

1. INTRODUCTION

Fruit flies (Diptera: Tephritidae) are one of the most fascinating and diversified group of insects often referred to as 'peacock flies' due to their habit of strutting and vibrating wings, and rank among the world's most serious pests of horticultural crops (Kapoor 1993; Agarwal and Sueyoshi 2005; Satarkar *et al.* 2009).

The family Tephritidae of order Diptera consists of over 4,448 species or subspecies of fruit flies, classified in 481 genera (Agarwal and Sueyoshi 2005) of which 800 species belong to Dacinae fruit flies (Fletcher 1987; Drew 1989a). They have global distribution, covering tropical, subtropical and temperate regions and occupy habitats ranging from rainforests to open savannah except in Arctic and Antarctic regions (Kapoor *et al.* 1980; Drew 1989a; 1989b; Norrbom *et al.* 1998; Michaux and White 1999).

In India, fruit flies have been identified as one of the ten most serious problems of agriculture because of their polyphagous nature and cause a huge economic loss to fruits and vegetables which varies from 2.5 -100 per cent depending upon the crop and season (Verghese *et al.* 2004; Dhillon *et al.* 2005). Of the 243 species of fruit flies recorded from India (Agarwal and Sueyoshi 2005), nine species *viz.* melon fly, *Bactrocera cucurbitae* (Coquillett); oriental fruit fly, *Bactrocera dorsalis* (Hendel); peach fruit fly, *Bactrocera zonata* (Saunders); pumpkin fly, *Bactrocera tau* (Walker); guava fruit fly, *Bactrocera correcta* (Bezzi); lesser pumpkin fly, *Dacus ciliatus* (Loew); ber fly, *Carpomyia vesuviana* (Costa) and seed fly, *Acanthiophilus helianthi* (Rossi) are major economically important species which cause a loss of Rs 7000 crore per annum (Sardana *et al.* 2005). Owing to their enormous damage potential and faster acclimatization and adaptability, many fruit fly species have been recommended for domestic and international quarantine to restrict their entry into new habitats. Some alien species *viz.* *Bactrocera minax* (Enderlein), *Bactrocera latifrons* (Hendel) and *Bactrocera oleae* (Gmelin) have also been reported to cause damage in Bhutan, South India, and Jammu & Kashmir, respectively (Sardana *et al.* 2005).

Among various tephritids, *B. cucurbitae*, *B. tau* and *D. ciliatus* are the most important and serious pests of cucurbits in India causing significant reduction in qualitative and quantitative yield of crops (Srinivasan and Narayanaswamy 1961; Prabhakar *et al.* 2007; 2009a) which is estimated to the tune of Rs 4,705 crore per annum (Sardana *et al.* 2005).

In Himachal Pradesh, *B. tau*, *B. cucurbitae* and *B. scutellaris* (Bezzi) were reported on many vegetable crops (Narayanan and Batra 1960; Gupta *et al.* 1992; Sood and Nath 1999; Prabhakar *et al.* 2007; 2009a). *Bactrocera tau*, in particular was rated as the most serious pest of cucurbits in Himachal Pradesh (Gupta *et al.* 1992; Sood and Nath 1999; Prabhakar *et al.* 2009a).

The fruit flies are the most difficult pests to control as they attain peak activity with the onset of rains; as a result, the residual insecticides applied for their control get washed away. Even no effective bioagent is known which can keep the population of fruit flies under check. Moreover, most of the available insecticides fail to target the eggs as well as the developing maggots in fruits and tender vegetables. Even the repeated application of insecticides may pose serious health hazards to the consumers.

Symbiotic association with bacteria among tephritid fruit flies is known since 1909, when it was first observed in the olive fly, *B. oleae* (Gmelin) by Petri (1909). At present, a number of bacteria have been found to be symbiotically associated with fruit flies which play an important role in physiology of insect especially with reference to protein hydrolyzation (Murphy *et al.* 1988; Behar *et al.* 2005), degradation of xenobiotics (Bousch and Matsumara 1967) and as attractants to fruit flies (Lauzon *et al.* 1998; 2000).

The control of fruit flies by the symbionts is not a new idea as it was first conceived in Florida in 1930 to control Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann) by foliar application of copper carbonate, which reduced symbiont population (Baker *et al.* 1944). The role of symbionts associated with different stages of fruit flies is only partially understood and even today it is not been fully explained. A comprehensive understanding of the fruit fly biology in an ecological pretext with associated bacteria as an important component of the system is of utmost importance for envisaging this multitrophic interaction.

Himachal Pradesh is a north-western Himalayan state of India comprising massive variety of inimitable flora and fauna in diverse agroclimatic zones like sub-tropical, sub-temperate, temperate and cold desert. The changing climate scenario, land utilization pattern, cropping system approach and increasing international trade and tourism have, however, made it vulnerable to biological invasion by alien species. This is leading to weakened ecosystem stability, affecting farmer's livelihoods & consumer confidence, and at the end, loss of resident species. Therefore fruit flies are indeed the excellent candidates for studies on biodiversity, adaptability in changing climate and invasion to new areas because of their capability to fly to long distances, polyphagous in nature and vast host range, homoplasmy in taxonomic characters, high reproductive potential, wide range of distribution due to their high adaptability and great economic importance as a pest.

Among different DNA markers, two sets of markers i.e. multilocus microsatellite loci and mitochondrial DNA sequences have been used extensively to study the recent history of insect populations, including population structure, phylogeography and invasion biology (Roderick 1996; 2004; Sunnucks 2000). Microsatellites, being nuclear, co-dominant loci, with high levels of variability, are particularly informative in the study of recent population phenomena such as biological invasions. In contrast, the unique properties of nucleotide sequence polymorphism of mitochondrial DNA (mtDNA) can provide high resolution information on the evolutionary relations between taxonomically bound families as mitochondrial genes evolve approximately 10 times faster than single-copy nuclear DNA (Brown *et al.* 1979). Therefore, mtDNA sequence is a useful molecular marker (Brown and Simpson 1981; Barton and Jones 1983; Aquadro *et al.* 1984; Palumbi and Cipriano 1998). Also *mitochondrial cytochrome oxidase I (mtCOI)* gene is reasonably well conserved, and has been sequenced in various invertebrate taxa (Brown 1985; Bermingham and Lessios 1993; Brower 1994a; Hu *et al.* 2008). Nevertheless, *mtCOI* sequences are at the base of the barcoding identification system (Hebert *et al.* 2003; Hajibabaei *et al.* 2006) that,

besides being a valuable tool for species identification and discovery, have also been proposed as a powerful methodology in biosecurity and invasive species identification (Armstrong and Ball 2005).

Therefore, the present investigation was planned to generate information on biodiversity, geographical distribution and genetic relationship among fruit fly species with *mitochondrial cytochrome oxidase I (mtCOI)* gene sequencing, and also to explore their gut associated bacteria. It is imperative to know about the species which are trying to invade new areas especially from quarantine purpose. This study would be helpful in understanding the true distribution of fruit fly species in the region and their gut associated bacteria which may be useful in devising alternative eco-friendly management strategies for this devastating pest. Therefore the present study was undertaken with the following objectives:

- i) Molecular characterization of fruit fly species infesting cucurbits in Himachal Pradesh,
- ii) isolation, identification and characterization of predominant fruit fly gut bacteria, and
- iii) to evaluate the role of predominant gut bacteria in management of fruit fly.