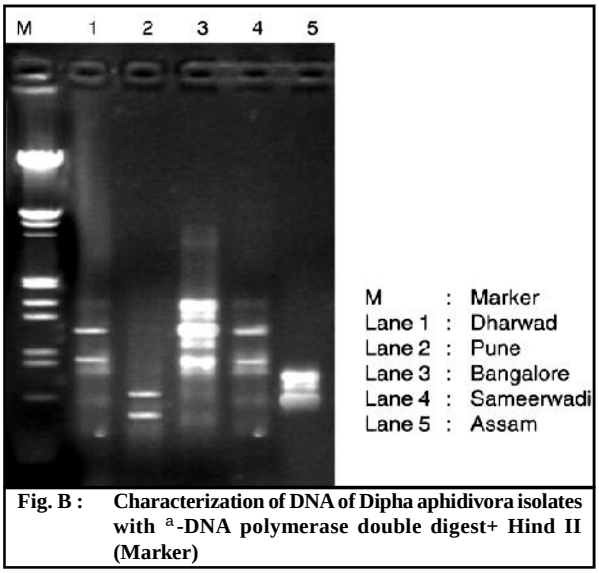

Fig. B : Characterization of DNA of Dipha aphidivora isolates with [a]-DNA polymerase double digest+ Hind II (Marker)

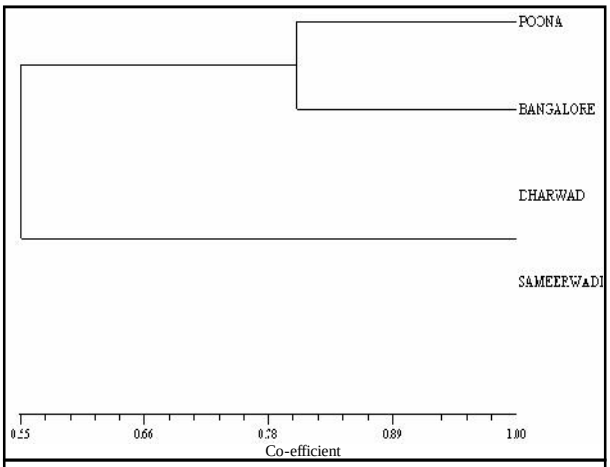

Fig. C : Dendrogram of different *Micromus igoratus* isolates

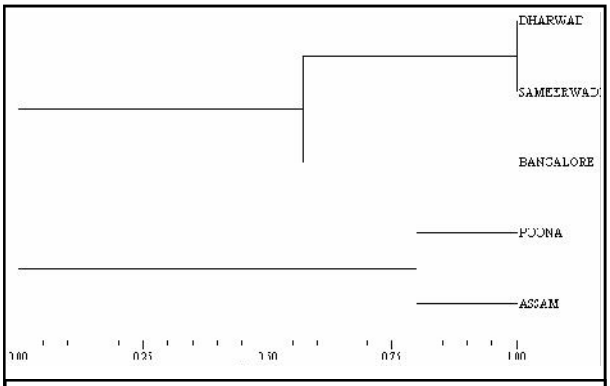

Fig. D : Dendrogram of different *Dipha aphidivora* isolates

RESULTS AND DISCUSSION

The dendrogram constructed using symmetric matrix of different isolates resulted into two major clusters (Fig. C). The similarity matrix pertaining to different isolates of *Micromus igoratus* that the similarity co-efficient ranged from 0.5 to 0.80. The highest genetic similarity index of 0.80 was seen between the isolates from Bangalore and Poona followed by 0.5 between the isolates from Poona and both from Dharwad and Sameerwadi (Bagalkot) (Table 2).

The highest genetic similarity of 0.8 was seen between the isolates of *Dipha aphidivora* from Assam and Poona, even both places are different in their cropping pattern, weather factors (temperature, relative humidity, rainfall etc.) followed by 0.57 was seen between Bangalore and Dharwad, and also from Bangalore and Sameerwadi (Bagalkot) (Table 1 and Fig. D). All 10 primers selected for the study produced unique banding patterns that could differentiate all the 5 geographical populations. The polymorphism revealed by RAPD serves as a dominant Mendelian marker (Williams *et al.,* 1990). As heterozygotes are not normally detectable, results are not readily usable for computing Hardy-Weinberg gene frequencies or Nei's standard genetic distance (Lynch and Milligan, 1994). RAPD-PCR has proved useful for comparing closely related species, subspecies or populations of *Trichogramma* sps. The identification of *Trichogramma* sps, a minute egg parasiting wasp is difficult because of the occurrence of various strains/types of the same species. A new taxanomic tool, several different molecular technique, such as RAPD-PCR and sequencing of r-DNA, ITS were tested to construct a phylogenetic tree (Silva *et al.,* 1995). RAPD procedures also revealed species-specific banding patterns and appeared promising for a rapid and easy identification of *Trichogramma* species. Moreover, it was through that, for some *Trichogramma* species, RAPD banding patterns might be informative of the phylogenetic relatedness (Vanlerberghe Masutti, 1994).

RAPD markers have been used to try to identify species, strains, biotype and geographic variation of insects. Among the insect groups examined are aphids and parasitoids (Putreka *et al*., 1993) microhymenopteran parasitoids of lepidoptera (Landry *et al.,* 1993). High sequence divergence

Table 1 : Similarity Matrix of different isolates of *Dipha aphidivora*

	Dharwad	Poona	Bangalore	Sameerwadi	Assam
Dharwad	1.00				
Poona	0.00	0.00			
Bangalore	0.57	0.00	1.00		
Sameerwadi	1.00	0.00	0.57	1.00	
Assam	0.00	0.80	0.00	0.00	1.00

Table 2 : Similarity Matrix of different isolates of *Micromus igorotus.*

	Poona	Bangalore	Dharwad	Sameerwadi
Poona	1.00			
Dharwad	0.80	1.00		
Bangalore	0.50	0.60	1.00	
Sameerwadi	0.50	0.60	1.00	1.00

and species-specific restriction patterns clearly differentiate both species pairs of *Encarsia* sp. (Monti *et al.*, 2005). Use of RAPD-PCR can detect the presence of parasitoid patterns which are species-specific. Technology could then be developed to identify parasitoids using RAPD-PCR pattern keys (Black *et al.*, 1992). A similar technology is already being used with esterase in the field of survey of parasitoids (Walton and Dejong, 1990 a&b). It is possible that RAPD-PCR could be used to detect the presence of parasitoids earlier during their development cycle than the esterase system allows. Further more, the technique offers a viable alternative to electrophoresis in monitoring parasitoid colonies for purity (Morgan *et al.*, 1988). Until recently monitoring genetic variation in arthropod enemies had been limited to the use of enzyme electrophoresis, but many natural enemies such as parasitoids (Hymenoptera) lack substantial variation in these proteins (Packer and Owen, 1992). New DNA based methods for monitoring genetic variation are now available, including mitochondrial DNA analysis and DNA sequencing. The use of PCR (polymerase chain reaction) to identify DNA markers, particularly markers identified by a random sample of the genome (such as RAPD-PCR), offers a highly efficient method for detecting genetic changes in arthropod populations (Arheim *et al.*, 1990 and Hadrys *et al.*, 1992).

The variation in the genome level may be due to the difference in the sequence of nucleotides. It is interestingly to note that isolates from two major clusters (Dharwad and Sameerwadi, also from Bangalore and Poona) not only grouped into a single cluster but also possessed highest similarity. Interestingly all these isolates were from regions, which have almost common cropping pattern, weather parameters in terms of temperature, relative humidity and rainfall. Perhaps these factors might have strong bearing on their evolution and hence might be sharing more nucleiotide similarity when compared to isolates of other locations *i.e.* Assam (which is hilly area where cropping pattern and weather differ). The high genetic variability will help species to evolve and adapt faster to different environments; rapid evolution and dispersal of natural enemies.

REFERENCES

Anonymous (2005). Annal progress report for 2004-05. All India Co-ordinated Research Project on Biological Contorl of Crop Pests and Weeds. Project Directorate of Biological Control., Bangalore. Part-I.

Arkaki, N. and Yoshiyasu, Y. (1988). Notes on biology, taxonomy and distribution of the aphidophagous pyralid, *Dipha aphidivora* (Meyrick) comb. Nov. (Lepidoptera:Pyralidae). *Appl. Ent. Zool.* **23**(3):234-244.

Arheim, N., White, T. and Rainey, W.E. (1990). Applicatiion of PCR organismal and population biology. *Bioscience.*, **40**: 174-182.

Ballinger-Crabtree, M.E., Black, W.C. IV and Miller, B.R. (1992). Use of genetic polymorphisms detected by random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) for differentiation and idefication of *Aedes aegypti* subspecies and populations. *Am. J. Trop. Med. Hyg.*, **47**: 893-901.

Black, W.C., DuTeau, N.M., Putreka, G.J., Nechola J.H. and Pettroni, J.M. (1992). Use of the random amplified polymoephic DNA-polumerase chain reaction (RAPD-PCR) to detect DNA polymorphisms in aphids (Homoptera:Aphididae). *Bull. Ent. Res.*, **82**: 152-159.

CABI (2001). Crop Protection Compendium, Global Module, Wallingford, Commonwealth Agricultrual Bureau Intrenational, UK.

Canis, J.L., Perez, P. and Fereres, A. (1993). Idefication of aphid (Homoptera: Aphididae) species and clones by random amplified polymorphic DNA. *Ann. Entomol. Soc. Am.*, **86**: 151-159.

Chapco, W., Ashton, N.W., Martel, R.K.B., Antonishyn, N. and Crosby, W.L. (1992). A feasibility study of the use of random amplified polymorphic DNA in the population genetics and systemics of grasshoppers. *Genome*., **35**: 569-574.

Cheng, N.Y., Wang, Z.T., Hung, T.H. and Hung, J.K. (1994). Factors and their effect on the occurrence of sugarcane woolly aphid. *Rep. Taiwan Sug. Res. Inst*., **145**: 1-24.

Hadrys, H., Balick, M. and Schiewater, B. (1992). Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Mol. Ecol.*, **1**: 55-63.

Harry, M., Robin, S. and Lachaise, D. (1998). Use of polymorphic genetic markers (RAPDs) in evolutionary and applied entomology. *Anales Societe Entomogique France*, **34**: 9-32.

Henry, T.J. (1988). Anthocoridae. In: Henry, T.J. and Froeschner, R.C. Catalog of the Heteroptera, or True Bugs of Canada and the Continental United States. E.J. Brill. Leiden. 958 pp.

Joshi, S. and Viraktamath, C.A. (2004). The sugarcane wooly aphid, *Ceratvocuna lanigera* Zehntner (Homoptera:Aphididae): its biology, pest status and control. *Curr. Sci.*, **87**(3): 307-316.

Kambhampti, S., Black, W.C. and Rai, K.S. (1992). Random amplified polymorphic DNA of mosquito species and populations (Diptera: Culicidae): technique, statistical analysis and applications. *J. Med. Entomol.*, **29**: 939-945.

Landry, B.S., Dextraze, L. and Bovin, G. (1993). Random amplified polymorphic DNA markers for DNA markers for DNA fingerprinting and genetic variability assessment of minute parasitic wasp species (Hymenopetra: Mymaridae and Trichogrammatidae) used in biological control programs of phytophagous insects. *Genome.*, **36**: 580-587.

Lingappa, S., Patil, R.K., Vidya, Mulimani and Ramegowda, C.K. (2004). Brown lacewing, *Micromus igorotus* Banks, a potential predator of sugarcane woolly aphid, *Curr. Sci.*, **87**(3): 307-316.

Lynch, M. and Milligan, B.G. (1994). Analysis of population genetic structure with RAPD markers. *Molec. Ecol.*, **3**: 91-99.

Morgan, P.B. Jones, C.J., Patterson, R.S. and Milne, DS. (1988). Use of electrophoresis for monitoring purity of laboratory colonies of exotic parasitoids. (Hymenoptera: Pteromalidae). *Advances in parasitic Hymenoptera Res.* pp. 525-531.

Mote, U.N. and Puri, S.N. (2003). Status of sugarcane woolly aphid, *Ceratovacuna lanigera* Zehntner. A new pest problem on sugarcane in Maharashtra. Paper presented at brain storming session on sugarcane woolly aphid, 10 June 2003.

Murray, M.G. and Thompson, W.S. (1980). Rapid isolation of high molecular weight of plant DNA. *Nucleic Acid Res.*, **8**: 19-20.

Packer, L. and Owen, R.E. (1992). Variable enzyme systems in the Hymenoptera. *Biochemical Systematics & Ecology*, **20**: 1-7.

Patil, N.G. (2003). Record of natural enemies fauna of sugarcane woolly aphid, *Ceratovocuna lanigera* Zehntner (Aphididae: Homoptera) in western Maharastra. *Insect Environ.*, **9**(3): 138-139.

Roehrdanz, R.L and Degrugillier, M.E. (2000). Mitochandrial gene order and arthopod evolution entomological Society of America North Central Branch Meeting. March 23-26, 2000, Minneapolis. M.N.

Rohlf, F.J. (1998). NTSYS-PC: numerical taxonomy and multivariate analysis system version 2.0 Department of Ecology and Evolution. State University of New York.

Silva, J.M.M.S., Vantteest, J.P.N.F., Van Kan, F.J.P.M., Strippentow, P., Takaes, K. and Stouthamer, R. (1995). phylogeny of egg parasitoids *Trichogramma* spp. (Hymenoptera : Trichogrammatidae) In: M. J. Soummeijier (Ed.,), 11th meetings of Experimental and Applied Entomolgists in the Netherlands, Wageningen, Netherlands, 17 December 1999. Proceedings of the section Experimental and Applied Entomology of the Netherlands. *Entomological Soc.*, **6** : 195-196.

Sneath, P.H.A. and Sokal, R.R. (1973). Numerical taxonomy. pp. 147-157. W.H. Freeman and Company, San Francisco.

Vanlerberghe, Maustti, F. (1994). Molecular identification and phylogeny of parasitic wasp species (Hymenoptera: Trichgrammatidae) by mitochondrial DNA, RFLP and RAPD markers. *Insect. Mole. Biology,* **3**: 229-237.

Walton, E.F. and Dejong, T. M. (1990a). Comparison of 4 methods calculating the seasonal pattern of plant-growth efficiency of a Kiwifruit Berry. *Annl. Bot.,* **66**(3): 299-307.

Walton, E.F. and Dejong, T.M. (1990b). Estimating the bioenergetic cost of a developing Kiwifruit Berry and its growth and maintenance respiration components. *Annl. Bot.,* **66**(4): 417-424.

Welsh, J. and McClellard. M. (1990). Finger printing genomes using PCR with arbitrary primers. *Nucleic Acids Res.*, **18**:1713-1718.

Welsh, J., Petersen, C. and McClelland, M. (1991). Polymorphism generated by arbitrarily primed PCR in the mouse: application to strain identification and genetic mapping. *Nucl. Acid Res.*, **19** : 303–306.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafolski, J.A. and Tingey, S.V. (1990). DNA polymorphims amplified by arditrary primers as usefull as genetic markers. *Nucleic Acids. Res.*, **18**:6531-6535.