Morphological identification of cucurbitaceous fruit flies in Bihar and genetic diversity of *Bactrocera cucurbitae*

A THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE

REQUIREMENTS FOR THE AWARD OF THE DEGREE

OF

MASTER OF SCIENCE IN AGRICULTURE

(ENTOMOLOGY)

BY

MANEESH PAL SINGH

(REG. NO. M/ENTO/249/BAC/2015-16)

DEPARTMENT OF ENTOMOLOGY

BIHAR AGRICULTURAL UNIVERSITY, SABOUR

BHAGALPUR-813 210, BIHAR, INDIA

2017

THE GRACE OF RABBI UL AALMEEN

ALL PRAISE BELONG TO HIM

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CERTIFICATE-I

This is to certify that the thesis entitled **"Morphological identification of cucurbitaceous fruit flies in Bihar and genetic diversity of *Bactrocera cucurbitae*"** submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE IN (AGRICULTURE)** in the subject of **ENTOMOLOGY** of the faculty of Agriculture, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, is a genuine record of *bonafide* research work carried out by **Mr. Maneesh Pal Singh, Reg. No.: M/ENTO/249/BAC/2015-16** under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been fully acknowledged.

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CERTIFICATE-II

We, the undersigned members of the Advisory Committee of **Mr. Maneesh Pal Singh**, **Reg. No.: M/ENTO /249/BAC/2015-16** a candidate for the degree of **MASTER OF SCIENCE IN AGRICULTURE (ENTOMOLOGY)** have gone through the manuscript of the thesis and agree that the thesis entitled **"Morphological identification of cucurbitaceous fruit flies in Bihar and genetic diversity of *Bactrocera cucurbitae*"** may be submitted in partial fulfilment of the requirement for the award of the degree.

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All are not mentioned, but none is forgotten.

For any errors or inadequacies that may remain in this work, of course, entirely I responsible for them.

Place: Sabour, Bhagalpur **(MANEESH PAL SINGH)**

Date: June, 2017

BIHAR AGRICULTURAL UNIVERSITY, SABOUR

Title of the thesis	**Morphological identification of cucurbitaceous fruit flies in Bihar and genetic diversity of *Bactrocera cucurbitae***
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ABSTRACT

Cucurbits are the major vegetable crops belonging to the family Cucurbitaceae, primarily comprising species consumed as food worldwide. The family consists of about 118 genera and 825 species (Prabhakar *et al*., 2012). In Bihar major growing cucurbits including bottle gourd, pointed gourd, bitter gourd and ash gourd. The production of cucurbits is hindered due to several factors like insect pests and diseases and out of those; fruit flies are one of the major limiting factor of cucurbits production occurring not only in Bihar but also in India. In India, fruit flies have been identified as one of the ten most serious problems of agriculture, because of their polyphagous nature and causes a huge economic loss to fruits and vegetables which varies from 2.50 -100 per cent depending upon the crop and season (Dhillon *et al*., 2005). There are approximately 4,500 species of fruit flies (Tephritidae) represented one of the largest families of Diptera and at around 243 species of fruit flies were recorded from India (Agarwal and Sueyoshi, 2005). Among those, *Bactrocera cucurbitae*, *B. tau* and *Dacus ciliatus* are the most diverse. Fruit flies are indeed the excellent candidates for studies on biodiversity, adaptability in changing climate and invasion to new areas because of their capability of flying long distances, polyphagous in nature, 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. Proper insect pests' management leads to better production in agroecosystem and a better management practice not supposed to be completed without proper identification of a particular pest. Therefore, the present investigation was conducted to study the morphological identification of predominant cucurbitaceous fruit flies and to find out the genetic diversity of *Bactrocera cucurbitae* through gene specific marker.

After a roving survey at four different agroclimatic zones of Bihar, it was observed that a total of six major fruit fly species associated with cucurbitaceous crops *viz. Bactrocera. (Zeugodacus) cucurbitae* (Coquillett), *B. (Z.) tau* (Walker), *B. (Z.) caudata* (Fabricius), *B. (Bactrocera) nigrofemoralis* White & Tsuruta, *B. (Hemigymnodacus) diversa* (Coquillett) and *Dacus (Didacus) ciliates* Loew. Their distribution pattern in different agroclimatic zones with their associated hosts was also recorded. The collected fruit fly species were identified taxonomically according to keys provided by White and Elson-Harris, 1992 and Drew and Raghu, 2002. Significant achievements of our studies were i. *Bactrocera (Bactrocera) nigrofemoralis* White & Tsuruta will be the first report from Bihar as new fruit fly species and also identified new host of *Dacus (Didacus) ciliatus* Loew, *Bactrocera (Hemigymnodacus) diversa* (Coquillett) from pointed gourd and flowers of *Cucurbita moschata* respectively. On the basis of amplified PCR product of DNA of *B. cucurbitae*, the expected product length of MCOX-I gene was 700 bp and the product obtained was nearly between 700 to 750 bp. The expected product length of MCOX-II gene was 600 bp and the product obtained was nearly between 500 to 600 bp. It revealed that there were not much difference in the banding pattern but very small difference noticed in the bands and which would be helpful for future studies in molecular diversity analysis after getting complete sequencing.

(Maneesh Pal Singh)
Signature of student

(Tamoghna Saha)
Chairman Advisory Committee

List of Symbols & Abbreviations Used

et al. - and others

e.g. - example

mt - metric ton

viz. - namely

m - meter

mm - milli meter

ml - milli litre

˚C - degree centigrade

mM - milli molar

µg - micro gram

bc - basal costa

bp - base pair

R_{2+3} - Radial 2+3

cup - posterior cubitus

i.e. - is that

% - percent

t/ha - ton per hectare

mtCOI - mitochondrial cytochrome oxidase

RE - Refractive index

cm - centimeter

µl - micro liter

rpm - rotation per minute

V - Volt

npl - notopleural

PCR - Polymerase Chain Reaction

UV - Ultra Violet

R_{4+5} - Radial 4+5

r-m - radio medial cross vein

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INTRODUCTION

The fruit flies are classical international pests. They are not only the production deterrent to fresh fruits and vegetables, but indeed a formidable export barrier limiting to a potential multimillion dollar worldwide trade (Mahmood, 2002). The family Tephritidae is one of the largest families of insect order Diptera, comprising of predominantly medium sized, pictured-winged and highly ornamented flies commonly known as “Fruit flies” and Peacock flies as a number of species infest a wide variety of fruits, vegetables, flower heads, seeds, leaves and other plant parts (Prabhakar *et al.,* 2012).

Cucurbits are the vegetable crops belonging to the family Cucurbitaceae, primarily comprising species consumed as food worldwide. The family consists of about 118 genera and 825 species. Cucurbits are consumed in various forms, *i.e.* salad (cucumber, gherkins, long melon), sweet (ash gourd, pointed gourd), pickles (gherkins), deserts (melons) and culinary purpose. Some of them, e.g. bitter gourd is well known for its unique medicinal properties. Among the cucurbits, cucumber and melon are the 20 most important vegetable crops worldwide (Prabhakar *et al*., 2012). Cucurbits share about 5.6% of the total vegetable production of India and are cultivated in about 4,290,000 ha with the productivity of 10.52 t/ha (FAO, 2012). In India, cucurbits production *viz*. musk melon 9,57,000 mt, water melon 2,36,2000 mt, bitter gourd 10,53,000 mt, cucumber 12,59,000 mt, pointed gourd 3,00,000 mt, pumpkin 15,09,000 mt, bottle gourd 2,51,200 mt (IIHR, 2017). In Bihar cucurbits production *viz*. bottle gourd 7,03,132 mt, sponge gourd 5,35,726 mt, cucumber 26,160 mt, ridge gourd 60,202 mt, bitter gourd 76,839 mt, ash gourd 17,437 mt, water melon 34,154 mt, musk melon 16,452 mt, pointed gourd 10,0245 mt, pumpkin 8,097 mt.(SHM, Bihar 2011-12).

Among the different pests of cucurbits, fruit flies have been identified as one of the most serious pests of cucurbits owing to their polyphagous nature and huge economic losses to vegetables varying from 30 to 100% depending upon the crop and season (Prabhakar *et al*., 2012). The family Tephritidae under the 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. 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. At about 4,500 species of fruit flies (Tephritidae) represent one of the largest families of Diptera. This family can be distinguished from the other families of Diptera by the combination of well developed mesoclinate frontal setae and subcostal vein bent sharply anteriorly at right angle before the apex, weakened or evanescent beyond the bend. In addition, the costa has three breaks *viz*., costal, humeral and subcostal; vein R1 dorsally with setulae; wing usually with color pattern; cell bcu usually with an acute extension. (David and Ramani, 2011).

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 of 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).

Approximately 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. Many insect pests attack fruits and vegetables, none have garnered greater notoriety than Tephritid fruit flies and they are recognized worldwide as the most important threat to the horticultural industry. However, during the last decade, there has been a slow but steady increase in awareness of the importance of fruit flies in the region partly due to their direct damage and their importance in export fruits and vegetables as well as the recent invasions into areas where they were not present. This region represents the endemic habitat of fruit fly belonging to subfamily Dacinae in India. Consequently, an accurate knowledge of the species identification in this region will be significant to our current understanding of regional Dacinae biogeography (Ganie *et al.,* 2013).

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 (Prabhakar *et al.,* 2007) which is estimated approximately of Rs 4,705 crore per annum (Sardana *et al*., 2005). Damage is done by the maggots of the fruit flies, female fruit fly lay eggs just below the skin of the fruit using oviscapt. After hatching, maggots feed on the inner content of the fruit resulting the fruit becoming unfit for human consumption. Afterwards full fed maggots come out of the fruit and pupate inside the soil.

The fruit flies are the most difficult pests to control as they attain peak activity during the onset of rains, as a result, the residual insecticides applied for their control get washed away. Even though no effective bio-agent is known this 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 cause serious health hazards to the consumers.

Bihar is a central eastern state of India and growing cucurbits in mass, specially pointed gourd, bottle gourd and ash gourd. 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 weaken ecosystem stability, affecting farmer's livelihoods and consumer confidence, and at the end, loss of resident species. Therefore, fruit flies are indeed the suitable 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 having 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.

Therefore, the present investigation was planned to generate information on biodiversity, taxonomic identification, geographical distribution and genetic relationship among *B. cucurbitae* from different zones of Bihar with *mitochondrial cytochrome oxidaseI* (*mtCOI*) gene PCR amplification. 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 recommendation of management practices.

The present study was undertaken with the following objectives:

- Morphological identification of predominant cucurbitaceous fruit flies.
- Genetic diversity of *Bactrocera cucurbitae* through gene specific marker.

REVIEW OF LITERATURE

The literature pertaining to the title and objectives of the study are described here under the following sub headings.

2.1 Morphological identification

Ganie *et al* (2013) identified the four major species of fruit flies from cucurbits in Kashmir at six locations namely, Batamaloo, Dal and Shalimar in district Srinagar, Chadoora, Bugam and Narkara in district Budgam. They identified *Bactrocera cucurbitae, B. dorsalis, B. tau* and *B. scutellaris* on the basis of taxonomical characters, while *B. scutellaris* was first time reported from Kashmir valley.

Kovac *et al* (2013) described the biology and morphologyof the third instar larva and puparium of *Ichneumonopsis burmensis* hardy (Diptera: Tephritidae: Dacinae: Gastrozonini), a bamboo-breeding fruit fly from the oriental region (North Thailand). Only one internode of a particular bamboo shoot was infested by *I. burmensis*, and this internode was inhabited by a single larva. The feeding activities of the larva caused the 5–6 apical internodes located above the infested internode to fall off, resulted in a typical damage pattern. In contrast to other Gastrozonini, *I. burmensis* larvae did not skip, and they constructed a puparial chamberinside the bamboo internode. Remarkable morphological characters of *I. burmensis* larvae include the large and strongly ramified anterior spiracles consisting of more than 100 papillae, a labial lobe covered by strong, outward oriented spines, a labial sense organ and a facial mask consisting mainly of accessory plates. These morphological characters are interpreted as an adaptation to the specific bamboo microhabitat utilized by *I. burmensis*. The association of *I. burmensis* with bamboo indicates that *Ichneumonopsis* belongs to the primarily bamboo inhabiting tribe Gastrozonini. However, the strong differences between the larval characters of *I. burmensis* and other *Gastrozonini* suggested that this genus has an isolated position within this group.

Leblanc *et al* (2013) surveyed the fruit flies (Diptera: Tephritidae: Dacinae) of Bangladesh province, Thirteen species of *Bactrocera* and one species of *Dacus* were collected during field survey in Bangladesh, included eight new country records, for a total of fifteen species confirmed to occur in the country. Colour variation in Bangladesh *B. dorsalis* is similar to that observed in *B. invadens* in Africa and Sri Lanka.

Prabhakar *et al* (2012) provided pictorial key of 13 species of fruit flies under two genera namely *Bactrocera* and *Dacus* of subfamily Dacinae (Diptera: Tephritidae) which was based on actual photographs of fruit flies collected from north western Himalaya of India during 2009-2010 and reported *Bactrocera diversa* (Coquillett), *Bactrocera scutellaris* (Bezzi), *Bactrocera tau* (Walker), *Bactrocera cucurbitae* (Coquillett), *Bactrocera zonata* (Saunders), *Bactrocera correcta* (Bezzi), *Bactrocera dorsalis* (Hendel), *Bactrocera latifrons* (Hendel) and *Dacus ciliatus* Loew were the pests of agricultural and horticultural ecosystems and *Bactrocera latifrons*, *Bactrocera nigrofemoralis* White & Tsuruta, *Dacus longicornis* Wiedemann and *Dacus sphaeroidalis* (Bezzi) were the new records from the region of which host range has yet to be investigated.

Prabhakar *et al* (2012) recorded *Bactrocera (Bactrocera) latifrons* (Hendel), *B. (B.)nigrofemoralis* White and Tsuruta, *Dacus (Callantra) longicornis* Wiedemann, *Dacus (Callantra) sphaeroidalis* (Bezzi), *Cyrtostola limbata* (Hendel) and *Pliomelaena udhampurensis* Agarwal and Kapoor, for the first time from Himachal Pradesh from cucurbit ecosystem. They also described *Bactrocera tau*, *Bactrocera cucurbitae*, *Bactrocera dorsalis*, *Bactrocera zonata*, *Bactrocera scutellaris*, *Bactrocera diversa* and *Dioxyna sororcula* (Wiedemann) and depicted the distribution of *B. (B.) dorsalis* (Hendel), *B. (B.) zonata* (Saunders), *Bactrocera (Hemigymnodacus) diversa* (Coquillett), *B. (Zeugodacus) cucurbitae* (Coquillett), *B. (Z.) scutellaris* (Bezzi) and *B. (Z.) tau* (Walker).

David and Ramani (2011) provided keys for 126 species of fruit flies under 46 genera in four subfamilies namely Dacinae, Phytalmiinae, Tephritinae and Trypetinae. Among these, *Acroceratitis striata* (Froggatt), *Rhochmopterum venustum* (de Meijere) and *Themara yunnana* Zia were new records from India. *Bactrocera yercaudiae* Drew was placed as a synonymn of *Bactrocera digressa* Radhakrishnan from Peninsular India and Andaman and Nicobar Islands.

Kitthawee and Rungsri (2011) studied on *Bactrocera tau* complex contains fruit fly pest which damage several species of cucurbit crops. Two cryptic species, A and C, of the *B. tau* complex both occur on the host fruit *Momordica cochinchinensis*. They analysed the wing geometry to differentiate the *B. tau* complex on *M. cochinchinensis*. A total of 586 wings (297 males, 289 females) were discriminated into two groups corresponding to the reference species A and C of the *B. tau* complex. *B. tau* A and C were reclassified at higher than 96% and 99% accuracy, respectively. *B. tau* C had larger wings than *B. tau* A. Interspecific differentiation was significant due to wing size, indicating that wing shape can be used to separate the species. The *B. tau* C was found to be the dominant species on *M. cochinchinensis*. A classification tree based on Mahalanobis distances suggested that the variation within *B. tau* C is related to seasonal variation. The variation among seasonal populations was similar in males and females.

Sumrandee *et al* (2011) studied ovipositor morphology and host relations of the *Bactrocera tau* complex (Diptera: Tephritidae) in Thailand. Ovipositor morphology was compared among the seven members of the *B. tau* complex using scanning electron microscopy. The flies could be placed into two main groups based on the shape of the aculeus apex. The first group comprised *B. tau* forms C and I which have trilobed aculeus apices. The second group included *B. tau* forms A, D, E, F and G, all of which have single-pointed apices. The latter five forms were further divided on the basis of the sharpness of the aculeus apex into "medium" (A and E), "sharp" (D and G) and "blunt" (F) apices. Host fruit associations, fly aculeus apex shape and geographical region were overlain into a molecular phylogeny previously published for the *B. tau* group in Thailand. Cucurbitaceae fruits appear to be ancestral hosts for the *B. tau* complex, whereas, the use of fruits of other plant families appeared late in the evolutionary history of this group. Forms with trilobed and single pointed aculeus apices separated early in *B. tau* evolutionary history, but the split does not seem host related. Flies with medium, sharp and blunt, simple-pointed aculeus apices showed no evident. Associations, being randomly distributed across phylogenetic tree, *B. tau* form A which infested fruits of nine Cucurbitaceae species was found in all five surveyed regions, whereas, each of the other forms, which were restricted to 1-3 fruit species, were found in 1-2 regions.

Copeland (2009) reported new species of *Munromyia* Bezzi (Diptera: Tephritidae) reared from *Chionanthus battiscombei* (Oleaceae) in northern Kenya. He distinguished from the two known congeners by the thoracic and abdominal colour patterns, structure of the male and female terminalia, and host plants. Larvae are seed predators, developing within the green, aborted fruits of *Chionanthus battiscombei*.

Meyer (2009) studied the afrotropical fruit fly genus *Perilampsis.* A total of 17 species were recognized out of which three were new to science: *P. deemingi* sp. nov., *P. incohata* sp. nov., and *P. rubella* sp. nov. *Perilampsis thyene* Munro considered a junior synonym of *P. amazuluana* Munro. All species were described or re-described, with illustrations of wing patterns and female terminalia where deemed necessary for unambiguous identification. Their host-specificity is briefly discussed. An identification key of these species was also provided.

Ohno *et al* (2008) first detected the fruit fly, *Bactrocera tau* (Diptera: Tephritidae), in the field of Japan and gave evidence of multiple invasions of Ishigaki Island and failure of colonization. These results indicated that *B. tau* had invaded Ishigaki at least twice but failed to colonize. *B. tau* might have come from Taiwan, considering its geographic proximity to Ishigaki and the abundance of the species in Taiwan. The invasions could not be fully explained by natural factors alone; it was possible that human-induced means were also involved.

Drew *et al* (2007) reported twenty-nine species of *Bactrocera* Macquart and *Dacus* Fabricius from Bhutan, including two new species, *Dacus (Mellesis) dorjii* Drew & Romig and *Dacus (Mellesis) fletcheri* Drew. Information was given on location of type specimens, host plants, attractant records and geographic distributions for all species.

Norrbom and Hancock (2004) reported new species and new records of Tephritidae (Diptera) from New Caledonia. The 18 species represented include 3 new species, *Austronevra irwini*, *Ceratitella schlingeri*, and *Euphranta hardyi*, and 6 species reported from New Caledonia for the first time. The total number of Tephritidae from the island was increased from 16 to 25.

Freidberg and Copeland (2006) reported *Notommima parallela*, a new genus and species of fruit fly from Kenya allied to *Notomma bezzi* (Diptera: Tephritidae). Important characters were illustrated, including drawings and photographs. *Notommima* compared to several other superficially similar genera and found most similar to *Notomma*. However, these two genera differ in several small but significant morphological characters as well as an important biological trait (*Notommima* is frugivorous; *Notomma* is a twig-gall inducer), that justified establishing the new genus.

Kapoor (2005) studied on taxonomy of fruit flies and he was found that the major fruit fly pests in India belong to the genus *Bactrocera: B. cucurbitae* (Coquillett), *B. dorsalis* (Hendel) and *B. zonata* (Saunders). *Dacus ciliatus* Loew sometimes becomes a serious pest of squash melons, dominating *B. cucurbitae*. Till date, five or six species of the *B. dorsalis* complex have been recorded in India, and at least ten species may occur there, as well as three or four species of the *B. zonata* complex. *B. tau* (Walker) and *B. scutellaris* (Bezzi) have not been recognized even as moderate pests, whereas, *B. caudate* (Walker) was still not fully confirmed in India. The useful fruit flies include some species that damage local weeds. *Procecidochares utilis* (Stone), a native of Mexico, was well established on crofton weed. Several other useful fruit fly species, such as *Dacus persicus* Hendel, *Ensina sonchi* (Linnaeus), *Urophora stylata* (Fabricius), and *D. sororcula* (Wiedemann), attack weeds belonging to Calotropis, Sonchus, Cirsium and Bidens, respectively.

Meyer *et al* (2005) described the new species of genus *Ceratitis* MacLeay and also five new species are hereby described namely *Ceratitis (Pardalaspis) millicentae sp. n.*, *C. (Ceratalaspis) oraria sp. n.*, *C. (C.) perisae sp. n.*, and *C. (C.) perseus, sp. n.* all from Kenya, and *C. (C.) ealensis sp. n.* from the Democratic Republic of Congo. Their relationship with closely allied species within the respective subgenera was discussed and differentiating characters were given.

Mahmood (2004) studied to differentiate pest species *B. carambolae* from *B. occipitalis, B. papayae* from *B. philippinensis and B. dorsalis* from *B. papayae,* and *B. philippinensis* (in oriental fruit fly complex) was presented. These methods / techniques were based on aculeus length, aedeagus length, discal cell length, and scanning electron microscopy of tomentum pattern on prescutum. The character "scales on the distal end of eversible membrane of ovipositor" was reviewed. However, morphological characters could not differentiate the two species *B. papayae* and *B. philippinensis*.

Drew and Raghu (2002) reported twenty-one species, were from the New Amarambalam Forest in the Western Ghats of India, eight of which was new species. Two new species based on earlier surveys in Southern India was also included. New species were *Bactrocera (Bactrocera) apiconigroscutella, B. (B.) amarambalensis*, *B. (B.) neoarecae*, *B. (B.) neonigrotibialis*, *B. (B.) paraosbeckiae*, *B. (B.) paraverbascifoliae*, *B. (B.) penecorrecta, B . (B.) pseudoversicolor*, *B. (Daculus) yercaudiae*, *B. (Sinodacus) binoyi*. Information was given on location of type specimens, host plants, attractant records and geographical distribution.

Knio *et al* (2002) described twenty thistle species belonging to the Asteraceae yielded eighteen species of tephritids. Out of the eighteen reared tephritids, fifteen were reported for the first time from Lebanon. Seventeen new host species and eleven new host genera for those tephritids were also reported for the first time.

Adsavakulchai *et al* (1999) studied on wing morphometry of *B. dorsalis* complex included *B. dorsalis, B. arecae,* B. *propinqua, B. pyrifoliae, B. verbascifoliae,* and three new species complexes were species E, species K and species P. *B. tau* was used as an out-group. They used 424 adults, which emerged from pupae collected from natural populations in Thailand, were prepared for wing measurements. They got 89.6% accurate identification compared with the formal description of these species. After clustering, the percentage of "grouped" cases yielded 100.0%, 98.9%, 98.1%, 95.2% and 84.6% accurate identification between the *B. dorsalis* complex and *B. tau; B. arecae* and Species E, *B. dorsalis* and *B. verbascifoliae; B. propinqua* and *B. pyrifoliae;* and species K and species P, respectively.

Hancock and Drew (1999) described Bamboo-shoot flies of Asia (Diptera: Tephritidae: Ceratitidinae). The bamboo-shoot breeding Gastrozonini (Ceratitidinae) of Asia was reviewed, with 86 species recognized in 17 genera. One new genus and new species were proposed: *Cyrtostola* gen. nov., *Acroceratitis hardyi* sp. nov., *Acroceratitis tenmalaica* sp. nov., *Chaetellipsis kinabaluensis* sp. nov., *Chaetellipsis maculosa* sp. nov., *Paragastrozona trivittata* sp. nov.

Adsavakulchai *et al* (1999) conducted a study on morphometry of *B. dorsalis* Hendel complex included *B. dorsalis*, *B. arecae*, *B. propinqua*, *B. pyrifoliae*, *B. verbascifoliae*, and three new species complexes were species E, species K and species P. *B. tau* was used as an out-group. A total of 424 adults, which emerged from pupae collected from natural populations in Thailand, were prepared for wing measurements. Morphometric analysis was performed on measurements of wing vein characters. Wing images were captured in digital format and taken through digital image processing to calculate the Euclidean distance between wing vein junctions. Discriminant and cluster analyses were used for dichotomy of classification processes. All 424 wing specimens were classified to species in terms of the percentage of "grouped" cases which yielded about 89.6% accurate identification compared with the formal description of these species. After clustering, the percentage of "grouped" cases yielded 100.0%, 98.9%, 98.1%, 95.2% and 84.6% accurate identification between the *B. dorsalis* complex and *B. tau*; *B. arecae* and Species E; *B. dorsalis* and *B. verbascifoliae*; *B. propinqua* and *B.*

pyrifoliae; and species K and species P, respectively. This method of numerical taxonomy may be useful for practical identification of other groups of agricultural pests.

2.2 Genetic diversity of *Bactrocera cucurbitae*

Asghar *et al* (2015) reviewed on DNA Extraction from insects by using different techniques was compiled through a thorough review of many research articles published in various journals of international repute. To identify insect, and for research on their role, different molecular techniques were used. For each molecular technique, high quality DNA was required, which was extracted from the insects by different techniques. The selection of DNA extraction technique was depended upon specimen under study, time required for extraction, economical stander of technique due to reagents and equipment used for extraction and most importantly extracted DNA quality.

Delomen *et al* (2013) studied on morphometric analysis and DNA barcoding using the 5' region of the mitochondrial cytochrome coxidase subunit 1 (*mtCOI*) gene, to distinguish the fruit flies *Bactrocera occipitalis* (Bezzi) and *B. philippinensis* Drew and Hancock. They concluded that *mtCOI* clustering did not support morphological ratings, with *B. occipitalis*, hybrid, and *B. philippinensis* samples grouped together. Low bootstrap values at certain branches suggested the lack of phylogenetic differentiation among the morphological species delineations. Pair wise distances of consensus sequences ranged from 0.00 to 0.033, which were lower than the standard threshold of 0.5% utilized for species delineation in fruit flies. Therefore, DNA barcoding failed to delineate *B. occipitalis* and *B. philippinensis*.

Prabhakar *et al* (2013) studied the population genetic structure of the pumpkin fruit fly, *Bactrocera tau*, a fruit fly pest of cucurbits, in Himachal Pradesh (India) used mitochondrial cytochrome oxidase I (mtCOI) gene sequences. Levels of differentiation (genetic distances and FST values) among the samples from different locations are minimal, suggesting the local occurrence of a large and geographically undifferentiated population, with the possible exception of population Solan. The phylogenetic analysis of local *B. tau* samples in the context of the different sibling species that constitute the *B. tau* complex in its South-East Asia region of origin revealed that local *B. tau* is closely related to B. tau species A from Thailand. They suggested marked local genetic uniformity and predominance of one single species of the species complex further suggest that the sterile insect technique (SIT) may be a viable option.

Asokan *et al* (2013) conducted study on life stage independent molecular identification using 28S rDNA. They evaluated that the utility of 28S rDNA for the quick and accurate species diagnosis of eggs, larvae, pupae and adults of all the *Bactrocera* spp. The phylogram for the *Bactrocera* spp. suggested that *B. tau* is phylogenetically distant from the rest of the three species viz. *B. dorsalis*, *B. zonata* and *B. correcta*, which was supported by 100% bootstrap value. Moreover *B. dorsalis*, *B. zonata* and *B. correcta* had maximum sequence identity (98%) with very few variable sites in the 28S rDNA sequences. Even though, 28S rDNA region had high reliability for species identification in these species studied and it was never reported before. This study indubitably proved the utility of 28S rDNA for the quick and accurate species diagnosis of egg, larva, pupa and adult of *B. zonata* Saunders, *B. tau* Walker and *B. dorsalis* Hendel.

San jose *et al* (2013) sequenced one mitochondrial and two nuclear genes from 73 specimens, belonging to 19 species to construct phylogenies and examine species relationships and limits within the genus *Bactrocera* and several species of the *B. dorsalis* complex, specifically addressing the placement of *B. invadens*. Their results indicated the *B. dorsalis* complex was polyphyletic. *B. invadens* and several other species within the *B. dorsalis* complex (*B. dorsalis*, *Bactrocera papayae*Drew&Hancock, and *Bactrocera philippinensis*Drew & Hancock) were also paraphyletic with respect to each other and probably represent a single genetically indistinguishable, phenotypically plastic, pest species that has spread throughout the world.

Khemakhem *et al*. (2012) described the use of molecular technique in diagnosis of invasive alien insect pests, having quarantine importance. They used PCR-RPLF COX-I gene for identification of 3 sample species viz. *B.cucurbitae*, *B. zonata* and *C. capitata*. They used *Dde*I and*Xmn*I enzyme to digest the COI PCR products. They concluded that the simplicity and relatively low cost of this molecular approach replaced the need to rear immature stages of adults for identification and will facilitate rapid quarantine decisions providing greater plant protection.

Prabhakar *et al* (2012) used COX-I to understand genetic structure of melon fruit fly. They suggested that melon fly populations across the globe were homogeneous with non-significant variation of 0.000–0.003 base substitutions per site. Test isolates represents various geographic situations across the world were placed in 26 mitochondrial haplotypes based on variations associated with a maximum of three mutational steps and the predominant haplotype i.e. H1 was present in all melon fly populations except Hawaiian population. Evolution of *mtCOI*gene suggested that the fly could have originated some 0.4 million years ago.

Wu *et al* (2012) studied genetic relationship of the melon fly, *Bactrocera cucurbitae* (Diptera: Tephritidae) from 23 locations covering nine countries based on DNA sequences of the mitochondrial cytochrome oxidase I (COI) gene. Forty-two polymorphic sites were described among 38 haplotypes. The most common haplotype, H1, was observed in 73% of the samples distributed among all populations. Highest genetic diversity was seen within populations, and no isolation-by-distance was detected. The western regions (Nepal, Bangladesh, Thailand, Burma and China-west) showed higher haplotype diversity than eastern regions (China-east). China-Yunnan showed highest levels of genetic diversity in China. Haplotype diversity decreased with longitude from west to east. All together, these analyses suggested that *B. cucurbitae* was expanded from west to east within a limited geographic scale and recently invaded China through Yunnan Province.

Lim *et al* (2012) described genetic lineages of *Bactrocera caudata* (Insecta: Tephritidae) Revealed by COI and 16S DNA Sequences. Their molecular phylogenetic analysis indicates that the *B. caudata*lineages are closely related to *B. ascita* sp. B, and form a clade with *B. scutellata*, *B. ishigakiensis*, *B. diaphora* and *B. ascita*sp. A. Their study provides additional baseline for the phylogenetic relationships of Bactrocera fruit flies of the subgenus *Zeugodacus*. Both the COI and 16S genes could be useful markers for the molecular differentiation and phylogenetic analysis of tephritid fruit flies.

Khamis *et al* (2012) studied the morphometry of invasive fruit fly *B.invadens* and DNA barcoding in kenya. In their study the morphometry and DNA Barcoding of different populations of *B. invadens* distributed across the species range of tropical Africa and a sample from the pest's putative aboriginal home of Sri Lanka was investigated. Morphometry using wing veins and tibia length were used to separate *B. invadens* populations from other closely related *Bactrocer*a species. The Principal component analysis yielded 15 components which corresponded to the 15 morphometric measurements. The first two principal axes contributed to 90.7% of the total variance and showed partial separation of these populations. Canonical discriminant analysis indicated that only the first five canonical variates were statistically significant. The first two canonical variates contributed a total of 80.9% of the total variance clustering *B.*

invadens with other members of the *B. dorsalis* complex while distinctly separating *B. correcta*, *B. cucurbitae*, *B. oleae* and *B. zonata.* They studied evolutionary history inferred by the neighbour - joining method clustered the *Bactrocera* species populations into four clusters. First cluster consisted of the *B. dorsalis* complex (*B. invadens*, *B. kandiensis* and *B. dorsalis*), branching from the same node while the second group was paraphyletic clades of *B. correcta* and *B. zonata*. The last two were monophyletic clades, consisting of *B. cucurbitae* and *B. oleae*, respectively.

Ashokan *et al* (2011) conducted a study on molecular identification and phylogeny of *Bactrocera* species (Tephritidae). They evaluated the utility of COX-I for the quick and accurate species diagnosis of eggs, larvae, pupae and adults of *B. zonata* Saunders*, B. tau* Walker*, and B. dorsalis* Hendel. Furthermore the utility of species-specific markers in differentiating *B. zonata* (500bp) and *B. tau* (220bp) was shown. Phylogenetic relationships among five subgenera, viz., *Austrodacus, Bactrocera, Daculus, Notodacus* and *Zeugodacus* have been resolved employing the 5´ region of COX-I (1490-2198); where COX-I sequences for *B. dorsalis* Hendel, *B. tau* Walker*, B. correcta* Bezzi and *B. zonata* Saunders from India were compared with other NCBI-GenBank accessions. Phylogenetic analysis employed maximum parsimony (MP) and Bayesian phylogenetic approach (BP) showed that the subgenus *Bactrocera* is monophyletic.

Liu et al (2011) identified the *Bactrocera (Bactrocera) invadens* Drew based on morphological characteristics and DNA barcode. Microscopic observations showed morphological character states that were congruent with the diagnosis of *B. invadens*. The mitochondrial DNA (mtDNA) cytochrome c oxidase I (COI) gene sequence alignment demonstrated the similarity between specimens 1 and 2 and *B. invadens* is 99.47%, between specimen 3 and *B. invadens* 98.77%, between specimen 4 and *B. invadens* 99.82%, and between the other 10 specimens and *B. invadens* 100%. Therefore, all samples were identified as *B. invadens* based on morphological characteristics and DNA barcode of COI gene. This study represented the first report of *B. invadens* in the Burundi District.

Samie and Fiky (2011) studied genetic structure of the Egyptian peach fruit fly (*Bactrocera zonata* (Saunders) (Diptera: Tephritidae)) population was analyzed using total RNA from adult females. A portion of mitochondrial cytochrome oxidase I (COI), 369 bp was amplified using RT-PCR, and was sequenced and analyzed to clarify the phylogenetic relationship of *B. zonata* established in Egypt. The data suggested that the gene shared a similarity in sequence compared to *Bactrocera* COI gene found in GenBank. Molecular phylogenetic analyses were performed based on nucleotide sequences in order to examine the position of the Egyptian population among many other species of fruit flies. The results indicated that four accession numbers of *B. zonata* (three from New Zealand and one from India) were closely related, while the Egyptian *B. zonata* was close to the 71 accession numbers of *Bactrocera* include one *B. zonata* from New Zealand. These two *B. zonata* from Egypt and New Zealand showed a close relationship in eighbour–joining analysis using the seven accession numbers of *B. zonata*.

Singh *et al* (2011) evaluated genetic characterization of *Bactrocera (dacus)* flies (diptera: tephritidae) based on rapd-pcr. They showed *B. cucurbitae* and *B. dorsalis* twenty four and twenty one fragments were amplified ranging from 150bp-2000bp, respectively. Four out of ten primers were monomorphic. Mean heterozygosity of *B. cucurbitae* and *B. dorsalis* were 0.119 and 0.093, respectively. Nei's genetic identity value (0.831), calculated using tools for Population Genetic Analysis (TFPGA) software, reveals very close genetic similarity between these two species.

Wan *et al* (2011) studied genetic diversity of *B. dorsalis* in china. Three mitochondrial genes (nad1, cytb and nad5) were used to infer the genetic diversity, population structure and demographic history of the oriental fruit fly from its entire distribution range in China. High levels of genetic diversity, as well as a significant correspondence between genetic and geographic distances, suggested that the invasion process might have been gradual, with no associated genetic bottlenecks. Three population groups could be identified; nevertheless the overall genetic structure was weak. They suggested the species originated in the coastal region facing the South China Sea and gradually expanded to colonize mainland China, expanding here to high population numbers.

Zhang *et al* (2010) studied molecular phylogeny of *Bactrocera* species (Diptera: Tephritidae: Dacini) inferred from mitochondrial sequences of *16s rDNA* and *COI* sequences. They explored the phylogenetic relationship among the 8 subgenera *Afrodacus*, *Austrodacus, Bactrocera*, *Daculus, Gymnodacus, Paratridacus, Tetradacus* and *Zeugodacus* based on the sequences of two mitochondrial DNA fragments with a combined length of 1034 base pairs. The two mtDNA fragments are a 689-bp segment of the *COI* gene and a 345- bp segment of the *16S rDNA* gene. Thirty-five individuals represented seven *Bactrocera* species found in the Chongqing region in China were sequenced for both fragments, and sequences of the same gene regions were acquired from GenBank for another 20 *Bactrocera* species and 2 other tephritid species, *Anastrepha ludens* and *Ceratitis capitata,* which were used as outgroups for the phylogenetic analyses. They reported *Bactrocera* (*Tetradacus*) *minax* and *Bactrocera* (*Zeugodacus*) *diaphora* sequences for the first time, and the subgenus *Bactrocera* (*Tetradacus*), here represented by *B.* (*T.*) *minax* and *B.* (*T.*) *tsuneonis,* were included for the first time in an analysis of the genus *Bactrocera* phylogeny. Their results of phylogenetic analyses based on maximum parsimony method supported that subgenus *Bactrocera* (*Bactrocera*) And *Bactrocera* (*Zeugodacus*) are paraphyletic. The subgenus *Zeugodacus* , *Bactrocera* (*Zeugodacus*) *Caudate* , *Bactrocera* (*Zeugodacus*) *diaphora,* and *Bactrocera* (*Zeugodacus*) *Scutellata* are closely related to *Bactrocera* (*Zeugodacus*) *tau* and *Bactrocera* (*Zeugodacus*) *Cucurbitae* . Their results indicated that subgenus *Austrodacus* and *Zeugodacus* , which attack the cucurbit plants, were closely related to species of the subgenus *Afrodacus* , *Bactrocera* , and *Gymnodacus* , which attack plants of numerous families.

Hu *et al* (2008) studied the population genetic structure of the melon fly, *Bactrocera cucurbitae* (Diptera: Tephritidae), from China and Southeast Asia. The population structure of seven geographic populations from coastal China, as well as samples from other regions of South East Asia and Japan, including lab colonies were studied by using a 782 bp fragment of mitochondrial cytochrome oxidase I (COI) gene sequence. Their genetic diversity was exceedingly low, considering the geographic scale of the sampling, and one single haplotype was found to be predominant from Sri Lanka to China. They concluded *Bactrocera cucurbitae* exists in South East Asia as a single phyletic lineage, that Chinese populations were genetically uniform, and that no apparent genetic differentiation exists between these and three available Japanese melon fly sequences.

Asokan *et al.* (2007) reported the *mtCOI* based identification of three fruit flies, *B. dorsalis*, *B. correcta* and *B. zonata*, where molecular identification has corroborated the morphological identification. A single fragement of approximately 500 bp was amplified for *B. dorsalis, B. correcta* and *B. zonata*. Sequencing results showed that the total nucleotide length obtained was 440 bases, for all the three species of fruit flies. Alignment of the above sequences in Bioedit revealed that there was 92% similarity between *B. dorsalis* and *B. correcta* and also between *B. correcta* and *B. zonata*. The number of nucleotides that were different between *B. dorsalis* and *B. correcta* and between *B. correcta* and *B. zonata* were 32 and 28, respectively. Highest variation (11%) was observed between *B. dorsalis* and *B. zonata*, where there was difference in 45 nucleotides.

Barbosa *et al* (2005) studied morphological and molecular characterization of three species of *Anastrepha* Schiner and of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) from Rio de Janeiro. Three from genus *Anastrepha*: *A. obliqua* (Macquart), *A. sororcula* Zucchi and *A. serpentina* (Wiedemann), and one from genus *Ceratitis, C. capitate* (Wiedemann) were compared based on puparium morphology and application of the Heteroduplex Mobility Assay (HMA). Puparia were characterized for the first time using the spiracular posterior plate morphology. Application of HMA allowed the detection of variability in the D2 domain from 28S rRNA gene in all four species (confirmed by sequencing).

Jamnongluk *etal* (2003) described molecular phylogeny of tephritid fruit flies in the *Bactrocera tau* complex using the mitochondrial *COI* sequences, from Thailand. They compared the sequences of the mitochondrial *cytochrome oxidase I* gene of eight species of the *Bactrocera tau* complex using *Bactrocera dorsalis*, *Bactrocera pyrifoliae*, *Ceratitis capitata*, *Anopheles gambiae*, and *Locusta migratoria* as outgroups. A 639-bp variable region was sequenced. The sequence divergence between species in the *B. tau* complex ranged from 0.06 to 28%, and up to 29% between the complex and its tephritid outgroups, *B. dorsalis* and *C. capitata*. According to the phylogenetic relationships, these members of the *B. tau* complex could be classified into four clades. Thus, species A and D form clades 1 and 3, respectively, while species C and I belong to clade 4.

Jamnongluk *et al* (2003)studied molecular evolution of tephritid fruit flies in the genus *Bactrocera* based on the cytochrome oxidase I gene. They investigated phylogenetic relationships using mitochondrial cytochrome oxidase I gene (COI) sequences, among four subgenera, *Asiadacus*, *Bactrocera*, *Hemigymnodacus*, and *Zeugodacus*. Their phylogenetic analysis of mitochondrial COI sequences suggested that tephritid fruit fly species, which attack on cucurbit plants, that is, *Asiadacus*, *Hemigymnodacus* and *Zeugodacus*, were more closely related to each other than to fruit fly species of the subgenus *Bactrocera*, which attack plants of numerous families.

Muraji and Nakahara (2002) evaluated the relation among pest species of *Bactrocera* (Diptera: Tephritidae) based on PCR-RFLP of the mitochondrial DNA mainly distributed in the Asia-Pacific region. Based on the results, a scheme for the *Bactrocera* pest species identification was proposed. The PCR-RFLP analysis using 83 individuals revealed that the scheme correctly identified most of the 18 species except for two closely related sympatric species, *Bactrocera carambolae* and *B. papayae*; the majority of the individuals of the former species showed the same banding patterns as the latter species.

Armstrong *et al* (1997) described molecular method to identify the fruit flies at larval stages for quarantine purpose using simple restriction patterns of ribosomal DNA (rDNA) as diagnostic markers. They were amplified 18S and 18S plus internal transcribed spacer (ITS) regions from larval DNA by the polymerase chain reaction (PCR). They screened 22 restriction enzymes but diagnostic RFLPs have yet to been found from six out of the ten *Bactrocera (Bactrocera)* species; *B. passiflorae* (Froggatt) neither be distinguished from *B. facialis* (Coquillet), nor *B. kirki* (Froggatt) from *B. trilineola* (Froggatt) or *B. neohumeralis* (Hardy) from *B. tryoni* (Froggatt).

MATERIALS AND METHODS

The research work of the present investigation entitled "**Morphological identification of cucurbitaceous fruit flies in Bihar and genetic diversity of *Bactrocera cucurbitae*"** was carried out in the Departments of Entomology and Plant Pathology (Virology laboratory), Bihar agricultural University, Sabour, Bhagalpur (Bihar), during 2016 - 2017. Geographically, the experimental site was situated at N 25°14′26″ latitude and E 87°2′56″ longitude and at an elevation of 36.567 m above mean sea level.

3.1 Survey and Taxonomic identification of fruit flies infesting cucurbits

A survey was undertaken from different agroclimatic zones of Bihar with reference to extreme directions like extreme north, extreme south, etc (Table 3.1). Adult fruit flies were collected by using lure (para-pheromone i.e. Cuelure), sweep net and larvae collected from infested fruits (Plate 3.1) for taxonomic studies and for maintenance of Stock culture. After proper processing of those specimens, the taxonomic keys were studied as provided by White and Elson-Harris (1992); Kapoor (1993); Drew and Raghu (2002).

The specimens of *Bactrocera cucurbitae* (Coquillett) preserved in 99% ethanol and kept in different vials at -20 ºC until process for DNA extraction.

Table 3.1 Surveyed locations for collection of fruit fly samples

Sl. No.	District(s)	Place(s)	Latitude*	Longitude*	Sample collected/ Method
1	Bhagalpur (Zone IIIA)	Sabour	25°14′26″	87°2′56″	Infested fruits & trap
2	Kishanganj (Zone II)	Gachhpara	26°5′59″	87°57′39″	Infested fruits & trap
3	Baxur (Zone IIIB)	Dumraon	25°34′0″	84°7′42″	Infested fruits & trap
4	Nalanda (Zone IIIB)	Pawapuri	25°5′32″	85°32′17″	Infested fruits & trap
5	East Champaran (Zone I)	Motihari	26°64′70″	84°90′89″	Infested fruits & trap

***Lat_lon (DM) were provided by mobile GPS inbuilt software (Lenovo A7000).**

3.2 Processing of sample and dissection of male genitalia for Taxonomic purpose

Collected specimens were side pinned with A-1 size micropins, larger micropins, pinned specimen (Plate 3.2) stage on a strip of plastazote measuring 4 x 4 x 15 mm. A general account of curating a Diptera collection was given by Irwin (1978). Than adult flies were pinned and staged on plastazote strip like given in photo, with three paper strips having information regarding the Specimen *viz*. Place of collection, host or plant from specimen obtained with name of collector and in last identity of specimen were given if identified.

Permanent slides of every species of fruit flies were also made by dissection of male specimen (Ganie *et al*., 2013). The abdomen of male separated and kept on hot plate for 20-30 minutes with 10 per cent KOH solution to get the musculature sufficiently relaxed. Later KOH was removed by washing these specimens in distilled water for two or three times. The dissection was performed on glass slide, with the help of fine forceps and dissection needles under a binocular microscope MS24 (Magniüs). The dissected body parts were transferred to glacial acetic acid in another cavity block and at this stage acid fuschin was also used for staining. After 10-15 minutes, specimen parts were again washed with fresh glacial acetic acid to remove the excess stain. Then the same was transferred to clove oil for 15 minutes. After getting clean the body parts of specimens were mounted on a plain ground edges microscope slide (76mm×26mm×1.25mm, RE 1.53±0.02) Borosil glass work ltd., Cover glass (18mm×18mm) in Canada balsam. Photographs were taken from field camera and digital camera attached with Olympus CX21i compound microscope.

3.2.1 Trap

In this study, a modified self made trap (Plate 3.3) was used, which was made by using the empty plastic water bottles generally through away by the people after used and also used Cuelure with 99.99% ethanol in 1:1 ratio, no insecticide is added in to it. The purpose of making this trap was to catch live flies for proper study. In readily available traps flies died quickly due to use of insecticides and dry soon.

In this trap the material used was empty water bottle, 4 hollow cylindrical plastic tube with 8 cm length and 1.8 cm inner hollow diameter, any soaking material for lure 5 ml 1:1 (Cuelure : Ethanol) and cotton thread or iron wire for hanging the bottle in field.

Four holes were made at the bottom of the bottle by hot needle or with the help of match stick and thereafter plastic tubes were fitted inside these holes in a manner so that maximum length remain inside and fixed with fast glue. At the inner side of bottle a thin Iron hook was stacked with fast glue so that the lure soaked cotton or sponge was hanged. The neck of the bottle iron wire tied to hang in field.

This design of the trap made to catch the live flies. Flies entered in to the bottle through the tubes due to smell of lure but due to their upward flying tendency they always try to fly upside of bottle and never come down towards tubes once enter. It was better than the poison lures provides live flies which was collected from the bottle using the aspirator. During study the average catch per day in this trap was 39.25 flies per day.

3.3 Genetic diversity of *Bactrocera cucurbitae*

3.3.1 DNA Extraction

Total genomic DNA of each isolate was extracted according to Wu *et al* (2011) with minor modifications. For extracting total genomic DNA, the individual fruit fly from each zone (*B. cucurbitae*) was ground to fine paste using micro pestle.

In each tube, 700 µl of CTAB extraction buffer (Table 3.2) was added after warm up. All tubes were incubated at 65^{o}C for 1h in a water bath (Secor India, Scientific Engineering Corporation). After incubation, each tube was filled with equal volume (700 µl) of chloroform: isoamyl alcohol (24:1). The contents were mixed thoroughly and the tubes were spun at 12,000 rpm for 12 min. (Eppendorf, 5430 R) at 25^{o}C. Aqueous phase was transferred to new tubes and 450 µl pre-chilled isopropanol was added and kept at -20^{o}C for 1 hour to precipitate the DNA. Then the tubes were spun at 10,000 rpm for 12 min. and supernatant was decanted. The DNA pellet was washed thrice with 70 percent ethanol, dried and dissolved in 100 µl of Tris EDTA (10mM TrisHCl and 1mM EDTA pH 8.0). The amount of DNA was quantified by agarose gel electrophoresis. DNA was further stored at -20^{o}C for further use.

Table 3.2 Reagent and concentration of DNA extraction buffer

Reagent	Stock concentration	Working concentration	Working solution (100 ml)
TrisHCl (pH- 8.0), 100 Mm	1 M	100 mM	10 ml
NaCl 1.4 M	5 M	1.4 M	28 ml
EDTA (pH- 8.0)	0.5 M	20 mM	4 ml
CTAB (2%)	-	-	2 g
PVP (1 %)	-	-	1 g
Water (RNAase and DNAase free)	-	-	55 ml
Total	-	-	100 ml

3.3.2 Primers used

Mitochondrial cytochrome oxidase subunit I gene (*mtCOI* gene) was amplified using the forward primer MCOX-I and MCOX-II developed by Lunt *et al* (1996). The base sequences of primers (Table 3.3) were custom synthesized (Life Technologies India Pvt. Ltd.).

Table 3.3 Base sequences of MCOX-I and MCOX-II primer

Name of the Primer	Sequence (5’ to 3’)
MCOX-I (Forward)	5’ GGTCAACAAATCATAAAGATATTG 3’
MCOX-I (Reverse)	5’ TAAACTTCAGGGTGACCAAAAAATCA 3’
MCOX-II (Forward)	5’ TACAGTTGGAATAGACGTTGATAC 3’
MCOX-II (Reverse)	5’ TCCAATGCACTAATCTGCCATATTA 3’

3.3.3 PCR amplification

The PCR amplification was carried out using 1X PCR pre-mix procured from Genet Bio, Korea. The total volume of the reaction mixture was 20µl. 1X PCR pre-mix consists following reaction mixture (Table 3.4):

\

Table 3.4 : Reagent and concentration for 1X PCR reaction

Reaction Mixture	Quantity (µl)
PCR pre-mix (Composition- Taq Buffer, $MgCl_2$, dNTPs, Taq DNA polymerase)	10 µl
Forward Primer (10 µM) 20 pmol	1.0
Reverse Primer (10 µM) 20 pmol	1.0
Water (SDW)	6.0
DNA	2.0
Total Volume	20.0

Reaction mixture was vortexed and centrifuged in a microfuge (Bangalore Genei, India). Amplifications were performed using thermal cycler (Eppendrof, USA) with following temperature transitions:

Steps	Temperature (^oC)	Time (minute)
1. Initial denaturation	94	3.00
2. Denaturation	94	1.00
3. Annealing	50	1.00
4. Elongation	72	1.00
5. Final extension	72	30.00

The thermal cycler was programmed for 30 cycles with one cycle of initial denaturation and steps 2-4 were repeated 30 times and a final extension at 72^{o}C for 30 min using fastest ramp time between the temperature transitions.

3.3.4 Agarose gel electrophoresis of PCR product

The PCR products were resolved on 1.5% agarose gel in 0.5X Tris borate EDTA buffer. DNA ladders of 100 bp was used as marker. The gels were run at 90V for 45 minutes using Genetix Biotech Asia Pvt. Ltd. system, stained with ethidium bromide (0.5 µg / ml), after electrophoresis, viewed and images were captured using UVITEC (Uvitec Cambridge GeNei^{tm}) gel documentation system.

EXPERIMENTAL FINDINGS

An intensive survey for collection of fruit flies in commercial cucurbits growing areas were conducted across five districts *viz.* Bhagalpur, Kishanganj, Nalanda, East Champaran and Buxar of four different agro climatic zones (Zone-I, II, IIIA and IIIB) of Bihar during 2016-2017 and the data were compiled to draw inferences as described under following sub headings:

4.1. Fruit fly infestation in cucurbits

The observation of fruit flies was taken at district and place wise and their per cent infestation was also recorded (Table 4.1). A total of four species namely *Bactrocera cucurbitae, B. tau, Dacus ciliatus* and *B. diversa* were recorded in different host crops *viz.* cucumber, bitter gourd, bottle gourd, pumpkin and pointed gourd (Plate 4.1 and 4.2). In Kishanganj (Gachhpara), *B. cucurbitae* and *B. tau*; in Bhagalpur (Sabour), *B. cucurbitae, B. tau, Dacus ciliatus and B. diversa;* in Nalnda (Pawapuri), only *B. cucurbitae;* in East Champaran (Motihari), *B. cucurbitae* and *B. tau;* and in Buxar (Dumraon), *B. cucurbitae* and *B. tau* were recorded from different cucurbitaceous crop during survey programme. The infestation of fruit flies ranges between 31.05 to 81.19 %. Maximum infestation was recorded at Sabour region (81.19 % in pointed gourd and 78.39 % in cucumber and bittergourd). Minimum infestation (45.14 %) of fruit flies was observed at Pawapuri region of Nalanda district. The mean per cent damage of different cucurbits by fruit flies was 58.59 %.

Table 4.1 Per cent infestation of fruit flies in different host crops during survey at different districts of Bihar

Sl. No.	District	Place	Species Recorded	Host	Per cent infestation
1	Kishanganj	Gachhpara	*B. cucurbitae* *B. tau*	Cucumber, bitter gourd, bottle gourd,Bitter gourd	72.81 % 48.22%
2	Bhagalpur	Sabuor	*B. cucurbitae* *B. tau*	Cucumber, bitter gourd, pumpkin, bottle gourd	78.39% 63.09%
			Dacus ciliatus *B. diversa*	Pointed gourd Pumpkin flowers	81.19% 46.45%
3	Nalanda	Pawapuri	*B. cucurbitae*	Bottle gourd	45.14%

4	East Champaran	Motihari	*B. cucurbitae*	Cucumber, bitter gourd	63.71%
			B. tau	Cucumber	31.05%
5	Buxar	Dumraon	*B. cucurbitae*	Bottle gourd	-
				Mean	**58.89 %**

4.1.2 Diversity of fruit flies captured in pheromone traps

Pheromone traps were installed at five locations *viz.* Sabour (Bhagalpur), Gachhpara (Kishanganj), Pawapuri (Nalanda), Motihari (East Champaran) and Dumraon (Buxar) to assess the diversity of fruit flies in different cucurbitaceous crop. Three species (*B. cucurbitae, B. tau* and *B. nigrofemoralis*) of fruit flies from Motihari (East Champaran) and four species (*B. cucurbitae, B. tau, B. caudata, B. nigrofemoralis*) of fruit flies from Sabour (Bhagalpur) were collected (Table 4.2). Whereas, only one species (*B. cucurbitae*) was collected from traps installed at Dumraon (Buxar). The latitude and longitude of different locations were also mentioned in table 4.2.

Table 4.2 Diversity of fruit flies captured in cuelure trap

Place	Latitude	Longitude	Species of fruit Fly collected
Sabour	25°14′26″	87°2′56″	*B. cucurbitae, B. tau, B. caudata, B. nigrofemoralis,*
Gachhpara	26°5′59″	87°57′39″	*B. cucurbitae, B. tau*
Dumraon	25°34′0″	84°7′42″	*B. cucurbitae*
Pawapuri	25°5′32″	85°32′17″	*B. cucurbitae, B. tau*
Motihari	26°64′70″	84°90′89″	*B. cucurbitae, B. tau, B. nigrofemoralis*

4.2 Species of fruit fly associated with cucurbits in Bihar

After an intensive survey of different locations of Bihar and collection of infested cucurbit samples, it was observed that two species *viz. B. cucurbitae* and *B. tau* were the predominant fruit fly species infesting in majority of the cucurbits at different locations. These two species were predominant at Kishanganj, Bhagalpur and Motihari (Table 4.2) districts of Bihar. However, in Nalanda and Buxar district, only *B. cucurbitae* infestation was observed in cucurbitaceous crop.

B. diversa was the lone species reared from the infested samples of flowers and tender fruits of pumpkin. It was collected from two locations namely, Sabour and Motihari district of Bhagalpur and East Champaran.

During the course of survey, *B. tau* and *B. cucurbitae* were recorded as the key species infesting majority of the cucurbits in Bihar. But nowadays, *Dacus ciliatus* gained a major status in cucurbits regarding damage point of view and it was collected from Sabour on pointed gourd and damages were more than the *B. cucurbitae*. However, *B. cucurbitae* was earlier considered as one of the major fruit fly species infesting cucurbits in the state, but nowadays *D. ciliatus* and *B. tau* were also gaining the status of greater damage. In our experimental findings it was observed that the infestation of *D. ciliatus* especially on Pointed gourd becoming more serious.

In earlier literature, Agarwal and Sueyoshi (2005) reported that *B. cucurbitae* was widely distributed among the cucurbits in India. Agarwal (1984) reared this species from infested cucurbit samples collected from Bihar.

4. 3 Identification of fruit fly species

As we know that different species of fruit flies infest the cucurbitaceous crop and its damage the crop either individually or collectively, hence an attempt was made to identify the associated species using the morphological characteristics. The specimens were studied taxonomically according keys provided byWhite and Elson-Harris (1992); Kapoor (1993) and Drew and Raghu (2002).

4.3.1 Morphological characteristics of fruit fly species identified

Subfamily : DACINAE

Tribe : DACINI

1. Genus ***Bactrocera*** Macquart

Subgenus ***Zeugodacus*** Hendel

Bactrocera* (*Zeugodacus*) *cucurbitae (Coquillett)

Material examined:

District Bhagalpur 3 ♂, 5 ♀, Sabour, 28 August 2016, ex *cucumis sativus*, 1 ♂, 3 ♀, Sabour, 22 August 2016, ex *Trichosanthes dioica*, 2 ♂, 2 ♀, Sabour, 30 August 2016, ex *Lagenaria siceraria*, 2 ♂, 5 ♀, Sabour, 28 May 2017, ex *Momordica chatantia*, 1 ♂, 2 ♀, Sabour, 11 March 2017, ex *Trichosanthes cucumerina*, 2 ♂, Sabour, 21 August 2016, Cuelure, District Kishanganj, 2 ♂, 3♀, Gachhpara, 24 September 2016, ex *Lagenaria siceraria*, 1 ♂, 2 ♀, Gachhpara, 24 September 2016, ex *Momordica Charantia*, 2 ♂, Gachhpara, 24 September 2016, Cuelure, District Buxar, 2 ♂, 2 ♀, Dumraon, 22 October 2016, ex *Lagenaria siceraria*, 2 ♂, Dumraon, 22 October 2016, Cuelure, District

Nalanda 1 ♂, 2 ♀, Pawapuri, 25 October 2016, ex *Lagenaria siceraria*, 2 ♂, Pawapuri, 25 October 2016, Cuelure, District East Champaran, 1 ♂, 2 ♀, Motihari, 28 April 2017, ex *Cucumis sativus*, 1 ♂, 2 ♀,Motihari, 28 April 2017, ex *Momordica charantia*, 1 ♂, Motihari 28 April 2017, Cuelure.

♂, Dumraon, 22 October 2016, Cuelure, District Nalanda 1♂, 2 ♀, Pawapuri, 25 October 2016, ex *Lagenaria siceraria*, 2 ♂, Pawapuri, 25 October 2016, Cuelure, District East Champaran, 1 ♂, 2 ♀, Motihari, 28 April 2017, ex *Cucumis sativus*, 1 ♂, 2 ♀,Motihari, 28 April 2017, ex *Momordica charantia*, 1 ♂, Motihari 28 April 2017, Cuelure.

Diagnosis:

Costal band with large apical spot, almost covering apical part of cells r 2+3 and r 4+5; crossveins R-M and DM-Cu thickly infuscated; 3 frontal setae; scutellum yellow

Abdomen: Orange brown abdominal terga, except a narrow transverse black band across anterior margin of tergum III which expands over anterolateral corners. A narrow medial longitudinal dark fuscous to black band over all three terga and anterolateral corners of terga IV and V were fuscous. On the 3rd terga male have pecten (Raw of Setae). Female with needle like aculeus and male have long coiled aedegus or surstylus (Plate 4.3).

Attractant: Cuelue

Host: Cucurbits

Dacus cucurbitae Coquillett, 1899. Entomol. News, 10: 129. Lectotype ~· Hawaii(Honolulu) (USNM).

Dasyneura caudata: Walker, 1849. List Dipt. Ins. Coli. Brit. Mus., 4: 1073.

Dacus fulvidus Froggatt, 1909. In Official report on fruit fly and other pests invarious countries 1907-1908. Report on parasitic and injurious insects.

N.S.W., Dept. Agric., Sydney, p. 99. ? Type?Sex. India (UMO). (Nomennudum, attributed to Bigot).

Bactrocera cucurbitae: Bezzi, 1913. Mem. Indian Mus., 3: 96.

Chaetodacus cucurbitae: Bezzi, 1916. Bull. Entomol. Res., 7: 109.

Strumeta cucurbitae: Perkins, 1938. Proc. R. Soc. Queens! 49(11): 127.

Dacus (Strumeta) cucurbitae: Swezey, 1946. Bull. B. P. Bishop Mus., 10: 199.

Dacus (Zeugodacus) cucurbitae: Drew, 1973. Queens!. Dep. Indus., Div. PlantIndus. Bull., 652, p. 23.

Bactrocera (*Zeugodacus*) *cucurbitae*:Drew and Raghu, 2002. Raffles Bull. Zool., 50(2): 348-349.

2. *Bactrocera (Zeugodacus) tau* (Walker)

***Material examined*:**

District Bhgalpur, 1 ♂, 2 ♀, Sabour, 12 December 2016, ex *Momordica charantia*, 1♀, 1 ♀, Sabour, 11 March 2017, ex *Trichosanthes cucumerina*, 2 ♂, Sabour, 25 March 2017, Cuelure, District Kishanganj, 1 ♂, 1 ♀, Gachhpara, 26 September 2016, ex *Lagenaria siceraria*, 1 ♂, 1 ♀, 22 September 2016, ex *Momordica Charantia*, 2 ♂, Gachhpara, 24 September 2016, Cuelure, District East Champaran, 1 ♂, 1 ♀, Motihari, 28 April 2017, ex *Cucumis sativus*, 1 ♂, Motihari, 28 April 2017, Cuelure, District Nalanda, 2 ♂, Pawapuri 25 October 2016, Cuelure.

***Diagnosis*:**

Head: Face fulvous with a pair of medium sized circular to oval black spots.

Thorax: Postpronotal lobes and notopleura yellow, scutum black with large areas of red brown centrally and anterocentrally, lateral and medial postsutural vittae were present, yellow spot anterior to mesonotal suture in front of lateral postsutural vittae, mesopleural stripe reaching midway between anterior margin of notopleuron and anterior *npl*. seta; scutellum entirely yellow (Plate 4.4).

Wing: wing with a narrow dark fuscous costal band overlapping R2+3 and expanding into a distinct apical spot and broad dark fuscous cubital streak, cells bc and c colourless, microtrichia in outer corner of cell c only.

Abdomen: Abdominal terga III-V fulvous with a black 'T' pattern and anterolateral corners of terga IV and V with broad black markings, male with pectin (Plate 4.4).

Atrractant: Cuelure

Host: Cucurbits

Dasyneura tau Walker, 1849: 1074.

Dacus hageni de Meijere, 1911: 375, type locality, Sumatra.

Dacus caudatus var. *nubilus* Hendel, 1912: 16, type locality Taiwan.

Dacus nubilus ssp. *femoralis* Hendel, 1934: 11, type locality China.

Dacus (Zeugodacus) tau – Hardy, 1977: 60.

Bactrocera (Zeugodacus) tau – Liang et al., 1993: 138; Wang, 1996: 72; Norrbom et al., 1998: 104.

3. ***Bactrocera (Zeugodacus) caudata*** (Fabricius)

***Material examined*:**

District Bhagalpur, 1 ♂, Sabour, 22 November 2016, Cuelure.

***Diagnosis*:**

Head: Face with a black line across mouthpart opening, or with black spot in the antennal furrow extended laterally and almost forming a line across the mouth opening (Plate 4.5).

Thorax: Scutum predominantly black with lateral and median yellow strips or vittae, with anterior supra alar setae, prescutellar acrostichal setae, 4 scutellar setae.

Wing: Costal band expended in to an apical spot (Plate 4.5).

Abdomen: Male with pectin, abdominal terga III-V fulvous with a black ‘T’ pattern

Attractant: Cuelure

Host: Cucurbits

Dacus caudatus Fabricius, 1805. Syst. Antliat., p. 276. Lectotype ~. Indonesia(Java) (ZMUC).

Dacus (Bactrocera) maculipennis Doleschall, 1856. Natuurk. Tijdschr. Ned.-Indie, 10: 412. ?Type. ?Sex. Indonesia (Java: Bogor) (?NMW, ?RNHL).

Chaetodacus cauda/us: Bezzi, 1916. Bull. Entomol. Res., 7: 110.

Chaetodacus maculipennis: Bezzi, 1916. Bull. Entomol. Res., 7: 109.

Zeugodacus caudatus: Shiraki, 1933. Mem. Fac. Agric. Taihoku Imp. Univ., 8: 88.192.

Dacus (Zeugodacus) caudatus: Hardy, 1954. Pacif. Sci., 8(2): 186.

Bactrocera (Zeugodacus) caudata: Kapoor, 1993. Indian Fruit Flies. Oxford & IBHPubl. Co., New Delhi: 80.

Subgenus ***Bactrocera*** Macquart

4. ***Bactrocera (Bactrocera) nigrofemoralis*** White & Tsuruta

***Material examined*:**

District Bhagalpur, 3 ♂, Sabour, 13 December 2016, Cuelure, District East Champaran, 2 ♂, Motihari, 28 April 2017, Cuelure.

***Diagnosis*:**

Head: Face entirely black except narrow fulvous lateral margins and dorsally below antennal sockets (Plate 4.6).

Thorax: Scutum entirely black, postpronotal lobes and notopleura yellow, mesopleural stripe reaching anterior notopleural seta dorsally, narrow parallel sided lateral postsutural vittae reaching to intra alar seta, medial postsutural vitta was absent, with 2 scutellar, 2 prescutellar, 2 notopleural and 4 scapula setae. Scutellum yellow except for narrow to medium black basal band; legs with fore femora shining black on entire outer surfaces, fulvous on inner surfaces and on basal and apical extremities, mid femora was entirely shining black except dark fulvous on basal and apical extremities, hind femora fulvous except shining black on apical 1/3, fore tibiae fuscous, mid tibiae dark fulvous tending pale fuscous basally, hind tibiae dark fuscous, tarsal segments entirely fulvous tending darker fulvous on apical four segment (Plate 4.6).

Wing: Wings with cells bc and c colourless, microtrichiain outer corner of cell c only, a narrow fuscous costal band confluent with R2+3 and remaining very narrow around costalmargin to end at apex of R4+5 (Plate 4.6), a very narrow fuscous cubital streak, supernumerary lobe weak (Plate 4.6).

Abdomen : Tergum III dark fuscous to black except red-brown posterocentrally either side of a narrow medial longitudinal black band, tergum IVfuscous to dark fuscous except red-brown posterocentrally either side of a narrow medial longitudinal black band (theposterocentral red-brown markings extend towards the lateralmargins), tergum V red-brown with dark fuscous anterolateral corners and a narrow medial longitudinal fuscous to black band, a pair of oval dark fuscous to black shining spots ontergum V.

Attractant: Cuelure

Host: *Terminalia catappa* (Family Combretaceae)

Bactrocera (Bactrocera) nigrofemoralis White & Tsuruta, in Tsuruta& White, 2001: 79. Holotype male in BMNH.

Subgenus ***Hemigymnodacus*** Hardy

5. *Bactrocera (Hemigymnodacus) diversa* (Coquillett)

***Material examined*:**

District Bhagalpur, 1 ♂, 2 ♀, Sabour, 11 May 2017, ex *Cucurbita moschata*, 2 ♀, Sabour, 17 May 2017, ex *Lagenaria siceraria*, East Champaran, 1 ♂, 2 ♀, Motihari, 28 April 2017, ex *Cucurbita peppo*.

***Diagnosis*:**

Head: Female face fulvous with two transverse black lines on the face just above mouth opening, Male face entirely fulvous without dark markings.

Thorax: Scutum entirely black, postpronotal lobes and notopleura yellow, mesopleural stripe almost reaching anterior notopleural seta dorsally, broad parallel sided lateral postsutural vittae beginning with a small spot anterior to mesonotal suture and ending just behind intra alar seta, a narrow medial longitudinal postsutural vitta was present (Plate 4.7). Scutellum yellow except for narrow black basal band; legs with femora fulvous with dark fuscous to black subapical spots on outer surfaces of all femora (on the mid femora they cover approximately 1/2 the outer apical surface), fore and mid tibiae fulvous tending dark fuscous basally, hind tibiae dark fuscous, tarsal segments entirely fulvous.

Wing: Wings with cells bc and c colourless, microtrichia in outer corner of cell c only, narrow dark fuscous costal band confluent with R2+3 and widening across apex of wing, medium width dark fuscous cubital streak was present, supernumerary lobe strong and rounded (Plate 4.7).

Abdomen: Abdominal terga III-V red-brown with distinct 'T' patterns on terga III and IV consisting of a transverse black band across anterior margin of each tergum and a medium width medial longitudinal black band running to hind margin of each tergum, tergum V red brown with large anterolateral dark fuscous to black corners and a narrow medial longitudinal dark fuscous to black band on anterior 1/2, a pair of red-brown oval shining spots on tergum V. Male abdomen without pectin.

Attractant: Methyl eugenol

Host: Flowers of cucurbits especially pumpkin and bottle gourd and other cucurbits.

Dacus diversus Coquillett, 1904: 139. Syntypes in USNM.

Dacus quadrifidus Hendel, 1928: 343.

Dacus citronellae Kapoor & Katiyar, 1969: 123.

Dacus (Hemigymnodacus) diversus – Hardy, 1973: 19.

Bactrocera (Hemigymnodacus) diversa – Liang et al., 1993: 138.

Bactrocera (Paratridacus) diversa – Norrbom et al., 1998: 99.

2. Genus ***Dacus*** Fabricius

Subgenus ***Didacus*** collart

6. *Dacus (Didacus) ciliates* Loew

***Material examined*:**

District Bhagalpur, 1 ♂, 1 ♀, Sabour, 16 August 2016, ex *Trichosanthes dioica*, 1♂, Sabour, 22 August 2016, on *Cucumis sativus*.

***Diagnosis*:**

Head: Black round to oval facial spot, face fulvous.

Thorax: Body and thorax predominantly orange, a yellow spot covering most of the katatergite, anatergite orange, midfemur orange yellow, postpronotal setae was absent. Presutural dorsocentral setae, Presutural supra-alar setae, Postsutural supra alar, Acrostichal setae and Postsutural dorsocentral setae were absent. Intra alar setae were present, well developed, similar to postalar setae. The single pair of scutellar bristles was apical. Anterior notopleural setae were present (Plate 4.8).

Wing: Wing with costal band extremely narrow in cell r_{2+3} but dilated apically near and beyond vein $R_{4+5,}$ Cell bc microtrichia was absent. Cell c microtrichia was present in apical area (Plate 4.8).

Abdomen: Abdominal tergites fused to form hemispherical capsule, with oval black spots on either side of tergite III, Pecten of dark bristles on tergite III of male was present.

Attractant: Not known

Host: Cucurbits, in Bihar predominant on pointed gourd.

Dacus ciliatus Loew, 1862. Ofvers. K. Svenska Vetenskapakad. Forh., 19: 7.

Syntype♂, ♀,'Guinea'; South Africa (Cape) (NRS, ZMUC).

Dacus sigmoides Coquillett, 1901. Proc. U. S. Natl. Mus., 24: 29. Holotype ♀Mauritius. (USNM).

Dacus brevistylus Bezzi, 1908. Bull. Soc. Entomol. Ital., 39: 149. Syntype♂, ♀, Eritrea (Dintomi di Adi Ugri; Cheren) (MZLS, MCSNM).

Dacus apoxanthus var. *decolor* Bezzi, 1924. Ann. S. Afr. Mus., 19: 467. Syntype ♀South Africa (Cape: Grahamstown) (SAN C).

Tridacus mallyi Munro, 1925. Entomol. Mem. S. Afr. Dep. Agric., [1]3: 42. (Nomennudum, Published without a diagnosis or indication. Attributed to Bezzi).

Dacus insistens Curran, 1927. Bull. Am. Nus. Nat. Hist., 57: 85. Holotype♀Zaire(Bas-Zaire: Boma) (AMNH).

Dacus (Bactrocera) coccinae Premlata & Awtar Singh, 1987. J. Bombay. Nat. Hist.Soc., 84: 401. Holotype♂India (Chandigarh) (PUC).

Dacus (Afrodacus) coccinae: Agarwal *et al.*, 1992. J. Insect Sci., 5(1): 20.

Dacus (Didacus) ciliatus: Hardy, 1977. Cat. Diptera Oriental Reg., 3: 53.

Keys to identify the fruit flies associated with cucurbits in Bihar

1. Abdomen oval or scutum with vittae ……………………........................ 3 ....**(Genus *Bactrocera*)** - Abdomen petiolate and Orange colour .... 2 .... **(Genus *Dacus*)**

2. Scutum orange without vittae……………………………………………… - Yellow spot covering most of the katatergite…………… ***Dacus (Didacus) ciliatus*** Loew

3. Wing with large dark spot on apex……………………………………………….. 4

- dm-cu cross vein and cubital streak with darkening…….…………………………

………………………………***Bactrocera* (*Zeugodacus*) *cucurbitae*** (Coquillett)

4. Dark facial line just above mouth ……….…………………..…………………… 5

- Face with round black spot and three thoracic vittae..

…………………………………………***Bactrocera (Zeugodacus) tau*** (Walker)

5. Scutum dark black with three yellow vittae……………………………………….. 6

- Wing apex with black spot………...***Bactrocera (Zeugodacus) caudate*** (Fabricius)

6. Female with mustache like line on face ……………………………………………

- Male without facial line and pectin…..***Bactrocera (Hemigymnodacus) diversa*** (Coquillett)

7. Scutum black with two thoracic vittae…………………………………………… 8

- Femora of all legs dark black…………………………………………………… 8

8. Face dark black………………......***Bactrocera (Bactrocera) nigrofemoralis***White &Tsuruta

4.4 Genetic diveristy of *Bactrocera cucurbitae* through gene specific marker

Genomic DNA was isolated by CTAB method and purified using RNAse and Proteinase K treatment. Purified genomic DNA was analyzed with 1.5 % agarose gel and observed under UV transilluminator after staining with ethidium bromide. Intact DNA was visible as discrete band of high molecular weight.

The amplification of mitochondrial *cytochrome oxidase* genes from genomic DNA was carried out by polymerase chain reaction using (MCOX-II and MCOX-II) forward and reverse primers (Plate 4.9 and 4.10). The PCR amplified product obtained after 30 cycles were run on 1.5% agarose gel and observed under UV transilluminator. The expected product length of MCOX-I gene was 700 bp and the product obtained was nearly between 700 to 750 bp. The expected product length of MCOX-II gene was 600 bp and the product obtained was nearly between 500 to 600 bp (Plate 4.9 and 4.10).

Amplified product of Dumraon, Sabour, Nalanda and Motihari using MCOX-I gene were showing the similar pattern indicating that product length was near to 700-750 bp. Further, amplified product of Kishanganj was showing little variation with slightly heavier compare to others (Plate 4.9 and 4.10). Similar results were also found in case of MCOX-II gene and its most of the amplified band shows the similar pattern except Kishanganj. Their product length was observed between 500-600 bp. There were not showing much difference in the banding pattern but very small difference noticed in the bands that gave very small information regarding diversity.

Plate 3.1 Collection of Samples using Cuelure Trap and Infested Fruits

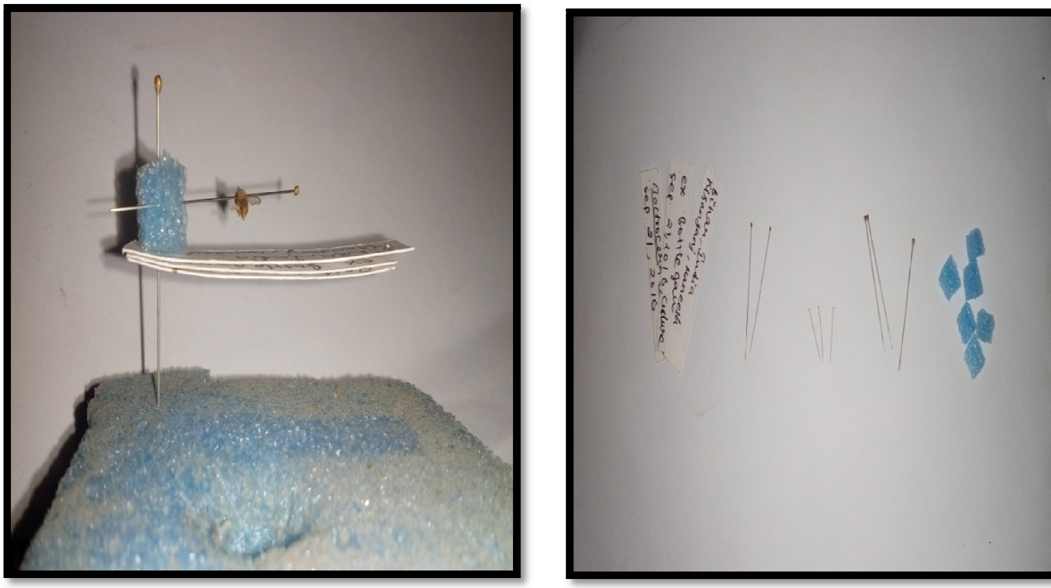
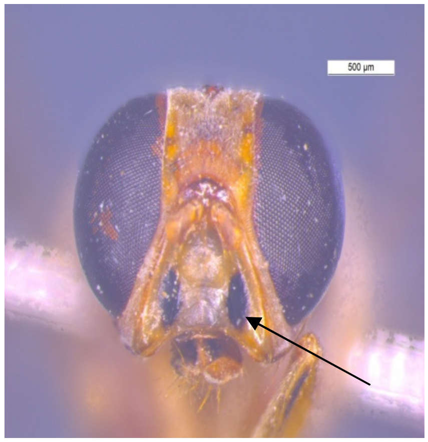


Plate 3.2 Pinning of specimen and Pinning Material

Plate 3.3 Plastic water bottle Trap

Plate 4.1 A – infested fruit of spine gourd, B – dorsal view of *Dacus ciliatus*, C, F – infested bottle gourd D, E, – infested bitter gourd G – collected fruit flies, H – bottle trap

Plate 4.2 M, O – infested bottle gourd, K, P – infested bitter gourd, I, J – stock culture, L – infested cauliflower, N- infested Bitter gourd field



Face **Thorax**

Wing **Abdomen**

Lateral view **Male Genetalia**

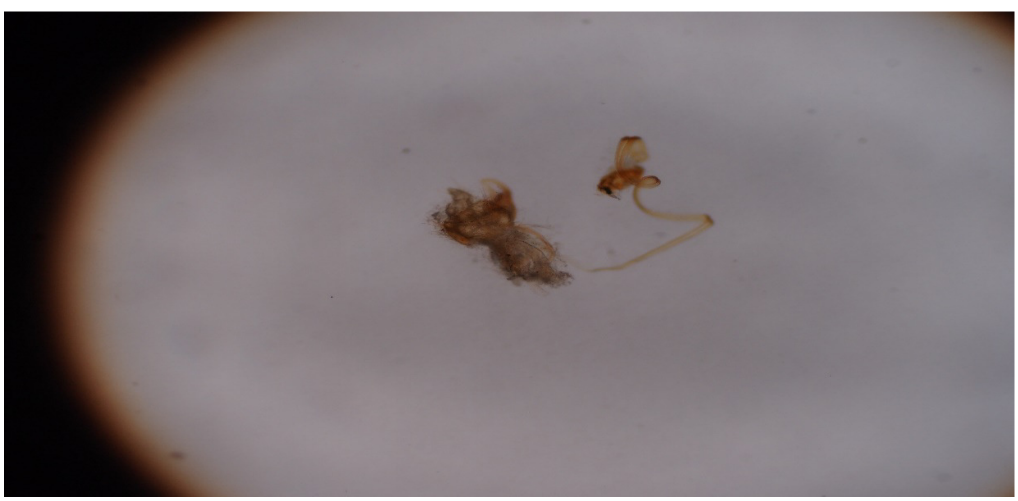
Plate 4.3 Morphographs of *Bactrocera* (*Zeugodacus*) *cucurbitae* (Coquillett)

Face

Thorax

Abdomen

Wing



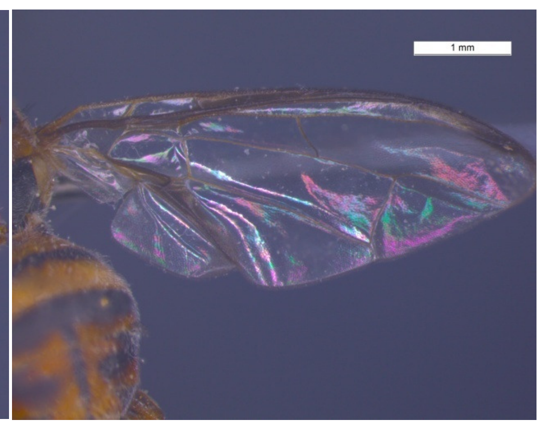
Male Genetalia

Plate 4.4 Morphographs of *Bactrocera (Zeugodacus) tau* (Walker)

Face

Thorax

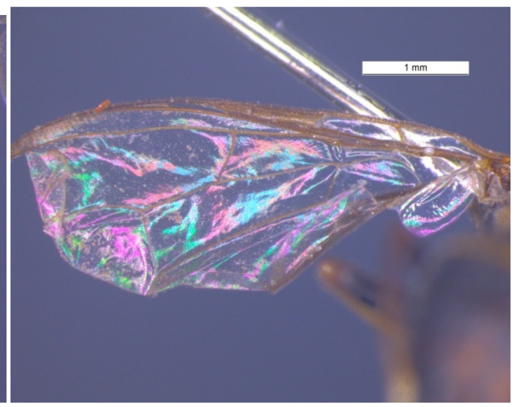
Abdomen



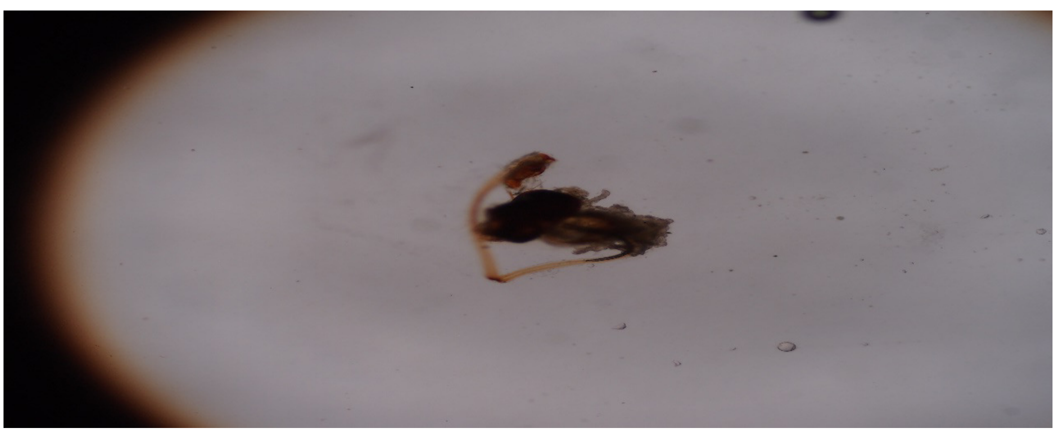
Wing

Plate 4.5 Morphographs of *Bactrocera (Zeugodacus) caudata* (Fabricius)

Face **Thorax**



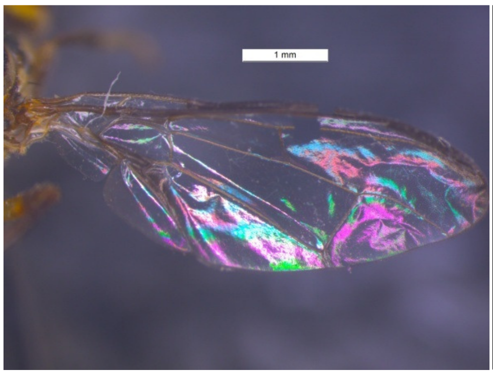
Abdomen **Wing**

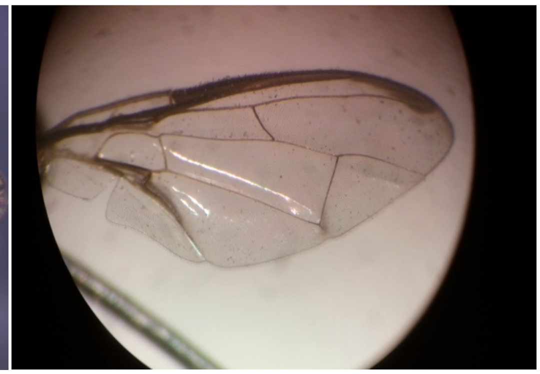


Male Genetalia

Plate 4.6 Morphographs of *Bactrocera (Bactrocera) nigrofemoralis* White & Tsuruta

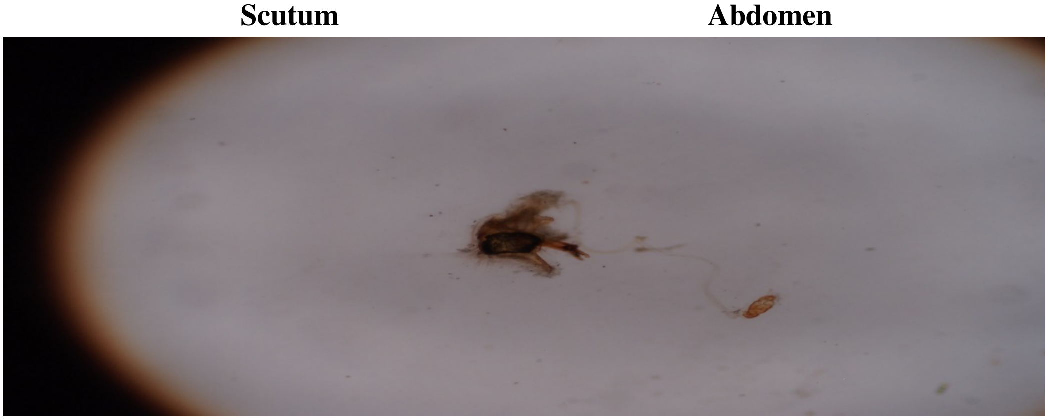
Face **Thorax**





Wing

Scutum **Abdomen**



Male Genetalia

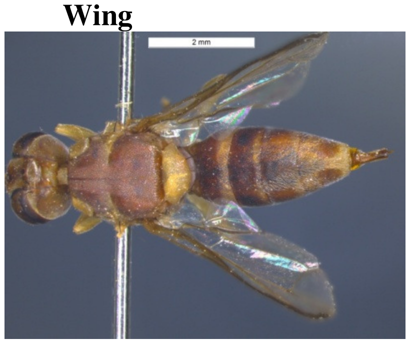
Plate 4.7 Morphographs of *Bactrocera (Hemigymnodacus) diversa* (Coquillett)

Face

Scutum

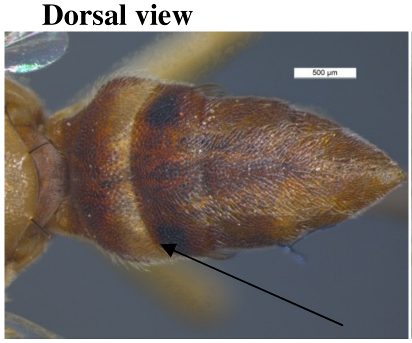
Wing

Female Terminalia



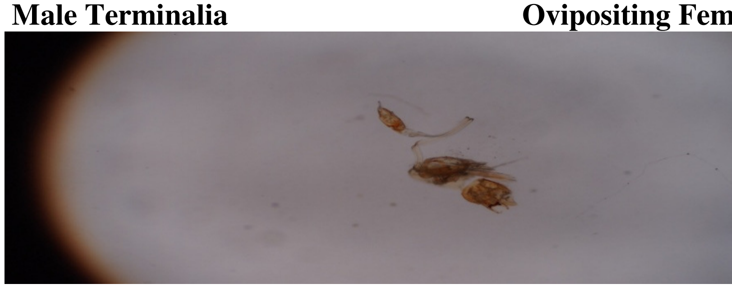
Dorsal view

Lateral view



Male Terminalia

Ovipositing Female



Male Genetalia

Plate 4.8 Morphographs of *Dacus (Didacus) ciliatus* Loew

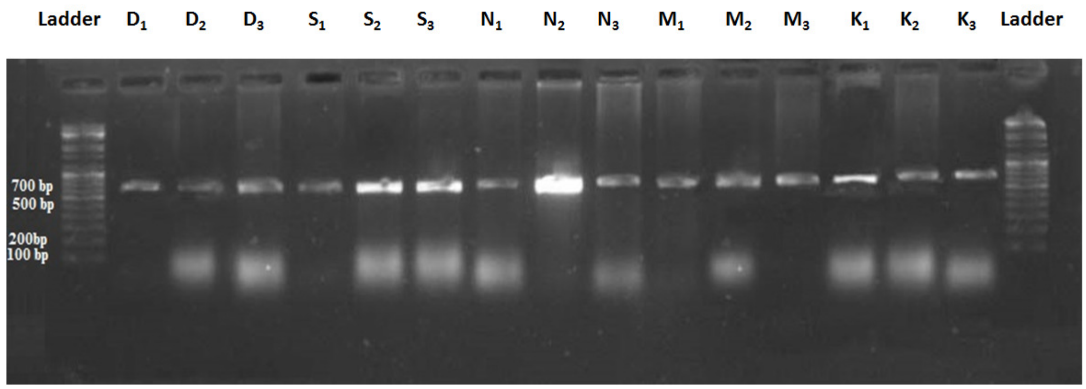


Plate 4.9 PCR Product of Cytochrome oxidase subunit 1 using MCOX-I primers ($D_1D_2D_3$ are the DNA samples were taken from the Dumraon, S_1 S_2 S_3 are the DNA samples were taken from the Sabour, $N_1N_2N_3$are DNA samples were taken from the Nalanda, $M_1M_2M_3$ and $K_1K_2K_3$ were taken from Motihari and Kishanganj respectively)

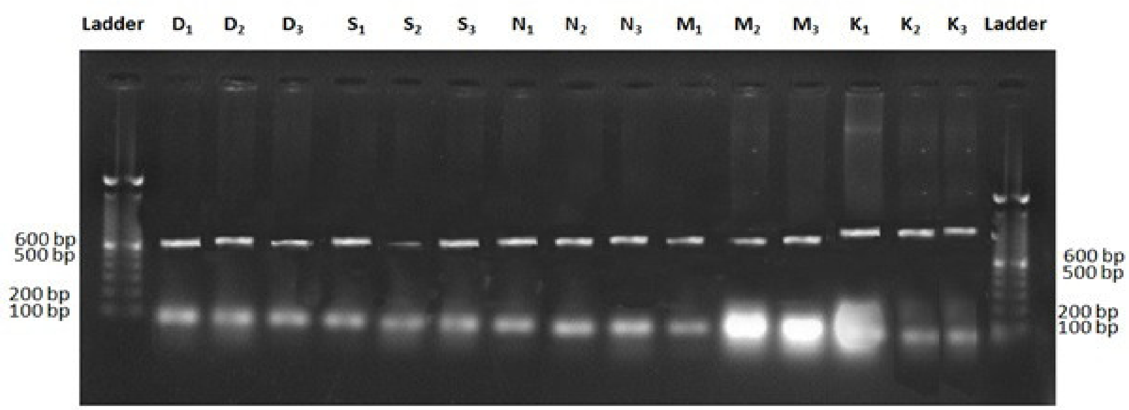


Plate 4.10 PCR Product of Cytochrome oxidase subunit 1 using MCOX-II primers

($D_1D_2D_3$ are the DNA samples were taken from the Dumraon, S_1 S_2 S_3 are the DNA samples were taken from the Sabour, $N_1N_2N_3$are DNA samples were taken from the Nalanda, $M_1M_2M_3$ and $K_1K_2K_3$ were taken from Motihari and Kishanganj respectively)

DISCUSSION

The discussion regarding the experimental finding is described here under the following sub headings:

Fruit flies are one of the most amazing insects under the order Diptera and the family Tephritidae. It is the largest family under the order Diptera comprising of nearly 4500 species. This family can be distinguished from the other families of Diptera by the combination of well developed mesoclinate frontal setae and subcostal vein bent sharply anteriorly at right angle before the apex, weakened or evanescent beyond the bend. In addition, the costa has three breaks viz., costal, humeral and subcostal, vein R1 dorsally with setulae; wing usually with colour pattern; cell bcu usually with an acute extension.

Approximately 243 species of fruit flies have been so far recorded from India (Agarwal and Sueyoshi 2005). Three species *viz*. melon fly, *Bactrocera cucurbitae* (Coquillett), pumpkin fly, *Bactrocera tau* (Walker) and lesser pumpkin fly, *Dacus ciliatus* (Loew) are the major economically important fruit fly species on cucurbits.

5.1 Morphological identification of fruit flies

Experimental finding revealed that in Bihar, major species that attacks mainly cucurbits was *B. cucurbitae* and during summer especially *B. cucurbitae* attacks on pointed gourd with a complex species, *Dacus ciliatus*. *D. ciliatus* also infests other cucurbits *viz*. pumpkin and bitter gourd but also found that it prefers pointed gourd.

B. cucurbitae is a light brown yellow coloured fruit fly easily recognized by the presence of three thoracic vittae, Costal band with large apical spot, almost covering apical part of cells r_{2+3} and r_{4+5}; cross veins R-M and DM-Cu thickly infuscated. While, *B. tau* was identified on the presence of three thoracic vittae and costal band dark and expanded into spot near apex of cell r_{2+3} extending across vein $R_{4+5}$with slightly darker than the *B. cucurbitae*. However, *B. caudata* was not easily recognised because it comes under tau complex and differentiates from *B. tau* by the face without brown or black spot in antennal furrow, with black line across mouth opening; costal band thinner and slightly expanded at vein R_{4+5}.

B. nigrofemoralis and *B. diversa* both are black in colour approximately similar in size but can be easily distinguished from each other by presence of moustache like line on the face of *B. diversa* but in case of *B. nigrofemoralis*, face have no marking or lines and face of *B. nigrofemoralis* slightly dark. It was also recognized by a narrow fuscous costal band confluent with R_{2+3} and remaining very narrow around costal margin to end at apex of R_{4+5}. Males of *B. diversa* have no lines on the face and abdominal pecten also absent in it and costal band not expanded near apex of vein R_{4+5}.

D. ciliatus is predominantly orange with black facial spots; a yellow spot covering most of the katatergite, anatergite orange, midfemur orange yellow, and wing with costal band extremely narrow in cell r_{2+3} but dilated apically near and beyond vein R_{4+5}, abdominal tergites fused to form hemispherical capsule, with oval black spots on either side of third tergite.

Kapoor (2005) also found the same situation and he reported that during the hot summer months (*i.e.*, April-June), *B. cucurbitae* attacks on cucurbits *often* occur together with *Dacus (Didacus) ciliatus Loew*. Nowadays dominance of *B. cucurbitae* has not allowed to increase in number, but recent observations in certain northern and central territories of India have observed that *D. ciliatus* has begun to dominate over *B. cucurbitae and* it started to increase in number.

Beside these two, other fruit flies were also causes considerable damage and *B. diversa* was still not reported from cucurbits in Bihar. Same as in case of *D. ciliatus*, it was still not reported from pointed gourd in Bihar. Earlier Agrawal (1984) reported that *B. diversa* from citrus and *Eugenia jambolana* and *D. ciliatus* from *Cephalenra indica*. *B. nigrofemoralis* was still not reported from Bihar. In earlier literature Agarwal and Sueyoshi (2005) reported that *B. nigrofemoralis* from Kerala, Karnataka and Tamilnadu. Prabhakar *et al* (2012) also reported from Himachal Pradesh. They found that *B. cucurbitae*, *B. tau*, *B. diversa* and some other important species of tephritids from Himachal Pradesh.

Ganie *et al* (2013) was also reported that *B. cucurbitae* and *B. tau* from the Kashmir valley. Drew and Raghu (2002) found that *B. cucurbitae*, *B. tau, B. nigrofemoralis*, and *B. caudata* from the Amrambalam forests of Western Ghat.

5.2 Genetic diversity of *Bactrocera cucurbitae*

As per the earlier reports, the primer used in this study could amplify expected product length of 700 bp in case of MCOX-I gene, while the product obtained was nearly between 700 to 750 bp. The expected product length of MCOX-II gene was 600 bp, while the product obtained was nearly between 500 to 600 bp. The amplified product of Dumraon, Sabour, Nalanda and Motihari using MCOX-I gene shows the similar pattern means the product length was very similar, which is 700-750 bp. Amplified product of Kishanganj shows less variation with slightly heavier than the others. Similar result also occurs in case of MCOX-II gene and its most of the band shows the similar pattern except Kishanganj. Their product length was observed between 500-600 bp. Results were not showing much difference but very small difference noticed in bands that gave very small information regarding diversity. On the basis of band pattern of MCOX-I and MCOX-II genes of *B. cucurbitae* collected from different regions of Bihar, it may be confirm that there might be slight variation in the amplified region which could be further confirmed through sequencing of isolated genes and further analysis of those genes using bioinformatics tools.

In this study, the variable region of the mitochondrial cytochrome oxidase subunit I (COI) gene was used to obtain better estimates of divergence from two species of the *B. dorsalis* complex. The COI gene was used in this study because it appears to be among the most conservative protein-coding genes in the mitochondrial genome of animals (Brown, 1985). The COI gene was the slow-evolving gene in the mitochondrial protein coding gene (Simon *et al*., 1994). The conserved sequence of COI gene allow the researchers to use it as a 'universal' primers, and it has been widely used to investigate multiple different taxa and for interspecific analysis.

Prabhakar *et al* (2012) reported that the *mtCOI-I* based identification of thirty three (33) *B. cucurbitae* individuals collected from 20 locations with universal primer pair (UEA7 and UEA10) and it was amplified in 611 bp region of cytochrome oxidase gene.

Abdullah (2012) analyzed the genetic diversity of *Bactrocera dorsalis* using the same mtCOI-II gene and obtained 570 bp regions after PCR amplification. The above result shows that there was significant diversity in mtCOI gene between different *Bactrocera* species. In this study, it was amplified that 700-750 bp of mtCOI-I and 500-600bp of mtCOI-II gene of different species of *B. cucurbitae* collected from different agroclimatic region of this state and it reveals that a significant genetic diversity among the *B. cucurbitae* species.

Zhang *et al* (2010) studied 689 bp nucleotide sequences of the mitochondrial *cytochrome oxidase I* gene of thirty-five individuals represented seven *Bactrocera* species found in the Chongqing region of China and sequences submitted to GenBank for another 20 *Bactrocera* species and 2 tephritid species, *Anastrepha ludens* and *Ceratitis capitata,* which were used as outgroups for the phylogenetic analysis.

Asokan *et al* (2007) reported that *mtCOI* based identification of three fruit flies namely *B. dorsalis*, *B. correcta* and *B. zonata* and their molecular identification has corroborated the morphological identification. A single fragement of approximately 500 bp was amplified for *B. dorsalis, B. correcta* and *B. zonata*.

SUMMARY AND CONCLUSION

The results obtained in the present investigation entitled "Morphological identification of cucurbitaceous fruit flies in Bihar and genetic diversity of *Bactrocera cucurbitae*" are summarized here under:

- As we know Bihar is one of the states of India growing lot of cucurbits and the main reason behind it is situated near Indo gangetic plains that provide better production of cucurbits in this region. Cucurbits are one of the major vegetable crops not only in Bihar but also in India. They are attacked by several insect pests. The fruit fly is one of the most serious pests of cucurbits causing 30-100% yield loss.
- In Bihar, *Bactrocera cucurbitae* and *Dacus ciliatus* were observed to be serious on cucurbits. However, *B. cucurbitae* has more host range than the other fruit flies in cucurbits.
- In Bihar, mean per cent infestation of fruit flies was recorded to be 58.89 per cent in cucurbits. The maximum infestation of 81.19 per cent was recorded at Sabour (Bhagalpur) and minimum of 31.05 per cent at Motihari (East Champaran).
- In the present study, six species of tephritid fruit flies from two genera were recorded namely, *Bactrocera cucurbitae, B. tau, B. caudate, B. ngrofemoralis, Dacus ciliatus* and *B. Diversa*.
- One fruit fly species was identified for the first time from Bihar *i.e.* *Bactrocera nigrofemoralis* White & Tsuruta, from subfamily Dacinae and it was not previously reported from Bihar. It was found in large number from cucurbits field of Bihar region.
- Pointed gourd and flowers of pumpkin were recorded as new host crop of *D. ciliatus* and *B. diversa* for the first time from Bihar.
- There are chances of presence of more fruit flies associated with cucurbits in Bihar region that need more extensive study and survey. Because in Bihar diversity of cucurbitaceous crop is very large that means there is probability of association of more species of fruit flies with cucurbits.

- Fifteen isolates of *B. cucurbitae* collected from different agroclimatic zones of Bihar, were genetically characterized with *mtCOI* gene and on the basis of gel picture diversity was interpreted.
- Amplified product of Dumraon, Sabour, Nalanda and Motihari using MCOX-I gene shows the similar pattern means product length very similar that near to 700-750 bp, amplified product of Kishanganj shows slightly variation that it was slightly heavier than the others. Same result also occurs in case of MCOX-II gene and most of the band shows the similar pattern except Kishanganj. The product length was 500-600 bp. Results were not showing so much difference but very small difference noticed in a band that gives very small information regarding diversity.
- Information obtained using gel documentation picture reveals that some level of diversity which would be very useful for further study and may provide complete information regarding diversity after sequencing.

The present study provided valuable information regarding the species diversity of fruit flies associated with cucurbits in the state of Bihar. A total of six species of fruit flies were associated with cucurbits namely *Bactrocera cucurbitae, B. tau, Dacus ciliatus*, *B. nigrofemoralis B. caudate* and *B. Diversa* and further they were identified based on key provided by White and Elson-Harris (1992); Kapoor (1993); Drew and Raghu (2002). *B. nigrofemoralis* (White and Tsuruta) will be first time report from Bihar and regarding new host is concerned, pointed gourd and flowers of pumpkin were recorded as new host crop of *D. ciliatus* and *B. diversa* for the first time from Bihar. Therefore, the present study provided a platform to make aware of proper management practices of different species of fruit flies and also provides the level of biodiversity of tephritids in Bihar. Genetic diversity of *B. cucurbitae* was also studied among the different zones of Bihar and found that there were not showing so much difference very small difference occurs in a band that gives very small information regarding diversity.

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